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**High energy density nutritional supplements; impact
on appetite, appetite regulation and energy intake in
underweight and malnourished individuals**

by

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Doctor of Philosophy

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College of Medicine, Veterinary and Life Sciences

School of Medicine

Abstract

This thesis describes the impact of high energy density nutritional supplement drinks (HENSsDs) on appetite regulation, energy intake and cardiovascular risk factors in lean healthy females. It also explores the impact of Solid Ready-To-Use Foods (RTUF) and a milk based Liquid Ready-To-Use proprietary Supplement (LRUS) on weight gain and appetite in mild to moderate underweight children from Pakistan. The thesis consists of a literature review (Chapter 1), general methods (Chapter 2), three experimental chapters (Chapter 3- Chapter 5), each describing an independent research study, and a general discussion and conclusion chapter (Chapter 6).

Accumulating evidence suggests that oral HENSsDs increases energy intake and are beneficial for the treatment of malnutrition. Their effectiveness however, may be diminished by acute suppression of appetite. Therefore, the first experimental study aimed to investigate the extent to which the consumption of the HENSsD in the fasted state reduces energy intake during a consecutive breakfast and lunch and whether this reduction relates to changes in appetite and metabolic appetite regulators. Twenty three young females with BMI of $18.2 \pm 0.8 \text{ kg/m}^2$ consumed either a HENSsD or a low energy drink (PLACEBO) after fasting, in a single blind randomized cross-over study. Appetite was tracked, and blood taken, prior to the intake of the supplement and 240 minutes afterwards. Energy intake was recorded during an *ad libitum* buffet breakfast served 60 minutes and an *ad libitum* buffet lunch served 240 minutes post supplementation. Energy intake during the breakfast was significantly higher in the PLACEBO than in the HENSsD trial. No significant difference was found in energy intake during the lunch between the two trials. When energy provided by supplements was added to energy intake during breakfast and lunch, the energy intake in the HENSsD trial was significantly higher. The net effect was that total energy intake was increased by $1.07 \pm 0.34 \text{ MJ}$ in the HENSsD trial. During the pre-breakfast, feelings of hunger and a desire to eat were significantly lower; satiety and fullness were significantly higher in the HENSsD trial. After breakfast, none of the appetite measures differed between the trials regardless of plasma PYY, CCK, and insulin concentrations being significantly higher in the HENSsD trial.

The second experimental study investigated the time scale of compensation after HENSsD supplementation. Over a five day period, energy intake was measured after the supplementation during the evening meal, and during the *ad libitum* breakfast, lunch and

dinner consumed on the consecutive day. Since, consumption of HENSD due to the promotion of energy was expected to promote positive energy balance, this study also aimed to investigate the impact of HENSD supplementation on cardio-metabolic risk factors. Twenty-three young healthy females with a BMI of $18.7 \pm 1.2 \text{ kg/m}^2$ participated in a single blind randomised, controlled, crossover study. Participants consumed either HENSD or a PLACEBO for five days in the evening. Participants were asked to record their dietary intake during the days of supplementation. On the sixth day plasma lipids, insulin and glucose concentrations were measured in the fasted state and at 30, 60, 90 and 120 minutes after the *ad libitum* buffet breakfast and lunch. The findings showed that the average daily energy intake was significantly higher in HENSD trial and that consumption of HENSDs in the evening induced immediate and short-lasting reduction in energy intake. Fasting plasma concentrations of insulin and HOMA_(IR) were significantly higher in the HENSD as compared to the PLACEBO trial. No significant differences were detected in fasting plasma concentrations of TAG, total-, HDL- and LDL-cholesterol between the HENSD and the PLACEBO trials.

The third experimental study explored the efficacy of RTUFs and LRUS in promoting weight gain and their effect on appetite regulation in mild to moderate malnourished children. An open labelled randomized controlled trial was conducted in primary schools of Pakistan. Sixty eight mild to moderate underweight children aged 8.2 ± 1.2 years were randomly allocated to receive either RTUF or LRUS providing 500 kcal/ day in addition to regular diet in their school for four weeks. The children's height, weight, and skinfolds were measured before supplementation and at the end of the supplementation. The children marked visual analogue scale questionnaires before the provision of the first and the last supplement. The findings from this study indicated that after four weeks of supplementation the average weight gain, change from the baseline in weight-for-age Z score (WAZ), height-for-age Z score (HAZ) in the RTUF and LRUS were not significantly different between the two groups. The difference in the appetite measures before the provision of the first and the last supplement between the two groups were also not significantly different. The total extra energy supplied for 4 weeks would have been expected to lead to an excess gain of 2kg. Thus, at least 2/3 of the energy ingested appeared to have been compensated by less intake at other times.

Based on the data obtained the following conclusions have been drawn:

- Following oral intake of HENSIDs, the appetite suppressive action of the metabolic and hormonal appetite modulators is short lived.
- HENSIDs consumption for five consecutive days in the evening induced compensation, which happens immediately, disappears quickly and is short-lived which allows only partial compensation for the energy provided by HENSID.
- Short-term supplementation with HENSID is safe in relation to the impact on cardiometabolic risk factors such as plasma concentration of fasting and postprandial lipids but can be expected to reduce insulin sensitivity.
- RTUF and LRUS given to the community has similar impact on improving the nutritional status in mild of moderate underweight children but the overall rate of weight gain was lower than expected.

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Author's Declaration

I declare that the work contained in this thesis is original, and is the work of one author, Dr Sadia Fatima except where otherwise stated. The information reported from other authors has been quoted with their name and source of publication. The relative contributions in terms of study design, data collection and analysis have been highlighted at the beginning of each research chapter.

Some of the results presented in the experimental chapter of this thesis have been published as follows:

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Fatima, S., Gerisimidis, K., Tsiountsioura, M., Arvantidou, I., Wright, C. & Malkova, D. (2012). Impact of high energy nutritional supplement drinks on energy intake, appetite measures, appetite hormones, and rate of gastric emptying. Yorkhill Research Day, Glasgow, UK, 9th November 2012.

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Fatima, S., Gerisimidis, K., Tsiountsioura, M., Arvantidou, I., Wright, C. & Malkova, D. (2013). Impact of high energy nutritional supplement drinks on energy intake, appetite measures, appetite hormones, and rate of gastric emptying. ESPEN 2013 Congress. Leipzig, Germany, 31st August – 3rd September 2013.

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List of Abbreviations

BMI	Body Mass Index
HENSD	High Energy Nutritional Supplement Drinks
WHO	World Health Organization
WHZ	Weight- for-height Z score
WAZ	Weight-for-age Z score
MAM	Moderate Acute Malnutrition
RTUF	Ready to use food
LRUS	Liquid Ready To Use Supplement
CCK	Cholecystokinin
PYY	Peptide YY
DIT	Diet Induced Thermogenesis
AUC	Area under the curve
EE	Energy Expenditure
CHO	Carbohydrates
CVD	Cardiovascular Disease
NEFA	Non-esterified Fatty Acids
TAG	Triglycerides
HDL	High Density Lipoproteins
VLDL	Very Low Density Lipoprotein
FFA	Free Fatty Acids
LDL	Low Density Lipoprotein
VAS	Visual Analogue Scale
RMR	Resting Metabolic Rate
VO ₂	Oxygen uptake
VCO ₂	Carbon dioxide production
ELISA	Enzyme-linked Immunoassay
HOMA _{IR}	Homeostasis Model Assessment of Insulin Resistance
IR	Insulin Resistance

1 Introduction and Literature Review

1.1 Malnutrition

1.1.1 Prevalence, causes and consequences

Malnutrition is a condition in which nutrients such as proteins, vitamins, minerals and energy are deficient or in excess (imbalance). This causes severe measurable adverse effects on body composition, function and clinical outcomes (Meier and Stratton 2008, Nieuwenhuizen, Weenen *et al.* 2010). There are the controversies and confusion regarding the definition of malnutrition and its recognition. There is no universally accepted definition of malnutrition (Alberda, Graf *et al.* 2006). According to the World Health Organization (WHO) malnutrition is defined as “*The cellular imbalance between the supply of nutrients and energy and the body’s demand for them to ensure growth, maintenance, and specific functions*” (Alberda, Graf *et al.* 2006).

It is suggested that for defining malnutrition, deficiencies of energy, protein, fat free mass, and function should be included, and for the operational definition BMI, involuntary weight loss and nutritional intake should be incorporated. Malnutrition can be explained as either over or under-nutrition with inflammation (Meijers, van Bokhorst-de van der Schueren *et al.* 2010). In this context, we are using under-nutrition. The WHO cites malnutrition as the single greatest threat to the world’s public health (Nieuwenhuizen, Weenen *et al.* 2010). One out of twelve people is malnourished worldwide. According to estimates, 148 million children in the world are underweight, out of which 78 million are from South Asia and 36 million are from Sub-Saharan Africa. Approximately 19 million are severely malnourished (Walton and Allen 2011). Undernutrition is a direct cause of about 300,000 deaths per year and is indirectly responsible for about half of all the deaths in young children

(Nemer, Gelband *et al.* 2001, Black, Morris *et al.* 2003, Müller, Garenne *et al.* 2003).

Developed world

Malnutrition is a common condition affecting millions of individuals in the developed world. Its victims are not age specific. In hospitalized patients, almost 50% are affected by under-nutrition, and the incidence further increases with an increase in the duration of hospital stay. Fifty-five percent of elderly people are undernourished when they are hospitalized (Milne, Avenell *et al.* 2006). In Australia, the rate of malnutrition in hospitals is between 30-40% (NICE 2006). In the UK more than 3 million people are malnourished or at risk of malnutrition of which 1.3 million people were aged over 65 years (Elia, Russell *et al.* 2010, Cawood, Elia *et al.* 2011). Ninety-three percent of these malnourished people are living in the community, 5% in care homes and 3% in hospitals or in other NHS settings (Elia, Russell *et al.* 2010, Stratton and Elia 2010).

Hospital admissions at risk of malnutrition range from 10-60% and 15-30% of outpatients are at risk of malnutrition (Stratton and Elia 2010). The risk of death is directly related to the degree of malnutrition (Pelletier, Frongillo Jr *et al.* 1993, Black, Morris *et al.* 2003, Müller and Krawinkel 2005). Disease related malnutrition is prevalent across all health settings, including: hospitals, social care settings and sheltered homes (Cawood, Elia *et al.* 2011). Over the last ten years the prevalence of acute malnutrition in hospitalized children in the UK, France, Germany and USA varied from 6-14% while in Turkey its prevalence was up to 40% (Joosten and Hulst 2011). 37% of the institutionalized elderly in European countries are malnourished (Manders, de Groot *et al.* 2009). According to a campaign Fight Against Malnutrition, it is estimated that about 5% of the European Union population is at risk of developing malnutrition (Ljungqvist, van Gossum *et al.* 2010). The British

Association of Parenteral and Enteral Nutrition (BAPEN) estimate that this corresponds to 20 million people (Ljungqvist, van Gossum *et al.* 2010).

It has been estimated that disease-related malnutrition costs more than £13x 10⁹ annually to the UK (Stratton 2005, Elia and Russell 2009, Elia and Stratton 2009, Elia, Russell *et al.* 2010, Stratton, Hébuterne *et al.* 2013). In several countries around Europe, for example, the Netherlands, Ireland and Germany, similar calculations have been done at the national level (Rice and Normand 2012). In England and Wales, in 2007, the cost of nutrition prescriptions was £157x10⁶. Of this, about £99x10⁶ was for oral nutritional supplements (Stratton and Elia 2010). On average the under-nourished hospitalized patients incurred 19% more cost to the hospital as compared to the well-nourished patients. Some studies have found that the treatment cost associated with malnutrition is 75% or even 300% higher (Nieuwenhuizen, Weenen *et al.* 2010). According to one study it was found that the treatment of malnourished patients costs more than twice that of treating non-malnourished patients (Guest, Panca *et al.* 2011).

Developing world

Malnutrition is a highly pervasive and damaging condition in low and middle income countries, and is prevalent in community settings (Black, Allen *et al.* 2008). Childhood malnutrition and under nutrition is common in low and middle-income countries. According to UNICEF 129 million children (25%) under 5 years of age in these countries are underweight and approximately 195 million children (28%) are stunted (Imdad, Sadiq *et al.* 2011). According to the UN, malnutrition kills 10 children every minute (Blössner and de Onis 2005). WHO estimates, that by 2015, the worldwide prevalence of malnutrition will be 17.6%, and 29% of the population will have stunted growth due to under nutrition. Not surprisingly in the low income countries 112 million (20%) out of 556 million children under 5 years of age are underweight and 36 million (6.4%) are suffering from moderate wasting

(Golden 2009, Michaelsen, Hoppe *et al.* 2009). The data from 139 countries showed that about 10.2 % of the deaths are attributable to wasting, and wasted children have a three times higher risk of death compared to well nourished children (Black, Allen *et al.* 2008, Huybregts, Houngré *et al.* 2012). South Central Asia has the highest estimated point prevalence (19%) of moderate acute malnutrition, and the highest absolute number of affected children (30 million) (Lazzerini, Rubert *et al.* 2013). In South Asian countries, the most prevalent public health issue is malnutrition among children under five years of age. It is documented that more than 50 percent of the world's malnourished children are residing in Pakistan, India and Bangladesh (Gillespie and Haddad 2003, Hirani 2012).

The major underlying cause of malnutrition and its determinants is poverty (Müller and Krawinkel 2005, Imdad, Sadiq *et al.* 2011). In a given population the degree and distribution of malnutrition depends upon many factors, such as socio-economic, political, seasonal, climatic, sanitation conditions, educational level, food production, prevalence of infectious diseases, breast feeding habits, and non-availability of health services (Brabin and Coulter 2003, Young, Borrel *et al.* 2004, Müller and Krawinkel 2005, Grover and Ee 2009, Imdad, Sadiq *et al.* 2011). Poor dietary intake along with repeated infections especially in the underprivileged population is the main contributor to malnutrition. Inadequate calorie intake due to less dietary intake or decreased diet assimilation, stress due to critical acute illness, or chronic inflammation extensive burns or postoperative sepsis, gastrointestinal disease, mixed metabolic abnormalities like AIDS, cancer or chronic liver diseases are considered to be important etiological factors for malnutrition (Alberda, Graf *et al.* 2006, Imdad, Sadiq *et al.* 2011).

Despite the high global prevalence of malnutrition, it remains under-treated, or undetected. This causes an enormous detrimental effect on the health of each individual and imposes a financial burden on both the individual and the health care system (Cawood, Elia *et al.* 2011). Malnourished children are at increased

risk of mortality and morbidity as compared to well-nourished children. There is an increased risk of death with increasing severity of malnutrition (Imdad, Sadiq *et al.* 2011).

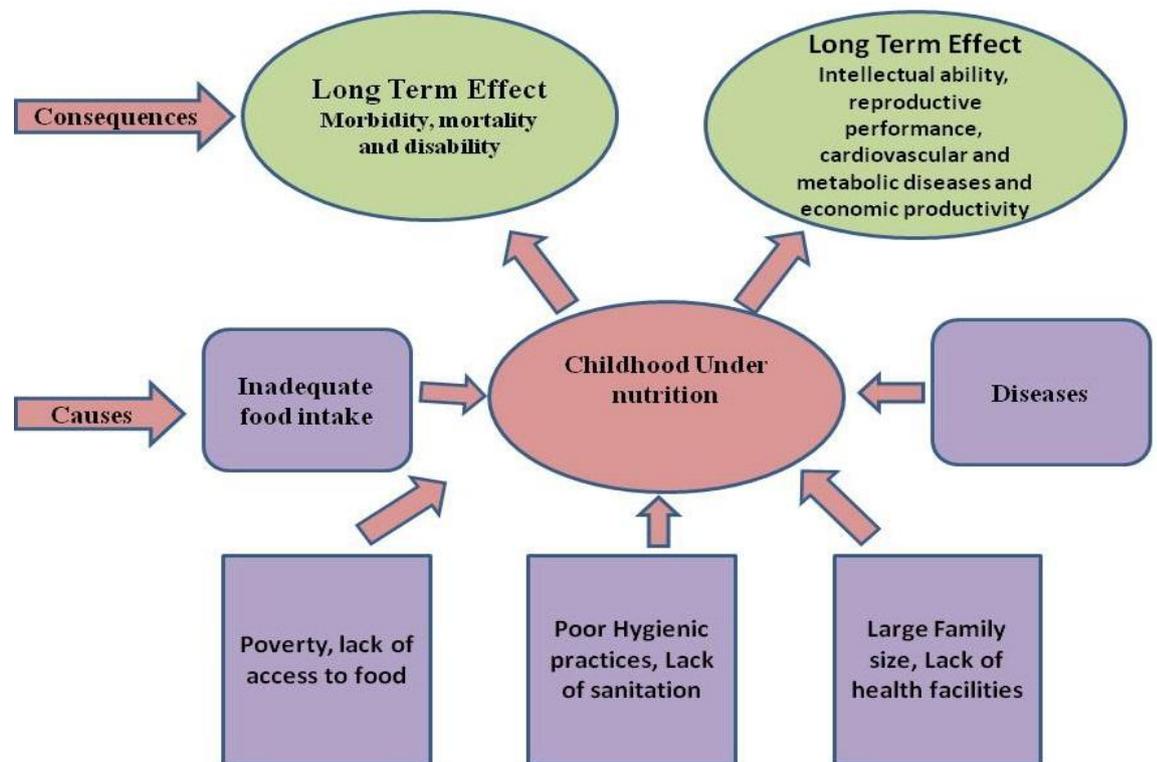


Figure 1.1: Causes and consequences of malnutrition

Malnutrition predisposes the body to the risk of different diseases, and also causes severe adverse outcomes of disease in a variety of ways (Cawood, Elia *et al.* 2011). Disease related malnutrition is very harmful physiologically and clinically, by delaying recovery from illnesses and impairing the quality of life (Stratton and Elia 2007). If malnutrition is not treated or inefficiently treated, it will lead to poor quality of life of the patients, increase complications, delay recovery from diseases, result into more use of healthcare facilities, more health care expenses and more rehabilitation needs (Alberda, Graf *et al.* 2006, Nieuwenhuizen, Weenen *et al.* 2010, Stratton and Elia 2010, Cawood, Elia *et al.* 2011). Malnutrition leads to severe adverse metabolic events which compromise the body's immunity, impairs body function, composition and its ability to acclimatize, recover and to survive (Alberda, Graf *et al.* 2006,

Cawood, Elia *et al.* 2011). Malnutrition also has severe adverse effects on the cognitive development of children, decreased productivity, reduced ability to work and small adult physique (Imdad, Sadiq *et al.* 2011). Taking into consideration the enormous costs of malnutrition, a condition that is largely preventable and treatable, a timely identification followed by the most suitable, effective, efficient, evidence-based treatment is recommended (Stratton and Elia 2010).

1.1.2 Nutritional supplements and their use in the treatment of malnutrition in developing countries

Interventions to prevent protein energy malnutrition include food supplementation, dietary diversification and fortification of salt with iodine (Müller and Krawinkel 2005). Other preventive measures include maternal nutritional education, reduction in the price of food items, high immunization coverage and correct management of infectious diseases (Müller and Krawinkel 2005). In the majority of cases of malnutrition, energy intake is low therefore supplements may be given to provide extra energy and macronutrients which in turn increases the body weight (Lawson, Doshi *et al.* 2000).

Supplements are products which are used in conjunction with a diet containing vitamins, minerals, proteins and amino acids to enhance the diet (Maughan, Depiesse *et al.* 2007). Oral nutritional supplements are usually multinutrient mixtures containing macronutrients (proteins, carbohydrates and fats) and micronutrients (minerals, vitamins and trace elements). The oral nutritional supplements are energy dense, mostly containing 1.5kcal/ml to 2.4kcal/ml (Stratton and Elia 2010). They are usually available in liquid form or in powder form which is mixed with whole milk before drinking (Stratton and Elia 2010). Usually, the supplements available in liquid form are ready to use. Health Care professionals find them convenient to administer in hospital and community settings (Stratton and Elia 2010). A variety of supplements are available which may be nutritionally complete or incomplete (lacking some of

essential micronutrients or fatty acids) and may be modular (containing only one or two energy sources). Specialised supplements are also available for treating malnourishment in specific diseases such as cancer, diabetes, hepatic or renal diseases (Stratton and Elia 2007). High-energy nutritional supplement drinks (HENSsDs) have substantially higher energy content and are supplemented with a large variety of micronutrients. For example, Scandishake[®], a mainstream dairy HENSsD has 2.5 kcal/ml, 5% protein, 13% fat, and 61% carbohydrates, 230 mg of phosphorus and an osmolarity of 880 mOsmol/L.

In the 1960's supplements were first used for prevention of malnutrition in Guatemala, where children under 3 years of age, and pregnant women, were provided with either a moderate energy supplement (90kcal/ 100 ml), or, a low energy supplement (33kcal/100 ml). It was found that the nutritional interventions were associated with greater improvement in height, weight and mental development of the children (Rivera and Habicht 2002). In low and middle-income countries, moderate acute malnutrition was treated by providing food supplements to improve dietary habits (Lazzerini, Rubert *et al.* 2013). Two main categories of food supplements include lipid based nutrient supplements (with high lipid content as ready- to- use foods and blended food supplements which are food mixtures for example corn-soy blends, or wheat-soy blends that can be cooked at home to make porridge or soup (Lazzerini, Rubert *et al.* 2013).

According to the WHO, recommendations for the treatment of malnutrition, during the nutritional rehabilitation phase the children should receive an energy and protein dense diet fortified with vitamins and minerals, to promote rapid weight gain. For this purpose, a solid, ready-to-use-food (RTUF), has been developed which is made up of peanut butter (Diop, Dossou *et al.* 2003). RTUF was developed as an alternative to the F-100 formula or milk oil formula to be used in the hospitals and nutritional rehabilitation centres after initiation of cure (Brewster 2006). In RTUF, the skimmed milk was replaced

by groundnut paste and lacto-serum. RTUF is an energy dense paste which does not require cooking and can be stored for several months (24 months shelf life) without spoiling (Briend 2001, Ashworth 2006). These RTUF have low osmolarity and can be eaten directly from the silver foil package by the child without the addition of water or milk which reduces risk of bacterial contamination (Diop, Dossou *et al.* 2003, Lazzerini, Rubert *et al.* 2013). In hospital settings the major limitation of RTUF is that it cannot be administered easily through a naso-gastric tube (Brewster 2006). RTUF have the following salient features: good nutritional characteristics, low cost, resistant to bacterial contamination, long shelf life, does not need refrigeration, highly palatable, does not require any further processing, prior feeding and its consistency is suitable for feeding infants and children (Ciliberto, Sandige *et al.* 2005, Brewster 2006).

Malnutrition is a potentially preventable disease and is a chief contributor to child mortality and the overall global disease burden (Black, Allen *et al.* 2008, Hendricks 2010). Childhood malnutrition is associated with a number of socio-economic and environmental factors such as poverty leading to inadequate food intake, lack of access to food, poor hygienic practices, lack of sanitation, poor health, recurrent infections and large family size (Golden 2009, Babar, Muzaffar *et al.* 2010).

Pakistan is the world's seventh most populous country and 45% of its population is under 15 years of age. Evidence suggests that malnutrition in children between 5-12 years is high in Pakistan and is a very serious impediment in the development of the nation (Mian, Ali *et al.* 2002, Pappas, Agha *et al.* 2008). In Pakistan, 23.9% of the population lives below the poverty line and 38% of all children under 5 years of age are underweight (Pappas, Agha *et al.* 2008, Akram, Arif *et al.* 2010). According to National Health Survey of Pakistan, one out of three children is malnourished, 6.2-8.3 million (30-40%) Pakistani children have stunting (low height for their age), and more than 2.9 million (> 14%) children have wasting (low weight for their

height) (Gillespie and Haddad 2003, Mujib, Kazmi *et al.* 2006). In Pakistan, there is a complex interplay between poverty, hunger and uncomplicated malnutrition coupled with un-acceptable treatment cost to the family. In such circumstances, the family does not consider uncomplicated malnutrition as a health problem. That is why health care is approached only when the problem is associated with co-morbidities, which lead to increased mortality.

Malnutrition is identified using standard deviation scores (Z-scores), which are a statistical measure identifying how body weight and height compares to reference values. The World Health Organization (WHO) Global Database on Child Growth and Malnutrition uses a Z-score cut off point of $<-2SD$ to classify undernutrition and a cut-off of $>+2SD$ to classify overweight in children (Wang and Chen 2012). Moderate acute malnutrition (MAM) is defined as weight- for-height Z-score (WHZ) <-2 and ≥-3 without oedema (Chang, Trehan *et al.* 2013). Children with MAM are at three times greater risk of deaths as compared to the well-nourished children and are prone to morbidity from infectious diseases and suffer from delayed cognitive and physical development (Black, Allen *et al.* 2008, Chang, Trehan *et al.* 2013). Worldwide 11% of the children under 5 years of age are suffering from MAM (Black, Allen *et al.* 2008). It is estimated that in developing countries 32% (178 million) of children had weight-for-age Z score (WAZ) of less than -2 (Black, Allen *et al.* 2008). A mildly underweight child with (WAZ) between -1.0 and -2.0 have twice the risk of death as compared with WAZ >-1.0 , and the relative risk increases to five times and eight times for the moderately underweight (WAZ between -2.0 and -3.0) and severely underweight children (WAZ <-3.0), respectively (Caulfield, de Onis *et al.* 2004, Thakwalakwa, Ashorn *et al.* 2010). More detailed information regarding the use of RTUF in community settings in low- and middle-income countries in children is explained later in this chapter.

1.1.3 Use of nutritional supplements in developed countries

As major cause of malnutrition is reduced dietary intake, in 2006 the National Institute for Health and Clinical Excellence (NICE) recommended an increase in dietary intake using a variety of nutrition support strategies including oral nutritional supplements, artificial nutritional support and dietary counselling. All these strategies are aimed to reverse inadequate food intake by increasing energy intake and to improve awareness, knowledge, practices and attitudes related to healthy diet (Cawood, Elia *et al.* 2011, Lazzerini, Rubert *et al.* 2013). Inadequate energy, protein and micronutrient intake mostly occurs in disease related malnutrition as appetite is poor due to diseases and patients ingest less food with less proteins and less nutrients, and in some cases protein requirements are also higher and there is the need for more protein to encourage damaged tissues repair and to facilitate body repletion (Cawood, Elia *et al.* 2011).

The current evidence suggests that the nutritional supplements have several beneficial effects (Table 1.1). A number of meta-analyses and systematic reviews indicate the beneficial clinical outcomes while using nutritional supplements (Stratton and Elia 2007, Cawood, Elia *et al.* 2012). There is a significant reduction in the rates of complications like pressure ulcers, surgical wound healing, chronic obstructive airways disease, liver diseases and infections with the use of nutritional supplements in hospitals (Olofsson, Stenvall *et al.* 2007), community (Tidermark, Ponzer *et al.* 2004) and in both hospitals and community settings (Stratton and Elia 2007, Botella-Carretero, Iglesias *et al.* 2008, Cawood, Elia *et al.* 2012). In the majority of cases the liquid ready to use multi-nutrient nutritional supplements with a reported intake of 1.05-2.52 MJ/d (250- 600kcal) were used. The use of nutritional supplements during peri-operative periods have constantly indicated fewer complications including major complications, infectious complications and intra-abdominal/thoracic complications before, during and after hospitalization (Stratton and Elia 2010, Cawood, Elia *et al.* 2011).

In elderly patients with a range of diseases: respiratory, acute illnesses, hip fracture, gastrointestinal diseases, and cancer were reported to have improved functional outcomes with the use of nutritional supplements (Norman, Kirchner *et al.* 2008, Norman, Pirlich *et al.* 2011, Piantadosi, Visvanathan *et al.* 2011). Functional outcomes include handgrip strength, physical and mental health, walking distance and activities of daily life, quality of life and mobility (Bourdel-Marchasson, Barateau *et al.* 2000, Stratton, Green *et al.* 2003, Cawood, Elia *et al.* 2011).

The systemic review and meta-analysis by Cawood *et al.* 2012 assessed thirty-six randomized controlled trials (n=3790) and a series of meta-analysis of high protein oral nutritional supplements provided evidence that oral nutritional supplements produce clinical, nutritional and functional benefits. This systemic review and meta-analysis reported that an overall mean weight change was greater in the supplement group (2.1kg; -1.3 to 11.0 kg) as compared to the control group (-1.6kg; -2.4 to 2.7 kg) (Cawood, Elia *et al.* 2012). Studies have also reported information regarding the beneficial impact of the nutritional supplements on body composition including the triceps skinfolds, mid upper arm circumference, fat mass measured by DEXA and bone mineral density (Gariballa, Forster *et al.* 2006, Gariballa and Forster 2007, Norman, Kirchner *et al.* 2008, Cawood, Elia *et al.* 2012).

Studies have shown that the improvement after the supplementation in total energy intake, body weight and body functions, occurs more commonly in those patients with a BMI of less than 20kg/m² compared to those with a BMI of more than 20kg/m² (Stratton and Elia 2000). Some of the studies have shown that in older adults the nutritional supplements have improved the proteins, vitamins, minerals and energy intake (Gray-Donald, Payette *et al.* 1994, Turic, Gordon *et al.* 1998, Edington, Barnes *et al.* 2004). It is also found from the meta-analysis that the oral nutritional supplements also reduce the length of hospital stay and readmission rates thus improving the health

economy (Stratton, Green *et al.* 2003, Norman, Kirchner *et al.* 2008, Cawood, Elia *et al.* 2011, Stratton, Hébuterne *et al.* 2013).

Table 1.1 A summary of studies, assessing the beneficial clinical and functional outcomes of the oral nutritional supplement.

Systemic Review	Study design	Patient group	Settings	Interventions	No. of trials/No. of Patients	Significant benefits to clinical and functional outcomes with supplements
(Stratton, Hebuterne <i>et al.</i> 2013)	Systemic review and meta-analysis	Adults of any nutritional characteristics (mostly elderly patients)	Community settings	All ONS types, energy density 1.00–2.48 kcal/ml, (475 to 1200 kcal/day) for 6wks - 1 yr,	Systematic review (9 RCT, n = 1190) Meta-analysis (6 RCT, n = 852)	Hospital (re)admission: Significant reductions with ONS vs. routine care (OR 0.59, 95% CI 0.43–0.80, $P = 0.001$)
(Collins, Elia <i>et al.</i> 2013)	Systemic review and meta-analysis	Stable patients with a diagnosis of COPD	All settings	ONS and ETF	12 RCT (n = 448)	Respiratory muscle strength: Significantly improved with ONS (pressure +3.86 standard error (SE) 1.89 cm H ₂ O, $P = 0.041$; maximal expiratory mouth pressure +11.85 SE 5.54 cm H ₂ O, $P = 0.032$) Handgrip strength: Significantly improved (+1.35 SE 0.69 kg, $P = 0.05$) with ONS and ETF Weight gains: Weight gain of ≥ 2 kg with ONS. QoL: Improved with supplementation
(Cawood, Elia <i>et al.</i> 2012)	Systemic review and meta-analysis	Elderly with hip fractures, pressure ulcers, COPD, cancer, gastro-intestinal disease, and a range of critical and acute illnesses	Hospital and community settings	ONS energy densities (0.75–3.85 kcal/ml) and the percentage energy from protein ranged from 20–54% (149 to 995 kcal/day)	36 RCT (n=3790)	Complications: ONS significantly reduced the incidence of complications (pressure ulcers, wounds, non healing fracture, infections, or a combination of complications) compared to control (OR 0.68 (95% CI 0.55–0.83)). Functional: ONS improved grip strength (1.76 kg (95% CI 0.36–3.17) Energy Intake: ONS increased intake of protein ($p < 0.001$) and energy ($p < 0.001$)

						<p>Improvements in weight: ONS significantly improve weight</p> <p>Readmissions: ONS, significantly reduce hospital readmissions compared to control (OR 0.59 (95% CI 0.41–0.84)).</p>
(Norman, Pirlich <i>et al.</i> 2011)	RCT	Malnourished with benign GI disease	Hospital	ONS (~200ml/day) for 3 months	n= 114	<p>QoL: Improved with ONS</p> <p>Cost effective: Treatment is cost effective according to international benchmarks</p>
(Norman, Kirchner <i>et al.</i> 2008)	RCT	Patients with benign GI disease	Hospital setting	ONS (200 ml) per day (nutritionally complete, 150 kcal and 10 g protein/ 100 ml, 27% energy from protein) for three months compared with DC	n= 80	<p>The following outcome measures significantly improved with ONS as compared to DC</p> <p>Energy intake: Total energy and protein intake ($p<0.0001$)</p> <p>Hand-grip Strength: 26.1 ± 11.3–31.5 ± 10.1 kg, ($p<0.0001$)</p> <p>Peak flow: 329.2 ± 124.0–388.9 ± 108.4 l/min ($p=0.004$)</p> <p>QoL: Improved</p> <p>Hospital readmissions: Significantly less ($p=0.041$).</p>
(Olofsson, Stenvall <i>et al.</i> 2007)	RCT	Patients with femoral neck fracture aged ≥ 70 years	Hospital Settings	Nutritional and protein drinks (400 ml/day) and protein-enriched meals for at least 4 days postoperatively during hospitalization.	n=157	<p>Post-operative complications:</p> <p>a) Fewer patients in the intervention group developed post-operative delirium (46 patients in the intervention group vs. 54 patients in the control group, $p=0.022$).</p> <p>b)The number of days with delirium was significantly fewer ($p<0.001$) in intervention group</p> <p>c) Significantly less number of patients developed decubitus ulcers in intervention group</p> <p>d) Hospitalization period was shorter in intervention</p>

						group ($p = 0.019$)
(Botella-Carretero, Iglesias <i>et al.</i> 2008)	RCT	Geriatric patients aged ≥ 65 years submitted to surgery for hip fracture	Hospital settings	2 types of ONS: a) 37.6 g of protein (500 kcal/ day) b) 36 g of protein (152 kcal/day)	n=90	Small effect of ONS on serum albumin was detected in patients with post-surgical complications.
(Stratton and Elia 2007)	Review of reviews	Gastrointestinal surgical patients	All settings – mostly hospitalized patients	ONS (single nutrient and multi-nutrient) 250-600 kcal/day for 7 days-10 weeks	18 RCT (n=907) of ONS and ETF 6RCT (n=418) of ONS	Mortality: Significantly lower in supplemented group with OR 0.61 (95% CI 0.48-0.78). Morbidity: Supplements significantly reduced postoperative complications including wound and lung infections, postoperative ileus, unresolved peritonitis and wound dehiscence with OR 0.37 (95% CI 0.26-0.53).
(Gariballa and Forster 2007)	Prospective , double-blind, placebo-controlled trial	Acutely ill elderly patients aged ≥ 65 years	Hospital setting	ONS (995 kcal/day) (carbohydrate 45%, fat 35% and protein 20%) and 100% of the Reference Nutrient Intakes for 6 weeks	n=225	Nutritional status: Serum albumin concentration, red-cell folate and plasma vitamin B12 concentrations significantly improved in the supplement group as compared to placebo group. Symptoms of depression: In the supplement group there was a significant increase in the number of patients with no symptoms of depression and a decrease in those with symptoms of mild or severe depression as compared to placebo group.
(Milne, Avenell <i>et al.</i> 2006)	Meta-analysis	Older people aged ≥ 65 years (excluding critically ill patients and	All settings	Commercial ready-made ONS and other milk-based supplements 175-1000 kcal/day	55 trails(n =9187)	Mortality: Improved survival with supplementation in : a) Undernourished people (17 trials; 2093 participants) (Peto OR, 0.73 [CI, 0.56 -0.94]) b) People aged ≥ 75 years (18 trials; 1611

		patients recovering from cancer treatment)		Duration of Supplementation : 10 days – 18 months		participants) (Peto OR, 0.64 [CI, 0.49 - 0.85]), c) Ill people (22 trials; 6630 participants) (Peto OR, 0.86 [CI, 0.74 - 1.00]). Morbidity and Complications: In hospitalized patients supplements significantly decrease complications (Peto OR, 0.72 [CI, 0.53 to 0.97]) like infective complications, incomplete wound healing etc.
(Gariballa, Forster <i>et al.</i> 2006)	RCT	Patients aged 65 -92 years.	Hospital setting	ONS (995 kcal/day) and 100% of the Reference Nutrient for 6 weeks	n=445	Readmission: 29% patients in the supplements group were readmitted to the hospital compared with 40% in the placebo group (adjusted hazard ratio 0.68 [95% CI 0.49-0.94]). Mean Length of hospital stay: was 9.4 days in supplement group compared with 10.1 days in placebo group. Nutritional Outcomes: Serum albumin concentration increased significantly in the supplement group
(Stratton, Bircher <i>et al.</i> 2005)	Systemic review and meta-analysis	Patients with chronic kidney disease receiving maintenance dialysis	Any setting (hospital, outpatient or home)	ONS and enteral tube feeding	18 studies: RCT (n=5), non-RCTs (13)	Biochemical outcomes: Enteral nutritional support increased serum albumin concentration by 0.23g/dl. Energy Intake: Increased total energy intake Clinical outcome: May improve clinical outcomes
(Stratton, Ek <i>et al.</i> 2005)	Systemic review and meta-analysis	Patients with, or at risk of developing, pressure ulcers	All settings – mostly hospitalized elderly, post-surgical patients.	ONS (250–500 kcal/day) Duration of supplementation: 2–26 weeks	15 studies: RCT (n=8), CCTs (n=1), CTs (n=1) and cohort studies (n=5)	Prevention of pressure ulcers: ONS were associated with significantly lower incidence of development of pressure ulcers compared to routine care with OR 0.75(95% CI 0.62-0.89, n=1224) Healing of existing pressure ulcers: A tendency of improved wound healing

(Stratton 2000) (Stratton and Elia 2000)	Systemic review	Patients with specific diseases	Community settings	ONS (were used in 80% of the studies) with energy density (3.25–16.0 kJ/ml), ranged from < 0.42 MJ/d to > 10.5 MJ/d. Duration of supplementation: 1 week -over 2 years.	84 trials; 45 RCT (n=1728) and non-39 RCT (n=842). Studies grouped according to disease: COPD (n=14), Crohn's disease (n=9), cystic fibrosis (n=11), elderly (n=12), HIV and AIDS (n=15); liver disease (n=2), malignancy (n=15); other conditions (n=6).	<p>Weight change: The mean percentage weight change of patients receiving ONS (2.93 %) was greater than that of the control patients (1.15 %). Patients with a mean BMI < 20 kg/m² had a greater percentage weight change (4.7 % of the body weight) than patients with a mean BMI > 20 kg/m² (2.4 % of the body weight).</p> <p>Total energy intake: Mean increase in energy intake = 67 % of the energy of the ONS consumed), which varied considerably according to the disease state and the BMI of patients.</p> <p>Functional benefits:</p> <p>COPD patients: Improved muscle strength, walking distance and well-being.</p> <p>Children with cystic fibrosis: Improved growth performance.</p> <p>Elderly: reduced falls and increased activities of daily living</p>
(Lauque, Arnaud-Battandier <i>et al.</i> 2000)	RCT	Elderly people aged ≥ 65 years	Community setting (private nursing home)	ONS (300-500 kcal/day) for 60 days	n=88	<p>ONS improved:</p> <p>a) Mini-nutritional assessment score: in subjects at risk of malnutrition and in malnourished subjects (from 13.9± 2.6 to 17.1 ± 3.9).</p> <p>b) Weight gain: (1.4 ± 0.5kg) in subjects at risk of malnutrition and (1.5 ± 0.4kg) in malnourished subjects.</p>

Abbreviations: ONS, Oral nutritional supplements; RCT, Randomized controlled trial; ETF, Enteral tube feeding; OR; Odds ratio; CI, Confidence interval; CCTs, Controlled clinical trials; CTs, Clinical trials; GI disease, Gastrointestinal disease; COPD, Chronic obstructive pulmonary disease; QoL, Quality of life; DC, dietary counselling.

Stratton found that the energy from the supplements was additional to that taken from the habitual food intake with an increase in mean energy intake to 67% of the energy consumed by the nutritional supplements although this contribution varied with the disease state and BMI of the patients. In the patients with a BMI $<20 \text{ kg/m}^2$ the proportion of supplement energy additional to the food intake was approximately 79% compared to 28% in those with a BMI greater than 20 kg/m^2 . The individuals with a BMI $<20 \text{ kg/m}^2$ showed more improvement in the total energy intake, body weight and the body function compared to the individuals with a BMI $>20 \text{ kg/m}^2$ (Stratton 2000, Stratton and Elia 2007).

The evidence suggests that there was slight suppression of normal food intake with the oral nutritional supplements but they effectively increased the total energy and nutritional intake (Stratton, Green *et al.* 2003, Stratton 2005, Milne, Avenell *et al.* 2006, Stratton and Elia 2007, Cawood, Elia *et al.* 2011, Hubbard, Elia *et al.* 2012). In one prospective randomized trial based on interviews with patients about their dietary habits and appetite, no significant difference was found in appetite between the two groups; one on the nutritional supplement, and another without the supplement, in older non-demented females with hip-fracture (Carlsson, Tidermark *et al.* 2005). Studies have also found that there was significantly more protein and energy intake with the supplements (Edington, Barnes *et al.* 2004), and there was no significant reduction in food intake (Norman, Kirchner *et al.* 2008). A multi-centre trial to look at the effects of nutritional supplements in critically-ill elderly patients found that, whilst oral nutritional supplements increased energy and protein intake of elderly patients, the actual intake was low compared to what was expected (Bourdel-Marchasson, Barateau *et al.* 2000). Another study found that there was slight decrease in the voluntary intake of food in the supplement group compared to controls but the total energy intake including the supplement was significantly higher in the supplement group (Lauque, Arnaud-Battandier *et al.* 2000).

A recent meta-analysis suggested that the consumption of oral nutritional supplements could increase the daily energy intake by a mean of 375 kcal/d (Hubbard, Elia *et al.* 2012). However, another systematic review suggested that this translated into an average total weight gain of only 1.7 kg, with wide variability (Cawood *et al.* 2012). A rare longer term trial in children and adolescents with cystic fibrosis showed no net gain in BMI (Poustie *et al.* 2006). The difference between the intake and the net weight gain thus must reflect some degree of energy compensation. It is important to study how consumption of HENSD affects energy intake during subsequent meals.

No study has formally assessed the impact of high energy nutritional supplement drinks on appetite sensations (such as hunger, satiety, fullness or desire to eat), however a range of anecdotal observations were documented ranging from stimulation of appetite to loss of appetite (Stratton 2000, Stratton and Elia 2000). As energy content, nutrient composition, concentration of electrolytes and osmolarity (i.e. gastric emptying speed and gastrointestinal absorption) are well-established regulators of appetite, it is important to consider how consumption of HENSD affects energy intake during subsequent meals. Although existing evidence suggests the clinical beneficial effects of nutritional supplements, it is imperative to perform further research to determine the mechanisms responsible for the partial compensation of the energy provided by the nutritional supplements.

1.1.4 Specially formulated therapeutic foods for treatment of moderate acute malnutrition in children from low- and middle-income countries

Moderate acute malnutrition (MAM) affects approximately 10% of the children under five years of age in low and middle income countries. Different approaches have been used for the nutritional recovery of the children in these settings such as lipid based nutrient supplements (food with high energy density and high lipid content) or blended foods (dry food mixtures without high lipid content), which can be provided in a low dose or full dose as a

supplement to their habitual diet (Müller and Krawinkel 2005, Lazzerini, Rubert *et al.* 2013). The provision of these supplements to MAM children increased the recovery rate by 29% and significantly improved weight-for-height as compared to standard care (Lazzerini, Rubert *et al.* 2013).

An early study by Walker *et al.* (1991) performed on stunted children (< -2 SD of the National Centre for Health Statistics (NCHS) reference) aged 9-24 months, were randomly assigned to four groups: a nutritional supplement group (milk based supplement providing 750 kcal per day), psychosocial stimulation group, both the supplement and stimulation group and a control group for 12 months. Supplementation significantly increased weight, head circumference, mid upper arm circumference and triceps skinfold in the first 6 months, but no significant increase was reported in the subsequent six months. In this study dietary intake was measured by two 24 hour dietary recalls before the intervention and then after 6 months of the intervention. The baseline dietary intakes were similar in the stunted and non stunted children, while at 6 months, dietary intake was significantly reduced in the supplement group (Walker, Powell *et al.* 1991). Lack of further improvement in weight, head circumference, mid upper arm circumference and triceps skinfold in the last six months of the study might be due to decreased food intake or/and to a decline in the intake of the supplement. The compliance of subjects in taking the supplements was not measured in this study.

On the other hand, a study by Gershoff *et al.*, (1988) observed no demonstrable changes in anthropometric indices as a result of high caloric supplementation (300 kcal) provided to pre-school children, in Thailand, from December 1981 to October 1983. No impact of supplementation on the anthropometric indices of the children in the intervention group was thought to be due to increased physical activity and reduced intake of habitual food, although neither were not measured in that study (Gershoff, McGandy *et al.* 1988). The disparity of the results in these studies may be due to differences in the energy density of the supplements provided and duration of the intervention.

The WHO recommends that during treatment of malnutrition in the nutritional rehabilitation phase, children should receive an energy and protein dense diet fortified with vitamins and minerals to promote rapid weight gain. This phase of rehabilitation requires 3-4 weeks and is usually carried out in hospital or residential therapeutic centres and the mother has to stay with the child (Diop, Dossou *et al.* 2003). This has some limitations as there are more chances of cross-infections and secondly it is unfavourable for other family members (Collins 2001). Therefore, community based rehabilitation is suggested as an alternative (Ashworth and Khanum 1997).

Ready to use foods in the treatment of severe malnutrition

For this purpose, a solid ready to use food (RTUF) has been developed which is made up of peanut butter (Diop, Dossou *et al.* 2003). RTUF was developed as an alternative to the F-100 formula (a liquid milk based diet, recommended by WHO during the rehabilitation phase of the treatment of severe malnutrition, which has high energy and protein content, 100 energy units/100 mL, 418 J; 135-200 mL/kg body weight/24 hours and 2.9 g proteins/100 ml) or milk oil formula to be used in the hospitals and nutritional rehabilitation centres after initiation of the cure (Brewster 2006). In RTUF, the skimmed milk was replaced by groundnut paste and lacto-serum. RTUF is an energy-dense paste which does not require cooking and can be stored for several months (24 months shelf life) without spoiling (Briend 2001, Ashworth 2006). These RTUF has low osmolarity and can be eaten directly from the silver foil package by the child without the addition of water or milk which reduces the risk of bacterial contamination (Diop, Dossou *et al.* 2003).

Systemic reviews and randomized controlled trials have showed that RTUFs are very effective and safe in the treatment of severe malnutrition in the children (Briend, Lacsala *et al.* 1999, Collins and Sadler 2002, Dossou, Ndour *et al.* 2003, Sandige, Ndekha *et al.* 2004, Ciliberto, Sandige *et al.* 2005,

Brewster 2006, Bhutta, Ahmed *et al.* 2008, Gera 2010). Although these systemic reviews assessed the efficacy and effectiveness of therapeutic nutritional products in the treatment of severe acute malnutrition they cited different studies (Ashworth 2006, Gera 2010). In a systemic review of thirty three community based rehabilitation studies 5 trials utilized RTUF and found them to be very effective (Ashworth 2006). In another review of two systemic reviews, 7 randomized controlled trials and 7 observational studies, it was revealed that home based management of uncomplicated severe acute malnutrition with RTUF was as effective as F-100 and was more effective than home based dietary therapies (Gera 2010).

A randomized controlled trial conducted on 60 severely malnourished Senegalese children, in a therapeutic feeding centre, compared RTUF with F 100 formula (high energy milk) and demonstrated that the increase in energy intake (808 vs. 573 kJ/kg/day) and weight gain (15.6 vs.10.1g/kg/day) achieved by the supplementation with RTUF was significantly higher in comparison to changes induced by the consumption of a traditional high-energy drink based on milk (F-100). This study provided evidence that RTUF is superior to F 100 for the management of severe malnutrition during the rehabilitation phase for the rapid catch up growth (Diop, Dossou *et al.* 2003). A controlled comparative clinical effectiveness trial in Malawi on 1178 malnourished children compared home based therapy with RTUF with standard in patient therapy and demonstrated better weight gain (3.5 compared with 2.0 g/kg/day), lower relapse rates (8.7% compared with 16.7%) and lesser rates of cross infection with RTUF (Ciliberto, Sandige *et al.* 2005).

A study on 282 HIV negative Malawian children of greater than 1 year of age compared RTUF with two home based treatments (RTUF supplement and blended maize soy flour). After two weeks of hospital treatment, the children were monitored fortnightly for 6 months. The RTUF group had rapid gain in weight (5.2 g/kg/day compared to 3.1g/kg/day) and rapid recovery from wasting (WHZ >0 in 35 days compared to 56 days) as compared to the home

based treatment with maize/soy and RTUF supplement groups (Manary, Ndekeha *et al.* 2004). The better outcomes with RTUF in these studies were related to fivefold higher energy density of RTUF than F-100 and maize/soy (Brewster 2006). In the developing world, due to prevalence of childhood under-nutrition, local production of RTUF is needed in order to make the therapy more widely available. Therefore, Sandige *et al.* (2004) conducted a study on 260 severely malnourished Malawian children, to compare a locally produced RTUF with imported RTUF, and observed a similar weight gain with the imported RTUF (5.2 g/kg/day) and locally produced RTUF (4.8 g/kg/day). This is evidence that both locally produced and imported RTUF have similar efficacy and effectiveness (Sandige, Ndekeha *et al.* 2004). Recently a study demonstrated that a whey protein based RTUF in which all the dried skimmed milk is replaced by whey protein concentrate, is an effective, cheaper alternative to the milk based RTUF (Bahwere, Banda *et al.* 2014).

Ready to use foods in the treatment and prevention of moderate malnutrition

RTUF have also been used to supplement the dietary intake of the moderately malnourished (Kuusipalo, Maleta *et al.* 2006, Defourny, Seroux *et al.* 2007, Matilsky, Maleta *et al.* 2009, Nackers, Broillet *et al.* 2010, Patel, Sandige *et al.* 2011), as shown in Table 1.2. RTUF was found to be effective in preventing malnutrition in non-wasted children in areas of food insecurity (Defourny, Minetti *et al.* 2009, Isanaka, Nombela *et al.* 2009, Isanaka, Roederer *et al.* 2010, Huybregts, Houngbé *et al.* 2012). It has been recommended that the nutritional intervention to prevent malnutrition might be more effective than the curative treatment of malnutrition (Ruel, Menon *et al.* 2008). A cluster-randomized trial of children aged 6-60 months, found that 3 months of supplementation with 1 packet of RTUF per day (500 kcal/day) reduced the incidence of wasting and severe wasting over a period of 8 months of follow-up. Although, a significant reduction in the incidence of wasting was observed

in the intervention group, no difference in mortality was found between the intervention and control group (Isanaka, Nombela *et al.* 2009). Here it is worth mentioning that two different growth standards were used in that study. For inclusion, NCHS growth standards were used while for the analysis outcomes WHO growth standards were used. In the WHO growth reference the estimates of wasting tend to decrease (de Onis, Garza *et al.* 2007) and the use of WHO growth reference for analysis of outcomes may have showed exaggerated improvements in weight for height.

Similarly, Defourny *et al.* (2009) evaluated a large scale distribution of ready-to-use food (RUF 1000kcal/day), during the 2007 hunger gap in Maradi region, Niger and reported that the incidence of severe acute malnutrition remained extremely low. Recovery rates were higher in approximately 60,000 moderately malnourished children who received a blanket supplementation (Defourny, Minetti *et al.* 2009). Although it is important to mention here that, the moderate malnourished children consisted of a group with severe malnutrition without complications.

Similarly, Grellety *et al.* (2012), performed a non-randomized 4- months cohort study on 2238 children, aged 6-23 months, and found a positive effect on anthropometric status as presented in Table 1.2 and prevention of wasting by RUSF (250 kcal/day) supplementation. However, no difference in length gain was observed between the two groups. In the intervention group, fewer initially non-wasted children also developed moderate wasting compared to the group (without supplementation) (Grellety, Shepherd *et al.* 2012). Although the baseline anthropometry of the children was not significantly different between the two groups, the children in the intervention group had slightly lower mean weight-for-length (Table 1.2). They were younger, and came from larger families, which might have increased the chances of sharing the supplement and could have an impact on the findings of this study. Likewise, Huybregts *et al.* (2012) performed a cluster-randomized controlled pragmatic intervention study on 6-36 months, non-wasted children ($WHZ \geq 80$

% of NCHS reference median and absence of bilateral pitting oedema) from city Abache. The intervention group was provided with 46 grams of ready-to-use supplementary food (RUSF =247 kcal) daily for 4 months. It was found that the intervention group had significantly higher gain in height-for-age Z score, haemoglobin concentration accompanied by reduction in fever episodes, and lower risk of diarrhoea, compared to control group, at the end of the study. However, no significant differences were detected in weight increase, mean change in WHZ, between the two groups. The provision of RUSF packets in the intervention group did not result in a reduction in cumulative incidence of wasting. Compared to baseline mean WHZ increased slightly in both arms while mean HAZ was slightly lower in both arms. This study could not establish clear evidence that addition of RUSF to the household food ration was effective in the prevention of acute malnutrition (Huybregts, Houngré *et al.* 2012). In this study, a general food ration was also provided to both groups, which might dilute the effects of the intervention, and may be a possible reason for not finding the effect of the supplementation on nutritional status of the children in the intervention group.

For treating children with MAM, besides RTUF, other supplements, such as fortified blended flours, like corn-soy blend and corn-soy blend plus oil, and milk (CSB++) are also used (Chang, Trehan *et al.* 2013). The evidence regarding the comparison of the efficacy of RTUF with the corn-soy blend in children with MAM has conflicting results. The majority of the studies reported higher recovery rates with RTUF compared to the corn-soy blend (Matilsky, Maleta *et al.* 2009, Nackers, Broillet *et al.* 2010, Thakwalakwa, Ashorn *et al.* 2010, Patel, Sandige *et al.* 2011, Karakochuk, van den Briel *et al.* 2012). However, other studies demonstrated that CSB++ and RTUF are equally effective (LaGrone, Trehan *et al.* 2012).

A randomized clinical effectiveness trial in rural Malawi, on moderately wasted children (WHZ <-2 but \geq -3), found that 8-week intervention with milk/peanut fortified spread, soy peanut fortified spread, or corn soy blend,

each providing 314 kJ/kg/day, had higher and faster recovery rates with the fortified spread groups compared to CSB group. However, no difference was detected in the rates of the length gain among three groups, and 8% of children in each group developed severe malnutrition (Matilsky, Maleta *et al.* 2009). Karakochuk *et al* (2012) performed a cluster randomized effectiveness trial on 1125 Ethiopian children aged 6–60 months with moderate acute malnutrition (MAM) (WHZ between -2 and -3). They reported that after 16 weeks of supplementation with Ready-to-use supplementary food (RUSF) 92 g (500 kcal) and corn-soy blend (CSB) 300g (1413 kcal) daily resulted in the higher recovery rate with RUSF, however this difference was not statistically significant (Karakochuk, van den Briel *et al.* 2012).

Likewise, Patel *et al* (2011) found greater rates of weight gain 3.1g/kg/day, higher recovery rates and lower relapse rates with RTUF than corn/soy blend (1.4g/kg/day), after 8 weeks of supplementation. Nackers *et al* (2010) performed a field randomized trial in Niger. Four hundred and fifty one children aged between 6 to ≥ 36 months measuring 65 to <110 cm, with moderate acute malnutrition (WHM% between 70% and $<80\%$ of the NCHS median) were randomized to receive either RUTF (Plumpy' Nut 1000 kcal/day) or CSB premix (1231 kcal/day). Two hundred and fifteen children were recruited in RTUF group and 236 in the corn/soy-blend (CSB-based-premix). Children were assessed weekly until their recovery (discharge criteria: WHM % $\geq 85\%$ for 2 consecutive weeks). Children who recovered after the intervention were additionally followed up for 6 months.

Although RUTF group resulted in higher weight gain, higher recovery rates (79% versus 64% in the CSB group) and shorter length of stay as compared to the CSB group, during follow up, height and height-for-age gains were similar in both groups. Also, and one fifth of the cured children relapsed (Nackers, Broillet *et al.* 2010). On the other hand, a study by Lagrone *et al* (2012) reported that 12 weeks of supplementation with 75kcal of either soy whey RUSF, soy RUSF and CSB++ are equally effective and there were no

significant differences in the recovery rate of the children in each group (LaGrone, Trehan *et al.* 2012). During the 12-months following period, this study demonstrated that only 63% of children who recovered from the MAM remained well-nourished, 17% relapsed to MAM and 10% developed severe acute malnutrition (Chang, Trehan *et al.* 2013).

Limitation of existing research on moderate acute malnutrition

The majority of the existing evidence on the use of community based management of uncomplicated, moderately acute malnutrition, has emerged from the studies conducted in Africa in emergency settings. There is little evidence exploring the impact of RTUF with other commercially available milk-based proprietary supplement in settings where food is available, and nutritional habits, nutritional education and sanitation are the main determinants of malnutrition. There are no studies on MAM from Asia where MAM is mainly prevalent (Lazzerini, Rubert *et al.* 2013). Furthermore, while assessing the impact of the supplementation on child nutritional status, other factors should also be taken into account, including appetite suppression, replacement of habitual food intake and compliance to the intervention. There is a need to generate more evidence regarding the efficacy of RTUF and other locally available, ready-to-use supplements in non-emergency settings, particularly in Pakistan.

Table 1.2 Studies assessing the comparison of effectiveness of specially formulated therapeutic foods for the treatment malnutrition in children.

	Study	Study Design	Study participant	Intervention duration	Results				
1.	(Huybregts, Hougbe <i>et al.</i> 2012) City of Abache Central Africa	Cluster RCT	n=1,038, non-wasted children (≥ 80 % of NCHS reference) between 6-36 mths.	RTUF (Plumpy' Doz) = 247 kcal/day for 4 mths		Intervention (n= 598)	Control (n=440)	P value	
					WHZ (SD)	-1.05 (0.93)	-1.09 (0.95)		
					Intervention effect (95% CI) WHZ	-0.002 (-0.03,0.03)		0.89	
					HAZ (SD)	-1.79 (1.46)	-2.06 (1.39)		
					Intervention effect(95% CI) HAZ	0.03 (0.01,0.04)		<0.001	
					Prevalence of stunting (%) (n)	46.2 (276)	52.3 (230)		
					MUAC (cm)(SD)	14.3 (1.1)	14.1 (1.2)		
					Intervention effect(95% CI) MUAC	0.01(-0.02,0.04)		0.49	
2.	(Thakwalakwa, Ashorn <i>et al.</i> 2012) Malawi	RCT	Underweight (WAZ< -2) age 6-15 mths	12 wks an average daily 71 g CSB= 1188kJ or 43g LNS =920kJ and no supplement (control) group.		LNS (n=99)	CSB (n=106)	Control (n=77)	P value
					↑Weight(kg) (SD)	0.75 (0.41)	0.68(0.50)	0.63(0.40)	0.21
					↑ Length (cm) (SD)	3.6 (1.3)	3.3(1.3)	3.4(1.2)	0.29
					↑ MUAC (cm) (SD)	0.4 (0.7)	0.3(0.9)	0.3(0.8)	0.74
					Δ WAZ(SD)	-0.12 (0.60)	-0.13 (0.71)	-0.13(0.61)	0.40
					Δ WHZ(SD)	-0.10(0.64)	-0.14 (0.81)	-0.25(0.71)	0.40
					Δ HAZ(SD)	0.02 (0.47)	-0.02 (0.47)	0.06 (0.44)	0.45

3.	(Bahwere, Banda <i>et al.</i> 2014) Malawi	RCT	n=600 age 6 -59 mths SAM (MUAC <11.0 cm or pitting edema +1 or +2).	P-RTUF and WPC-RTUF =175kcal/kg. till recovery (weight gain of at least 15%, MUAC >11.0cm)		WPC-RUTF (n=308)	P-RUTF (n=292)	
average weight gain (g/kg/d)						3.1	2.9	
recovery rate						84.8%	84.2%	
defaulter rate						12.2%	12.2%	
Mortality rate						1.6%	0.7%	
In both groups recovery rate exceeds the SPHERE minimum standards of > 70%.								
4.	(Bisimwa, Owino <i>et al.</i> 2012) Democratic republic of Congo	Prospective, non blinded, RCT	n=1331 full term born infants (gestational age >37 weeks) when became 4-5 mths.	RUCF 280 kcal or UNIMIX 275 kcal daily for six mths.		RUCF (n= 656)	UNIMIX (n=675)	P value
Weight gain (kg)						1.2 (1.2,1.3)	1.3 (1.3, 1.4)	0.08
Underweight						20%	18%	0.42
Stunting						48%	46%	0.31
Length gain (cm)						5.2 (5.0, 5.3)	5.4 (5.3, 5.6)	0.039
MUAC gain (mm)						5.3 (4.6, 6.1)	5.2 (4.5, 6.0)	NS
Diff HAZ						-1.1 (-1.2, -1.0)	1.0 (-1.1, -0.9)	NS
Diff WAZ						-0.3 (-0.4, -0.2)	0.3 (-0.3, -0.2)	NS
Diff WHZ						0.1 (0.0, 0.2)	1 (0.0, 0.2)	NS
5.	(LaGrone, Trehan <i>et al.</i> 2012) Malawi	Prospective, RCT	n= 2712 MAM (WHZ <-2 and ≥ -3) children aged 6-59 mths	75 kcal/ kg/day of CSB ++, locally produced soy RUSF or an imported soy/whey RUSF for ≤ 12 wks		CSB++ (n=888)	Soy RUSF (n= 906)	Soy/whey RUSF (n=918)
Weight gain (g/kg/d)						3.1±2.4 ¹	3.4 ± 2.6	3.6 ± 2.8
Length gain (mm/d)						0.13±0.46	0.13±0.44	0.15 ±0.47
MUAC gain(mm/d)						0.13±0.40 ¹	0.13±0.43	0.21±0.44
WHZ						-1.68±0.67	-1.61±0.63	-1.59±0.60
Recovered % (n)						85.9 (763)	87.7 (795)	87.9 (807)
Develop SAM % (n)						6.6 (59) ¹	5.2 (47)	4.2 (39)
Continue MAM % (n)						0.9 (8)	0.6 (5)	0.9 (8)
¹ SG from soy/whey RUSF.								

