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African starchy foods, gastric emptying, and starch digestion in Malian stunted children

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AFRICAN STARCHY FOODS, GASTRIC EMPTYING, AND STARCH DIGESTION IN MALIAN STUNTED CHILDREN

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Fatimata Cisse

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I dedicate this thesis to my husband Dioukamady Diallo, my daughters Aminata, Fatou, and Niamoye, my father Madiou Cisse, my mother Fatoumata Boussoura for their prayers, understanding, support, and confidence in me.

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ABSTRACT

Cisse, Fatimata. Ph.D., Purdue University, December 2014. African Starchy Foods, Gastric Emptying, and Starch Digestion in Malian Stunted Children. Major Professor: Bruce R. Hamaker.

Starch serves as the main energy source in cereal and tuber-rich diets, and its glycemic response profile has been associated with health-related conditions. Sorghum and millet are known to have relatively low starch digestibility, a potentially desirable property for controlling blood glucose response and providing sustained energy. Gastric emptying rates of traditional sorghum and millet-based African foods of the Sahelian region (couscous, thick and thin porridges made from millet and/or sorghum) were compared to those of non-traditional "modern" foods that are mostly consumed in urban areas using a non-invasive ^{13}C-labelled octanoic acid breath test in healthy volunteers. The obtained results showed that traditional sorghum and millet-based solid African foods had markedly slower in gastric emptying rate compared to rice, potatoes, and pasta as measured by lag phase and half-emptying time ($P < 0.0001$). Factors that regulate gastric emptying of an ingested food are of interest since their understanding may help in controlling overall energy intake. In a second study, Polycose® solution (rapidly absorbed glucose) and slow digesting, cooked and washed alginate-based waxy starch-entrapped microspheres (of variable digestion rates) were consumed as a preload, followed 20 minutes later by a 13C-labeled non-nutritive paste mixture. A comparatively fast emptying rate was observed when the paste was consumed alone whereas the slowest digesting starch-entrapped microspheres preload presented the slowest paste emptying rate as measured by the lag phase and the half emptying time parameters of the gastric emptying test ($P < 0.05$).

Starch is also a dominant source of dietary energy in complimentary feeding of growing toddlers. After weaning, dietary glucose is generated mostly from starch which is the main component of most complementary foods. A new, non-invasive modified ^{13}C-breath test was used to assess pancreatic α-amylase activity, and the ability to digest sorghum porridge starch in healthy and moderately stunted toddlers from 18 – 30 months of age in Bamako, Mali. α-amylase insufficiency was present in both Malian healthy and stunted toddlers. However, children with α-amylase insufficiency digested, absorbed, and oxidized the released glucose from normal sorghum porridge starch at least as well as, and in some cases even better, than the healthy group, indicating that the α-glucosidases compensate for the α-amylase insufficiency, and particularly well in the stunted group. A thicker porridge and its α-amylase thinned counterpart were also digested well by the stunted group.

Overall, this work shows value of traditional African sorghum and millet foods and could lead to increased demand for local foods and, thus, provide better markets for smallholder farmers. The clinical study conducted on toddlers suggests that thick energy dense porridges supply digestible carbohydrates to stunted children, and that pending further study could be considered for supplemental feeding programs.

INTRODUCTION

African traditional cereal crops and tubers represent an important source of dietary carbohydrates, proteins, fibers, vitamins and minerals. They are the major energy source to the vast majority of African people. These crops and tubers are processed to a large variety of foods and beverages with improved texture, taste, aroma, nutritional and microbial qualities, and digestibility using some basic techniques such as fermentation. They are important for the low income population as they ensure food security in those regions. Crops such as sorghum and millets can grow in difficult semi-arid climatic conditions and still give fairly high yields. However, in the last ten years, sorghum and millet in particular have seen only a very slow increase in production compared to rice and maize.

Factors such as rapid and uncontrolled urbanization, and improved economic status, have prompted changes in the dietary habits of many Africans with a substitution of traditional foods for imported or Western foods. This dietary trend has resulted in reduced consumption and demand for sorghum and millet, and a concomitant increased rice and wheat consumption, and has been noted to be a possible factor in the increasing prevalence of obesity and chronic diseases (diabetes and cardiovascular disease).The risk factors for these diseases are more common in urban than rural areas.

Nonetheless, sorghum and millet as well as the other minor grains (fonio, teff, amaranth, and quinoa) are not only staple foods for African people, but they also present some useful quality characteristics. They are rich in phytochemicals which have antioxidant properties.

An improved understanding of the health-associated attributes of traditional African foods could serve to minimize this nutrition transition in Africa. Furthermore, identification of potential advantages or attributes of traditional African diets could lead

to better promotion of local foods leading to better markets for smallholder farmers through increased productivity, demand, and utilization.

Digestible carbohydrates, which are mainly from starchy foods, are the main source of dietary energy in growing children after weaning; the period when non-breast milk food is introduced to the child until the stoppage of breastfeeding. Weaning foods in Sahelian West Africa are cereal-based and starch is their major component. Glucose is needed for a developing weaned child, not only as the principal dietary energy source, but also for brain development. After weaning, glucose mainly comes from starch in complementary foods. Young infants lack secreted α-amylase until weaning and after this period malnourished toddlers have been shown to continue to have reduced α-amylase activity.

Chapter 1 of this dissertation is a recapitulation of the pertinent information in the field of traditional African diets, and physiological properties of their major component – starch, weaning foods in West Sahelian Africa, starch digestion, and problems associated with malnutrition in children.

Sorghum starch has been known to have slow digestible property, a potentially desirable property for controlling blood glucose response after meal consumption and to provide energy to the body in an extended manner. This important property may make sorghum beneficial to diabetics and moreover, a source of sustained energy. Blood glucose response has been related to the way starchy food empties from the stomach, called gastric emptying, as slow emptying rates are associated with lower glucose responses and fast emptying with higher responses. Chapter 2 of this dissertation illustrates such a study on the implication of traditional African foods on gastric emptying and satiety. An interesting small survey has been conducted to gather the impressions subjects have about traditional African foods and so called “imported” or modern, non-traditional foods (rice, potatoes and pasta). Comparison has been made between their gastric emptying rates accompanied with assessment of satiety parameters.

Slowly digested and absorbed carbohydrates have been shown to reduce the rise of postprandial glucose response, as well as influence the gastric emptying rate of foods. The presence of nutrients in the distal part of small intestine is a key factor in the

stimulation of hormones from enteroendocrine L-cells which in turn regulate the motility of the upper gastrointestinal tract. In the past, infusion of different carbohydrate solutions (glucose, hydrolyzed starch) into the ileal part of small intestine showed notable reduction in gastric emptying time. Therefore, factors that regulate gastric emptying of an ingested food is of interest since their understanding may help in controlling overall energy intake. Chapter 3 of this dissertation focuses on studying the effect of the pre-ingestion of slowly digestible carbohydrates on gastric emptying. The gastric emptying rate of a non-nutritive thick paste has been assessed using preloads with different starch digestion rates.

It is known that malnutrition causes insufficiency of pancreatic enzyme production, including α-amylase that digests starch. Later studies provided additional evidence of α-amylase insufficiency in children with moderate malnutrition. The low luminal pancreatic α-amylase concentration in young children resulted in high amount of resistant starch which may have contributed to diarrhea through high osmotic load. An extension of the above information is that marginally and severe malnourished children with low pancreatic function may have starch digestion problems which interfere with their development and proper growth. Glucose is the only energy molecule utilized by the brain, and in children, 40% of the glucose needed for brain development comes from the diet. Chapter 4 of this dissertation presents a study on the use of a new, non-invasive modified ^{13}C-breath test to assess α-amylase activity, the ability to digest sorghum starch, and to evaluate the gastric emptying of sorghum porridge in healthy and malnourished stunted children in Mali.

Overall, this series of studies on African starchy foods, gastric emptying, and starch digestion in stunted children show the value of traditional African sorghum and millet foods and, moreover, that thick energy dense porridges adequately supply digestible carbohydrates to stunted children.

CHAPTER 1. LITERATURE REVIEW

1.1 Introduction

In West African Sahelian countries (Mali, Burkina Faso, Niger, Senegal, Northern Nigeria, and Chad), sorghum [*Sorghum bicolor (L.)* Moench] and millet (Pennisetum glaucum) are two of the most important and widely consumed food crops. They constitute a main source of macronutrients (carbohydrate, protein) and, in some cases micronutrients (vitamins, minerals) in many African diets. They usually provide more than 50% of the total calories in the diet, and up to 70-80% (FAO, 1995).

The African traditional diet is mainly based on cereal grains (sorghum, millet), tubers and roots (sweet potato, cassava, yam), and plantain, depending on the climatic zone. They are usually complemented by soups, sauces, or stews. In the Sub-Saharan semi-arid regions, cereal crops are the more dominant, while tubers and roots are prevalent in the humid tropical zones. Apart from the majority starch component, foods made from cereal grains, tubers, roots, and plantains are significant sources of other components like proteins (7 – 11% for grains, and 1 – 3% for tubers and roots), fats (1 – 5% for grains), non-starch polysaccharides , some vitamins (mainly B vitamins), and minerals (calcium, zinc, iron) (FAO, 1995; Dicko et al., 2006; Obilana www.afripro.org.uk/; Taylor, www.afripro.org.uk/).

In Mali, sorghum and millet are important staple cereal crops, not only because of their high production and the farmed area (FAOSTAT, 2013), but also for their per capita consumption (FAO 1995). The average consumption of millet/sorghum and maize is around 148 Kg/capita/year or 75% of cereal consumption. This represents a 1 bag of 100 Kg for an average family of 8 people per month, including children. Rice consumption is 44 Kg/capita/year or 22% of total cereal consumption. (Marjon, 2006).

1.2 Traditional African food

African traditional foods can be divided in different groups like breads, thick (stiff) and thin porridges, grits and couscous (steamed products), snacks, and beverages. The composition and preparation methods of African traditional foods have been addressed in the past by many authors (FAO, 1995; Aboubacar et al., 1999; Aryee et al., 2005; Aboubacar et al., 2006; Taylor et al., 2006; Kajuna, www.fao.org/; Kamal-Eldin et al., http://projekt.sik.se/). These food products can be fermented or unfermented. A result of fermentation is the improvement of some nutritional (digestibility, quality) and physicochemical and sensory attributes (the shelf life, aroma, texture, taste) of traditional foods.

1.2.1 Steps of making traditional African thick and thin porridges

The difference between thick and thin porridges is the concentration of the flour. Generally, in West Africa thick porridges (tô or tuwo) are "solid" and can be eaten with the hand, while thin porridges (moni, bouillie or koko) are "fluid" and are drinkable or are eaten with utensils. Porridges prepared with malted sorghums have significantly lower viscosities than those of non-malted sorghums due to the presence of amylases (Malleshi et al., 1989; Dicko et al. 2006). These porridges are particularly useful for the formulation of weaning foods for infants, because of their comparatively high energy density (Traore et al., 2004). There are many other types of porridges found in Africa, including fermented porridges such as nasha, a traditional weaning food (infant porridge) prepared by fermentation of sorghum flour (Graham et al., 1986), and ogi, an example of a traditional fermented sorghum food used as weaning food, which has been commercialized to a semi-industrial scale (Achi, 2005).

Scheuring et al. (1982) described the procedure of thick porridge (tô) preparation in Mali. Approximately 4 l of water is boiled in a pot. Wood ash extract (10 g) is mixed with about 650 ml of cold water in a calabash or bowl, and about 750 g of flour (sorghum or millet) is added. The mixture is stirred until homogenous and then swirled into the boiling water in the pot. The boiling mixture is stirred for about 8 min, and at this point the thin solution is called "bouillie" (thin porridge). Heat is then reduced by removing

part of the burning wood. About 1,300 ml of the thin porridge is removed from the pot and set aside in a calabash. About 1,250 g of sorghum flour is added, a handful at a time, to the boiling thin porridge in the pot. After each addition of flour, the paste is vigorously stirred with a flat wooden spoon. When the paste is too thick for stirring, a small amount of thin porridge from the calabash is added. The process of addition of flour followed by stirring and further addition of bouillie continues for about 9 min until all the bouillie from the calabash is used and the paste is thick and homogeneous. The fire under the pot is reduced, and the thick paste is allowed to cook for about 12 min. Thick porridge is usually prepared in the afternoon and served as an evening meal. The left over portion is stored overnight in a bowl covered with a piece of cloth. The following morning it is eaten cold or reheated. Tô is consumed by tearing off a handful and dipping it into sauce made with meat or fish and vegetables such as tomatoes, onions, chilies, okra, garlic, and baobab leaves. Okra contains mucilaginous gums that facilitates the swallowing of the porridge (Murty et al., 1995).

The most important characteristics of thick porridge (tô) as noted by consumers are a thick (consistent) and firm texture, and non-sticky with good keeping quality. Regarding raw grain quality attributes, the color of the pericarp, presence or absence of a sub-coat, and the texture of the endosperm of sorghum and millet grain affect the quality of thick porridge (Murty et al., 1995). There is a good deal of varietal variation in grains that affects different porridge quality attributes (Murty et al., 1995). Certain cultivars produce an acceptable porridge when cooked in an acid medium, but not in an alkaline medium. Some cultivars produce good porridge with acceptable sensory quality, but present poor storage quality. Porridge made with tamarind extract or in acid medium is usually firmer in texture and lighter in color than that made with alkaline extract. In general, the taste of thick porridge is masked by the sauces and stews or soups consumed along with it, and thus might not be as important as other qualities.

Various traditional fermented and unfermented thin porridges are prepared from millet and sorghum grains; they can be made with or without granules (Murty et al., 1995). For the thin porridge without granules, a thick flour slurry is made and added to boiling water, the thin boiling solution is left on the fire and stirred for some minutes

until a change in color is seen indicating the completed cooking process. For the thin porridge with granules, 2/3 of the flour is mixed with water to make the granules. The granules are then cooked in boiled water, and slurry is made from the rest of the flour and added to the cooked granules. Lemon juice or tamarind extract is added to the thin porridges for taste and flavor purposes, and to improve the storage quality. In many African countries, fermented porridges are more popular than unfermented ones. Fermentation is believed to improve the flavor and storage quality of the traditionally made thin porridges.

Factors such as urbanization and improved economic status have prompted changes in the dietary habits of many Africans causing a substitution of traditional foods with imported or Western foods (Boughton et Reardon, 1997; Popkin et al., 2001; Popkin, 2003; Dorelien, 2008). This dietary trend has resulted in reducing consumption and demand for sorghum and millet, and concomitant increased rice and wheat consumption (Rashcke et al., 2007). However, this progressive increase of rice consumption has not prevented the traditional cereal products to continue to be significant in the overall food consumption pattern. This is because sorghum and millet are still predominantly consumed in the rural areas and, even in the urban areas, the traditional foods are still often consumed for breakfast and dinner (Reardon, 1993). The consequences of the dietary transition could be the expansion of diet-related metabolic diseases such as Type 2 diabetes (Mbanya et al., 2010) and cardiovascular disease (Mbewu, 2009).

1.3 Starch as primary source of energy

The rate of starch digestion has a number of implications that are related to the improvement of energy delivery. Glucose serves as the most important form of energy for the brain. Studies have shown that glucose enhances cognitive function and that even the rate at which starch is digested to glucose molecules can have a positive impact on memory and mood (Benton and Nabb 2003).