6.	(Karakochuk, van den Briel <i>et al.</i> 2012) Ethiopia	Cluster randomized effectiveness trial.	n=1125 age 6–60 mths with MAM (WHZ between -2 and -3)	92 g RUSF =500 kcal 300g CSB =1413 kcal daily for 16 wks	RUSF (n= 351)	CSB (n=698)	P value		
					Recovery rate % (n)	73 (265)	67 (482)	0.056	
					Defaulted % (n)	2 (7)	2 (12)	NS	
					Non responsive % (n)	24 (86)	30 (216)	NS	
					Met sphere target	No	No		
7.	(Singh, Kang <i>et al.</i> 2010) India	RCT	n=128, 18-59 mths children with WAZ of ≤-2SD.	RTUF 50g/child /day (550 cal/ 100 g) HCCM (187 calories/ 100ml)	RTUF (n= 51)	HCCM (n=45)	P value		
					Weight gain (kg)	0.54 (0.44-0.65)	0.38 (0.25-0.51)	0.047	
8.	(Nackers, Broillet <i>et al.</i> 2010) South Niger	Field Randomized trial	MAM (WHM% between 70% and <80% of the NCHS median) age 6 to ≥ 36 mths.	RTUF =1000 kcal/d or CSB =1231 kcal/day. until recovery (WHM% ≥ 85% for 2 wks).	RTUF (n= 215)	CSB (n=236)	P value		
					Weight gain(g/kg/d)	5.67 ± 3.02	4.59 ± 2.59	<0.001	
					MUAC gain (mm/d)	0.37 ± 0.29	0.32 ± 0.24	0.11	
					LOS (weeks)	4 (2-16)	6 (2-16)	<0.001	
					Recovered % (n)	79.1(170)	64.4 (152)	<0.001	
					Non responder % (n)	6.0 (13)	8.9 (21)	0.25	
9.	(Thakwalak wa, Ashorn <i>et al.</i> 2010) Malawi	Clinical randomized trial	n=182 underweight children (WAZ <-2) between 6-15 mths	43g/day LNS =1189 kJ, 71 g/day CSB =921kJ control group= No supplement.	LNS (n=99)	CSB (n=106)	Control (n=77)	P value	
					↑Weight(kg)	0.62 ± 0.47	0.51±0.35	0.47 ± 0.35	0.11
					↑ Length (cm)	3.4 ± 1.1	3.5 ± 1.1	3.3 ± 1.2	0.60
					↑ MUAC (cm)	0.2 ± 0.8	-0.1 ± 0.6	0.0 ± 0.6	0.06
					ΔWAZ	0.02 ± 1.11 ¹	-0.31 ± 0.59	-0.32±0.54	0.03
					ΔWHZ	-0.34 ± 0.77	-0.58 ± 0.76	-0.55±0.73	0.16
					ΔHAZ	0.29 ± 1.07	0.14 ± 0.37	0.11 ± 0.42	0.29
					¹ LNS vs Control SG				

10.	(Phuka, Thakwalakwa <i>et al.</i> 2009) Malawi	RCT	6-18 mths with low (WAZ < -2.0)	50g/d FS or 71 g/day LP for 12 wks.		LP (n=86)	FS (n=90)	P value
					Δ Weight(kg)	0.84 \pm 0.46	0.89 \pm 0.38	NS
					Δ Length (cm)	2.65 \pm 1.1	2.50 \pm 1.3	NS
					Δ MUAC (cm)	0.3 \pm 0.8	0.4 \pm 0.8	NS
					Δ WAZ	0.22 \pm 0.69	0.29 \pm 0.51	NS
					Δ WHZ	0.39 \pm 0.85	0.52 \pm 0.63	NS
					Δ HAZ	-0.08 \pm 0.41	-0.13 \pm 0.42	NS
11.	(Patel, Sandige <i>et al.</i> 2011) Malawi	Controlled clinical effectiveness trial	Weight for height <85% but >80% of the international standard.	7 kg of RTUF/mth and 50 kg of CSB/mth for 8 wks		RTUF (n= 331)	CSB (n=41)	P value
					Rate of weight gain (g/kg/d)	3.1 \pm 2.7	1.4 \pm 2.5	<0.001
					Rate of height gain (mm/d)	0.28 \pm 0.27	0.17 \pm 0.21	0.003
					Rate of MUAC gain (mm/d)	0.30 \pm 0.31	0.18 \pm 0.29	0.02
12.	(Defourny, Minetti <i>et al.</i> 2009) Maradi	Evaluation of large scale distribution of nutritional supplement on prevention of wasting.	n= 60,000 age between 6-36 mths with a height between 60 and 85 cm	Six monthly distribution of RTUF) 1000kcal/day for children < 8kg and 1500 kcal/day for children > 8kg		RTUF (n= 3,362)	No RTUF (n=2,949)	P value
					Weight gain (g/kg/d)	5.1 \pm 4.6	5.5 \pm 4.7	0.005
					LOS (days)	44.4 \pm 29.8	44.4 \pm 29.6	0.951
					Cured % (n)	92.3 (3054)	90.1 (2621)	0.003
					Died % (n)	1.8 (60)	2.2 (65)	0.23
					Non respondent % (n)	1.1 (37)	1.4 (41)	0.30

13.	(Isanaka, Nombela <i>et al.</i> 2009) Niger	Cluster randomized trial	n= 3533 children aged 6-60 months with weight for height 80% or more of the NCHS reference.	RTUF (92g(500kcal/day) for three months	<p>RTUF (n= 1671) No RTUF (n=1862) P value</p> <p>Rate of ΔWHZ <0.001</p> <p>WHZ differences between groups at baseline= -0.10 z (-0.23-0.03) WHZ differences between groups at end = 0.12 z (0.02-0.21) HAZ differences between groups at baseline = -0.06z (-0.18-0.06) HAZ differences between groups at end = 0.08 (-0.18-0.06)</p> <p>↓ incidence of wasting with RTUF =36% (17%-50%)</p> <p>↓incidence of severe wasting with RTUF =58% (53%-68%)</p>
14.	(Matilsky, Maleta <i>et al.</i> 2009) Malawi	Randomized clinical effectiveness trial	1362 moderately wasted children WHZ <-2 but \geq -3	Milk/ peanut FS=314kj/kg/day, Soy/peanut FS =314kj/kg/day or CSB for 8 wks.	<p>Milk /Peanut FS (n=465) Soy/Peanut FS (n=450) CSB (n=447)</p> <p>Recovered % (n) 79 (369) 80 (360) 72 (323)</p> <p>Remained wasted % (n) 8 (35) 7 (29) 15 (67)</p> <p>WHZ at discharge \sim1.6 \pm 0.7 \sim 1.7 \pm 0.7 \sim1.8 \pm 0.8</p> <p>HAZ at discharge \sim 2.7 \pm 1.3 \sim2.6 \pm 1.5 \sim 2.8 \pm 1.9</p> <p>Duration of Supplementation (days) 14 (14,42) 14 (14,42) 28 (14,56)</p>
15.	(Phuka, Maleta <i>et al.</i> 2008) Malawi	RCT	n=182 age between 5.5-6.69 mths	One year of daily supplementation with 50 g (FS50) =256 kcal, 25g FS (FS25)=127kcal or 71 g LP=282 kcal	<p>LP (n=61) FS50 (n=61) FS25 (n=60) P value</p> <p>ΔWeight(kg) 2.37\pm0.60 2.47\pm0.77 2.37 \pm 0.61 0.66</p> <p>ΔLength (cm) 12.7\pm 1.7 13.5 \pm 2.9 13.2 \pm 2.9 0.23</p> <p>ΔMUAC (cm) 1.1\pm0.9 1.0\pm1.1 1.0\pm0.8 0.64</p> <p>ΔWAZ -1.29 \pm 0.63 -1.18\pm0.90 -1.32 \pm0.65 0.53</p> <p>ΔWHZ -0.98 \pm0.83 - 1.05\pm0.86 -1.13\pm0.75 0.62</p> <p>ΔHAZ -0.74 \pm0.95 -0.59 \pm 1.22 -0.64 \pm0.8 0.71</p>
16.	(Adu-Afarwuah, Lartey <i>et al.</i> 2007) Ghana	Community based randomized trial	All infants attending weight monitoring session were potentially eligible.	Complementary foods with SP = 1sachet/d NT= 1 tablet/d or NB =20g/d from 6-12 months of age.	<p>NB group had a significantly higher WAZ (-0.49 \pm 0.54) LAZ (-0.20 \pm 0.54) as compared to NT group (WAZ:-0.67 \pm 0.54; LAZ: -0.39 \pm 0.54)</p> <p>SP (n=96) NT (n=101) NB (n=97) NI (n=81)</p> <p>WAZ -0.53\pm1.1^{a,b} -0.88\pm1.1^a -0.40\pm1.1^b -0.74 \pm 1.1^{a,b}</p> <p>HAZ -0.40 \pm 1.0 -0.44\pm1.0 -0.14 \pm 1.0 -0.40 \pm 1.0</p>

					<p>WHZ $-0.45 \pm 1.1^{a,b}$ -0.89 ± 1.1^a -0.43 ± 1.1^b $-0.74 \pm 1.1^{a,b}$</p> <p>Values in the same row with different superscripts are significantly different $P < 0.05$</p>																		
17.	(Kuusipalo, Maleta <i>et al.</i> 2006) Malawi	RCT	n= 128 6-17 mths underweight infants (WAZ<-2, WHZ greater than -3).	12 wks supplementatio n 1 of 8 supplements, nothing, 5, 25, 50, or 75 g/day milk based fortified spread (FS), or 25, 50, or 75 g/day soy- based fortified spread.	<p>Change in WAZ score, HAZ, and WHZ score was not significantly different between the groups.</p> <p>Average gain in weight and height was higher among infants receiving FS (daily 25 to 75 g) than among those receiving only 0 to 5 g FS. The maximum weight gain was 0.83 kg with 50g of milk base fortified spread</p>																		
18.	(Ciliberto, Sandige <i>et al.</i> 2005) Malawi	Controlle d, comparat ive clinical effective ness trial	n=1178 children aged between 10- 60 mths with wasting (WHZ < -2)	Home based therapy with RTUF (260g plastic jar/day) for 8 weeks. Standard therapy (F100).	<table border="0"> <thead> <tr> <th></th> <th>F100 (n= 186)</th> <th>RTUF (n=992)</th> </tr> </thead> <tbody> <tr> <td>Rate of weight gain (g/kg/d) 4 wks</td> <td>2.0 ± 6.9</td> <td>3.5 ± 3.7*</td> </tr> <tr> <td>Rate of height gain (mm/d) 8 wks</td> <td>0.12± 0.29</td> <td>0.19±0.59*</td> </tr> <tr> <td>Rate of MUAC gain (mm/d) 4wks</td> <td>0.23±0.33</td> <td>0.32±0.41*</td> </tr> <tr> <td>Children died %(n)</td> <td>5.4 (10)</td> <td>3.0 (30)</td> </tr> <tr> <td>Children relapsed % (n)</td> <td>16.7 (31)</td> <td>8.7 (87)*</td> </tr> </tbody> </table>		F100 (n= 186)	RTUF (n=992)	Rate of weight gain (g/kg/d) 4 wks	2.0 ± 6.9	3.5 ± 3.7*	Rate of height gain (mm/d) 8 wks	0.12± 0.29	0.19±0.59*	Rate of MUAC gain (mm/d) 4wks	0.23±0.33	0.32±0.41*	Children died %(n)	5.4 (10)	3.0 (30)	Children relapsed % (n)	16.7 (31)	8.7 (87)*
	F100 (n= 186)	RTUF (n=992)																					
Rate of weight gain (g/kg/d) 4 wks	2.0 ± 6.9	3.5 ± 3.7*																					
Rate of height gain (mm/d) 8 wks	0.12± 0.29	0.19±0.59*																					
Rate of MUAC gain (mm/d) 4wks	0.23±0.33	0.32±0.41*																					
Children died %(n)	5.4 (10)	3.0 (30)																					
Children relapsed % (n)	16.7 (31)	8.7 (87)*																					
19.	(Manary, Ndkeha <i>et al.</i> 2004) Malawi	RCT	n=282 children > 1 year old discharged from nutrition unit were systematicall y allocated to treatment	RTUF, blended maize/soy flour =730kJ/kg/d, or RTUF supplement=21 00 kJ/d Recovery WHZ >0. provided	<table border="0"> <thead> <tr> <th></th> <th>RTUF (n=69)</th> <th>RTUF-S (n=96)</th> <th>Maize/soy (n=117)</th> </tr> </thead> <tbody> <tr> <td>Average weight gain (g/kg/d)</td> <td>5.2*</td> <td>3.1</td> <td>3.1</td> </tr> <tr> <td>Children likely to reach WHZ>0 (1.1%-1.3%)</td> <td>95%</td> <td>78%</td> <td>78%</td> </tr> </tbody> </table>		RTUF (n=69)	RTUF-S (n=96)	Maize/soy (n=117)	Average weight gain (g/kg/d)	5.2*	3.1	3.1	Children likely to reach WHZ>0 (1.1%-1.3%)	95%	78%	78%						
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20.	(Sandige, Ndekeha <i>et al.</i> 2004)	RCT	n=260 with severe malnutrition	Imported RTUF or local RTUF=175	<table border="0"> <thead> <tr> <th></th> <th>Local RTUF (n= 135)</th> <th>Imported RTUF (n=125)</th> </tr> </thead> <tbody> <tr> <td>Weight gain (g/kg/d)</td> <td>5.2 ± 4.6</td> <td>4.8 ± 4.0</td> </tr> </tbody> </table>		Local RTUF (n= 135)	Imported RTUF (n=125)	Weight gain (g/kg/d)	5.2 ± 4.6	4.8 ± 4.0												
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Weight gain (g/kg/d)	5.2 ± 4.6	4.8 ± 4.0																					

	Maawi			kcal/kg/d) till they reach WHZ> -0.5 or after 16 wks	Height gain (mm/d) MUAC gain (mm/d)	0.33 ± 0.29 0.35 ± 0.30	0.24 ±0.23 0.36 ± 0.31	
21.	(Diop, Dossou <i>et al.</i> 2003) Senegal	Open-labeled randomized trial	n=70 severely malnourished (WFH Z score < -2) age 6-30 mths	The children received either 3 meals of F100 or 3 meals of RTUF daily <i>ad libitum</i>		F100 (n= 30)	RTUF (n=30)	
					Rate of weight gain (g/kg/d)	10.1 (8.7, 11.4)	15.6 (13.4, 17.8)*	
					Average duration of rehabilitation	17.3 (15.6,19.0)	13.4(12.1, 14.7)*	
					Daily energy intake (kJ /kg body wt/d)	573± 201	808 ±280*	
22.	(Yebyo, Kendall <i>et al.</i> 2013) Ethiopia	A retrospective cohort study	n= 628 age 6-59 mths treated for SAM (MUAC < 110 cm or weight for height ratio <70%)	Plumpy'Nut sachets according to their body weight	Weight gain= 5.24 (4.98, 5.63) gm/kg/day. Recovery rate =61.78%, Defaulter rate=13.85% Mortality rate= 3.02% Weight increase= 21.4% Mean length of stay under intervention = 6.48 weeks (95% CI=6.25, 6.72). The overall rate of weight gain and recovery rate < sphere standards			
23.	(Grellety, Shepherd <i>et al.</i> 2012) Niger	Cohort study	n=2238 age 6 -23 mths between 60 - 80 cm in length	RUSF (Plumpy'Doz) 4 x325 g pots (4 pots = 1 mth) for 4 mths.		RUSF (n= 1400)	Control (n=838)	P value
					Δ Weight (g)	395 (364-425)	327 (281-372)	0.05
					Δ Length (cm)	2.7 (2.6-2.8)	2.8 (2.6-2.9)	0.24
					Δ WHZ	-0.2 (-0.2-0.2)	-0.3 (-0.4-0.3)	0.006
					Δ MUAC (mm)	-2.8 (-3.2-2.3)	-4.0(-4.7-3.3)	0.002

24.	(Isanaka, Roederer <i>et al.</i> 2010) Niger	Cohort study	n=1645 age 6-36 mths	RUSF= 247 kcal / day for 6 mths, RTUF =500 kcal /day for 4 mths.	Reduction in wasting Incidence of wasting Incidence of severe wasting Reduction in incidence of stunting	RUSF 46% (6%-69%) NS NS	RTUF 59% (17%-80%) NS NS ↓
25.	(Ali, Zachariah <i>et al.</i> 2013)	Cross-sectional semi-structure d question naire survey	Care givers and community health workers of malnourished children age 6-59 mths	149 care givers interviewed	Expressed problems with acceptability = 60% Perceived child dissatisfaction with taste = 43% Attributed side effects to PPN = 64% Children took PPN willingly = 48% Needed encouragement or had to be forced = 47% children completely rejected PPN after 3 weeks = 5%		

Abbreviations: RCT, randomized control trial; P-RTUF, Peanut based RTUF; WPC-RTUF, whey protein concentrate RTUF; RUCF, Lipid based ready to use complementary foods; UNIMIX , fortified corn soy blend porridge; LNS, lipid based nutrient supplement; SAM, severe acute malnutrition; MUAC, mid-upper arm circumference; RTUF, ready-to-use therapeutic food; WAZ, weight-for-height Z-score; HAZ, height-for-age Z-score; WHZ, weight-for-height Z- score; WLZ, weight-for-length Z- score; LAZ, length-for-age Z- score; CSB, corn-soy blend; CSB++, corn-soy blend “plus-plus”; LNS, A lipid-based nutrient supplement; RUSF, ready-to-use supplementary food; FS, fortified spread; LP, micronutrient- fortified maize-soy flour ; PPN, peanut-based ready-to-use therapeutic food; HCCM , High Calorie Cereal Milk; NB, Nutributter; NT, Nutritabs; SP, Sprinkle powder; NI, non intervention; LOS, Length of stay; SG, significantly different; NS, Not statistically significant; Diff, Difference; Δ, change; wks, weeks; months, mth.

1.2 Appetite regulation in relation to energy provision

1.2.1 Principles of appetite regulation

The internal driving force for food ingestion is called appetite. Appetite has two definitions; a) covers the entire field of food intake, motivation, selection and preference, and, b) refers particularly to the qualitative features of eating, sensory characteristic or responsiveness to the environmental stimulation which can be contrasted with conditions based on eating in response to physiological stimuli or energy deficit (Blundell, De Graaf *et al.* 2010).

Appetite is divided into three components i.e. hunger, satiation and satiety (Mattes, Hollis *et al.* 2005). Hunger is a motivational state that enhances food intake and reflects the body state in which metabolic fuels for example free fatty acids and glucose, are low and is a principal variable that connotes the drive to eat (Blundell, De Graaf *et al.* 2010). Satiation is the process that leads to the termination of eating and it takes place when food is being eaten. It governs the size and duration of the meal. It is also called as intra-meal satiety (Blundell, De Graaf *et al.* 2010). Following meal initiation, hunger subsides and satiation is dominant. Then, satiation feelings contribute to eating cessation and a period of eating abstinence begins.

The feelings that determine the inter-meal fasting period is known as satiety, it is the process which leads to the inhibition of further eating. It reduces the feelings of hunger and increases fullness after the meal has finished (Mattes, Hollis *et al.* 2005, Blundell, De Graaf *et al.* 2010, Geraedts, Troost *et al.* 2011). Satiety is influenced by a number of factors such as food mass, palatability, energy density and glycaemic index (Halton and Hu 2004). Satiety is a complex interaction of behavioural, psychological and physiological mechanisms (Halton and Hu 2004). Satiety leads to increase in fullness and a decline in hunger after the meal has finished (Blundell, De Graaf *et al.* 2010).

Appetite control is a complex process that involves the interaction between the central and peripheral organs. This influences the short term feeding behaviour, as well as the long term adaptive process that responds to the energy input and energy expenditure (Delzenne, Blundell *et al.* 2010). A number of different physiological, psychological, cultural, emotional and social factors interact in a very intricate manner. This influences the sensations of hunger, satiety and desire to eat (Melanson, Westerterp-Plantenga *et al.* 1999, Flint, Raben *et al.* 2000). Therefore, appetite control is a complex process, and it involves both central and peripheral organ interaction (Delzenne, Blundell *et al.* 2010) and is a combination of neuro-endocrine, behavioural, and psychological influences which are involved in controlling the appetite and food intake (Huda, Wilding *et al.* 2006).

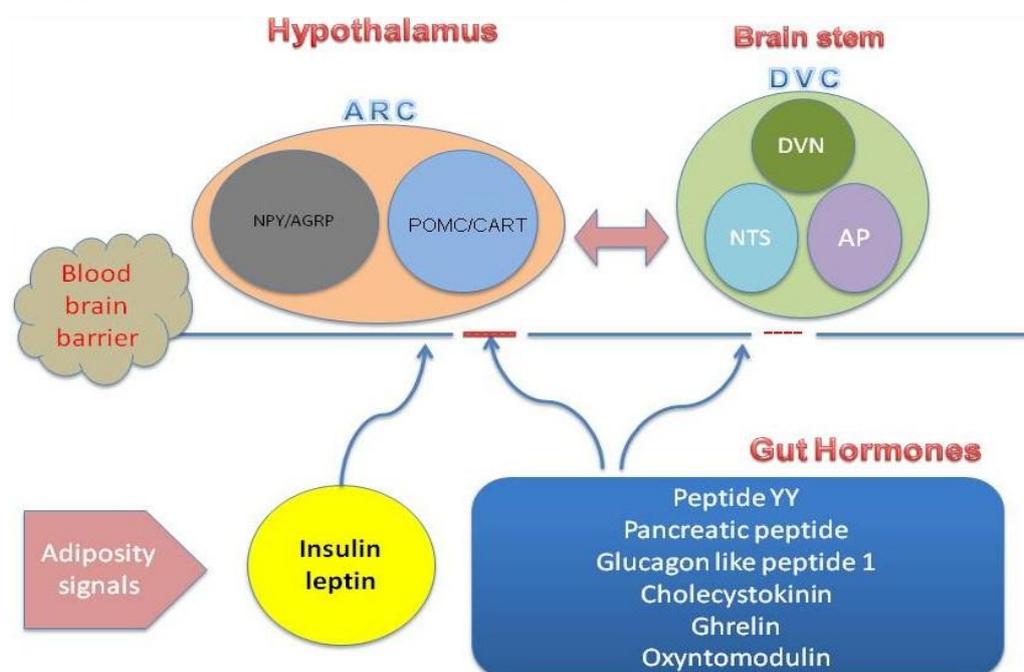


Figure.1.2 Schematic diagram of appetite regulation by central appetite circuits and circulating factors.

Abbreviations: ARC- Arcuate Nucleus, NPY- Neuropeptide Y, AgRP - Agouti-Related Peptide, CART- Cocaine Amphetamine Regulated Transcripts, POMC- proopiomelanocortin, DVC- Dorsal Vagal Complex, consisting of dorsal motor nucleus, AP- Area Postrema and NTS- Nucleus Tractus Solitarius

1.2.2 Role of gut peptides in appetite control

There are numerous peptides and hormones produced by the gut and the pancreas. These include peptide YY (PYY), cholecystokinin (CCK), ghrelin and a glucagon-like peptide 1 (GLP-1) which regulate the food intake and directly influences satiety and hunger (Huda, Wilding *et al.* 2006, Crespo, Cachero *et al.* 2014).

In this section, the role of ghrelin, PYY and CCK is discussed in detail. Gastrointestinal hormones which regulate acute satiety are GLP-1, Peptide YY, Oxyntomodulin and glucose-dependent inhibitory polypeptide (Wynne, Stanley *et al.* 2005). In 1912, stomach contractions were thought to be involved in regulating appetite. In 1975, the duodenum was considered as the "pituitary of gastrointestinal tract", as it controls gut hormones (Le Roux and Bloom 2005). After the discovery of gut peptides in the hypothalamus and presence of hypothalamic hormones in the gut, the relationship between the gut and brain became evident (Cowley, Smith *et al.* 2003). Endocrine cells are scattered throughout the gut mucosa, which ensure the diverse and important endocrinological capacity of the gut. Nutrients in contact with the gut mucosa release gut hormones, which regulate different gastrointestinal functions. Examples of these are; motility, secretion, absorption and positive feedback to the central nervous system in response to the nutrients' availability. All these factors play an important role in regulating the food intake.

Ghrelin

Ghrelin is the only endogenous peripheral hormone that has powerful orexigenic properties (appetite stimulant; which induces hunger and increases food intake). In 1996, the growth hormone secretagogue (GHS) receptor was identified, and surprisingly ghrelin was discovered as an endogenous ligand for the GHS receptor in the stomach (Kojima, Hosoda *et al.* 1999). Ghrelin is a

28- amino acid peptide released primarily by the oxyntic glands of the gastric mucosa, and from the duodenum, ileum, caecum and colon (Sakata, Tanaka *et al.* 2002, Huda, Wilding *et al.* 2006, Crespo, Cachero *et al.* 2014). Ghrelin was also isolated from tissues of the hypothalamus (Cowley, Smith *et al.* 2003), anterior pituitary gland (Korbonits, Bustin *et al.* 2001), lungs (Volante, Fulcheri *et al.* 2002), pancreas (Volante, Allia *et al.* 2002), kidneys, adrenal glands, thyroid, placenta, gonads, lymphatic tissue, myocardium, adipose tissues, and the bones (Huda, Wilding *et al.* 2006). This widespread distribution of ghrelin may explain its many endocrine and non-endocrine effects.

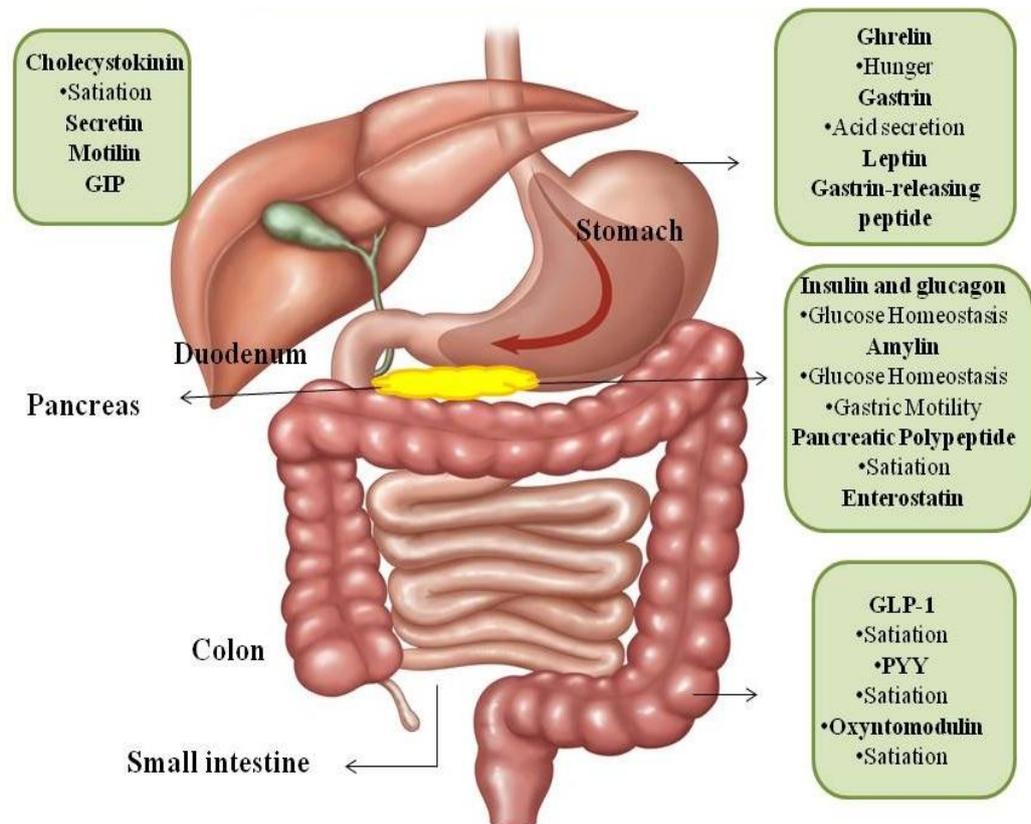


Figure 1.3 Schematic presentation of principal sites of gastrointestinal peptides and their role in appetite regulation. *Modified from* (Cummings and Overduin 2007)

Table 1.3. Main Neuropeptides and Peripheral Appetite Regulating hormones and their Role in Appetite Regulation

Neuropeptides and Neurotransmitters	Main site of synthesis/production	Action on appetite	Other functions	Effect of peripheral peptides on food intake
Anorexigenic factors				
PYY	Ileum and colon (gastrointestinal L cells)	<ul style="list-style-type: none"> • Inhibition of gastric emptying • Increases absorption of fluid and electrolytes from ileum after meal • May acts as “ileal brake” • Direct appetite suppressing effect on brain by activation of anorexigenic POMC neurons and inhibition of NPY neurons 	<ul style="list-style-type: none"> • Inhibits bile acid secretion • Inhibits secretion of digestive enzymes • Inhibits gall bladder emptying 	↓
CCK	Duodenum and proximal jejunum	<ul style="list-style-type: none"> • Inhibits gastric emptying • Appetite suppressing action on brain (hypothalamus through neural pathway to 	<ul style="list-style-type: none"> • Stimulates gallbladder contraction • Stimulates release of digestive enzymes 	↓

	(Intestinal I cells)	<p>brain stem).</p> <ul style="list-style-type: none"> • CCK1 receptors are present in the areas of CNS involved in food regulation such as dorsomedial hypothalamus, NTS and AP 	<ul style="list-style-type: none"> • Stimulates pancreatic enzyme secretion • Regulates nutrient delivery rate from stomach to small intestine • CCK has numerous effects on memory, analgesia, sexual behaviour, anxiety and seizure threshold • May be involved in long-term body weight regulation. 	
GLP-1	Ileum and colon (gastrointestinal L cells)	<ul style="list-style-type: none"> • Inhibits gastric emptying • Important role in “ileal brake” • Direct appetite suppressing action on brain. It exert its effects via GLP-1 receptors containing neurones of NTS project to POMC/CART neurones in arcuate nucleus and to dorsomedial and para-ventricular nucleus. The central effect of GLP-1 are 	<ul style="list-style-type: none"> • Stimulates glucose dependent insulin secretion and inhibits glucagon release 	↓

		mediated through AP and ascending brain stem pathway.		
Oxyntomodulin	Ileum and colon (gastrointestinal L cells)	<ul style="list-style-type: none"> • Delays gastric emptying • Inhibit gastric acid secretion 	<ul style="list-style-type: none"> • Glucose dependent insulin release 	↓
Pancreatic Polypeptide	Peripheries of pancreatic islets (endocrine type F cells)	<ul style="list-style-type: none"> • Stimulation of gastrointestinal motility • Stimulation of gastric acid secretion 	<ul style="list-style-type: none"> • Inhibit pancreatic exocrine function • Inhibit gall bladder contraction 	↓
Leptin	Adipocytes	<ul style="list-style-type: none"> • Signals the brain stem and arcuate nucleus of hypothalamus when fat stores are low • Regulate body weight and energy homeostasis. It inhibits NPY and AgRP and activates α-MSH. It also acts on IGF-1 and growth hormone • Involved in neuroendocrine response to 	<ul style="list-style-type: none"> • Expression of NPY and AgRP is up-regulated in leptin deficiency due to fasting • Regulation of lipid metabolism • Regulation of inflammatory response • Glucose homeostasis • Puberty and ovulation 	↓

	starvation	<ul style="list-style-type: none"> • Fetal growth and metabolism • Bone metabolism 	
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Orexigenic Factors				
Ghrelin	Gastric mucosa (X/A like cells)	<ul style="list-style-type: none"> • Direct appetite stimulating effect. It stimulates neurons expressing AgRP and NPY, which are orexigenic peptides. • Increase gastric emptying • It also increases gastric motility gastric acid secretion and pancreatic exocrine secretion 	<ul style="list-style-type: none"> • Powerful releasing stimulator of growth hormones from anterior pituitary gland • It also influences secretion of other endocrine hormones like cortisol and insulin 	↑
Orexin A and B	Cell bodies of lateral hypothalamic area	<ul style="list-style-type: none"> • Excitatory neuropeptides • Appetite stimulating neuropeptides 		↑

NPY	Arcuate nucleus of hypothalamus	<ul style="list-style-type: none"> • Stimulate food intake • Decrease energy expenditure 	↑
AgRP	Arcuate nucleus of hypothalamus	<ul style="list-style-type: none"> • Stimulate food intake 	↑

Abbreviations: *Cholecystinin, CCK; neuropeptide Y, NPY; agouti-related peptide, AgRP; peptide YY, PYY; glucagon-like peptide 1, GLP-1; insulin like growth factor-1, IGF-1; Growth hormone, GH; proopiomelanocortin, POMC; α -melanocyte-stimulating hormone, α -MSH; area postrema, AP; nucleus tractus solitaries, NTS*

Ghrelin has numerous physiological effects on the human body due to its extensive expression in various tissues (Table 1.3). Through GHSR-1a activation, it is a powerful releasing stimulator of growth hormones from the anterior pituitary gland. Ghrelin also influences the secretion of the other endocrine hormones such as cortisol (Takaya, Ariyasu *et al.* 2000) and insulin (Wierup, Svensson *et al.* 2002, Date, Toshinai *et al.* 2005). The most important function of ghrelin is the regulation of food intake and energy balance. Ghrelin also increases gastric motility, gastric acid secretion and pancreatic exocrine secretion (Masuda, Tanaka *et al.* 2000, Asakawa, Inui *et al.* 2001). After binding to its receptors GHSR-1a, it stimulates neurons expressing AgRP and NPY, which are orexigenic peptides (Crespo, Cachero *et al.* 2014).

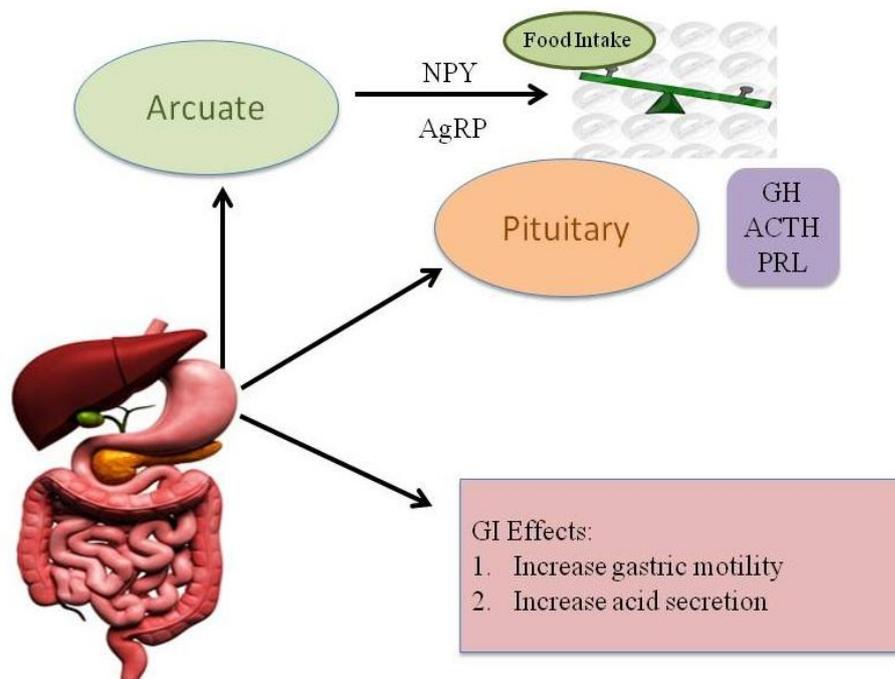


Figure 1.4 Schematic presentation of the role of ghrelin in appetite regulation

Ghrelin secretion from the stomach is largely dependent upon the nutritional state. Under fasting conditions before a meal ghrelin, plasma concentration is highest, suggesting its role in meal initiation (Cummings, Purnell *et al.* 2001).

Its level decreases tremendously after a meal (within 15-20 minutes of food intake) and again, its plasma concentration increases before the next meal after gastric emptying (Cummings, Foster-Schubert *et al.* 2005). Ghrelin responses are dose dependently associated to the amount of calories ingested. They are also dependent on the type and composition of the macronutrient ingested (Callahan, Cummings *et al.* 2004).

Ghrelin is more effectively suppressed by proteins and carbohydrates as compared to isocaloric-rich fat meals (Blom, Lluich *et al.* 2006, Foster-Schubert, Overduin *et al.* 2008). Prolonged suppression of ghrelin after consumption of dietary proteins is found in many studies showing relationship between higher satiety and high protein intake (Latner and Schwartz 1999, Bowen, Noakes *et al.* 2006).

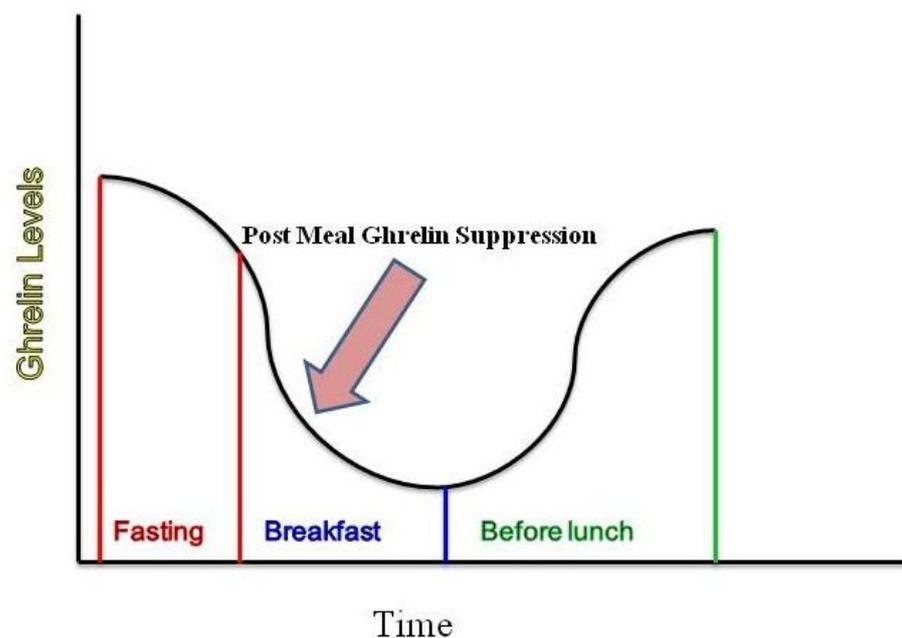


Figure 1.5 Schematic presentation of ghrelin release in response to food intake

Plasma ghrelin levels are higher in lean people, low in obese people, and its level increases with the loss of weight in obesity (Hansen, Dall *et al.* 2002). There is a difference in appetite regulation and ghrelin in overweight and lean

subjects (Hellstrom, Geliebter *et al.* 2004). In obese subjects fasting ghrelin levels are reduced (Tschöp, Weyer *et al.* 2001) and post-prandial ghrelin levels are not suppressed in obese subjects, which demonstrates that ghrelin may be involved in obesity (English, Ghatei *et al.* 2002). Moreover, intravenous administration of ghrelin in obese subjects resulted in the consumption of more food showing that obese subjects are not resistant to ghrelin (Druce, Wren *et al.* 2005). On the other hand, ghrelin levels rises when weight loss is associated with cachexia and anorexia (Shimizu, Nagaya *et al.* 2003, Itoh, Nagaya *et al.* 2004).

An understanding of the fundamental role of ghrelin, in appetite regulation, in humans, comes mainly from two independent clinical trials. One in which synthetic ghrelin was intravenously administered to the subjects and then appetite and food intake was measured prior to, during and after infusion which was then compared with the infusion of placebo (saline). In other trials, the ghrelin was investigated in pre-load studies in which ghrelin concentrations were studied prior to, during and after consumption of a pre-load, and then compared with the reference product. In healthy subjects intravenous administration of ghrelin caused 28% increase in energy intake from *ad libitum* buffet breakfast, and increased the subjective ratings of hunger (Wren, Seal *et al.* 2001).

Similarly, intravenous infusion of ghrelin in lean and obese humans has shown increased energy intake from buffet meals. In healthy lean and obese individuals intravenous administration of ghrelin at doses of 1 pmol/kg/min (low dose) and 5 pmol/kg/min (high dose) increased the mean energy intake by $36.6 \pm 9.4\%$ on the low dose day and $70.1 \pm 15.5\%$ on the high dose day in obese subjects. In the lean subjects, the mean increase in energy intake was $20.2 \pm 10.8\%$ on the low dose day and $20.1 \pm 10.6\%$ on the high dose day (Druce, Neary *et al.* 2005, Druce, Wren *et al.* 2005), demonstrating its relevance to food intake behaviour (Delzenne, Blundell *et al.* 2010).

Studies have documented, that after ghrelin infusion, the palatability of the food was increased (Druce, Wren *et al.* 2005). Moreover, ghrelin administration increased the neural response to food pictures, particularly in areas, involved in the hedonic values of visual cues (Malik, McGlone *et al.* 2008).

The pre-load studies also demonstrated that suppression of ghrelin resulted in decreased energy intake from subsequent an *ad libitum* buffet meal. In lean and obese subjects, it was observed that high protein and adequate protein meals resulted in decreased energy intake by approximately 14 and 22% respectively from subsequent an *ad libitum* buffet meal, which was associated with the suppression of ghrelin (Brennan, Luscombe-Marsh *et al.* 2012). In overweight male subjects, consumption of a glucose based liquid pre-load, compared with lactose, resulted in higher *ad libitum* energy intake and an earlier return of ghrelin to pre-prandial concentrations (Bowen, Noakes *et al.* 2006).

There are some studies where this is not the case. This suggests that ghrelin suppression may postpone initiation of the next meal. However, evidence also suggests that ghrelin levels are conditioned by the habitual patterns of meals and rises in anticipation of a meal (Frecka and Mattes 2008). On the other hand, studies have documented that time-blinded spontaneous meal requests are directly related to the caloric content of the previous meal and not to the ghrelin response. When the macronutrient distribution, volume and all other features are kept constant, and the caloric content of meals is varied, the duration and depth of prandial ghrelin suppression are dose-dependently associated to the number of calories ingested (Callahan, Cummings *et al.* 2004). Therefore, it can be suggested that large meals suppresses both ghrelin and hunger more comprehensively as compared to small meals. Additionally, the degree of the consequent pre-prandial recovery of ghrelin levels has also been reported to correlate with the number of calories consumed in the

subsequent meal (Callahan, Cummings *et al.* 2004, Cummings 2006). All the above studies provide evidence of ghrelin as a meal initiator.

Peptide tyrosin-tyrosin

Peptide tyrosin-tyrosin (PYY) was first isolated from porcine jejunal mucosa by Tatemoto and his colleagues (Huda, Wilding *et al.* 2006). Peptide YY is a thirty-six amino acid linear peptide synthesized and released from endocrine cells (L-cells) of the intestine (ileum and colon) (Delzenne, Blundell *et al.* 2010, Crespo, Cachero *et al.* 2014). Peptide YY is present throughout the gastrointestinal tract, but its highest concentration is present in the distal segment of gastrointestinal tract, i.e. ileum, colon and rectum. It is also found in the pancreas and hypothalamus. Peptide YY is a member of the PP fold peptide family and is co-secreted with glucagon like peptide 1 (Huda, Wilding *et al.* 2006, Cooper 2014). PYY affects gastrointestinal tract motility and inhibits gastric acid secretion, pancreatic enzyme secretion and gall bladder emptying (Pittner, Moore *et al.* 2004, Wren and Bloom 2007, Cooper 2014). It may act as part of the “ileal brake” by slowing down gastric emptying and intestinal transit so that nutrients, fluid and electrolytes absorption from the ileum increases after a meal (Huda, Wilding *et al.* 2006).

The ileal brake is the primary inhibitory feedback mechanism to control meal transit through the gastrointestinal tract to optimise nutrient digestion and absorption (Maljaars, Peters *et al.* 2008). Activation of the ileal brake leads to reduced frequency of antral and duodenal peristaltic waves. It stimulates an increase in pyloric sphincter pressure which contributes to delayed gastric emptying (Fone, Horowitz *et al.* 1990). Moreover, ileal brake activation also reduces the frequency of jejunal contractions (percentage of jejunal contractions and distance of propagation) which contributes to an increased small intestinal transit time. In addition to that, ileal brake activation has been shown to decrease the meal stimulated secretion of pancreatic enzymes,

inhibits gastric acid secretion and also reduces bile acid output in a dose dependent manner (Keller, Holst *et al.* 2006, Maljaars, Peters *et al.* 2008).

Plasma PYY levels are lowest in the fasted state in the morning, rise after breakfast, increase further after lunch, and peak a few hours after the evening meal (Delzenne, Blundell *et al.* 2010, Crespo, Cachero *et al.* 2014). PYY is secreted into the circulation after the intake of food and its levels are elevated within 30 minutes of food reaching the small intestine (Batterham, Cohen *et al.* 2003, Crespo, Cachero *et al.* 2014). This suggests neural regulation of PYY in the intestine as most PYY is released from the small intestine and colon in advance of the arrival of the nutrients to this region of the intestine. The highest plasma concentration of PYY occurs postprandially, one hour after the meal (Batterham, Cowley *et al.* 2002, Batterham, Cohen *et al.* 2003), and its levels remain high for about 6 hours after the meal (Batterham, Cohen *et al.* 2003, Crespo, Cachero *et al.* 2014).

The increase in PYY levels is directly proportional to caloric intake (Degen, Oesch *et al.* 2005, Crespo, Cachero *et al.* 2014). The size of the meal is also important in relation to the PYY response (Degen, Oesch *et al.* 2005, Le Roux, Batterham *et al.* 2006). As HENSD supplements are energy rich and provide 2.49 MJ of energy, and increase the caloric intake, it is therefore important to determine how HENSD supplementation impacts on PYY levels.

PYY release is stimulated more by fat intake than protein and carbohydrate meals with similar energy content (Onaga, Zabielski *et al.* 2002, Huda, Wilding *et al.* 2006, Cooper 2014). Protein rich meals cause the greatest elevation in PYY levels compared to other macronutrients (Batterham, Heffron *et al.* 2006, Crespo, Cachero *et al.* 2014).

Evidence for the causal role of PYY in appetite regulation in humans mainly comes from two independent clinical trials; one in which synthetic PYY was exogenously administered to the subjects and then appetite was measured

(appetite ratings and or *ad libitum* food intake after fixed time period) prior to, during and after infusion which was then compared with the infusion of a placebo (saline). In other clinical trial the PYY was investigated in pre-load studies in which serum PYY concentrations were studied prior, during and after consumption of test meals and then compared with the reference product (Mars, Stafleu *et al.* 2012).

Peripheral administration of PYY₁₋₃₆ in both rodents and man, inhibited food intake for several hours (Batterham, Cowley *et al.* 2002, Challis, Pinnock *et al.* 2003). Similarly, a study by Sloth *et al.* (2007) on 12 lean and 12 obese males observed a decreased energy intake of 19% during lunch. This was compared to PYY₁₋₃₆ and a 22% decrease in the energy intake after saline infusion. A statistically significant lower rating of perceived ability to eat were observed after both PYY₃₋₃₆ and PYY₁₋₃₆ infusion but no significant differences were observed in other ratings of appetite (Sloth, Holst *et al.* 2007). The effect of exogenous administration of PYY₃₋₃₆ was also studied in both lean and obese subjects. After 2 hours of intravenous infusion of PYY₃₋₃₆ the energy intake at an *ad libitum* buffet was decreased by 30% in obese subjects and 31% in lean subjects (Batterham, Cohen *et al.* 2003). Lower fasting plasma levels of PYY have been reported in obese people in comparison with lean subjects (Batterham, Cohen *et al.* 2003, Le Roux, Batterham *et al.* 2006). Several other studies have also demonstrated reduction in energy intake following exogenous PYY administration (Degen, Oesch *et al.* 2005, Neary, Small *et al.* 2005).

All the above mentioned exogenously administered PYY studies demonstrate that the intravenous infusion of PYY reduced food intake. However, evidence regarding the role of endogenously produced PYY on energy intake is less conclusive and is less frequently studied (Cooper 2014). Ducet *et al.* (2008) observed no association between *ad libitum* energy intake and PYY levels in twenty-five pre-menopausal women (Ducet, Laviolette *et al.* 2008). Guo *et al.* (2006) observed that fasting and postprandial PYY levels were not

associated with the *ad libitum* food intake over 24 hours in twenty-nine non-diabetic subjects (Guo, Ma *et al.* 2006). However, in a study to evaluate the impact of barley β -glucans on short-term appetite and satiety related hormones, it was found that β -glucan enriched bread (β GB) resulted in a 16% higher total AUC of the PYY response, resulting in 19% reduction of energy intake at an *ad libitum* lunch subsequent to β GB, as compared to the control bread (Vitaglione, Lumaga *et al.* 2009). There is also controversy regarding the role of endogenous PYY on appetite control. With some studies showing that plasma PYY is positively correlated with the feelings of fullness and inversely related with the feelings of hunger (Guo, Ma *et al.* 2006), while other studies report that PYY levels were not correlated with the feelings of fullness and hunger (Cooper, Watras *et al.* 2011, Heden, Liu *et al.* 2013, Kozimor, Chang *et al.* 2013). The fact that higher endogenous PYY levels are not always associated with a decreased energy intake, or subjective ratings of fullness, or hunger, highlights the fact that appetite control is a very complex process. When food is ingested, numerous physiological, hormonal, social and psychological processes are triggered in an intricate in a very complex manner. Therefore, it can be said that PYY is just one piece, which contributes to the complex process of appetite control.

HENSD are energy-rich supplements, rich in both carbohydrates and fats. Therefore, it is imperative to investigate how the intake of HENSD impacts on post-prandial PYY levels, and how this contributes to the appetite regulation and energy intake during the consecutive meal.

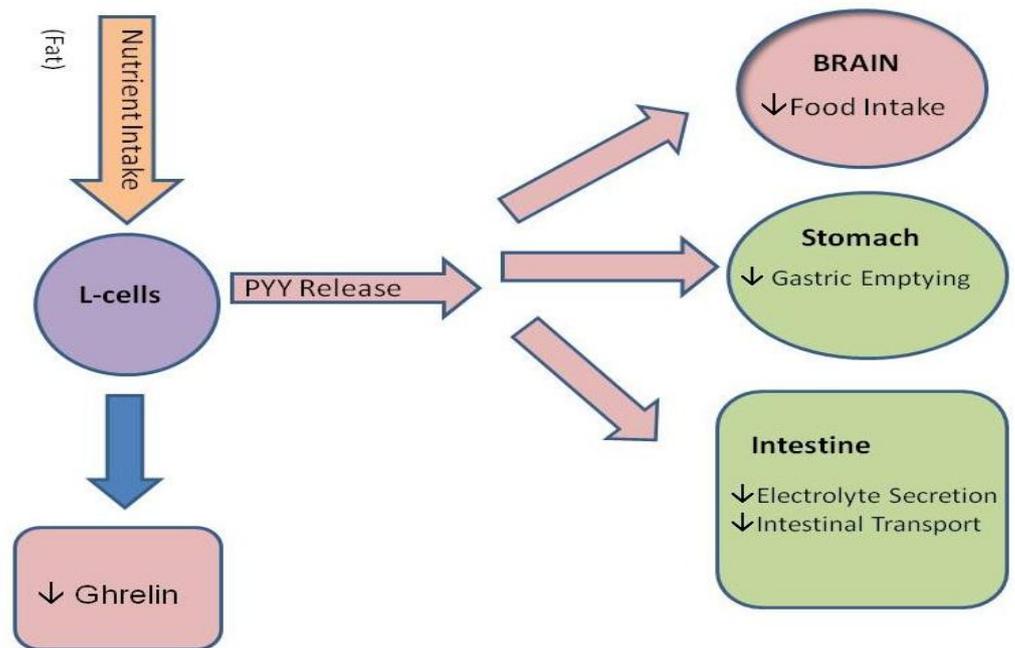


Figure 1.6 Schematic presentation of the effects of peptide YY (PYY) on gastrointestinal system

Cholecystokinin

Cholecystokinin (CCK) was discovered by Ivy and Oldberg in 1928. It is a hormone that contracts the gall bladder (Huda, Wilding *et al.* 2006). In 1973, Gibbs and his colleagues discovered that CCK had effects on the appetite and it was the first gut hormone found which had this property. CCK is a gut hormone found mainly in the duodenum and jejunum. CCK is also present in parts of brain, for example, the amygdala, cortex, hippocampus, thalamus, hypothalamus, septum, dorsal hindbrain and basal ganglia, and acts as a neurotransmitter (Huda, Wilding *et al.* 2006, Delzenne, Blundell *et al.* 2010, Crespo, Cachero *et al.* 2014).

The main effect of CCK on the gastrointestinal system is to facilitate nutrient absorption, stimulate gall bladder contraction, enhance pancreatic enzyme secretion and slow gastric emptying. The nutrient delivery rate from the stomach to the small intestine is regulated by CCK (Wren and Bloom 2007, Delzenne, Blundell *et al.* 2010, Zac-Varghese, Tan *et al.* 2010) (Table 1.3).

Cholecystokinin levels increase 10-30 minutes after the initiation of a meal. The levels fall gradually and require 3-to-5 hrs to return to baseline levels. Proteins and fats (rather than equal calories of carbohydrates) have been shown to stimulate the CCK release (Delzenne, Blundell *et al.* 2010).

It has been reported that fasting CCK levels were higher in obese women compared to lean women. In anorexia nervosa fasting CCK levels were found to be lowered (Baranowska, Radzikowska *et al.* 2000). Results of another study are at variance (Milewicz, Bidzinska *et al.* 2000). In one study on Prader-Willi Syndrome (PWS), which is characterised by hyperphagia, it was found that the fasting CCK levels were not significantly different from non PWS obese subjects, which suggests that CCK may not be associated with the hyperphagia of this PWS (Butler, Carlson *et al.* 2000).

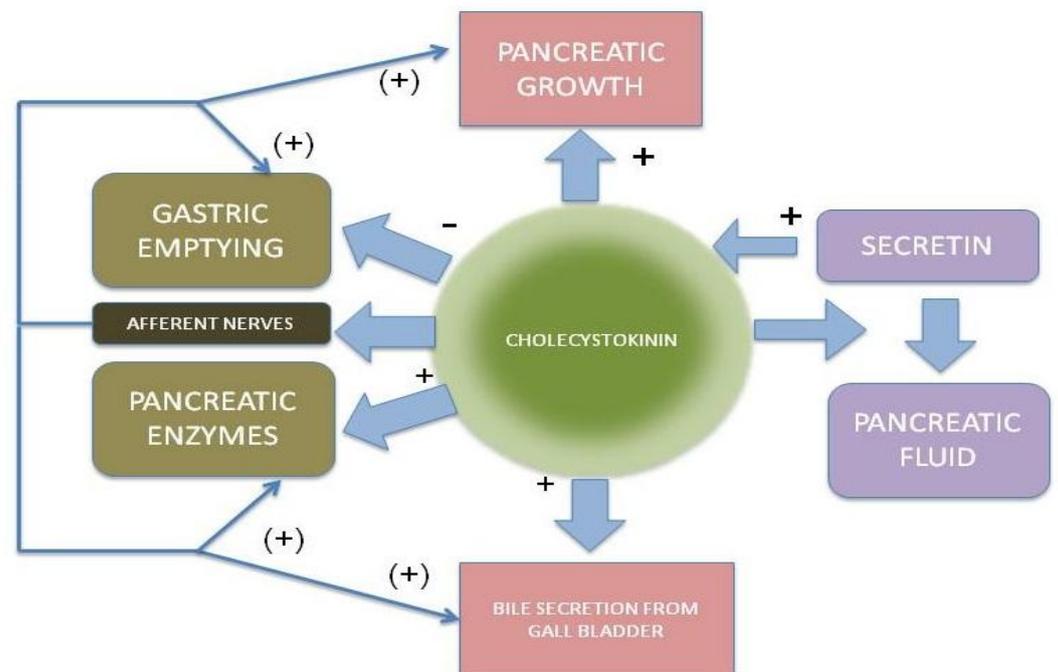


Figure 1.7 Schematic presentation of the effects of Cholecystokinin on gastrointestinal system

As with PYY, evidence for the causal role of CCK in appetite regulation comes mostly from two types of clinical trials, infusion studies and pre-load studies. Intravenous administration of physiological doses of CCK in humans reduces food intake and also enhances the perception of fullness (Crespo, Cachero *et al.* 2014). Several studies have been performed regarding the impact of CCK on appetite. A study by Ballinger *et al.* (1995) observed a statistically significant reduction of 20% in food intake, after administration of CCK-8. This produced a similar plasma concentration to that of the meal (Ballinger, McLoughlin *et al.* 1995). Similarly, an infusion of CCK-33 also reduced food intake in the subsequent meal by 20% (Lieverse, Jansen *et al.* 1994). Brennan *et al.* (2005) also demonstrated that exogenous administration of CCK-8 in the nine healthy males increased the subjective rating of fullness, decreased ratings of desire to eat and subsequent energy intake at the *ad libitum* buffet meal (Brennan, Feltrin *et al.* 2005). Exogenous administration of CCK-8 in ten healthy men reduced energy intake during an *ad libitum* buffet meal, and reduced the desire to eat after 90 minutes of the intravenous administration of CCK-8 (Brennan, Little *et al.* 2008).

Evidence also suggests that the intravenous administration of CCK-8 in healthy older adults also suppressed energy intake significantly at a subsequent *ad libitum* buffet meal by 11% (Tai, Feinle-Bisset *et al.* 2010). This indicates that CCK has a fundamental role in appetite regulation (Delzenne, Blundell *et al.* 2010). Generally, all these studies provide a consistent picture that CCK suppresses food intake. The suppression of food intake varied considerably between 0% and 63% depending upon the subject characteristics, dose and other experimental conditions. For the appetite suppressing impact of CCK a full stomach is considered to be a necessary condition, which indicates that the delayed gastric emptying may be the possible mechanism by which CCK suppresses appetite (De Graaf, Blom *et al.* 2004).

Evidence regarding the role of the endogenously produced CCK on the energy intake and appetite is controversial, with some demonstrating, that the higher

CCK levels, compared to the reference, was associated with decreased energy intake during an *ad libitum* meal, and decreased ratings of hunger and increased fullness (Hall, Millward *et al.* 2003, Pasman, Blokdijsk *et al.* 2003). While some studies demonstrated no effect of CCK on satiety (Sanggaard, Holst *et al.* 2004) another demonstrated the opposite effect (Zijlstra, Mars *et al.* 2009). Hall *et al.* 2005 demonstrated that there was significantly less energy intake from the *ad libitum* buffet meal after 90 minutes, after whey protein, compared to casein pre-load, with a 60% increase in the plasma CCK and greater subjective feeling of satiety (Hall, Millward *et al.* 2003). In another pre-load study, performed on overweight subjects, it was observed that the higher responses of CCK were correlated with satiety, but had no impact on energy intake (Bowen, Noakes *et al.* 2006).

The exogenous administration of CCK may achieve concentrations above physiological values (the increase in blood levels may be larger than that which can be evoked by food) and therefore we see a link between CCK, appetite and food intake. However, the higher endogenous CCK levels are not always associated with increased subjective ratings of fullness, or decreased hunger, or decreased energy intake, which identifies the fact, that when food is ingested numerous physiological processes are triggered, including hormonal responses which interact in a very complex manner. Therefore, we can say that CCK is just a single factor, which also contributes to the complex process of appetite control.

So far, there are no studies on CCK and PYY responses after HENSND supplementation, and therefore contribution of their responses to the partial energy compensation seen with supplementation remains to be investigated.

1.2.3 Role of glucose and insulin in appetite regulation

There are many theoretical ideas regarding hunger, meal initiation and control of food which were preceded by the concept that glucose intake and its utilization plays central role in regulating the energy balance of the body, and

in control of satiety and hunger (Campfield and Smith 2003, Flint, Gregersen *et al.* 2007). In 1955, Mayer proposed the glucostatic theory, whereby increases in the concentration of blood glucose levels increases the satiety feelings, whereas decreased levels of blood glucose concentration produces the opposite effect. Mayer postulated that post-prandial hyperglycemia and increased glucose utilization in gluco-sensitive sites of brain leads to decreased feeling of hunger and termination of food intake (a period of satiety). Mayer postulated that the reduced glucose utilization (metabolic hypoglycemia) point at which the peripheral arteriovenous difference of blood glucose becomes negligible, and glucose is not entering “metabolizing cells” was the point for meal initiation (Flint, Gregersen *et al.* 2007).

After the glucostatic theory, numerous studies were performed to look at the impact of decreased intracellular glucose concentration as the meal initiation stimulus (Anderson and Woodend 2003, Campfield and Smith 2003, Flint, Gregersen *et al.* 2007). In the 1980s, experiments were performed on free feeding rats, with continuous computerized monitoring of blood glucose levels. It was found that transient decline in blood glucose levels, was associated with meal initiation (Thorens 2008). This decline in blood glucose concentration did not predict meal size or timing of meal termination (Flint, Gregersen *et al.* 2007). However, studies have found that the changes in blood insulin and glucose concentrations ran parallel to changes in visual analogue scale scores of fullness (Lemmens, Martens *et al.* 2011).

Whether only blood glucose levels are associated with short-term regulation of appetite is still unclear. It is also controversial that decline in blood glucose concentration before meal is the causal factor for initiation of meal. Some studies demonstrated that decrease in blood glucose concentration causes meal initiation (Anderson and Woodend 2003, Campfield and Smith 2003). One other study demonstrated that there is no significant role of blood glucose levels on short term regulation of appetite (Porte Jr, Baskin *et al.* 2002, Flint, Gregersen *et al.* 2007). Some studies have shown that the concentrations of

blood glucose levels, following a meal, are inversely associated with subjective measures of appetite and food intake (Anderson, Catherine *et al.* 2002), while another study found no association between satiety and blood glucose concentrations after raising the blood glucose concentrations by intravenous infusion (Lavin, Wittert *et al.* 1996). It has been postulated that the potential impact of insulin as a satiety signal may require increased blood glucose concentrations (Blaak, Antoine *et al.* 2012). Moreover, along with post-prandial glucose concentration there are numerous factors which co vary, making it difficult to determine that whether it is only blood glucose or other factors that are responsible for promoting the satiety effect (Holt, Brand *et al.* 1996, Raben, Holst *et al.* 1996).

In one study, 18 healthy adults were housed in a room isolated from time cues. Blood glucose concentration was monitored continuously (blood withdrawn from antecubital vein at the rate of 25 μ l/min) following an overnight fast over 2-6 hours period. It was found that both meal request and changes in hunger were preceded by brief transient decrease in the blood glucose levels. This association between changes in hunger rating, meal request and decline in the blood glucose levels was significant. No changes were found in hunger rating associated with stable blood glucose levels (Campfield, Smith *et al.* 1996).