In order to be utilized as an energy source, starch must be broken down to glucose in the small intestine. Starch digestion begins in the mouth where it is to some degree

hydrolyzed by salivary α-amylase, and then undigested and digested materials travel to the small intestine. There they are cleaved by pancreatic α-amylase resulting in smaller oligosaccharide products composed of linear maltooligosaccharides (maltose, maltotriose, and small amount of maltotetraose and maltopentose) and branched α-limit dextrins. This collection of maltooligosaccharides act as substrate for the mucosal small intestine brush border enzymes, maltase-glucoamylase and sucrase-isomaltase, which completely hydrolyzes them into glucose which is transported through the enterocytes into the portal vein where it is taken to the liver for processing to energy, converted to the storage molecule glycogen, or transported into the blood system.

1.3.1 Physiological and metabolic effects of starch

The rate of starch digestion affects the rate of glucose release and absorption in the small intestine, and this may have some health-related consequences. The postprandial blood glucose excursion is called the glycemic response (Gropper et al. 2005). Rapid postprandial glucose response and sharp initial peak in the glycemic curve have been linked to higher risk for developing diseases such as diabetes, obesity and heart disease (Ludwig, 2002). It is theorized that slowly digestible starch can play a role in controlling the postprandial glycemic response in individuals with diabetes mellitus as well as to help maintain better overall glucose homeostasis (Björck and Asp, 1994).

1.3.2 Starch classification

The digestibility of starch is a measure of the rate and degree to which starches are hydrolyzed through the gastrointestinal tract. Starch digestibility in cooked foods is influenced by factors such as amylose and amylopectin contents, and interaction with non-starch components. Amylose content of starch is inversely proportional to its digestibility (Rooney and Pflugfelder 1986). Starch can be classified into three different categories: rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (Englyst et al., 1992; Englyst et al., 1999; Lehmann and Robin, 2007). Each type of starch is based on its rate of digestion through the small intestine.

Rapidly digestible starch

Starches that are accessible to digestive enzymes are typically referred to as rapidly digestible starch. This starch fraction leads to a sharp increase in the postprandial

glucose response, because it is hydrolyzed to glucose very quickly. Rapidly digestible starch is digested and glucose is released within 20 minutes in the in vitro assay (Englyst et al., 1992; Englyst et al., 1996). Examples of foods high in RDS include cooked white rice (Englyst et al., 1996) or boiled potatoes.

Slowly digestible starch

The slowly digestible starch fraction is digested slower and releases glucose at a slower rate than rapidly digestible starch. Glucose release occurs between 20 and 120 minutes after ingestion in the in vitro assay. Starches high in SDS function as a source of prolonged energy and as a means of regulating the postprandial glucose response (Brand et al., 1991; Fontvielle et al., 1992). Examples of SDS are pearled barley (Englyst et al., 1999).

Resistant starch

Resistant starch is defined as starch that is not hydrolyzed in 2 h digestion using the Englyst assay, or more generally starch that escapes digestion in the small intestine and is deposited in the large intestine. It is considered, both scientifically and by regulations, as dietary fiber. It is usually fermented in the colon, providing energy to the microbiota and produced short chain fatty acids with particularly high content of butyrate. Examples of RS include raw starchy foods, such as uncooked pasta or potatoes, and foods with retrograded amylose (Topping, 2001).

1.4 Starches in the African diet

The African diet is composed of starchy foods from cereals, roots, tubers, legumes, and plantains. Cereal starches are the easiest to digest, followed by legume starches, and root and tuber starches having the lowest digestibility (Rooney and Pflugfelder 1986). However, much variability in digestion also exists within each food type. The digestibility of these types of starches will be discussed in further detail.

1.4.1 Digestibility of starch in African cereals foods

Common sources of cereal starch in Africa are sorghum, maize, millet, and rice. Because the processing of foods made from these cereals is so different in Africa, their starch digestibility cannot be generalized.

Cereal endosperms have vitreous and flour endosperm, the former which has a complex starch-protein composition in which the starch granules are embedded within a protein matrix. In raw grain, the protein matrix makes accessibility of the starch more difficult and reduces its digestibility. Therefore, in order for the starch to be accessed, the protein matrix of the endosperm must first be degraded. Compared with corn, sorghum has lower digestibility as it contains more protein and higher amounts of peripheral endosperm which is resistant to water and enzymes. After cooking, sorghum proteins are even harder to digest and appear to contain the gelatinized starch granules in a still harder to digest form than in other cereals (Hamaker and Bugusu, http://www.afripro.org.uk/). The amylose content of millet starch has been found between 20 and 22% (Beleia 1980), within the typical range of cereal starches.

1.4.2 Digestibility of root and tuber starch

Cassava, sweet potatoes, and yam are among the commonly consumed root and tuber starches in Africa. The raw starches of roots and tubers have been observed to have low digestibility in comparison with other starch types and contain more resistant starch. Roots and tubers contain B-type crystallite structure with longer double helices, making them more resistant to enzymatic digestion. *In vitro* studies show that these starch granules did not contain surface pores as previously found on some cereal starch granules, and which facilitate digestion of the raw starches (Fannon et al., 1992).

Cassava, the plant from which tapioca starch is derived, has been found to have about 14 to 24% amylose (Moorthy, 2002). *In vitro* studies have shown that raw cassava starch is more susceptible to enzyme hydrolysis than other tuber starches such as sweet potato, carrot, and potato (Rocha 2010).

Sweet potatoes vary in amylose content depending on type, but generally are found to to be in the range between 20 and 25% (Moorthy 2002). The raw starch exhibits A-type arrangement and is also more likely to be hydrolyzed by α-amylase. In

comparison to regular potato starch, sweet potato starch has smaller granule size which increases its digestibility.

Yam shows lower digestibility compared with other commonly used root and tuber starches and a higher amylose content at approximately 30% (Freitas et al 2004). The starch granules of yam are larger and are classified as B-type (Wickramasinghe 2009). Yam starch, along with sweet potato starch, appears to have shorter branch chains within their amylopectin structures.

Cooked Sorghum and millet pastes and foods have been shown to contain starch with slow digesting properties (Aryee et al., 2005; Lichtenwalner et al., 1978; Zhang et al., 1998; Archana et al., 2001; Shin et al., 2004). This slow digesting property of starches is desirable nowadays as it may relate to the management and prevention of obesity and diabetes. Slowly digestible starch is digested and absorbed releases glucose more slowly for extended absorption, and may digest into the ileal part of the small intestine (Liu et al., 2006; Aprianata et al., 2009). An extended release and absorption of glucose by the body implies a way to provide energy in a sustained manner. Moreover, African traditional crops in general, and sorghum and millet specifically, do not contain gluten, and therefore they can be used as potential source of carbohydrate for people suffering from celiac and other allergic diseases. Slowly digestible carbohydrates reduce the initial rise in blood glucose, and hold the possibility of reducing the gastric emptying rate of foods through the ileal brake mechanism (Lee et al., 2013).

1.5 Gastrointestinal tract

1.5.1 Functions of the stomach

The stomach has 4 major regions (fundus, body, antrum, and pylorus) and 3 main motor functions (storage, mixing and emptying). The fundus and the body act as storage compartments of the undigested foods, while the antrum represents the grinder, mixer, and siever of solid foods. The stomach has a flexible volume and can accommodate up to 4 L of food. It plays an important role in controlling the rate of nutrient delivery to the body through regulating gastric emptying (Delzenne et al., 2010). The stomach secretes

gastric juice (gastric acid, bile salts, and digestive enzymes) which dilutes the food bolus and helps in its mixing and homogenization. The homogeneous food bolus or chyme will continue to be mixed and is directed from the antrum to the pylorus under the effect of peristaltic waves of the stomach wall which are initiated by its contractions. The peristaltic waves spread out toward the antrum and continue down to the pyloric valve (Urban et al., 1990; Schulze 2006). The pylorus contracts and slows down the emptying rate from the stomach to the duodenum. As the peristaltic waves get more and more intense, antrum emptying is favored as well as the movement of the chyme with small particles (less than 1 – 2 mm) (Thomas 2006) and its passage through the pylorus and to the duodenum. Comparatively large particles greater than 1 – 2 mm will return back into the stomach and are ground again before they reach the required size to pass through the pylorus. This is a continual process until all the ingested food gets reduced in size, mixed, and converted into the correct particle-sized chyme (**Figure 1.1**).

1.5.2 Ileal brake mechanism

Food intake is tightly regulated by many neural and hormonal factors in the gastrointestinal tract that signal and influence locally gut motility and the hypothalamus of the central nervous system (Leibowitz et al., 2004; Murphy et al., 2006; Cummings et al., 2007). The gut-brain axis and ileal brake mechanisms, which are controlled by several factors including gut hormones, are central to the regulation of food intake. The ileal brake mechanism is a distal small intestinal feedback to the proximal gastrointestinal tract (stomach and proximal small intestine (**Figure 1.2**) that inhibits stomach emptying rate and overall gut motility. This is through the release of gut hormones, such as peptide YY (PYY), glucagon-like peptide-1 (GLP-1), and cholecystokinin (CCK) (Konturek et al., 2004; Murphy et al., 2006; Woods et al., 2004). It helps to control the transit of the ingested food in order to optimize nutrient digestion and absorption. These gut hormones, which are secreted from intestinal endocrine L-cells into the blood circulation, are released in response to the presence of macronutrients (glucose, fatty acids, and peptides) in the gut. They act on the afferent nerves or directly on the hypothalamic arcuate nucleus to inhibit the expression and release of the neuropeptide Y (NPY) and agouti related protein (AgRP) known to stimulate food intake (Konturek et al., 2004; Leibowitz et al.,

2004). Therefore, gut hormones play a key role in communicating between the gut and the brain, a link called the gut-brain axis. Accordingly, there is the possibility that the gastrointestinal system can be targeted to stimulate the secretion of its hormones using appropriate food-based approaches to regulate food intake.

1.5.3 Peptide YY – PYY

PYY, secreted by the L-cells in the gut with higher secretion in the ileal part of the small intestine and the colon, is a 36 amino acid peptide involved in the ileal brake mechanism, as well as the gut-brain axis. It is released into circulation after food consumption in proportion to the size of the meal, the amount of calories ingested and its levels rise 1 – 2 hours after meal ingestion. It presents inhibitory properties on gut motility during digestion and absorption of meals. PYY, which is secreted mainly in response to lipids and carbohydrates (Kim et al., 2005; Layer et al., 1995), is promoted by ileal absorption and reduces food intake. Degen et al. (2005) reported change in plasma PYY levels and its inhibitory effect on food intake using different doses in an exogenous infusion. The higher the PYY dosage resulted in greater plasma level and the lower food intake. Peripheral administration of PYY_{3-36} significantly reduced food intake and body weight in rodents and suppressed appetite and food intake when intravenously infused into humans. This resulted in an increase in the expression of the early response gene *c-fos* in the ARC (Batterham et al., 2002).

1.5.4 Glucagon-like peptide 1 – GLP-1

GLP-1 is secreted by the same L-cells that secrete PYY. L-cells are more abundant in the distal small intestine and colon. As an incretin hormone, GLP-1 has several functions such as stimulation of insulin secretion, suppression of glucagon, and stimulation of β-cells neogenesis. GLP-1 released in response to the presence of nutrients in the gut, and specifically to carbohydrates (Ritzel et al., 1997; Kim et al., 2005), is also one of the hormones that triggers the ileal brake mechanism (Layer et al., 1995; Degen et al., 2006). Therefore, it participates in regulation of appetite and energy intake. Intravenous infusion of GLP-1 (50 pmol/Kg.h) showed enhanced satiety and reduced energy intake by 12% in normal weight healthy men, respectively, after an energy fixed breakfast and an ad libitum lunch compared to a saline control (Flint et al., 199). It was

shown to slow gastric emptying, and decrease the feeling of hunger and increase satiety (Naslund et al., 1998; Little et al., 2006). GLP-1 as it regulates blood glucose concentration (Orskov, 1992), is known to be metabolized very rapidly (Deacon et al., 1996; Kieffer et al., 1995). Degen et al. (2006) showed that GLP-1 first peaks 15 – 30 min after meal ingestion and a second peak occurs several hours later.

1.5.5 Gastric emptying rate

The rate at which nutrients are digested and absorbed in the small intestine depends on the emptying rate from the stomach of the ingested food. Gastric emptying is known to play a major role in the regulation of postprandial glucose and energy intake. Slow emptying rate has been associated with low blood glucose levels (Horowitz et al., 1993). It also has been shown to be directly proportional to satiety and hunger (Bergmann et al., 1992).

Several factors influence the rate of emptying of a food, including viscosity, nutrient content, physical properties, composition, and volume. A number of studies have investigated the effect of high viscosity of polysaccharide gums on gastric emptying rate and postprandial blood glucose response. Addition of guar gum to isocaloric meals reduced gastric emptying rate and glycemic response (Torsdottir et al., 1989), while mixing guar gum and starch decreased starch hydrolysis rate, but had no effect on postprandial blood glucose (Leclere et al., 1994). In another study, test meals containing nutrients compared to non-nutrients ones had greater influence on gastric emptying than viscosity, while high viscosity positively affected satiety (Maricani et al., 2001). By comparing homogenized and solid-liquid meals, the physical form of a food was found to impact gastric emptying rate and satiety (Bergmann et al., 1992), with viscosity and volume more associated with satiety (Santangelo et al., 1998; Marciani et al., 2000; Rolls et al., 1998).

Gastric emptying is in part regulated by the gut hormones (PYY, GLP-1) which are secreted in response to the presence of nutrients in the GI tract. The role of the small intestine on gastric functions (secretions and emptying) was studied in the past using perfusion techniques. Findings showed that different macronutrients perfused into the ileum have a regulatory effect on gastric functions (Miller et al., 1981; Azpiroz et al.,

1985; Layer et al., 1990; Siegle et al., 1990). This indicated that the intestinal phase is a major regulator of digesta to the duodenum (gastric emptying) to make digestion and absorption more efficient. PYY and GLP-1 are secreted in response to macronutrients in the ileal part of the small intestine and are responsible for the ileal brake and are involved in the gut-brain axis mechanisms.

Gastric emptying rate has been shown to be affected by the concentration and exposure time of glucose in the small intestine (Lin et al., 1989). Infusion of glucose solutions (0.06 – 2.0 mol/L) or hydrolyzed starch in proximal and distal parts of the small intestine of dogs resulted in reduced gastric emptying rates of a solid test meal with slower emptying for the distal infusion (Lin et al., 1992). The perfusion of carbohydrate solution (75% rice starch + 25% glucose) showed a reduction in gastric emptying of a homogenized mixed meal (60% carbohydrate, 20% protein, 20% fat) and this effect was more pronounced when an amylase inhibitor was added (Jain et al., 1989). The inhibition of intestinal amylase in humans showed a reduction in gastric emptying time of rice starch and decrease in peak rise of postprandial plasma glucose by 85% (Layer et al., 1986). Compared to lipid (oleate) and protein (amino acid), carbohydrate (glucose) had the most potent effect in slowing the motility of SI, when isoenergetic amounts were infused (Siegle et al., 1990) (**Figure 1.3**).

1.5.6 Satiety

Carbohydrate ingestion has been shown to influence eating behavior including satiety and food intake. It is useful to note the difference between satiation and satiety. Satiation is the feeling involved in meal termination, whereas satiety is the feeling of fullness that persists after eating (**Figure 1.4**). It has been shown that dextrose infusion in the stomach at fast rate resulted in food intake reduction compared with saline, whereas intravenous infusion did not show any difference (Shide et al., 1995). Glucose infused intraduodenally also has shown a decrease in subsequent food intake, hunger suppression, increase in fullness and overall satiety ratings compared to when it was infused intravenously (Lavin et al., 1996). The authors speculated that this effect was due to the release of insulin or intestinal hormones via small intestine stimulation. The consumption

of 250 ml of glucose drink (30%) with added guar gum (2%) by healthy volunteers resulted in a decrease in hunger and increase in satiety ratings. The guar gum was added to delay gastric emptying and absorption the glucose (Lavin et al., 1995).