Similarly Dewan *et al.* (2004) demonstrated that hypoglycemia induced by exogenous insulin, significantly increased the feelings of hunger over a 20 minutes interval, and also increased food intake at the successive test meal in healthy men (Dewan, Gillett *et al.* 2004). The relatively early and sharp decline in the blood glucose levels seems to be very important in the early return of the appetite and hunger and disappearance of satiety (Niwano, Adachi *et al.* 2009). Evidence from previous studies demonstrates that glucose may be used as a biomarker of satiety in certain conditions. The evidence suggests that high concentrations of glucose in the blood are associated with lower appetite, however, absolute glucose concentrations have a complicated relation to appetite (De Graaf, Blom *et al.* 2004). A study by Arumugam *et al.*

(2008) also demonstrated link between postprandial changes in blood glucose levels and appetite changes supporting the glucostatic theory (Arumugam, Lee *et al.* 2008).

Glucose triggers insulin secretion by β cells of the pancreatic islets of Langerhans (Thorens 2008, Crespo, Cachero *et al.* 2014). It has been suggested that insulin is involved in short term appetite regulation (Flint, Gregersen *et al.* 2007, Blaak, Antoine *et al.* 2012). Insulin secretion increases rapidly after food intake and acts to control blood glucose levels (Benelam 2009). Studies have demonstrated that, in normal weight subjects, the concentration of insulin before a test meal are inversely related to subsequent *ad libitum* energy intake (Speechly and Buffenstein 2000) while this association is not found in obese people (Speechly and Buffenstein 2000).

From one meta-analysis it seems that the postprandial insulin response is one of the best predictors of *ad libitum* energy intake and satiety (Flint, Gregersen *et al.* 2007). In order to clarify the role of blood glucose and insulin in short term regulation of appetite, Flint *et al.* 2007, conducted a meta-analysis on seven single test meal studies on healthy or overweight subjects conducted in their department, having individual participant data on blood glucose, insulin and subjective appetite sensations, using visual analogue scales (Flint, Gregersen *et al.* 2007). The results from this meta-analysis suggested that in healthy subject's insulin, but not glucose, is associated with short-term regulation of appetite. However, this relationship is disrupted in obese or overweight subjects. Therefore, this study does not support a significant role for blood glucose in short term appetite regulation. However, it has been postulated that the portable effect of insulin as a satiety signal might require increased blood glucose concentrations.

Studies have demonstrated that both insulinemic and glycaemic response are associated with the subjective feelings of hunger, and /or satiety and appetite irrespective of age, sex and weight. The relative sharp and early decline of

blood glucose below the baseline may be a key to the earlier return of hunger and appetite and earlier disappearance of satiety (Niwano, Adachi *et al.* 2009). Therefore, the presence of both hyperglycaemia and hyperinsulinaemia may be essential to inhibit appetite and food intake (Blaak, Antoine *et al.* 2012).

Several mechanisms may be involved if insulin acts as a short-term satiety signal (Flint, Gregersen *et al.* 2007). Insulin could act directly on the hypothalamic area through insulin receptors (Verdich, Toubro *et al.* 2001) as central insulin receptors plays an important role in appetite regulation (Porte Jr, Baskin *et al.* 2002). A central insulin effect could be mediated indirectly through its interaction with other satiety inducing hormones like peptide YY, CCK and GLP-1 (Schwartz and Morton 2002, Flint, Moller *et al.* 2004). Blood insulin and glucose levels are known to stimulate leptin production (Crespo, Cachero *et al.* 2014). Leptin levels are negatively correlated with food intake and appetite, when subjects are not in energy balance, while the leptin relationship with appetite during energy balance is less clear. When subjects are not in energy balance, leptin is appropriate as a long term biomarker of satiety. However, leptin cannot act as a simple short term satiety biomarker (De Graaf, Blom *et al.* 2004). Some of the studies have shown that insulin may affect the intracellular ATP levels in the liver by altering the transport of energy yielding substrate to the liver (Flint, Gregersen *et al.* 2007). According to Flint *et al.* 2007, the insulin infusion studies do not reflect normal circumstances. The intravenous infusion of insulin glucose is also infused concomitantly in order to prevent hypoglycemia. As a result of which, insulin effect on the energy status of the liver could be bypassed (Flint, Gregersen *et al.* 2007).

Since insulin and glucose levels may contribute to the suppression of appetite prior to a consecutive meal, thus diminishing energy intake in response to the HENSD supplementation. Therefore, research is required to investigate whether blood glucose and insulin levels contribute to the appetite suppressive action of HENSD supplementation prior to a consecutive meal.

1.2.4 Role of thermogenesis in appetite regulation

Diet induced thermogenesis (DIT) is the increase in the energy expenditure above basal fasting levels consequent to meal consumption, and it reflects the amount of energy required for the processing, digestion, absorption and storage of the food consumed per day (Westerterp 2004, Tentolouris, Pavlatos *et al.* 2008, Tentolouris, Alexiadou *et al.* 2011, Ravn, Gregersen *et al.* 2013). The two main determinants of DIT are the nutrient composition of food and the level of energy intake. DIT is increased by increasing the carbohydrate or protein content of food and by increasing energy intake (Westerterp-Plantenga, Wijckmans-Duijsens *et al.* 1997, Westerterp 2004). There are two distinctive components of DIT: a) obligatory component which is involved in the postprandial nutrient metabolism and comprises of all the energy expenditure involved in the breakdown, digestion and storage of the nutrients in the body, and b) a facultative component which is partly mediated through the increased sympathetic nervous system activity, and could be determined by means of sensory characteristics of food (van Baak 2008).

DIT is commonly expressed as a percentage and accounts for 10-15 % of the total daily energy expenditure (Donahoo, Levine *et al.* 2004, Westerterp 2004, Scott and Devore 2005, Tentolouris, Alexiadou *et al.* 2011). However, in studies, it varies considerably between individuals and between specific groups of individuals (Nagai, Sakane *et al.* 2005, Petzke and Klaus 2008) and also varies depending upon the macronutrient composition of the meals (Westerterp 2004, Scott and Devore 2005, Compher, Frankenfield *et al.* 2006, Tentolouris, Alexiadou *et al.* 2011). There is convincing evidence that protein exerts more thermic effect (20-30% of the energy content of the protein consumed) as compared to fats (0-5%) and carbohydrates (5-10 %) (Tentolouris, Pavlatos *et al.* 2008, Acheson, Blondel-Lubrano *et al.* 2011, Tentolouris, Alexiadou *et al.* 2011).

Raben *et al* (2003) found that protein and alcohol produce greater effects on thermogenesis, compared to carbohydrates and fats. However, this study did not find significant differences in the appetite rating and food intake after ingestion of these macronutrients with equal energy. Therefore this study did not support the relationship between macronutrient oxidation hierarchy and the satiety hierarchy (Raben, Agerholm-Larsen *et al.* 2003). There are controversies regarding the thermic effect of fat and carbohydrate in the studies comparing high carbohydrate and high fat meals with a constant protein content. Some studies observed significantly higher thermogenic responses after high carbohydrate meals (Rasmussen, Larsen *et al.* 2007) while others found no significant differences between both the meals (Verboeket-Van De Venne and Westerterp 1996, Labayen, Forga *et al.* 1999).

DIT, as has been suggested previously, is one of the mechanisms that influence appetite sensations including hunger, satiety and energy intake. Studies have demonstrated that increased DIT has been directly associated with reduced hunger and increased fullness (Luscombe, Clifton *et al.* 2003, Raben, Agerholm-Larsen *et al.* 2003, Lejeune, Westerterp *et al.* 2006). Borbeck postulated thermostatic hypothesis in which he postulated that the changes in visceral, skin and core temperature could stimulate behaviours that alter energy balance and match energy expenditure, energy intake and body fat mass over a prolonged period of time. Therefore, hypothermia and reduced thermogenesis and its metabolic effects initiates food intake, while on the other hand postprandial hyperthermia leads to feeding cessation and satiety (Campfield and Smith 2003, Stratton, Hébuterne *et al.* 2013) .

Westerterp-Plantenga *et al* (1999) proposed that the basis of the association between DIT and satiety may be that the increased energy expenditure at rest after intake of food causes increased oxygen consumption and body temperature. This may give rise to the body feeling deprived of oxygen and could be translated into the feeling of satiety (Ravn, Gregersen *et al.* 2013).

Several studies have measured DIT and the appetite responses to a specific meal (Raben, Christensen *et al.* 1994, Westerterp-Plantenga, Wijckmans-Duijsens *et al.* 1997, Crovetti, Porrini *et al.* 1998, Westerterp-Plantenga, Rolland *et al.* 1999, Raben, Agerholm-Larsen *et al.* 2003, Keogh, Lau *et al.* 2007, Leidy, Mattes *et al.* 2007, Ravn, Gregersen *et al.* 2013). Some of these studies (de Graaf, Hulshof *et al.* 1992, Raben, Agerholm-Larsen *et al.* 2003, Keogh, Lau *et al.* 2007, Leidy, Mattes *et al.* 2007, Ravn, Gregersen *et al.* 2013) did not find any significant differences in the DIT, appetite, or both among their comparative test meals. Therefore, from these studies it is difficult to form a relationship between DIT and postprandial appetite.

While there are some studies (Raben, Christensen *et al.* 1994, Westerterp-Plantenga, Wijckmans-Duijsens *et al.* 1997, Crovetti, Porrini *et al.* 1998, Westerterp, Wilson *et al.* 1999) that have found a significant correlation between the postprandial subjective ratings of satiety and DIT, while comparing meals of the different compositions. A significant relationship between DIT and the postprandial AUC for satiety was found by Westerterp-Plantenga *et al.* (1997) while comparing a full-fat lunch (41% fat, 44% carbohydrate and 15% protein) and isoenergetic reduced fat lunch (26% fat, 59% carbohydrate and 15% protein) and a reduced -fat reduced- energy lunch (26% fat, 58% carbohydrate and 15% protein) (Westerterp-Plantenga, Wijckmans-Duijsens *et al.* 1997).

Similarly, Crovetti *et al.* (1998) examined the association between appetite sensations and the thermic effect of food after three isoenergetic meals, a high protein meal (HP) (68% of energy from protein), high carbohydrate meal (HC) (69% of energy from carbohydrate) and high fat meal (HF) (70% energy from fat). There was highest thermogenesis with the HP meal and it determined the highest fullness, while no differences in the appetite sensations and the thermic effect between the carbohydrate and the fat meals were detected. A significant positive correlation was found between DIT and the postprandial area under

the curve (AUC) for fullness, but no correlation was detected between the desire to eat and satiety and DIT (Crovetti, Porrini *et al.* 1998).

A recent individual participant data meta-analysis found no association between the DIT and satiety (Ravn, Gregersen *et al.* 2013). According to a review by Veldhorst *et al.* (2008) in a single meal, the acute satiating impact of protein is present when the content of 25-81% of energy is from protein (Veldhorst, Smeets *et al.* 2008). In a Ravn *et al.* (2013) the protein intake was approximately 16% of the energy from protein and may be too low to trigger a satiating effect. De Graaf *et al.* (2004) suggested that the rise in body temperature resulting from the DIT might be a biomarker of satiety and satiation. They proposed that DIT and body temperature are dependent on the nutrient oxidation and may indicate an integrative measure of energy balance (De Graaf, Blom *et al.* 2004).(De Graaf, Blom *et al.* 2004). Since HENS are energy rich supplements, their consumption can be expected to significantly enhance thermogenesis and thus contributes to the suppression of appetite prior consecutive meal, and thus diminish impact of supplements on promotion of energy intake. Thus further investigation is imperative to find out whether DIT contributes to the appetite suppressive action of HENS supplementation prior consecutive meal.

1.2.5 Role of Gastric emptying in appetite regulation

Gastric emptying is a complex process, which is determined by the coordinated gastric and proximal intestinal motor activity, myogenic, neural and hormonal factors (Malagelada and Azpiroz 2010).

Evidence from previous studies suggests that gastric emptying is associated with appetite, energy intake (Sepple and Read 1989, Jones, Doran *et al.* 1997, Sturm, Parker *et al.* 2004, Nair, Brennan *et al.* 2009) and glycaemic control (Horowitz, Edelbroek *et al.* 1993, Wishart, Horowitz *et al.* 1998, Beckoff, MacIntosh *et al.* 2001). The role of gastric emptying in appetite control is

complex (Janssen, Vanden Berghe *et al.* 2011). Very few studies directly investigated the relationship between gastric emptying and appetite (Janssen, Vanden Berghe *et al.* 2011).

Physiological delay in gastric emptying, or artificially induced delayed gastric emptying, was found to be associated with increased subjective feelings of fullness and satiety and cessation of food intake (Hunt 1980, Di Lorenzo, Williams *et al.* 1988, Wisén and Hellström 1995). A delayed rate of gastric emptying is observed in patients with anorexia nervosa (Dubois, Gross *et al.* 1979). Some studies have documented rapid rate of gastric emptying in obese subjects (Johansson and Ekelund 1976). Gastric distension influences the appetite as it triggers stretch and tension mechanoreceptors, which transmit information to the brain (Grundy 2002, Powley and Phillips 2004). There is a strong relationship between antral distension and sensation of fullness. This suggests that a slower rate of gastric emptying, and hence increased gastric distension, might predispose to satiety. Previous studies have demonstrated that increased gastric distension due to delayed gastric emptying is related with the delayed return of hunger and increased satiety (Geliebter 1988). Similarly, another study found a significant correlation between the time required for 90% of the food to empty and increased postprandial hunger ratings (Sepple and Read 1989). Experiments using ultrasonographic and scintigraphic techniques have found that satiety and satiation are inversely correlated to gastric emptying (Hveem, Jones *et al.* 1996). Gastric emptying is inversely correlated with the caloric content of the food (Calbet and MacLean 1997).

Existing evidence suggests that in response to the varying combination of intraduodenal fat and/or carbohydrate, inter-related gastrointestinal factors like glucagon-like peptide -1 (GLP-1), CCK and PYY are released which provides feedback inhibition of gastric emptying and suppresses the energy intake (Feinle, O'Donovan *et al.* 2003, Seimon, Feltrin *et al.* 2009, Seimon, Lange *et al.* 2010, Little and Feinle-Bisset 2011). The studies on humans suggests that the fat slows down the rate of gastric emptying, suppresses the

appetite and stimulates the secretion of CCK, which is dependent upon the digestion of triglycerides to fatty acids (Matzinger, Degen *et al.* 2000, Feinle, Rades *et al.* 2001, Feinle, O'Donovan *et al.* 2003). Intra-duodenal infusion of fat in healthy subjects slows gastric emptying, reduces hunger and food intake (Lieverse, Jansen *et al.* 1994, Chapman, Goble *et al.* 1999) and these effects are in part mediated by CCK (Fried, Erlacher *et al.* 1991, Lieverse, Jansen *et al.* 1994). Moreover, gastric emptying is also influenced by the individual's characteristics like age, sex and time of the day.

In addition to gastric distension, the presence of nutrients in the small intestine is also vital for satiety. In response to intestinal nutrients numerous gut peptides are released that act directly by entering the bloodstream, and indirectly through the vagus nerve, to inhibit appetite (Cummings and Overduin 2007, Savastano and Covasa 2007). After eating, CCK, PYY and a glucagon-like peptide-1 (GLP-1) are released (Huda, Wilding *et al.* 2006, Wren and Bloom 2007). Both PYY and GLP-1 show a biphasic response to meal ingestion (Cummings and Overduin 2007). It is proposed that duodenal nutrients initiate a humoral and/or neural signal to the distal gut which contributes to their early release (Pilichiewicz, Chaikomin *et al.* 2007), then followed by direct nutrient stimulation of the distal gut (Cummings and Overduin 2007). During early postprandial period, more rapid gastric emptying is directly associated with increases in plasma CCK (Nguyen, Fraser *et al.* 2007), PYY (Nguyen, Fraser *et al.* 2007) and GLP-1.

Evidence suggests that a threshold rate of gastric emptying exists which should be exceeded to stimulate the release of GLP-1. Upon release GLP-1, CCK and PYY in turn inhibit gastric emptying (Camilleri 2009). However, the inhibition of gastric emptying is not essential for the control of food intake by intestinal stimulation (Ritter 2004). On the other hand, ghrelin accelerates the rate of gastric emptying (Levin, Edholm *et al.* 2006). However, a more rapid gastric emptying is associated with the lower postprandial ghrelin concentrations (Blom, Lluch *et al.* 2006), which in turn is correlated with a reduced appetite

(Heath, Jones *et al.* 2004). All these findings suggest that a slow rate of gastric emptying might enhance and prolong gastric distension, delay or reduce release of PYY, CCK, and GLP-1 and reduce ghrelin suppression.

Gastric distension by the food may play a vital role in the feelings of fullness. Reduced sensations of hunger, after a meal, may result from nutrient interaction with receptors in the small intestine (Näslund, Hellström *et al.* 2001). Moreover, factors like the inter-meal interval also influence the relative contribution of the intestinal and gastric signals to appetite control. Differences in the initial versus subsequent rate of gastric emptying (Little, Russo *et al.* 2007) might explain why accuracy in compensation for prior energy intake reduces as the time interval to the subsequent meal increases, thus showing the importance of considering the temporal pattern and the kinetic of gastric emptying in relation to appetite control. From the current literature it appears that there is a vital integrative relationship between gastric emptying and gut peptides in appetite control.

There is no evidence about how gastric emptying is influenced by HENSD supplementation, therefore further research is necessary to find out how gastric emptying impacts on the compensation induced by HENSD supplementation.

1.2.6 Appetite regulation and amendments in energy intake after high energy nutritional supplement drinks

There are no studies investigating the impact of specific nutritional supplements use in the treatment of malnutrition on appetite and appetite hormones. So far, the role of metabolic and hormonal appetite modulators in amendments of energy intake after high energy nutritional supplements was investigated only in tube feeding studies (Stratton and Elia 1999, Stratton, Stubbs *et al.* 2008).

Stratton *et al* performed a study on six healthy men who received 3 days of bolus tube feeding (6.93 ± 0.38 MJ/day of 4.18 kJ/mL multinutrient feed). For two days before and after tube feeding placebo boluses (<0.4 MJ/day) were provided by the tube. Bolus tube feeding significantly increased the total energy intake as compared with the placebo periods before and after tube feeding. Overall, oral food intake was significantly reduced with the bolus tube feeding as compared to the placebo periods. With bolus tube feeding the suppression of food intake was equivalent to 39.7% of the total tube feed energy infused leaving 60.3% additive to oral energy intake. During three days of bolus tube feeding the oral energy intake significantly declined by 15%. Therefore, in total only 40% of the tube feed energy was additive. No significant differences were detected in the first hour VAS rating, except for the preoccupation with the thoughts of food rating, which was significantly different during the bolus tube feeding. Significant increase in some of the putative satiety mediators like insulin, leptin, glucagon, resting energy expenditure and respiratory exchange rates and reduction in ghrelin was observed with the bolus tube feeding (Stratton, Stubbs *et al.* 2008).

The intermittent delivery of tube feeding as bolus reflected a physiological liquid meal-like pattern. This was regardless of the variety of significant metabolic and hormonal changes involved in satiation. However, this induced only partial compensation for energy provided by the bolus (Stratton, Stubbs *et al.* 2008). However, studies on continuous tube feeding suggested that the continuous tube feeding did not suppress appetite and food intake (Stratton, Stubbs *et al.* 2003). A study conducted by Stratton *et al* (2003) on six healthy subjects showed that 3 day administration of liquid feeds (6.9 MJ) containing a mixture of macro and micronutrients, continuously for 12- 24 hours, irrespective of whether the feed was administered diurnal or nocturnal, had remarkably little impact on appetite sensations (measured by visual analogue scale) and on the potential metabolic and hormonal mediators of appetite. Although there was a marked increase in total energy intake during tube

feeding, and a reduction in the oral energy intake equivalent to <40% of the tube feed energy (Stratton, Stubbs *et al.* 2003).

Studies have demonstrated that distressing appetite sensations may occur even when all the nutrient requirements were fulfilled by enteral tube feeding, or by parenteral nutrition (Stratton and Elia 1999). Moreover, enteral tube feeding and parenteral nutrition appears to only partially suppress oral food intake. Therefore, when nutritional supports are used to supplement food intake, an increase in total energy intake is observed (Stratton 2001). Indeed, tube feeding delivers nutrients directly into stomach and thus by-passes the sensory aspects of oral consumption and the cephalic phase response. In addition, one bolus of tube feeding provides nearly twice as much energy in comparison to typical oral high-energy supplement (Stratton and Elia 1999, Stratton, Stubbs *et al.* 2008). However, interaction between appetite, food intake, and the metabolic and hormonal mediators of appetite and satiety, following tube feeding and oral supplementation, can be different.

Impaired adjustment of energy intake due to previous ingestion has also been reported by studies investigating satiating effects of liquid beverages. These studies report weak satiating effects of these beverages and suggest that the liquid calories are not well perceived by the body (DiMeglio and Mattes 2000, Mattes 2006, Drewnowski and Bellisle 2007). This suggestion receives support from the evidence available from pre-load and appetite regulation studies. These studies report that preloads consumed in liquid form, elicit greater postprandial hunger and lower fullness sensations, more rapid gastric-emptying and attenuated insulin and glucagon-like peptide release, and lower ghrelin suppression, than do preloads consumed in solid form. This is related to a lower energy intake during the consistent meal (Cassady, Considine *et al.* 2012). Therefore, HENSD supplements may permit enhancement in daily energy intake, due to diminish appetite responses. Further research is required to more fully understand the impact of oral nutritional supplements on the putative measures of appetite, metabolic and hormonal variables, and appetite sensations, and to investigate the mechanisms which are related to partial

compensation for the energy provided by high-energy nutritional supplements, and which permit an increase in energy intake.

1.3 Impact of acute changes in energy intake and energy balance on plasma lipids, glycaemia and insulinemia

1.3.1 Insulin resistance

Insulin is very important anabolic hormone and is vital for appropriate tissue development, growth, and maintenance of body glucose homeostasis. Insulin is secreted by the β cells of the pancreas located in the islets of Langerhans, in response to elevated levels of glucose and amino acids after meal (Pessin and Saltiel 2000, Shulman 2000, Thorens 2008, Crespo, Cachero *et al.* 2014). Insulin regulates glucose homeostasis at several sites; it reduces the hepatic glucose output (decreases gluconeogenesis and glycogenolysis) and increases the rate of glucose uptake, mainly into striated muscle and adipose tissue. Insulin also has an effect on the lipid metabolism, increases lipid synthesis in both liver and fat cells, and attenuates the release of fatty acid from triglycerides in fat and muscle (Shulman 2000). Insulin resistance occurs when normal circulating concentration of insulin are insufficient to regulate all these processes properly (Pessin and Saltiel 2000).

Insulin resistance is described as a condition in which greater concentration of insulin is required than normal to elicit a given metabolic response to glucose (Frayn 2002). Therefore, insulin resistance is characterized by decreased insulin ability to promote peripheral glucose disposal and suppress hepatic glucose production. As a result, a higher concentration of insulin in the blood is required to clear glucose. Insulin resistance is a growing worldwide problem, which develops progressively over the years, if unchecked predisposes to type 2 diabetes mellitus and CVD (Haag and Dippenaar 2005,

Reaven 2012). Insulin resistance is tightly coupled with obesity and cardiovascular pathology (Eckel, Grundy *et al.* 2005, Li, Fu *et al.* 2014).

Initially, insulin resistance is reflected by hyper-insulinaemia in response to food, if untreated, progresses to the stage where hyper-insulinaemia and hyperglycaemia both are experienced in response to food (Kendrick 2003). If the individuals suffering from the latter stage are not treated, insulin resistance will progress to next stage in which fasting insulin and glucose levels are elevated (Kendrick 2003). If all the former stages are not adequately dealt with, it results into type 2 diabetes (Kendrick 2003).

In type 2 diabetes, initially the pancreas is able to secrete insulin effectively although it is characterised by the resistance of the body tissue to insulin impact. However, constant high insulin demand can eventually cause β cell failure assisting the transition starting from insulin resistance to diabetes. Increased risk of cardiovascular disease is independently associated with low insulin sensitivity (Rutter, Meigs *et al.* 2005). Studies have highlighted the role of hyper-insulinaemia (insulin resistance) as a major determinant for the development of cardiovascular events (Bonora, Kiechl *et al.* 2007) in pre-diabetic women who developed type 2 diabetes later on (Hu, Stampfer *et al.* 2002) and in established type 2 diabetic patients (Bonora, Formentini *et al.* 2002). Insulin resistance is not only a key cofounder of type 2 diabetes, CVD risk, but is also associated with other metabolic disorders such as dyslipidaemia (Chahil, Reyes *et al.* 2008, Li, Fu *et al.* 2014), chronic low grade inflammation (Pradhan, Cook *et al.* 2003) and obesity (Ritchie and Connell 2007). Therefore, insulin resistance is a generalized metabolic disorder which is characterized by ineffective insulin function in liver, skeletal muscle, vascular endothelium and adipocytes (Haag and Dippenaar 2005). All these changes are the consequence of insulin resistance and contribute to insulin resistance so it is difficult to separate the causes from effects.

1.3.2 Lack of adipose tissue and insulin resistance

The metabolic consequences of having too little fat (lipodystrophy) are astonishingly similar to those of having too much fat (obesity) (Vatier, Bidault *et al.* 2013). Adipose tissue is a crucial metabolically active site of hormone synthesis and energy balance (Havel 2004, Harwood Jr 2012). Adipose tissue produces adipocytokines, which have both paracrine and endocrine functions, so adipose tissue has the ability to influence other tissues such as skeletal muscle and liver through tissue derived hormones and the dynamic flux of fatty acids. An increase in adipose tissue compartment is associated with enhanced lipolysis and increased non-esterified fatty acids (NEFA) in the circulation, which is suggestive of adipose tissue insulin resistance (Delarue and Magnan 2007).

Elevated levels of NEFA in the circulation induces chronic skeletal muscle insulin resistance through decreased insulin stimulated glucose uptake and carbohydrate oxidation (Boden and Shulman 2002). In insulin resistance adipose tissue fails to suppress lipolysis in response to insulin which may lead to inappropriate NEFA release during the postprandial state producing additional 'lipid overflow' to skeletal muscle and liver (Arner 2005). Moreover, NEFA induce hepatic insulin resistance which is regarded as unchecked hepatic glucose production (Roden, Stingl *et al.* 2000). However, in insulin resistance the NEFA concentrations are not always elevated, therefore lipotoxicity may be associated with decreased fat oxidative capacity of the target organs (Corpeleijn, Saris *et al.* 2009).

On the other hand, post-prandial NEFA concentrations may have a comparatively greater impact on insulin sensitivity. An increased concentration of circulating NEFA in response to the dietary fat may be associated with 'lipid overflow' (Jackson, Wolstencroft *et al.* 2005). Visceral adipose tissue is supposed to have greater influence on insulin sensitivity as compared to subcutaneous fat (Macor, Ruggeri *et al.* 1997, Boyko, Fujimoto *et al.* 2000). An enlarged visceral adipose tissue compartment may elevate total

circulating NEFA (Arner 2002). This may impact the skeletal muscles insulin resistance (Corcoran, Lamon-Fava *et al.* 2007), and may increase NEFA delivery to liver through splanchnic circulation (Basu, Basu *et al.* 2001). This may cause pancreatic beta cell toxicity (Taylor 2008) and may be a source of pro-inflammatory adipokines. This in turn promotes insulin resistance by directly acting on insulin signalling, and indirectly by the induction of endothelium dysfunction (Kahn, Hull *et al.* 2006, Montecucco, Steffens *et al.* 2008). Adipose tissue hormones also contribute to insulin sensitivity, plasma leptin concentrations are directly associated with the adipose tissue mass (Mantzoros, Magkos *et al.* 2011). Plasma adiponectin levels are inversely associated with adipose tissue mass (Arita, Kihara *et al.* 1999). In obesity, pre-diabetic states and type 2 diabetes, the circulating adiponectin levels are lower (Pellme, Smith *et al.* 2003, Kowalska, Straczkowski *et al.* 2008).

Deficiency of adipose tissue as in lipodystrophy (either total or partial) is also associated with insulin resistance (Freitas and Carvalho 2013). Interestingly, the same pattern of TAG accumulation and insulin resistance is observed in lipodystrophy as shown in the Figure 1.5.

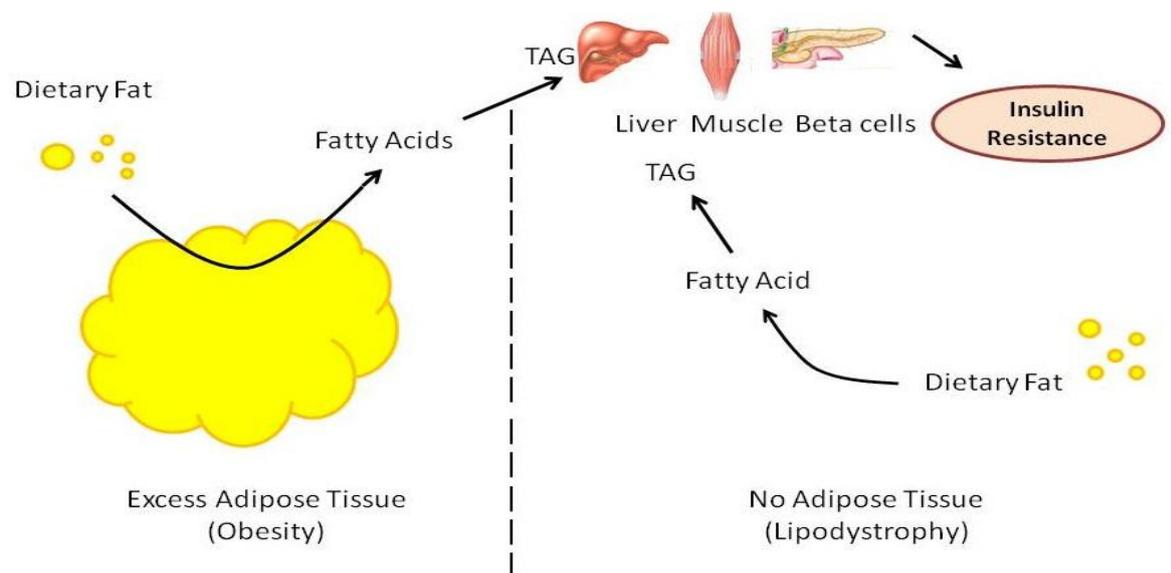


Figure 1.8 Loss of normal buffering action of adipose tissue against daily influx of dietary fat in obesity and lipodystrophy *Modified from (Frayn 2001)*

Human lipodystrophy, whether partial or total is an insulin resistant state and is usually associated with type 2 diabetes (Garg 2004). The metabolic consequences of having too little fat (lipodystrophy) are similar to those of having too much fat (obesity) (Vatier, Bidault *et al.* 2013). The normal role of adipose tissue is to buffer the daily influx of dietary fat in the circulation in the post-prandial period. This is similar to the roles of the skeletal muscle and liver in buffering the post-prandial glucose flux. Adipose tissue provides buffering action by increasing TAG clearance and by suppressing the release of non-esterified fatty acids into the circulation. If this buffering action is impaired then extra adipose tissues (liver, skeletal muscle and pancreatic beta cells) are exposed to excessive flux of fatty acid leading to insulin resistance (Frayn 2001, Frayn 2002).

In obesity, the buffering action of adipose tissue is impaired through defects in the ability of adipose tissue to respond to dynamic postprandial situation. The buffering action of adipose tissue is also impaired in lipodystrophy. There is not enough adipose tissue to provide necessary buffering capacity. Therefore, the phenotype with regard to insulin resistance is similar in both deficiency and excess of adipose tissue and other tissues are exposed to an excessive flux of fatty acids (Frayn 2002). This results in fatty acid accumulation (in the form of TAG) and interference with insulin mediated glucose disposal (Frayn 2001).

Lipodystrophies are characterized by the selective loss of body fat in an insulin resistant state (Garg 2011, Vatier, Bidault *et al.* 2013). It remains unclear whether very lean individuals, due to low adipose tissue mass, have impaired insulin sensitivity. Nor is it clear how supplements expected to promote positive energy balance impact insulin sensitivity in those with low adipose tissue mass.

1.3.3 Insulin resistance and dyslipidaemia

It has been suggested that insulin resistance and dyslipidemia may have a reciprocal, positive feedback correlation, and may reinforce each other. Low

HDL-C and hypertriglyceridemia are associated with insulin resistance, whereas the consequences of insulin resistance also involve dyslipidemia. This vicious cascade of events accentuates the development of insulin resistance (Li, Fu *et al.* 2014). In insulin resistance, three major components of dyslipidaemia occurs; a) elevated triglyceride levels, b) decreased HDL cholesterol (HDL-C), and c) compositional changes in LDL (Howard 1999). Triglyceride and HDL-C may be a causal factor of insulin resistance, rather than LDL-C (Li, Fu *et al.* 2014). The Framingham Heart Study supports this idea. It demonstrated that low HDL-C and hypertriglyceridemia were more prevalent in type 2 diabetic patients as compared to the normal population, whereas high LDL-C did not differ significantly between the two groups. From this it can be demonstrated that low HDL-C and hypertriglyceridemia may be the causal factors of type 2 diabetes and numerous studies support this inference (von Eckardstein and Sibling 2011, Drew, Rye *et al.* 2012, Mineo and Shaul 2012, Li, Fu *et al.* 2014).

HDL can enhance insulin secretion from β cells, and also augment insulin sensitivity in the target tissues such as muscles, adipose tissues and liver. Moreover, HDL has a positive effect on β cell survival (Li, Fu *et al.* 2014). A few of the benign effects of HDL may be due to its ability to suppress inducible fatty acid synthase and nitric oxide synthase. Other effects can be attributed to the role of HDL on cholesterol homeostasis. It was also found that the primary apolipoprotein moiety of HDL, APOA-I induces the phosphorylation of AMP kinase, which facilitates the glucose uptake into myocytes and inhibits gluconeogenesis in hepatocytes (von Eckardstein and Sibling 2011). Recent evidence shows that HDL also regulates the storage of fat in adipocytes. All these properties of HDL signify that, HDL plays a role in β cell function and insulin resistance. There is a substantial heterogeneity between the HDL sub-classes ranging from small HDL to large HDL subclasses. Usually, a larger HDL sub-class illustrates more favourable effects on insulin sensitivity (Filippatos, Rizos *et al.* 2013).

There is a strong inverse relationship between HDL and VLDL levels which suggests that VLDL also plays a role in insulin resistance. VLDL may have direct and indirect influence on insulin sensitivity. The exact mechanism by which hypertriglyceridemia and low HDL-C could lead to insulin resistance is still unclear. However, three generally accepted mechanisms known to induce insulin resistance are inflammation, lipotoxicity and endoplasmic reticulum stress (Glass and Olefsky 2012, Samuel and Shulman 2012). Evidence suggests that higher serum triglycerides levels may provoke a substantial amount of fatty acids to be deposited in cells resulting into ectopic lipid storage. Therefore, following dyslipidaemia, lipotoxicity may be the major causal mechanism in insulin resistance (Drew, Rye *et al.* 2012). However, it is also suggested that even in the absence of lipotoxicity, dyslipidaemia may directly causes inflammation, endoplasmic reticulum stress or some other mechanisms may lead to insulin resistance (Li, Fu *et al.* 2014).

Many studies have documented the mechanisms by which insulin resistance causes increased VLDL and plasma free fatty acid levels. Insulin resistance stimulates cholesterol synthesis and lipogenesis. Therefore, excess amounts of free fatty acids are produced in the adipose tissue. Then, free fatty acid are transferred to the liver, which consequently leads to the overproduction of VLDL, higher plasma triglycerides and a decline in HDL-C levels through the action of cholesterol ester transfer protein (CETP) and increased HDL-C clearance by the kidney (Reaven 2012). The LDL role in insulin resistance is less clear, but if it plays a role in insulin resistance, it may be a very modest one. Despite the fact that, LDL does not seem to amend insulin sensitivity, it has the capability to affect β -cell function, as it has been found that elevated levels of plasma LDL promotes β -cell loss (Brunham, Kruit *et al.* 2008).

1.3.4 Impact of acute changes in energy balance on fasting and post-prandial lipaemia

Fasting and postprandial triglyceride concentrations are linked closely to energy balance. A long-term positive energy balance can lead to weight gain

and an elevated levels of plasma triglyceride concentrations (Terán-García, Després *et al.* 2004). A study on twelve pairs of monozygotic twins found that a positive energy balance achieved by overfeeding of 4.2 MJ (1000kcal) above the daily energy needs, 6 days a week, for a period of 100 days significantly increased body weight, fat mass, plasma TAG levels, VLDL-TAG, LDL cholesterol whereas HDL-cholesterol levels decreased significantly. This suggests that a chronic positive energy balance is associated with significant weight gain. Adiposity has a detrimental impact on plasma lipoproteins levels and most likely on CHD risk (Terán-García, Després *et al.* 2004). Similarly, another study on healthy male subjects demonstrated that overfeeding by 4.2MJ per day above their individual energy requirement for 100 days resulted in undesirable changes in adiposity and HDL-C levels (Terán-García, Després *et al.* 2008).

A negative energy balance associated with weight loss induces a decline in plasma triglyceride concentrations. Studies have demonstrated that hypocaloric diets lower post-prandial triacylglycerolemia during the period of active weight loss, with more improvement seen when diet is low in carbohydrates (Sharman, Gómez *et al.* 2004, Volek, Sharman *et al.* 2004).

Mittendorfer *et al* (2003) observed that the rate of VLDL-TAG measured with the stable isotope tracer technique, was decreased by approximately 40% in six lean women and seven obese women after modest weight loss of 10% (Mittendorfer, Patterson *et al.* 2003). However, studies have shown that even a single day of diet induced energy deficit also reduces the postprandial TAG concentrations (Maraki, Magkos *et al.* 2010). This suggests that the observed decline in the post-prandial TAG concentrations after low carbohydrate diets in weight loss studies mentioned by Volek *et al* (2004) and Sharman *et al* (2004) may be due to acute negative energy balance than the weight loss itself.

A few studies have assessed postprandial lipemia after weight stabilization following weight loss of 5 kg (Abbasi, Chen *et al.* 2008), or 10 kg (James, Watts *et al.* 2003), induced by several weeks on a hypocaloric diet. But these did not significantly influence postprandial plasma TAG concentrations after 2 weeks (Abbasi, Chen *et al.* 2008) or 4 weeks (James, Watts *et al.* 2003) of weight stabilization i.e. when the impact of diet induced acute negative energy balance was no longer present. On the other hand, 10% weight loss followed by ≥ 4 weeks of weight maintenance decreased the plasma TAG concentrations (Maraki, Aggelopoulou *et al.* 2011). The inconsistency of these results may be attributed to the fact that the different approaches were used to induce weight loss.

Many studies have investigated the acute impact of reduction in energy intake on the plasma triglyceride concentrations, through moderate dietary restrictions, and, or, increasing the energy expenditure through single bout of exercise. Studies have shown that an acute moderate energy deficit, independent of its origin (exercise, diet or combination of both), decreases fasting and post-prandial triacylglycerolemia in females (Maraki, Magkos *et al.* 2010). Accumulating evidence suggests that a single bout of aerobic exercise reduces fasting and postprandial TAG concentration the next day (Gill and Hardman 2000, Harrison, O'Gorman *et al.* 2009, Maraki, Magkos *et al.* 2010), and is more evident when accompanied with a negative energy balance (Harrison, O'Gorman *et al.* 2009).

On the other hand a recent study Bellou *et al.* (2013b), found no impact of acute dietary energy restriction of the equivalent energy deficit, of approximately 2MJ, on the very low density lipoprotein (VLDL) (Bellou, Siopi *et al.* 2013). This study failed to observe any changes in VLDL-TAG kinetics, might be due to the energy deficit being inadequate in these participants (Bellou, Maraki *et al.* 2013). While on the other hand, a study by Yamada *et al.* (2008) found that a negative energy balance achieved by reduction in the dietary energy intake for a short period of 5 days (energy

deficit of 4428 ± 250 kcal/ 5 days) can decrease the fasting plasma triglyceride concentrations (Yamada, Kurasawa *et al.* 2008).

Combining different types of energy deficit (light exercise with mild hypoenergetic diet) have also shown to reduce the postprandial lipemia. Maraki *et al.* (2009), performed two oral fat tolerance tests in the morning on eight healthy sedentary pre-menopausal women, once after a single bout of light exercise (100 min at 30% of peak oxygen consumption, net energy expenditure 1.04 MJ) coupled with mild restriction in the energy intake (1.39 MJ), and once after resting, coupled with iso-energetic feeding (control) on the preceding day. Fasting plasma TAG concentrations and TAG in the TAG –rich lipoproteins were 18% and 34% lower respectively after the exercise plus diet, as compared to the control. Postprandial plasma TAG and TRL-TAG concentrations were 19 and 27% less after the exercise plus diet, than the control (Maraki, Christodoulou *et al.* 2009).

Since supplementation with HENSD is expected to promote energy intake and induce a positive energy balance, evidence on the impact of positive energy balance on triglyceride metabolism will be discussed in the following text. There is some evidence regarding the acute impact of positive energy balance, independent of changes in body weight and composition, on triglyceride metabolism. A positive energy balance induced by an increase in dietary energy intake also impacts upon plasma triglyceride concentrations (Hill, Peters *et al.* 1990, Faeh, Minehira *et al.* 2005, Bortolotti, Kreis *et al.* 2009, Brøns, Jensen *et al.* 2009). The impact of short term overfeeding on triglyceride metabolism is inconclusive. The majority of the studies on dietary macronutrient content were manipulated with the dietary energy intake. Many studies reported an increase in VLDL-TAG concentrations after 4 to 7 days of consuming hyper-caloric high carbohydrate diet (Ngo Sock, Lê *et al.* 2010, Sobrecases, Lê *et al.* 2010). Studies about overfeeding with high fat diet lowered VLDL-TAG concentrations (Brøns, Jensen *et al.* 2009, Sobrecases, Lê *et al.* 2010). The impact of hypercaloric diet on TAG concentration appears

to be dependent upon the macronutrient content that is ingested to excess. Whereas, some studies found no impact of hypercaloric feeding on VLDL-TAG concentrations in healthy women (Bellou, Maraki *et al.* 2013) and in obese subjects (Smith, Magkos *et al.* 2013).

A recent study by Bellou *et al.* (2013) found that there was no effect on basal VLDL-TAG metabolism when a positive energy balance was induced by an acute dietary energy surplus of approximately 3MJ, as compared to an isocaloric diet (providing estimated daily energy needs for weight maintenance), or hypocaloric diet (providing estimated daily energy needs for weight maintenance minus 3MJ). The additional energy required to achieve a positive energy balance was provided by asking the subjects to consume two high-calorie (6kJ/ml) fibre-free energy drinks; the percentage energy from macronutrients was 50% from carbohydrates, 35% from fat, and 15% from protein. While an acute negative energy balance achieved by an acute dietary energy deficit of approximately 3MJ leads to hypotriglyceridemia i.e., decreased in fasting plasma VLDL-TAG concentrations by approximately 26%, owing to 21% decline in hepatic VLDL-TAG secretion rate, and 12% increase in the plasma clearance rate of VLDL-TAG, than isocaloric feeding (Bellou, Maraki *et al.* 2013).

Therefore, it can be presumed that an induction of acute positive energy balance in a manner mimicking free living conditions (over consumption of all macronutrients) has no impact on VLDL-TAG secretion and clearance (Bellou, Maraki *et al.* 2013). Smith *et al.* (2013) performed a study to find out whether the increased energy intake (and resulting positive energy balance) *per se* contributes to abnormalities in the VLDL-TAG metabolism, or whether they develop a secondary to elevated free fatty acid release from the adipose tissues and insulin resistance in obese people. They found that one day of moderate overfeeding (30% excess energy intake) has no impact on the hepatic and adipose tissue fatty acid metabolism in obese men. Using isotope-labeled tracer methods, 8 obese men were studied on two occasions in a randomized

cross over design once after they consumed balanced diet that provided 30% excess calories (hypercaloric diet) and another occasion, after they consumed a balanced diet providing estimated daily energy needs for weight maintenance (isocaloric diet). Total plasma TAG and VLDL-TAG were not different in the isocaloric and hypercaloric conditions, while VLDL-apoB-100 concentrations was significantly different after hypercaloric but not isocaloric feeding (Smith, Magkos *et al.* 2013).

Similarly, a study by Despres *et al* (1987) on six pairs of male monozygotic twins found that an acute positive energy balance achieved by the ingestion of 4.2 MJ (1000 kcal) above individual daily energy needs, from a mixed diet (percentage energy from macronutrients was 50% energy from carbohydrates, 35% energy from fat and 15% energy from protein), for 22 days, significantly increased the total serum cholesterol and LDL-cholesterol, and had no impact on the serum triglyceride levels (Després, Poehlman *et al.* 1987). In a similar study, Mc Devitt *et al* (2001) found no significant difference in the plasma concentrations of TAG after 96 hours of dietary treatment, in eight lean and five obese women. The dietary treatment comprised of two overfeeding treatments and a control treatment. In the control treatment, the energy intake was calculated as basal metabolic rate multiplied by a fixed constant of 1.3, and the participants were fed to the nearest 0.5 MJ/day. The two overfeeding treatments provided 50% more energy as compared to the control treatment. The overall macronutrient composition of the overfeeding treatment was 42% of energy as fat, 50% of energy as carbohydrate and 8% of energy as protein (McDevitt, Bott *et al.* 2001).

In summary, the evidence from the previous studies reported changes in the VLDL-TAG concentrations following hypercaloric feeding for a few days (resulting in mild weight gain), with specific macronutrients, such as carbohydrate or fat (Hill, Peters *et al.* 1990, Minehira, Bettschart *et al.* 2003, Faeh, Minehira *et al.* 2005, Brøns, Jensen *et al.* 2009, Ngo Sock, Lê *et al.* 2010, Sobrecases, Lê *et al.* 2010). These studies demonstrated that excess

carbohydrate intake of mainly fructose (Minehira, Bettschart *et al.* 2003, Faeh, Minehira *et al.* 2005, Sobrecases, Lê *et al.* 2010) increases VLDL-TAG concentrations, whereas excess fat intake decreases VLDL-TAG concentrations (Bortolotti, Kreis *et al.* 2009, Brøns, Jensen *et al.* 2009, Sobrecases, Lê *et al.* 2010). However, overfeeding with both fat and carbohydrate had no impact on VLDL-TAG concentrations (McDevitt, Bott *et al.* 2001, Sobrecases, Lê *et al.* 2010, Bellou, Maraki *et al.* 2013), which demonstrate that the increased caloric intake in a mode mimicking free living conditions (that is over-consumption of all the macronutrients) has no impact on basal VLDL-TAG metabolism (Bellou, Maraki *et al.* 2013). However further research is required to verify the validity of the results when linking plasma lipids to an acute positive energy balance. In this thesis, the impact of HENSD supplementation (which provides 2.49 MJ excess energy above the habitual diet) via mixed overfeeding (46% energy from carbohydrate, 46% from fat and 8% from proteins) for five days will be investigated.

1.3.5 Acute modification of the macronutrient composition: impact on plasma lipids and insulin sensitivity

The impact of acute positive energy balance on the TAG, HDL, VLDL-TAG, insulin and glucose levels depends upon the macronutrient that is ingested in excess. Several studies have confirmed that high carbohydrate (CHO) diets have detrimental impact on the risk of CVD. A dietary excess in carbohydrates, particularly fructose, for a short duration of 4-6 days increases the total plasma triglyceride concentrations and VLDL-TAG concentrations (Minehira, Bettschart *et al.* 2003, Faeh, Minehira *et al.* 2005, Ngo Sock, Lê *et al.* 2010). In healthy sedentary individual's high CHO low fat diet have shown to increase the fasting and postprandial TAG concentrations and reduced HDL- cholesterol (Koutsari, Malkova *et al.* 2000, Roberts, Bickerton *et al.* 2008, Culling, Neil *et al.* 2009).

The detrimental impact of high carbohydrate intake on plasma lipids has been reported in iso-energetic controlled trials. Study by Koutsari *et al.* (2000) found

that a high carbohydrate diet (68% energy from CHO) consumed for three days by nine normolipidemic men significantly increased both the fasting and postprandial TAG, decreased fasting HDL-cholesterol, decreased plasma glucose levels, and had no impact on the serum insulin response as compared to an isoenergetic high fat diet (66% energy from fat) (Koutsari, Malkova *et al.* 2000). Similarly, in Robert *et al.* (2008), by applying stable isotope tracer technique showed that both the fasting and postprandial TAG concentrations were significantly increased after a high CHO diet as compared to high fat diet (Roberts, Bickerton *et al.* 2008). Likewise, Culling *et al.* (2009), observed that the short term TAG raising impact of high CHO diet depends upon the nature of the CHO, with a greater impact of a sugar rich than a complex CHO rich diet (Culling, Neil *et al.* 2009). This provides evidence that different types of CHO produce a difference in the lipid profile and could be important in preventing the risk of CVD.

Evidence suggests that the replacement of saturated fat with an increased CHO intake (particularly refined CHO) can aggravate atherogenic dyslipidaemia. This is associated with an increased TAG and small LDL particles and reduced HDL-cholesterol (Siri-Tarino, Sun *et al.* 2010). In a study by Arefhosseini *et al.* (2009), twelve healthy postmenopausal women were advised to increase their proportions of energy derived from CHO and reduce their fat intake for 4 weeks. It was found that by increasing the CHO energy intake, by up to 50% the results were a in the reduction of body weight, significant increase in fasting TAG concentration, and significant decrease in HDL cholesterol, favouring an increased risk of CVD (Arefhosseini, Edwards *et al.* 2009).

The detrimental impact of high carbohydrate intake on plasma lipids has been reported in hypercaloric studies. In a study by Sevastianova *et al.* (2012), sixteen overweight subjects were placed on high carbohydrate hypercaloric diet (> 1000kcal with 98% of energy from carbohydrates) for three weeks and thereafter on a hypocaloric diet for 6 months. During 3 weeks of carbohydrate overfeeding body weight increased by 2%, fasting serum concentrations of

triglycerides increased significantly, whereas HDL cholesterol decreased significantly (Sevastianova, Santos *et al.* 2012). Faeh *et al.* (2005) also observed, a significantly increased plasma TAG concentrations by 79% in their fructose overfeeding study (diet supplemented with 3g/kg/day fructose resulting in hyperenergetic diet (800-1,000 kcal/day), for six days (Faeh, Minehira *et al.* 2005). Similarly Le *et al.* (2006) found that in seven healthy subjects high fructose supplementation (1.5g fructose.kg body wt⁻¹.d⁻¹) within one week significantly increased the fasting VLDL-TAG (72%) and total triacylglycerol (36%) concentrations (Lê, Faeh *et al.* 2006). Sobrecases *et al.* (2010) studied thirty healthy male subjects to assess the effect of 7-day high fructose diet (Fruc: +3.5gfructose/kg fat-free mass/day, +35% energy), 4-days high fat diet (Fat; + 30%energy as saturated fat) or a 4- days high fructose high fat diet (Fruc fat:+3.5g fructose/kg fat-free mass/day, +30% energy as fat,+ 65% total energy) on intrahepatocellular lipid. It was found that hypercaloric high fructose diet increased VLDL –TAG concentrations (+ 58%) whereas VLDL-TAG concentrations decreased after high fat diet (-22%) while no changes were observed after high fructose high fat diet (Sobrecases, Lê *et al.* 2010).

Evidence regarding carbohydrate overfeeding studies has conflicting results on fasting plasma glucose and serum insulin concentrations. Some studies reported an increase in the concentration of both insulin and glucose (Silbernagel, Machann *et al.* 2011) some in insulin only (Lê, Ith *et al.* 2009, Sobrecases, Lê *et al.* 2010) or in glucose only (Lê, Faeh *et al.* 2006), and others found no change in insulin or glucose levels (Ngo Sock, Lê *et al.* 2010). A study by Koltzman *et al.* (1979) found that the consumption of high carbohydrate diet (75% energy from CHO) for 2 weeks in 10 healthy males improved insulin sensitivity, increase the ability of insulin to stimulate glucose removal from the plasma (Kolterman, Greenfield *et al.* 1979). These findings were not confirmed by other studies. A study conducted by Borkman *et al.* (1991) on eight healthy subjects found that a high CHO diet (more than 50% energy from CHO) for three weeks had no impact on fasting blood glucose and

serum insulin concentration, and thus did not enhance the insulin sensitivity (Borkman, Campbell *et al.* 1991). These findings were supported by another study by Garg *et al.* (1992) in which no impact on the glycemic control or insulin sensitivity was detected when 8 men with mild non insulin dependent diabetes consumed a high carbohydrate diet (60% energy from CHO) for a period of 21 days (Garg, Grundy *et al.* 1992).

Conversely, postprandial hyperinsulemia was found by Coulston *et al.* (1983) in 11 healthy individuals on high CHO diet (60% energy from CHO) for 10 days which was not present when individuals were on the control diet (40% energy from CHO) (Coulston, Liu *et al.* 1983). Ngo Sock *et al.* (2010) studied eleven healthy men in a randomized order after 7 days of the hypercaloric diet enriched with either fructose (3.5g fructose/kg fat free mass per day, +35% energy intake) or glucose (3.5g fructose/kg fat free-mass per day, +35% energy intake) showed a significant increase in the fasting VLDL-TAG (high fructose diet: 59% and high glucose diet 31%), while no changes were observed in the fasting glucose and insulin concentrations as compared to weight maintenance controlled diet (Ngo Sock, Lê *et al.* 2010). Similarly, Silbernagel *et al.* (2011) observed that post-prandial TAG, fasting and post-prandial concentration of LDL were significantly increased in response to 2 weeks consumption of 25% of energy as fructose, and high fructose corn syrup, but not with the consumption of glucose in forty-eight younger, normal weight and overweight subjects. AUC for 24-hour glucose and 24-hour insulin and the post meal insulin peaks were significantly higher in the participants consuming glucose, significantly lower in the subjects consuming fructose while it remains unchanged in the subjects consuming high fructose corn syrup (Silbernagel, Machann *et al.* 2011).

Evidence regarding fat overfeeding studies has also conflicting results on the total plasma triglyceride and VLDL concentrations. Some studies reported decrease in the concentration of the both plasma triglycerides and the VLDL (Bortolotti, Kreis *et al.* 2009, Brøns, Jensen *et al.* 2009, Sobrecases, Lê *et al.*

2010), some reported significant increase in the plasma triglyceride (van der Meer, Hammer *et al.* 2008) while other reported no impact of high fat diet on plasma triglyceride (Schrauwen-Hinderling, Kooi *et al.* 2005, Westerbacka, Lammi *et al.* 2005).

Fat and fructose have an opposite effect on the plasma triglycerides indicating a complex interaction between these two nutrients classes (Sobrecases, Lê *et al.* 2010). A study on 10 subjects found that after 4 days of hyper-caloric high fat diet, the total triglycerides and VLDL-TAG concentrations were reduced as compared to the control diet. While high fat diet has no impact on the plasma insulin and glucose levels (Bortolotti, Kreis *et al.* 2009). Likewise, Brons *et al.* (2009) investigated the effects of 5 days high fat (60%) overfeeding (+50%) versus a control diet (50% of the energy coming from CHO, 35% from fat and 7.5% from protein), on the glucose and insulin metabolism of twenty-six young healthy volunteers. They found that a high fat- high calorie- diet (containing 50% extra energy, where 60% energy is from fat, 32.5% from CHO and 7.5% from protein) decreased the fasting plasma TAG, LDL- and VLDL- cholesterol concentration. Whereas HDL- cholesterol was increased compared to the control diet. Fasting glucose levels were significantly higher while borderline increase in the insulin levels were observed in high-fat high-calorie diet (Brøns, Jensen *et al.* 2009).

Van der Meer *et al.* (2008) found that after a 3-day high-fat high-energy diet (total energy intake was approximately 4732 kcal/d: 69% fat, 20% carbohydrate and 11% protein) the plasma triglyceride, plasma NEFA, and postprandial plasma insulin concentrations were significantly higher. While the plasma glucose levels remained unchanged (van der Meer, Hammer *et al.* 2008). Westerbacka *et al.* (2005) placed 10 overweight apparently healthy women on two successive 2-week isocaloric diets (16% and 56% energy from fat) in a randomized cross- over study. No significant changes were observed in the fasting plasma triglycerides, LDL-, HDL-cholesterol and fasting plasma

glucose concentrations. However, fasting plasma insulin concentrations were significantly higher during the high fat diet (Westerbacka, Lammi *et al.* 2005).

The impact of acute positive energy balance achieved by overfeeding with both carbohydrate and fat has controversial findings on plasma glucose and insulin levels. Some studies reported an increase in the concentration of both insulin and glucose (Tam, Viardot *et al.* 2010, Bellou, Maraki *et al.* 2013), some insulin only (Sobrecases, Lê *et al.* 2010, Cornford, Hinko *et al.* 2012) and others found no change in insulin or glucose levels (Després, Poehlman *et al.* 1987, McDevitt, Bott *et al.* 2001).

A study performed by Bellou *et al.* (2013) found that acute positive energy balance of approximately 3MJ during hypercaloric diet significantly increased the fasting plasma glucose concentration, but not after an acute negative energy balance of approximately 3MJ (hypocaloric diet) or isocaloric diet (zero energy balance). Serum insulin levels were significantly higher after the hypercaloric diet than after the hypocaloric diet while no difference was detected between the hypercaloric and the isocaloric diet (Bellou, Maraki *et al.* 2013). Similarly a study conducted by Tam *et al.* (2010) on thirty six healthy individuals found that, after 28 days of overfeeding by 1,250 kcal/day above baseline energy requirements (45% fat, 40% carbohydrate and 15% protein), there was a significant increase in both fasting insulin and glucose concentrations (Tam, Viardot *et al.* 2010). A study by Cornford *et al.* (2012), on healthy non-obese subjects, found that after two-weeks, over-feeding (approximately 4000 kcal/day (50% carbohydrate, 35% fat and 15%protein) doubled fasting plasma insulin concentrations. As a result the HOMA_(IR) was also significantly elevated, although fasting plasma glucose concentration was well maintained (Cornford, Hinko *et al.* 2012).

Similarly, an overfeeding study conducted by Adochio *et al.* (2009) also found that, 5-days of 40% overfeeding of a high carbohydrate (60% carbohydrate, 20% fat) diet induced fasting insulin levels, but had no impact on fasting

glucose levels (Adochio, Leitner *et al.* 2009). In one study, no change in insulin and glucose responses were detected after a short term caloric excess of daily surplus of 4.2 MJ (1000 kcal) for 22 days (Després, Poehlman *et al.* 1987).

Studies have shown that short-term over feeding with modest weight gain, increases plasma insulin concentrations and induced insulin resistance (Tam, Viardot *et al.* 2010, Brands, Swat *et al.* 2013). Brands *et al.* (2013) demonstrated that in lean healthy men the positive energy balance induced by short term over feeding ($1.4 \times$ caloric intake from eucaloric diet), resulted in weight gain, induced whole-body insulin resistance, fasting and glucose stimulated hyperinsulinaemia. The surplus energy was provided by liquid drink that contained 36.8g carbohydrates, 12g protein and 11.2g fat (300 kcal per 200ml). The mechanisms responsible for hyperinsulinaemia may be explained by the combined effect on the increased insulin secretion and decreased insulin clearance (Brands, Swat *et al.* 2013). A study conducted by Erdmann *et al.* (2008) demonstrated that hyperinsulinaemia after modest weight gain was explained by the hepatic clearance only and did not observe any difference in the insulin secretion (Erdmann, Kallabis *et al.* 2008).

The difference in the Brands *et al.* (2013) and Erdmann *et al.* (2008) study might be due to the difference in study design. Erdmann *et al.* (2008) studied their subjects after a hypercaloric diet of 3-4 months with a weight stable period of 4 weeks; however, Brands *et al.* studied their subjects during a hypercaloric diet. This implies that during hypercaloric feeding insulin secretion is enhanced and insulin clearance is reduced, whereas during weight stability after weight gain, only insulin clearance is increased. Increased insulin secretion is related to the insulin resistance induced elevated glucose concentrations, and to increased dynamic β -cell responsivity (higher response to a change in glucose concentrations). Though glucose sensitivity of the β -cells did not change. The impact of hypercaloric diet on dynamic insulin responsivity index indicates that the hypercaloric feeding enhances β -cell

response (Brands, Swat *et al.* 2013). However, it is worth mentioning here that during a hypocaloric diet, insulin sensitivity, insulin clearance, glucose stimulated insulin secretion and the dynamic β -cell responsitivity returned to baseline. These findings suggest that the metabolic disturbances in the early phase of hypercaloric intake, with intentional weight gain, are entirely reversible by a caloric restriction (Brands, Swat *et al.* 2013).

Overfeeding studies have demonstrated that, during hypercaloric state fasting glucose concentrations did not increase significantly, while fasting insulin secretions increased (Brands, Swat *et al.* 2013, Smith, Magkos *et al.* 2013). As fasting glucose concentrations were mostly determined by endogenous glucose production (Turner and Holman 1976), it might be possible that the increase in portal insulin inhibited the increase in the glucose concentration by reducing glucose output. The stable glucose concentrations might represents a new steady state at the expense of higher basal insulin secretion (Brands, Swat *et al.* 2013).

To sum up the literature, the isocaloric high carbohydrate diets, particularly fructose, for a short duration of 4-6 days increases the total plasma triglyceride concentrations and VLDL-TAG, and have no impact on the fasting plasma glucose and serum insulin concentrations. The overfeeding study with an excess fat intake (decreased carbohydrate diet) reduces total plasma triglyceride and increases insulin and glucose concentrations. Therefore, further research is required to look at the impact of acute positive energy balance induced by HENS_D supplementation for five days, on fasting plasma insulin and glucose concentrations.

1.4 Aims and objectives

Accumulating evidence suggests that multi-nutrient, oral high-energy nutritional supplements (HENS_Ds), increases energy intake, and are beneficial for the treatment of malnutrition. Their effectiveness, however, may be diminished by the acute suppression of appetite. The existing knowledge of the

hormonal and metabolic effects of HENSD is mostly based on the work done in tube feeding studies. However, interaction between appetite, food intake, metabolic and hormonal mediators of appetite and satiety, following tube feeding, and oral HENSD can be different. Therefore, further studies are required to investigate the mechanisms which are related to the partial compensation for the energy provided by HENSD and which causes an increase in energy intake.

Furthermore, there is a need to investigate if supplementation in the evening, for a longer duration, induces a positive energy balance and also investigate the time scale of compensation after the HENSD supplementation for five consecutive days. Since, the energy intake with consumption of HENSD is expected to promote a positive energy balance; there is a need to investigate the impact of HENSD supplementation on the cardio-metabolic risk factors.