Variable digestion and absorption profiles are observed in different types and forms of starches. For example, starches that have a resistant component that reaches the large intestine undigested caused greater satiety feeling than low fiber foods and rapid digestible starch (Willis et al., 2009; Raben et al., 1994; Kendall et al., 2010). Satiety and satiation were assessed in preschool children (24 to 48 months) using three rice soups with different starch levels (4, 8, and 12%). The results showed that higher satiation and satiety were observed with the highest level of starch (Alvina et al., 2000).

1.6 Malnutrition: definition and causes

Malnutrition is defined as "a state of nutrition in which a deficiency, excess or imbalance of energy, protein, or other nutrients, including minerals and vitamins, causes measurable adverse effects on body function and clinical outcome" (Puntis, 2010). The prevalence of undernourishment in the world is estimated at 11.3% (805 million people) (FAO, 2014). Worldwide, malnutrition is associated with more than 50% of the 10 million annual deaths occurring in children under 5 years (Pelletier et al., 1993). Malnutrition of children (0 – 59 months) is a public health concern in Africa, particularly in the Sahelian countries where 40% of the children are stunted (UNICEF, 2013).

There are four different types of malnutrition: stunting, wasting, underweight, and overweight which are classified based on weight and height indices. Whereas stunting (low height for age) indicates long-term malnutrition and poor health, wasting (low weight for height) refers to a short-term response to insufficient intake. Overweight (high weight for height) implies obesity while, underweight (low weight for age) may be considered as stunting and/or wasting. Stunting, wasting, and underweight have different forms such adequate, mildly malnourished, moderately malnourished, and severe malnourished (**Table 1.1**). The different malnutrition forms are interrelated (Richard et al., 2012).

In 2011, 26 % (around 165 million) of the world's children were stunted, whereas 16 % (101 million) were underweight and 8 % (52 million) were wasted (WHO/UNICEF, 2011). Out of the 42 countries facing food crisis, 36 are in Africa, this a major problem because it is estimated that one child in three is underweight on the African continent. Although these different prevalences showed an overall decrease in stunting among children under 5 years, the general progress is still insufficient and children remain at risk. Interestingly, the trend for overweight has increased in Africa from 4 to 7% from 1990 to 2011 (WHO/UNICEF, 2011). In Mali, 1 in 3 children were shown to suffer from chronic malnutrition. Stunting among children 3 – 36 months was 30% in 1995 – 1996, and became 33% in 2001 for the same age group (PSNAN, 2005). In Sudan, Kenya, and Tanzania, 6%, 19%, and 28% of children with severe malnutrition who entered in the hospital died (Mahgoub et al., 2012).

In Mali, malnutrition is an important factor leading to high infant mortality and morbidity. According to the results of the Demographic Health Survey III (DHS III-Mali 2001), the nutritional situation is often exacerbated by economic crises such as drought and locust invasions, and led to a rather alarming health situation with child and maternal mortality rates of 113 in 1000 and 582 per 1000 live births, respectively. The results of the same DHS III showed that 38% of children under 5 years in Mali suffer from some kind of chronic malnutrition and half of those from severe chronic malnutrition. A survey conducted in 2011 using the methodology SMART (Standardized Monitoring and Assessment of Relief and Transitions) on children 6 – 59 months showed that at the national level, 11% had acute malnutrition, with 2% severe cases; and there was 27% with chronic malnutrition, of which 9% were severe cases and 20% were underweight. Although, the Sikasso region is the wettest part of Mali, with good agricultural productivity, it had the highest prevalence level of malnutrition among children under five years. The results of the survey SMART 2011 showed that the Sikasso region is below the emergency threshold for wasting 6.5% with 1% of severe cases.

This high prevalence of malnutrition in Mali stems from many interdependent factors such as medical, behavioral, and economic. (Tefft et al., 2003).

Malnutrition is often associated with decreased pancreatic function (Sauniere & Sarles, 1988), poor cognitive development, and frequent infectious diseases (Rytter et al., 2014). Knowing the causes, consequences, and the possible means to prevent malnutrition will help to develop strategies to fight this global problem (Imdad et al., 2011).

1.7 Energy and nutrient needs of infants and young children

During the first few months of life, breast milk represents a sufficient source of nutrition (energy and nutrient) for the newborn. However, starting 4 – 6 to 24 month's natural milk becomes insufficient to support the growth of the child. At this age range, children have increased needs for energy and nutrients to support their growth and development. Thus, complementary food is necessary to be added to the breast milk in order to help cover energy and nutrient deficits (Rowland & Whitehead, 1978). According to WHO/UNICEF (1998), the total energy requirements are classified based on age range and are 682, 830, and 1092 kcal/day for 6 – 8, 9 – 11, and 12 - 23 months of age, respectively. Energy required from the complementary (weaning) foods is 269, 451, and 746 kcal/day for these age groups (Dewey and Brown, 2003). A recently conducted US longitudinal study proposed new total energy requirements that are 25% to 32% less than the 1998 WHO/UNICEF requirements. Requirements from complementary foods take into account that the child is breastfed. If the child is not breastfed, then the total energy requirement will necessarily be supplied by the complementary foods. Nutrient density of complementary foods can be problematic during the weaning period Complementary foods should have a high nutrient density in order to satisfy the children needs. Also, micronutrients must in some cases be provided in relatively high amounts. For example, 6 – 8 months breastfed children need 9 times more iron and 4 times more zinc than an adult (Stephen et al., 2012; Dewey, 2013). Achieving the necessary energy requirement depends on meal frequency. The minimum daily meal number varies from 3 to 5 (Stephen et al., 2012).

1.8 Starch digestion and absorption in early infancy

Starches are the main source of dietary energy for the developing infant and growing child, particularly in developing countries. There are different enzymes that are responsible for the digestion of starch and lactose and sucrose in the human. These enzymes are salivary α-amylase, pancreatic α-amylase, and the brush border α-glucosidases for starch (and starch-derived product) digestion, lactase for lactose, and sucrase for sucrose. There is α-amylase in human milk which may help children in starch digestion from weaning foods (Lindberg and Skude, 1982; Dewitt et al., 1990). Salivary α-amylase has low activity at birth and increases rapidly and, in a normal infant, reaches a high level by the third month after birth. Hodge et al., (1983) found that salivary α-amylase is present in the gastric aspirates of premature infants and the enzyme was highly active. On the other hand, pancreatic α-amylase is not detectable during the gestation period and its activity starts after 1 month after birth. Pancreatic α-amylase reaches an adequately high level after 24 months of age (weaning age) (McClean and Weaver, 1993) (**Figure 1.5**). Small intestine brush border α-glucosidases are present in infants one month and at a high level (Lebenthal et Lee 1980), which indicates that young infants have the ability to digest starch and absorb glucose. α-Glucosidases were shown to have the ability to digest glucose polymers directly to glucose even when pancreatic secretion is absent (Kerzner et al., 1981).

1.9 Traditional weaning foods

In developing countries, traditional complementary foods are generally starch-based prepared from local available cereals such as millet, sorghum, maize, rice, or wheat. During cooking, the starch granules swell in the presence of the heat and gelatinize to form a viscous, bulky paste on cooling which is difficult to consume by children, particularly if they have immature pancreatic function; which in case starch may not be fully digested (McClean & Weaver, 1993). On the other hand the presence of other sources of amylase may also help in starch digestion such as from saliva and breast milk (if infants are still breastfed) (Dewit et al., 1990), and as well the small intestinal α-

glucosidases (Lebenthal & Lee, 1984). In order to make these traditional weaning foods easy to consume and be digested by young infants, less flour is often added to make the viscosity acceptable (1000 – 3000 cP), therefore decreasing the energy delivered in the food (Mosha and Svanberg, 1983). Traditional weaning foods are low energy dense, low nutrient content, and possible infected by bacteria due to unhygienic preparation conditions (Weaver 1994). An ideal weaning food type should be high in energy and nutrient content, microbiologically safe, easily ingested and digested, able to be frequently consumed, culturally acceptable, locally available, and cheap (Weaver, 1994).

Increase of the flour content or addition of fat, oil, sugar or complex carbohydrates, or fortification with legumes, are ways to increase the energy and nutrient density of the traditional weaning foods which also makes them thick, bulky, viscous and difficult to digest (Hellstrom et al., 1981; Nout & Ngoddy, 1997). Germination or malting, fermentation, extrusion, and fortification are different techniques used to increase the energy and/or nutrient densities of traditional weaning foods.

Malt is used to partially digest gelatinized starch, thus reducing the viscosity of bulky traditional weaning foods through the action of amylases. Cereal grains are used to make malt and this amylase-rich flour helps to lower the viscosity of the thick gruels, increase their ingestion, as well as their further digestion and absorption (may double the energy density) (Nout, 1993, Weaver et al., 1995). Amylase partially digests the starch to easily digestible molecules like dextrins and maltose which have less water-binding capacity (Hellstrom et al., 1981).

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Table 1.1 The different degrees of malnutrition

	Weight for height	Height for age	Weight for age
Adequate	≥ -1.0	≥ - 1.0	≥ -1.0
Mild	<-1.0 - ≥ -2.0	<-1.0 - ≥ -2.0	<-1.0 - ≥ -2.0
Moderate	< -2.0 - ≥ -3.0	< -2.0 - ≥ -3.0	< -2.0 - ≥ -3.0
Severe	< -3.0	< -3.0	< -3.0

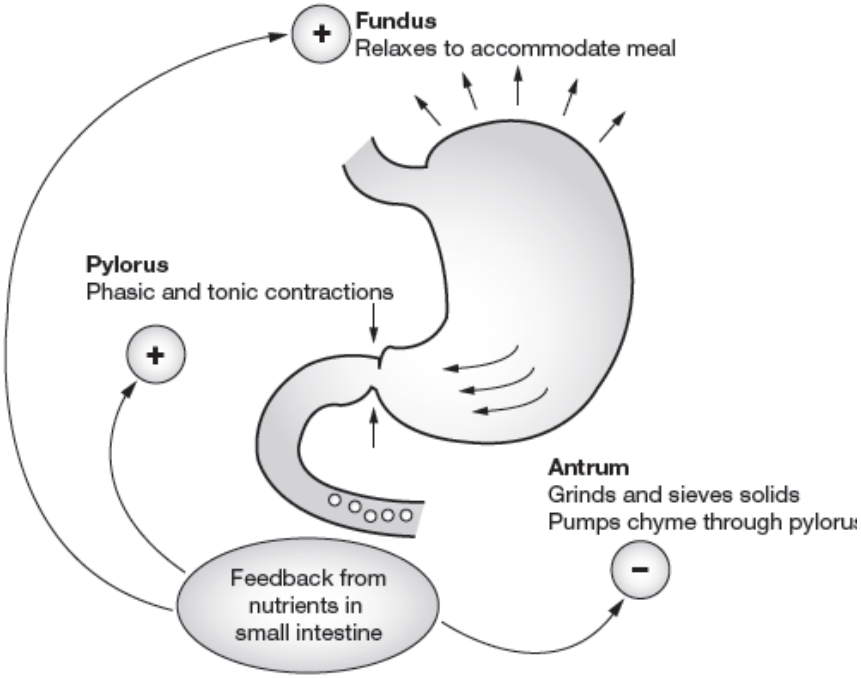

Figure 1.1 Different parts of the stomach and their functions.
Adapted from Rayner and Horowitz, 2005

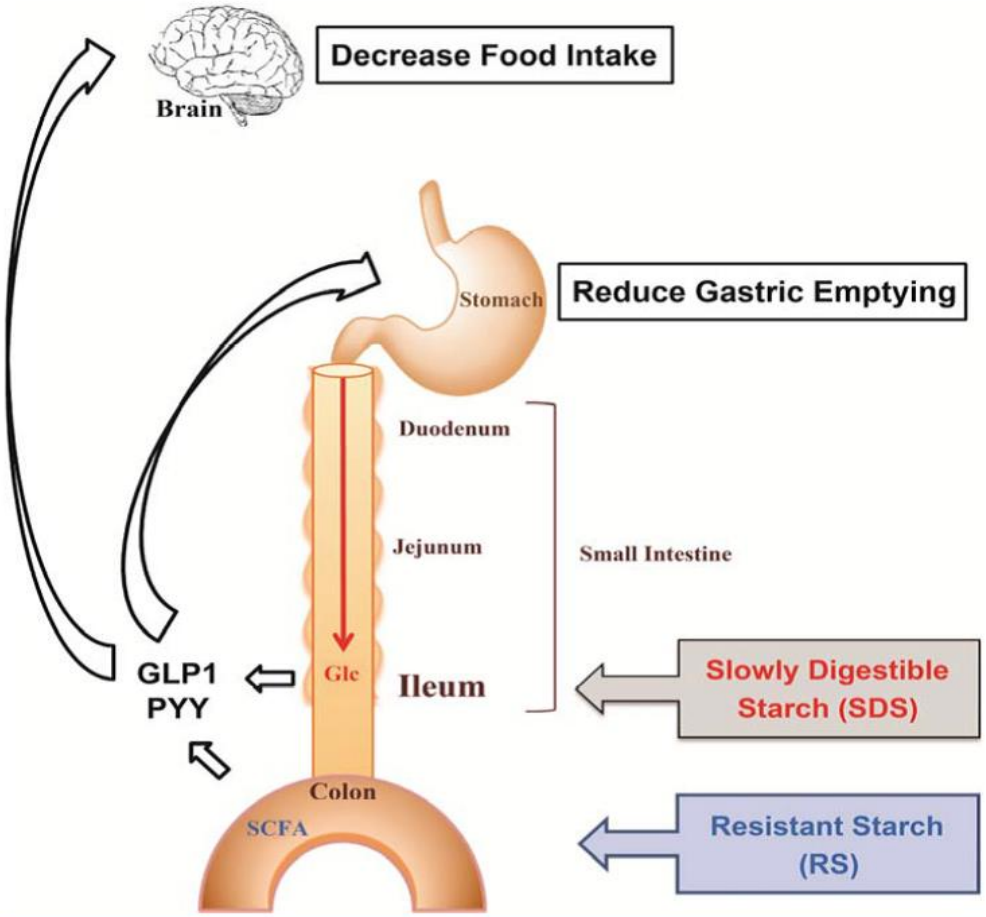

Figure 1.2 Ileal brake activation.
Adapted from Lee et al., 2013

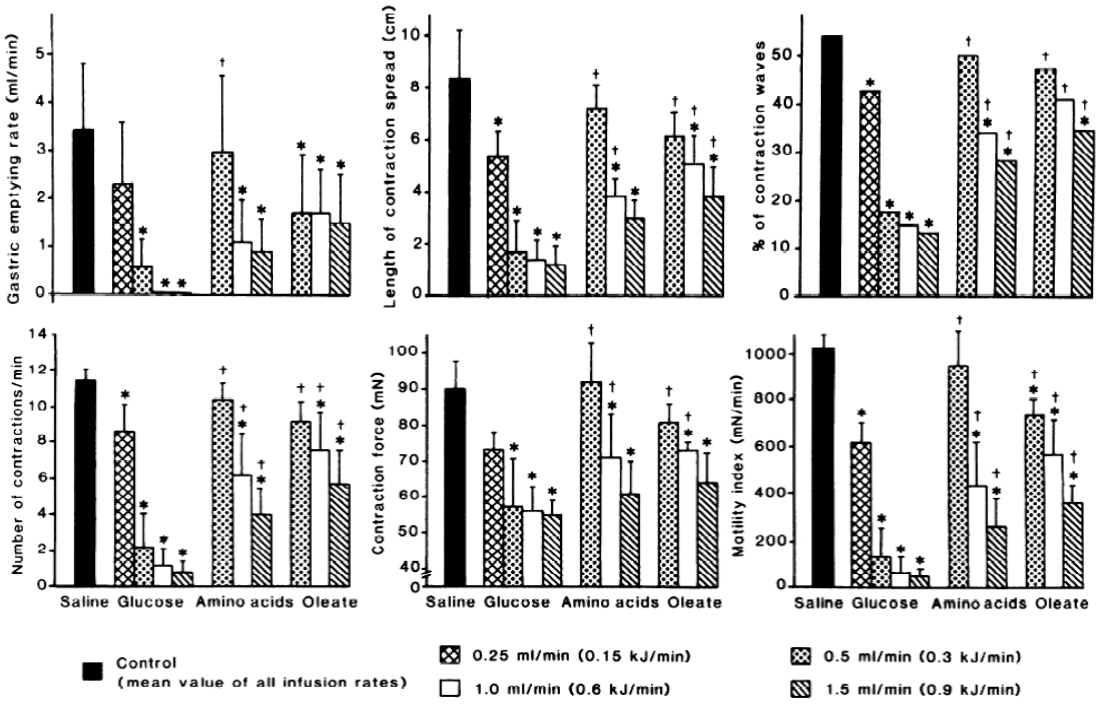

Figure 1.3 Changes in gastric emptying rate and gut motility after infusion of nutrients. Bars represent means + SD of 4 dogs. Adapted from Siegle et al., 1990.

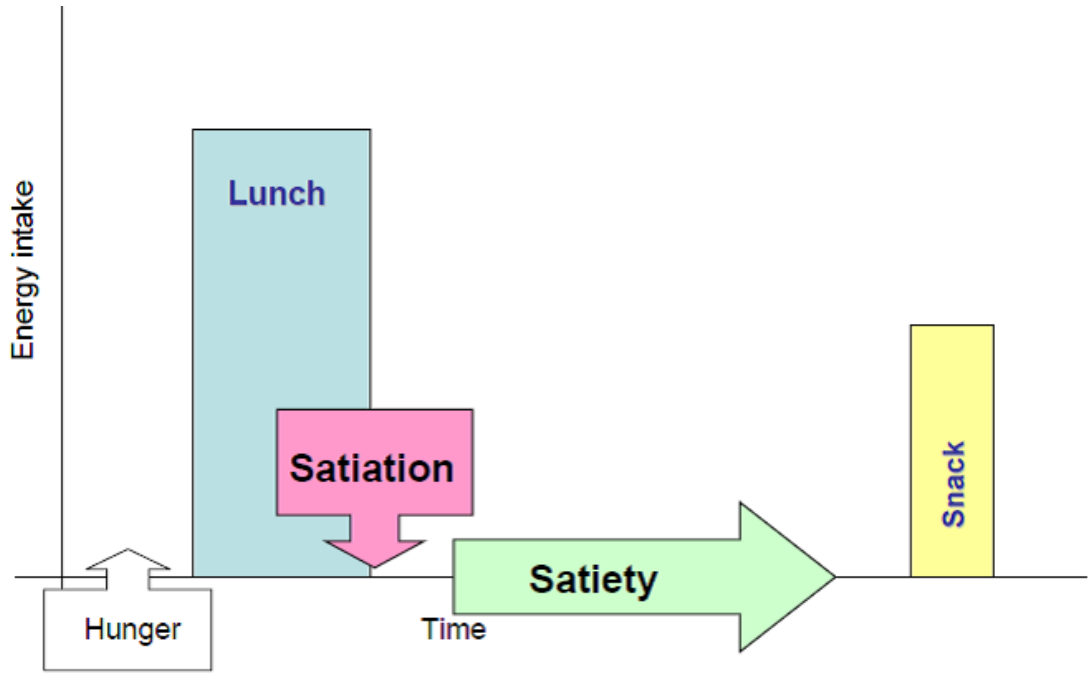

Figure 1.4 Satiation and satiety
Adapted from Benelam, British Nutrition Foundation. www.nutrition.org.uk/satiety

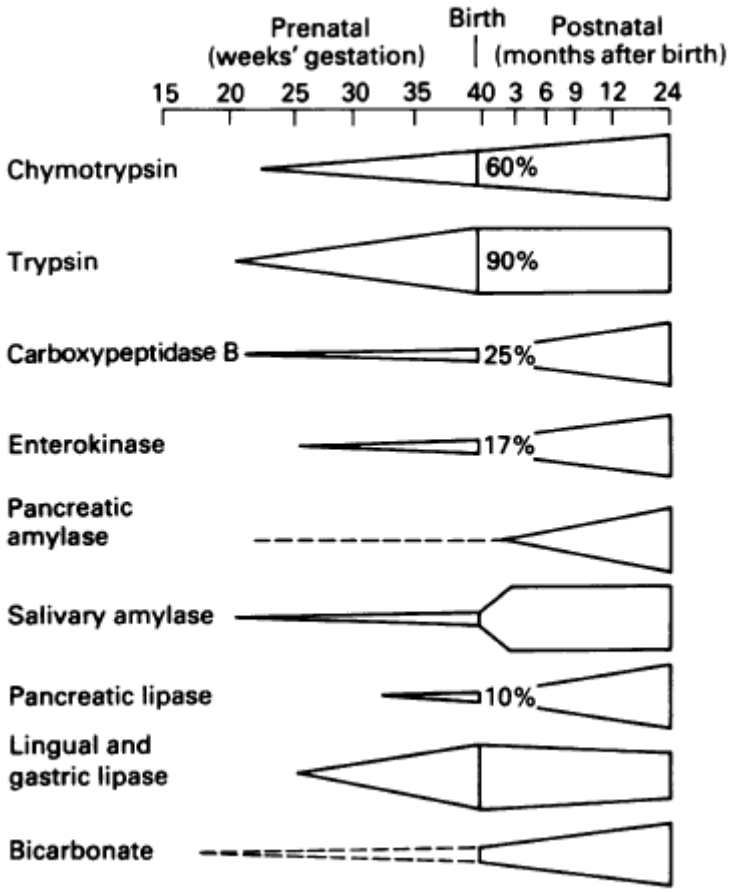

Figure 1.5 Pre and post natal pancreatic enzymes distribution.
Adapted from McClean and Weaver, 1993

CHAPTER 2. IMPLICATION OF AFRICAN TRADITIONAL FOODS ON GASTRIC EMPTYING AND SATIETY

2.1 Abstract

Starch serves as the main energy source in cereal and tuber-rich diets, and its glycemic response profile has been related to health-related conditions. Sorghum and millet are known to have comparably low starch digestibility, a potentially desirable property for controlling blood glucose response and providing sustained energy. It was postulated that these effects may be through a slowing of gastric emptying. Thus, the aim of this study was to assess gastric emptying rates of traditional sorghum and millet-based African foods of the Sahelian region (couscous, thick and thin porridges made from millet and/or sorghum) versus non-traditional "modern" foods that are mostly consumed in urban areas, under the hypothesis that these indigenous foods provide a sustained energy delivery to the body that is related to a slower gastric emptying and concomitant moderated glycemic response, and may be related to satiety. A non-invasive ^{13}C-labelled octanoic acid breath test method and subjective pre-test and satiety response questionnaires were used. An initial study was done to assess and compare the gastric emptying rate of traditional African sorghum and millet based foods (sorghum thick porridge, millet thick porridge, millet couscous, and two thin millet thin porridges) to that of rice, boiled potatoes and pasta. Fourteen healthy volunteers [mean ± SD age: 22.79 ± 2.11 y; BMI (in Kg/m^2) 20.48 ± 1.73] participated in the initial study. However, in the initial study, we did not take into account the ^{13}C enrichment difference that exists between C4 (sorghum, and millet) and C3 (rice, potatoes, and wheat) plants which can affect the gastric emptying rate assessment. Actually, C4 plants contain inherently high amount of endogenous ^{13}C which is a confounding factor in their baseline ^{13}C enrichment levels.

To overcome this, we conducted a second study with six volunteers [mean ± SD age: 24.33 ± 3.27 y; BMI (in kg/m^2) 22.52 ± 1.54] to assess gastric emptying rate of the different test meals made with and without the ^{13}C tracer (^{13}C-octanoic acid). Both studies were done in Bamako, Mali. Participants consumed test meals mixed with 100 mg of ^{13}C-labelled octanoic acid and collected breath samples were analyzed. Traditional sorghum and millet-based solid African foods were markedly slower in gastric emptying rate compared to rice, potatoes, and pasta as measured by lag phase and half-emptying time ($P < 0.0001$). Millet couscous and rice had higher fullness and lower hunger scores compared to others (all $P < 0.05$), suggesting a bias towards satiety scoring in the case of rice as seen in a pre-test questionnaire.

Traditional sorghum and millet-based African foods were concluded to provide same meal slow gastric emptying to provide sustained energy delivery to the body more than non-traditional, modern foods that are nowadays considered desirable by urban consumers.

2.2 Introduction

Sorghum and millet, as two of the most important and widely consumed food crops in Africa, constitute a main source of macronutrients (carbohydrate, proteins) and, in some cases, micronutrients (vitamins, minerals) in many African diets. Since these crops are able to grow in semi-arid conditions and are mostly consumed by disadvantaged people, they are considered by FAO to be "crops for poor people" (FAO, 1995). Traditional West African sorghum and millet foods are served in different forms, such as liquid (thin porridges, with and without granules) and solid (very thick and medium thick porridges, and couscous) foods (Rooney et al., 1987).

Factors such as urbanization and improved economic status have prompted changes in the dietary habits of many Africans with a substitution of traditional foods for imported or Western foods (FAO, 1995; Raschke et al., 2007; Popkin et al., 2001; Dorelien, 2008). This dietary trend has resulted in reduced consumption and demand for sorghum and millet, and a concomitant increased rice and wheat consumption (Raschke et al., 2007), and has been noted to be a possible factor in the increasing prevalence of obesity and chronic diseases (diabetes and cardiovascular disease) (Folake et al., 2008). For example, in one study rapid health deterioration and increases of obesity and chronic diseases in sub-Saharan African migrants to Australia were attributed to changes in dietary habits from traditional foods (Renzaho et al., 2006). An improved understanding of the health-associated attributes of traditional African foods could serve to minimize this nutrition transition in Africa. Furthermore, identification of potential advantages or attributes of traditional African diets could lead to better promotion of local foods leading to better markets for smallholder farmers through increased demand.

Traditional African diets are rich in starch, comprised largely by foods such as porridges, flatbreads, and agglomerated products made from cereal grains and tubers, whereas Westernized diets contain higher proportions of animal-source and processed foods, the latter often with rapidly digestible carbohydrates, as well as added sugars and salt. Starch serves as the main energy source for cereal-rich diets; and sorghum and millet are known to have comparably low starch digestibility, a potentially desirable

property for controlling blood glucose response after meal consumption and to provide energy to the body in an extended manner (Lichtenwalner et al., 1978; Zhang et al., 1998; Archan et al., 2001; Lee et al., 2013). Blood glucose response of starchy foods can be related to gastric emptying rate, as slow emptying rates are associated with lower glucose responses and fast emptying with higher responses (Torsdottir et al., 1984; Mourot et al., 1988).

Several factors influence the rate of gastric emptying of a food, including nutrient content, physical properties, composition, and volume. A number of studies have investigated the effect of high viscosity caused by polysaccharide gums on postprandial blood glucose response, glucose diffusion rate, and gastric emptying rate in relation to satiety and hunger. For example, addition of guar gum to meals of the same composition was shown to reduce gastric emptying rate and glycemic response (Torsdottir et al., 1989). Contrarily, mixing of guar gum and starch caused a decrease of starch hydrolysis rate, but had no effect on postprandial rise in blood glucose (Leclere et al., 1994). In another study, test meals with varied viscosity and nutrient content (low-viscosity no nutrient, low-viscosity nutrient, high-viscosity no nutrient, high-viscosity nutrient) were shown to have a cumulative effect in slowing gastric emptying and enhancing satiety (Marciani et al., 2001). However, test meals containing nutrients compared to the non-nutrients ones had greater influence than viscosity on gastric emptying, while high viscosity had high effect on satiety. Gastric emptying rate has been shown to be directly proportional to satiety and hunger (Bergmann et al., 1992). Although the physical form of a food has been reported as having an impact on gastric emptying rate and satiety by comparing homogenized and solid-liquid meals (Santangelo et al., 1998), viscosity and volume were more associated with satiety (Marciani et al., 2000; Rolls et al., 1998).

Gastric emptying is also well known to be regulated by gut hormones which are triggered in the small intestine by different macronutrients. The ileal-secreted gut hormones, glucagon like peptide-1 (GLP-1) and peptide YY (PYY), in response to the presence of glucose, fatty acids, and peptides are responsible for the ileal brake mechanism (Spiller et al., 1984; Siegle et al., 1990; Avesaat et al., 2014). This distal

small intestinal feedback to the proximal gastrointestinal tract (stomach and the proximal small intestine) is known to inhibit gut motility including gastric emptying rate.

This study aimed to test the hypothesis that traditional African foods made from sorghum and millet have slower gastric emptying than non-traditional, modern foodstuffs such as rice, potatoes, and wheat pasta that are commonly eaten today in African cities. The overall goal of this work was to understand whether traditional sorghum and millet foods of West Africa have positive attributes, such as low glycemic response and the providing of sustained energy to the body, which can be used to promote these grains in its urban areas for health reasons and to provide better markets for local smallholder farmers.

An initial study was done to assess and compare the gastric emptying rate of traditional African sorghum and millet based foods (sorghum thick porridge, millet thick porridge, millet couscous, and two thin millet thin porridges) to that of rice, boiled potatoes and pasta. Subjects' impressions about African traditional foods, and satiety were also assessed. However, in the initial study, we did not take into account the ^{13}C enrichment difference that exists between C4 (sorghum, and millet) and C3 (rice, potatoes, and wheat) plants which can affect the gastric emptying rate assessment. Actually, C4 plants contain inherently high amount of endogenous ^{13}C which is a confounding factor in their baseline ^{13}C enrichment levels. To overcome this, we conducted a second study testing the test meals made with sorghum and millet with and without the tracer (^{13}C-octanoic acid).

2.3 Subjects/Materials and methods

2.3.1 Subjects eligibility

Eligibility criteria were: males or females aged 20 – 50 years, normal body mass index (18 kg/m2 ≤ BMI ≤ 25 kg/m2), not under any medication, no history of any gastrointestinal disease or surgery, no diabetes, and no smoking. After the screening for eligibility, 14 healthy volunteers (12 men and 2 women) participated in the initial study. For the second study, 6 subjects (3 men and 3 women) were recruited. For both studies, participants were asked to avoid intense physical activity the day before and during the

test days as well as reduce or avoid, if possible, the consumption of naturally ^{13}C-enriched foods, for example corn, sorghum, millet, and cane sugar-based products during the testing period. A written consent form approved by the Institutional Review Board of Purdue University and the National Ethical Committee for Health and Life Sciences in Mali was obtained from each subject before his or her participation in the study.