Studies have shown that the nutritional supplements such as Solid ready to use food (RTUFs) are very effective in the treatment of severe malnutrition in children. Community-based management of acute malnutrition using RTUF has been associated with higher recovery rates, greater weight gain and lower case fatalities. However, there is little evidence from exploring the impact of RTUF and milk based 'Liquid Ready To Use' proprietary Supplement (LRUS) on weight gain in mild to moderate underweight children from Asia. Therefore, the main aims and objectives of the following research were as follows:

- To investigate the level of energy intake compensation in lean adult women following HENSD consumption and find out how it relates to responses of a range of hormonal and metabolic appetite regulators. (Chapter 3).
- To investigate the impact of HENSD supplementation on proxy measure of gastric emptying and to evaluate the extent by which the

supplementation-induced increase in energy-intake is diminished by the thermogenic effect (Chapter 3).

- To investigate the impact of supplementation of HENSD in the afternoon for 5 consecutive days on energy intake and to find out whether it has any detrimental impact on plasma lipids, glycaemia and insulinaemia Chapter 4).
- To explore the efficacy of RTUF and LRUS in promoting weight gain and appetite regulation in mild to moderate underweight children between the ages of 5-10 years from Pakistan (Chapter 5).

2 General Material and Methods

2.1 Participants

The experimental studies described in chapter 3 and 4 of this thesis involved healthy women with a body mass index of 17- 20 kg/m². Both experimental studies were approved by the College of Medicine, Veterinary and Life Sciences, University of Glasgow, Research Ethics Committee and were performed in accordance with the Helsinki Declaration.

The eligible inclusion criteria for the subjects to participate in the experiments described in chapter 3 and 4 were: females aged between 18-35 years, generally in good health, and with a BMI of less than 20kgm⁻². All the participants were non-smokers, had stable weight for one month prior to the study, not pregnant, having a regular menstrual cycle, were not on any medication or any nutritional supplement, and were not following a special diet. The participants were recruited by word of mouth in the campus of the University of Glasgow, at Yorkhill hospital and in the University gym. The subjects who agreed to take part in the study were provided with the detailed volunteer information sheet (Appendix IIa, b) regarding the study, to take away before making their decision.

The volunteer information sheet described all the details of the purpose of the study, inclusion criteria for the study, screening procedures of the study, what the participant would do prior to the study, experimental tests involved in the study, possible benefits for taking part in the study and possible disadvantages and risks of taking part in the study. It was essential for all the subjects who agreed to take part in the study to attend a screening visit at the metabolic room of the Human Nutrition department of Yorkhill Hospital. During this visit they were screened by detailed medical history (Appendix IVa) to exclude chronic illnesses,

eating disorders and any major gastrointestinal surgery. This was done by them completing a health questionnaire describing the subjects past illnesses, present illnesses and the health status and family history of any diseases. Furthermore, anthropometric measurements were also taken prior to the study, to make sure that the participants fulfilled the inclusion criteria of the study. All the subjects were provided with written, informed consent (Appendix IIIa, b). Ethics documents for chapter 3, 4 and 5 are shown in (Appendix I a, b, c).

2.2 Anthropometry and body composition

2.2.1 Height and Weight Measurement

The height of the participants was measured with a portable stadiometer (Seca, Leicester, UK), using a stretch stature method. The stature is the maximum distance from the ground to the uppermost point of the skull when the position of head is held in the Frankfort plane position (Marfell-Jones, Stewart *et al.* 2006). The participants height were measured barefooted, with their back placed against a fixed backboard, and their arms placed in a position lateral to their body. The head was positioned adjacent to the backboard, in such a way that the line of eyesight was perpendicular to the backboard. When the volunteer was properly positioned and was relaxed then the measurement was carried out. The headboard was lowered on the top of head with slight pressure to compress the hairs. The researcher applied mild upwards pressure below the angle of mandible and the measurement was performed to the nearest of 0.01m. The same height scale was used by the researcher throughout the experimental study.

Body mass was measured with the help of a digital scale (TBF-300, TANITA, Cranlea, UK). The weight of the participants was measured wearing lightweight cloths but without shoes. Prior to taking the

measurements, extraneous clothing and jewellery were removed. The participants stood with both feet placed flat on the balance, without socks, and with arms positioned laterally along the side of the body. The measurements were performed to the nearest of 0.01kg. To calculate their BMI, the derived values of height and weight were used (Marfell-Jones, Stewart *et al.* 2006).

2.2.2 Body Composition

Body-fat mass and fat-free mass was measured with bioelectrical impedance scales (TBF-300, TANITA, Cranlea, UK). Two pressure contacts (footpad electrodes) were incorporated into the platform of the Tanita scales. The participant's measurements were taken in a standing position with bare feet in contact with the electrodes. Tanita scales determine the opposition to the flow of electric current through the body tissues i.e. bioelectrical impedance, which is then used to estimate the total body water. Total body water is then used to calculate fat-free body mass, and through difference in body weight, body fat is estimated.

2.3 *Ad libitum* Meals

Ad libitum buffet meals consisted of a wide variety of standardised foods, and were presented at about three times in excess from what participants were expected to consume. For the *ad libitum* breakfast a variety of breakfast cereals such as corn flakes, fruit & fibre or chocolate-flavoured cereals were offered. Milk (semi-skimmed, skimmed and whole cream), croissants, jam (apricot, mixed fruit jam, black-current), butter, apple and orange juice were presented. Banana and apples were also offered (Figure 2.1). The *ad libitum* lunch included two filled white bread sandwiches, two filled whole meal bread sandwiches, mixed leaf salad, yoghurt, banana, grapes and apple-juice or orange-juice (Figure 2.2). Before offering meals to the participants, all foods were weighed and the left-

overs from the meal were weighed to estimate the amount of food consumed. Water was available throughout the trial. Reading and watching television were not allowed during *ad libitum* meals as these activities could influence the food intake (de Castro 2000).



Figure 2.1 *Ad libitum* Breakfast

The *ad libitum* buffet meals were identical in the HENSD and PLACEBO trials, containing the same energy and macronutrient contents and providing a variety of carbohydrate, fat and protein content. The food was cut into smaller pieces in order to eliminate the portion related cues. Furthermore all the *ad libitum* meals were served in a standardised setting, serving the same type of food in the same dishes (coloured), scheduling meals at the same time, and on the same table in order to avoid any bias in the eating behaviour. The participants were given 30 minutes to consume their meal and were advised to eat according to their appetite until they were satisfied and were comfortably full. Moreover, additional food was also available if desired. The researchers were not present when the participants consumed the *ad libitum* buffet meals in order to curtail any potential influence by the presence of the researcher on the feeding behaviour. The participants were blinded to the actual purpose of

ad libitum buffet meals i.e. measurement of the food intake, and were instead informed that biomarkers in the blood will be investigated for the food consumed. This approach avoided conscious eating and reduced the potential bias that could take place if the participants were aware of the fact that food consumption was being monitored (de Castro 2000). As the effects of social context on eating behaviour and patterns is very complex and different psychological processes, cognitive and emotional beliefs and restrains influence the volume and type of food eaten. Furthermore, the presence of observers can induce people to discontinue eating (Herman, Roth *et al.* 2003, Herman and Polivy 2005, Hetherington, Anderson *et al.* 2006).



Figure 2.2 *Ad libitum* Lunch

2.4 Energy Intake Measurement

The researcher weighed all food and drinks offered and left-over during the *ad libitum* buffet breakfast and the *ad libitum* buffet lunch, with an electronic kitchen scales (Salter Housewares Ltd., Tonbridge, U.K.) to estimate the amount of food being consumed. The type and the amount of each food item

were then entered into the dietary software Windiets 2005 (The Robert Gordon University, Aberdeen, Scotland, UK), a computerized version of *McCance and Widdowson's The Composition of Foods* (Krebs 2002), by way of calculating the energy and macronutrient intake during the *ad libitum* buffet breakfast and lunch. These values included intake during the *ad libitum* breakfast, intake during the *ad libitum* lunch, and intake of meals, including either the HENSD or PLACEBO. The macronutrient intake (CHO, protein and fat) for each *ad libitum* meal and the macronutrient intake, together with the supplements, were also taken into consideration. Three researchers carried out this analysis of dietary intake independently to reduce inputting errors and researcher bias. Then the mean values of each of their results were taken into account to enhance the accuracy of the results.

2.5 Subjective Appetite Sensations

Visual analogue scales (VAS) are most commonly used for the assessment of subjective appetite sensations. VAS helps to evaluate a subject's desire to eat by reporting hunger, desire to eat, prospective food consumption, satiety and fullness rating (Flint, Raben *et al.* 2000). The feelings of prospective food consumption and the desire to eat are considered as the initial phase of the drive to eat. Eating related stimuli such as smell of food, view of food etc can trigger these feelings and motivate eating even when subject is satiated.

Visual analogue scales consist of lines of the same length (usually 100mm) with words at each end of the line, which describe extremes such as I am not hungry at all/ I have never been hungrier. The negative respective feelings are anchored on the left and positive feelings on the right. The subjects were requested to rate their appetite sensations including hunger, satiety, fullness, prospective food consumption and the desire to eat by making a vertical line at the point which corresponded to their feeling. The measurements were quantified by measuring a distance on the line from left end to the mark (Flint, Raben *et al.* 2000). Visual analogue scales are more accurate and sensitive

with in subject comparison then to the comparison between the subjects (Flint, Raben *et al.* 2000). VAS questionnaire used in the study is presented in Appendix V.

For a subjective measure of the appetite response, the total area under the curve (AUC) was calculated using the Trapezium Rule. The time averaged AUC was calculated as total AUCs divided by the total duration of observation time (240 minutes). Time averaged AUC was measured for hunger, satiety, fullness, prospective food consumption and desire to eat for both PLACEBO and HENSD trials. AUC was calculated by the equation:

$$[(a+b)/ 2 \times \text{Time}]$$

(a=trapezium 1), (b=trapezium 2)

The time-averaged AUC was calculated which provided a measure of the average value over the period of observation.

2.6 Resting Metabolic Rate and Diet Induced Thermogenesis

The resting metabolic rate (RMR) and diet-induced thermogenesis were measured by indirect calorimetry, with a computerised open-circuit ventilated hood system (Oxycon Pro, Jaeger GmbH, Hoechberg, Germany). The Oxycon Pro consisted of a high-speed paramagnetic oxygen sensor and an infrared carbon dioxide analyser. A transparent ventilated plastic hood which was connected to the gas mixing chamber by a flexible plastic tube. It had a bi-directional digital volume sensor consisting of an amplifier, Triple V and a pressure transducer, and a gut mixing chamber, turbine and twin tubing. Before the start of each test the volume calibration was completed. For volume calibration, a 3-liter syringe was connected to the assembly of Triple V. Six complete pumps of the syringe was repeated so that the percentage difference

between the recent and previous volume calibration was less than 1 % (James Carter *et al* 2002).

For gas calibration, and delay time calibration, the Jaeger Oxycon Pro had an automatic calibration process. For gas calibration 16% Oxygen and 5% carbon dioxide was introduced to Oxycon Pro through a gas cylinder. The automatic process was repeated until the existing and prior data, delay time and offset were within 1 % (Carter and Jeukendrup 2002).

The RMR of the participant was measured in the morning at 8.30 am in the metabolic suite, after 12 hours of fasting and abstinence from physical activity, for two days prior to the main trial. The metabolic suite of the Yorkhill Hospital was a quiet, semi-dark room, where background noise was kept to a minimum. It had a thermo-neutral environment (21-24⁰ C). Before starting the main trial in the metabolic room, the subjects were asked to lie in a supine position with straight legs and arms placed straight on the side. Then after 10min, the transparent ventilated plastic hood was placed over the subject's head. The ventilated hood was connected to the gas-mixing chamber through corrugated plastic tubes and the ventilation was run by means of a flow volume sensor unit through the system. The rate of Oxygen consumption (VO₂) and rate of carbon dioxide production (VCO₂) were recorded, at intervals of 1 minute. Measurements were recorded for 10 minutes prior to placing the canopy on the participant and these measurements were called pre-drift. Then, 20 minutes of measurements were recorded with the participant inside the canopy. After that, 10 minutes of measurements were taken when the participant was outside the canopy, which acts as a post-drift. Then mean of pre-drift and post drift was taken for the correction of drift. Throughout the experiment, participants were closely monitored so that talking, excess movement and sleeping would be avoided.

VO₂ and VCO₂ were continuously recorded when the participant was outside the canopy for calculating the pre-drift. The participants lay down after meals and the canopy was placed on them for 20 minutes. After 20 minutes, the

participants came out of the canopy for 10 minutes during which they were allowed to perform their sanitary activities.

The mean values obtained from VO_2 and VCO_2 were corrected for the drift by taking the mean of the pre-drift and post-drift values. Then these values were used to calculate respiratory exchange ratio and rate of energy expenditure by using the indirect calorimetry equations described by Frayn and Macdonald (Frayn and Macdonald 1997) .

Rate of fat oxidation (g/min) = $(\text{VO}_2 - \text{VCO}_2) / 0.57$

Rate of carbohydrate oxidation (g/min) = $(1.40 \times \text{VCO}_2 - \text{VO}_2) / 0.30$

Rate of energy expenditure (kJ/min) = (rate of carbohydrate oxidation $\times 15.6$) + (rate of fat oxidation $\times 39$).

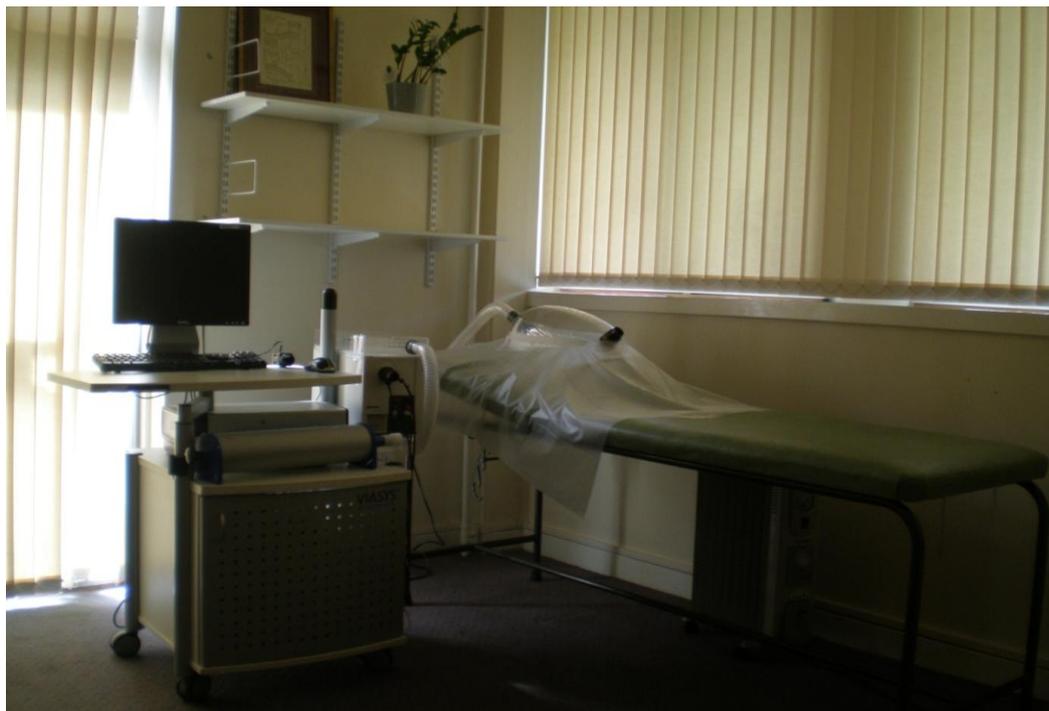


Figure 2.3 Oxycon Pro (Oxycon Pro, Jaeger GmbH, Germany) with ventilated hood system

2.7 Blood sampling and Plasma Preparation

On the days of the main experimental trial, venous blood samples were collected from the participants. Participants arrived in the morning at the metabolic room after an overnight fast. After recording the resting-metabolic rate measurement, the participant rested in a supine position and was informed about the intravenous cannulation. Then an indwelling 18G cannula (Venfoln, BOC, Helsingborg, Sweden) was inserted into the antecubital vein of the forearm. Fasting or baseline venous blood sample was collected into a 10 ml ethylenediamine tetra-acetic acid (EDTA) Vacutainer™ tube (BD Vacutainer Systems, Plymouth, UK). About 10mls of venous blood was collected at 0, 30, 60, 120, 150, 180, 210 and 240 minutes during the entire period of study.



Figure 2.4 Intravenous cannulation during experimental trials

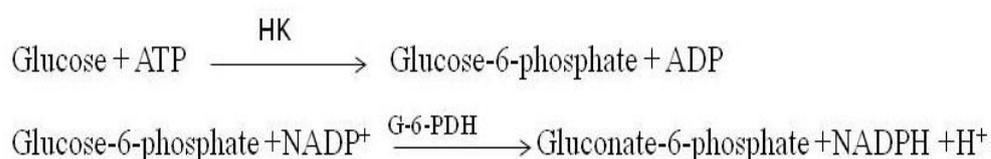
Blood samples for the analysis of insulin, glucose, plasma lipids concentrations and for the measurement of plasma paracetamol levels (as an indirect measure of gastric emptying) were centrifuged at 4⁰C, 3000 rpm for 15mins in a refrigerated centrifuge. After centrifugation the plasma supernatant was aspirated using a pipette and then 0.3ml of plasma aliquoted into three labelled 2ml Eppendorf (Alpha laboratories Ltd, UK) and frozen at -

80°C for the analysis. The blood samples used for the determination of gut peptides were split into two aliquots: a) 1ml of blood was aliquoted into three Eppendorf tubes each containing 80µl aprotonin (0.4TIU/ 500KIU) (Sigma-Aldrich, UK). Then samples were centrifuged in a smaller centrifuge machine for 4mins at maximum 14000 rpm. Subsequently, 300µL of plasma was aliquoted into 3 Eppendorf tubes labeled for CCK/ PYY. Immediately all the aliquoted samples were frozen at -80°C until assayed.

2.8 Plasma Analysis

2.8.1 Glucose Analysis

Plasma glucose concentrations were determined in the Exercise Physiology Laboratory, Western Infirmary, University of Glasgow by the enzymatic calorimetric method using commercially available glucose analysis kits. Glucose analysis kits consist of reagents (Glucose HK CP Reagent ABX Pentra, Horiba ABX, France) and the analysis was performed on the spectrophotometric analyser an automated Roche Cobas Mira (Horibra ABX, Montpellier, France). The precision and accuracy of the assays was monitored with quality control sera (Horiba ABX, Montpellier, France). The analysis of all the samples from each subject were carried out on a single run and in duplicate with the co-efficient of variation of less than 3%. The glucose determination principle is based on the calorimetric reaction



Where HK is hexokinase and G-6-PDH is glucose-6-phosphate-dehydrogenase

2.8.2 Insulin Analysis

Insulin analysis was performed in the Steroid II laboratory, at the Human Nutrition, Royal Hospital for Sick Children Yorkhill, University of Glasgow. Quantitative insulin analysis was performed by using a commercially available Enzyme-linked immunosorbent assay (ELISA) kit with pro-insulin (Merckodia AB, Uppsala Sweden) with less than 0.01% cross-reactivity. For analysis Interassay and intrassay coefficient of variance were 3.4% and 3.6%. Merckodia insulin ELISA is based on the principle of solid phase two-site enzyme immunoassay. Monoclonal antibodies were directed against the antigenic determinants of insulin molecules in a 'sandwich' technique. Insulin present in the samples reacts with the per-oxidase conjugated anti-insulin antibodies, and these anti-insulin antibodies then bound to the micro-titration well. Unbound enzymes labelled anti-bodies are then removed by simple washing step. Bound conjugates are detected by their reaction with 3, 3', 5, 5'-tetramethylbenzidine (TMB). Finally, the acid was added to stop the reaction and to get a calorimetric end point which was read spectrophotometrically.

Before use, all samples and reagents were brought to room temperature. Sufficient micro-plate wells were prepared to accommodate the Calibrators and samples in the duplicate. Plasma samples (25 μ L) and Calibrators (25 μ L) were poured into appropriate assay wells. Then 100- μ L of freshly prepared enzyme conjugate was added to each well. Afterwards, incubation of the plates for one hour at room temperature (18-25⁰C) was done on a plate shaker (700-900 rpm). Insulin in the samples during incubation period reacts with peroxidase-conjugated anti-insulin antibodies and these anti-insulin antibodies attach to the plate wells. Using an automated and wash buffer solution (700 μ L), the plates were washed and dried six times to eliminate any unbound enzyme labelled antibody. After that 200 μ L of 3, 3', 5, 5'-tetramethylbenzidine (TMB) was added to the wells to detect bound conjugates in the well. To permit the reaction between bound conjugates and TMB, the plates were again incubated for 15 min at room temperature. The

reaction was stopped after incubation in each well by adding 50 microlitres of stop solution containing 0.5M sulphuric acid. Then the plate was placed again on the shaker for 5 seconds approximately to ensure the proper mixing. According to the conjugate-substrate complex concentration a yellowish tint colour developed. Subsequently by means of a Plate reader the optical density of every well was read. By comparing the optical density of the samples to the standard curve for every plate insulin concentration in the samples was determined. The coefficients of variation for assay were <5%.

All the samples from one subject were analysed in a single run. Quality control sera (Mercodia Diabetes Antigen Control (Low, High)/human) (Mercodia AB, Uppsala Sweden) was used to determine the precision and accuracy of assays.

2.8.3 Human Peptide YY Analysis

Human Peptide YY analysis was performed in the Steroid II laboratory, at the Human Nutrition, Royal Hospital for Sick Children Yorkhill, University of Glasgow. For determination of PYY concentrations in all the samples commercially available ELISA kit (Millipore 96-well plate, Cat. # EZHPYYT66K) was used according to the manufacturer's instructions.

This is a "Sandwich" ELISA, used for non-radioactive quantification of Human PYY molecule in the plasma and serum. Its sensitivity is 1.4pg/ml for 20µl sample. It involves binding the PYY molecules (PYY1-36 and PYY3-36) in the sample by the rabbit anti-human PYY IgG. The resulting complex was then immobilized to the microtiter plate wells which were coated with the anti-rabbit IgG antibodies. Simultaneously, a second biotinylated antibody was bound to the PYY molecules. At this step unbound materials were removed by washing the wells, followed by conjugation of the immobilized biotinylated antibodies with horseradish peroxidase. Unbound enzymes labelled antibodies were removed by washing. The quantification of immobilized antibody-enzyme conjugates was done in the presence of the substrate TMB

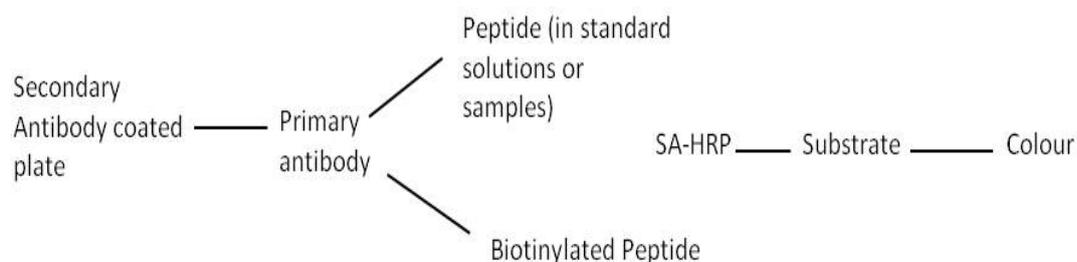
by monitoring horseradish peroxidase activities. In the last step acid was added to stop the reaction and to get a calorimetric end point where the blue colour of the assay turned to yellow. Afterwards enzyme activity was measured with a spectrophotometer (Thermo Scientific multi-scan spectrum). The automated analyser for the specific assay was set up at two wavelengths; one at 450 nm for the increased absorbency and other at 590nm for correction. The increasing absorbance was then directly proportional to the amount of PYY; therefore, a standard curve was formed (by using reference standards with known concentrations) to calculate the PYY concentrations.

2.8.4 Cholecystokinin Analysis

Cholecystokinin (CCK) analysis was performed in the Steroid II laboratory, at the Human Nutrition, Royal Hospital for Sick Children Yorkhill, University of Glasgow. Cholecystokinin (CCK) was analysed by using an Enzyme Immunoassay kit (EIA kit) (Phoenix, Pharmaceuticals, Inc., Burlingame, CA, USA) according to the manufacturer's instructions.

This EIA kit was based on the principle of "competitive" enzyme immunoassay. The immunoplate provided in the kit was pre-coated with the secondary antibody while the non-specific binding sites were blocked. The secondary antibody bound to the Fc fragment of the primary antibody whereas Fab fragment binds was bound competitively by the peptide standards and biotinylated peptide or targeted peptides in the samples. Streptavidin-horseradish peroxidase (SA-HRP) catalyzed the substrate solution. The biotinylated peptide interacts with SA-HRP. There was a competitive binding of biotinylated peptide with the peptide antibody of the samples (primary antibody) or with the standard peptide. The intensity of yellow colour was directly proportional to the biotinylated peptide SA-HRP complex and was inversely proportional to the amount of peptides in the samples or standard solutions. A standard curve of known concentration was developed accordingly and an unknown concentration of the samples was determined by

extrapolation of the standard curve. For analysis inter-assay and intra-assay coefficient of variance were < 6.6% and <5.4% respectively.



2.8.5 Gastric Emptying

The paracetamol (acetaminophen) absorption test was used to assess the gastric emptying. The paracetamol absorption test was based on the pharmacokinetic principle (Clements, Heading *et al.* 1978) that gastric emptying was the rate-limiting step in the absorption of paracetamol because the paracetamol was not absorbed in the stomach but was completely and rapidly absorbed from the small intestine after passage through the pylorus (Kim, Myung *et al.* 2000). Therefore, the plasma paracetamol had been shown to correlate with the rate of gastric emptying.

On the morning of the experimental trial, after the baseline measurements, following the supplement intake, two paracetamol tablets (1000mg with 100ml of water) were given to the participants. Then, venous blood samples were collected at 0, 30, 60, 120, 150, 180, 210 and 240 minutes during the entire period of study for measuring plasma paracetamol concentrations.

For assessment of gastric emptying plasma paracetamol concentrations were assayed by using Acetaminophen Assay kits (Cambridge Life Sciences, Cambridge, U.K.). An enzyme specific for amide bond of acylated aromatic amines was used for the cleavage of the paracetamol molecule and produced

p-aminophenol. The p-aminophenol reacted with o-cresol in ammoniacal copper solution, which gave a blue color. The instructions of the kit were followed and an alternative protocol was used for this assay, which has been validated by Cambridge Life Sciences and allowed the use of reduced volumes.

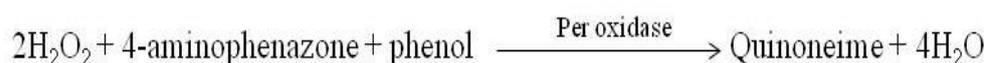
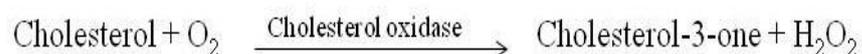
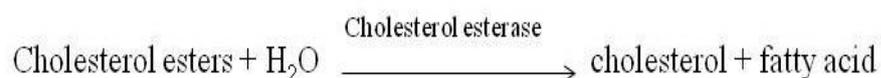
The accuracy of the paracetamol absorption test greatly depends on the absorptive index used (Willems, Quartero *et al.* 2001). Several pharmacokinetic parameters have been used as the rate metrics in the paracetamol absorption test such as a fixed-time concentration, maximal plasma concentration (C_{max}), the time of maximal plasma concentration (T_{max}), and the area under the curve (AUC). However, the most appropriate index for the paracetamol absorption remains inconclusive (Willems, Quartero *et al.* 2001). The AUC is considered as an accurate estimation of the rate of paracetamol absorption (Willems, Quartero *et al.* 2001). A study by Nimmo *et al.* (1975) also obtained a significantly high correlation ($r = 0.94$) between AUC and Scintigraphy (Nimmo, Heading *et al.* 1975).

We calculated the total areas under the curves of concentration versus time (AUC) using the trapezium rule for plasma paracetamol concentrations. AUC from 0-60 minutes (AUC₆₀) were calculated on responses at 0, 30, and 60 minutes and AUC from 0-180 minutes were calculated using values at 0, 30, 60, 120, 150 and 180 minutes.

2.8.6 Cholesterol Analysis

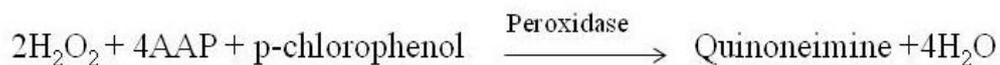
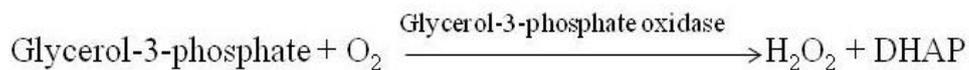
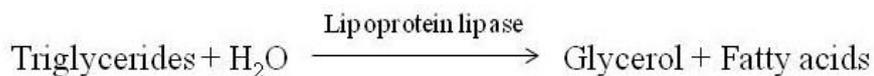
The determination of total cholesterol was performed on an automated Roche Cobas Mira Plus spectrophotometric analyser (Cobas Mira Plus (ABX Diagnostics, France), by enzymatic calorimetric method using commercially available kits (Cholesterol CP Reagent, ABX Pentra, Horiba ABX, France). All samples from each participant were analyzed on a single analyser run. The accuracy and precision of the assays were monitored using quality control sera

(Roche diagnostics GmbH, Mannheim, Germany. Randox laboratories Limited Co., Antrim Ireland, Wako chemicals GmbH, Germany). The quantitative determination of cholesterol is usually done for screening purposes and can be performed by an enzymatic photometric test “CHOD-PAP”. The principle of the cholesterol determination is based on the calorimetric equation as follows:



2.8.7 Triglyceride Analysis

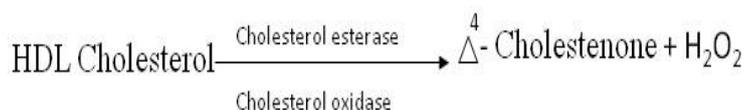
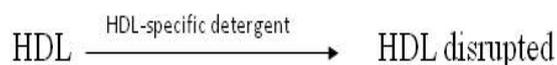
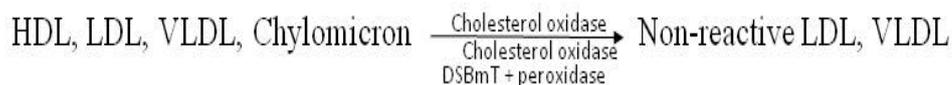
The measurement of plasma triglycerides was performed on an automated Roche Cobas Mira Plus spectrophotometric analyser (Horiba ABX, Montpellier, France) using a commercially available kit (Triglycerides CP Reagent ABX Pentra, Horiba ABX, France). All samples from each participant were performed on a single analyser run. The principle of enzymatic determination of triglycerides was based on the following colorimetric equation;



(DHAP = Dihydroxyacetone phosphate, 4-AAP = 4-aminoantipyrine)

2.8.8 High-density lipoprotein Analysis

The measurement of high-density lipoprotein (HDL) was performed on an automated Roche Cobas Mira Plus spectrophotometric analyser (Horiba ABX, Montpellier, France) using the commercially available kit (HDL Direct Reagent ABX Pentra, Horiba ABX, France). All samples from each participant were performed on a single analyser run. The principle of enzymatic determination of HDL was based on the following colorimetric equation;



DSBmT: N,N-bis(4 sulphobutyl)-m-toluidine-disodium; 4-AAP: 4-aminoantipyrine

The above methods were used to obtain the values of plasma TAG, HDL-cholesterol, and the total cholesterol. LDL-cholesterol within the plasma was then calculated by using the standardized formula (Friedewald, Levy *et al.* 1972);

$$\text{LDL-cholesterol} = (\text{Total cholesterol} - \text{HDL cholesterol} - \text{Total triglyceride}) / 2.19$$

2.9 Statistical Analysis

The Anderson-Darling test was used to determine the normality of the data using *Minitab 16* statistical software. Depending upon the distribution of the data, parametric or nonparametric analyses were applied. Time-averaged AUC for subjective measures of appetite, for energy intake, and for post-meal energy expenditure were calculated using trapezium rule. Statistical

significance was accepted for P-values of less than 0.05. Differences in energy intake between both trials were tested for pre-breakfast, pre-lunch and over the whole duration of the trial. All the data was presented as Means \pm SE. All the analysis of the data was carried out using the Minitab software (Minitab Inc., State College, Pennsylvania). Please refer to each experimental chapter for detailed information regarding the calculation of sample size and the statistical analysis of the respective experimental chapter.

3 Effects of high energy nutritional supplement drinks on appetite, appetite hormones, postprandial energy expenditure & energy intake

3.1 Introduction

Studies in the published literature suggest that oral multi-nutrient high-energy liquid supplements (HENSND) can improve energy intake and thus increase body weight and have a variety of clinical and functional benefits in malnourished patients, and patients at risk of becoming malnourished (Stratton and Elia 2007, Stratton and Elia 2010, Cawood, Elia *et al.* 2011, Hubbard, Elia *et al.* 2012, Stratton, Hebuterne *et al.* 2013). Studies have demonstrated that HENSND supplementation increases daily energy intake (Huynh, Devitt *et al.* 2014). A recent meta-analysis of 8 RCTs (total patients: $n = 771$) revealed that, due to consumption of oral nutritional supplements daily energy intake increased by a mean of 375 kcal/d (Hubbard, Elia *et al.* 2012). Thus, the oral nutritional supplements, (typically containing a mixture of protein, carbohydrate and fat and some micronutrients, and providing from 6.3 kJ/ml to 10.1 kJ/ml per typical serving of 125–220 ml) form an integral part of the management of malnutrition (Stratton and Elia 2010).

It is obvious that the ability of oral HENSND to promote an increase in the daily energy intake and induce a positive energy balance is related to incomplete energy compensation and lack of adjustment of energy intake due to previous ingestion. The standard explanation for lack or diminished compensation can be based on the results from studies which report weak satiating effects of soft drinks, and their inability to induce precise compensation for the energy provided. This suggests that liquid calories are not perceived by the body (DiMeglio and Mattes 2000, Mattes 2006, Drewnowski and Bellisle 2007). In addition, contrary to expectations, the higher energy preloads were associated with less compensation (Almiron- Roig, Palla *et al.* 2013). The mechanisms

responsible for little suppression of food intake following oral intake of HENSD remain to be investigated.

Current evidence indicates that the postprandial release of insulin and gastrointestinal factors such as peptide YY (Le Roux and Bloom 2005), Cholecystokinin (CCK) (Simpson, Parker *et al.* 2012), provide inhibitory feedback that suppresses appetite and food intake. Postprandial release of CCK also slows gastric emptying (GE) and by this mechanism contributes further to energy intake suppression (Kleibecker, Beekhuis *et al.* 1988, Konturek, Kwiecien *et al.* 1990). So far, the role of metabolic and hormonal appetite modulators, in amendment of energy intake, due to malnutrition treatment has been investigated only in tube-feeding studies (Stratton and Elia 1999, Stratton, Stubbs *et al.* 2008). The intermittent delivery of tube-feeding as a bolus, reflecting a physiological liquid meal-like pattern, regardless of the variety of significant metabolic and hormonal changes involved in satiation, induce only partial compensation for the energy provided by the bolus (Stratton, Stubbs *et al.* 2008). Indeed, tube feeding delivers nutrients directly into the stomach, and thus bypasses the sensory aspects of oral consumption and cephalic-phase response. In addition, one bolus of tube feeding provides nearly twice as much energy in comparison to a typical oral high-energy supplement (Stratton and Elia 1999, Stratton, Stubbs *et al.* 2008). However, interaction between appetite, food intake, metabolic and the hormonal mediators of appetite and satiety, following tube-feeding and oral supplementation can be different.

This study aimed to confirm that consumption of oral HENSD induces only a partial suppression of *ad libitum* food intake. In addition, study investigated impact of HENSD supplementation on the proxy measure of gastric emptying which is most probably an important mediator of both appetite and the energy intake (Jones, Doran *et al.* 1997, Sturm, Parker *et al.* 2004) and evaluate extent by which supplementation induced increase in the energy intake is diminished by thermogenic effect.

3.2 Material and Methods

Participants

Twenty-three healthy women with a mean (\pm SD) age of 24.4 ± 2.8 years (range: 18-35 years) with a body mass index (BMI) in (kg/m^2) of 18.6 ± 0.9 kg/m^2 (range: 17-20 kg/m^2) and body fat mass in (kg) of 9.1 ± 3.3 were recruited into this study. We have chosen “underweight” healthy women (BMI: 17-20 kg/m^2) to simulate relative under-nutrition. This allows a sampling regime and study design which would not be ethical in a paediatric patient group. Participants were recruited by means of advertisement leaflets on notice boards at the university and word of mouth in the campus of the University of Glasgow and in other public places. All the participants were non-smokers, had stable weight for one month prior to the study, not pregnant, having a regular menstrual cycle, were not on any medication or any nutritional supplement, and were not following a special diet. Before enrolling in the study participants underwent a screening visit which included height and weight measurement and filling out a detailed health screen questionnaire regarding the participant’s health, to exclude chronic illness, eating disorders and gastrointestinal operations, which could interfere with the results of the study. All participants were required to give written, informed consent. The study was approved by the College of Medical Veterinary and Life Sciences, University of Glasgow, Research Ethics Committee.

Study Design

This study used a single blind crossover design with two randomly sequenced experimental trials, separated by 4 weeks. A schematic representation of the study design is shown in Figure 3.1.

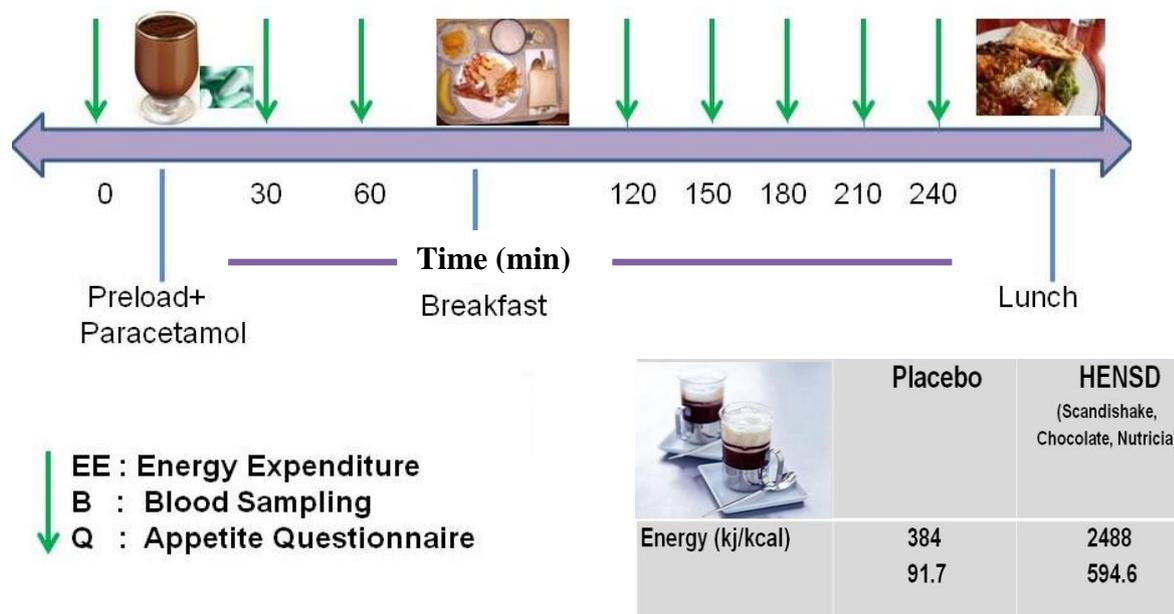


Figure 3.1 Schematic representation of the study design

On the morning of the experimental trial, participants reported to the metabolic investigation room between 0800 and 0900 after a 12 hour fast. Height (Seca, Leicester, UK) body mass and body fat (TBF-300, TANITA, Cranlea, UK), and resting metabolic rate (RMR) were measured. A venous cannula was then inserted and after an interval of 10 min, a base-line blood sample was obtained. Subsequently an appetite questionnaire was completed. Then, within 10 minutes, the study participants were asked to consume 240 ml of either high energy liquid mainstream nutritional supplement (Scandishake, Chocolate, Nutricia) prepared with full fat milk (HENS D trial), or a low calorie drink made up with skimmed milk, cocoa and sweeteners (PLACEBO trial).

Table 3.1 Energy (MJ) and proportion (percentage) from carbohydrate (CHO), fat and protein provided by PLACEBO and HENSND drink.

	PLACEBO	HENSND
CHO (g)	11.3g	68.8g
Fat (g)	1.3g	30.4g
Protein (g)	9g	11.9g
Energy (MJ)	0.38	2.49
Energy from CHO (%)	48	46
Energy from Fat (%)	13	46
Energy from Protein (%)	39	8

Abbreviations: CHO, Carbohydrates

The participants were blinded to the preload drink, and they were not told which chocolate drink they would consume. The order in which drinks were provided was randomly allocated. Each participant was allocated an alphanumeric subject code by the researcher. A block method of randomization was used to randomly allocate participants for taking the HENSND or PLACEBO drink first (Block size of 3 was taken). The first block participants were given HENSND drink and next block was given PLACEBO first. The HENSND and PLACEBO drinks had the same colour, volume, flavour and texture and were not distinguishable. Following supplement intake, two paracetamol tablets (1000mg with 100ml of water) were given to the participants. The participants marked further appetite questionnaires and blood samples were collected at 30 and 60 min after supplement intake, while the metabolic rate was measured every minute for the duration of 20 minutes after each blood sample. In the 60 minutes following the preload drink an *ad libitum* buffet style breakfast was served to the participants.

Subsequently appetite questionnaires were completed, blood samples were collected at 120, 150, 180, 210 and 240 minutes and metabolic rate was recorded as explained previously in Chapter 2.

Measurement of food and energy intake

All food and drink items (excluding water) were covertly weighed before and after each breakfast and lunch using the standard electronic kitchen scales (accurate to ± 1 g). The macronutrient and energy intake with and without macronutrients and energy provided by the supplements, was calculated over the whole experimental trial using the dietary software Windiets 2005 (The Robert Gordon University, Aberdeen, Scotland, UK). The calculations on energy and macronutrient intake were independently conducted by two researchers and mean values of each of their results was taken into account to enhance the accuracy of results.

Appetite Measurements

Visual Analogue Scale (VAS) questionnaires, with a line of 100 mm (Flint, Raben *et al.* 2000) were used throughout the trial for measuring the appetite sensations of the participants. Participants were asked to express their feeling of hunger, satiety, fullness, prospective food consumption and the desire to eat on 100mm lines by placing a vertical mark on the horizontal line at a point, which corresponds to their feelings at that time. The lines were anchored by negative respective feeling words (I am not hungry at all) on the left and by positive feeling words (never being hungrier) on the right respectively. Quantification of the measurement was made by measuring distance from the left end of the line to the participant's mark.

Metabolic Rate

Metabolic rate was measured by means of computerised open-circuit ventilated hood system (Oxycon Pro, Jaeger GmbH, Hoechberg, Germany). The rate of oxygen consumption (VO_2) and rate of carbon dioxide production

(VCO₂) were recorded at every minute for the duration of 20 minutes. Then these values were used to calculate the rate of energy expenditure by using the indirect calorimetry equations described by Frayn and Macdonald (Frayn and Macdonald 1997).

Blood Analysis

Venous blood samples were collected into a 10 ml ethylenediamine tetraacetic acid (EDTA) Vacutainer tube (BD Vacutainer Systems, Plymouth, UK). Blood samples used for the analysis of insulin and glucose were centrifuged at 4°C, 3000 rpm for 15mins in a refrigerated centrifuge. After centrifugation the plasma supernatant was aspirated and frozen at -80°C until analysed. The blood samples used for the determination of gut peptides were aliquoted into three Eppendorf tubes each containing 80µl Aprotinin (0.4TIU/ 500KIU) (Sigma-Aldrich, UK). As 0.6 TIU activity per ml of the blood is provided by 80µl of Aprotinin. Each bottle contains 4.4 TIU activity per mg protein and for 1.7 mg/protein per ml it has 7.48 TIU activity per ml (1.7 mg x 4.4 TIU), thus 0.6 TIU activity will be supplied by 80µl of Aprotinin (0.6 TIU x 1ml/7.48 TIU). Then samples were centrifuged in a smaller centrifuge machine for 4mins at maximum 14000 rpm. Subsequently 300µL of plasma was aliquoted into three Eppendorf tubes labelled for CCK/ PYY.

Blood glucose analysis was performed on a spectro-photometric analyser which was an automated Roche Cobas Mira (Horibra ABX, Montpellier, France) with co-efficient of variation < 3%. Quantitative insulin analysis was performed using commercially available Enzyme-linked immunosorbent assay (ELISA) kit (Mercodia AB, Uppsala Sweden). For analysis, the inter-assay and intra-assay coefficients of variance were 3.4% and 3.6%. The PYY were measured according to the manufacturer's instructions using Millipore ELISA kit (Merck, Millipore, Bioscience Division, UK), with an inter-assay coefficient of variance of < 7.6% and an intra-assay coefficient of variance of < 4%. Cholecystinin (CCK) was also analysed using ELISA kit (Phoenix, Pharmaceuticals, Inc., Burlingame, CA, USA). For analysis, inter-assay and

intra-assay coefficients of variance were $< 6.6\%$ and $< 5.4\%$ respectively. For the assessment of gastric emptying, plasma paracetamol concentrations were assayed by using Acetaminophen Assay kits (Cambridge Life Sciences, Cambridge, U.K.). The instructions of the kit were followed and an alternative protocol was used for this assay which had been validated by Cambridge Life Sciences, which allowed the use of reduced volumes.

Calculations and Statistical Analysis

The Anderson-Darling test was used to determine the normality of the data and it was found that the data was normally distributed.

For the subjective measure of appetite responses (hunger, satiety, fullness, prospective food consumption and desire to eat), plasma concentration of glucose, insulin, peptide YY and CCK the total areas under the curve (AUC) were calculated using the Trapezium Rule. Paired t-test was used to identify the differences between the PLACEBO and the HENSD trials. For subjective measure of the appetite responses (hunger, satiety, fullness, prospective food consumption and desire to eat), glucose levels, insulin levels and appetite hormones, the total areas under the curve (AUC) were calculated using the Trapezium Rule. Then time averaged AUC was calculated as total AUCs divided by the total duration of observation time (240 minutes).

The time-averaged AUC was calculated for the pre-breakfast (60 minutes), pre-lunch (150 minutes) and over the entire duration of the trial (240 minutes). This provided a measure of the average value over the period of observation. AUC for pre-breakfast (0-60min) values were calculated on the responses at 0, 30, and 60 time points. The AUC for pre-lunch values were calculated using values at 60, 90, 120, and 240. The calculated AUC were divided by time of observation to obtain the time averaged AUCs.

We calculated the total areas under the curves of concentration versus time (AUC) using the trapezium rule for plasma paracetamol concentrations. AUC from 0-60 minutes (AUC₆₀) were calculated on responses at 0, 30, and 60

minutes and AUC from 0-180 minutes were calculated using values at 0, 30, 60, 120, 150 and 180 minutes.

The metabolic rate pre-breakfast and pre-lunch energy expenditure and energy expenditure (EE) for the whole duration of the trial (240 minutes) was calculated in (kJ/min). After that the pre-breakfast increase in the energy expenditure (kJ) above RMR was calculated for a period of 60 minutes. Then pre-lunch increase in EE was calculated for 150 minutes and again for the whole duration of the trial (240 minutes). This was calculated by subtracting the mean value of energy expenditure rate (pre-breakfast, pre-lunch and over the full trial) from RMR and then multiplied by the time of observation. The percentage increase in energy expenditure above the resting metabolic rate was calculated for pre-breakfast, pre-lunch and for the whole duration of the trial. All the data was presented as means \pm SE. All the analysis of the data was carried out using the Minitab¹⁶ statistical software (Minitab Inc., State College, Pennsylvania).

3.3 Results

Energy Intake

Data on energy and macronutrient intake during the HENSD and PLACEBO trials are presented in Figure 3.2 and Table 3.2.

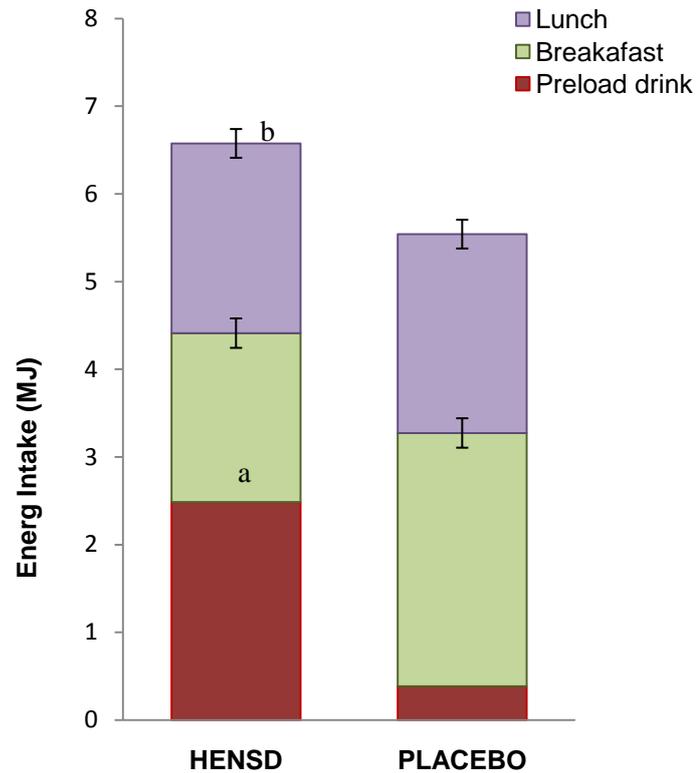


Figure 3.2 Energy provided by preload drinks and energy intake during *ad libitum* breakfast and *ad libitum* lunch in HENSD and PLACEBO trials.

^a Significant ($P=0.001$) difference during breakfast between HENSD and PLACEBO trials; ^b Significant ($P=0.006$) difference in summary energy intake between HENSD and PLACEBO.

Energy intake during the *ad libitum* breakfast was significantly ($P=0.001$) lower in the HENSD compared with the PLACEBO trial, but no significant difference between the two trials was found in the energy intake during the *ad libitum* lunch. Due to energy intake reduction during breakfast in the HENSD trial, energy intake during the breakfast and lunch combined was also significantly ($P=0.005$) lower in the HENSD than the PLACEBO trial. In the HENSD trial, the suppression of energy intake was equivalent to $43 \pm 14\%$ (1.07 ± 0.34 MJ) of energy provided by the supplement. Thus, total energy intake calculated as a sum of energy intake during breakfast and lunch, and energy provided by supplements was significantly ($P=0.006$) higher in the HENSD than the PLACEBO trial.

Table 3.2 Energy from carbohydrate (CHO), protein and fat intake in HENSD and PLACEBO trial

Meal		HENSD	PLACEBO
Breakfast	Energy (MJ)	1.92 ± 0.17	2.89 ± 0.23**
	CHO (g)	80 ± 8	113 ± 1*
	Protein (g)	11 ± 1	16 ± 1*
	Fat (g)	12 ± 1	21 ± 2**
Lunch	Energy (MJ)	2.16 ± 0.16	2.27 ± 0.20
	CHO (g)	69 ± 5	72 ± 7
	Protein (g)	22 ± 2	24 ± 2
	Fat (g)	18 ± 1.6	18 ± 1.9
Breakfast + Lunch	Energy (MJ)	4.09 ± 0.26	5.16 ± 0.32**
	CHO (g)	150 ± 11	186 ± 13**
	Protein (g)	33 ± 3	40 ± 3*
	Fat (g)	30 ± 2	40 ± 3**
Breakfast + Lunch + Supplement	Energy (MJ)	6.58 ± 0.26	5.54 ± 0.32**
	CHO (g)	219 ± 11	197 ± 13
	Protein (g)	45 ± 3	50 ± 3
	Fat (g)	60 ± 2	41 ± 3**

*Values are presented as Mean ± SE. Significantly different (*P<0.05, **P>0.001) from HENSD*

Appetite Responses

Responses of appetite measures during the HENSD and the PLACEBO trials are presented (Figure 3.3).

In the fasted state, VASs for hunger, desire to eat, fullness, and satiety were not significantly different between the HENSD and the PLACEBO trials. During the pre-breakfast period (0-60 min) mean VASs for hunger (HENSD,

41 ± 4 mm; PLACEBO, 52 ± 4 mm, $P = 0.03$) and desire to eat (HENSND, 46 ± 4 mm; PLACEBO, 62 ± 4 mm, $P= 0.007$), were significantly lower, and for satiety (HENSND, 46 ± 4 mm; PLACEBO, 34 ± 4 mm, $P=0.03$) and fullness (HENSND, 46 ± 4 mm; PLACEBO, 32 mm ± 4 mm, $P=0.006$) significantly higher in the HENSND than the PLACEBO trial. During the pre-lunch period (60-240 min), mean VASs for hunger (HENSND, 28 ± 3 mm; PLACEBO 30 ± 3 mm, $P=0.61$), desire to eat (HENSND 48 ± 4 mm; PLACEBO, 53 ± 5 mm, $P=0.28$), fullness (HENSND 64 ± 3 mm; PLACEBO, 62 ± 3 mm, $P=0.43$), and satiety (HENSND 64 ± 3 mm; PLACEBO, 60 ± 3 mm, $P=0.15$) were not significantly different between the HENSND and the PLACEBO trials.

Metabolic Rate

The resting metabolic rate, measured before consumption of the supplement was not significantly different between the two trials (HENSND, 3.1± 0.1 kJ/min; PLACEBO 3.2 ± 0.1 kJ/min). Energy expenditure (EE) above resting metabolic rate (RMR) after supplement intake during the pre-breakfast period (0-60 min) and pre-lunch period (60-240 min) in the HENSND and the PLACEBO trials is presented (Figure 3.4). The percentage increase in energy expenditure during the pre-breakfast period, was measured for 60 minutes after consumption of the supplements (HENSND, 15.5 ± 4.03%; PLACEBO, 4.84 ± 3.85%, $P=0.03$) and during 180 min of pre-lunch period (HENSND, 33.87 ± 6.2%; PLACEBO, 21.71± 3.78%, $P=0.03$) was significantly higher in the HENSND than the PLACEBO trial. The amount of energy expended above the resting metabolic rate during the pre-breakfast period, measured for 60 minutes after consumption of the supplements (HENSND, 27.54 ± 5.24 kJ; PLACEBO, 10.73± 6.3 kJ, $P=0.04$), during 180 min of pre-lunch period (HENSND, 142.97 ± 19.11 kJ; PLACEBO, 95.91± 16.11 kJ, $P=0.01$) and during the whole duration (0-240 min) of the experimental trials (HENSND, 296.6 ± 37.8 kJ; PLACEBO, 171.8± 40.0 kJ, $P=0.003$) was significantly higher in the HENSND trial with difference in energy expended above the metabolic rate between trials being 124.74 ± 35.95 kJ.

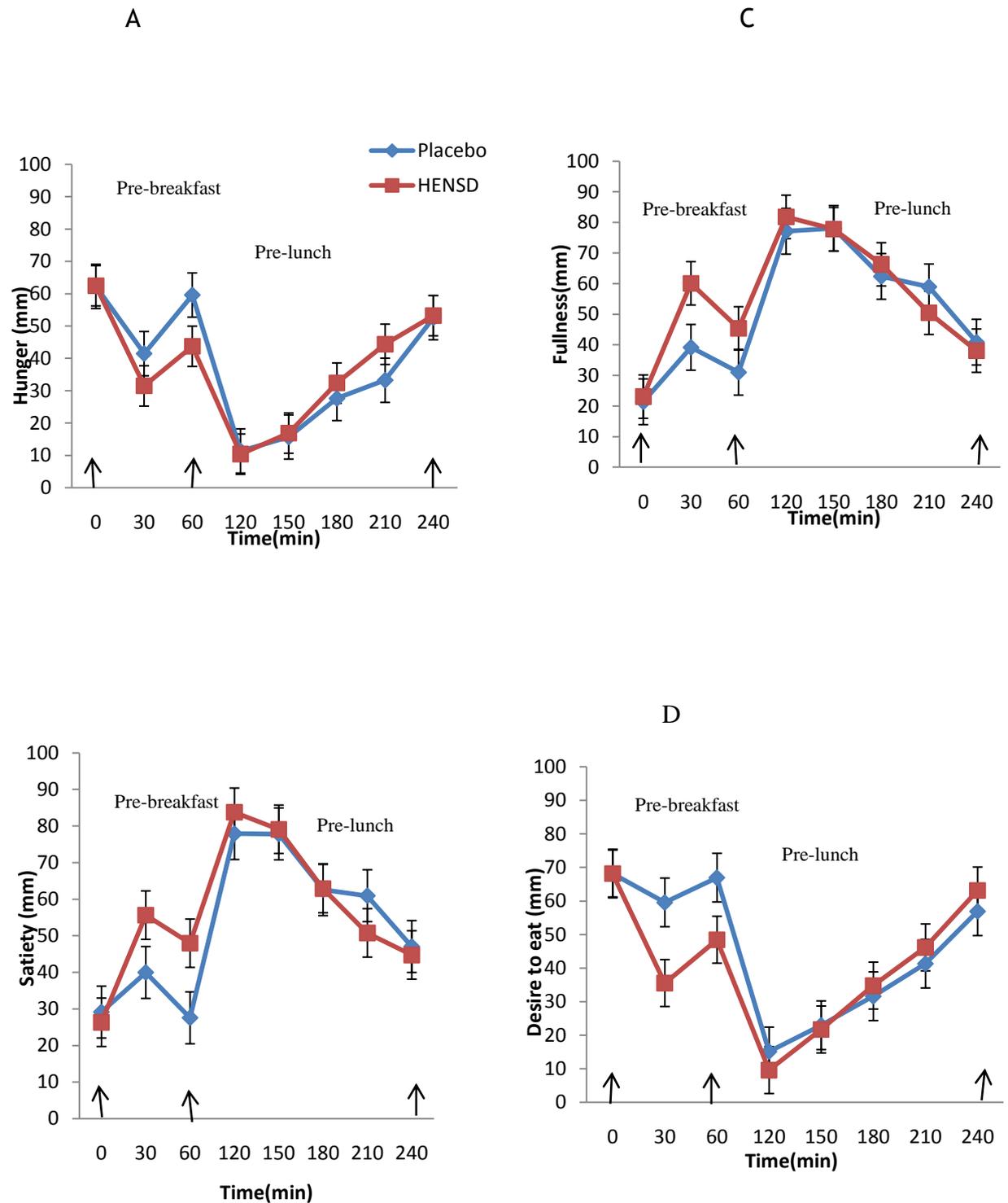


Figure 3.3 Measures of hunger, satiety, fullness, and desire to eat in the fasted state (0 min), during pre-breakfast period (0-60 min) and pre-lunch periods (60-240 min) in HENSD and PLACEBO trials

The preloads were consumed immediately after the fasting measurements. Values are presented as mean \pm SE.

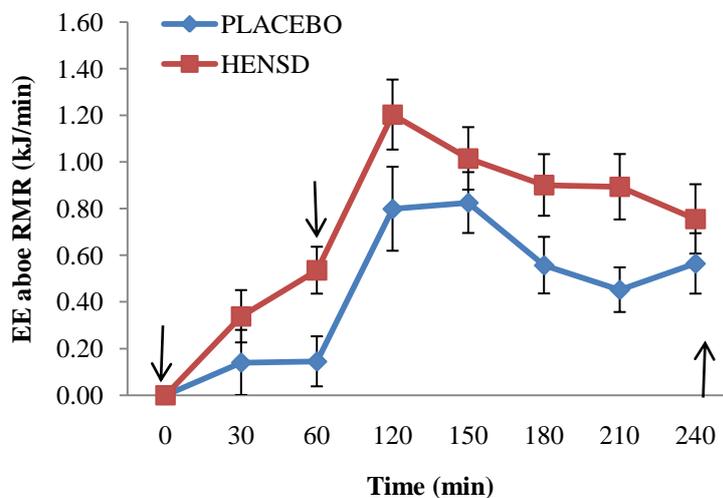


Figure 3.4: Energy expenditure (EE) above resting metabolic rate (RMR) after supplement intake during pre-breakfast period (0-60 min) and pre-lunch periods (60-240 min) in HENSD and PLACEBO trials. Values are expressed as mean \pm SE.

Metabolic and Hormonal Responses

Metabolic and hormonal responses during the HENSD and PLACEBO trials are presented in (Figure 3.5., Table 3.3). Mean concentration of plasma glucose during pre-breakfast (HENSD, 6.37 ± 0.25 mmol/l; PLACEBO, 5.51 ± 0.18 mmol/l, $P=0.0001$) was significantly higher in HENSD than the PLACEBO trial. During pre-lunch period (HENSD, 6.76 ± 0.21 mmol/l; PLACEBO, 6.09 ± 0.29 mmol/l, $P=0.06$) mean concentration of the plasma glucose was not significantly different between HENSD and PLACEBO trial.

During the pre-breakfast period, mean concentration of plasma insulin (HENSD, 30.27 ± 7.03 mU/l; PLACEBO, 7.94 ± 1.43 mU/l, $P=0.006$), CCK (HENSD, 2.2 ± 0.19 ng/ml; PLACEBO, 2.0 ± 0.22 ng/ml, $P=0.04$) and peptide YY (HENSD, 112 ± 10 pg/ml; PLACEBO, 76 ± 9 pg/ml, $P=0.006$) were significantly higher in the HENSD trial than the PLACEBO trial. During the pre-lunch period, the mean concentration of plasma insulin (HENSD, 62.71 ± 13.47 mU/l; PLACEBO, 36.74 ± 6.13 mU/l, $P=0.01$), CCK (HENSD, 2.43 ± 0.17 ng/ml; PLACEBO, 2.06 ± 0.13 ng/ml, $P=0.001$) and peptide YY

(HENS D, 136 ± 8 pg/ml; PLACEBO, 101 ± 11 pg/ml, $P=0.006$) were also significantly higher in the HENS D than the PLACEBO trial.