2.3.2 Test meals

Six starch-based solid meal staples were tested, rice, boiled potatoes, wheat pasta, sorghum thick porridge, millet thick porridge, and millet couscous; and two millet thin porridges (with and without granules made from millet flour). Solid test meals were served with a tomato sauce made with onions, tomato paste, and fresh tomatoes, plus a seasoning of salt, black pepper, celery and green pepper. The two thin porridges were served as is. All test meals were prepared locally based on typical preparation methods used in Mali. Rice, potatoes, and pasta were cooked in slightly salted water. Thick porridges and thin porridge without granules were prepared similar to the conventional cooking methods as described by Scheuring et al. (1982). For the thin porridge with granules, 2/3 of the flour was mixed with water to make the granules. The granules were then cooked in boiled water, and slurry was made from the rest of the flour and added to the cooked granules. Lemon juice was added to the thin porridges for taste purposes. Millet couscous was prepared by mixing the flour with a small amount of water in order to make small fine particles. The particles were sieved with a medium diameter traditional sieve for uniformity after which they were steamed three times consecutively. Before adding the sauce (200 g), 100 mg of ^{13}C-octanoic acid (Sigma-Aldrich, St. Louis, MO) was mixed into each subject's weighed test meal portion (500 g) as a tracer, therefore the total amount of test meal given was 700 g. Test meals were cooled for 10 to 20 min before serving. After ingestion of the test meals, each subject was allowed 150 mL of water.

2.3.3 Procedure

This study was performed in Bamako, Mali at the Sotuba Agricultural Research Center of the Institut d'Economie Rurale (IER). Gastric emptying was assessed using the ^{13}C-octanoic acid breath test (Ghoos et al., 1993; Choi et al., 1997; Clegget al., 2010).

Subject impressions (n=14) of differences between traditional and imported foods were assessed by a pre-test questionnaire (**Table 2.1**) following completion of the consent form. Information gained from the questionnaire included subject food preferences, consumption frequency, impressions of satiety effects of traditional and "imported" foods, gender, and age.

One test meal experiment was performed on each day in a random order, with all subjects provided the same meals. Subjects were asked to come to the Sotuba Center at 9:30 AM for nine consecutive days and were instructed to fast overnight from midnight to 10:00 AM prior to the test. Each test meal was given at 10:00 AM and eating time was less than 15 min, with subjects asked to eat as much as they would like until they felt full and well satiated (Rolls et al., 1990; Erdmann et al., 2007). Breath samples were taken in duplicate before eating the test meal (to be used as a baseline value) and during the 4 h period after eating, in 15 min intervals during the first two h, then every 30 min for the final 2 h. The test meals were weighed before and after they were presented to the subjects and from this the energy intake was calculated using food composition tables. The amount of test meal consumed on dry weight basis was converted into energy intake by multiplying the weight of the food by its caloric value (USDA, National Nutrient Database for Standard Reference Release 24, 2011; FAO, 1995).

Visual analogue scales (VAS, in mm) were used to assess fullness, hunger, desire to eat, and prospective food consumption in both studies (Rolls et al., 1990; Cassady et al., 2012), and were performed before testing, right after eating, and 2 and 4 hours after test meal consumption. For example, fullness was rated by placing a mark on a 100 mm VAS at a position that showed degree of fullness between "Not at all full" on the left to "Extremely full" on the right. All other parameters (hunger, desire to eat, and prospective consumption) were rated in the same manner. Breath samples were analyzed using a ^{13}C

breath analyzer (POCone, Otsuka Co., Japan), an infrared spectrophotometer that determines the ratio of $^{13}CO_2$ to $^{12}CO_2$ (Sanaka et al., 2007; Braden et al., 1999; Schadewaldt et al., 1997). The breath analyzer POCone automatically conducts two self-diagnoses when its turns on and the precision of the instrument was checked once as described in its instruction manual before starting the test.

In the second, the gastric emptying rate assessment was repeated (n-6) in order to take into account the endogenous ^{13}C in C4 plants. The sorghum and millet thick and thin porridges, and the millet couscous, were tested on two occasions with and without the ^{13}C-octanoic acid. This was to provide baseline reference data for ^{13}C in breath CO_2 associated with the inherently higher amount of endogenous ^{13}C in C4 plants which includes sorghum and millet (Schoeller et al., 1980). Rice, potatoes and pasta were tested only with the ^{13}C-octanoic acid, because their endogenous ^{13}C contents were considered negligible.

Calculation of gastric emptying parameters

The breath analyzer provides data in terms of the change in the $^{13}CO_2$ delta over baseline (DOB, ‰), where the $^{13}CO_2/^{12}CO_2$ ratio of a sample gas is compared to the corresponding ratio of a reference gas (i.e. baseline value). For sorghum and millet thick and thin porridges, and millet couscous, the $^{13}CO_2$ DOB values obtained without tracer was subtracted from the corresponding values of $^{13}CO_2$ DOB obtained with tracer. This difference was used in the calculations. The amount of ingested tracer for each subject and each test meal was calculated by subtracting the remaining amount of tracer from the initial dose given. Using the appropriate $^{13}CO_2$ DOB for each test meal, CO_2 production, the amount of tracer ingested percent dose ^{13}C recovery per hour (PDR), and cumulative percentage dose recovery over time (CPDR) was calculated (Ghoos et al., 1993; Braden et al., 2007; Haycock et al., 1978). CO_2 production was assumed to be 300 mmol/(m^2 body surface area x hour), with body surface area calculated using the formula developed by Haycock et al. (1978). After calculating PDR and CPDR values from the obtained data set and these functions were modeled using the following equations to discern parameters related to the gastric emptying rate.

$y = at^b c^{-ct}$ Where y = percentage dose recovery per hour, t = time in hours, a, b, and c = constants.

$y = m(1 - e^{-kt})^{\beta}$ Where y = cumulative percentage dose recovery over time, t = time in hours, m, k, and β = constants and m = total cumulative dose recovery when time is infinite.

Modeling of data was achieved by nonlinear regression using SAS statistical analysis software (SAS 9-3TS1M1, Institute Inc., Cary, NC) and was confirmed using a macro program in Excel (Microsoft Corp., Redmond, WA). Gastric emptying parameters were then calculated using the following formulas:

Lag phase (i.e. time required for the $^{13}CO_2$ excretion rate to attend its maximal level) (Sanaka et al., 2010).

$T(Lag) = (\ln \beta) / k$

Half emptying time (i.e. time necessary for half of the ^{13}C dose to be metabolized) (Sanaka et al., 2010).

$(T_{1/2}) = (-\frac{1}{k}) \times \ln(1 - 2^{-\frac{1}{\beta}})$

Statistical analyses

The pre-test questionnaire results were reported as percentage of participants (n=14) responding.

For purpose of comparison of gastric emptying data, test foods were separated into solid and liquid groups. Solid foods were rice, boiled potatoes, pasta, sorghum thick porridge, millet thick porridge, and millet couscous. Liquids were the two thin porridges. Comparisons of lag phase, half-emptying times and values across foods, the amount of test meal consumed, the energy intake as well as the PDR curves at each time point were analyzed by one-way repeated-measure ANOVA with post-hoc Tukey tests used to form statistical groupings (α=0.05) using the statistical package SAS 9-3TS1M1.

Fullness, hunger, desire to eat, and prospective consumption scores of the different test meals were also compared at α=0.05 using repeated measures analysis of variance with Bonferroni correction for multiple comparisons using IBM SPSS Statistics v.19.0 Window software package. The different ratings at each time point were compared

using one-way ANOVA with post-hoc Tukey tests to form statistical groupings (α=0.05) using IBM SPSS Statistics v.21 software package.

All values were reported as mean values ± SEM (standard error of means) unless otherwise stated and compared at significance level α=0.05. The means with same letter are not significantly different whereas those not sharing the same letter are significantly different when $p < 0.05$.

2.4 Results

The gastric emptying data from the initial study is not given here since it was not valid because of the inherent endogenous ^{13}C in sorghum and millet based foods. The pre-test questionnaire results are from the initial study and the satiety data is shown for both studies.

2.4.1 Subjects characteristics

Fourteen healthy volunteers (12 men, 2 women) between the ages of 20 – 26 years old (mean ± SD 22.79 ± 2.11) with a mean (± SD) BMI of 20.48 ± 1.73 Kg/m^2 were recruited by local advertisement in the area of the research center at Sotuba, a periurban section of Bamako where the study was conducted. All subjects were free of any gastrointestinal disease and were not using any medication throughout the duration of the study.

The characteristics of the six subjects (3 men, 3 women) used to do the second study were mean (± SD) age=24.33 ± 3.27 and mean (± SD) BMI=22.52 ±1.54 kg/m^2.

2.4.2 Pre-test questionnaire

In Mali, solid foods (rice, boiled potatoes, pasta, sorghum and millet thick porridges, and millet couscous) are consumed mostly for lunch and/or dinner, while thin porridges with granules are taken for breakfast. Results revealed that 100% of the participants consumed thin porridge with granules once per day as breakfast, while thin porridge without granules was never eaten. This would differ in other West Africa Sahelian countries where plain thin porridges are commonly consumed. The majority of participants reported consumption of rice and couscous more than once in a week (around

43% and 36%, respectively) (**Table 2.2**). Thick porridges were most often eaten once per week or more than once per week (50% and 43% of participants, respectively) (**Table 2.2**). Rice was the preferred lunch option for 71% of the participants (**Table 2.3**). Thin porridge with granules as well as potatoes was preferred for breakfast with equal responses of 43% of the participants, while millet thick porridge was preferred by 36% of the participants for dinner (**Table 2.3**). In terms of perceived satiating properties, millet couscous and rice were rated highest in the pre-test questionnaire with values 43% and 36%, respectively (**Table 2.4**).

2.4.3 Consumption amount and energy intake

Table 2.5 reports the amount of test meal consumed in the second study (n=6) (subjects consumed meals until full). There was no statistically significant difference among the solid types of food (rice, 593 ± 54.7 g; boiled potatoes, 620.3 ± 50.4 g; pasta, 446.3 ± 47.1 g; sorghum thick porridge, 426 ± 36.5 g; millet thick porridge, 522 ± 54.8 g; and millet couscous, 464 ± 39.8 g). However when analyzed together with the thin porridges, the later had significantly high amount consumed (**Table 2.5**). Among the tested foods, the mean energy intake was significantly lower for boiled potatoes (2031.5 ± 89.5 kJ) (485.5 kcal) compared to rice (3222.4 ± 135.6 kJ) (770.2 kcal), pasta (2635 ± 78.5 kJ) (629.8 kcal), sorghum thick porridge (2269.1 ± 102.3 kJ) (542.3 kcal), millet thick porridge (3571.3 ± 202.6 kJ) (853.6 kcal), and millet couscous (2609.2 ± 104.9 kJ) (623.6 kcal).

2.4.4 Gastric emptying of the different test meals

Table 2.6 reports the baseline breath $^{13}CO_2$ content of each subjects during the testing period. **Table 2.7** reports the baseline reference data for ^{13}C in breath CO_2 associated with the inherently amount of endogenous ^{13}C in sorghum and millet based test meals. **Figure 2.1** shows the mean rate of recovery of ^{13}C in breath after ingestion of labeled octanoic acid in solid foods. Traditional African sorghum and millet solid foods (sorghum thick porridge, millet thick porridge, millet couscous) were characterized by a later peak time of recovery at two hours after ingestion of the test meal followed by a plateau, whereas the non-traditional, modern ones (rice, pasta and potatoes) had a very

early peak time around one h after ingestion followed by a decrease of the PDR curves back to baseline. During the first and last hours, there was a significant difference in the rate of recovery of ^{13}C between the traditional foods and the non-traditional ones (P <0.0001 at 15 min, 210 min, 240 min, P = 0.0002 at 30 min, P = 0.0163 at 45 min, P = 0.0064 at 60 min). The two thin porridges showed similar ^{13}C label recovery rate (**Figure 2.3**) and their gastric emptying parameters did not show any significant difference (P=0.6583 and P= 0.6198 respectively for lag phase and half emptying time) (**Figure 2.6**). The gastric emptying parameters of thin porridges were slightly lower than the traditional solid foods. **Figures 2.2** and 2**.4** represent the cumulative percentage dose of $^{13}CO_2$ recovered curves after consumption of the solid foods and the thin porridges. **Figures 2.5** and 2**.6** show the mean values of the gastric emptying parameters [lag phase, T (Lag) and half-emptying time, $T_{1/2}$] of solid foods and thin porridges respectively. Rice [T(Lag) = 1.3 ± 0.2, $T_{1/2}$ = 2.6 ± 0.3], boiled potatoes [T(Lag) =1.5 ± 0.1, $T_{1/2}$ = 2.9 ± 0.3], and pasta [T(Lag) =1.2 ± 0.04, $T_{1/2}$ = 2.8 ± 0.2] were comparably fast emptying and did not differ significantly from one another for either parameter; while traditional sorghum and millet foods (sorghum thick porridge, millet thick porridge, and millet couscous) were substantially higher, showing slower gastric emptying than the non-traditional foods and did not differ from one another for the half-emptying time (5.4 ± 0.4, 4.5 ± 0.1, and 5.3 ± 0.06, respectively), and lag time (2.5 ± 0.04, 2.1 ± 0.1, and 2.5 ± 0.06, respectively). Thus, the studied solid foods could be clustered in two groups:

- Fast gastric emptying group: rice, boiled potatoes, and pasta
- Slow gastric emptying group: sorghum and millet thick porridges, and millet couscous

2.4.5 Satiety

Figures 2.7 and **2.8** display mean fullness and hunger scores of the solid test meals after 4 h following consumption for all 14 participants. A main effect of meal type and meal type by time interaction were observed for subjective hunger scores (P < 0.001, P < 0.001), fullness (P = 0.009, P = 0.012), desire to eat (P = 0.008, P < 0.001), but not prospective consumption (P = 0.05, P = 0.005). Rice and millet couscous presented the

highest postprandial fullness score and lowest hunger and desire to eat scores compare to all other solid test foods ($P < 0.05$).

Figures 2.9 and **2.10** display mean fullness and hunger scores of the test meals after four hours following consumption (n=6). **Figures 2.11**, and **2.12** exhibit the mean ratings for "desire to eat", and "prospective consumption" of all test meals for four hours after ingestion respectively. Fasting scores for all satiety parameters at the beginning of the experiment did not differ among the different foods (fullness P=0.893, hunger P=0.899, desire to eat P=0969, prospective consumption P=0.986). Consumption of the test meals resulted in a significant increase in fullness and decrease in hunger, desire to eat, and prospective consumption in all treatments (P<0.05). After ingestion of the different test meals, no significant differences were observed in fullness and prospective consumption scores between them at 2 and 4 hours. However, there were significant differences in hunger at 2 and 4 hours (P=0.021 and P=0.015 respectively). Rice and sorghum thick porridge were significantly different from thin porridge w/o granules (P=0.021 and P=0.047). The different test meals were significantly different in "desire to eat" scores only at 4 hours after eating (P=0.030).

Overall, statistical significant difference was seen in all parameters between the treatments in the big group (n=14) when compared to the small group (n=6).

2.5 Discussion

Among the foods tested, the traditional solid African foods (sorghum and millet thick porridges, and millet couscous) had markedly slower gastric emptying rates compared to the non-traditional, modern foods more commonly consumed in urban areas (rice, boiled potatoes, and pasta). Among the fast gastric emptying modern foods, none had significantly different gastric emptying or lag times. Pasta has been reported to have relatively slow gastric emptying (Torsdottir et al., 1984; Mourot et al., 1988), though this could be because the pasta in this study was cooked longer resulting in a softer and more rapidly digesting material.

It may seem apparent that the two thick porridges would slow gastric emptying due to a viscosity effect that has been shown in several studies (Russell et al., 1985;

Torsdottir et al., 1991; Leclere et al., 1994), however it is notable that millet couscous was as slow as the two thick porridges. Couscous is a granular, non-viscous food. We speculate that the mechanism of slowing gastric emptying may be related to the ileal brake mechanism caused by slowly digesting starch associated with dense small particulates that are likely to be still present, though in smaller size, at the entry point of the pylorus into the duodenum. In this scenario, associated distal glucose release in the ileum could trigger the ileal brake and slow gastric emptying (Layer et al., 1990; Torsdottir et al., 1991; Lin et al., 1992). Mechanistically, the thick porridges might also trigger the ileal brake due to a comparably slow digestion of starch related to the viscosity effect. Indeed, sorghum and millet foods are generally known to have comparably low starch digestibility that may deposit glucose more distally in the small intestine (Lichtenwalner et al., 1978; Zhang et al., 1998; Archan et al., 2001; Lee et al., 2013), and may explain why foods made from them have slow gastric emptying rate.