Table 3.3: Time-averaged AUC for responses of glucose, insulin, peptide YY (PYY), cholecystokinin (CCK) during pre-breakfast, pre-lunch period and during the entire period of trial. Values are presented as Mean \pm SE. (n=9)

Time points	HENS D	PLACEBO	P-Values
Glucose (mmol/L)			
Pre-breakfast	6.37 ± 0.25	5.51 ± 0.18	0.0001*
Pre-lunch	6.76 ± 0.21	6.09 ± 0.29	0.06
Over 240 mins	6.66 ± 0.17	5.95 ± 0.24	0.01*
Insulin (mU/L)			
Pre-breakfast	30.27 ± 7.03	7.94 ± 1.43	0.006*
Pre-lunch	36.74 ± 6.13	62.71 ± 13.47	0.018*
Over 240 mins	38.93 ± 7.71	29.54 ± 4.86	0.05*
PYY (pg/ml)			
Pre-breakfast	112 ± 10	76 ± 9	0.006*
Pre-lunch	136 ± 8	101 ± 11	0.006*
Over 240 mins	130 ± 8	95 ± 10	0.003*
CCK (ng/ml)			
Pre-breakfast	2.2 ± 0.19	2.0 ± 0.22	0.04*
Pre-lunch	2.43 ± 0.17	2.06 ± 0.13	0.001*
Over 240 mins	2.39 ± 0.17	2.05 ± 0.15	<0.0001

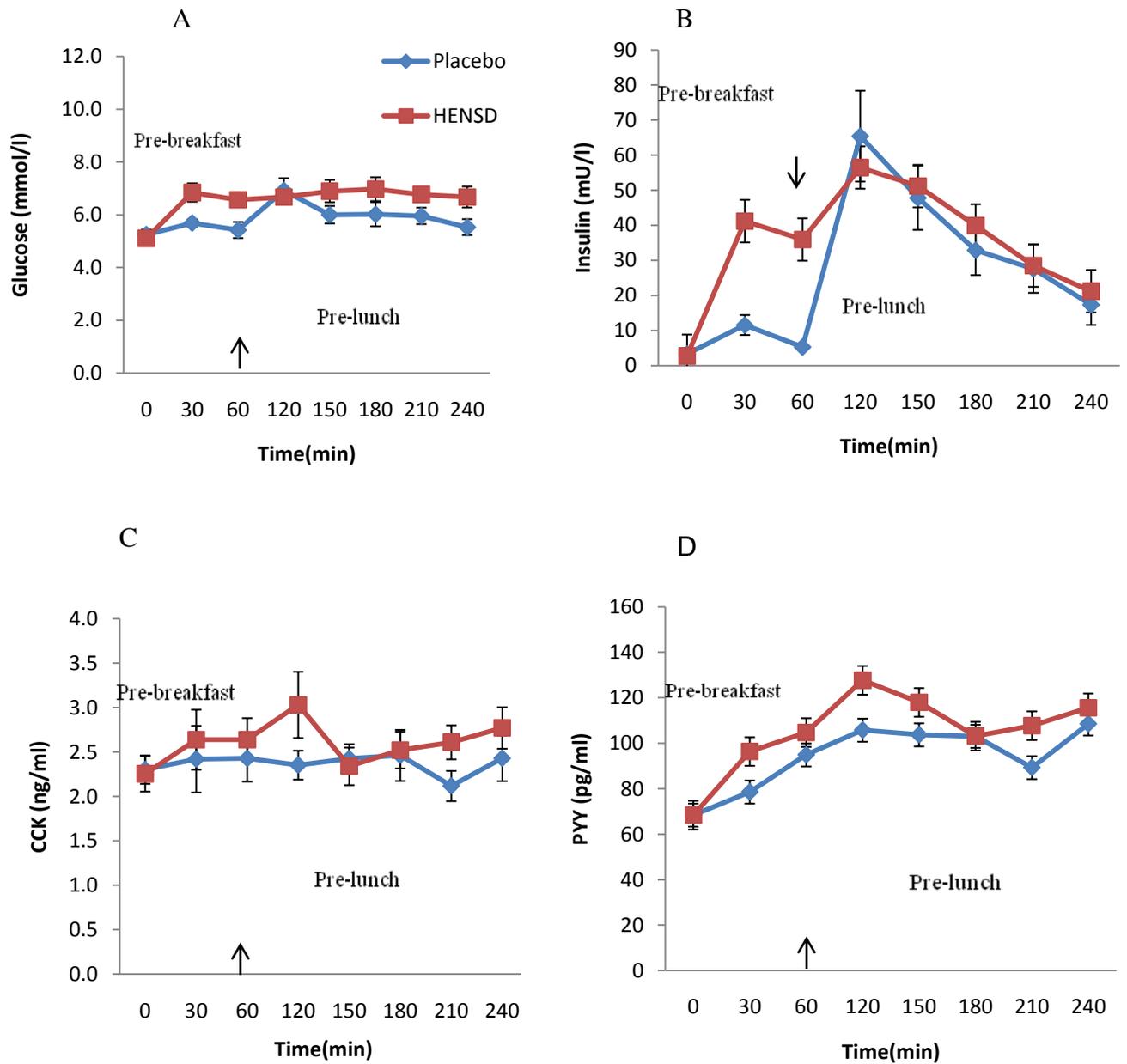


Figure 3.5: Plasma concentration of glucose, insulin, cholecystokinin (CCK), peptide YY (PYY) in the fasted state (0 min), during pre-breakfast period (0-60 min) and pre-lunch periods (60-240 min) in HENS D and PLACEBO trials.

Values are expressed as mean \pm SE.

Gastric emptying was a little faster in the PLACEBO trial as compared to HENSD trial. Time taken to reach peak plasma paracetamol concentration in PLACEBO trial was 120 minutes whereas time taken to reach peak plasma paracetamol concentration in HENSD trial was delayed until 150 minutes (Figure 3.6).

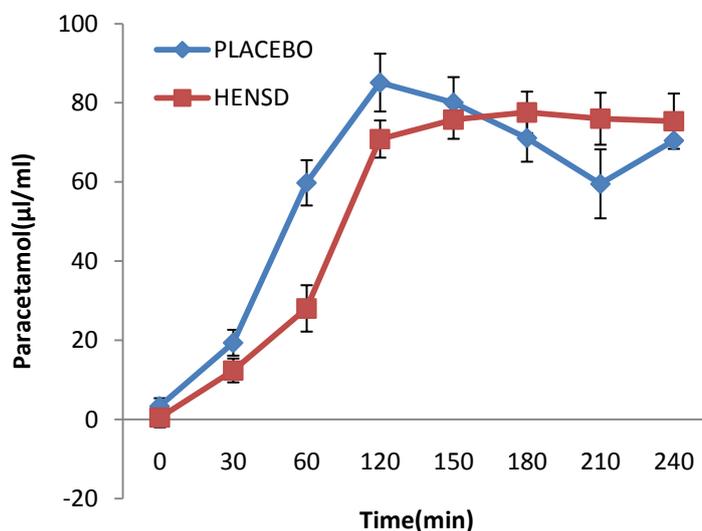


Figure 3.6: Plasma concentration of paracetamol in fasted state (0 min), from 0-60 minutes (AUC_{60}) and from 0-180 minutes (AUC_{180}) in HENSD and PLACEBO trials.

Values are expressed as mean \pm SE.

AUC of the plasma paracetamol concentration from 0-60 minutes (AUC_{60}) (HENSD, $816 \pm 224 \mu\text{l/ml}$; PLACEBO, $1556 \pm 146 \mu\text{l/ml}$, $P=0.01$) and from 0-180 minutes (AUC_{180}) (HENSD, $8508 \pm 551 \mu\text{l/ml}$; PLACEBO, $10979 \pm 637 \mu\text{l/ml}$, $P=0.008$) was significantly lower in the HENSD trial than the PLACEBO trial.

Table 3.4 The area under plasma paracetamol concentration-time curve from 0-60 minutes (AUC_{60}) and the area under plasma paracetamol concentration-time curve from 0-180 minutes (AUC_{180}) in HENSD and PLACEBO trials. Values are presented as Mean \pm SE. (n=9)

Time points	HENSD	PLACEBO	P-Values
Paracetamol ($\mu\text{l/ml}$)			
(AUC_{60})	816 ± 224	1556 ± 146	0.01*
(AUC_{180})	8508 ± 551	10979 ± 637	0.008*

3.4 Discussion

This study demonstrated that the metabolic and hormonal appetite regulators following HENSD consumption responded in a way, which is expected to facilitate the suppression of appetite and energy intake. Regardless of this, suppression of energy intake was only partial and is evident during the first meal. Inability to induce precise compensation for the energy provided by the HENSD even in circumstances of enhanced satiety hormones suggests that calories provided by the HENSD are not fully perceived by the body, which justifies this type of supplement for the treatment of malnutrition.

Due to higher energy provision with the HENSD in comparison to PLACEBO during pre-breakfast period, we observed that PYY, CCK, insulin and glucose levels were higher which is in consistence with findings from other studies (Lieverse, Jansen *et al.* 1993, Verdich, Toubro *et al.* 2001, De Graaf, Blom *et al.* 2004, Degen, Oesch *et al.* 2005, Stratton, Stubbs *et al.* 2008). The appetite suppressing impact of the elevated levels of CCK becomes evident by suppression of food intake during the breakfast in the HENSD trial. Since the studies on humans showed that intravenous administration of CCK suppresses food intake by 19% in a test meal (Lieverse, Jansen *et al.* 1993, De Graaf, Blom *et al.* 2004) and it also induced a significant decrease in the feelings of hunger whereas fullness feelings tended to be increased (Degen, Matzinger *et al.* 2001). Intravenous infusion of PYY was shown to suppress the food intake by approximately 30%, while the subjective feelings of hunger and fullness were in line with a decline in food intake during the breakfast (Batterham, Cowley *et al.* 2002, Batterham, Cohen *et al.* 2003). Insulin also had an appetite suppressing impact, and its levels immediately before *ad libitum* meals were inversely correlated with the energy intake in lean subjects (Verdich, Toubro *et al.* 2001).

Appetite after preload before breakfast coincided with all hormonal appetite regulators including CCK, PYY, insulin and glucose. We found that 40% of the energy provided by HENSD was compensated for by eating less during the breakfast in the HENSD trial. Our findings were similar to the previous studies in which liquid preloads interventions reported, energy compensation levels of 75% or less (Mattes 2006, Mattes and Campbell 2009, Almiron-Roig, Palla *et al.* 2013). In one of these studies food intake with bolus feeding was equivalent to 39.7 % of the tube feed energy infused and the food energy intake was declined by 15% (Stratton, Stubbs *et al.* 2008).

During the pre-lunch period, which started after breakfast and lasted for 180 minutes, appetite measures and energy intake during lunch were not different between the trials regardless of participants of the study in HENSD trial after breakfast being in more positive energy balance. Conversely, concentration of hormonal appetite regulators during pre-lunch period such as CCK, PYY and insulin were significantly higher in the HENSD trial.

Therefore, it seems that dissociation between appetite measures and the expected action of these appetite regulators is in contradiction with some studies (French, Murray *et al.* 1993, Delzenne, Blundell *et al.* 2010), but in consistence with other studies (Woo, Kissileff *et al.* 1984, Doucet, Laviolette *et al.* 2008) which found that appetite scores were not related to CCK concentration (De Graaf, Blom *et al.* 2004) PYY concentration (Doucet, Laviolette *et al.* 2008) and insulin concentration (Gielkens, Verkijk *et al.* 1998).

Having no difference in the energy intake during lunch most likely was related to the gastric emptying. It has been found that the mechanism by which CCK suppresses appetite is by slowing the gastric emptying (Kleibeuker, Beekhuis *et al.* 1988, Konturek, Kwiecien *et al.* 1990, Melton, Kissileff *et al.* 1992). Since gastric emptying is one of the most important appetite regulator (Jones, Doran *et al.* 1997, Sturm, Parker *et al.* 2004), we also measured the rate of gastric emptying. We found that gastric emptying coincides with appetite and food intake induced after HENSD supplementation. Gastric emptying was slower in HENSD trial, and energy intake and appetite was lower during pre-breakfast period while during pre-lunch period no difference in gastric emptying coincides with no difference in energy intake and appetite measures. Therefore, short-lived impact of HENSD on gastric emptying may be a central facilitator in increase in the energy intake during the lunch period. Thus having no appetite and energy intake suppression during second meal consumed during 240 minutes after HENSD intake played an important role in the promotion of positive energy balance. Therefore having short-lived action of HENSD on energy regulation can be very important for promotion of energy intake.

The thermic effect of food is another potential appetite and energy intake regulator (Crovetti, Porrini *et al.* 1998, De Graaf, Blom *et al.* 2004). Previous evidence demonstrated that higher diet induced thermogenesis was correlated with reduced feelings of hunger and higher values for fullness (Luscombe, Clifton *et al.* 2003, Raben, Agerholm-Larsen *et al.*

2003, Lejeune, Westerterp *et al.* 2006). We aimed to link the appetite responses and the energy intake during meals following HENSD consumption with the thermic effect of food. Increased energy expenditure above the resting metabolic rate during the pre-breakfast period also coincided with reduced feelings of hunger and desire to eat, but higher values for satiety, and fullness as found by other studies (Luscombe, Clifton *et al.* 2003, Raben, Agerholm-Larsen *et al.* 2003, Lejeune, Westerterp *et al.* 2006). The enhanced thermic effect of food was significant after the intake of HENSD and after breakfast, but energy intake was suppressed during breakfast. We found 15% increase in energy expenditure after the HENSD supplementation, as compared to the thermic effect for proteins (20-30%), carbohydrates (5-10%) and fat (0-3%) (Tappy 1996, Raben, Agerholm-Larsen *et al.* 2003). The participants ate less during breakfast; the enhancement in the energy expenditure lasted longer and was found to be 34% during the 180 minutes of the pre-lunch period, suggesting that the effects of these supplements might be more prolonged. It has been found that the postprandial rise in energy expenditure lasts for several hours (Reed and Hill 1996). In spite of the increased energy expenditure during the pre-lunch period in the HENSD trial, we did not observe any significant difference in the appetite measures and *ad libitum* energy intake during the lunch. Findings that showed an increase in the metabolic rate was not related to appetite measures during the pre-lunch period. This is not surprising, since other studies have also demonstrated that subjective sensations of hunger, satiety, fullness or prospective food consumption are sometimes, but not always, related to energy expenditure (Raben, Agerholm-Larsen *et al.* 2003). We found that diet-induced thermogenesis was 269kJ (64kcal) over 240 minutes after the consumption of HENSD and approximately 171kJ (41kcal) after the consumption of the PLACEBO, a difference of 98kJ (23kcal). Therefore, the total energy intake on the day of the HENSD trial was significantly higher even when corrected for post-prandial energy expenditure (EE).

Energy compensation is the adjustment of energy intake provoked by the previous ingestion of supplement (Almiron-Roig, Palla *et al.* 2013). Inter-meal interval (IMI) is one of the strongest contributors to the level of compensation (Almiron-Roig, Palla *et al.* 2013). In lean subjects, satiety hormones regulated the IMI (Chapelot, Aubert *et al.* 2000). We considered an IMI of 60 minutes in our study as a physiological factor. A minimum interval of 20 minutes is required for the initial absorptive effect of the preload to influence

the energy level (Booth, Toates *et al.* 1976) and it is speculated that short intervals allows the detection of orosensory and gastric effects (Blundell, De Graaf *et al.* 2010).

The results of this study are highly reliable and accurate as the energy intake was measured under strict laboratory conditions and all foodstuffs were measured by the researcher. Three researchers analyzed the energy and macronutrient intake increasing the validity of results. In this study while calculating energy expenditure the values were corrected by taking the mean of both pre and post drift and it was the first study in which the energy expenditure was measured in this way. In order to measure the appetite and metabolic responses of the females in the same phase of menstrual cycle, both the trials were set apart by four weeks or according to the duration of the menstrual cycles as appetite control and energy intake vary considerably across menstrual cycle (Buffenstein, Poppitt *et al.* 1995).

This study has some limitations; firstly, the trial was set for a short duration of time under laboratory conditions so short term impact of the preload drink might not be representative of clinical settings where HENSDs are prescribed daily. Therefore, this trial might not effectively reflect the long-term effects of HENSD. Additionally participants remained sedentary in the metabolic suite for the whole duration of the trial which was not in accordance with habitual interaction (Poppitt, McCormack *et al.* 1998). Moreover, the accuracy of self-reported appetite responses like hunger, satiety and fullness depends on the participant's ability to provide representative responses. Due to the expenses involved in the measurements of appetite regulating hormones, and difficulty in obtaining blood samples from some of the participants, data on PYY, CCK, glucose levels and insulin levels were obtained for only nine participants. However, despite of these numbers of participants we were able to find statistically significant differences between HENSD and the PLACEBO trials. In fact, during the pre-breakfast period time averaged AUC in case of PYY, CCK, insulin and glucose was significantly higher. The current findings apply to healthy under-weight women and may not necessarily be generalized to the malnourished population so the results should not be over-interpreted.

In summary our study suggest that only partial compensation for energy provided by HENSD may be at least, in part, explain why the suppressive action of metabolic and hormonal appetite regulators was short lived.

3.5 Conclusions

In this study we found that in underweight healthy young females with a BMI of less than 20kg/m^2 the consumption of ultra HENSD at 60 minutes allows an increase in energy prior to breakfast, reduced hunger, increased satiety, increased post prandial energy expenditure and enhanced blood glucose, increased insulin and PYY concentrations, which all suppressed appetite. This increase in satiety leads to partial compensation for energy consumed by HENSD as compared to the PLACEBO, and 43% of the energy provided was compensated by eating less during the breakfast. Furthermore, this suppression of appetite is short lived and has its greatest impact on energy intake during the breakfast. Therefore, the daily energy provision in the HENSD trial remained much higher even when corrected for the increase in post- prandial energy expenditure. Consequently HENSD does not induce full compensation for energy provided and can be expected to increase daily energy intake, thus the improving nutritional status of lean females. However, the overall benefit of HENSD is lower than expected.

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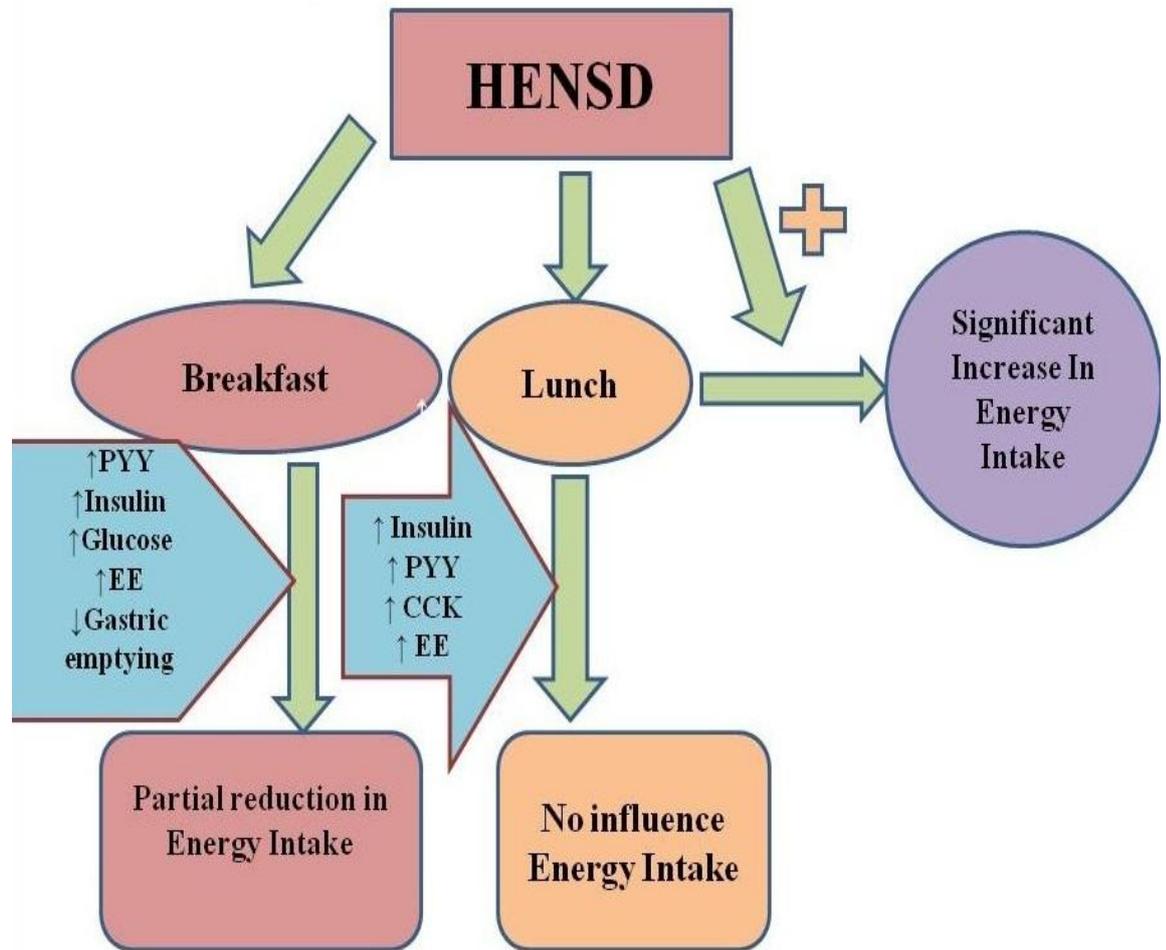


Figure 3.7 Schematic presentation of the results, showing the impact of HENSD supplementation on partial reduction in energy intake during *ad libitum* buffet breakfast and having no impact on energy intake during *ad libitum* buffet lunch

4 Impact of High Energy Nutritional Supplement Drink (HENSND) consumed for five consecutive days on appetite, energy intake and cardio-metabolic risk factors in underweight females

4.1 Introduction

The evidence suggests that high energy nutritional supplement drinks (HENSNDs) can improve energy intake and prevent or correct disease associated under-nutrition and improve clinical outcomes (Stratton and Elia 2007, Stratton and Elia 2010, Cawood, Elia *et al.* 2011, Hubbard, Elia *et al.* 2012, Stratton, Hebuterne *et al.* 2013). Many studies demonstrated that provision of HENSND to malnourished patients increased their energy intake, promoted rapid recovery, reduced morbidity and improved their quality of life (Potter, Langhorne *et al.* 1998, Milne, Potter *et al.* 2005, Stratton and Elia 2007, Vanderkroft, Collins *et al.* 2007, Nieuwenhuizen, Weenen *et al.* 2010, Philipson, Thornton *et al.* 2013, Stratton, Hebuterne *et al.* 2013).

In our previous study (chapter 3) we found that in slim young females, HENSND consumed 60 minutes before breakfast reduced hunger, increased satiety, and enhanced blood glucose, insulin and peptide YY (PYY) concentrations which all are known as appetite suppressors (Huda, Wilding *et al.* 2006, Wren and Bloom 2007). This increase in satiety and potential appetite regulators led to compensation for energy consumed by HENSND. However, the compensation was only partial, although energy intake during the *ad libitum* breakfast was significantly lower in the HENSND than the PLACEBO trial. When energy provided by supplements was added to the energy intake from breakfast and lunch, the energy intake in the HENSND trial was significantly higher. We also found that in the HENSND trial energy provision remained higher even when corrected for the increase in post-prandial energy expenditure. Thus, in a well-controlled study we demonstrated that in young, healthy, slim women, consumption of HENSND in the morning

does not induce full compensation for energy provided by this drink. We concluded that the compensation for the energy provided by HENSD supplementation happens immediately and disappears quickly and increases daily total energy intake. Therefore, HENSD might be beneficial for the treatment of malnutrition.

In clinical practice, high-energy supplements are usually provided in the evening, rather than prior to breakfast, and prescribed for several days or weeks. Therefore, from a practical point of view, it is important to find whether enhancement in daily energy intake can also be achieved when HENSD is consumed on a day-by-day basis and when intake of supplements takes place in the evening. There is a need to investigate if supplementation in the evening induces positive energy balance and to confirm the findings from our first study that compensations with HENSD supplementation happens immediately, disappears quickly and is short lived (Fatima, Gerasimidis *et al.* 2013).

Therefore, this study aimed to investigate the time scale of compensation after HENSD supplementation for five days by measuring energy intake after supplementation during the evening meal, and during the *ad libitum* breakfast, lunch and dinner consumed on the consecutive day. Since, consumption of HENSD due to promotion of energy intake is expected to promote positive energy balance which as discussed in chapter 1 may have deleterious effects in the malnourished, , this study also aimed to investigate the impact of HENSD supplementation on cardio-metabolic risk factors.

Evidence from data originating from previous studies investigating the impact of acute negative energy balance achieved through moderate dietary restrictions and/or increasing energy expenditure through a single bout of exercise on reducing lipoprotein metabolism, insulin and glucose concentrations are available in abundance (Maraki, Magkos *et al.* 2010, Maraki and Sidossis 2010, Bellou, Siopi *et al.* 2013). However, evidence relating to an acutely induced positive energy balance in relation to

postprandial lipid profiles, insulin and glucose concentrations is lacking. A positive energy balance induced by an increase in dietary energy intake impacts on plasma triglyceride, insulin and glucose concentrations (Hill, Peters *et al.* 1990, Faeh, Minehira *et al.* 2005, Bortolotti, Kreis *et al.* 2009, Brøns, Jensen *et al.* 2009). Bortolotti *et al.* (2009) in a cross-over study observed that after 4 days of either hypercaloric high fat diet, hypercaloric high fat high protein diet, or an isocaloric diet (control). The total LDL-cholesterol and HDL-cholesterol concentrations were significantly higher in both high fat diet and high fat high protein diet. However, total triglycerides and VLDL-TAG were slightly reduced with a high fat diet as compared to the control diet, while a high fat diet had no impact on plasma insulin and glucose levels (Bortolotti, Kreis *et al.* 2009). Likewise, Brøns *et al.* (2009) investigated the effects of a 5 day high fat (60%) overfeeding (+50%) versus a control diet (50% of the energy coming from CHO, 35% from fat and 7.5% from protein) in twenty-six young healthy volunteers. They found that a high fat- high calorie- diet (containing 50% extra energy, where 60% energy is from fat, 32.5% from CHO and 7.5% from protein) decreased fasting plasma TAG, LDL- and VLDL- cholesterol concentration. Whereas HDL- cholesterol was higher than the control diet. Fasting glucose levels were significantly higher while borderline increase in insulin levels were observed on the high fat high calorie diet (Brøns, Jensen *et al.* 2009).

The impact of short term overfeeding on triglyceride metabolism is uncertain since it is difficult to distinguish between changes in energy intake and changes in the macronutrient composition. Some studies reported elevated VLDL-TAG concentrations after 4 to 7 days of consuming hypercaloric high carbohydrate diet (Ngo Sock, Lê *et al.* 2010, Sobrecases, Lê *et al.* 2010) while overfeeding studies with high fat diet found that VLDL-TAG concentrations were reduced (Brøns, Jensen *et al.* 2009, Sobrecases, Lê *et al.* 2010). Other studies found no impact of hypercaloric feeding on very low density lipoprotein triglyceride (VLDL-TAG) concentrations in healthy women (Bellou, Maraki *et al.* 2013), and in obese subjects (Smith, Magkos *et al.*

2013). A study conducted by Bellou *et al* (2013) found that a single day with a positive energy balance induced by overfeeding (surplus of approximately 3MJ), with a mixed diet (balanced diet), did not have an acute effect on VLDL-TAG concentration. Two high-calorie fibre-free energy drinks (6kJ/ml) provided the surplus energy (Bellou, Maraki *et al.* 2013). Smith *et al* (2013) confirmed the results from Bellou *et al* (2013), and demonstrated that a single day of an acute positive energy balance achieved by increasing the daily energy intake by 30% and providing 55% energy from carbohydrate, 30% from fat and 15% from proteins in both normal and overweight subjects had no impact on the VLDL-TAG concentrations (Smith, Magkos *et al.* 2013).

The impact of acute positive energy balance achieved by overfeeding with a mixed diet (balanced diet) produced controversial findings of effects on plasma glucose and insulin levels. Some studies reported an increase in the concentration of both insulin and glucose (Tam, Viardot *et al.* 2010, Bellou, Maraki *et al.* 2013), some insulin only (Sobrecases, Lê *et al.* 2010, Cornford, Hinko *et al.* 2012) and others found no change in insulin or glucose levels (Després, Poehlman *et al.* 1987, McDevitt, Bott *et al.* 2001).

The evidence from previous studies demonstrated that, by manipulating the macronutrient composition and keeping the intervention isocaloric there was a detrimental impact on the postprandial lipid responses. Koutsari *et al* (2000) found that a high carbohydrate diet (68% energy from CHO) consumed for three days by nine normolipidemic men significantly increased both fasting and postprandial TAG, decreased fasting HDL-cholesterol, decreased the plasma glucose levels, and had no impact on serum insulin response in comparison with an isoenergetic high fat diet (66% energy from fat) (Koutsari, Malkova *et al.* 2000). Similarly, Robert *et al* (2008) found that when eight healthy individuals consumed either an isocaloric high CHO diet (75% energy from CHO), or, a high-fat low-carbohydrate diet (40% of energy from fat) for three days, a stable isotope tracer technique showed that both fasting and

postprandial TAG concentrations were significantly increased after the high CHO diet as compared to high-fat diet (Roberts, Bickerton *et al.* 2008).

Likewise, in a randomized crossover study by Culling *et al.* (2009), eight non-diabetic subjects consumed three isoenergetic diets: high fat (50% energy from fat), high sugar and high starch (70% energy from CHO) each for three days. On day 4, following each dietary period fasting TAG concentrations were highest following a high sugar diet and lowest when following a high fat diet. There were no significant differences between high-fat and high-starch diet. Fasting glucose levels were not affected by the prior diet but, the postprandial glucose concentrations were significantly higher after the high-fat diet as compared to high-sugar diet. It was suggested by this study, that the short term TAG raising impact of high CHO diet depends upon the nature of the CHO, with a greater impact of a sugar rich than a complex CHO rich diet (Culling, Neil *et al.* 2009).

These isocaloric studies showed that, by manipulating the macronutrient composition of the intervention, there was a detrimental impact on postprandial lipids and insulin, the intervention with isocaloric high carbohydrate diet significantly increased the postprandial TAG concentrations. The high-fat diet had no impact on postprandial TAG concentration but had a detrimental impact on glucose homeostasis. However, further research is necessary to verify these results when linking postprandial TAG concentrations, plasma insulin and plasma glucose concentrations to induce a positive energy balance.

Therefore, this study aimed to investigate the impact of 5 days of supplementation with HENSD, in the evening, on the energy intake following HENSD supplementation, and during the consecutive day. We also aimed to find out if consumption of HENSD in the evening for five days has a detrimental impact on the plasma lipids, glycaemia and insulinaemia.

4.2 Material and Methods

Participants

The participants of this study were twenty-three healthy females with a mean (\pm SD) age of 25 ± 5 years (range: 18-34 years) with a BMI in (kg/m^2) of 18.7 ± 1.2 (range: 17-20 kg/m^2) and body fat mass in (kg) of 8.5 ± 3 . The participants had a stable weight for one month prior to the study, had regular menstrual cycle and were not on medication, nutritional supplements and were not following specific diets. Participants were recruited from the campus of the University of Glasgow or from other public places. All participants gave written informed consent. The study was approved by College of Medical Veterinary and Life Sciences, University of Glasgow, Research Ethics Committee.

Study Design

This was a single blinded, randomised, controlled, crossover study. Participants commenced upon two experimental trials of a high-energy nutritional supplement drink (HENSND) and PLACEBO trial, lasting for six days. The trials were conducted in a counter balance order and were separated by at least 7 days of wash out period. During each evening for five days, participants came to the metabolic suit at Yorkhill hospital, and consumed a) HENSND (Scandishake, Chocolate, Nutricia) made up with 240g of full fat milk, according to the manufacturer instructions (Nutricia, 2009) b) PLACEBO (a low calorie drink prepared with 240 g of skimmed milk, 4g of cocoa and 2 sweeteners). The participants were blinded in relation to the drink offered and the drinks were consumed under supervision of the researcher, ensuring compliance. The order in which drinks were provided was randomly allocated. The PLACEBO and HENSND drinks had the same volume, colour, texture and flavour and were not distinguishable. The researcher allocated an alphanumeric subject code to each participant. Then block method of randomization was used to randomly allocate participants for taking HENSND

and PLACEBO drink first (Block size of 3 was taken). First block participants were given HENSND drink and next block was given PLACEBO first, and so on. The energy content (MJ) and the percentage energy from the macronutrient content of the HENSND and the PLACEBO are presented in Table 4.1.

Table 4.1 Energy (MJ) and proportion (percentage) from carbohydrate (CHO), fat and protein provided by PLACEBO and HENSND drink.

	PLACEBO	HENSND
CHO (g)	11.3	68.8
Fat (g)	1.3	30.4
Protein (g)	9	11.9
Energy (MJ)	0.38	2.49
Energy from CHO (%)	48	46
Energy from Fat (%)	13	46
Energy from Protein (%)	39	8

Abbreviations: CHO, Carbohydrates

After supplementation, the participants were allowed to go home and consume their evening meal at home. Participants recorded their dietary intake during the four days of supplementation. The participants were asked to record, in detail, the weight of all the food consumed and any leftover after eating, for four days. They were asked to document the weight of each item of food separately and time of their consumption. The participants were provided with the food diary, oral and written instructions on how to keep their dietary record and the electronic scales (Salter Housewares Ltd., Tonbridge, U.K.) to weigh their food accurately. The researchers of the study inspected the dietary records to make sure the dietary records were completed with sufficient details.

On day six, the participants were requested to come to the metabolic investigation suite at Yorkhill Hospital at ~ 08:30am in a fasted state.

Participant height was measured with a portable stadiometer (Seca, Leicester, UK). Body weight and fat mass was measured to the nearest 0.01 kg using a digital bioelectrical impedance scale (TBF-300, TANITA, Cranlea, UK). Approximately 10-15 minutes were given to the participant to rest and acclimatize with the environment. Then a venous cannula was inserted and, after an interval of 10 min, a base line blood sample was obtained and the participants were asked to mark the appetite questionnaire regarding their appetite sensations. Then an *ad libitum* buffet breakfast was served and following breakfast, blood samples were collected and appetite questionnaires were completed at 30, 60, 90 and 120 minutes. An *ad libitum* buffet lunch and *ad libitum* buffet dinner was provided three and seven hours after breakfast, respectively. After lunch, blood samples were collected and appetite was evaluated in the same manner as after breakfast. The amount of food offered and leftover during the *ad libitum* buffet breakfast, *ad libitum* buffet lunch, and *ad libitum* buffet dinner was recorded by the researcher. A schematic representation of the study design is shown in Figure 4.1.

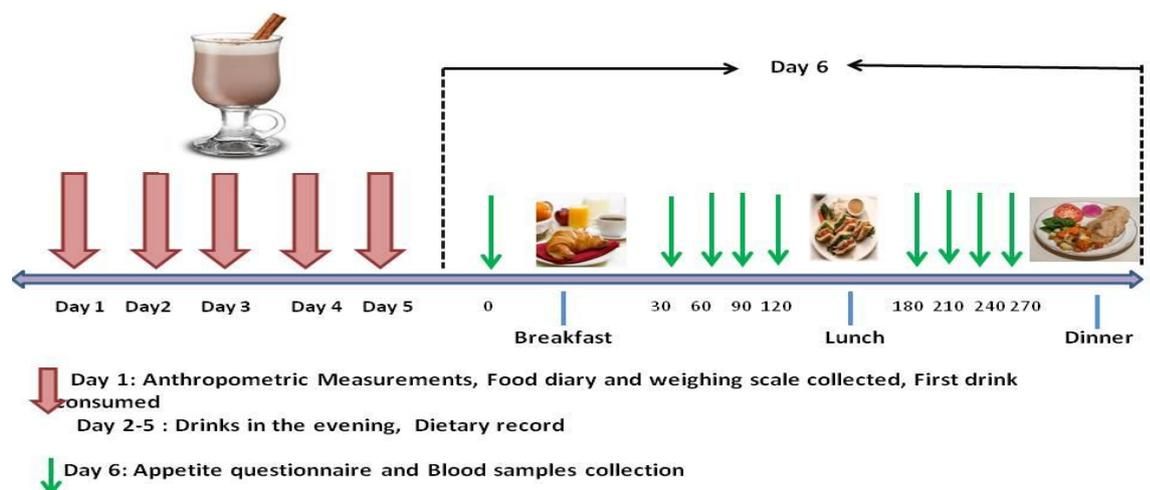


Figure 4.1 A schematic presentation of the study design

Anthropometry and Body Composition

The height of the participants was measured with a portable stadiometer (Seca, Leicester, UK) using a stretch stature method. Body weight and fat mass were measured using a bioelectrical impedance scale (TBF-300, TANITA, Cranela, UK) to the nearest 0.01kg.

***Ad libitum* Buffet Meals**

This study offered *ad libitum* buffet meals to the participants in order to provide them an opportunity to self-select from a large variety of normal every day food, and to ensure that the voluntary food intake were not constrained by the choice or quantity. *Ad libitum* buffet meals consisted of a wide variety of standardised foods and were presented about 3 times in excess from what participants were expected to consume. In the *ad libitum* breakfast, a variety of breakfast cereals (corn flakes, fruit & fibre or coco pops), milk (semi-skimmed, skimmed and whole cream), croissants, jam (apricot, mixed fruit jam, black-current), butter, juice (apple and orange) and fruits (apple, banana, pineapple and grapes) were offered. The *ad libitum* lunch included two filled white bread sandwiches, two filled whole meal bread sandwiches, mixed leaf salad, yogurt (plain, flavoured), fruits (apple banana, grapes) and juice (apple, orange, and blackcurrant). In the *ad libitum* dinner, participants selected the meal of their choice from the cafeteria at the Yorkhill Hospital. This was then served with fruits (apple, banana, grapes and pineapple), yogurt (plain, flavoured) and juice (apple, orange, and blackcurrant) (Figure 4.2). Before offering meals to the participants, all foods were weighed and recorded by the researcher, at the end of each meal the leftovers were weighed and recorded again to calculate the energy intake. The total food intake during the *ad libitum* buffet breakfast, lunch, dinner, and the left over after the intake was weighed with an electronic kitchen scale, accurate to 1g.

Throughout the trial water was available *ad libitum*. During *ad libitum* meals reading, listening music and watching television were not allowed as all these activities might influence the food intake (de Castro 2000). The *ad libitum* buffet meals served during HENSD and PLACEBO trials were identical, containing the same energy and macronutrient contents, and provided a variety of carbohydrate, protein and fat. In order to eliminate the portion related cues the food offered was cut into smaller pieces. Additionally all the *ad libitum* meals were served in standardised settings for example, scheduling meals at the same time, on the same table, and the same type of food was served in the same coloured dishes to avoid any bias in the eating behaviour. The participants were allowed 30 minutes to consume their meals. The participants were given advice to eat according to their appetite until they are comfortably full and satisfied. The participants were left alone during the consumption of *ad libitum* buffet meals, in order to curtail any potential impact of the presence of the other person on the feeding behaviour, as the presence of observers may induce subjects to discontinue eating (Herman, Roth *et al.* 2003, Herman and Polivy 2005, Hetherington, Anderson *et al.* 2006). The participants were not aware of the actual purpose of *ad libitum* buffet meals (measurement of food intake) and were instead told that the biomarkers of blood will be investigated for the food consumed. This approach avoids the potential bias and conscious eating that might occurs if the participants were aware of the reality that the consumption of food was being monitored (de Castro 2000).



Figure 4.2 *Ad libitum* buffet breakfast, lunch and dinner

Dietary Intake Analysis

The energy intake was measured by calculating the weight of all the foods served and left over from the *ad libitum* breakfast, *ad libitum* lunch and *ad libitum* dinner in the metabolic suite. Then the data was analysed by using the dietary software Windiets 2005 (The Robert Gordon University, Aberdeen, Scotland, UK) by two researchers and average values of each of their results was taken into account. These values included the energy and macronutrient intake during *ad libitum* breakfast, *ad libitum* lunch, *ad libitum* dinner and total intake of all the meals. Energy and macronutrient intake during four days of dietary record before and after the provision of a drink was also analysed by two researchers.

Appetite Profile

The appetite profile of the participants was assessed by using a validated Visual Analogue Scale (VAS) questionnaires (100 mm) throughout the trial (Flint, Raben *et al.* 2000). The VAS were anchored with negative feeling words, “not at all” at one end and a positive feeling word “extremely” at the other end. Participants were instructed to mark the horizontal line with a vertical line at a point that most accurately reflects their feeling of hunger, fullness, satiety, desire to eat and prospective food consumption at that time.

Plasma preparation and Blood Analysis

Venous blood samples were collected into a 10 ml ethylenediamine tetraacetic acid (EDTA) Vacutainer tube (BD Vacutainer Systems, Plymouth, UK). Blood samples were centrifuged at 4⁰C, 3000 rpm for 15mins in a refrigerated centrifuge. After centrifugation the plasma supernatant was aspirated and frozen at -80⁰C until analysed. Blood samples were used for the analysis of plasma lipids, insulin, and glucose concentrations.

The analytical measurements of plasma lipid concentrations of the assay were carried out by Roche Cobas Mira (Horibra ABX, Montpellier, France) through

a calorimetric method using commercially available kits (ABX Pentra). The precision and accuracy of the assays were monitored by quality controls (Roche Diagnostics GmbH, Mannheim, Germany. Randox Laboratories Limited Co., Antrim Ireland, Wako Chemicals GmbH, Germany). The values for plasma TAG, HDL-cholesterol and total cholesterol were measured with a calorimetric method. LDL-cholesterol concentration was calculated by using a standardised formula (Friedewald, Levy *et al.* 1972);

$$(\text{Total cholesterol} - \text{HDL cholesterol} - \text{Total triglyceride}) / 2.19$$

Blood glucose analysis was performed on an automated Roche Cobas Mira (Horibra ABX, Montpellier, France) with co-efficient of variation < 3%. Quantitative insulin analysis was performed by using the commercially available Enzyme-linked immunosorbent assay (ELISA) kit (Mercodia AB, Uppsala Sweden). For analysis inter-assay and intrassay coefficient of variance were 3.2% and 3.4%.

The homeostasis model assessment of insulin resistance (HOMA_{IR}) was used to measure the insulin resistance. HOMA_(IR) score calculate an estimated insulin resistance by using the following formula (Matthews, Hosker *et al.* 1985):

$$\text{HOMA}_{(IR)} = \text{Fasting glucose} * \text{Fasting insulin} / 22.5$$

Statistical Analysis

All the data was analysed using Minitab16 statistical software (Minitab Inc., State College, Pennsylvania). The normality of the data was determined by using the Anderson-Darling test.

For the pre-breakfast, pre-lunch, and pre-dinner appetite responses, the time averaged area under the appetite measure versus time curve (AUC) was used as a summary measure. The AUCs for TAG, insulin and glucose vs. time

curves were calculated using time averaged values for pre-lunch, pre-dinner and over the whole postprandial periods. Then a paired t-test was used to detect any statistical difference between the PLACEBO and the HENSD trials. Any significant differences in fasting plasma TAG concentration, total-, HDL-, LDL- cholesterol, insulin and glucose were also analyzed through a paired t-test. The energy and macronutrient intake was recorded for four days of supplementation and on the day following five days of supplementation, results were compared using a paired t-test. As the body weight and fat mass data was not distributed normally, a Mann-Whitney test was performed. All the data obtained was presented as means \pm SE, significance was set at $P < 0.05$.

4.3 Results

Energy Intake and Appetite

The average daily energy intake (MJ) and the percentage energy intake from carbohydrate (CHO), protein and fat during four days of supplementation with and without addition of the energy and macronutrients provided by HENSD and PLACEBO is presented in (Table 4.2).

The average daily energy intake during four days of supplementation without the energy provided from a supplement was significantly lower in HENSD as compared to the PLACEBO trial. The average daily energy intake after consumption of a drink in the evening meal was significantly lower in HENSD than PLACEBO trial. This may reflect that an immediate compensation happens after the intake of HENSD due to which energy intake during the evening meal was lower in HENSD trial. While before the provision of supplement drink, no significant difference was found in the energy intake during four days of supplementation. During HENSD supplementation, the suppression of energy intake was equivalent to $43 \pm 19\%$ (1.07 ± 0.47 MJ) of energy provided by the supplement. However, during four days of supplementation the average total energy intake including energy provided by the drink was significantly higher in HENSD (HENSD, 9.2 ± 0.3 MJ;

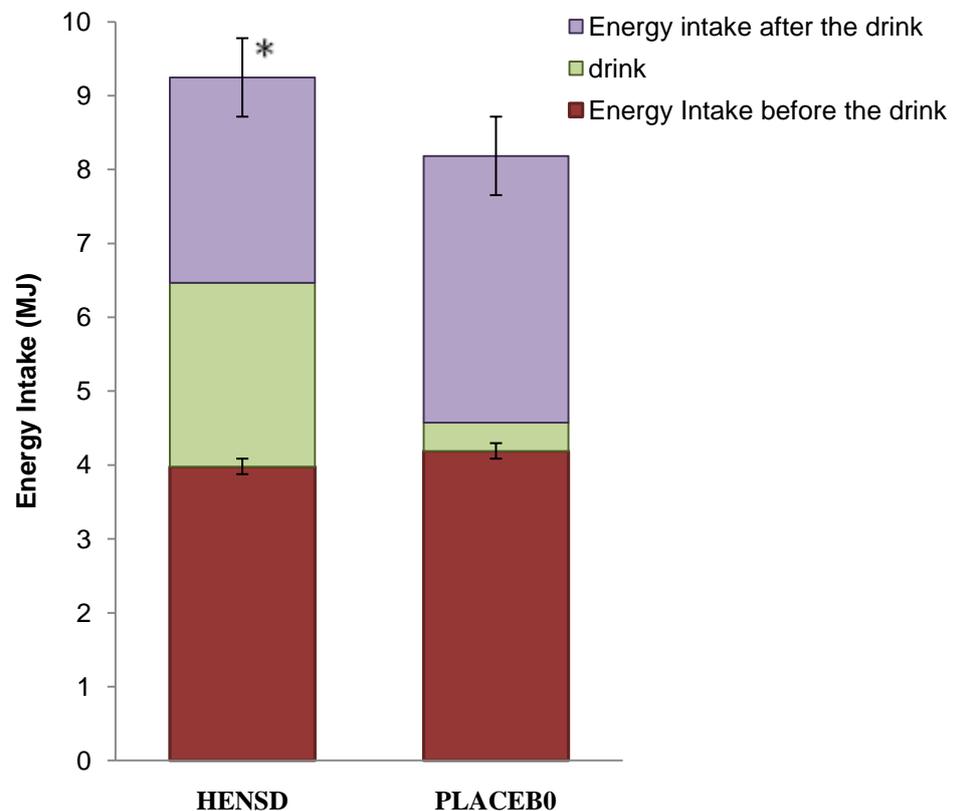
PLACEBO, 8.2 ± 0.4 MJ, $P=0.03$) than PLACEBO trial. The averaged daily energy intake (MJ) total, before and after provision of the drink during four days of supplementation are shown in (Figure 4.3).

Table 4.2 Averaged daily energy intake (MJ) and macronutrient intake during 4 days of supplementation with and without energy and macronutrients provided by HENSD and PLACEBO drinks. Values are presented as Mean \pm SE (n=21)

	HENSD	PLACEBO
Without Drink		
Energy Intake (MJ)	$6.8 \pm 0.3^*$	7.7 ± 0.3
Carbohydrate (g)	217 ± 11	237 ± 13
Protein (g)	$57 \pm 3^{**}$	70 ± 4
Fat (g)	61 ± 6	70 ± 4
Energy from CHO (%)	54 ± 3	51 ± 3
Energy from Fat (%)	34 ± 3	34 ± 2
Energy from Protein (%)	14 ± 1	15 ± 1
With Drink		
Energy Intake (MJ)	$9.2 \pm 0.3^*$	8.2 ± 0.4
Carbohydrate (g)	$286 \pm 11^*$	249 ± 13
Protein (g)	$69 \pm 3^*$	79 ± 4
Fat (g)	$91 \pm 6^{**}$	71 ± 4
Energy from CHO (%)	52 ± 2	51 ± 3
Energy from Fat (%)	37 ± 2	33 ± 2
Energy from Protein (%)	$12 \pm 1^*$	17 ± 1

Significantly different ($P < 0.05$, ** $P > 0.001$) from HENSD*

During four days of supplementation, the daily average consumption of carbohydrates (CHO) and fat without the supplementation drink was not significantly different between PLACEBO and the HENSND trial. When the CHO and fat provided by the supplementation drink was added then the consumption of carbohydrate and fat was significantly higher in the HENSND compared to the PLACEBO trial. The consumption of protein from the diet *alone* and after addition of protein from the supplementation drink was significantly higher in the PLACEBO trial days.



*Significant difference in energy intake after drink ($P < 0.05$)

Figure 4.3 Averaged total daily energy intake (MJ), including energy intake before and after supplementation, and energy provided by supplements in HENSND and PLACEBO trials.

The energy intake (MJ) and percentage energy intake from macronutrients during the *ad libitum* breakfast, lunch, and dinner and total energy intake

during experimental trial conducted on the day following five days of supplementation are given in Table 4.3. Energy intake during *ad libitum* breakfast, *ad libitum* lunch, *ad libitum* dinner and total energy intake was not significantly different between the HENSD and the PLACEBO trials. Similarly, no significant difference was found in the carbohydrate, fat and protein intake during *ad libitum* breakfast, lunch and dinner between HENSD and PLACEBO trials.

Table 4.3 Energy Intake (MJ) and energy intake from macronutrients during *ad libitum* breakfast, lunch, and dinner on the day following five days of supplementation. Values are presented as Mean \pm SE (n=21)

Meal		HENSD	PLACEBO
Breakfast	Energy Intake	3.19 \pm 0.21	3.19 \pm 0.18
	CHO (g)	128 \pm 7	129 \pm 7
	Protein (g)	17 \pm 1	17 \pm 1
	Fat (g)	23 \pm 3	24 \pm 3
Lunch	Energy Intake	2.65 \pm 0.21	2.52 \pm 0.19
	CHO (g)	81 \pm 7	77 \pm 6
	Protein (g)	28 \pm 3	27 \pm 3
	Fat (g)	24 \pm 3	22 \pm 2
Dinner	Energy Intake	2.97 \pm 0.24	2.90 \pm 0.25
	CHO (g)	100 \pm 10	97 \pm 8
	Protein (g)	23 \pm 2.4	22 \pm 2.5
	Fat (g)	26 \pm 3	27 \pm 3
Total	Energy Intake	8.93 \pm 0.53	8.75 \pm 0.49
	CHO (g)	310 \pm 20	301 \pm 15
	Protein (g)	68 \pm 5	66 \pm 5
	Fat (g)	72 \pm 5	73 \pm 6

Results obtained from the data on the subjective measures of appetite sensation over time, using VAS questionnaires between the HENSD and the PLACEBO

trial on the day following five days of supplementation are shown in (Table 4.4; Figure 4.4).

Time-averaged area under the curve for appetite measurement over the time was used to detect statistical differences between HENSD and the PLACEBO trials. Time-averaged area under the curve was calculated for values on the appetite responses over the time obtained during pre-breakfast (0-30min), pre-lunch (30-120 min) and pre-dinner (180-270 min) period. No significant difference was detected in any of the appetite responses during the pre-breakfast, pre-lunch, pre-dinner period on the day following five days of supplementation between HENSD and the PLACEBO trials.

Plasma Glucose, Insulin and Lipids

Fasting and postprandial plasma glucose, insulin and triglycerides (TAG) concentrations in the HENSD and the PLACEBO trials are presented in (Figure 4.5 and Table 4.5).

Fasting plasma concentration of insulin ($P= 0.01$) and HOMA_(IR) ($P= 0.01$) were significantly higher in HENSD as compared to the PLACEBO trial. Fasting plasma glucose levels were not significantly different between HENSD and the PLACEBO trials. The mean concentration of plasma glucose and insulin evaluated as time averaged areas under concentration versus time response during the postprandial period (pre-lunch and pre-dinner period), were not significantly different between HENSD and the PLACEBO trial.

No significant differences were detected in fasting plasma concentration of TAG, total-, HDL- and LDL-cholesterol between HENSD and the PLACEBO trials. Postprandial TAG concentrations were also not significantly different in the HENSD and the PLACEBO trials.

Table 4.4: Time-averaged area under the curve (AUC) for responses of hunger, satiety, fullness, prospective food consumption and desire to eat during pre-breakfast, pre-lunch and pre-dinner period. Data is presented as Mean \pm SE.

	HENSD	PLACEBO
Hunger (mm)		
Pre-breakfast	38 \pm 3	38 \pm 3
Pre-lunch	27 \pm 3	29 \pm 3
Pre-dinner	20 \pm 3	22 \pm 3
Satiety (mm)		
Pre-breakfast	57 \pm 3	54 \pm 3
Pre-lunch	71 \pm 3	68 \pm 3
Pre-dinner	78 \pm 3	76 \pm 3
Fullness (mm)		
Pre-breakfast	55 \pm 3	55 \pm 2
Pre-lunch	70 \pm 3	68 \pm 3
Pre-dinner	79 \pm 3	77 \pm 3
Prospective food consumption (mm)		
Pre-breakfast	59 \pm 2	56 \pm 2
Pre-lunch	68 \pm 3	67 \pm 2
Pre-dinner	77 \pm 3	73 \pm 3
Desire to eat (mm)		
Pre-breakfast	39 \pm 3	42 \pm 3
Pre-lunch	29 \pm 3	32 \pm 3
Pre-dinner	21 \pm 3	24 \pm 3

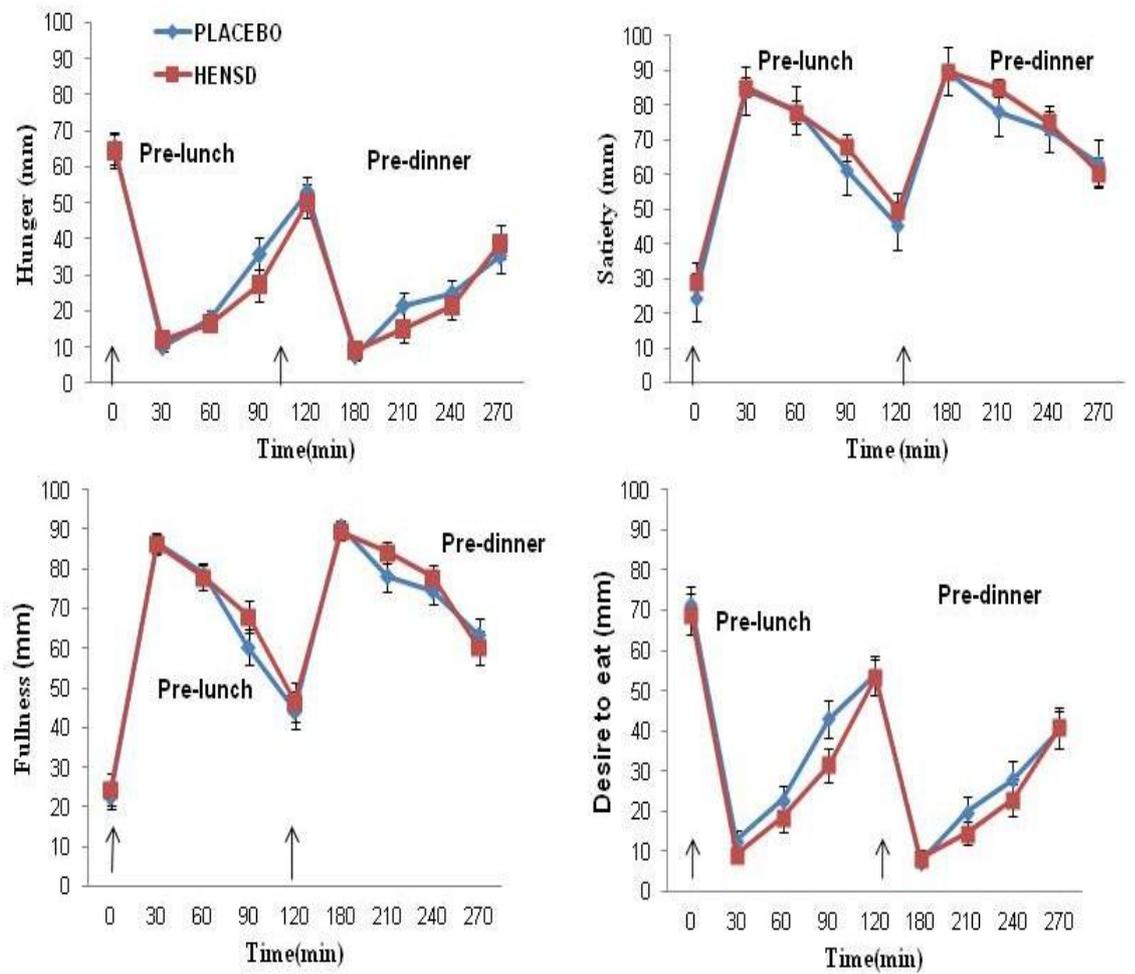


Figure 4.4: Responses of hunger, satiety, fullness and desire to eat on the trial day.

Ad libitum breakfast and ad libitum lunch were provided at 120 and 270 minutes respectively.

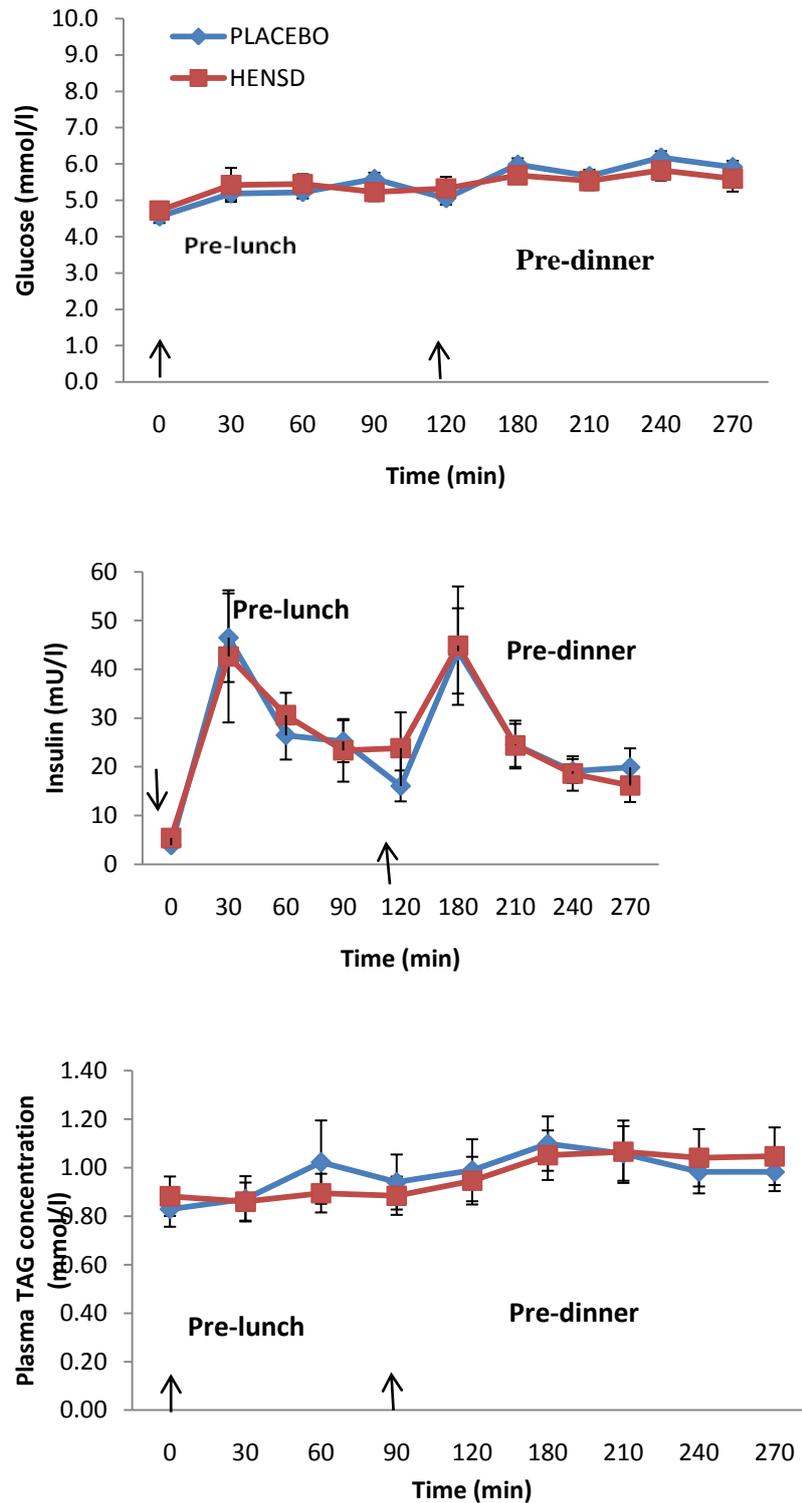


Figure 4.5 Plasma concentration of glucose, insulin and triglycerides (TAG) in the fasted state (0 min) and postprandial period in HENSD and PLACEBO trials. Values are expressed as Mean \pm SE

Table 4.5: Fasting plasma insulin, glucose, triglycerides (TAG), total-, HDL-, LDL-cholesterol and postprandial responses of glucose, insulin and TAG. Values are expressed as Mean \pm SE

	HENSD	PLACEBO
Fasting Insulin (mU/L)	5.19 \pm 0.56*	3.47 \pm 0.27
Fasting Glucose (mmol/L)	4.72 \pm 0.19	4.55 \pm 0.29
HOMA _(IR)	1.08 \pm 0.13*	0.68 \pm 0.05
Fasting Total Cholesterol (mmol/L)	5.56 \pm 1.13	5.30 \pm 1.04
Fasting HDL-Cholesterol (mmol/L)	1.76 \pm 0.31	1.62 \pm 0.29
Fasting LDL-Cholesterol (mmol/L)	3.39 \pm 0.84	3.23 \pm 1.01
Fasting TAG (mmol/L)	0.88 \pm 0.30	0.83 \pm 0.27
Insulin AUC (mU/L)	24.22 \pm 3.67	26.24 \pm 4.11
Glucose AUC (mmol/L)	5.46 \pm 0.21	5.51 \pm 0.34
TAG AUC (mmol/L)	0.97 \pm 0.35	0.99 \pm 0.38

*Values are presented as Mean \pm SE (n=11). *Significantly different (P<0.05) from HENSD. Postprandial concentrations are presented as AUCs (time-averaged areas under concentrations vs. time curves).*

Body weight and Fat Mass

The body weight and fat mass measurements were taken before and after five days of supplementation in HENSD and the PLACEBO trials. There was no significant difference in the magnitude of change in the body weight (mean difference 0.1 (95% confidence interval -0.30 to 0.40), P = 0.67) and fat mass (mean difference 0.4 (95% confidence interval -0.50 to 1.40), P = 0.37) between the HENSD and the PLACEBO trials.