Slow gastric emptying rates are directly associated with lower glycemic responses (Torsdottir et al., 1984; Mourot et al., 1988), as well as higher satiating properties (Marciani et al., 2001; Santangelo et al., 1998). Slowly digestible and absorbed carbohydrates have been shown to reduce the rise of postprandial blood glucose response (Jenkins et al., 1978), and this could well be related to slower gastric emptying which portions out food slowly for digestion. Our results suggest that the types of traditional African foods used in this study with their slowly digesting starch property may trigger the ileal brake and result in slow gastric emptying of the same meal. This also would provide sustained energy to the body in the postprandial period. We speculate further that our results may help to explain the possible association between the changes in dietary habits from traditional to non-traditional foods, also known as the nutrition transition, and the increase in obesity prevalence that has been noted by Renzaho et al. (2006) and others.

It should be noted that volume has been shown in some reports to have greater effect on satiety than gastric emptying rate (Rolls et al., 1998; Marciani et al., 2000). In our study, there was not a statistically significant difference between the amounts of test meal consumed among the studied foods, but our test meals presented differences in

gastric emptying rate. Therefore, for the foods studied, volume was not a factor in controlling gastric emptying.

The pre-test questionnaire results showed thin porridge with granules as the most widely consumed meal type for breakfast. Thick porridges, rice, and couscous were more consumed during lunch or dinner. One hundred percent of the participants noted in the pre-test questionnaire that rice, couscous, and potatoes are considered to be most satiating, followed by pasta (85.7%) and then thick porridges (57.1%). Participants ranked millet couscous and rice as the most perceived satiating foods.

Subjective postprandial satiety scores were similar to rankings from the pre-test questionnaire. It seems probable, and we speculate here, that fullness and hunger feelings may be influenced by a preconceived idea that one has about a food. Satiety is a complex feeling that is influenced by both physiological and cognitive factors. Livingstone et al. (2000) noted that the methodology used to evaluate satiety parameters is subjective and, thus, has an impact on the obtained results and their interpretation. Indeed, a recent study reported that satiety might be influenced by people's preconceived ideas about the food such as beliefs, and expectations (Brunstrom et al., 2011). They found that satiety scores after meal consumption were affected by the expected satiety when they gave the same amount of smoothies made after showing two different portions of fruits (small and large fruits). The group that saw the large portion of fruits gave high ratings for fullness after consumption of the smoothie, whereas the other group that saw the small portion rated high hunger and low fullness. Cassady et al. (2012) showed that the same test meal presented different physiological responses and satiating ratings when subjects had different perceptions related to the behavior of the test meal during the gastric phase. Higher energy intake and hunger ratings, faster gastric emptying and reduced fullness ratings were found when subjects perceived that liquids were ingested compared to when perceived solids. Brunstrom et al. (2008) reported that expected satiety is directly proportional to familiarity with foods.

2.6 Conclusions

Our findings show that traditional African foods made from sorghum and millet have markedly slower gastric emptying rates, assessed by the ^{13}C octanoic acid breath test, compared to non-traditional, modern foods that are today commonly consumed in urban areas. We conclude that the slow emptying rate and concomitant sustained energy delivery of these traditional West African staple foods might be important in understanding the nutrition transition from traditional to non-traditional foods in urban areas of developing countries associated with the rise in obesity and metabolic syndrome diseases. Moreover, this desirable attribute of traditional sorghum and millet foods in West Africa might be used to enhance their image in urban areas and to promote consumption to provide better market access for local smallholder farmers. Preconceived ideas about a food's satiety quality may influence its subjective satiety scores.

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Table 2.1 Pre-test questionnaire

Questions	**Possible Answers**
How often do you eat (test meal name)?	a. Once in a day:…Yes ( )…No ( ) b. More than once a day….Yes ( )….No ( ) c. Once in a week:…Yes ( )…No ( ) d. More than once a week...Yes ( )…No ( ) e. Once in a month Yes ( )…No ( ) f. More than once a month .Yes ( )…No ( ) g. Once in a year....Yes ( )…No ( ) h. More than once a year….Yes ( )....No ( ) i. Have never eaten Tô....Yes ( )….No ( ) j. If the answer is "d" or "e", ask why?
Does the consumption of (test meal name) provide you fullness?	(a) Yes (b) No (c) I don't know
Which food do you prefer to eat for breakfast? Which food do you prefer to eat for lunch? Which food do you prefer to eat for dinner? Which food do you think will provide you more fullness? Rank these foods according to their satiating effect?	(a) Rice (b) Sorghum Tô (c) Millet Tô (d) Millet couscous (e) Thin porridge with granules (f) Thin porridge without granules (g) Pasta (h) Potatoes (boiled)

Table 2.2 Frequency of consumption of the different test meals
(n = 14)

Frequency of consumption	Percentage						
	Rice	Boiled potatoes	Pasta	Thick porridge	Millet couscous	Thin porridge w/o granules	Thin porridge with granules
Once a day	28.57	0	7.14	7.14	0	0	100
More than once a day	28.57	0	0	0	0	0	0
Once a week	0	21.43	21.43	50	28.57	0	0
More than once a week	42.86	14.29	14.29	42.86	35.72	0	0
Once a month	0	42.86	21.43	0	7.14	0	0
More than once a month	0	21.43	28.57	0	7.14	0	0
Once a year	0	0	7.14	0	21.43	0	0
More than once a year	0	0	0	0	0	0	0
Have never eaten	0	0	0	0	0	100	0
Total	100	100	100	100	100	100	100

Table 2.3 Frequency of preference of the different test meals
(n = 14)

	Percentage			
	Breakfast	Lunch	Dinner	More fullness
Rice	7.14	71.43	14.28	35.72
Boiled potatoes	42.86	0	21.43	7.14
Pasta	7.14	7.14	7.14	7.14
Sorghum thick porridge	0	0	0	0
Millet thick porridge	0	7.14	35.72	7.14
Millet couscous	0	14.29	14.29	42.86
Thin porridge w/o granules	0	0	7.14	0
Thin porridge with granules	42.86	0	0	0
Total	100	100	100	100

Table 2.4 Responses to "Does the consumption of (test meal name) provide you fullness (n = 14)

	Percentage			
	"Yes"	"No"	"Don't know"	Total
Rice	100	0	0	100
Boiled potatoes	100	0	0	100
Pasta	85.71	0	14.29	100
Thick porridge	57.14	42.86	0	100
Millet couscous	100	0	0	100
Thin porridge w/o granules	14.29	85.71	0	100
Thin porridge with granules	14.29	85.71	0	100

Table 2.5 Amount of test meal consumed

Values are means of measurements from 6 subjects. Means not sharing the same letter are significantly different at α=0.05.

Meal type	Amount consumed, g	SEM
Rice	593^{bac}	54.7
Boiled potatoes	620^{ba}	50.4
Pasta	446.3^{bc}	47.1
Sorghum thick porridge	426.8^{c}	36.5
Millet thick porridge	522^{bac}	54.8
Millet couscous	464^{bc}	39.8
Thin porridge without granules	700^{a}	-
Thin porridge with granules	700^{a}	-

Table 2.6 Subjects' daily baseline $^{13}CO_2$ values prior to testing over the whole testing period (n = 6)

Subject / Days	1	2	3	4	5	6
1	0	0	0.1	0.6	-0.5	0
2	0	0.1	0.5	-0.3	-0.3	-0.1
3	0	-0.3	-0.1	-0.2	0.2	-0.1
4	-0.2	-0.6	0	-0.1	0.1	0.3
5	-0.1	0.2	0	-0.1	0.1	-0.3
6	0.6	-0.3	0	0	0.4	0.3
7	0.8	-0.3	0.1	0.1	0	-0.2
8	0.3	-0.4	0.1	0.2	-0.6	0.1
9	0	0.1	-0.5	0.2	-0.1	0
10	-0.3	0.4	-0.1	0.1	-0.2	0.1
11	0.1	-0.6	0.5	0.8	0.2	-0.2
12	-0.6	0	-0.2	-0.3	-0.1	-0.6
13	0.2	0.1	-0.8	-0.1	0	-0.1

Table 2.7 Mean values for postprandial $^{13}CO_2$ associated with the higher endogenous ^{13}C found in sorghum and millet-based test meals
These were treated as baseline reference samples and values were subtracted from ^{13}C-octanoic acid gastric emptying test values for these foods.
Values are means of breath $^{13}CO_2$ (DOB, ‰) of the sorghum and millet-based test meals for all subjects (n=6).

Time	Sorghum thick porridge	Millet thick porridge	Millet couscous	Thin porridge without granules	Thin porridge with granules
0	-0.1	0.0	-0.1	0.0	0.0
15	0.3	-0.4	-0.2	0.3	0.4
30	0.8	-0.1	0.2	0.6	0.7
45	0.9	0.1	0.2	1.4	1.3
60	1.1	0.4	0.6	2.0	1.9
75	1.5	0.8	0.9	2.4	2.5
90	2.0	1.2	0.9	2.8	2.8
105	1.8	1.7	1.6	3.3	3.3
120	1.9	2.2	2.3	3.5	4.0
150	2.9	2.6	2.8	3.6	4.7
180	2.8	3.0	2.9	3.3	4.7
210	3.3	3.4	3.2	3.2	4.8
240	3.3	3.3	3.5	3.0	5.2

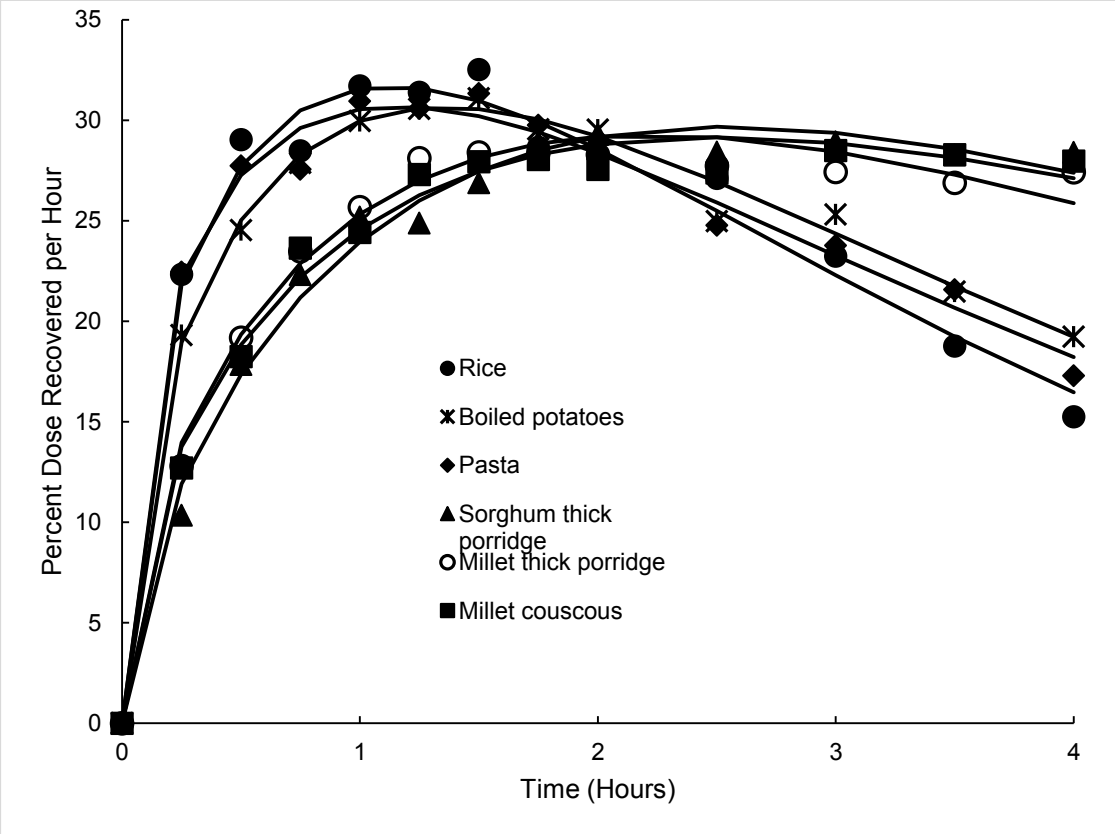

Figure 2.1 Curves of $^{13}CO_2$ excretion (%dosee/h) after ingestion of the different solid test meals and the corresponding fitting curves (solid lines).
Values are mean of excretion measured in 6 subjects.

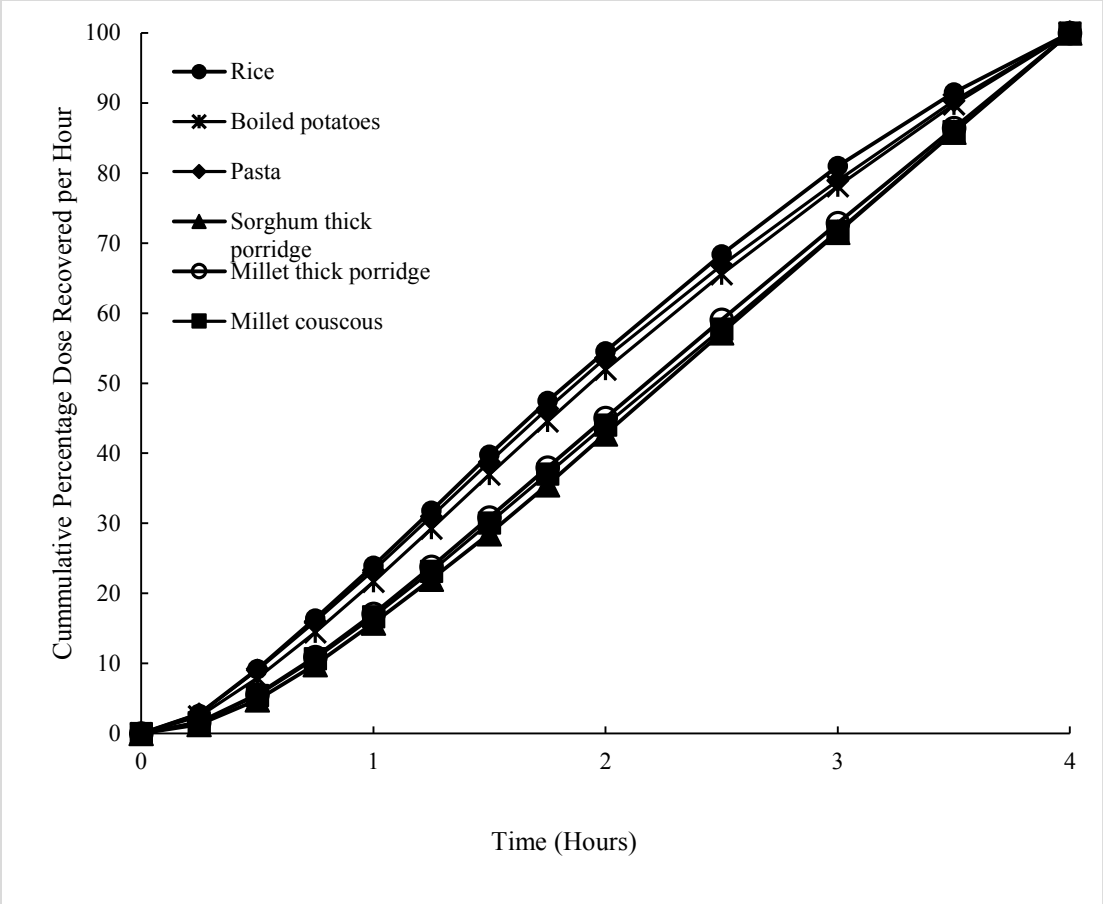

Figure 2.2 Curves of the cumulative breath $^{13}CO_2$ excretion over time of the different solid test meals.
Values are means of cumulative breath $^{13}CO_2$ excretion in 6 subjects