4.4 Discussion

This study aimed to measure the immediate impact of HENSD provided to slim healthy women in the evening, for five consecutive days, on energy intake, and on appetite sensations and the energy intake on the consecutive day. Impact of supplementation on cardio-metabolic risk factors was also assessed in this study. The obtained data confirmed that consumption of HENSDs for five consecutive days in the evening induced immediate compensation for energy provided and that the modifying impact of supplement on energy intake was short-lived. Furthermore, HENSD supplementation for five consecutive days in the evening significantly increased the total energy intake regardless of partial compensation. It demonstrated that HENSD supplementation for five consecutive days increased the fasting plasma insulin concentrations and HOMA_(IR) but have no impact on the fasting plasma lipids, postprandial lipemia, insulinaemia and glycaemia.

During four days of the supplementation with HENSD the averaged total energy intake including the energy provided from the drink was significantly higher (1.06 ± 0.47 MJ). Increased energy with supplementation has been observed in other studies (Lauque, Arnaud-Battandier *et al.* 2000, Stratton and Elia 2000, Norman, Kirchner *et al.* 2008, Stratton, Stubbs *et al.* 2008, Manders, de Groot *et al.* 2009, Stratton and Elia 2010, Hubbard, Elia *et al.* 2012, Huynh, Devitt *et al.* 2014) and in our previous study we have also found that HENSD supplementation is beneficial for increasing the energy intake (Fatima, Gerasimidis *et al.* 2013). Previous studies have demonstrated that energy provision by the oral nutritional supplements is additive to that taken from habitual food, which produces a mean increase in the energy intake equivalent to 67% of the energy from the supplement consumed. However, the proportion of energy provided by supplement that was additional to the habitual food intake varied considerably from an average increase of 79% in

patients with BMI $< 20\text{kg/m}^2$ as compared to 28% increase in those with BMI $> 20\text{kg/m}^2$ (Stratton and Elia 2000).

During five days of supplementation, acute suppression of energy intake immediately after HENSD supplementation has been observed in the evening meal. We observed that 43% of the energy provided by HENSD was compensated by eating less during the evening meal after HENSD supplementation. Previous studies have also reported energy compensation (Mattes 2006, Stratton, Stubbs *et al.* 2008, Mattes and Campbell 2009, Almiron-Roig, Palla *et al.* 2013) and in our previous study we have found that exactly the same 43% of energy was compensated by eating less during breakfast after HENSD supplementation (Fatima, Gerasimidis *et al.* 2013). However, it is worth mentioning that a significant decrease in the energy intake of the evening meal was seen during four days after consumption of the HENSD, no differences in the energy intake during the *ad libitum* breakfast, lunch and dinner was observed on the consecutive day.

This suggests that the provision of the HENSD in the evening for five consecutive days suppressed intake only immediately during the evening meal, and did not suppress appetite on the following day. Therefore, it can be concluded that the appetite suppressive action of HENSD is short lived and induced only an acute compensation, as it did not impact energy intake the next day. The findings of this study are consistent with our previous study (Fatima, Gerasimidis *et al.* 2013) in which we observed partial compensation for the energy provided by the HENSD due to acute appetite suppression, which was only short lived as it occurs only in the meal that followed the HENSD consumption. In this study, we did not measure appetite hormones but in our previous study we found that energy intake was related to appetite. Appetite measures reflect better energy intake rather than appetite hormones. Measuring the appetite hormones may not be quantitatively related to satiety. (Delzenne, Blundell *et al.* 2010). Therefore, in this study for this reason and

due to appetite hormone measurements being very expensive we did not measure appetite hormones.

No suppression of energy intake on the next day after five days of HENSD supplementation coincides with the measurement of appetite sensations during the pre-breakfast, pre-lunch and pre-dinner period that too demonstrated no significant difference in the appetite sensations of hunger, satiety, fullness, prospective food consumption and desire to eat.

We appreciate that the measurement of the energy intake in the dinner during five days of supplementation was conducted at home as representative of actual eating behavior. Later on, subjects remained in the laboratory for further measurement of the energy intake during *ad libitum* breakfast, lunch and dinner the following day. Evidence from the previous studies have shown that the correlation was significantly high between the energy intake evaluated from 4 day *ad libitum* energy intake in the laboratory and 4 day dietary records kept at home. In that study, no difference in body weight was observed during either condition, which suggests that food consumed in a laboratory setting under controlled conditions is a reasonable approximation of the energy intake measured under free living conditions (Obarzanek and Levitsky 1985). Moreover, in our study the free-living conditions also provided time flexibility for dinner, which varies considerably between the individuals and is not possible under the laboratory conditions.

In our study, we found no difference in the magnitude of change in body weight and fat mass between HENSD and the PLACEBO trials. Here it is worth mentioning that the duration of supplementation lasted only for 5 days, which was expected to increase the weight of the body by 300 grams only. That value is within the range of the mistake of the measurements moreover 43% compensation was also observed during HENSD supplementation. Increased body weight may be observed if HENSD supplements were provided for a longer duration.

The present study indicated that five days of positive energy balance induced by the HENS supplement does not have an acute impact on fasting and the postprandial lipid profiles. The results in this study are corroborated with a study conducted by Bellou *et al* (2013), which demonstrated that the acute positive energy balance achieved by hypercaloric feeding with a dietary energy surplus of 2.91 ± 0.32 MJ from a mixed diet does not affect VLDL-TAG concentration and metabolism in healthy women (Bellou, Maraki *et al.* 2013). Likewise a study by Smith *et al* (2013) found that only one day of hypercaloric feeding (total energy intake exceeded the total daily energy requirement by 30% (from mixed diet) had no impact on VLDL-TAG secretion and plasma clearance rates, VLDL-apoB-100 plasma clearance rate, and free fatty acid rate of appearance in plasma in overweight/obese men (Smith, Magkos *et al.* 2013). Another study by Sobrecases *et al* (2010) demonstrated that a short term hypercaloric diet in healthy male subjects with a mixture of fat and fructose combined resulted in no significant changes in VLDL TAG concentrations (Sobrecases, Lê *et al.* 2010).

However, studies have also reported the effect of hypercaloric feeding on VLDL-TAG metabolism but they have examined the impact of hypercaloric feeding with increase in a specific macronutrient like carbohydrate or fat (Hill, Peters *et al.* 1990, Minehira, Bettschart *et al.* 2003, Faeh, Minehira *et al.* 2005, Brøns, Jensen *et al.* 2009, Ngo Sock, Lê *et al.* 2010, Sobrecases, Lê *et al.* 2010). These studies demonstrated that excess carbohydrate intake mainly fructose (Minehira, Bettschart *et al.* 2003, Faeh, Minehira *et al.* 2005, Sobrecases, Lê *et al.* 2010) increased VLDL-TAG concentrations, whereas excess fat intake decreased the VLDL-TAG concentrations (Bortolotti, Kreis *et al.* 2009, Brøns, Jensen *et al.* 2009, Sobrecases, Lê *et al.* 2010). However, overfeeding with a mixed diet (balance diet containing all macronutrient) had no impact on VLDL-TAG concentrations (McDevitt, Bott *et al.* 2001, Sobrecases, Lê *et al.* 2010, Bellou, Maraki *et al.* 2013, Smith, Magkos *et al.* 2013). Findings from the overfeeding studies using a mixed diet are in

agreement with the results of our study. We have induced a positive energy balance by provision of HENSD with the energy surplus of 2.49 MJ (46% of energy from carbohydrate, 46% from fat and 8% from protein) showing that the positive energy balance achieved by increased caloric intake in a way mimicking free-living conditions (over consumption of all macronutrients) has no impact on fasting and the postprandial lipid profile.

The results from our study demonstrated a significant increase in fasting insulin concentrations and HOMA (IR) after only five days of overfeeding with HENSD supplementation. This confirmed that insulin resistance develops very rapidly during a period of positive energy balance irrespective of the macronutrient composition of the diet. However, we found no impact of HENSD supplementation on fasting plasma glucose concentrations. Our finding is in accord with previous studies showing that one day of moderate mixed meal overfeeding (30% excess energy intake) significantly increased the plasma insulin concentrations, but had no impact on the plasma glucose concentrations (Smith, Magkos *et al.* 2013). Our results are also in line with a recent study performed by Bellou *et al.* 2013 which showed that hypercaloric feeding with a dietary energy surplus of 2.91 ± 0.32 MJ increased plasma insulin levels (Bellou, Maraki *et al.* 2013). The findings from our study suggest that the deleterious effects of positive energy balance achieved by overfeeding on insulin homeostasis manifests rapidly. These results indicate that insulin resistance is sensitive to acute (5 days) moderate increase in the net energy balance, whereas lipid/ TAG metabolism are not. This increase in fasting insulin levels could be simply because of the higher carbohydrate load ingested during the HENSD supplementation, the mechanisms for this finding needs to be investigated further.

In our study, we found that acute positive energy balance did not impact on plasma glucose concentrations. Our findings are consistent with the findings from other overfeeding studies. Overfeeding studies have demonstrated that during a hypercaloric state, fasting glucose concentrations did not increase

significantly, while fasting insulin secretions increased (Brands, Swat *et al.* 2013, Smith, Magkos *et al.* 2013). As fasting glucose concentrations are mostly determined by endogenous glucose production (Turner and Holman 1976), it might be possible that an increase in portal insulin inhibits an increase in the glucose concentration by reducing the glucose output. The stable glucose concentrations may represent a new steady state at the expense of higher basal insulin secretion (Brands, Swat *et al.* 2013).

The strengths of this study include the participant's attendance, as none of the participants were eliminated due to the non-compliance of supplementation. All participants consumed both HENSD and the PLACEBO supplements in the presence of the researchers. This study being a blinded randomized control trial, it was expected that the participants would not be able to identify whether they were taking the supplement or the PLACEBO. This is endorsed by the fact that 61% of participants were unable to correctly guess about the type of drink they were given. This also ensures that there was no psychological influence on the food choices during the four days of dietary record.

The results of this study are highly reliable and accurate as on the day of main trial the energy intake was measured under strict laboratory conditions and all food stuffs were measured by the researchers. Two researchers analyzed the energy and macronutrient intake and the mean of the combined values were used for statistical analysis which further increased the validity of the results. Moreover the under and over-reporters were excluded from the study by using Goldberg cut offs, which was also confirmed by Johnson (Johnson 2002). Since it is implausible that habitual energy intake could be $<1.35X$ BMR or $>2.0 X$ BMR (Goldberg, Black *et al.* 1991). The reported four days dietary records of the participants were not included for statistical analysis if the reported energy intake was greater than basal metabolic rate (BMR) multiplied by 2.0 (over-reporter) or less than BMR multiplied by 1.35 (under-reporter). On this basis, dietary records of two participants were excluded, based on the suspected over-reporting (one participant) and under-reporting (one

participant). Moreover, this study was a randomised crossover study, in which each participant acted as her own control thus minimising the number of exterior confounding factors, which is vital while considering the results as the impact of confounding factor are considerably reduced and the findings are weighted more significantly than to other study designs.

Like other studies, our study has several limitations. Firstly, we studied only healthy, lean young females; therefore, it remains unclear that whether similar responses to the HENSD supplementation for a few days would occur in other study populations (i.e. healthy lean men, obese men, obese women and children). Thus, we cannot generalize our results for obese women, healthy and obese men, or hypertriglyceridemic subjects. Secondly, the positive energy balance achieved by the HENSD supplementation was relatively moderate and only for five days, therefore it remains unclear whether a longer duration of supplementation or different degrees of supplementation would result in similar responses or not. Furthermore, on day six, for the dinner, the participants had a free choice for their meal from the cafeteria of Yorkhill Hospital. Consequently, all twenty-three participants consumed different food with different composition and consistency. This introduced some limitation to the accuracy of the analysis during dinner as energy composition and macronutrient intake of the dinner was only estimated and was not hundred percent. Although this was expedient, as cooking facilities were not available at Yorkhill Hospital and it was necessary to provide participants the food which they could enjoy.

Future research is required to investigate the impact of longer duration of supplementation or different degrees of supplementation with HENSD on lipoprotein, glucose and insulin metabolism in healthy and obese subjects. The detrimental impact of continuous supplementation should also be considered; as an acute short-term positive energy balance was found to enhance insulin resistance, which if unchecked may predisposes to type 2 diabetes mellitus and

CVD even in malnourished or very thin people (Martin, Warram *et al.* 1992, Haag and Dippenaar 2005, Reaven 2012).

4.5 Conclusion

In summary, the results from our study confirm that regardless of compensation the provision of HENSD can be expected to increase the daily energy intake most likely because compensation for the energy provided is immediate, short lived and has no impact on the appetite and the energy intake during the next day. A positive energy balance induced by the HENSD supplementation can be expected to reduce insulin sensitivity but have no impact on the fasting lipids and the postprandial lipemia, insulinaemia and glycaemia.

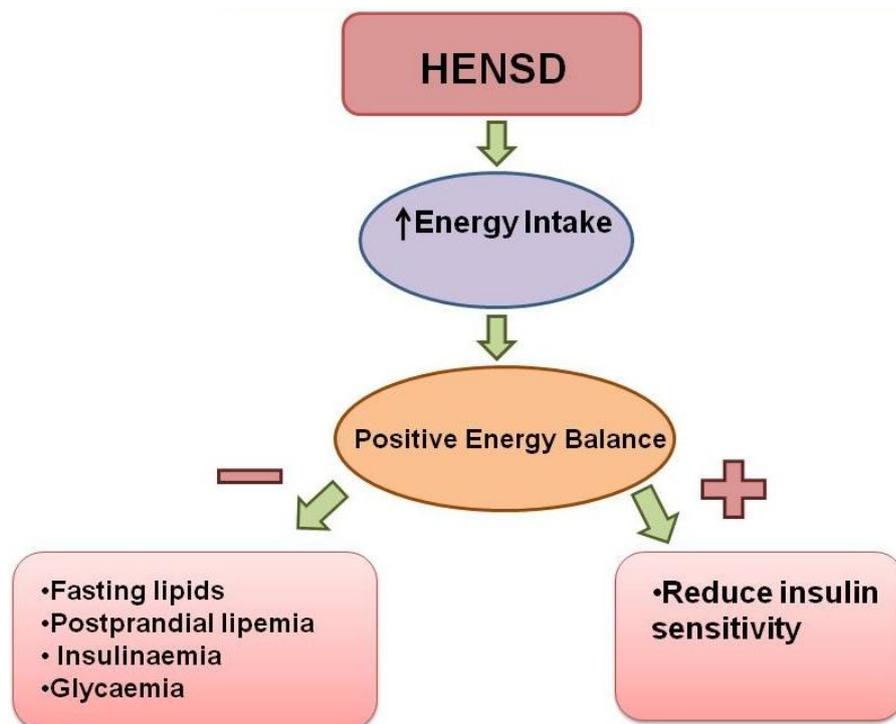


Figure 4.6 Schematic presentation of results showing that the provision of HENSD supplements induces positive energy balance which is expected to reduce insulin sensitivity but have no impact on postprandial lipaemia, insulinaemia and glycaemia

5 Limited effectiveness of Solid Ready-To-Eat and a Liquid Ready-To-Drink Supplements in Mild to Moderate Underweight Children from Pakistan

5.1 Introduction

Malnutrition is, potentially, a preventable condition and is the main contributor to child mortality and the overall burden of global disease (Black, Allen *et al.* 2008, Hendricks 2010). In developing countries childhood malnutrition is associated with numerous socio-economic and environmental factors such as poverty, poor hygienic practices, lack of sanitation, recurrent infections, poor health and large family size (Golden 2009, Babar, Muzaffar *et al.* 2010). Malnutrition is a highly pervasive and damaging condition in low and middle income countries (Black, Allen *et al.* 2008). Not surprisingly in low income countries 112 million out of 556 million children (20%) under 5 years of age are underweight and 36 million (6.4%) are suffering from moderate wasting (Golden 2009, Michaelsen, Hoppe *et al.* 2009). Malnutrition is the most prevalent health issue in South Asian countries which are predominantly classified as middle income countries. It is the most prevalent public health issue among children under five years of age (Hirani 2012). It is documented that more than 50 percent of the world's malnourished children reside in Pakistan, India and Bangladesh (Gillespie and Haddad 2003, Hirani 2012).

As it has been discussed in Chapter 1, children who are exposed to insufficient food intake and recurrent infections can easily enter into a vicious cycle of increased susceptibility to infections, weight loss, stunting and ever worsening nutritional status. Food interventions aim to reverse inadequate food intake. There are different strategies to address malnutrition with prepared foods for the nutritional recovery of malnourished children for example providing lipid-based nutrient supplements (RTUF) or blended foods to complement the usual diet.

Studies from the published literature suggests that RTUFs are very effective and safe in the treatment of severe malnutrition in children (Briend, Lacsala *et al.* 1999, Collins and Sadler 2002, Dossou, Ndour *et al.* 2003, Sandige, Ndekha *et al.* 2004, Ciliberto, Sandige *et al.* 2005, Ashworth 2006, Brewster 2006, Bhutta, Ahmed *et al.* 2008, Gera 2010). There is evidence to demonstrate that home based management of uncomplicated severe acute malnutrition with RTUF is as effective as F-100, and more effective compared to home based dietary therapies (Gera 2010). Some studies have demonstrated a higher increase in energy intake (808 vs. 573kJ/kg/day) and weight gain (15.6 vs.10.1g/kg/day) achieved by supplementation with RTUF compared to the changes induced by consumption of a traditional high-energy drink based on milk (F-100) (Diop, Dossou *et al.* 2003). A clinical effectiveness trial demonstrated better weight gain (3.5 compared with 2.0 g/kg/day), lower relapse rates (8.7% compared with 16.7%) and lesser rates of cross infection with RTUF (Ciliberto, Sandige *et al.* 2005).

RTUFs have been used to supplement the dietary intake of moderately malnourished children (Kuusipalo, Maleta *et al.* 2006, Defourny, Seroux *et al.* 2007, Matilsky, Maleta *et al.* 2009, Nackers, Broillet *et al.* 2010, Patel, Sandige *et al.* 2011). These studies define moderate malnutrition differently; Nackers *et al.* 2010 defined moderate acute malnutrition as WHM % between 70% and < 80% of the NCHS median and compared RTUF with CSB. Patel *et al.* 2011 defined children at risk of malnutrition as weight-for -height < 85% but > 80% of the international standard and their primary outcomes were recovery and rate of weight gain. Matilsky *et al.* 2009, defined moderate malnutrition as WHZ <-2 but \geq -3, and recovery rates (defined as having WHZ >-2) were compared among three intervention groups i.e. milk/peanut fortified spread (FS), soy/peanut FS and CSB. RTUF have been found to be effective in preventing onset of malnutrition in non-wasted children from the areas of food insecurity (Defourny, Minetti *et al.* 2009, Isanaka, Nombela *et al.* 2009, Isanaka, Roederer *et al.* 2010, Huybregts, Houngbé *et al.* 2012). It has been recommended that nutritional intervention to prevent malnutrition might be

more effective than the curative treatment of malnutrition (Ruel, Menon *et al.* 2008).

A recent review by Lazzerini *et al* 2013 evaluated the safety and effectiveness of specially-formulated food products for children with MAM in low- and middle-income countries. This review included eight randomized controlled trials, enrolling 10,037 children. In this review seven studies compared lipid – based nutrient supplements (LNS) to blended foods, two of the studies compared complementary LNS to blended foods, three studies compared specific blended foods (enriched blended foods CSB++) to other LNS and one study compared enriched blended food CSB++ to other blended foods. When LNS were compared to any blended foods, it significantly improved the number of children who recovered (RR 1.10, 95% CI 1.04-1.16; 6367 children, 5 trials), and reduced the number of non-recovering children (RR 0.53, 95% CI 0.40-0.69; 4537 children, 3 trials). LNS slightly improved the nutritional status of the recovered children with improved weight gain, weight-for-height, and mid-upper arm circumference (MUAC), and this improvement was regarded as modest as it came from moderate quality evidence. However, the comparison between LNS and blended foods did not show significant difference in mortality (RR 0.93, 95% CI 0.54-1.62; 6367 children, 5 trials), progression to severe acute malnutrition (RR 0.88, 95%CI 0.72 -1.07; 4537 children, 3 trials), or the number of defaulters (drop outs) from the nutritional programme (RR 1.14, 95% CI 0.62-2.11; 5107 children, 4 trials).

Only one study reported more children with vomiting when given LNS compared to blended food (RR 1.43, 95% CI 1.11-1.85; 2712 children, 1 trial). Enriched blended foods resulted in similar outcomes to LNS (4758 children, 3 trials) (Lazzerini, Rubert *et al.* 2013). The findings from this review demonstrated that both LNS and blended foods are effective in the treatment of the children with MAM although there are no studies evaluating the impact of improving adequacy and quality of local diet/home diet in the settings where food is available. However, Lazzerini *et al* 2013 also points to the fact

that there are “limitations in the completeness of evidence and its generalizability”

Similarly Lenters *et al* 2013 reviewed five studies investigating the impact of RTUF compared to CSB in moderately malnourished children under 5 years of age. Two of the studies were cluster randomized controlled trials and three were RCTs. This review found no significant difference in mortality and height gain between those children who received RTUF compared to those given CSB. Although, the children in the RTUF group were more likely to recover (RR: 1.11, 95% CI 1.04-1.18) and the non-response rate was also significantly lower in the RTUF group (RR: 0.65, 95% CI 0.47-0.90) compared to the CSB group. Children in RTUF group had a greater gain in MUAC (0.04 mm/day) (MD: 0.04, 95% CI 0.01 - 0.07) and an average weight gain of 0.61 g/kg/d than CSB group.

Although, this difference was statistically significant, it may not be an adequate difference to be clinically important. Lenters *et al* 2013, in their systemic review and meta-analysis observed considerable variations among the trials reviewed and that the effectiveness of overall treatment approaches for MAM was inconclusive. The authors concluded that the evidence generalization must be treated with caution due to gaps in their ability to estimate effectiveness (Lenters, Wazny *et al.* 2013).

The majority of the existing evidence on the use of community based management of uncomplicated severe acute malnutrition has emerged from studies conducted in Africa in emergency settings (Gera 2010). There are no studies from Asia exploring the effectiveness of RTUF and other industrialised foods in the treatment of children with moderate acute malnutrition (MAM), where MAM is most prevalent (Lazzerini, Rubert *et al.* 2013). There is a need to generate more evidence regarding the efficacy of RTUF and other locally available ready to use the supplements in non-emergency settings particularly in a Pakistani context.

Moreover, there is no evidence comparing the impact of RTUF and the proprietary liquid supplements on weight gain in mild to moderate malnourished children between the ages of 5-10 years.

The proposed study aimed:

- To explore the efficacy of RTUF and LRUS in promoting the weight gain and appetite regulation in mild to moderate underweight children between the ages of 5-10 years from Pakistan.

5.2 Material and Methods

Participants

The participants of this study were primary school children between the age of 5-10 years, with a mild to moderate malnutrition weight for age Z score (WAZ) (between -2 and -1 Z score). Before enrolling in the study, the participants underwent a screening visit, which included height and weight measurement. These anthropometric measurements were used to calculate Z-scores using the WHO standards. Children with mild to moderate malnutrition WAZ (between -2 and -1 Z-score) were selected for the study. A detailed health screen questionnaire was filled in by the participant parent/carer regarding participant's health to exclude chronic illness, eating disorders, any specific food intolerance (e.g., peanut allergy) and gastrointestinal operations, which could interfere with the results of the study.

Setting

This study was conducted in the primary school of the district of Abbottabad located in the Hazara region of Khyber Pakhtunkhwa province in Pakistan. Abbottabad is situated 50 kilometres northeast of the capital Islamabad. Recruitment and the follow up for the study were started in June and the intervention was started on the day of recruitment. The eligible children were identified by the researcher, on the basis of anthropometric status.

Anthropometric measurements were taken on day 1, day 14 and day 29, with a window of 2 days after planned date allowing for weekends and holidays.

Study Design

This study was an open labelled randomized controlled trial. The children were randomly allocated by computerized randomization using free software (Research Randomiser) (Urbaniak and Plous 2011) to receive either solid ready to use food (RTUF) or liquid ready to use supplement (LRUS). Height, weight, biceps and triceps skinfolds and mid-upper-arm circumference of the children were measured before the supplementation (baseline), two weeks after initiation of the supplementation and at the end of the supplementation. The children were provided with these supplements in their school for four weeks and were asked to consume the supplements in addition to their habitual diet. The supplements were delivered to the children by the researcher. The children were asked to keep the empty bottles/sachet of the supplements with them after drinking/eating, which were collected by the main researcher the same day in the afternoon in order to check the compliance. Any leftover in the bottle or sachet was also recorded.

The appetite questionnaires were marked by the children before the provision of the first and the last supplement. Visual Analogue Scale (VAS) questionnaires with a line of 100 mm (Flint, Raben *et al.* 2000) were used for measuring appetite sensations of the children before provision of first and last supplement. Furthermore, the parents/carers were requested to attend structured interview regarding the appetite of their child once before the start of supplementation and once at the end of the study. Moreover, the parents/carers were also encouraged to continue the regular habitual diet of their children. The children who were screened (n=128) and did not meet the criteria for recruitment ($WAZ > -1 SD$) were measured for the height and weight again after four weeks and acted as a control group (CG). The questions were also asked from the parents/carers of the children to obtain the information about their socio-demographic characteristics. The children who

were provided with the supplements were followed after mean (\pm SD) 7.13 ± 3.14 months when they were off the supplements.

Interventions

The energy, micronutrient and macronutrient contents of RTUF and LRUS are presented in Table 5.1. RTUF is a mixture of peanut, sugar, milk powder, minerals, vitamins and vegetable oil. It does not require cooking and is a microbiologically safe product and can be stored in the household conditions for several months (Isanaka, Nombela *et al.* 2009, Ali, Zachariah *et al.* 2013). RTUF and LRUS looked different; therefore, the study was not blinded. LRUS (Fortini, Strawberry, Nutricia) was a strawberry flavoured ready to drink sip feed available in 200 ml bottles with a flexible straw, and RTUF (Plumpy' Nut; Nutriset, Malaunay, France) was individually packaged in airtight alu-foil sachet, looked like a thick paste and tasted like a slightly sweeter peanut butter. Daily the children in RTUF group were provided with one sachet of RTUF (92 g, 500 kcal/d), and the children in LRUS group were provided with two bottles of LRUS (60ml of LRUS was removed daily from one of the two bottles in order to provide nearly 500 kcal/ day).

Measurements

All the measurements were performed by the same researcher. For each skinfold measurement two measurements were taken and the mean of both were considered for the accuracy of results.

Height Measurement

Height was measured with a portable stadiometer (Seca, Leicester, UK) using the stretch stature method. The stature is the maximum distance from the ground to the uppermost point of the skull when the position of head is held in Frankfort plane position (Marfell-Jones, Stewart *et al.* 2006). The measurements were performed to the nearest 0.01m. The same height

stadiometer was used throughout the study and the same researcher performed all the measurements.

Table 5.1 Comparison of nutritional composition of RTUF and LRUS

	Per 100 g		Distributed Per day	
	RTUF	LRUS	RTUF (92 g)	LRUS (340ml)
Energy (kcal)	545	150	500	510
Energy(kJ)	2281	630	2093	2142
Macronutrients				
Protein (g)	13.6	3.4	12.5	11.56
Carbohydrates (g)	35.0	18.8	32.2	63.92
Fat g	35.7	6.8	32.9	23.12
Minerals				
Sodium (mg)	189	67	<189	227.8
Potassium (mg)	1111	140	1051	476
Calcium (mg)	320	84	276	285.6
Phosphorus (mg)	394	75	276	255
Magnesium (mg)	92	17	84.6	57.8
Zinc (mg)	14	1.5	12.9	5.1
Iron (mg)	11.5	1.5	10.6	5.1

RTUF, solid ready to use food; LRUS, liquid ready to use supplement

Weight Measurement

Weight was measured with a regularly calibrated electronic scale (Seca 861, Hamburg, Germany). The weight of the children was measured wearing light weight clothes without shoes. Prior to the measurements extraneous clothing were removed. The children stood with both feet placed flat on the balance and arms positioned laterally along the side of the body. The measurements were performed to the nearest 0.1kg.

Mid upper arm circumference (nearest mm)

Mid upper arm circumference was measured with a mid upper arm circumference (MUAC) measuring tape which were colour coded (green, yellow and red) to indicate nutritional status. The measurements were performed on the left upper arm while the arm was relaxed and hanging down the side of the body.

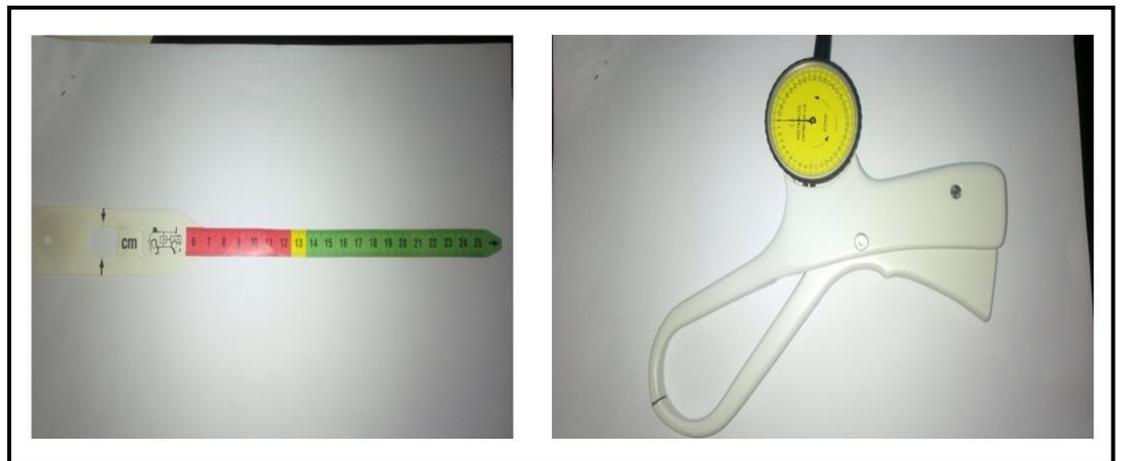


Figure 5.1 Holtain skinfold calliper and a mid upper arm circumference (MUAC) measuring tape

Skinfold Measurement

Triceps, biceps and sub scapular skinfolds were measured using Holtain skinfold calliper (Holtain LTD, Crosswell, United Kingdom) to the nearest 0.2mm. For measuring triceps skinfold the child was asked to bend the left arm at 90° at the elbow and place his/her forearm across the body. Then the Acromion process at the outer-most edge of the shoulder and tip of the Olecranon process of the ulna were located and the distance between the two was measured with the help of the measuring tape. Then the mid-point in line with the elbow and Acromion process was marked. After that the child's arm

was extended so that it hung loosely at the side. Using the thumb and forefinger a vertical fold of skin and underlying fat 1cm above the marked mid-point was grasped. Afterwards the skinfold was gently pulled away from the underlying muscle and calliper jaws were applied at right angles exactly at the marked mid-point. While taking the measurements the skinfold were held in the fingers. Two measurements were taken and the mean of both were considered for the accuracy of results.

For measuring biceps, skinfolds at the level of the mid-point between the Acromion process (bony tip of shoulder) and the proximal and lateral border of the radius bone (approximately the elbow joint), on the mid-line of the anterior surface of the arm (over the biceps muscle) was marked. Then the child was asked to relax his/her arm with the palm of the hand facing forwards. Then using thumb and forefinger a vertical fold of skin was grasped at the land mark, and calliper jaws were applied.

For measuring sub scapular skinfold the lower most tip of the scapula was marked. The skinfold was picked up obliquely following the natural skin contour and the calliper jaws were applied.

Appetite Measurement

Children were asked to rate their appetite on Visual Analogue Scale (VAS) questionnaires along a line of 100 mm. Children were asked to express their feeling of hunger, satiety, fullness, prospective food consumption and desire to eat by placing a vertical mark on the horizontal 100 mm line at a point which corresponds to their feelings at that time. The lines were anchored by negative respective feeling words (I am not hungry at all) on the left and by positive feeling words (never being hungrier) on the right. Quantification was made by measuring the distance from the left end of the line to the child's mark.

Structured interviews

In depth structured interviews were conducted with the parents/carers of the children who received RTUF and LRUS once before the start of supplementation and once at the end of the study. The main researcher led the structured interviews and each interview lasted for approximately 35 minutes. In each discussion, the parents/carers were asked questions regarding the appetite of their children before the start of supplements and after supplementation, acceptability of supplements, taste of supplement and any changes observed by them in their children after 4 weeks of the supplementation.

Calculations and Statistical Analysis

The Anderson-Darling test was used to determine the normality of the data. Power calculations were based on data available from the previous study (Diop, Dossou *et al.* 2003). We used the standard deviation (STDEV) for the difference in weight gain (which was measured by calculating the weight gain expressed in (g/kg/d) between solid ready to eat supplement and liquid ready to drink supplement groups, the main outcome of this study. We found that at 80 % power, considering STDEV of 0.7, 32 subjects in each group will allow us to detect a mean difference of 0.5kg between two groups. This number of participants should be sufficient to detect differences in other outcomes of the study. The difference between the body weight, MUAC, biceps, triceps, and sub scapular measurements at the baseline and after 28 days of supplementation between two groups was determined by using unpaired *t* test. The differences in the socio-demographic characteristics and structured interviews between the RTUF and LRUS groups were determined by using Chi-square test and significance was set at $P < 0.05$.

Ethics

The study was approved by College of Medical Veterinary and Life Sciences, University of Glasgow, Research Ethics Committee and the Research Ethics Committee of Ayub Medical College Abbottabad. Approval from the

principals of the school was received prior to the start of the study. The purpose of the study and study protocols were explained to the principals and parents/carers in local language. The parents/carers of all the participants were required to give written informed consent after clarification that their refusal would have no effect on how the child is treated in the school by the teachers and support staff.

5.3 Results:

A total of 239 children were eligible, 165 were excluded as they did not meet the inclusion criteria, 146 weight for age Z score ($WAZ > -1SD$) while 19 ($WAZ < -2SD$). A total of 74 children were eligible for the study, 2 refused enrolment, and 4 were excluded on the basis of health screen. A total of 68 children were enrolled in the study; 34 received RTUF and 34 received LRUS. The children who were screened ($n=128$) and did not meet the criteria for recruitment ($WAZ > -1 SD$) were measured for height and weight again after four weeks and acted as a control group as presented in figure 5.3.

Socio-demographic data

A total of 63 parents/carers (RTUF =32, LRUS= 31) were asked about socio-demographic characteristics. The socio-demographic characteristics are summarized in Table 5.2, which demonstrates that the parents of the supplement group earned little money, spent small amounts on food items; mothers were illiterate, had more children and had more people living in one small house. However, no statistically significant differences were detected in any of the socio-demographic characteristics between the RTUF and LRUS groups.

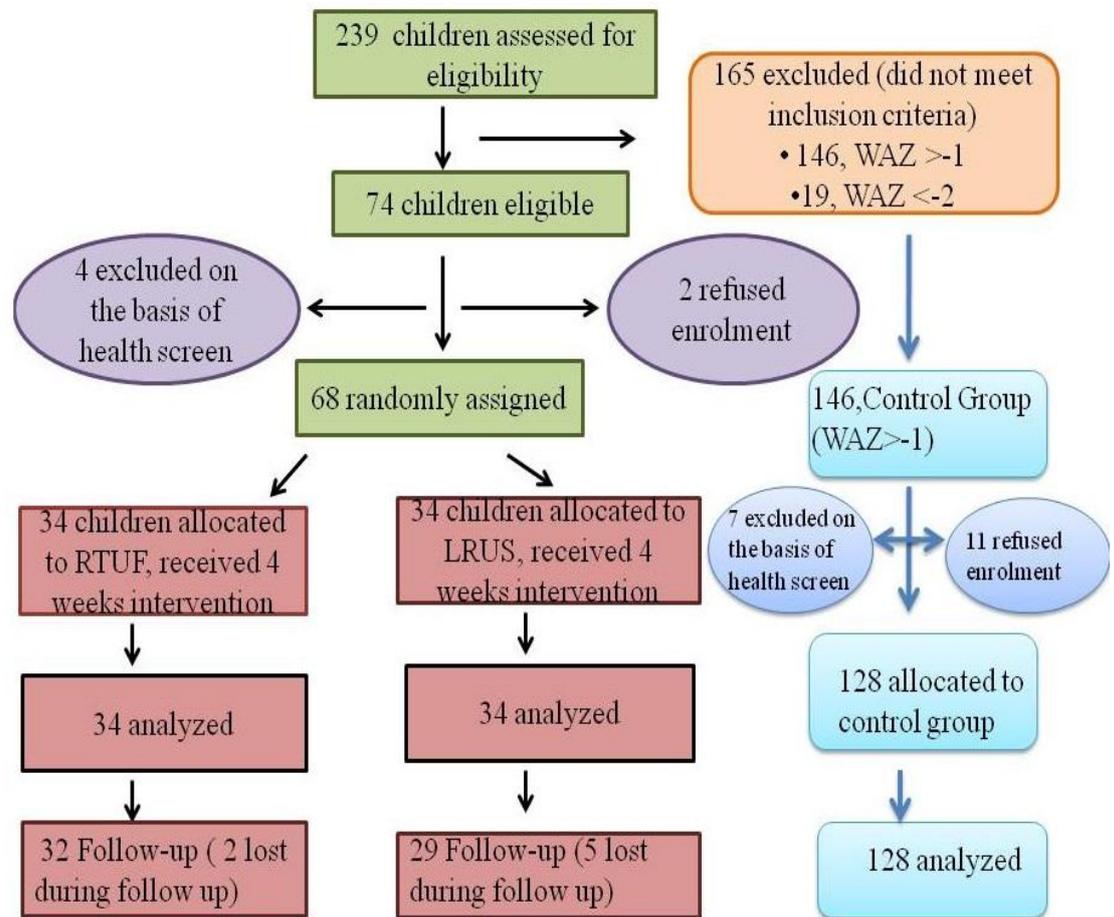


Figure 5.2 Flow chart of children's course throughout the trial

Table 5.2 Socio-demographic characteristics of the parents of schoolchildren who were provided with RTUF and LRUS.

Characteristics	RTUF (n=32) No (%)	LRUS (n=31) No (%)	P value
Father's Occupation			
Skilled	20 (62.5)	20 (64.5)	0.538
Unskilled	12 (37.5)	11 (35.5)	
Mother' Occupation			
Employed	5 (15.6)	7 (22.6)	0.35
Unemployed	27 (84.4)	24 (77.41)	
Father's Income (PKR per month)			
Up to 9000	25 (78.1)	23 (74.2)	0.508
9000-18000	6 (18.7)	8 (25.8)	
>18000	1 (3.1)	0	
Money Used on Food monthly (PKR per month)			
<3000	24 (75)	23 (74.2)	0.580
3000-6000	8 (25)	8 (25.8)	
Mother's Educational level			
Not educated	19 (59.4)	17 (54.8)	0.727
Incomplete primary (1-4 yrs)	3 (9.4)	3 (9.7)	
Complete primary (5 yrs)	4 (12.5)	2 (6.4)	
Incomplete secondary (6-10yrs)	6 (18.7)	8 (25.9)	
Complete secondary (11yrs)	0	0	
University (\geq 12yrs)	0	1 (3.2)	
House Ownership			
Owned by the family	16 (50)	17 (54.8)	0.924
Rented	10 (31.2)	9 (29.0)	

Shared	6 (18.7)	5 (16.1)	
House construction			
Pakkah (cemented)	22 (68.7)	17 (54.8)	0.503
Kacha (made of mud)	7 (21.9)	9 (29.0)	
Semi Pakkah	3 (9.4)	5 (16.1)	
Total number of rooms in the house			
0-3	22 (68.75)	25 (80.6)	0.517
4-6	9 (28.1)	5 (16.1)	
>6	1 (3.1)	1 (3.2)	
No. of siblings			
0-3	6 (18.7)	7 (22.6)	0.06
4-6	15 (46.9)	21 (67.7)	
>6	11 (34.4)	3 (9.7)	
No. of Family members residing in the house			
0-5	2 (6.2)	3 (9.6)	0.849
6-10	15 (46.9)	15 (48.4)	
>10	15 (46.9)	13 (41.9)	
Water source			
Tap water	12 (37.5)	15 (48.4)	0.780
Hand pump	8 (25)	5 (16.1)	
Wells	5 (15.6)	5 (16.1)	
Spring	7 (21.9)	6 (19.3)	
Type of Bathroom			
Flush system	7 (21.9)	5 (16.1)	0.517
Laterine	25 (78.1)	25 (80.6)	
Open space	0	1 (3.2)	
Garbage disposal			
In the dump	0	0	

Outside the house	32 (100)	31 (100)
Inside the house	0	0

Nutritional Characteristics

At the beginning of the trial the mean (\pm SD) age of the children (range: 5-10 years) (RTUF, 8.45 ± 1.22 years; LRUS, 8.19 ± 0.86 years, $P = 0.30$), weight-for-age Z score (WAZ) (RTUF, -1.33 ± 0.28 ; LRUS, -1.35 ± 0.29 , $P = 0.86$) and body mass index Z score (BMI Z score) (RTUF, -1.43 ± 0.73 ; LRUS, -1.58 ± 0.61 , $P = 0.37$) were not significantly different between the RTUF and LRUS groups. The baseline nutritional characteristics of the children at enrolment are presented in the Table 5.3.

The weight, height weight-for-age Z score (WAZ), and height-for-age Z (HAZ) score and BMI Z score at baseline, 14, and 28 days of supplementation and during the follow up in the RTUF and LRUS groups, are presented in Table 5.4 and Figure 5.4. Compared with baseline there was a significant increase in mean (\pm SD) weight gain (g/kg/day) in RTUF (0.96 ± 0.48 g/kg/day) and LRUS group (1.10 ± 0.69 g/kg/day), after four weeks of supplementation, however the difference in the mean weight gain (g/kg body weight/day) was not significantly different between RTUF and LRUS groups (0.96 compared with 1.10 g/kg/day difference: 0.14 ; 95% CI: $-0.45, 0.11$ g/kg/day, $P = 0.33$) (Table 5.5).

Table 5.3 Nutritional characteristics of children on enrollment

Characteristics	Control group (n=128)	RTUF (n=34)	LRUS (n=34)
Age (years)	8.73 ± 0.86 ^b	8.45 ± 1.22	8.19 ± 0.86
Female/ Male n (%)	124/4 (97/3)	30/4 (88/12)	33/1 (97/ 3)
Height (cm)	136.20 ± 8.71 ^{a, b}	126.31 ± 7.22	124.5 ± 4.32
Weight (kg)	28.60 ± 5.18 ^{a, b}	21.61 ± 2.72	20.77± 2.12
BMI (kg/m ²)	15.37 ± 1.67 ^{a, b}	13.85 ± 0.98	13.52 ± 0.78
WAZ (SD)	0.16 ± 0.72 ^{a, b}	-1.33 ± 0.28	-1.35 ± 0.29
HAZ (SD)	0.87 ± 1.05 ^{a, b}	-0.51 ± 0.66	-0.54 ± 0.56
BMI Z score (SD)	-0.48 ± 0.87 ^{a, b}	-1.43 ± 0.73	-1.58 ± 0.61
Skinfold Z score	-	-0.79 ± 0.51	-0.95 ± 0.57
MUAC (cm)	-	15.81 ± 1.03	15.56 ± 0.82
Triceps (mm)	-	7.62 ± 1.34	7.46 ± 1.46
Sub scapular (mm)	-	5.36 ± 0.79	5.03 ± 0.89

Abbreviations: RTUF, Solid ready to use food; LRUS, liquid ready to use supplement; CI, confidence interval; WAZ, weight-for-age Z-score; HAZ, height-for-age Z-score; BMI Z score, body mass index Z- score; MUAC, mid upper arm circumference; ^a Significantly different from RTUF group. ^b Significantly different from LRUS group. $P < 0.05$ (Student's *t* test).

Similarly there was a significant change in WAZ after 4 weeks of supplementation in the RTUF group (from -1.33 ± 0.28 to -1.21 ± 0.29 , $P < 0.0001$) and the LRUS group (from -1.35 ± 0.29 to -1.20 ± 0.31 , $P < 0.0001$). When the difference in change in WAZ after 28 days of supplementation in the RTUF group (RTUF: 0.12 compared with LRUS: 0.15; difference: 0.03; 95% CI: -0.03, 0.08) and the LRUS group was compared it was not significantly different. Likewise, when changes in WAZ, HAZ, and skinfold Z score were compared between RTUF and LRUS groups, no significant differences were

found between the two groups (Table 5.5). In the control group, a significant increase in height was observed after 28 days; however, no significant differences were detected in weight, WAZ, HAZ, and BMI Z score after 28 days (Table 5.4). A statistically significant lower mean weight gain (g/kg/day) and change in WAZ was detected in the control group as compared with the RTUF and LRUS groups (Table 5.5). Thirty two children in the RTUF group and 29 in LRUS group were followed after mean (\pm SD) 7.13 ± 3.14 months when they were off the supplements (Table 5.4). During follow up height and weight gain were similar in both RTUF and LRUS groups. No significant differences were detected in WAZ, HAZ, BMI Z-score; skinfold Z score when the end of supplementation was compared with the follow up (Table 5.4; Figure 5.4).

The changes in nutritional characteristics of mildly wasted (BMI $Z < -1$) and mildly stunted (HAZ < -1 SD) children of the supplemented group after four weeks of supplementation with the control group were also compared (Table 5.6). A statistically significant difference was detected in changes in WAZ, HAZ and BMI Z score when mildly wasted children of the supplement group (RTUF + LRUS) was compared with the control group after 28 days of the baseline measurements. However, when the mildly stunted children of the supplemented group after four weeks of supplementation was compared with control group after 28 days of baseline measurements a statistically significant difference was detected only in changes in WAZ (Table 5.6).

Table 5.4 Nutritional Characteristics after 4 weeks of supplementation

Characteristics	Control group		RTUF			LRUS		
	Baseline	After 28 days	Before Supplementation	After Supplementation	Follow up (n=32)	Before Supplementation	After Supplementation	Follow up (n=29)
Weight (kg)	28.60 ± 5.18	28.94 ± 5.51	21.78 ± 2.57	22.36 ± 2.67*	23.27 ± 2.75 ^a	20.97 ± 1.81	21.62 ± 1.94*	22.80 ± 1.94 ^a
Height (cm)	136.20 ± 8.71	136.64 ± 8.60*	126.31 ± 7.22	127.00 ± 7.25*	129.85 ± 6.83 ^a	124.50 ± 4.32	125.18 ± 4.41*	128.70 ± 5.01 ^a
WAZ (SDS)	0.16 ± 0.72	0.15 ± 0.76	-1.33 ± 0.28	-1.21 ± 0.29*	-1.31 ± 0.33	-1.35 ± 0.29	-1.20 ± 0.31*	-1.25 ± 0.36
HAZ (SDS)	0.87 ± 1.05	0.86 ± 1.05	-0.51 ± 0.66	-0.47 ± 0.65*	-0.52 ± 0.54	-0.54 ± 0.56	-0.50 ± 0.57*	-0.51 ± 0.59
BMI-Z score (SDS)	-0.48 ± 0.87	-0.49 ± 0.86	-1.43 ± 0.73	-1.45 ± 0.73*	-1.56 ± 0.74	-1.58 ± 0.61	-1.39 ± 0.60*	-1.47 ± 0.76
MUAC (cm)	-	-	15.81 ± 1.03	16.30 ± 1.12*	16.27 ± 1.15	15.56 ± 0.79	16.00 ± 0.83*	16.04 ± 0.87
Triceps (mm)	-	-	7.62 ± 1.34	7.91 ± 1.27*	7.69 ± 1.27	7.46 ± 1.46	7.89 ± 1.42*	7.63 ± 1.26
Sub scapular (mm)	-	-	5.36 ± 0.79	5.73 ± 0.84*	5.44 ± 0.96	5.03 ± 0.89	5.35 ± 0.90*	5.16 ± 0.82
Skinfold Z score (SD)	-	-	-0.79 ± 0.51	-0.62 ± 0.47*	-0.80 ± 0.57	-0.95 ± 0.57	-0.75 ± 0.54*	-0.92 ± 0.54

Abbreviations: *RTUF*, Solid ready to use food; *LRUS*, liquid ready to use supplement; *CI*, confidence interval; *WAZ*, weight-for-age Z-score; *HAZ*, height-for-age Z-score; *BMI Z-score*, body mass index Z- score. *Significantly different from baseline; ^a Significantly different from the end of supplementation, $P < 0.001$ (Student's *t* test).

Table 5.5 Comparison of gain in anthropometric outcomes of children receiving 4 weeks supplementation between RTUF and LRUS groups

	Control (n=128)	RTUF (n=34)	LRUS (n=34)	P value	Difference between RTUF and LRUS (95% CI)
Rate of weight gain (g/kg/day)	0.30 ± 1.64 ^{a, b}	0.96 ± 0.48	1.10 ± 0.69	0.33	0.14 (-0.45, 0.11)
Weight gain (kg)	0.26 ± 1.54	0.59 ± 0.30	0.65 ± 0.42	0.48	0.06 (-0.244, 0.12)
Height gain (cm)	0.44 ± 0.86	0.69 ± 0.49	0.68 ± 0.44	0.87	0.01 (-0.210, 0.25)
Change in WAZ (SDS)	-0.01 ± 0.20 ^{a, b}	0.12 ± 0.09	0.15 ± 0.13	0.33	0.03 (-0.0285, 0.08)
Change in HAZ (SDS)	0.00 ± 0.15	0.04 ± 0.08	0.04 ± 0.08	0.91	0.00(-0.039, 0.034)
MUAC (cm)	-	0.49 ± 0.39	0.44 ± 0.29	0.54	0.05 (-0.118, 0.22)
Triceps gain (mm)	-	0.29 ± 0.24	0.31 ± 0.23	0.80	0.02 (-0.101, 0.131)
Sub scapular gain(mm)	-	0.37 ± 0.29	0.31 ± 0.25	0.38	0.06 (-0.189, 0.073)
Change in Skinfold Z score		0.20 ± 0.13	0.17 ± 0.12	0.30	0.03 (-0.086, 0.025)

Abbreviations: RTUF, Solid ready to use food; LRUS, liquid ready to use supplement; MUAC, mid upper arm circumference; CI, confidence interval; WAZ, weight-for-age Z-score; HAZ, height-for-age Z-score; ^a Significantly different from RTUF group; ^b Significantly different from LRUS group; *Significantly different from RTUF group. $P < 0.001$ (Student's *t* test)

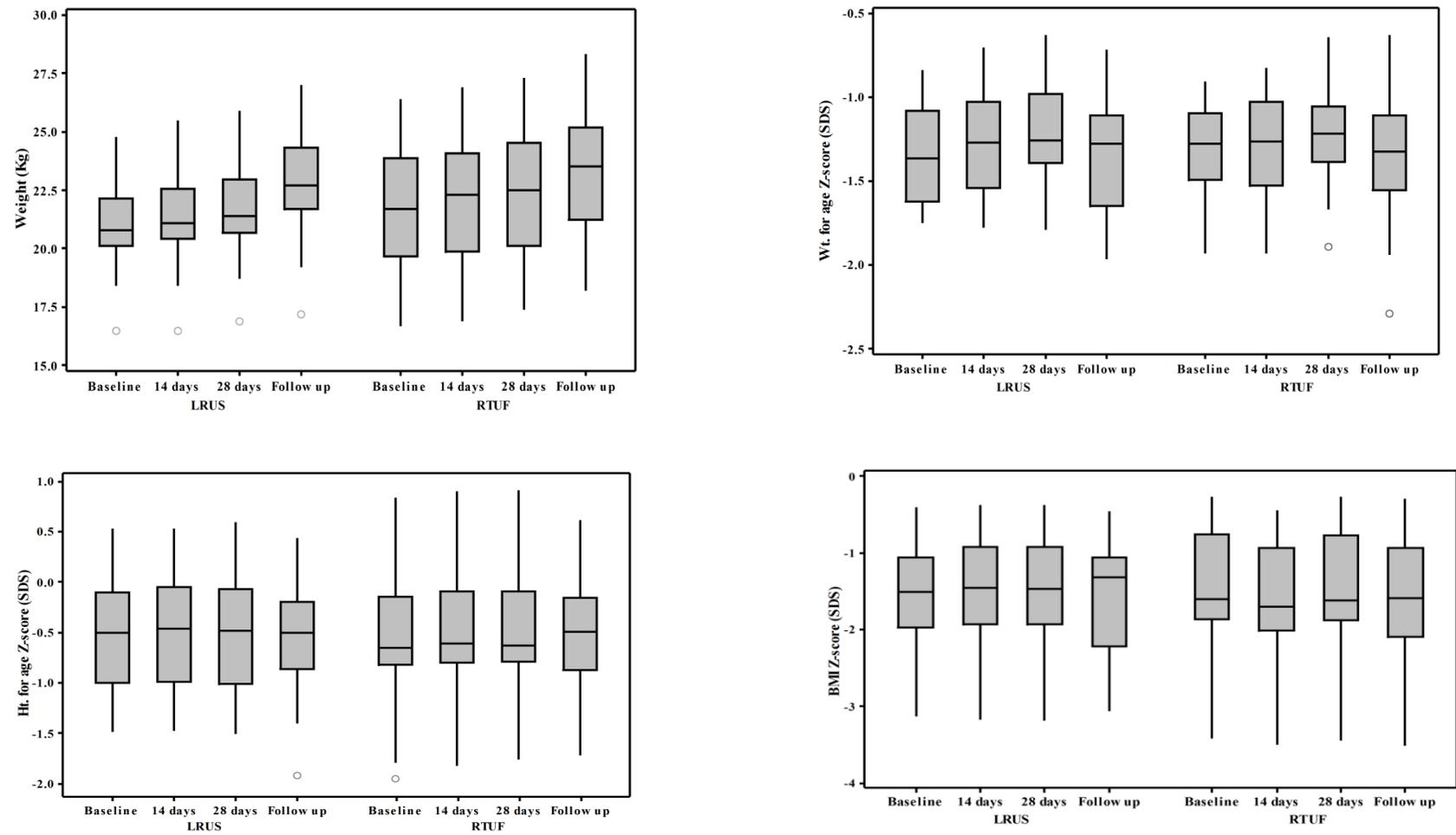


Figure 5.3: Box plots showing changes in weight, weight-for-age Z score (WAZ) and height-for-age Z score (HAZ) and BMI Z-score at baseline, 14 and 28 days of supplementation and during follow up in RTUF and LRUS groups

Table 5.6 Comparison of gain in anthropometric outcomes of mildly wasted children (<-1 Z score) and mildly stunted (<-1 Z score) children receiving 4 weeks of supplementation and control group.

	Control (n=128)	Wasted (n=49)	P Value	Stunted (n=14)	P Value
Change in WAZ (SDS)	-0.01 ± 0.20	0.13 ± 0.11	0.04	0.10 ± 0.14	0.04
Change in HAZ (SDS)	0.00 ± 0.15	0.04 ± 0.07	< 0.0001	0.05 ± 0.09	0.17
Change in BMI Z score (SDS)	-0.01 ± 0.34	0.10 ± 0.18	0.03	0.06 ± 0.20	0.48

Appetite Responses

The difference in the appetite measures before the provision of the first and the last supplement between the two groups for hunger (RTUF, 1.91 ± 1.76mm; LRUS, 2.14 ± 1.08 mm, $P=0.99$), desire to eat (RTUF, 4.53 ± 2.53 mm; LRUS, 2.32 ± 2.67 mm, $P=0.55$), satiety (RTUF, 0.71 ± 1.93 mm; LRUS, 1.44 ± 1.23 mm, $P=0.35$) and fullness (RTUF, 2.56 ± 2.94 mm; LRUS, 1.50 ± 2.15 mm, $P=0.77$) were also not significantly different between the two supplemented groups (Table 5.7).

Table 5.7 Appetite responses before the provision of the first and the last supplement between RTUF and LRUS groups. Data is presented as Mean \pm SE.

Appetite Responses	Before provision of first supplement	Before provision of last supplement	P value
Hunger (mm)			
RUTF	44.97 \pm 4.87	46.88 \pm 4.65	0.28
LRUS	29.00 \pm 4.24	31.14 \pm 4.08	0.06
Satiety (mm)			
RUTF	50.76 \pm 4.66	51.47 \pm 5.03	0.71
LRUS	69.88 \pm 4.36	68.44 \pm 4.01	0.25
Fullness (mm)			
RUTF	50.23 \pm 4.66	52.76 \pm 4.73	0.66
LRUS	70 \pm 4.52	71.5 \pm 3.95	0.49
Prospective Food Consumption (mm)			
RUTF	52.17 \pm 54.88	54.88 \pm 4.48	0.22
LRUS	70.88 \pm 4.11	70.91 \pm 3.96	0.98
Desire to Eat (mm)			
RUTF	45.97 \pm 4.7	50.5 \pm 4.88	0.08
LRUS	31.32 \pm 4.60	33.64 \pm 4.71	0.38

RTUF, Solid ready to use food; LRUS, liquid ready to use supplement

Structured interviews

All the parents/carers reported that their children ate/ drink the provided supplements, although 10/34 (29%) children from RTUF group and 14/34 (41%) children from LRUS, $P=0.310$ did not like the taste of the supplements much. 19/34 (56%) and 12/34 (35%) of the parents/carers observed height/weight gain after 4 weeks of supplementation with LRUS and RTUF respectively, $P=0.225$ (Table 5.8). Regarding appetite 6/34 (18%), parents/carers of both groups observed a loss of appetite for short duration in

their children. Moreover, 2/34 (6%) parents/ carers in RTUF group and 6/34 (18%) in LRUS group, P=0.132 attributed side effects to the intake of these supplements, the most common complaints were nausea, abdominal pain and diarrhoea.

The parents/carers from RTUF group 26/34 (76.4%) and LRUS 24/ 34 (70%) group, P=0.582 were pleased with the supplementary food and wanted to use the supplements again provided that they are cheap or supplied cost-free (Table 5.8).

Table 5.8 RTUF and LRUS perceived acceptability, benefits, and side effects among children according to their parents/carers in Abbottabad, Pakistan.

Variable	RTUF (%)	LRUS (%)	P value
Total parents/carers	34	34	
Taste unacceptable	10 (29.4%)	14 (41%)	0.310
Perceived Benefits			
Weight /height gain	14 (41%)	19 (56%)	0.225
Improved general health	24 (70%)	24 (70%)	1.000
Improved appetite	6 (18%)	4 (12%)	0.493
Perceived side effects			
Loss of appetite	6 (18%)	6 (18%)	1.000
Abdominal pain, nausea, diarrhoea	2/34 (6%)	6/34 (18%)	0.132
Wants to use supplements again in future	26 (76%)	24 (70%)	0.582

RTUF, Solid ready to use food; LRUS, liquid ready to use supplement

5.4 Discussion

To our knowledge, this is the first community based study to explore the effectiveness of solid ready to eat and liquid ready to drink supplements in the management of mild to moderate malnutrition in the children aged between 5 – 10 years. Commercially available high energy nutritional supplement drinks are very expensive and have not been tested in the community settings (Singh, Kang *et al.* 2010). Enrolment rate for this study was high and group

allocation was random. Groups were similar on enrolment, compliance with the supplementation appeared good, follow up for both the groups was identical and no loss to follow up was observed during the intervention.

In this study both RTUF and LRUS improved the nutritional characteristics of the children which has also been suggested by various other studies investigating RTUF (Diop, Dossou *et al.* 2003, Defourmy, Seroux *et al.* 2007, Isanaka, Nombela *et al.* 2009, Patel, Sandige *et al.* 2011). In our study, we have found that there was no significant difference in weight gain, change in WAZ, HAZ, triceps- and sub scapular-Z score between RTUF and LRUS groups. Our findings are consistent with other studies in which RTUF was compared with other nutritional supplements (Phuka, Thakwalakwa *et al.* 2009, Bisimwa, Owino *et al.* 2012, LaGrone, Trehan *et al.* 2012, Thakwalakwa, Ashorn *et al.* 2012). Similar to the results of this study, a RCT in 6-18 months old moderately underweight Malawian children provided with micronutrient-fortified maize –soy flour or ready to use fortified spread for 12 weeks. The primary outcome of the study was weight gain and other secondary outcomes were change in anthropometric indices HAZ, WAZ, WHZ and recovery from underweight, stunting and wasting. No statistically significant differences between the outcome measures were detected between the two groups after 12 weeks of supplementation (Phuka, Thakwalakwa *et al.* 2009).

Similarly other community based studies conducted in Malawi and other African countries did not observe a significant difference in the outcomes measures like weight gain, recovery rate and length of stay while comparing standard RTUF with whey protein concentrate RTUF (Bahwere, Banda *et al.* 2014), corn- soy blend “plus plus”, RTUF with locally produced soy RTUF, or an imported soy/whey RTUF for ≤ 12 weeks (LaGrone, Trehan *et al.* 2012) and locally produced and imported RTUF (Sandige, Ndekha *et al.* 2004). In our study, when the RTUF and LRUS group was compared with control group, a statistically significant difference was observed in some of the nutritional measures, more specifically, the rate of weight gain (g/kg/d) and change in WAZ score. Previous studies also observed a significant improvement in some of the nutritional outcome measures when the intervention group was

compared with a control group (Thakwalakwa, Ashorn *et al.* 2010, Grellety, Shepherd *et al.* 2012, Huybregts, Houngbé *et al.* 2012).

In our study, we observed that weight gain after 4 weeks of supplementation in RTUF group was 0.59 ± 0.30 kg and in LRUS group 0.65 ± 0.42 kg. Previous studies have also documented weight gain after supplementation (Phuka, Thakwalakwa *et al.* 2009, Singh, Kang *et al.* 2010, Thakwalakwa, Ashorn *et al.* 2012). In Phuka *et al.* mean (\pm SD) weight gain after 12 weeks of dietary supplementation with either maize-soya flour or fortified spread results was 0.89 ± 0.38 kg, and 0.84 ± 0.46 kg respectively (Phuka, Thakwalakwa *et al.* 2009), while Thakwala *et al.* observed a weight gain of 0.75 ± 0.41 kg in RTUF group during 12 weeks of supplementation. Here it is worth mentioning that the duration of supplementation lasted only for 4 weeks in our study. Moreover rapid weight gain occurs in severely wasted children, when children approach a normal weight for height Z score that rate of weight gain falls to 1-2 g/kg/day (Ashworth 2006).

The healthy children in the control group were measured for height and weight again after 4 weeks to observe temporal changes on growth and anthropometry. It was found that the mean weight gain of the children in the control group of same age was 0.26 ± 1.54 kg and the increase in height was 0.44 ± 0.86 cm after 4 weeks. A significantly higher rate of weight gain (g/kg/d) was observed in RTUF and LRUS groups as compared to the control group. This showed that the seasonal effect on the weight and height gain in the children was lower than the increase observed in this trial after 4 weeks of intervention. The mean gain in weight, height, and skinfold measurements observed in this study are comparable with other interventional studies of supplementary feeding as compared to non supplemented group (Kuusipalo, Maleta *et al.* 2006, Adu-Afarwuah, Lartey *et al.* 2007). Here it is worth mentioning that the excess mean weight gain in supplemented group was 0.62 ± 0.37 kg.

The total extra energy supplied (500 kcal/day) for 4 weeks would have been expected to lead to an excess gain of 2 kg. Thus, at least 2/3 of the energy ingested appears to have been compensated for by eating less at other times.

Previous studies have also found that the observed weight gain rate was substantially less as compared to their predicted growth rate. A study on children receiving RTUF observed a growth rate of 5.1g/kg/d, which was considerably less than their predicted 20 g/kg/d (based on theoretical model) (Manary, Ndkeha *et al.* 2004).

In contrast to weight gain, the gain in height seems to be very little by 4-weeks of supplementation with both RTUF and LRUS in our study. This is not surprising; as gain in height/length has been shown to follow the weight gain after a certain lag period (Walker and Golden 1988, Maleta, Virtanen *et al.* 2003). Therefore, a longer period of intervention might prevent stunting or mild to moderate malnutrition. In our study, we have found no significant difference in the height gain between RTUF and LRUS groups. Similarly, in another randomized clinical effectiveness trial on the moderately malnourished Malawian children it was found that the rates of the length gain was not different between corn/soy blend flour (CSB), milk peanut fortified spread (FS) and soy/peanut FS (Matilsky, Maleta *et al.* 2009).

In our study we have found that RTUF and LRUS both have good compliance among mild to moderate malnourished children, which was also demonstrated in other studies. Studies on RTUF in moderately underweight children showed good compliance (Flax, Maleta *et al.* 2008, Flax, Thakwalakwa *et al.* 2009, Hess, Bado *et al.* 2011). In this study all the supplements were provided by the main researcher herself to each child and then the empty sachet and bottles were collected again on the same day in the afternoon to look at the compliance but it is possible that the RTUF or LRUS may have been shared with other children or wasted in the school. However, if this happened to a large extent we may not have observed the full supplementation effect on the nutritional characteristics of the children. Moreover, during the distribution of supplements the researcher also emphasized that both the supplements (RTUF and LRUS) were intended for the targeted children only. In addition to that, both the supplements used in this study were ready to use and required no cooking, which further improved the compliance. In one of clinical effectiveness trial of two supplementary feeding regimens, one supplement was ready- to- use therapeutic food, and another was macronutrient fortified

corn/soy-blend, which required cooking and that may have made an impact on the compliance, due to inconvenience of the time consuming process needed to cook. This supplement resulted in poorer outcomes than with the micronutrient fortified corn/soy-blend (Patel, Sandige *et al.* 2011). Similarly in another study the same problem was observed when ready to use food (RTUF) was compared with maize/soy which required time and resources to prepare maize/soy a disadvantage over RTUF (Manary, Ndkeha *et al.* 2004).

In this study, the structured interviews with the parents/carers showed that 29% of the children from RTUF group and 41% of the children from the LRUS group were dissatisfied with the consistency and the taste of the supplement. In one of the previous study it was found that 43% of the children were dissatisfied with the taste of the RTUF which is higher than in our study (Ali, Zachariah *et al.* 2013). Despite the problems with taste of the supplements 70% of the parents/carers from the RTUF and LRUS group perceived these supplements to be beneficial for their children which is less than another study in which 91% of the caregivers perceived RTUF to be of a therapeutic benefit to their severe acute malnourished or moderate acute malnourished children (Ali, Zachariah *et al.* 2013). One limitation of the study is that the questions regarding the taste and acceptability of the supplements were put to the parents/carers of the children, and the reported responses did not reflect the actual perception of the children themselves. Thus, the impact of adult perception of actual acceptability in children is not known. Moreover, behavioural and cultural factors might also play a role in the acceptability of the ready to use nutritional supplements and we have not assessed such factors in this study. We are unable to validate any direct relation between the RTUF or LRUS intake and the attributed side effects.

As economic inequity is an independent determinant of childhood malnutrition, numerous studies have demonstrated that poor children are expected to be at greater risk of being undernourished (Van de Poel, Hosseinpoor *et al.* 2008, Janevic, Petrovic *et al.* 2010, Mushtaq, Gull *et al.* 2011, Stalin, Bazroy *et al.* 2013). Therefore, in this study we also determined the socio-demographic characteristics of the children from the supplement group. We found that the parents of children from the supplement groups had

low monthly income, spent less on food items, had larger family sizes and mothers were illiterate. Studies have documented that thinness and stunting was significantly higher among children with illiterate parents as compared to educated parents. Parental education is also identified as a predictor of under nutrition (Mushtaq, Gull *et al.* 2011, Emamian, Fateh *et al.* 2013). Similarly, thinness and stunting were also found to be significantly higher in the children living in crowded houses and having more siblings, both of these factors are considered to be an indirect predictor of lower socio-economic status (Mushtaq, Gull *et al.* 2011).

RTUF contains patent allergens , and these allergens may be enhanced during cooking (Maleki, Chung *et al.* 2000). We did not observe any incidence of peanut allergy in this study as we screened eligible children for food allergies with the help of the health questionnaire during screening. Furthermore, allergic reactions are rare in this population, as peanuts are the part of their traditional diet. Moreover clinical allergy is rare in developing countries (Abbassy, El-Din *et al.* 1974) and food allergy manifests in infancy and declines after three years of age (Husain and Schwartz 2013). This finding is consistent with another study in which peanut allergy was not observed with ready to use food (Manary, Ndkeha *et al.* 2004). In developing countries there is lower incidence of peanut allergy as compared to developed countries (Singh, Kang *et al.* 2010). It was found that 5.8% (2/34) of the parents/carers from RTUF and 21% (7/34) from LRUS group attributed side effects to the intake of the supplements, the most common complaints were nausea, abdominal pain and diarrhoea. Other studies have also demonstrated these side effects by the use of RTUF and therapeutic milk F-100 (Manary, Ndkeha *et al.* 2004, Talbert, Thuo *et al.* 2012, Ali, Zachariah *et al.* 2013).

In this study, the movement of the children into other schools at the end of the academic year was anticipated. Hence the study was performed in the mid part of academic year i.e. May to December. Thus all the children participated in this study and there were no drop outs in the study due to change in the school. A previous study performed in the early part of academic year (from January to the end of May 2008) to evaluate the effectiveness of locally made RTUF in mild to moderate malnourished pre- school children observed a higher dropout

rate due to movement of children into other schools (Singh, Kang *et al.* 2010). This study was not blinded as blinding would have been very difficult for two different interventions with one supplement being solid and other being liquid. The response of the children from both groups during four weeks of supplementation was exceptionally good and there was no dropout and all the 34 children from each group used the supplements as the researcher daily collected the empty bottles and sachet from each of the participants.

We found lower effect of RTUF and LRUS on weight for age Z score (WAZ) and height for age Z score (HAZ) than expected but is in the range reported in other trials assessing the efficacy of supplementary feeding in children (Isanaka, Nombela *et al.* 2009). The little effect of RTUF and LRUS on WAZ and HAZ may suggest acute compensation, which we have observed in our first two experimental studies. We observed that 43% of the energy provided by supplements is compensated by eating less during the consecutive meal (Fatima, Gerasimidis *et al.* 2013) and previous studies have also reported energy compensation (Mattes 2006, Stratton, Stubbs *et al.* 2008, Mattes and Campbell 2009, Almiron-Roig, Palla *et al.* 2013). It might be possible that children eat less from their habitual diet after the intake of supplements due to acute suppression of appetite following supplementation. Other factors may be short duration of supplementation, increased energy expenditure due to physical activity in community setting, sharing of supplements with other children in the school and wastage of the supplements as compared to inpatient therapeutic feeding centre. Other studies have demonstrated barriers to the use of RTUF and its inadequate compliance due to sharing within the households (Maleta, Kuittinen *et al.* 2004, Ickes, Jilcott *et al.* 2012). One 24 hour dietary study reporting on moderately malnourished children, found that the children only received 30% and 43% of the provided RTUF and the maize soy flour respectively (Maleta, Kuittinen *et al.* 2004). However, in our study we tried to ensure that the compliance with supplements was high, as empty containers were collected daily in the afternoon during supplementation.

In this study the appetite was measured using visual analogue scales before the start of the first supplement and before the provision of last supplement but we were unable to measure the dietary intake of the participants before the

intervention or during the intervention. Subsequently, we did not have the data on the habitual average energy, macronutrient and micronutrient intake. Therefore, we could not measure the average daily energy intake from their habitual diet at the baseline and during the intervention period to indicate whether liquid ready to drink supplement or RTUF supplemented or displaced habitual energy intake. Previous studies have observed that energy dense ready to use foods seems have little measurable impact on children's diet and breast milk intake (Galpin, Thakwalakwa *et al.* 2007, Owino, Bahwere *et al.* 2011) but assessment of habitual diet is difficult, so further dietary assessment studies are necessary, to investigate the impact of RTUF and liquid ready to drink supplement on habitual diet substitution in children.

In this study, we followed the children from the supplement group up to 12 months. Data on long term follow up after supplementation is available only from few previous studies (Nackers, Broillet *et al.* 2010, Chang, Trehan *et al.* 2013). Both these studies showed high rate of relapse during follow up in children with moderate acute malnutrition. The Malawian study (LaGrone, Trehan *et al.* 2012) was followed up for twelve months and was reported in Chang *et al.* 2013. During twelve months follow up it was found that 63% of the children remained well nourished, 17% relapsed to MAM, 10 % developed SAM and 4% die. A study by Nackers *et al.* 2010, found that during a six months follow up, 62% remained well nourished and 20% relapsed to MAM. In our study, we observed that, during the follow up, the children in the supplement group remained in WAZ between -1 and -2. Previous evidence also suggested that 30% to 40% of the children recovered from MAM relapsed in a short period of six to twelve months (Lazzerini, Rubert *et al.* 2013). This may suggest that provision of supplements may be a short-term solution and for a sustained, long-term impact focus should be placed on actual problem of food access, dietary habits, and treatment of underlying diseases, education, and sanitation.

We have found that the children who were provided with the supplements belong to low socio-economic status and were mildly malnourished. Supplementation with RTUF and LRUS for four weeks resulted in modest weight gain and during follow up it was found that there was a tendency to

relapse, which demonstrates that provision of nutritional supplements is a short-term solution. The findings from our study suggest that RTUF and liquid nutritional supplements are equally effective only for short-term nutritional improvement. However, for the long term emphasis should be placed on utilising local foods and re-educating food habits as compared to externally provided industrial food. Though our findings are consistent with the idea that both supplements promote recovery from mild to moderate malnutrition, poverty in Pakistan diminishes the possibility to purchase commercially available nutritional supplements. In spite of the fact that mild to moderate malnutrition is a contributing factor to morbidity in a huge proportion of child mortality in our country, it is not getting the attention, which it deserves. It is therefore, suggested that, in the developing countries like Pakistan, an integrated nutrition programs may have a considerable impact through combination of interventions involving education, health, sanitation and food. A school based food program focused on poor and socially disadvantaged population in developing countries like Pakistan is recommended.

5.5 Conclusion

Our results suggest that benefits of supplementation with RTUF and LRUS in relation to the treatment of moderate malnourished children of Pakistan are similar. However, the overall improvement in weight gain was less than expected.

6 General Discussion and Conclusions

Malnutrition is one of the many health inequalities, affecting millions of individuals worldwide, and is basically a treatable condition. Systematic reviews and meta-analyses consistently suggest that HENSD can improve the energy and nutritional intake, body weight, and have a variety of clinical and functional benefits in malnourished patients, and patients at risk of becoming malnourished (Stratton and Elia 2010, Cawood, Elia *et al.* 2011, Stratton, Hebuterne *et al.* 2013). Typically, HENSD contains a mixture of protein, carbohydrate and fat and some micronutrients and provide from 6.3 kJ/ml to 10.1 kJ/ml per typical serving of 125–220 ml (Stratton and Elia 2010). A recent meta-analysis of 8 RCTs suggest that they enhance daily energy intake increased by a mean of 1569 kJ/d (Hubbard, Elia *et al.* 2012). Increase in body weight due to HENSD supplementation however is usually lower than predicted. This implies that HENSD diminishes other food intake and that the energy provided by HENSD is only partially compensated by a reduction in the energy intake during habitual meals. Mechanisms responsible for only partial energy compensation during meals consumed after intake of the supplements used for malnutrition treatment remain to be investigated.

So far, the role of metabolic and hormonal appetite modulators in amendments of energy intake due to malnutrition treatment has been investigated only in tube feeding studies (Stratton and Elia 1999, Stratton, Stubbs *et al.* 2008). It has been reported that compensation for energy provided by the intermittent delivery of tube feeding as bolus was only partial regardless of significant enhancement in the metabolic and hormonal responses involved in satiation (Stratton, Stubbs *et al.* 2008). The interaction between appetite, food intake and metabolic and hormonal mediators of appetite and satiety following tube feeding and oral supplementation may be different. Therefore, the first aim of this thesis was to investigate the level of energy intake compensation in lean adult women following HENSD consumption, and to find out how it relates to

the response of a range of hormonal and metabolic appetite regulators as well as measure of gastric emptying.

In clinical practice, HENSD is not consumed on an acute basis, but is taken for a long duration (Hubbard, Elia *et al.* 2012). In addition, consumption happens in the evening, rather than prior to breakfast. Therefore, a first experimental study, applying a typical pre-load trial approach, with a requirement to consume the supplement in the morning prior breakfast, might not fully reflect the food compensatory nature related to supplement intake in the evening and for a longer duration. Therefore, one of the aims of the second study was to investigate the time scale and level of energy intake compensation after five days of supplementation with HENSD in the afternoon.

An enhancement in daily energy achieved by HENSD supplementation, is expected to increase body mass (Cawood, Elia *et al.* 2012) and thus induces a positive energy balance. A positive energy balance induced by an increase in dietary energy intake impacts plasma triglyceride, insulin and glucose concentrations (Hill, Peters *et al.* 1990, Faeh, Minehira *et al.* 2005, Bortolotti, Kreis *et al.* 2009, Brøns, Jensen *et al.* 2009). The impact of short term overfeeding on triglyceride metabolism is uncertain, since it is difficult to distinguish between changes in energy intake and changes in the macronutrient composition. Some studies reported an elevated VLDL-TAG concentrations after 4 to 7 days of consuming hyper-caloric high carbohydrate diet (Ngo Sock, Lê *et al.* 2010, Sobrecases, Lê *et al.* 2010) while overfeeding studies with high fat diet found that VLDL-TAG concentrations were reduced (Brøns, Jensen *et al.* 2009, Sobrecases, Lê *et al.* 2010). Whereas, some studies found no impact of hyper-caloric feeding on very low density lipoprotein triglyceride (VLDL-TAG) concentrations in healthy women (Bellou, Maraki *et al.* 2013) and in obese subjects (Smith, Magkos *et al.* 2013). The impact of an acute positive energy balance achieved by overfeeding with a mixed diet (balanced diet) has controversial findings with respect to plasma glucose and insulin levels. Some studies reported an increase in the concentration of both insulin and glucose (Tam, Viardot *et al.* 2010, Bellou, Maraki *et al.* 2013), some insulin only (Sobrecases, Lê *et al.* 2010, Cornford, Hinko *et al.* 2012) and

others found no change in insulin or glucose levels (Després, Poehlman *et al.* 1987, McDevitt, Bott *et al.* 2001). Therefore, another aim of the second experimental study was to investigate the impact of five days supplementation on plasma lipids and insulin sensitivity.

Systemic reviews and randomized controlled trials have showed that RTUFs are very effective and safe in the treatment of severe malnutrition in children (Briend, Lacsala *et al.* 1999, Collins and Sadler 2002, Dossou, Ndour *et al.* 2003, Sandige, Ndekha *et al.* 2004, Ciliberto, Sandige *et al.* 2005, Ashworth 2006, Brewster 2006, Bhutta, Ahmed *et al.* 2008, Gera 2010). Some studies have demonstrated a higher increase in energy intake (808 vs. 573kJ/kg/day) and weight gain (15.6 vs.10.1g/kg/day) achieved by supplementation with RTUF compared to the changes induced by consumption of a traditional high-energy drink based on milk (F-100) (Diop, Dossou *et al.* 2003). RTUFs have been used to supplement the dietary intake of moderately malnourished children (Kuusipalo, Maleta *et al.* 2006, Defourny, Seroux *et al.* 2007, Matilsky, Maleta *et al.* 2009, Nackers, Broillet *et al.* 2010, Patel, Sandige *et al.* 2011). RTUF have been found to be effective in preventing onset of malnutrition in non-wasted children from the areas of food insecurity (Defourny, Minetti *et al.* 2009, Isanaka, Nombela *et al.* 2009, Isanaka, Roederer *et al.* 2010, Huybregts, Houngré *et al.* 2012). The majority of the existing evidence on the use of community-based management of uncomplicated, severe acute malnutrition has emerged from studies conducted in Africa in emergency settings (Gera 2010). There are no studies from Asia exploring the effectiveness of RTUF and other industrialised foods in the treatment of children with moderate acute malnutrition (MAM), where MAM is most prevalent (Lazzerini, Rubert *et al.* 2013). There was a need to generate more evidence regarding the efficacy of RTUF and other locally available ready to use supplements in non-emergency settings particularly in a Pakistani context. There was no evidence comparing the impact of RTUF and proprietary liquid supplements on the weight gain in mild to moderate malnourished children between the ages of 5-10 years. Therefore, the third experimental study aimed to explore the efficacy of RTUF and LRUS in

promoting weight gain and appetite regulation in mild to moderate underweight children from Pakistan.

The first experimental chapter provides insight into the appetite, metabolic, hormonal and gastric emptying responses to the HENSD consumption and thus investigated mechanisms related to impaired adjustment of energy intake commonly seen during subsequent intake. We found that HENSD increased the net energy intake by about half the energy value of the supplement and thus confirmed previous evidence (Stratton, Stubbs *et al.* 2008) that suppression of energy intake during meals consumed after supplementation with HENSD is only partial. The novel finding of this study is that the appetite suppressive response following the HENSD intake was shorter than the response of metabolic and hormonal appetite regulators, with the result that compensation for the energy provided by the HENSD is partial and evident only during immediate hours after the supplementation. In consistence with other studies reporting an effect of energy content of preload on appetite responses (Karl, Young *et al.* 2013), we found that during 60 minutes post-supplementation hunger suppression and enhancement in satiety was more profound after HENSD than PLACEBO intake.

Several mechanisms could have been responsible for these differences. Enhanced satiety and reduced hunger found in the HENSD trial during the immediate post supplementation period can be attributed to significantly higher plasma concentrations of PYY and CCK, gastrointestinal hormones known to elicit anorexigenic effects, (Chaudhri, Field *et al.* 2008, Suzuki, Simpson *et al.* 2010), and insulin, known to play a role in appetite regulation of lean individuals (Flint, Gregersen *et al.* 2007). Even though the plasma concentration of potential appetite regulators such as CCK, PYY (Chaudhri, Field *et al.* 2008, Suzuki, Simpson *et al.* 2010) and insulin (Flint, Gregersen *et al.* 2007) persisted to be different beyond breakfast, neither subjective appetite measures during pre-lunch nor energy intake during lunch, differed between the HENSD and PLACEBO trials. The finding of dissociation between appetite measures and expected action of these appetite regulators is consistent with some other studies, which found that appetite scores are not always

related to CCK (De Graaf, Blom *et al.* 2004) or PYY (Doucet, Laviolette *et al.* 2008) and insulin concentration (Gielkens, Verkijk *et al.* 1998). Thus, data obtain in our and some other studies suggests that subjective appetite measures might be a better correlate of energy intake than circulating levels of appetite hormones.

The thermic effect of food is another potential regulator of the appetite sensations, including satiety. Some studies have found that higher-diet induced thermogenesis was correlated with reduced hunger and more satiety (Crovetti, Porrini *et al.* 1998, Mansour, Ni *et al.* 2012). While others have not (Ravn, Gregersen *et al.* 2013). Since even a small positive association between TEF and satiety could have clinical implications for weight gain during supplementation with HENSD, this study also aimed to find out whether following HENSD consumption appetite responses were related to the postprandial energy expenditure. We found that during 60 minutes post-supplementation (pre-breakfast period), energy expended above resting metabolic rate (a measure of thermogenesis) was significantly higher in the HENSD than the PLACEBO trial. This coincided with hunger and desire to eat being significantly lower, and satiety and fullness significantly higher in the HENSD than the PLACEBO trial. Thus, our data hardly support notion of the importance of thermogenesis in appetite and satiety regulation. Future research is required to look at how the composition and frequency of the HENSD supplementation may modify the compensation for the energy provided by the HENSD supplements in men and in the diseased men and women suffering from malnutrition.

To achieve the first aim of the second experimental study HENSD was supplemented for five days and the impact on energy intake consumed during immediate hours after supplementation, and during the *ad libitum* buffet breakfast, lunch and dinner consumed on the consecutive day was measured. It was found that during five days of supplementation with HENSD the averaged total energy intake, including energy provided from the drink, was significantly higher. However, during supplementation we observed an acute suppression of the energy intake immediately after HENSD supplementation

in the evening meal. It was observed that 43% of the energy provided by the HENSD is compensated by eating less during the evening meal in HENSD supplementation. Previous studies have also reported energy compensation (Mattes 2006, Stratton, Stubbs *et al.* 2008, Mattes and Campbell 2009, Almiron-Roig, Palla *et al.* 2013) and the first experimental study of this thesis found that exactly the same 43% of energy was compensated by eating less during the breakfast after HENSD supplementation (Fatima, Gerasimidis *et al.* 2013). A significant decrease in energy intake of the evening meal was seen during four days after consumption of the HENSD but no differences in the energy intake during the *ad libitum* breakfast, lunch and dinner was observed on the consecutive day. This suggests that the provision of HENSD in the evening for five consecutive days suppresses intake only immediately during the evening meal and does not suppress the appetite on the following day. Therefore, the results from second experimental study confirmed that the provision of the HENSD could be expected to increase daily energy intake as compensation for the energy provided was partial, immediate, short lived and has no impact on the appetite and energy intake during the next day.

The second experimental study investigated the impact of HENSD supplementation for five consecutive days on cardio-metabolic risk factors as the HENSD supplements are expected to induce a positive energy balance. The results of the second experimental study demonstrated that positive energy balance induced by HENSD supplementation can be expected to reduce insulin sensitivity, but have no impact on fasting lipids and postprandial lipaemia, insulinaemia and glycaemia. The results of our study are in corroboration with other studies which demonstrated that the acute positive energy balance achieved by hypercaloric feeding does not affect VLDL-TAG concentration and plasma insulin concentrations (Bellou, Maraki *et al.* 2013, Smith, Magkos *et al.* 2013). The findings from our study suggest that the deleterious effects of the positive energy balance, achieved by overfeeding, on insulin homeostasis manifests rapidly. These results indicate that insulin resistance is sensitive to acute (5 days) moderate increase in net energy balance whereas lipid/ TAG metabolism are not. This increase in fasting insulin levels could be simply because of higher carbohydrate load ingested during the HENSD

supplementation, the mechanisms for this finding need to be investigated further. The results of our first two experimental studies provide information for clinical practice, while prescribing supplements to the malnourished individuals. As in both our studies, we observed that 43% of the energy provided by the HENSD is compensated by eating less during the consecutive meal.

Current evidence suggests that the use of nutritional supplements increases fat body mass (Lazzerini, Rubert *et al.* 2013). Rapid gain in weight due to adipocyte deposits (rather than increase in lean body mass) may lead to adult obesity, adiposity, diabetes and cardiovascular risk factors especially in malnourished subjects (Adair, Fall *et al.* 2013). Unfortunately, there is little evidence to evaluate possible side effects of energy dense foods and long-term outcomes of the treatment in malnourished children (Lazzerini, Rubert *et al.* 2013). As HENSD consumption is expected to induce the positive energy balance, we investigated the impact of short-term supplementation (five days) with HENSD on cardio-metabolic risk factors. However, more research is required to investigate the impact of longer duration of supplementation or different degrees of supplementation with the HENSD on lipoprotein, glucose and insulin metabolism in the healthy and obese subjects. It should be noted that we have performed this experimental study on very healthy young participants and they had very high insulin sensitivity even after five days of the HENSD supplementation. Therefore, future studies are required to investigate the impact of these HENSD supplements on elderly ill patients in which insulin sensitivity and lipid profile is in most cases already disturbed due to aging (Chang and Halter 2003, Refaie, Sayed-Ahmed *et al.* 2006). The detrimental impact of continuous supplementation should also be considered; as acute short-term positive energy balance have found to enhanced the insulin resistance which if unchecked may predisposes to type 2 diabetes mellitus and CVD.

The third experimental study on mild to moderate underweight children from Pakistan explored the efficacy of RTUF and liquid supplements, on promoting weight gain and appetite regulation, in mild to moderate malnourished children

between the ages of 5-10 years. We found that both RTUF and LRUS given in the community showed similar impact on promoting weight gain, height, and skinfold thickness in mild to moderate underweight children. However, the overall rate of the weight gain was lower than expected. Previous studies have also documented that the observed growth rate was substantially less as compared to their predicted growth rate after the supplementation (Manary, Ndkeha *et al.* 2004). The reason might be the acute suppression of appetite immediately after the supplementation as has been observed by us, in our first two experimental studies. It was found that the children might eat less than from their regular habitual diet. Other factors might be the sharing of supplements in the school and increased energy expenditure because of physical activity. However, when compared to a control group a significantly higher rate of weight gain (g/kg/d) was observed in RTUF and LRUS groups.

Studies have documented significant improvement in nutritional characteristics when the intervention group was compared with a control group (Thakwalakwa, Ashorn *et al.* 2010, Grellety, Shepherd *et al.* 2012, Huybregts, Houngré *et al.* 2012). This suggests that although RTUFs led to clinically significant benefits in the number of children recovering. However, RTUFs did not reduce mortality or progression to severe acute malnutrition and also induced vomiting (Lazzerini, Rubert *et al.* 2013). We also found that the children in the supplement group belong to low socio-economic status. Their parents had a low monthly income, spent less on food items, had larger family sizes and the mothers were illiterate. Previous studies have documented that thinness and stunting was significantly higher among the children with illiterate parents as compared to educated parents and parental education is also identified as a predictor of under nutrition (Mushtaq, Gull *et al.* 2011, Emamian, Fateh *et al.* 2013). Similarly thinness and stunting were also found to be significantly higher in children living in crowded houses and having more siblings, both of these factors are considered to be an indirect predictor of lower socio-economic status (Mushtaq, Gull *et al.* 2011). The structured interviews with the parents/carers showed that 29% of the children from RTUF group and 41% of the children from LRUS group were dissatisfied with the consistency and the taste of the supplement. In one of the previous study it was

found that 43% of the children were dissatisfied with the taste of the RTUF which is higher compared with our study (Ali, Zachariah *et al.* 2013).

Despite of the problems with the taste of the supplements 70% of the parents/carers from RTUF and LRUS group perceived these supplements to be beneficial for their children. This is less compared with another study in which 91% of the caregivers perceived RTUF to be of therapeutic benefit to their severe, acutely malnourished or moderate acutely malnourished children (Ali, Zachariah *et al.* 2013). We found that there was no significant difference in any of the appetite measure between RTUF and LRUS groups before and after the supplementation. This finding is also in corroboration of our first two experimental studies that the appetite suppressive action of the supplements was only short-lived. Further dietary assessment studies are necessary, to investigate the impact of RTUF and the liquid ready to drink supplement on the habitual diet substitution in children suffering from different disease conditions as the appetite suppressive action of the HENSD might be different in the children suffering from diseases as compared to the mildly malnourished but healthy children from the community settings. As currently very little evidence is available on the possible side effects of energy rich, lipid based nutritional supplements such as RTUF and their long-term outcomes on the malnourished children so future research is also required in this regard.

We chose “underweight” healthy young females (BMI: 17-20 kg/m²) to simulate relative under-nutrition and to allow a sampling regime and study design which would not be ethical in a paediatric patient group. The first experimental study investigated the level of energy intake compensation in the lean adult women following HENSD consumption. The question being how it relates to the response of a range of hormonal and metabolic appetite regulators. The second study investigated the time scale and level of the energy intake compensation after five days of supplementation with HENSD in the afternoon. From both these studies we observed that 43% of the energy provided by the HENSD is compensated by eating less during consecutive meal. This informs clinical practice about prescribing supplements to malnourished individuals. Later on, the third non-invasive experimental study

was performed on mild to moderate malnourished children to explore the efficacy of nutritional supplements in underweight school going children, between the ages of 5-10 years. It measured appetite before the start of the first supplement and before the provision of last supplements. We have observed that the overall rate of weight gain was lower than expected and at least 2/3 of the energy ingested appears to have been compensated for by eating less at other time, as has been observed in the first two experimental studies.

As all studies, the experimental studies presented in this thesis have some limitations. In first two experimental studies, we recruited slim but healthy young women in order to test the likely effect of the HENSD in younger malnourished patients. Most prior research has been done in the elderly. Thus, results obtained in this study are most likely to be relevant to adolescents and younger adults and cannot necessarily be generalized to truly malnourished individuals or to males. In first experimental study only short term regulation of appetite and energy intake was measured, which might not be generalizable to clinical or home settings where HENSD are prescribed for the long term. In the first experimental study, HENSD was consumed in the morning, prior to breakfast while in clinical practice intake happens during the day. However, the first experimental study investigated mechanisms responsible for the partial energy compensation and so we used a pre-load type design requiring the intake in the morning (Blundell, De Graaf *et al.* 2010).

We appreciate that some other potential short-term appetite regulators such as glucagon like peptide -1 and ghrelin could have also been investigated in the first experimental study. The decision to measure PYY and CCK was based on the fact that both PYY and CCK are related to the subjective measures of appetite and food intake (De Graaf, Blom *et al.* 2004, Crespo, Cachero *et al.* 2014). Numerous studies have found the suppressing impact of CCK on food intake or the subjective feelings of hunger (Delzenne, Blundell *et al.* 2010). Moreover PYY may be involved in the “ileal brake” by slowing down gastric emptying (Huda, Wilding *et al.* 2006) and CCK also slows gastric emptying rate (Delzenne, Blundell *et al.* 2010). Appetite hormones were not investigated in the second experimental study because the main aim of the study was to

confirm that the differences in appetite and energy intake (compensation) are short lived. In our first experimental study we observed that the supplement works most likely because of both partial compensation and the disturbance in the appetite measures were short lived. We also noted that responses of the metabolic and hormonal appetite regulators were measured only in a subset of the study participants. This decision was made based on the power calculations identifying that the investigation of nine participants would allow us to pick up physiologically meaningful and significant differences in postprandial PYY, CCK, glucose, insulin, concentrations at power of 85%. Moreover, studies have demonstrated that appetite scores are better correlates of energy intake than the circulating levels of appetite hormones like PYY or ghrelin (Doucet, Laviolette *et al.* 2008). For the same reason the appetite, regulators were not investigated in the third study.

The strengths of first experimental study was the study design which included the use of a preload paradigm. This is accepted as a useful methodology to assess the effects of food or drink on appetite and energy intake in the short term under controlled condition (Almiron-Roig, Palla *et al.* 2013). This design overcomes misreporting of food intake and thus allows precise measurement of energy intake. To further improve the quality of energy intake data, food included in breakfast and lunch and left over of breakfast and lunch were weighted and analyzed by two researchers. Differently from most of the other pre-load studies, our study investigated the impact of the HENSD consumption on the energy intake compensation in the longer term, and thus measured impact on energy intake during two consecutive meals. This allowed us to find out that appetite suppressive responses and energy compensation happens close to the HENSD supplementation, and disappears quickly with time. It should be noted however that the laboratory environment was artificial, and thus energy intake could have been influenced by deviations from normal eating behaviours, timing and food availability (Hetherington, Anderson *et al.* 2006). In the second experimental study the energy intake during the evening meal was measured under free living conditions and previous studies have documented that food consumed in a laboratory setting under controlled conditions is in a reasonable approximation of energy intake as measured

under free living conditions (Obarzanek and Levitsky 1985). Moreover, in our study the free-living conditions also provided time flexibility for dinner, which varies considerably among the individuals and is not possible under the laboratory conditions. Additionally participants remained to have sedentary in the metabolic suite for the whole duration of the trial which was not in correspondence to habitual interaction (Poppitt, McCormack *et al.* 1998).

The third experimental study was limited by the fact that we were unable to measure the average daily energy intake of the children from their habitual diet, at the baseline, and during the intervention period, to indicate whether liquid ready to drink supplement or RTUF supplemented or displaced habitual energy intake. Appetite was measured using visual analogue scales before the start of first supplement and before the provision of last supplement but we were unable to measure the dietary intake of participants before intervention or during intervention. Moreover, the questions regarding the taste and acceptability of the supplements were put to the parents/carers of the children and the reported responses might not reflect the actual perception of the children themselves. Great care should be taken while translating the results from this study to different local settings as contextual factors, such as local preferences might influence the adherence to the intervention. The results from this study are from a general population of children with mild to moderate malnutrition in the absence of any disease, therefore the results should not be translated directly to children with MAM with a diagnosis of some disease condition. The strength of this study was that the enrolment rate for this study was high and group allocation was random. Groups were similar at enrolment, compliance with the supplementation appeared well, follow up for both the groups were identical and no loss to follow up was observed during the intervention.

We have performed the HOMA technique for measuring insulin resistance. The impact of HENSD supplementation on insulin sensitivity can be checked with better techniques like euglycemic hyperinsulinemic clamp or the frequently sampled intravenous glucose tolerance test (FSIGT). Although HOMA technique is relatively inexpensive and easy it did not provide nearly

the same degree of information as FSIGT or clamp technique (Trout, Homko *et al.* 2007), but these latter techniques are invasive and unpleasant and not suitable for children in a population setting. The strengths of this study include the participants' attendance, as none of the participants was eliminated due to non-compliance of supplementation. All participants consumed both the HENSD and the PLACEBO supplements in the presence of the researchers ensuring compliance.

In the third experimental study the short term supplementation aimed only to assess the benefits of supplementation in mild to moderate malnourished children. As dietary counselling is a fundamental and effective part in the treatment of mild to moderate malnutrition (Ashworth and Ferguson 2009), the parent/carer obtained dietary counselling on the completion of the study. Parents of all the participants were educated about the role of proper dietary requirements in school-going children as well as the importance of healthy diet in growing children. Dietary supplementation beyond the duration of study was beyond the scope of this study, however, this study highlighted the level of malnourishment in the government schools and in addition parents were given much needed information about the nutritional level of the kids and ways to improve it by improving their dietary intake. For effective, long term benefits there is a need to place more focus on the improvement of the adequacy of home diet by nutritional education and by improving nutritional habits especially in the settings where food is available which may ensure maintained and sustainable improvement in prevention and treatment of mild to moderate malnutrition.

Poverty seems to be the major contributing factor for the malnourished status of these children. Therefore it is recommended that Government should start a programme for food provision, "healthy school meals" for the school going children and International donor agencies should help the economically crippled countries to address this manageable problem. This should also be augmented by educational programmes for the masses especially focusing on the parents having poor socioeconomic status.

Conclusions

- HENSD consumed either in the morning as a single dose or in the evening for several days in slim healthy females induces only partial reduction in habitual food intake and thus may benefit daily energy intake and body weight gain. Further studies are required to investigate the impact of these supplements on energy intake in malnourished ill individuals.
- In the short term HENSD, increased net energy intake by about half the energy value of the supplement. This finding should be taken into consideration while estimating the expected enhancement in weight gain of the malnourished individual.
- HENSD when consumed before breakfast by slim healthy females had partial and a relatively short-lived suppressive action on energy intake, but metabolic and hormonal effects persisted longer, though their impact on appetite or energy intake was only during the hours immediately after supplementation.
- Short-term supplementation with HENSD can be expected to reduce insulin sensitivity but had no impact on the fasting lipids and postprandial lipaemia, insulinaemia and glycaemia in slim healthy females.
- RTUF and LRUS given in a community setting in Pakistan had similar impact on improving the nutritional status in mild to moderate underweight children, but the overall rate of weight gain was lower than expected. This implies partial compensation for energy provided by the supplements.

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8 Appendices

Appendix I a, b, c

UNIVERSITY OF GLASGOW

FACULTY OF MEDICINE ETHICS COMMITTEE FOR NON
CLINICAL
RESEARCH INVOLVING HUMAN SUBJECTS

APPLICATION FORM FOR ETHICAL APPROVAL

NOTES:

THIS APPLICATION FORM SHOULD BE TYPED NOT HAND WRITTEN.

**ALL QUESTIONS MUST BE ANSWERED.
“NOT APPLICABLE” IS A SATISFACTORY ANSWER WHERE APPROPRIATE.**

FACULTY PROJECT CODE:

Project Title

Effect of high energy nutritional supplement drinks on appetite, appetite hormones and dietary intake

Date of submission 07.05.11

Name of all person(s) submitting research proposal

Dr Dalia Malkova¹, Dr Konstantinos Gerasimidis², Professor Charlotte Wright³

Position(s) held

¹Senior Lecturer,

²Konstantinos Gerasimidis- Lecturer

³Charlotte Wright- Professor

Department/Group/Institute/Centre

¹Clinical Specialities, Medical School, Human Nutrition Section

²Academic Unit of Life Course in Nutrition and Health, Human Nutrition Section

³Clinical Specialities, Medical Scholl, PEACH Unit

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Name of Principal Researcher (if different from above e.g., Student's Supervisor)

Position held

Undergraduate student project

No

Postgraduate student project

Yes, PhD degree

If Yes, please state degree being undertaken

1. Describe the purposes of the research proposed. Please include the background and scientific justification for the research. Why is this an area of importance?

High energy nutritional supplement drinks (HENSD) are widely promoted by food industry and used in malnourished children and adults with a significant cost to the UK National Health Service (£99 million pa) <http://www.ic.nhs.uk/pubs/prescostanalysis> 2007. These drinks aim to maximise energy/nutrient intake on top of the normal habitual diet of the patient, to prevent or correct disease associated undernutrition and improve clinical outcome, function and quality of life (1). Although these, mainly dairy supplements, mimic ordinary food their composition is significantly different. They usually have a substantially higher energy and protein content and are supplemented with large amounts of micronutrients. For example Scandishake[®], a mainstream dairy HENSD has 2.5 kcal/ml, 5% protein, 13% fat, 230 mg of phosphorus and an osmolarity of 880 mOsmol/L compared to cow's milk which has 0.7 kcal/ml, 3% protein, 3.7% fat and 95mg of phosphate and an osmolarity of 270 mOsmol/L. As energy content, nutrient composition, concentration of electrolytes and osmolarity (i.e. gastric emptying speed and gastrointestinal absorption) are well established regulators of appetite, it is important to study how consumption of HENSD impacts on energy intake during subsequent meals.

Although there is some evidence to support HENSD use in institutionalised elderly (2;3), their efficacy in paediatric patients has been barely studied, but one randomized trial found that use of HENSD in paediatric patients with cystic fibrosis was ineffective in improving markers of nutritional status or lung function (4). This suggests that HENSD can be partially effective and these findings may be attributed to the known tendency for healthy children to regulate their habitual dietary/energy intake more closely than adults, so that after the consumption of a high energy drink they eat less at meals in compensation (5). Relevant studies in paediatric patients are missing although one study in patient group found no satiating effect of a high energy preload in children with weight faltering (6). No study has yet specifically looked at the extent to which patients of any age regulate intake after taking HENSD. Evidence is also needed on how HENSD change appetite and food intake and their impact on regulators of appetite such ghrelin (7), peptide YY (8), leptin (9), insulin (10) as well as changes in postprandial energy expenditure (diet induced thermogenesis) (11) and digestion physiology (gastric emptying) (12).

We speculate that the use of high energy supplements may suppress voluntary oral intake, particularly in children, and this compensation is regulated through the impact of these supplements on objective and subjective markers of appetite, postprandial energy expenditure and digestion physiology. In view of the lack of prior work in this area, this proof of concept study aims first to describe the impact in adults of the bolus consumption of a mainstream HENSD on energy intake/expenditure, metabolic responses and appetite regulation. In particular we will try to answer the following questions:

1. To what extent do healthy, lean adults compensate for the energy consumed in an ultra high energy HENSD, and how does this relate to subjective measures of appetite.
2. What is the impact of an ultra high HENSD on the response of appetite hormones, gastric emptying and diet induced thermogenesis.
3. To what extent do changes in appetite hormones gastric emptying and diet induced thermogenesis correlate with or explain the extent of individual energy compensation?

2. Describe the design of the study and methods to be used. Include sample size and the calculation used to determine this. Statistical advice should be obtained if in doubt.

Study Design.

This will be a single blind randomised controlled trial, with participants acting as their own controls. Participants will undertake two main experimental tests, 1-4 weeks apart using two different preloads: a) A high energy liquid mainstream nutritional supplement (Scandishake, Chocolate, Nutricia®) made up with full fat milk and b) The same volume of a low calorie fat free milk drink. Both preloads will be composed using the same flavouring and colouring. The order of the drinks will be randomly allocated and the participants will be blinded to type of preload. In both experimental test blood samples will be collected and data on appetite obtained in the fasted state and at 30, 60, and 90 minutes after supplement and at 30, 60, 90, 120 minutes post-breakfast. Two hours post breakfast (3.5 hours post pre-load) the participants will be offered *ad libitum* buffet lunch and the amount of food ingested will be recorded. Participants will be asked to keep a diary of their dietary intake using weighed records for the rest of the day several time at after *ad libitum* buffet breakfast. Energy consumed during breakfast and lunch and the rest of the day will be also measured. Participants will record of all food and drink consumed and refrain from physical activity for 2 days preceding their first visit to the laboratory and will be asked to replicated this prior to the subsequent second visit.

Power calculation: 19 subjects will give 80 % power at 5% level to detect a difference of around 2/3 of standard deviation between supplements for any one measure. This would be sufficient to detect a difference of around 500 KJ energy intake.

Methods

Anthropometry

Body weight and height will be measured using electronic scales and a rigid height measure. Triceps and sub scapular skinfolds will be measured using Holtain callipers and summed.

Energy intake

The energy consumed in the preload and at each meal will be calculated using dietary analysis software (Windiets 2005, The Robert Gordon University, Aberdeen, Scotland, UK).

Appetite measurements

Appetite sensations will be measured with validated appetite questionnaires (13). Participants will be asked to rate measures of appetite on 100mm lines.

Metabolic rate and diet induced thermogenesis. Ventilated hood system (Oxycon Pro, Jaeger GmbH, Hoechberg, Germany). A ventilated hood will be placed over the volunteer's head to allow analysis of expired gas. Metabolic rate in the fasted state and after drink and after breakfast will be calculated.

Appetite hormones. Venous blood samples will be collected in 9 ml vacutainers containing EDTA. Plasma acylated ghrelin and peptide YY will be determined by radioimmunoassay (Millipore, Watford, UK).

Gastric emptying. Participants will be asked to consume of 1500 mg of plasma paracetamol will be measured as indirect marker for the rate of liquid and semisolid gastric emptying (15).

Statistical analysis. The total areas under the curve for appetite measurement or appetite biomarker response versus time curve (AUC) will be used as summary measures of the post load, the post-breakfast and post-lunch responses. Difference in energy intake measured in two tests will be compared by paired t-test or 1 Two-way repeated measures ANOVA (trial x time) will also be used to compare responses of appetite and appetite biomarkers, and metabolic rate between tests and evaluate changes over time. *Post hoc* Tukey's tests will be used to identify where differences lay.

3. Describe the research procedures as they affect the research subject and any other parties involved.

It should be clear exactly what will happen to the research participant, how many times and in what order.

On the day of the experimental test the participants will come to the metabolic investigation suite at ~ 08:30. The participants will be allowed 10 minutes to relax and acclimatize. Following measurements of anthropometry, body composition and resting metabolic rate (RMR), a cannula will be inserted in an antecubital vein, and a baseline blood sample will be collected and appetite questionnaires will be completed. Following this the participants will be offered 240 ml of one of the preload liquid meals and dose of oral paracetamol (1500 mg in 100 ml of water) and be asked to consume it within 5 minutes. Further appetite questionnaires will be completed and blood samples will be obtained at 30, 60, and 90 minutes. Metabolic rate will be measured for the duration of 20 minutes after each blood sampling. An *ad libitum* buffet breakfast will then be provided and the amount of food ingested will be recorded. Blood samples will be collected at 30, 60, 90, 120 minutes post-breakfast and metabolic rate will be measured after each sampling. Two hours post breakfast (3.5 hours post pre-load) the participants will be offered *ad libitum* buffet lunch and the amount of food ingested will be recorded by investigator. Participants will be asked to keep a diary of their dietary intake using weighed records for the rest of the day. Scales and diaries will be provided.

Thus, participants will be required to participate in two experimental trials lasting for ~6 hours. Prior their first trial they will be needed to come for anthropometric measurements, completion of health questionnaires and to obtain scales and food diaries (this will take ~45 minutes)

Tests will be conducted according to the ‘Code of Practice for Conducting Experiments on Non-patient Human Volunteers (including Handling and Disposal of Human Blood, Urine and Sputum), re-approved by the University Ethics Committee on October 26, 2001

4. How will potential participants in the study be (i) identified, (ii) approached and (iii) recruited?

Give details for cases and controls separately if appropriate:

Participants will be healthy adults with a body mass index $<20 \text{ kg/m}^2$. We will use “underweight” healthy adults to simulate relative under-nutrition and to allow a sampling regime and study design which would not be ethical in a paediatric patient group. Participants will be recruited by means of an advertisement leaflet and word of mouth in the campus of the University of Glasgow, and other public areas. Subjects who will be willing to participate will be screened by means of a detailed medical history to exclude chronic illness, eating disorders, and major gastrointestinal operations. All participants will provide written informed consent.

5. What are the ethical considerations involved in this proposal? (You may wish for example to comment on issues to do with consent, confidentiality, risk to subjects, etc.)

Blood sampling

Venous cannulation incurs a minor risk of bruising. There is also a risk of thrombophlebitis (inflammation of the vein) but this risk is small in non-smokers with no history of coagulation disorders. Plastic or air embolism can occur if incorrect cannulation technique is employed but good practice minimizes this risk. Some individuals may feel faint when giving blood.

6. Outline the reasons why the possible benefits, to be gained from the project, justify any risks or discomforts involved.

The risks associated with participating in this study are very small. Subjects will receive feedback on their habitual energy and macronutrient intakes and also on the impact of two supplements on energy intake during the rest of the day. Participants also will receive antropometric data so they will benefit from the study personally. In addition, the findings of the study will help understanding to what extent do healthy, lean adults compensate for the energy consumed in an ultra high energy HENSD, and how does this relate to subjective measures of appetite and appetite hormones.

7. Who are the investigators (including assistants) who will conduct the research? What are their qualifications and experience?

Dr Dalia Malkova, Dr K. Gerasimigis and Prof C. Wright have extensive experience in conducting in carrying out human studies, without incident. Dr Malkova has ~12 years of experience in conducting human metabolic studies and has ~7 years experience in venepuncture and cannulation. Dr Gerasimidis has extensive experience in carrying out dietary assessment. A PhD student Sadia Fatima and Prof Wright are a medical practitioner. Sadia Fatima is PhD student, working under the supervision of Dr Malkova, C. Wright and K. Gerasimidis will be trained in all testing procedures involved.

8. Are arrangements for the provision of clinical facilities to handle emergencies necessary? If so, briefly describe the arrangements made.

The risks associated with the procedures are extremely small. Tests will be conducted according to the 'Code of Practice for Conducting Experiments in Non-Patient Human Volunteers (including Handling and Disposal of Human Blood, Urine and Sputum)', re-approved by the University Ethics Committee on October 26, 2001.

9. In cases where subjects will be identified from information held by another party (for example, a doctor or hospital), describe how you intend to get this information. Include, where appropriate, which Multi Centre

Research Ethics Committee or Local Research Ethics Committee will be applied to.

N/A

10. Specify whether subjects will include students or others in a dependent relationship.

Some undergraduate or postgraduate students may be recruited but will not be placed under any pressure from staff to participate in the study.

11. Specify whether the research will include children or people with mental illness, disability or handicap. If so, please explain the necessity of involving these individuals as research subjects, and include documentation of the suitability of those researchers who will be in contact with children (eg Disclosure Scotland).

N/A

12. Will payment or any other incentive, such as a gift or free services, be made to any research subject? If so, please specify and state the level of payment to be made and/or the source of the funds/gift/free service to be used. Please explain the justification for offering an incentive.

No

13. Please give details of how consent is to be obtained. A copy of the proposed consent form, along with a separate information sheet, written in simple, non-technical language MUST ACCOMPANY THIS PROPOSAL FORM.

Subjects will be provided with a 'Volunteer Information Sheet' to read and will be given a verbal explanation of the study and the opportunity to ask questions before providing written consent to participate in the study. Subjects will also complete health history questionnaires at this time (formed attached).

14. Comment on any cultural, social or gender-based characteristics of the subject, which have affected the design of the project or may affect its conduct.

Participants will be “underweight” healthy adults with a body mass index <20 kg/m². The subjects will be aware that they have been invited to participate in this study on this basis.

15. Please state who will have access to the data and what measures will be adopted to maintain the confidentiality of the research subject and to comply with data protection requirements e.g. will the data be anonymised, how will it be stored, how will access be restricted, and for how long will it be retained?

The information obtained will be anonymised and individual information will not be passed on to anyone outside the study group.

16. Will the intended group of research subjects, to your knowledge, be involved in other research? If so, please justify.

Not during the duration of the study.

17. Proposed starting date

June 2011

Expected completion date

June 2015

18. Please state location(s) where the project will be carried out.

Subject testing take place in the Metabolic Investigation Suite at human Nutrition Section, Yorkhill Hospitals

19. Please state briefly any precautions being taken to protect the health and safety of researchers and others associated with the project (as distinct from the research subjects) e.g. where blood samples are being taken

All samples will be handled according to the ‘Code of Practice for Conducting Experiments on Non-patient Human Volunteers (including Handling and Disposal of Human Blood, Urine and Sputum)’.

20. Please state all relevant sources of funding or support for this study

The student who is a graduate in medicine has a secured PhD scholarship from the University in Kyber, Pakistan which pays tuition fees and monthly stipend for 3 years, but no bench fees.

21 a). Are there any conflicts of interest related to this project for any member of the research team? This includes, but is not restricted to, financial or commercial interests in the findings. If so, please explain these in detail and justify the role of the research team. For each member of the research team please complete a declaration of conflicts of interest below.

There are no conflicts of interest

Researcher:

Name: _____ conflict of interest

Yes / No

If yes, please detail below

Researcher:

Name: _____ conflict of interest

Yes / No

If yes, please detail below

Researcher:

Name: _____ conflict of interest

Yes / No

If yes, please detail below

Researcher:

Name: _____ conflict of interest

Yes / No

If yes, please detail below

21 b). If there are any conflicts of interest, please describe these in detail and justify conducting the proposed study.

N/A

22. How do you intend to disseminate the findings of this research?

We will use “underweight” healthy adults to simulate relative undernutrition and to allow a sampling regime and study design which would not be ethical in a paediatric patient group. Therefore

Findings will be disseminated as presentation in the Nutrition Society meeting and as peer reviewed paper.

Findings will also be disseminated to clinicians and dieticians.

I confirm that have read the University of Glasgow’s Data Protection Policy [<http://www.gla.ac.uk/services/dpfoioffice/policiesandprocedures/dpa-policy/>]

Please initial box

Signed _____

Date

(Proposer of research)

For student projects

I confirm that I have read and contributed to this submission and believe that the methods proposed and ethical issues discussed are appropriate.

I confirm that the student will have the time and resources to complete this project.

Signed _____

Date

(Supervisor of student)

Reference List

1. Stratton RJ, Elia M. Encouraging appropriate, evidence-based use of oral nutritional supplements. Proc Nutr Soc 2010;69:477-87.

2. Turic A, Gordon KL, Craig LD, Ataya DG, Voss AC. Nutrition supplementation enables elderly residents of long-term-care facilities to meet or exceed RDAs without displacing energy or nutrient intakes from meals. *J Am Diet Assoc* 1998;98:1457-9.
3. Stratton RJ, Elia M. Are oral nutritional supplements of benefit to patients in the community? Findings from a systematic review. *Curr Opin Clin Nutr Metab Care* 2000;3:311-5.
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6. Kasese-Hara M, Wright C, Drewett R. Energy compensation in young children who fail to thrive. *J Child Psychol Psychiatry* 2002;43:449-56.
7. Lim CT, Kola B, Korbonits M, Grossman AB. Ghrelin's role as a major regulator of appetite and its other functions in neuroendocrinology. *Prog Brain Res* 2010;182:189-205.
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9. Kolaczynski JW, Ohannesian JP, Considine RV, Marco CC, Caro JF. Response of leptin to short-term and prolonged overfeeding in humans. *J Clin Endocrinol Metab* 1996;81:4162-5.
10. Flint A, Gregersen NT, Gluud LL *et al*. Associations between postprandial insulin and blood glucose responses, appetite sensations and energy intake in normal weight and overweight individuals: a meta-analysis of test meal studies. *Br J Nutr* 2007;98:17-25.
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12. Bowen J, Noakes M, Trenerry C, Clifton PM. Energy intake, ghrelin, and cholecystokinin after different carbohydrate and protein preloads in overweight men. *J Clin Endocrinol Metab* 2006;91:1477-83.
13. Flint A, Raben A, Blundell JE, Astrup A. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *Int J Obes Relat Metab Disord* 2000;24:38-48.
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15. Naslund E, Bogefors J, Gryback P, Jacobsson H, Hellstrom PM. Gastric emptying: comparison of scintigraphic, polyethylene glycol dilution, and paracetamol tracer assessment techniques. *Scand J Gastroenterol* 2000;35:375-9.

Send completed signed form to

Stuart Morrison
 Room 330, Wolfson Medical School Building
 University Avenue
 Glasgow
 G12 8QQ
 Tel: 0141 330 5519
 Fax: 0141 330 4758

Please also send electronic versions of completed form and all other paperwork to

Stuart.Morrison@glasgow.ac.uk



**College of Medical, Veterinary & Life Sciences Ethics Committee for Non
Clinical Research Involving Human Subjects**

APPLICATION FORM FOR ETHICAL APPROVAL

NOTES:

THIS APPLICATION FORM SHOULD BE TYPED NOT HAND WRITTEN.

**ALL QUESTIONS MUST BE ANSWERED.
“NOT APPLICABLE” IS A SATISFACTORY ANSWER WHERE
APPROPRIATE.**

PROJECT CODE:

Project Title

Impact of High Energy Nutritional Supplement Drink (HENSND) consumed for five days on appetite, metabolic appetite regulators, resting and post prandial energy expenditure, and energy intake.

Has this application had been submitted previously to this or any other ethics committee?

No

If Yes, please state the title and reference number.

Is this project from a commercial source, or funded by a research grant of any kind?

No

If yes,

a) Has it been referred to Research & Enterprise?

Has it been allocated a project Number?

No

b) Give details and ensure that this is stated on the Informed Consent form.

Insurance Restrictions.

The University insurance cover is restricted in certain, specific circumstances, e.g. the use of hazardous materials, work overseas and numbers of participants in excess of 5000. All such projects must be referred to Research and Enterprise before ethical approval is sought.

Date of submission

10.03.2012

Name of all person(s) submitting research proposalDr Dalia Malkova¹, Dr Konstantinos Gerasimidis², Professor Charlotte Wright³, Dr Sadia Fatima**Position(s) held**¹ Dalia Malkova, Senior Lecturer,² Konstantinos Gerasimidis, Lecturer³ Charlotte Wright, Professor⁴ Sadia Fatima, PhD student**Department/Group/Institute/Centre**^{1, 2, 4} Human Nutrition Section, School of Medicine, MVLS³ Clinical Specialities, PEACH Unit School of Medicine, MVLS**Address for correspondence relating to this submission**

Dr Dalia Malkova
 School of Medicine
 Human Nutrition Section
 University of Glasgow
 Royal Hospital for Sick Children
 Glasgow, G3 8SJ

Email address: Dalia.Malkova@.gla.ac.uk

Name of Principal Researcher (if different from above e.g., Student's Supervisor)**Position held**Undergraduate student project **No**Postgraduate student project **Yes**

PhD degree being undertaken

1. Describe the purposes of the research proposed. Please include the background and scientific justification for the research. Why is this area of importance?

High energy nutritional supplement drinks (HENSND) are widely promoted by the food industry and used in malnourished children and adults with a significant cost to the UK National Health Service (£99 million pa) <http://www.ic.nhs.uk/pubs/prescostanalysis> 2007. These drinks aim to maximise energy/nutrient intake on top of the normal habitual intake of the patient and thus to prevent or correct disease associated under-nutrition and improve clinical outcome (Stratton and Elia 2010). However, evidence in relations to the benefits of HENSND remains unclear with some ((Delmi, Rapin *et al.* 1990, Potter, Langhorne *et al.* 1998, Green 1999, Lauque, Arnaud-Battandier *et al.* 2000) but not all (Poustie, Russell *et al.* 2006) studies reporting that the use of HENSND is beneficial.

In our previous study we found that in slim young females HENSND consumed 60 minutes before breakfast reduced hunger and increased satiety, and enhanced blood glucose, insulin and peptide YY (PYY) concentrations which all are known as appetite suppressors (Huda, Wilding *et al.* 2006, Wren and Bloom 2007). This increase in satiety and metabolites involved in appetite suppression led to compensation for energy consumed by HENSND. However the compensation was only partial: although energy intake during *ad libitum* breakfast was significantly lower in the HENSND than the PLACEBO trial (HENSND, 1924 ± 168 kJ; PLACEBO, 2889 ± 230 kJ, $P=0.001$), when energy provided by supplements (HENSND, 2505kJ; PLACEBO, 432 kJ) was added to energy intake from breakfast and lunch, the energy intake in the HENSND trial was significantly higher (HENSND, 6576 ± 258 kJ; PLACEBO, 5541 ± 318 kJ, $P=0.006$). We also found that in HENSND trial energy provision remained higher even when corrected for the increase in post-prandial energy expenditure. Thus, in a well controlled study we demonstrated that in young, healthy, slim women consumption of HENSND in the morning does not induce full compensation for energy provided by this drink and concluded that supplementation with HENSND may be beneficial for the treatment of malnutrition.

In clinical practice high energy supplements are usually provided in the evening rather than prior to breakfast and prescribed for several days or weeks. Therefore, from a practical point of view, it is important to find whether enhancement in daily energy intake can also be achieved when HENSND is consumed on a day by day basis and when intake of supplement takes place in the evening. In addition, further investigation is needed on the mechanisms involved in the amendments of energy intake regulation following HENSND. Therefore the proposed study will aim to:

- Investigate the effect of HENSND given in the evening for a period of 5 consecutive days on compensation for energy consumed in underweight young females
- Investigate impact of HENSND supplement consumed in the evening for a period of 5 consecutive days on subjective appetite measures and appetite regulating blood metabolites such as peptide YY, cholecystokinin, pancreatic polypeptide, insulin and glucose measured in the fasted state and after *ad libitum* breakfast, lunch and dinner.

2. Describe the design of the study and methods to be used. Include sample size and the calculation used to determine this. Statistical advice should be obtained if in doubt.

This study is a single blind randomized controlled, crossover study, in which participants will act as their own controls. The participants will conduct two main experimental trials, HENSD and Placebo. Study design is presented in figure 1. These trials will be conducted in counter balanced order (participants will receive drinks in different order) and be separated by at least 7 days of wash out period during which the impact of supplements on the measurements conducted in this study should disappear. During five days leading up to the main experimental trial, participants in the evening will come to the metabolic room and consume either HENSD (Scandishake, Chocolate, Nutricia) prepared with full fat milk, according to the manufacturer instructions or Placebo (a low calorie drink made up with skimmed milk, cocoa and sweeteners and having the same colour, volume, flavour and texture as HENSD). On the day of the experimental trial the rate of energy expenditure (kcal/min) and appetite will be measured, and blood samples will be taken in the fasted state and at 30, 60, 90 and 120 minutes after *ad libitum* breakfast and *ad libitum* lunch. A total of 90 ml of blood will be taken over the course of the day. Lunch will be provided three hours and dinner seven hours after the breakfast. Some fruits or snacks will be available in case participants feel hungry before dinner. The amount of food provided and consumed during the breakfast, lunch, snack and dinner will be recorded. For four days prior to the experimental trials, participants will record food and drink intake and their physical activity.

Power calculation: Power calculations are based on standard deviation (STDEV) for the difference in energy intake during breakfast and lunch between placebo and HENSD trials, the main outcome of our other study. We found that at 85 % power, considering STDEV of 278 kcal for energy intake difference, 22 subjects will allow us to detect a difference in energy intake of 195 kcal between two trials. This number of participants should be sufficient to detect differences in other imperative outcomes of the study.

Methods

Anthropometry

Body height will be measured with height measuring scale (portable stadiometer) (Seca, Leicester, UK). Body weight and fat mass will be measured to the nearest 0.01 kg using a bioelectrical impedance scale (TBF-300, TANITA, Cranlea, UK). Bioelectrical impedance measures the resistance (impedance) to the electrical signals when they travel through water which is found in muscle and fat. The greater the amount of water in a person's body, the easier it is for the current to pass through it. The more fat, the more resistance to the current as fat has less water content. This method is safe and it does not hurt.

Energy intake

The energy consumed during the days prior to experimental trial and during *ad libitum* meals will be calculated using dietary analysis software (Windiets 2005, The Robert Gordon University, Aberdeen, Scotland, UK).

Appetite measurements

Appetite sensations will be measured with validated appetite questionnaires (Flint, Raben *et al.* 2000). Participants will be asked to rate measures of appetite on Visual Analogue Scale (VAS) questionnaires with a line of 100 mm. Volunteers will be asked to express their feeling of hunger, satiety, fullness, prospective food consumption and desire to eat by placing a vertical mark on the horizontal 100 mm line at a point which corresponds to their feelings at that time. The lines are anchored by negative respective feeling words (I am not hungry at all) on the left and by positive feeling words (never being hungrier) on the right. Quantification of the measurement is made by measuring distance from the left end of the line to the participant's mark.

Rate of Energy expenditure and food induced thermogenesis. For measurement of rate of energy expenditure (EE) a ventilated hood system (Oxycon Pro, Jaeger GmbH, Hoechberg, Germany) will be used. A ventilated hood will be placed over the volunteer's head to allow collection of expired gas needed for measurements of oxygen consumption and carbon dioxide production. The rate of oxygen consumption (VO_2) and rate of carbon dioxide production (VCO_2) will be recorded every minute for the duration of 20 minutes. The mean values of VO_2 and VCO_2 will be corrected for the drift by taking the mean of pre-drift and post-drift values. The corrected values will be used to calculate respiratory exchange ratio and rate of energy expenditure by using indirect calorimetry equations (Fraysn and Macdonald 1997).