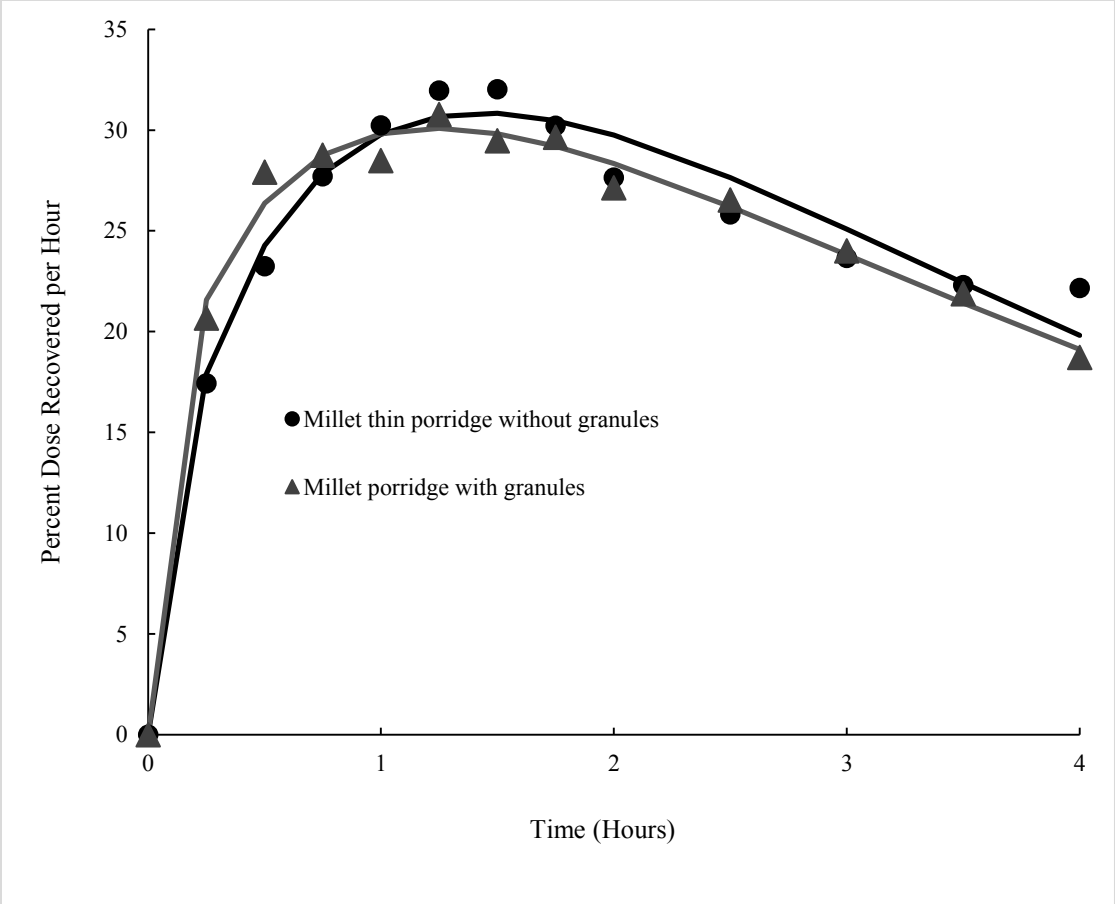

Figure 2.3 Curves of $^{13}CO_2$ excretion (%dose/h) after ingestion of the different liquid test meals and the corresponding fitting curves (solid lines).
Values are mean of excretion measured in 6 subjects.

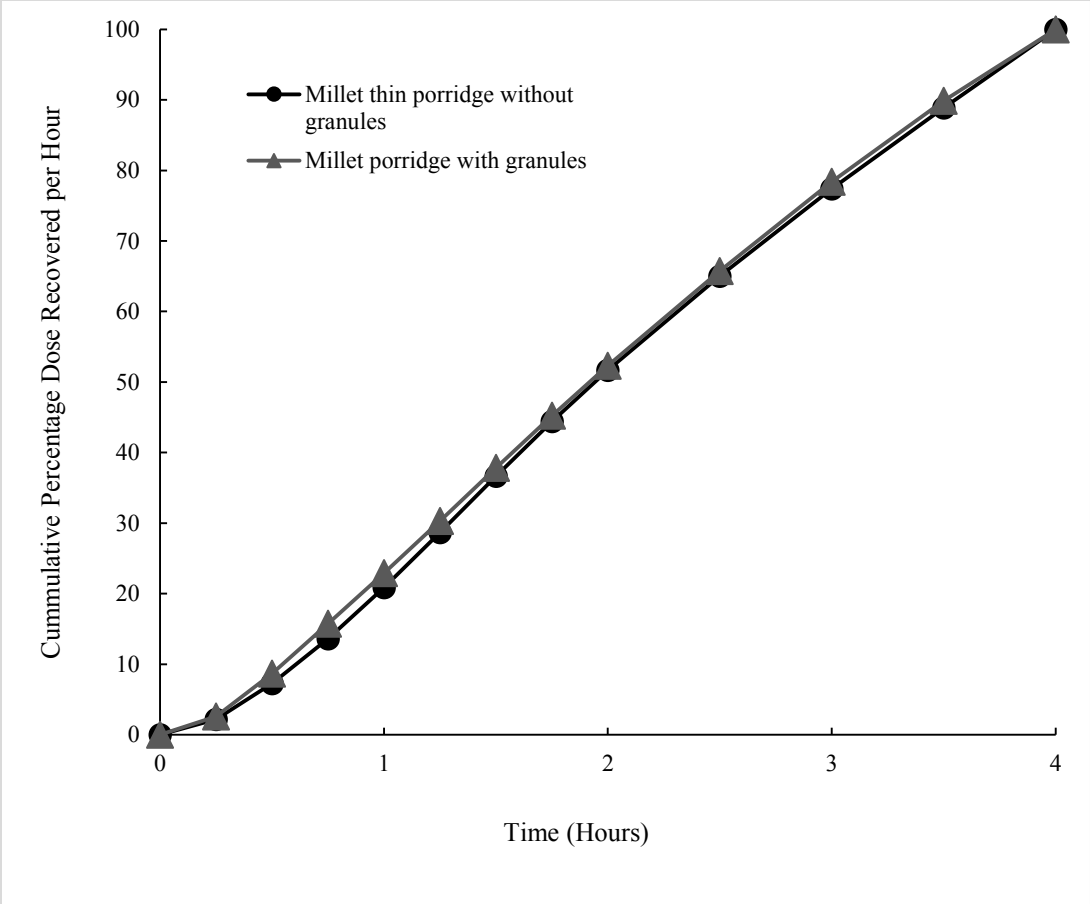

Figure 2.4 Curves of the cumulative breath $^{13}CO_2$ excretion over time of the different liquid test meals.
Values are means of cumulative breath $^{13}CO_2$ excretion in 6 subjects.

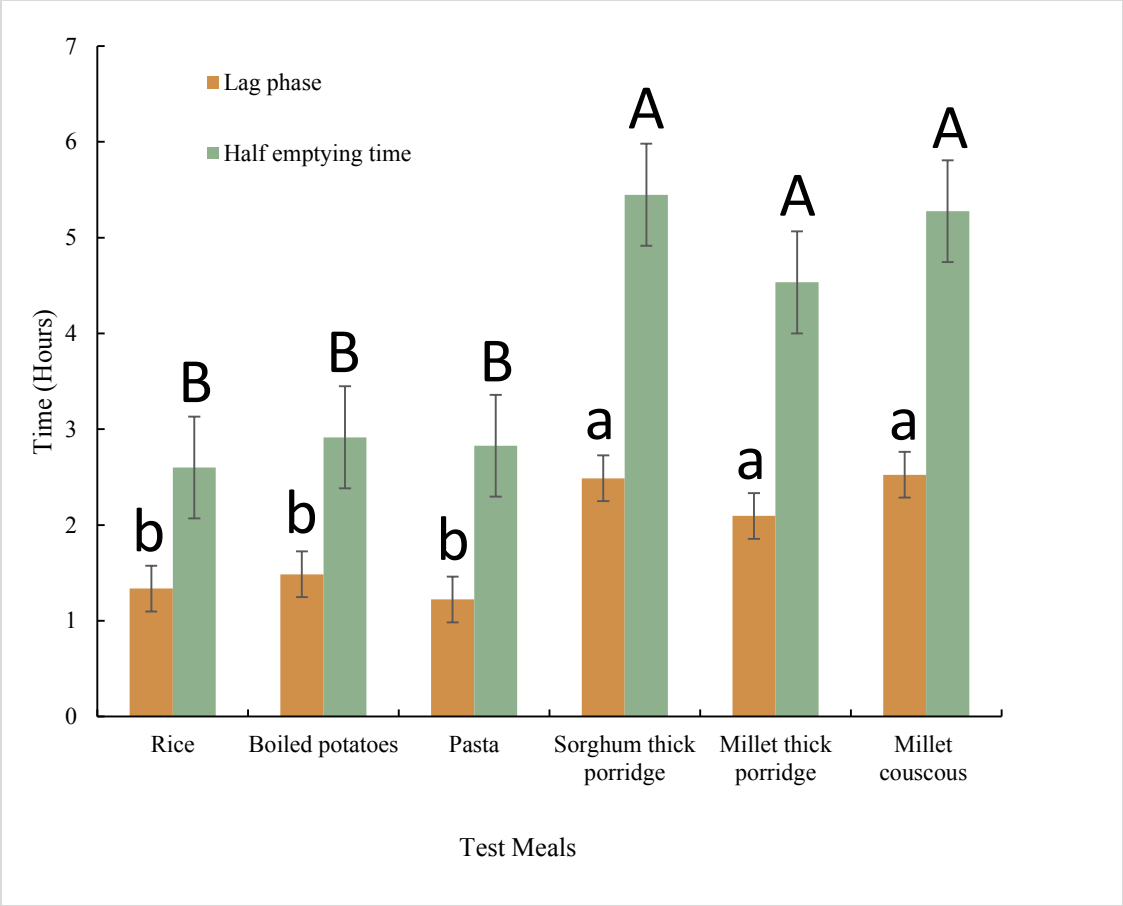

Figure 2.5 Gastric emptying parameters. Mean (± SEM) of lag phase and half emptying time of the different solid test meals.
Different letters indicate statistically significant differences between treatments.

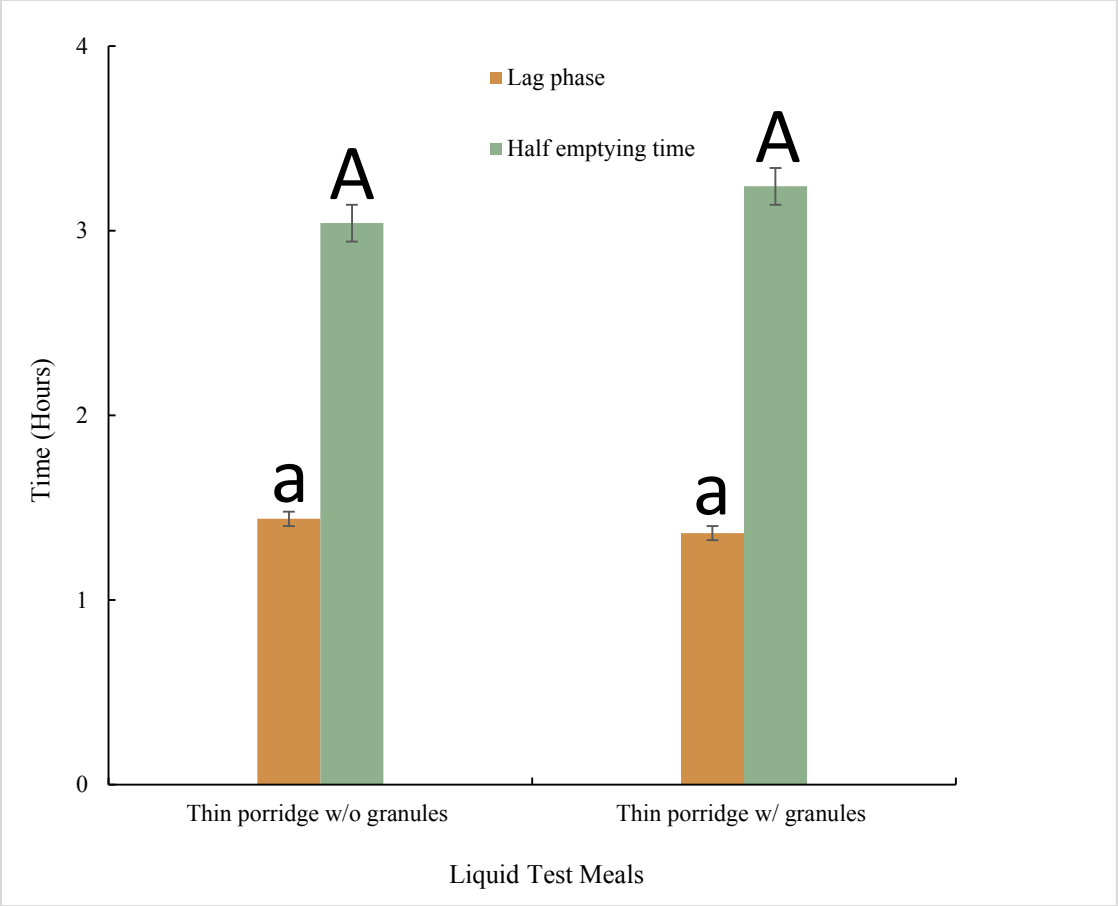

Figure 2.6 Gastric emptying parameters. Mean (± SEM) of lag phase and half emptying time of the different liquid test meals.
Different letters indicate statistically significant differences between treatments.