Appetite hormones. Venous blood samples will be collected in 9 ml vacutainers containing EDTA and then appropriately treated with chemicals prior to preparation of plasma. Enzyme-linked immunosorbent assay (ELISA) kits will be used to determine Peptide YY (Merck, Millipore, Bioscience Division, UK), Cholecystokinin (Phoenix Pharmaceuticals, INC Burlingame, CA, USA), Pancreatic polypeptide (Millipore, Missouri, USA), and insulin (Merckodia AB, Uppsala, Sweden)

Plasma glucose. Plasma glucose will be analysed using commercial assay kit (Life Sciences, Cambridge, UK) and automated Cobas Mira biochemical analyser.

Statistical analysis. The total areas under the curve (AUC) for appetite measures and appetite biomarkers will be used as summary measures of the post-breakfast, post-lunch and post-dinner responses. The difference in AUCs and energy intake between trials will be compared by paired t-test. Two-way repeated measure ANOVA (trial x time) followed by *Post hoc* Tukey's test will also be used to compare differences between two trials.

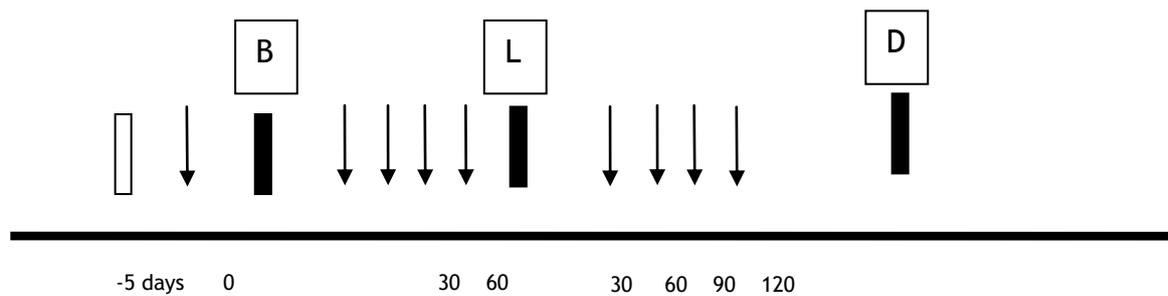
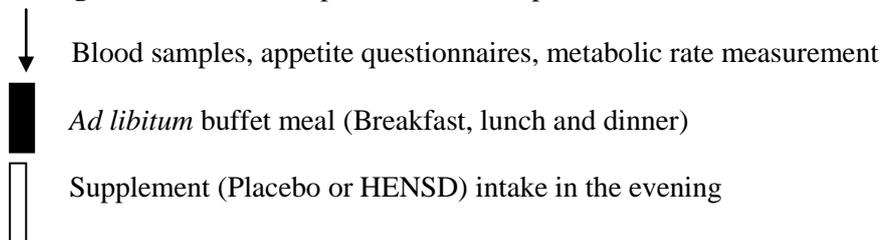


Figure 1. Schematic representation of experimental trial



3. Describe the research procedures as they affect the research subject and any other parties involved.

It should be clear exactly what will happen to the research participant, how many times and in what order

During the five days leading to the experimental trial, the participants will be asked to consume either HENSD or Placebo drinks. The consumption of drinks will take place in the evening, after the very last meal and in the presence of the researcher. The order in which the drinks will be consumed will be random with the participant being blinded in relation to the drink offered. For four days prior to the experimental trials, participants will record food and drink intake and their physical activity.

On each experimental day the participant will be requested to come to the metabolic investigation suite at Yorkhill Hospital at ~ 08:30am. Approximately 10 minutes will be given to the participant to rest and acclimatize with the environment. Then anthropometric measurements, body composition and rate of energy expenditure will be measured. After that a cannula will be inserted into an antecubital vein, and a baseline blood sample will be collected and appetite questionnaires will be completed. Breakfast will be served and following breakfast appetite questionnaires will be completed and blood samples obtained at 30, 60, 90 and 120 minutes. After each blood sample the rate of EE for the duration of 20 minutes will be measured. *Ad libitum* buffet lunch and dinner will be provided 3 and 7 hours after breakfast, respectively. After lunch appetite will be evaluated, blood obtained and metabolic rate measured in the same manner as after breakfast. Snacks or fruit will be available between lunch and dinner. The amount of food ingested during breakfast lunch, snack and dinner will be recorded by the researcher. The experimental trial will last for ~12 hours. Before the first experimental trial the participants will come to the metabolic suit for anthropometric measurements, completion of health questionnaires. During this visit they will be provided with the food intake diaries and scales for recording their dietary intake. This visit will take 45-50min.

Tests will be conducted according to the 'Code of Practice for Conducting Experiments on Non-patient Human Volunteers including Handling and Disposal of Human Blood, Urine and Sputum (www.legislation.gov.uk/ukpga/2004/30/contents), which has been re-approved by the University Ethics Committee on October 26, 2001.

4. How will potential participants in the study be (i) identified, (ii) approached and (iii) recruited?

Give details for cases and controls separately if appropriate:

Participants of the study will be healthy women with the body mass index of 17- 20 kg/m². The recruitment will be done by word of mouth; volunteers will be approached by PhD student Dr Sadia Fatima at the University library, canteen and University gym and asked if they would be interested to take part in human nutrition related research. If the answer is 'yes' then the volunteer information sheet will be given. Those willing to participate in the study will be screened by using health questionnaire to exclude any chronic illnesses, gastrointestinal problems or surgery or some eating disorders. Screening will be done by PhD student Dr Sadia Fatima. Written informed consent will be taken from the participants by Dr Sadia Fatima and Dr Dalia Malkova.

5. What are the ethical considerations involved in this proposal? (You may wish for example to comment on issues to do with consent, confidentiality, risk to subjects, etc.)

Blood sampling

There is a chance of minor bruising with venous cannulation. There is also a rare risk of thrombophelabitis which is very rare in non-smokers, and in healthy people with no coagulation disorders. With incorrect cannulation plastic or air embolism may occur but good technique minimizes the risks. Some people may faint while giving blood samples. Dr Sadia is a qualified medical doctor and she will be with the participants during the whole day of the experimental trial, and she will provide basic emergency care to the participants if required.

The information obtained from the participants and the data obtained after the analysis of the samples will be linked anonymised and individual information will not be passed on to anyone outside the researchers listed in the study. The data will be kept securely on papers and on appropriate electronic format with researchers of the study.

6. Outline the reasons why the possible benefits, to be gained from the project, justify any risks or discomforts involved.

The participants will benefit personally by receiving feedback on their dietary intake and their daily micronutrient and macronutrient consumption. They will also know their anthropometric measurement and receive information regarding their body composition i.e. fat mass, %fat in the body, BMI etc. This study also helps the participants to know about their resting metabolic rate and daily basal metabolic needs. Furthermore this study will help us to understand the impact of supplements on appetite and appetite markers when the supplements are given for a longer duration of time.

7. Who are the investigators (including assistants) who will conduct the research? What are their qualifications and experience?

Dr Dalia Malkova, Dr K. Gerasimidis and Prof C. Wright have extensive experience in conducting human studies. Dr Malkova has ~16 years of experience in conducting human metabolic studies and has ~8 years experience in venepuncture and cannulation. Dr Gerasimidis has extensive experience in carrying out dietary assessment. Dr Sadia Fatima PhD student and Prof Wright are medical practitioners. Dr Sadia Fatima is a PhD student, working under the supervision of Dr Malkova, C. Wright and K. Gerasimidis. She has recently completed her first study and is trained in all procedures.

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8. Are arrangements for the provision of clinical facilities to handle emergencies necessary? If so, briefly describe the arrangements made.

The risks associated with the procedures are extremely small. Tests will be conducted according to the 'Code of Practice for Conducting Experiments in Non-Patient Human Volunteers including Handling and Disposal of Human Blood, Urine and Sputum to the Human Tissue Act 2004 (Commencement No1) order 2005 (www.legislation.gov.uk/ukpga/2004/30/contents) re-approved by the University Ethics Committee on October 26, 2001

9. In cases where subjects will be identified from information held by another party (for example, a doctor or hospital), describe how you intend to get this information. Include, where appropriate, which Multi Centre Research Ethics Committee or Local Research Ethics Committee will be applied to.

N/A

10. Specify whether subjects will include students or others in a dependent relationship and where possible avoid recruiting students who might feel to be or be construed to be under an obligation to volunteer for a project. This is most likely to be where a student is enrolled on a course where the investigator is a teacher. In these circumstances the recruitment could be carried out by one of the other investigators or a suitably qualified third party.

Some post graduate and undergraduate students will be recruited from the University of Glasgow for the study but they will not be on courses taught by the applicants.

11. Specify whether the research will include children or people with mental illness, disability or handicap. If so, please explain the necessity of involving these individuals as research subjects, and include documentation of the suitability of those researchers who will be in contact with children (eg Disclosure Scotland).

N/A

12. Will payment or any other incentive, such as a gift or free services, be made to any research subject? If so, please specify and state the level of payment to be made and/or the source of the funds/gift/free service to be used. Please explain the justification for offering an incentive.

£40 will be given to the participants for partial compensation of their time and travelling expenses.

13. Please give details of how consent is to be obtained. A copy of the proposed consent form, along with a separate information sheet, written in simple, non-technical language MUST ACCOMPANY THIS PROPOSAL FORM.

Volunteer Information Sheet will be given to the subjects to read. Verbal explanation of the study will also be given by Dr Sadia Fatima and Dr Dalia Malkova. The participants will be provided with the opportunity to ask questions about the study before giving written consent. Participants will be asked to complete health history questionnaire to exclude those with any chronic illnesses, gastrointestinal problems or surgery or some eating disorders. The health questionnaire form is attached.

14. Comment on any cultural, social or gender-based characteristics of the subject, which have affected the design of the project or may affect its conduct.

Participants will be slim healthy women with a body mass index 17-20 kg/m². The subjects will be aware that they have been invited to participate in this study on this basis.

15. Please state who will have access to the data and what measures will be adopted to maintain the confidentiality of the research subject and to comply with data protection requirements e.g. will the data be anonymised, how will it be stored, how will access be restricted, and for how long will it be retained?

The information obtained from the participants will be linked anonymised and individual information will not be passed on to anyone outside the researchers listed in the study i.e. Dr Dalia Malkova, Dr Konstantinos Gerasimidis, Professor Charlotte Wright and Dr Sadia Fatima. The data will be coded by PhD student Dr Sadia Fatima. Each volunteer will be assigned a research code. The record matching the volunteer details with the code will be kept confidential with Dr Sadia Fatima. Linked anonymous data will be analyzed and stored in the form of an excel sheet in secure network of University of Glasgow which is virtual private network (VPN) set up by Glasgow University IT services and is accessible to Dr Sadia Fatima only. The data will be kept securely on papers in locked cabinets and on appropriate electronic format with Dr Sadia Fatima. The data will be securely held for the duration of ten years after the completion of research and will be disposed off thereafter as per University policy.

16. Will the intended group of research subjects, to your knowledge, be involved in other research? If so, please justify.

Not during the duration of the study.

17. Proposed starting date

November 2012

Expected completion date

June 2014

18. Please state location(s) where the project will be carried out.

Experimental trials shall be carried out in the Metabolic Investigation Suite at Human Nutrition Section, Yorkhill Hospital.

19. Please state briefly any precautions being taken to protect the health and safety of researchers and others associated with the project (as distinct from the research subjects) e.g. where blood samples are being taken

All samples will be handled according to the Human Tissue Act 2004 (Commencement No1) order 2005 (www.legislation.gov.uk/ukpga/2004/30/contents)

20. Please state all relevant sources of funding or support for this study

Dr Sadia Fatima has a secured PhD scholarship from the Khyber Medical University, Pakistan which pays tuition fees and monthly stipend for 3 years, and a small research bursary.

21 a). Are there any conflicts of interest related to this project for any member of the research team? This includes, but is not restricted to, financial or commercial interests in the findings. If so, please explain these in detail and justify the role of the research team. For each member of the research team please complete a declaration of conflicts of interest below.

There are no conflicts of interest

Researcher:

Name: _____ conflict of interest Yes / No

If yes, please detail below

Researcher:

Name: _____ conflict of interest Yes /

No

If yes, please detail below

Researcher:

Name: _____ conflict of interest Yes /

No

If yes, please detail below

Researcher:

Name: _____ conflict of interest Yes /

No

If yes, please detail below

--

21 b). If there are any conflicts of interest, please describe these in detail and justify conducting the proposed study.

N/A

22. How do you intend to disseminate the findings of this research?

The results of the study shall be disseminated through presentation to the clinical colleagues and dieticians through research symposium, and workshops. Findings will also be disseminated as presentation in the Nutrition Society meeting and as peer reviewed paper.

I confirm that have read the University of Glasgow's Data Protection Policy
[\[http://www.gla.ac.uk/services/dpfoioffice/policiesandprocedures/dpa-policy/\]](http://www.gla.ac.uk/services/dpfoioffice/policiesandprocedures/dpa-policy/)

Please initial box

Signed _____ Date _____
(Proposer of research)

For student projects

I confirm that I have read and contributed to this submission and believe that the methods proposed and ethical issues discussed are appropriate.

I confirm that the student will have the time and resources to complete this project.

Signed _____ Date _____
(Supervisor of student)

Send completed signed form to

Stuart Morrison
 Room 330, Wolfson Medical School Building
 University Avenue
 Glasgow
 G12 8QQ

Tel: 0141 330 5519
Fax: 0141 330 4758

Please also send electronic versions of completed form and all other paperwork to
Stuart.Morrison@glasgow.ac.uk

**College of Medical, Veterinary & Life Sciences Ethics Committee for Non
Clinical Research Involving Human Subjects**

APPLICATION FORM FOR ETHICAL APPROVAL

NOTES:

THIS APPLICATION FORM SHOULD BE TYPED NOT HAND WRITTEN.

**ALL QUESTIONS MUST BE ANSWERED.
“NOT APPLICABLE” IS A SATISFACTORY ANSWER WHERE
APPROPRIATE.**

PROJECT CODE:

Project Title

Comparison of the effectiveness of solid ready-to-eat and a liquid ready-to-drink supplements for management of mild to moderate malnutrition in children from Pakistan

Has this application had been submitted previously to this or any other ethics committee?

No

If Yes, please state the title and reference number.

Is this project from a commercial source, or funded by a research grant of any kind?

No

If yes,

a) Has it been referred to Research & Enterprise?

Has it been allocated a project Number?

No

b) Give details and ensure that this is stated on the Informed Consent form.

Insurance Restrictions.

The University insurance cover is restricted in certain, specific circumstances, e.g. the use of hazardous materials, work overseas and numbers of participants

in excess of 5000. All such projects must be referred to Research and Enterprise before ethical approval is sought.

Date of submission

03.04.2013

Name of all person(s) submitting research proposal

Dr Sadia Fatima¹, Dr Dalia Malkova², Dr Konstantinos Gerasimidis³, and Professor Charlotte Wright⁴

Position(s) held

¹Dr Sadia Fatima, PhD student

²Dr Dalia Malkova, Senior Lecturer,

³Dr Konstantinos Gerasimidis, Lecturer

⁴Prof Charlotte Wright, Professor

Department/Group/Institute/Centre

^{1, 2, 3}Human Nutrition Section, School of Medicine, MVLS

⁴Clinical Specialities, PEACH Unit School of Medicine, MVLS

Address for correspondence relating to this submission

Dr Dalia Malkova
School of Medicine, MVLS
University of Glasgow
Royal Hospital for Sick Children
Glasgow, G3 8SJ

Email address: Dalia.Malkova@.glagow.ac.uk

Name of Principal Researcher (if different from above e.g., Student's Supervisor)

Position held

Undergraduate student project **No**

Postgraduate student project **Yes**

PhD degree being undertaken

1. Describe the purposes of the research proposed. Please include the background and scientific justification for the research. Why is this an area of importance?

Malnutrition is a condition in which nutrients such as proteins, vitamins, minerals and energy are deficient or in excess (imbalance) which causes severe measurable adverse effects on body composition, function and clinical outcomes (Meier and Stratton 2008, Nieuwenhuizen, Weenen *et al.* 2010). The World Health Organization cites malnutrition as the single greatest threat to the world's public health (Nieuwenhuizen, Weenen *et al.* 2010). Childhood malnutrition is associated with a number of socio-economic and environmental factors such as poverty leading to inadequate food intake, lack of access to food, poor hygienic practices, lack of sanitation, poor health, recurrent infections and large family size (Golden 2009, Babar, Muzaffar *et al.* 2010). Not surprisingly in low income countries 112 million (20%) out of 556 million children under 5 years of age are underweight and 36 million (6.4%) are suffering from moderate wasting (Golden 2009, Michaelsen, Hoppe *et al.* 2009).

Pakistan is the world's seventh most populous country and 45% of its population is under 15 years of age. Evidence suggests that malnutrition in children between 5-12 years is high in Pakistan and is a very serious impediment to the development of the nation (Mian, Ali *et al.* 2002, Pappas, Agha *et al.* 2008). In Pakistan 23.9% of the population lives below the poverty line and 38% of all children under 5 years of age are underweight (Pappas, Agha *et al.* 2008, Akram, Arif *et al.* 2010).

Malnutrition is identified using standard deviation scores (Z-scores), which is a statistical measure identifying how body weight and height compares to reference values. The World Health Organization (WHO) Global Database on Child Growth and Malnutrition uses a Z-score cut off point of $<-2SD$ to classify undernutrition and a cutoff of $>+2SD$ to classify overweight in children (Wang and Chen 2012). Mildly underweight children (between -1 and -2 Z score have approximately a twofold higher risk of death as compared to well nourished children (Fishman, CAULFIELD *et al.* 2004).

According to the WHO recommendations for the treatment of malnutrition during the nutritional rehabilitation phase the children should receive an energy and protein dense diet fortified with vitamins and minerals to promote rapid weight gain. This phase of rehabilitation requires 3-4 weeks and is usually carried out in hospital or residential therapeutic centres and the mother has to stay with the child (Diop, Dossou *et al.* 2003). This has some limitations as there are more chances of cross-infections and secondly it is unfavourable for other family members (Collins 2001). Therefore, community based rehabilitation is suggested as an alternative (Ashworth and Khanum 1997). For this purpose, a solid ready to use food (RTUF) has been developed which is made up of peanut butter (Diop, Dossou *et al.* 2003). These RTUF can be eaten directly by the child without the addition of water or milk which reduces risk of bacterial contamination (Diop, Dossou *et al.* 2003).

Studies have showed that RTUFs are very effective in the treatment of severe malnutrition in children (Briend, Lacsala *et al.* 1999, Collins and Sadler 2002, Dossou, Ndour *et al.* 2003, Brewster 2006). The randomized controlled trial conducted on 60 severely malnourished Senegalese children in a therapeutic feeding centre demonstrated that increase in energy intake and weight gain achieved by supplementation with RTUF was higher in comparison to changes induced by consumption of a traditional high-energy drink based on milk. There is no evidence comparing the impact of RTUF and liquid supplements on weight gain in mild to moderate malnourished children.

Therefore the proposed study will aim :

- To compare the efficacy of solid ready to eat and liquid ready to drink supplements in promoting weight gain in mild to moderate malnourished children.

2. Describe the design of the study and methods to be used. Include sample size and the calculation used to determine this. Statistical advice should be obtained if in doubt.

This study will be open label randomized controlled trial. This study will be conducted in the primary schools of the district of Abbottabad located in the Hazara region of Khyber Pakhtunkhwa province in Pakistan. The purpose of the study will be explained to the principals and parent/carer in local language and informed consent will be obtained after clarification that their refusal will have no effect on how the child is treated in the school by the teachers and support staff. Then primary school children between the ages of 5-10 years will be randomly selected and then height and weight measurements will be done. These anthropometric measurements will be used to calculate Z-scores using WHO standards. Children with mild to moderate malnutrition (between -2 and -1 Z-score) will be selected for the study. After that the children will be randomly allocated by computerized randomization to receive either solid ready to use food (Plumpy' Nut) or liquid ready to use supplement (Fortini, Nutricia). The children will be provided with these supplements in their school for four weeks and will be asked to consume the supplements in addition to their habitual diet. The supplements will be delivered by the main researcher Dr Sadia Fatima daily. The children will be asked to keep the empty bottles/sachet of the supplements with them after drinking/eating, which will be collected by the main researcher next day in order to check the compliance. Any leftover in the bottle or sachet will be recorded. Height, weight, biceps and triceps skinfolds and mid-upper-arm circumference of the child will be measured before the supplementation (baseline), two weeks after initiation of the supplementation and at the end of the supplementation. The child will mark an appetite questionnaire before the provision of the first and the last supplement. Furthermore the parent/carer will be requested to attend a focus group regarding the appetite of their child once before the start of supplementation and once at the end of the study. The parents/carer of the children will be asked to continue regular normal habitual diet of their children during the days of supplementation as the supplements are not replacement to food. At the end of the study the children who were screened and did not meet the criteria for recruitment will be measured for height and weight.

Statistical Analysis: Power calculations are based on data available from previous study (Diop, Dossou *et al.* 2003). We used standard deviation (STDEV) for the difference in weight gain between solid ready to eat supplement and liquid ready to drink supplement groups, the main outcome of this study. We found that at 80 % power, considering STDEV of 0.7, 32 subjects in each group will allow us to detect a mean difference of 0.5kg between two groups. This number of participants should be sufficient to detect differences in other imperative outcomes of the study.

Prior data analysis Anderson-Darling test will be used to determine the normality of the data. Depending upon the distribution of the data, parametric or nonparametric analysis will be applied to the data. The difference between the body weight and appetite measures at the baseline between two groups will be determined using unpaired *t* test (for parametric data) and Mann-Whitney (for non-parametric data). The difference in the weight gain and appetite changes between solid ready to eat and liquid ready to drink supplement will be assessed using two way analysis of variance ANOVA (for parametric data) or Kruskal–Wallis (for non-parametric data). The data will be analysed using Minitab (version 15.1; Minitab, State College, PA, USA). P-value of ≤ 0.05 will be considered as significant

Methods

Height Measurement

Height will be measured with a portable stadiometer (Seca, Leicester, UK) using a stretch stature method. The stature is the maximum distance from the ground to the uppermost point of the skull when the position of the head is held in Frankfort plane position. The measurement will be performed to the nearest 0.01m. The same height scale will be used throughout the study and the same researcher will perform all the measurements.

Weight Measurement

The weight of the children will be measured wearing light weight clothes without shoes. Prior to the measurements extraneous clothing will be removed. The children will stand with both feet placed flat on the balance and arms positioned laterally along the side of the body. The measurements will be performed to the nearest 0.01kg.

Skinfold Measurement

Triceps and biceps skinfolds will be measured using the Harpenden skinfold calliper (Baty, International) to the nearest 0.2mm. For measuring triceps skinfolds the child will be asked to bend the left arm at 90° at the elbow and place the forearm across the body. Then acromion process at the outer-most edge of the shoulder and tip of olecranon process of the ulna will be located and the distance between the two will be measured with the help of the measuring tape. Then the mid-point in line with the elbow and acromion process will be marked. After that the child's arm will be extended so that it is hanging loosely by the side. Using the thumb and forefinger a vertical fold of skin and underlying fat 1cm above the marked mid-point will be grasped. Afterwards the skinfold will be gently pulled away from the underlying muscle and calliper jaws will be applied at right angles exactly at the marked mid-point. While taking the measurements the skinfold will be held in the fingers. Two measurements will be taken and the mean of both will be considered for the accuracy of results.

For measuring biceps skinfolds at the level of the mid-point between the acromion process (bony tip of shoulder) and the proximal and lateral border of the radius bone (approximately the elbow joint), on the mid-line of the anterior surface of the arm (over the biceps muscle) will be marked. Then the child will be asked to relax his/her arm with the palm of the hand facing forwards. Then using thumb and forefinger a vertical fold of skin will be grasped at the land mark, and calliper jaws will be applied. Again two measurements will be taken and the mean of both will be used

Mid upper arm circumference

Mid upper arm circumference will be measured with a flexible measuring tape on the left upper arm while the arm is relaxed and hanging down the side of the body.

Appetite measurements

Appetite sensations will be measured with validated appetite questionnaires (Flint, Raben *et al.* 2000). Participants will be asked to rate measures of appetite on Visual Analogue Scale (VAS) questionnaires with a line of 100 mm. Children will be asked to express their feeling of hunger, satiety, fullness, prospective food consumption and desire to eat by placing a vertical mark on the horizontal 100 mm line at a point which corresponds to their feelings at that time. The lines are anchored by negative respective feeling words (I am not hungry at all) on the left and by positive feeling words (never being hungrier) on the right. Quantification of the measurement is made by measuring the distance from the left end of the line to the participant's mark.

3. Describe the research procedures as they affect the research subject and any other parties involved. It should be clear exactly (i) what will happen to the research participant, (ii) how many times and (iii) in what order.

The study participants will be provided with either solid ready to eat food (Plumpy' Nut) or liquid ready to drink (Fortini, Nutricia) supplements and asked to consume these supplements daily for four weeks. Supplements will be provided by the main researcher Dr Sadia Fatima and consumed at school. The energy content of both the supplements will be same (500kcal/day). Height, weight, mid-upper-arm circumference and biceps and triceps skinfolds will be measured before the start of supplementation, two weeks after initiation and at the end of supplementation. These measurements will be conducted by the main researcher and will take place at the child's school. The child will mark an appetite questionnaire before the provision of the first and last supplement. The parent/carer will be asked to attend a focus group regarding their child's appetite once before start of supplementation and once at the end of supplementation. It will take only 15-20 minutes of the child and parent/carer to answer the questions.

This study will be conducted according to the 'Code of Practice for Conducting Experiments on Non-patient Human Volunteers including Handling and Disposal of Human Blood, Urine and Sputum (www.legislation.gov.uk/ukpga/2004/30/contents), which has been re-approved by the University Ethics Committee on October 26, 2001

4. How will potential participants in the study be (i) identified, (ii) approached and (iii) recruited? Give details for cases and controls separately if appropriate.

This study will be conducted in the primary schools of the district of Abbottabad located in the Hazara region of Khyber Pakhtunkhwa province in Pakistan. The participants of this study will be primary school children between the ages of 5-10 years with mild to moderate malnutrition. The recruitment will be done by the main researcher Dr Sadia Fatima in accordance with the principals of the primary schools in the vicinity of Abbottabad. The study protocols will be explained in detail to the principals of the schools and written permission will be taken from them to conduct the study in their schools. Then the parent/carer of all the children will be approached by the main researcher Dr Sadia Fatima. The purpose of the study will be explained to the parent/carer of the children in the local language. The eligible children will be screened by using health questionnaires to exclude any chronic illnesses, gastrointestinal problems or surgery or some allergy or eating disorders. Screening will be done by the main researcher Dr Sadia Fatima. Children less than 8 years of age will be given a pictorial form of the recruitment procedure along with the consent or assent form. Parent/carer will be requested to give consent in the case of participants who are unable to give consent on their own behalf. A Volunteer information sheets for the participants will be given along with a written informed consent in an easy to understand language (it will be translated to the local language Urdu) to parent/carer and to the participants. An explanation will be given to the participants and the parent/carer about all aspects of the research study by the researcher at the same time. The illiterate parents/carers will be asked to take the information sheet and request their educated relatives to read it for them so that they can understand the study. While the parents/carer who does not consent to take part or withdraw at any time will be provided with some dietary counselling and their children will not be included in the study. Then written informed consent will be obtained from the participants and their parent/carer by Dr Sadia Fatima after clarification that their refusal to participate will have no effect on how the child is treated in the school by the teachers and support staff. After taking permission, the children will be randomly selected from the school and then their height and weight will be measurement to identify mild to moderate malnourished children. Standard deviation scores (Z-score) for anthropometric measurements will be calculated based on international growth reference data (Cole 1990, H Pan 2012).

5. What are the ethical considerations involved in this proposal? You may wish, for example, to comment on issues to do with consent, confidentiality, risk to subjects, etc.

The purpose of the study will be explained to the parent/carer and children in local language and informed consent will be obtained before taking any measurement from the children. Those participants who do not meet the anthropometric criteria for inclusion will be encouraged to continue healthy diet and their height and weight will be measured again after 28 days. While those parents/carers who do not consent to take part or withdraw at any time will be provided with some dietary counselling and their children will not be included in the study.

A high energy diet given for a prolonged period of time could potentially lead to obesity (Golden 2009). We will provide these supplements with energy content of 500kcal/day for 28 days which may result in an approximate maximum weight gain of 1.8 kg. Thus a moderately malnourished child (-2 Z-score) is expected to improve towards the lower limit of normal and upper limit of mild malnutrition (-1 Z-score) and mildly malnourished children (-1Z-score) becomes normal (0Z-score). Therefore there will be no chance to induce obesity by using these supplements for 28 days.

There might be a risk of allergic reaction from the peanut butter used in the preparation of solid ready to use food. Peanut butter contains patent allergens, and these allergens may be enhanced during cooking (Maleki, Chung *et al.* 2000). An allergic reaction will be rare as peanuts are part of the traditional diet. Furthermore the eligible children will also be screened for food allergies with the help of the Health questionnaire. Moreover clinical allergy is rare in developing countries (Abbassy, El-Din *et al.* 1974) and food allergy manifests in infancy and declines after three years of age (Husain and Schwartz 2013).

The information obtained from the participants will be linked anonymised and individual information will not be passed on to anyone outside the researchers listed in the study. The data will be kept securely in appropriate electronic format with researchers of the study.

6. Outline the reasons why the possible benefits to be gained from the project justify any risks or discomforts involved.

The children participating in this study may benefit directly by receiving supplements which may improve their nutritional status. The parent/carer will find out about their child's anthropometric measurements and receive information regarding the child's nutritional status before and after supplementation. As dietary counselling is a fundamental and effective part in the treatment of mild to moderate malnutrition (Ashworth and Ferguson 2009), the parent/carer will obtain some counselling on the completion of the study.

Furthermore this study will help us to understand and compare the efficacy of solid ready to eat with liquid milk based supplements in promoting weight gain in mild to moderate malnourished children in community settings and the impact of these rich energy and nutrient supplements on appetite.

7. Who are the investigators (including assistants) who will conduct the research? What are their qualifications and experience?

Dr Dalia Malkova, Dr K. Gerasimidis and Prof C. Wright have extensive experience in conducting human research, including research studies in children. Dr Sadia Fatima (PhD student) and Prof Wright are medical practitioners. Dr Sadia Fatima is a PhD student, working under the supervision of Dr Malkova, Prof C. Wright and Dr K.

Gerasimidis. She has already completed her first two studies and therefore has extensive experience in all relevant methods and procedures.

8. Are arrangements for the provision of clinical facilities to handle emergencies necessary? If so, briefly describe the arrangements made.

The risks associated with this study are extremely small. The study will be conducted according to the 'Code of Practice for Conducting Experiments in Non-Patient Human Volunteers including Handling and Disposal of Human Blood, Urine and Sputum to the Human Tissue Act 2004' (Commencement No1) order 2005 (www.legislation.gov.uk/ukpga/2004/30/contents) re-approved by the University Ethics Committee on October 26, 2001

9. In cases where subjects will be identified from information held by another party (e.g., a doctor or hospital), describe how you intend to obtain this information. Include, where appropriate, which Multi Centre Research Ethics Committee or Local Research Ethics Committee will be applied to.

As the study will be conducted in the district of Abbottabad located in the Hazara region of Khyber Pakhtunkhwa province in Pakistan, the ethics will also be submitted to the research ethics committee of Ayub Medical College Abbottabad.

10. Specify whether subjects will include students or others in a dependent relationship and, where possible, avoid recruiting students who might feel to be, or be construed to be, under obligation to volunteer for a project. This is most likely to be when a student is enrolled on a course where the investigator is a teacher. In these circumstances, the recruitment could be carried out by one of the other investigators or a suitably qualified third party.

N/A

11. Specify whether the research will include children or participants with mental illness, disability or handicap. If so, please explain the necessity of involving these individuals as research subjects and include documentation of the suitability of those researchers who will be in contact with children (e.g., Disclosure Scotland or membership of the PVG Scheme).

The participants of this study will be primary school children from Pakistan aged between 5-10 years with mild to moderate malnutrition. In Pakistan prevalence of malnutrition including malnutrition in children between 5-12 years is very high and has serious impediment to the development of the nation (Mian, Ali *et al.* 2002, Pappas, Agha *et al.* 2008). Investigation of benefits of high energy supplements in children who have not yet developed severe malnutrition is of great importance. This study will provide evidence on the impact of solid ready to eat and liquid ready to drink supplements on weight gain in mild to moderate malnourished children and

suggests which type of supplement might be the best when supplementation takes place in community rather than hospital settings (Diop, Dossou *et al.* 2003). Children less than 8 years of age will be given a pictorial form of the recruitment procedure along with the consent or assent form. Parent/carer will be requested to give consent in the case of participants who are unable to give consent on their own behalf. A volunteer information sheets for the participants will be given along with a written informed consent in an easy to understand language (it will be translated to the local language Urdu) to parent/care and to the participants. An explanation will be given to the participants and the parent/carer about all aspects of the research study by the researcher at the same time.

The main researcher of the study, Dr Sadia Fatima will be in contact with the children. Dr Sadia Fatima is a qualified doctor and is a registered medical practitioner in Pakistan. She has experience of one and a half year of working as a medical officer in Pakistan. In addition Dr Sadia Fatima is the mother of two children and knows how to approach and talk to children. Dr Sadia Fatima has attended a Good Clinical Practice (GCP) Workshop in the first year of her PhD training, through which she developed an understanding of ethical issues and informed consent. This GCP workshop was conducted by Glasgow Clinical Research Facility in Tennet Building Church street Glasgow. This workshop mainly focused on the fact that good clinical practice is the rules or guidelines by which clinical trials are conducted. These rules provide guarantee that the data provided and results obtained are rational and accurate as well as protects participant's confidentiality, safety and well being.

12. Will payment or other incentive, such as a gift or free services, be made to any research subject? If so, please specify, and state the level of payment to be made and/or the source of the funds/gift/free service to be used. Please explain the justification for offering an incentive.

N/A

13. Please give details of how consent is to be obtained. A copy of the proposed consent form, along with a separate information sheet, written in simple, non-technical language MUST ACCOMPANY THIS PROPOSAL FORM.

Keeping in mind the age of legal capacity, Scotland Act 1991, all participants who are considered mentally competent to give consent on their own behalf will be asked to give an informed consent.

Competence is defined as;

“The ability of a person, given the necessary information, to understand the nature and the consequences of the proposed procedure or treatment, and to use that information to make a valid choice in accordance with their own fundamental values”

Children less than 8 years of age will be given a pictorial form of the recruitment procedure along with the consent or assent form. Legal guardians or parents will be requested to give consent in the case of participants who are unable to give consent on their own behalf.

Volunteer information sheets for the participants will be given along with a written informed consent in an easy to understand language (it will be translated to the local language Urdu) to parent/care. An explanation will be given to the participant and the carer about all aspects of the research study by the researcher at the same time.

Children or the parent/carer of the children will be asked to complete a health check questionnaire to exclude those with any chronic illnesses, gastrointestinal problems or surgery or some allergies to nuts, peanuts or milk. The health check questionnaire form is attached.

14. Comment on any cultural, social or gender-based characteristics of the subjects which have affected the design of the project or may affect its conduct.

The participants of this study will be primary school children between the ages of 5-10 years with mild to moderate malnutrition between -2 Z-score and -1 Z-score.

15. Please state (i) who will have access to the data and (ii) what measures will be adopted to maintain the confidentiality of the research subjects and to comply with data protection requirements. For example, will the data be anonymised, how will it be stored, how will access be restricted, and for how long will it be retained?

Every participant will be allocated an alphanumeric subject code and no names or personal details of the participants will be stored by electronic means. The information obtained from the participants will be linked anonymised and individual information will not be passed on to anyone outside the researchers listed in the study i.e. Dr Dalia Malkova, Dr Konstantinos Gerasimidis, Professor Charlotte Wright and Dr Sadia Fatima. Linked anonymous data will be analyzed and stored in the form of an excel sheet within the secure network of the University of Glasgow which is a virtual private network (VPN) set up by Glasgow University IT services and is accessible to Dr Sadia Fatima only. Initially the hard copies with the participant's details will be locked in a personal cabinet with Dr Sadia Fatima. Later on the hard copies will be shifted to the locked cabinet within the premises of the Human Nutrition Unit, University of Glasgow. For long term data storage, the data will be stored in locked cabinets under the supervision of the principal investigator. Data will be stored for 10 years after completion of study and will be disposed off thereafter as per hospital/University policy.

16. To your knowledge, will the intended group of research subjects be involved in other research? If so, please justify.

Not during the duration of the study.

17. Proposed starting date:

May 2013

Expected completion date:

June 2014

18. Please state location(s) where the project will be carried out.

This study will be conducted in 7-10 primary schools of the district of Abbottabad located in the Hazara region of Khyber Pakhtunkhwa province in Pakistan.

19. Please state briefly any precautions being taken to protect the health and safety of researchers and others associated with the project (as distinct from the research subjects), e.g., where blood samples are being taken.

No blood, urinary or stools samples will be collected in this study.

20. Please state all relevant sources of funding or support for this study.

Dr Sadia Fatima has a secured PhD scholarship from the Khyber Medical University, Pakistan which pays tuition fees and a monthly stipend for 3 years, and a small research bursary

21a). Are there any conflicts of interest related to this project for any member of the research team? This includes, but is not restricted to, financial or commercial interests in the findings. If so, please explain these in detail and justify the role of the research team. For each member of the research team please complete a declaration of conflicts of interest below.

There are no conflicts of interest

Researcher Name: _____ Dr Sadia Fatima _____ conflict of interest Yes / No ✓

If yes, please detail below

Researcher Name: ___ Dr Dalia Malkova _____ conflict of interest Yes / No ✓

If yes, please detail below

Researcher Name: _Dr Konstantinos Gerasimidis _____ conflict of interest Yes / No ✓

If yes, please detail below

Researcher Name: _Prof. Charlotte Wright _____ conflict of interest Yes / No ✓

If yes, please detail below

21b). If there are any conflicts of interest, please describe these in detail and justify conducting the proposed study.

N/A

22. How do you intend to disseminate the findings of this research?

The results of the study shall be disseminated through presentations to clinical colleagues and dieticians through research symposium, workshops and conferences. Findings will also be disseminated as a presentation in the Nutrition Society meeting and as peer reviewed papers.

I confirm that have read the University of Glasgow's Data Protection Policy.

[<http://www.gla.ac.uk/services/dpfoioffice/policiesandprocedures/dpa-policy/>]

Please initial box



Name _____ Dr Sadia Fatima _____ Date
_04.04.2013_____

(Proposer of research)

Please type your name on the line above.

For student projects:

I confirm that I have read and contributed to this submission and believe that the methods proposed and ethical issues discussed are appropriate.

I confirm that the student will have the time and resources to complete this project.

Name __Dr Dalia Malokva_____ Date
____04.04.2013_____

(Supervisor of student)

Please type your name on the line above.

Please upload the completed and signed form, along with other required documents by logging in to the Research Ethics System at - <https://frontdoor.spa.gla.ac.uk/login/>

Appendix II a, b, c, d**VOLUNTEER INFORMATION SHEET****Effect of high energy nutritional supplement drinks on appetite, appetite hormones and dietary intake**

You are being invited to take part in a research study. Before you decide whether or not to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. Thank you for reading this.

What is the purpose of the study?

High energy nutritional supplement drinks (HENSND) are commonly prescribed to under-nourished patients but it is not clear if they are effective, particularly in children, whose appetite tends to be spoiled by snacks and drinks between meals. We first need to find out what effect these ultra high energy drinks have on appetite, biochemical markers of appetite and food consumption during all day healthy slim adults, before going on to study their effect, using less invasive methods, in children. We will recruit 19 slim adults and to attend our lab twice each and eat two meals, once after a HENSND and once after a similar tasting low energy drink. Series of blood samples will be taken and appetite and energy intake consumed during these meals and for the rest of the day will be measured.

Why have I been chosen?

You have been chosen because you are a healthy adult aged between 18-45 years who is somewhat slimmer than the ideal weight for your height.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will happen to me if I take part?**A. SCREENING PROCEDURES**

Before enrolling in the study you will be asked to attend for a screening visit in which we will:

- discuss with you and complete confidential questionnaires regarding your health
- measure your height and weight and some of the skinfolds
- provide an opportunity for you to ask questions

These screening procedures will enable us to determine whether you fall into the group of people we wish to study

B. EXPERIMENTAL TESTS

We will ask you to undergo 2 main experimental tests lasting for the duration of ~ 8 hours. On the day of each experimental test you will be asked to come to the metabolic investigation suite at ~ 08:30. Upon arrival we will collect an expired air sample to measure how many calories you burn at rest. We will then place a tiny plastic tube called a 'cannula' into a vein in your forearm, from which we will take blood samples. This is no more painful than a simple blood test. A total of 200 ml of blood will be taken over the course of the day (a small cupful, less than half the amount when you donate a pint of blood). You will be given 240 ml of one of the preload liquid meals and be asked to consume it entirely within 5 minutes. You will be asked to take a standard dose of oral paracetamol (1500 mg in 100 ml of water) at the same time with the administration of the preload drink. In 90 minutes you will then be given breakfast, and later on lunch. Blood, expired air, and appetite ratings will be collected throughout the experimental test. You will be allowed to leave after lunch and instructed to record all food and drink consumed during the rest of the day.

What do I have to do?

Other than the specific tasks described above, we ask you to maintain your usual lifestyle (i.e. don't change your diet or exercise habits) for the duration of this study. We will ask you to record your food intake throughout the day before the first experimental test and to replicate the food intake prior to the second one. We also ask you to avoid alcohol and any planned exercise 2 days prior to each main trial.

What are the possible disadvantages and risks of taking part?

Blood sampling via the cannula may cause minor bruising, an inflammation of the vein or haematoma (a small accumulation of blood under the skin). Good practice, however, minimises this risk. Some people may feel faint when they give blood.

What are the possible benefits of taking part?

There may be no direct benefits to you but as a result of being involved in this study you will receive health information about yourself including body fat measurement. You will also receive information on your habitual intake of

energy, and nutrients. The findings of this study will be published in scientific journals so that understanding about high energy supplements can help slim people to improve their energy intake. This information may contribute towards guidelines on the consumption of high energy supplements

We will provide you with feedback about the main study findings and also about your own results and would be delighted to explain our findings and discuss their implications with you.

What if something goes wrong?

The chance of something going wrong is extremely small. All of the procedures involved in this study are low risk and our screening tests are designed to ensure that you will only participate if it is safe for you to do so. In the unlikely event that you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal University of Glasgow complaints mechanisms may be available to you.

Will my taking part in this study be kept confidential?

Any information which is collected about you during the course of the research will be kept strictly confidential. Any findings from the study that are published will have your name and address removed so that you cannot be recognised. We will not pass on confidential personal information to others.

Who has reviewed the study?

This study has been reviewed and approved by **Faculty of Medicine Ethics Committee for Non Clinical Research Involving Human Subjects**

Contact for Further Information

Any questions about the procedures used in this study are encouraged. If you have any doubts or questions, please ask for further explanations by contacting Sadia Fatima e- mail: s.fatima.1@gla.ac.uk

Dr Dalia Malkova, e-mail: Dalia.Malkova@clinmed.gla.ac.uk

You will be given a copy of this information sheet and a signed consent form to keep for your records.



UNIVERSITY
of Glasgow

VOLUNTEER INFORMATION SHEET

Impact of High Energy Nutritional Supplement Drink (HENSND) consumed for five days on appetite, appetite regulators, resting and post prandial energy expenditure, and energy intake

You are being invited to take part in a research study. Before you decide whether or not to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. Thank you for reading this.

What is the purpose of the study?

High energy nutritional supplement drinks (HENSND) are commonly prescribed to under-nourished patients but it is not clear if they are effective. We first need to find out what effect these ultra high energy drinks have on appetite, biomarkers of appetite and food consumption during all day in healthy slim individuals, before going on to study of their effect on those who are malnourished and have impaired health. In our previous study we found that consumption of HENSND in the morning by young, healthy, slim, young women benefited daily energy intake. In clinical practice high energy supplements are consumed in the evening rather than in the morning and are prescribed for several days or weeks. Therefore, this study aims to find whether enhancement in daily energy intake can be achieved when HENSND supplements are consumed on day by day basis and when intake of supplement takes place in the evening.

We will recruit 22 slim healthy women not suffering from any recognised eating disorder. Volunteers will be invited to attend our lab on two occasions and eat three meals, on one occasion after a five days supplementation with HENSND and on another after five days supplementation with similar tasting low energy drink. Series of blood samples will be taken and appetite and energy intake consumed during three meals will be measured.

Why have I been chosen?

You have been chosen because you are a healthy slim women aged between 18-45 years

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will happen to me if I take part?

A. SCREENING PROCEDURES

Before enrolling in the study you will be asked to attend for a screening visit in which we will:

- Discuss with you about this study and complete confidential questionnaires regarding your health
- Measure your height and weight. Height will be measured with a portable stadiometer. Body weight will be measured with the help of digital Tanita scale. Your weight will be measured wearing light weight cloths without shoes. You will stand with both the feet placed flat on the balance without socks and arms will be positioned laterally along the side of the body.
- Provide an opportunity for you to ask questions

These screening procedures will enable us to determine whether you fall into the group of people we wish to study

B. EXPERIMENTAL TESTS

We will ask you to undergo 2 main experimental tests lasting for the duration of ~ 12 hours. Prior both experimental tests, you will be provided with chocolate flavoured drink and asked to consume it for five consecutive days. On the day of the experimental test you will be asked to come in the fasted state to the metabolic investigation suite of the York hill Hospital at ~ 08:30. Upon arrival we will collect an expired air samples to measure how many calories you burn at rest. For these measurements we will ask you to lie in a supine position and relax for ~10 min. Then a transparent ventilated plastic hood will be placed over your head which is required for the measurements. You will be asked to stay under the ventilated plastic hood for the duration of 20 minutes. The measurements will be recorded on computer screen.

We will then place a tiny plastic tube called a 'cannula' into a vein in your forearm, from which we will take blood samples. This is no more painful than a simple blood test. A total of 90 ml of blood will be taken over the course of the day (less than one fifth of the amounts when you donate a pint of blood). You will then be given breakfast, and later on lunch and dinner. Blood, expired air, and appetite ratings will be collected throughout the experimental test.

What do I have to do?

Other than attending screening session and participating in two experimental trials, during 5 days leading to the main experimental trials you will be asked to consume chocolate flavoured drink. Prior the first experimental trial, you

will also be asked to record food and drink intake and your physical activity and then replicated it prior your second trial.

What are the possible disadvantages and risks of taking part?

Blood sampling via the cannula may cause minor bruising, an inflammation of the vein or haematoma (a small accumulation of blood under the skin). Good practice, however, minimises this risk. Some people may feel faint when they give blood.

What are the possible benefits of taking part?

There may be no direct benefits to you but as a result of being involved in this study you will receive some information about your current health. You will also receive information on your habitual intake of energy and nutrients. The findings of this study will be published in scientific journals so that understanding about high energy supplements can help slim people to improve their energy intake. This information may contribute towards guidelines on the consumption of high energy supplements. We will provide you with feedback about the main study findings and also about your own results and would be delighted to explain our findings and discuss their implications with you.

What if something goes wrong?

The chance of something going wrong is extremely small. All of the procedures involved in this study are low risk and our screening tests are designed to ensure that you will only participate if it is safe for you to do so. In the unlikely event that you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal University of Glasgow complaints mechanisms may be available to you.

Will my taking part in this study be kept confidential?

Any information which is collected about you during the course of the research will be kept strictly confidential. Any findings from the study that that are published will have your name and address removed so that you cannot be recognised. We will not pass on confidential personal information to others.

Who has reviewed the study?

This study has been reviewed and approved by Faculty of Medicine Ethics Committee for Non Clinical Research Involving Human Subjects.

Contact for Further Information

Any questions about the procedures used in this study are encouraged. If you have any doubts or questions, please ask for further explanations by contacting Sadia Fatima e- mail: s.fatima.1@research.gla.ac.uk

Dr Dalia Malkova, e-mail: Dalia.Malkova@clinmed.gla.ac.uk

You will be given a copy of this information sheet and a signed consent form to keep for your records.



INFORMATION SHEET

Impact of solid and liquid supplements on weight gain of children

(8-10 Years; CHILDREN)

We are asking you to join us in our research about the impact of solid and liquid supplements on weight gain in children. Before you join us, we want you to understand why this research is important and why you are being asked to join in. Please consider this pamphlet carefully and discuss it with your parents. We will be happy to answer your questions.

Why we are doing this research?

Solid and liquid supplements are very effective in the weight gain of thin, slim children. We want to find out which supplement is better for weight gain in children.



Why have I been chosen?



You have been chosen because you are a healthy slim child between 5-10 years. We will be inviting 64 other children of your age and health. This will be a four week study.

Do I have to take part?

It is up to you to decide whether or not to take part. If you agree to take part, you will be given a form which says you are happy. If you want to stop, you can do so by telling your teacher or your parents.



What will happen if I take part?

If you agree to take part, we will measure your height and weight and ask you some questions about your health.



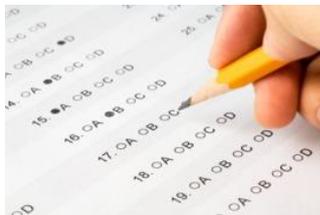
If you are eligible for the study, then we will measure your upper arm, and skinfold.



Then we will provide you supplements daily in the morning for 28 days.



We will measure your height, weight, skinfolds and mid-upper-arm circumference before the supplementation, two weeks after the start of supplementation, and after the completion of supplementation.



You have to mark a questionnaire about your appetite before the provision of first and last supplement.

We will not let anybody know that you are taking part in our research.

Are there any benefits or risks to taking part?

There are no benefits you if you participate research.

or risks to in this



Thank you for reading this

Study Design Presentation Using Pictures

Every day for four weeks in school



Or



Morning

Before, two weeks after, and at the end of the supplementation



Height



Weight



Mid arm circumference



Skin fold Measurement

Before the first and the last supplement



Appetite Questionnaire

College of MVLS
Ethics Committee

VOLUNTEER INFORMATION SHEET

Impact of solid and liquid supplements on weight gain and appetite of children between 5 to 10 years

Your child is being invited to take part in a research study. Before you decide whether or not your child should take part, it is important for you to understand why the study is being done and what it will involve. Please take time to read the following information carefully

and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish your child to take part. Thank you for reading this.

What is the purpose of the study?

It has been shown that solid ready to eat food supplements are very effective in the treatment of severe malnutrition. For example, a study conducted on severely malnourished Senegalese children in a therapeutic feeding centre demonstrated that increase in energy intake and weight gain achieved by supplementation with ready to use food was even higher in comparison to changes induced by consumption of a traditional high energy drink based on milk. This study aims to compare the efficacy of the high energy nutritional solid ready to eat and liquid milk based supplements on the weight gain of mild to moderate malnourished children aged between 5 and 10 years.

Why has my child been chosen?

Your child has been chosen because he/she is a healthy slim child between 5-10 years not suffering from any recognized eating disorders. This will be a four week study.

Does my child have to take part?

It is up to you to decide whether or not your child should take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part, you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care your child receives.

What will happen to my child if he/she takes part?

A. SCREENING PROCEDURES

The height of your child will be measured with a height measuring scale. Body weight will be measured with the help of an electronic scale. The weight of the child will be measured wearing light weight clothes without shoes. The child will stand with both feet placed flat on the balance and arms positioned laterally along the side of the body. These methods are

safe and it does not hurt. This screening procedure will enable us to determine whether your child falls into the group of children we wish to study.

B. INTERVENTION

It is still not clear which supplement is the best for the prevention of mild to moderate malnutrition in children. This is why one group of recruited children will receive a solid supplement and the other group a liquid supplement. The groups will be decided by chance.

The supplements will be provided to the child daily for the duration of four weeks. The supplements will be delivered to the school and given to your child on a daily basis. We will measure height, body weight, skinfolds and mid-upper-arm circumference of the child before supplementation, two weeks after initiation of supplementation, and after the completion of supplementation. The skinfolds of the upper arm will be measured with the help of a skinfold measuring caliper while mid-upper-arm circumference will be measured with the help of a measuring tape. The child will be asked to fill in the appetite questionnaire before the provision of the first and the last supplement. All these procedures will take no longer than 15-20 minutes and will be performed in the school. The parent/carer will be requested to come to school to discuss their child's appetite once before the start and once at the end of the supplementation. This will take no longer than 15-20 minutes.

What does my child have to do?

Other than attending the screening session, your child will be requested to consume liquid or solid nutritional supplements for four weeks. Height, body weight, skinfolds and mid-upper-arm circumference of the child will be measured before the supplementation, two weeks after the initiation of supplementation, and after the completion of supplementation. The child has to mark an appetite questionnaire before the provision of the first and the last supplement. Your child will be asked to keep the empty bottles/sachet of the supplements with him, which will be collected by the researcher next day. The parent/carer of the child will be requested to join two focus groups, and discuss their child's appetite.

What are the possible disadvantages and risks of taking part?

We do not anticipate any potential risk to children or their parent/carer in taking part in this study.

What are the possible benefits of taking part?

The children participating in this study will benefit directly by receiving supplements which will improve the nutritional needs of the children. Parent/carer will receive some information about their child's current health. Moreover dietary counselling for the parent/carer of the child will be done. We will provide parent/carer with feedback about the main study findings. The findings of this study will be published in scientific journals. This study will help to understand and compare the impact of liquid and solid nutritional supplements in preventing under-nutrition among children in their community. This information may contribute towards guidelines on the consumption of high energy supplements.

Will my child taking part in this study be kept confidential?

Any information which is collected about your child during the course of the research will be kept strictly confidential. Your child will be identified by an ID number. Any information about your child will not contain his/her name and address so your child cannot be recognised. Furthermore findings from the study that are published will contain only the results. We will not pass on confidential personal information to others.

Who has reviewed the study?

This study has been reviewed and approved by the College of Medical, Veterinary and Life Sciences Ethics Committee for Non Clinical Research Involving Human Subjects and the Research Ethics Committee of Ayub Medical College Abbottabad.

Contact for Further Information

Any questions about the procedures used in this study are encouraged. If you have any doubts or questions, please ask for further explanations by contacting

Sadia Fatima e- mail: s.fatima.1@research.gla.ac.uk

Dr Dalia Malkova, e-mail: dalia.malkova@glasgow.ac.uk

You will be given a copy of this information sheet and a signed consent form to keep for your records.

Appendix III a, b, c, d



Centre Number:

Study Number:

Subject Identification Number for this trial:

CONSENT FORM

Title of Project:

Effect of high energy nutritional supplement drinks on appetite, appetite hormones and dietary intake

Name of Researchers: Dr Dalia Malkova, Dr Konstantinos Gerasimidis, Professor Charlotte Wright Dr Sadia Fatima

Please initial box

1. I confirm that I have read and understand the information sheet dated for the above study and have had the opportunity to ask questions.
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my legal rights being affected.
3. I agree to take part in the above study.

Name of subject	Date	Signature
Name of Person taking consent (if different from researcher)	Date	Signature
Researcher	Date	Signature



Centre Number:
Project Number:
Subject Identification Number for this trial:

CONSENT FORM

Title of Project: Impact of solid and liquid supplements on weight gain of children

Name of Researcher(s): Dr Dalia Malkova, Dr Konstantinos Gerasimidis, Professor Charlotte Wright, Dr Sadia Fatima

To be completed by the child (or if unable, the parent on their behalf). Please circle your answers:

Have you read or has somebody else explained this project to you?	Yes / No
Do you understand what this project is about?	Yes / No
Have you asked all the questions you want?	Yes / No
Have you had your questions answered in a way that you understand?	Yes / No
Do you understand that it's OK to stop taking part at any time?	Yes / No
Are you happy to take part?	Yes / No

If any answers are 'no' or you don't want to take part, don't sign your name!!

If you do want to take part, you can sign your name below

Your name _____

Signature _____

Date _____

The researcher who explained this project to you needs to sign too

Print name _____

Signature _____

Date _____

Thank you for your help



Centre Number:
Project Number:
Subject Identification Number for this trial:

CONSENT FORM

Title of Project: Impact of solid and liquid supplements on weight gain of children.

Name of Researcher(s): Dr Sadia Fatima, Dr Dalia Malkova, Dr Konstantinos Gerasimidis, Professor Charlotte Wright.

Please initial box

1. I confirm that I have read and understood the information sheet dated.....
for the above study. I have had the opportunity to consider the information, ask
questions and have had these answered satisfactorily.
2. I understand that my child's participation is voluntary and that I and my child are
free to withdraw at any time, without giving any reason, without my child's legal
rights being affected.
3. I agree for my child to take part in the above study.

Name of carer/parent of participant

Date

Signature

**Name of Person taking consent
(if different from researcher)**

Date

Signature

Researcher

Date

Signature

1 for subject; 1 for researcher



HEALTH CHECK QUESTIONNAIRE FOR PARTICIPANTS

Name: **Sex**..... **Date of birth**.....

Date of Assessment.....

Anthropometry data;

Height (cm)..... **Weight (kg)**.....

It is important that volunteers participating in research studies are currently in good health and have had no significant medical problems in the past. This is to ensure (i) their own continuing well-being and (ii) to avoid the possibility of individual health issues confounding study outcomes.

Please complete this brief questionnaire to confirm fitness to participate:

- 1. Have you used antibiotics in the past month? Yes/No
- 2. Have you used any other medications apart from antibiotics? Yes/No

Please give details

.....

- 3. Have you had any recent illness or disease? Yes/No

Please give detail

.....

- 4. Are you allergic to any food like peanuts, nuts or milk? Yes/No

Please give detail

.....

- 5. Do you use dietary supplements like vitamins/protein shakes etc? Yes/No
- 6. Are you suffering from any chronic illness?

Please give detail

.....

7. Have you ever had gut surgery? Yes/No
Please give detail

.....
.....

8. Have you observed any recent weight changes? Yes/No

Appendix V

Appetite Questionnaire

Please answer the following questions by placing a vertical mark through the line for each question. Regard the end of each line as indicating the most extreme sensation you have ever felt and mark how you feel **NOW**.

Time: _____

1. How **hungry** do you feel (now)?

**I am not
hungry**

**Never been
hungrier**

2. How **satisfied** do you feel (now)?

**I am not
satisfied
at all**

**I cannot
eat another
bite**

3. How **full** do you feel (now)?

Not at all full

Totally full

4. How **much** do you think you **can eat** (now)?

A lot

**Nothing at
all**

5. How strong is your **desire to eat** (now)?

Not at all

Very

Appendix VI**FOOD INVENTORY INSTRUCTIONS**

It is important that you weigh and record everything that you eat and drink for the **two** days prior to your visit to the metabolic room at Yorkhill Hospital. Please do not take any alcohol on these days. Your last food and drink should be taken 12 hours before your arrival to the hospital.

Please (i) start a separate page for each day.
(ii) start a separate line for each item.

Column 1

Record meal and time and place of eating.

Column 2

Describe each item as accurately as possible, stating where relevant:

- (i) type and brand
- (ii) whether food is fresh, dried, canned, frozen, salted, smoked, etc.
- (iii) whether food is cooked, if so give method of cooking e.g. fried, baked, etc.

Column 3

Record the weight of each item after cooking:

- (i) place scales on a level surface
- (ii) place plate or container on top of scales
- (iii) press 'ON/Reset' button to turn on scales
- (iv) once zero appears, add first item of food
- (v) record weight displayed
- (vi) press reset button before weighing next item

Wherever possible, record weights in grams. If this is not possible, record weights in household measures (e.g. sugar or jam in teaspoons, stating whether level, rounded, or heaped).

Column 4

Record the weight of any leftovers, such as food remaining on plate, weight of container in which food has been weighed, apple cores, etc.

Columns 5 and 6

Please leave blank.

If food consists of several items, please list each on a separate line i.e. instead of writing 'one cheese sandwich', record separately the weights of bread margarine, cheese, etc.

Please remember to record all drinks, as well as food, giving weights where possible, or volumes if these are known. Record separately the weights of added milk and sugar.

An example is shown overleaf.

Food Inventory - Example

Name _____ Date _____

1. Time/Place	2. Description of food/drink	3. Weight of food/drink (g)	4. Weight of container/ leftovers (g)	Leave Blank	
Breakfast	Cornflakes (Kelloggs)	28			
8:30am	Milk (Sainsbury's virtually fat-free)	48			
Home	Bread (Mothers Pride, large white sliced, toasted)	76			
	Flora margarine	7			
	Robinsons lemon marmalade	12			
	Coffee (instant)	2			
	Milk (whole pasteurised)	10			
Lunch	Cheese (Cheddar)	55			
1:00pm	Bread (white, crusty)	76			
Pub	Butter	4			
	Chutney (2 teaspoons)				
Snack	Coffee (instant)	2			
3:30pm	Coffee-mate	6			
Office	Mars Bar	35			
	Apple	76	8 (core)		
Dinner	Turkey Fillet (frozen, grilled)	102			
6:30pm	Potatoes, old, boiled	320	74		
Home			(leftover)		
	Peas (Birds Eye, frozen, boiled)	50			
	Heinz tomato ketchup	14			
	Yoghurt (Ski strawberry thick and creamy)	162	10 (carton)		
	Coffee, filter	148			
	Milk (Sainsbury's virtually fat-free)	8			
Snack	Banana	107			
7:45pm	Orange Tango (can)	330			
Home					

Appendix VII



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Questionnaire Regarding Child's Appetite (A)

Read out the following statements and tick the boxes most appropriate to the child's eating behaviour

1) At present how is your child's appetite?

- a) Extremely good
- b) Very good
- c) Good
- d) Average
- e) Poor

2) Does your child leave food on his/her plate

- a) Always
- b) Often
- c) Sometimes
- d) Never



Questionnaire Regarding Child's Appetite (B)

Read out the following statements and tick the boxes most appropriate to the child's eating behaviour

1) At present how is your child's appetite?

- a) Extremely good
- b) Very good
- c) Good
- d) Average
- e) Poor

2) Does your child leave food on his/her plate

- a) Always
- b) Often
- c) Sometimes
- d) Never

3) Does your child like the taste of these supplements?

- a) Extremely
- b) Very much
- c) Little bit
- d) Not at all

4) Have you observed any changes in your child after the start of these supplements?

- a) Weight gain/growth
- b) Loss of appetite
- c) Behaviour changes
- d) Any other

5) How do you rate the supplements

- a) Very good
- b) Quite good
- c) Poor
- d) Very good
- e)

6) Would you like to use these supplements again?

- a) Definitely
- b) Maybe