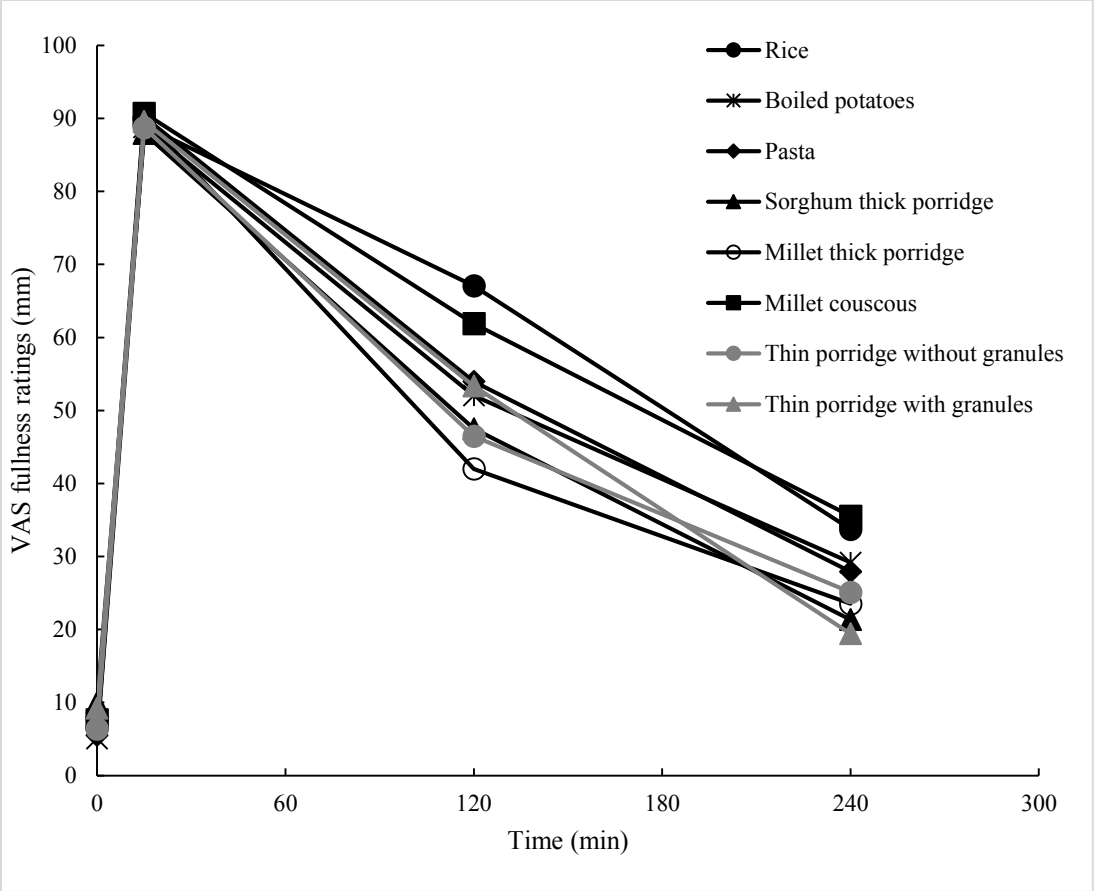

Figure 2.7 Subjective fullness ratings after ingestion of the different test meals, n = 14. Comparisons are based on repeated-measures ANOVA with post hoc Bonferroni multiple comparison tests. Significant main effects of treatment and treatment-by-time interactions were observed ($P < 0.001$).

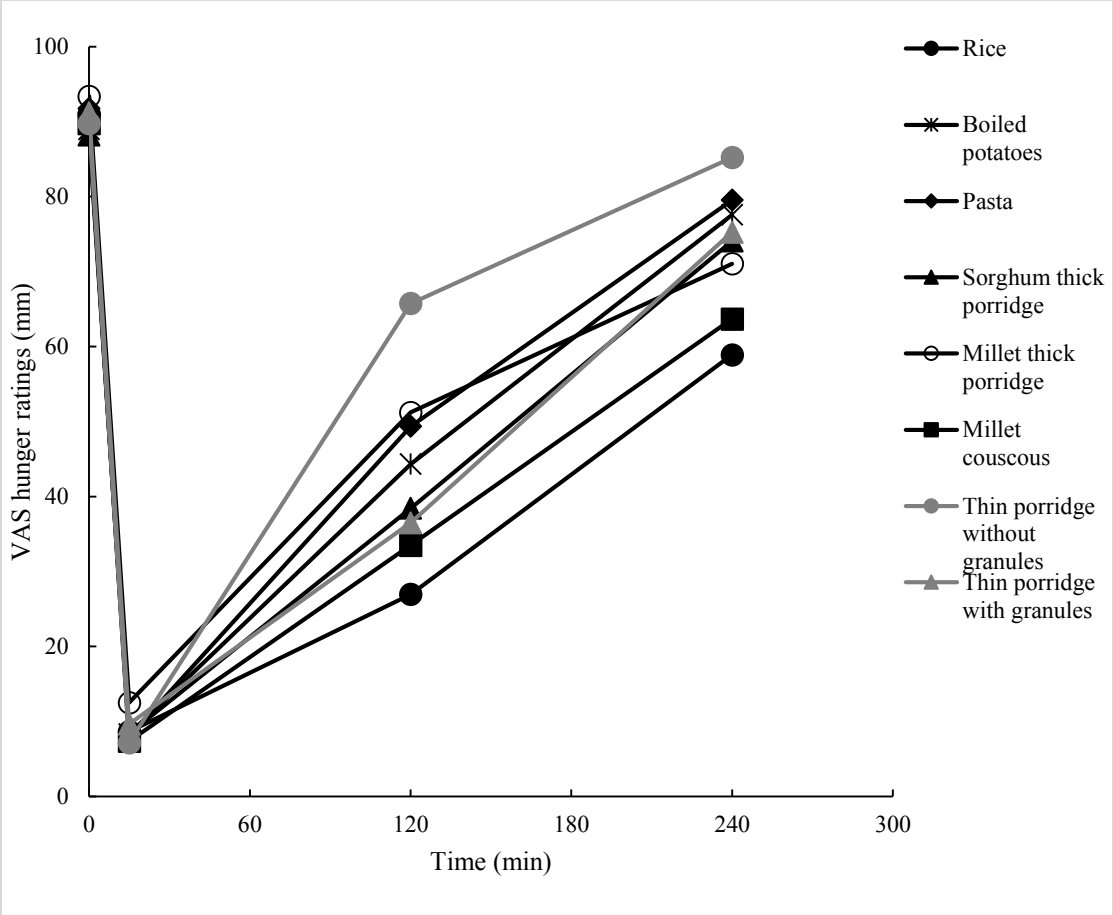

Figure 2.8 Subjective hunger ratings after ingestion of the different test meals, n = 14. Comparisons are based on repeated-measures ANOVA with post hoc Bonferroni multiple comparison tests. Significant main effects of treatment and treatment-by-time interactions were observed ($P < 0.001$).

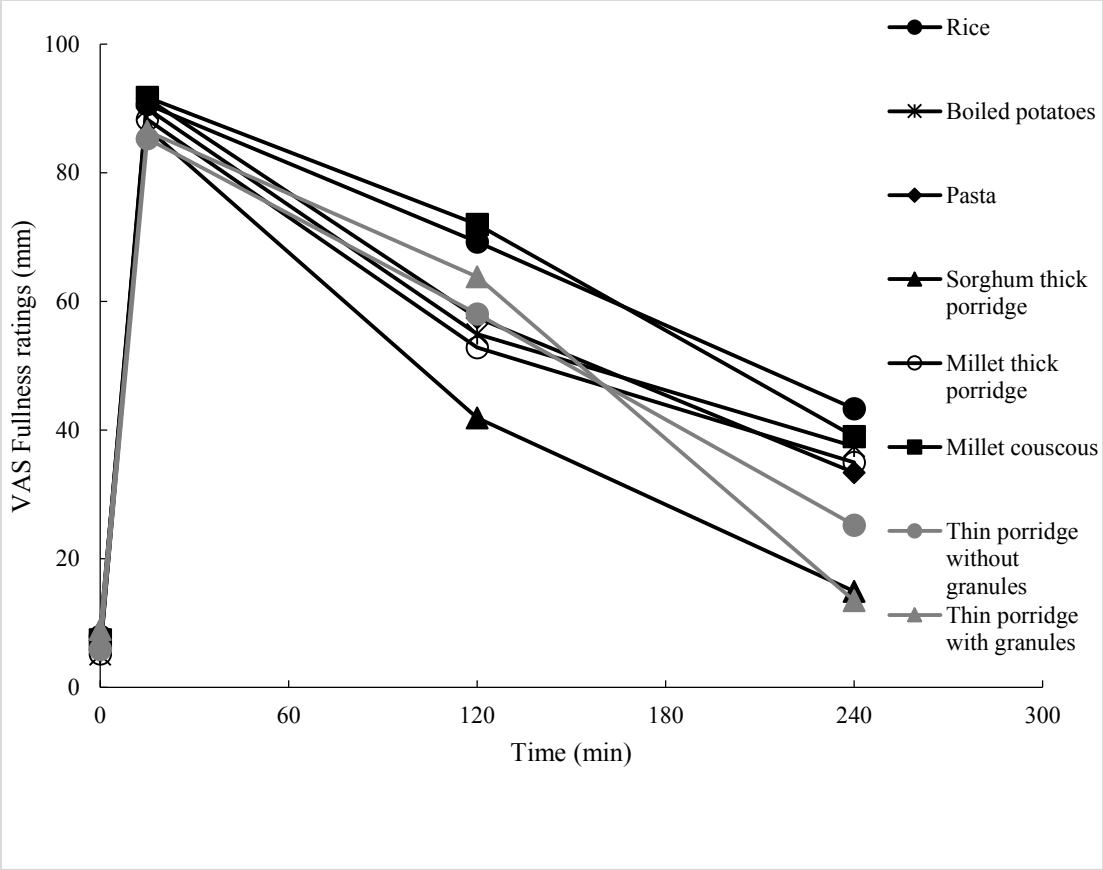

Figure 2.9 Subjective fullness ratings after ingestion of the different test meals, n = 6. Comparisons are based on repeated-measures ANOVA with post hoc Bonferroni multiple comparison tests. Significant main effects of treatment and treatment-by-time interactions were not observed (P =0.356 and P=0.460 respectively).

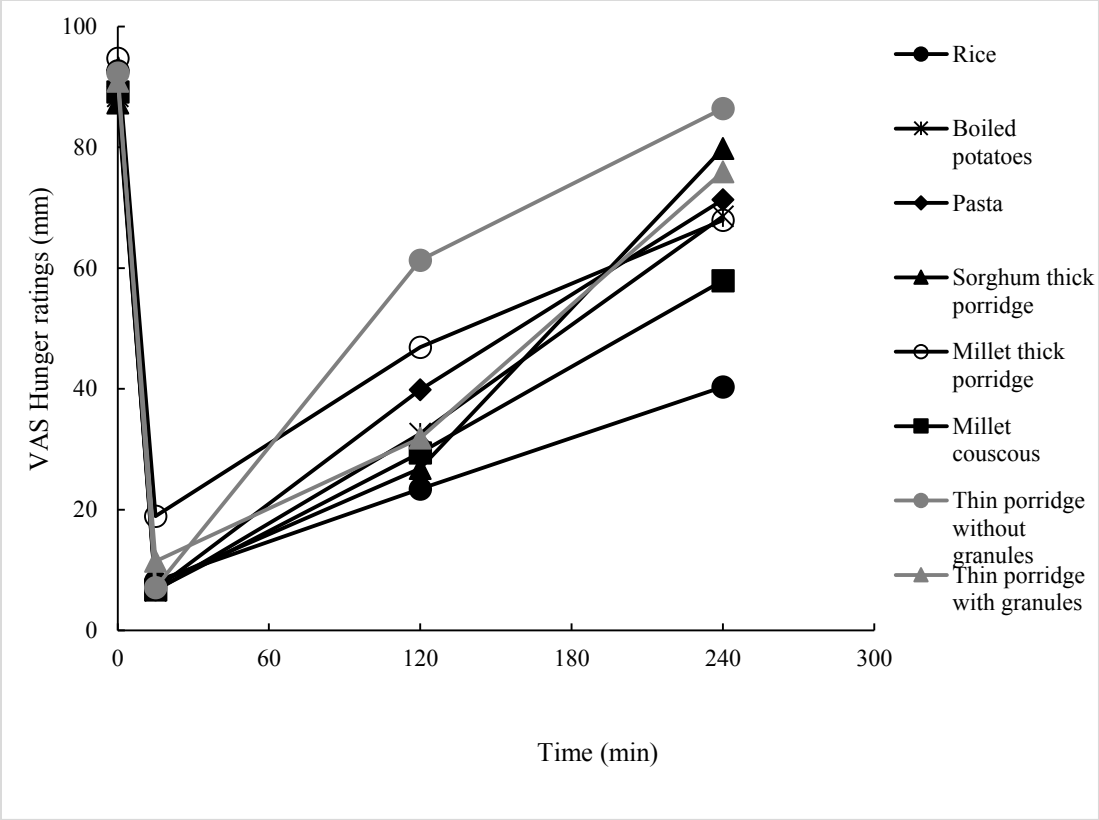

Figure 2.10 Subjective hunger ratings after ingestion of the different test meals, n = 6. Comparisons are based on repeated-measures ANOVA with post hoc Bonferroni multiple comparison tests. Significant main effects of treatment and treatment-by-time interactions were observed (P=0.002 and P < 0.001 respectively).

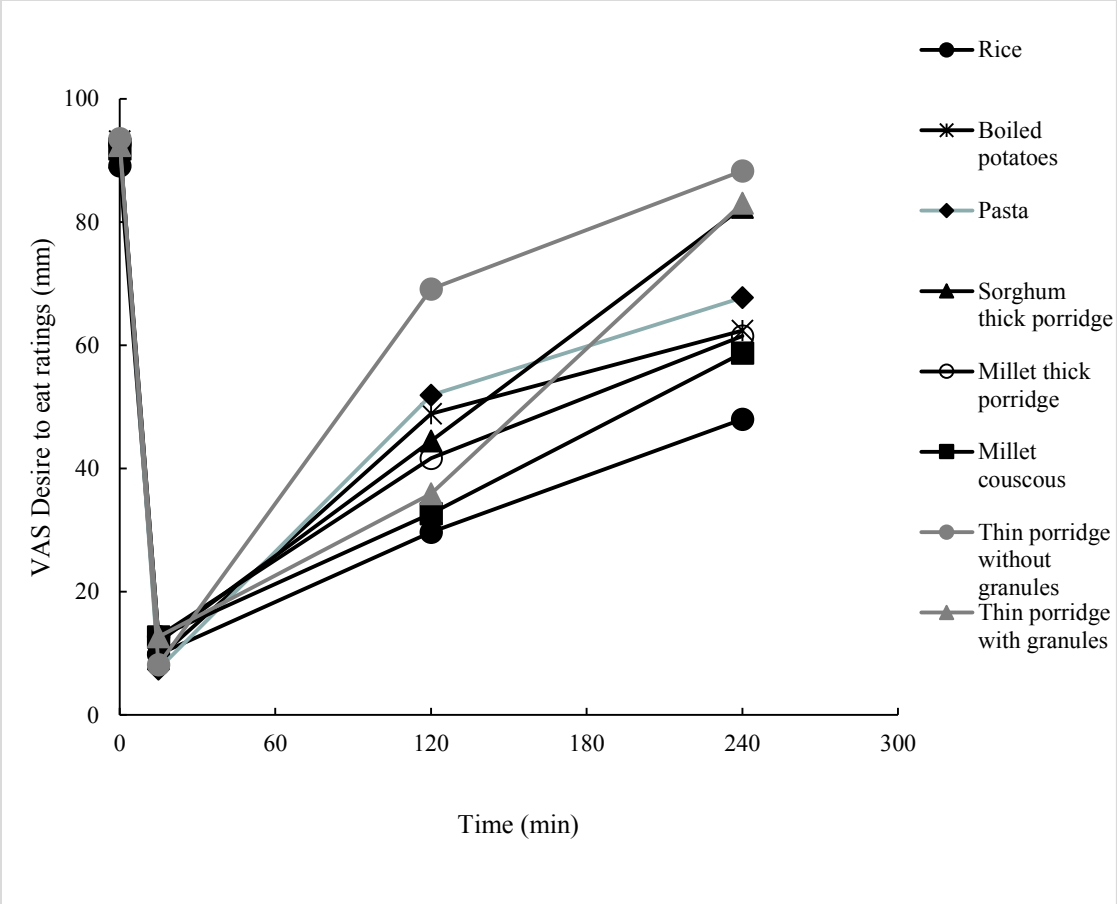

Figure 2.11 Subjective "desire to eat" ratings after ingestion of the different test meals, n = 6.

Comparisons are based on repeated-measures ANOVA with post hoc Bonferroni multiple comparison tests. No significant main effects of treatment (P=0.053) and significant effect treatment-by-time interactions were observed (P=0.005).

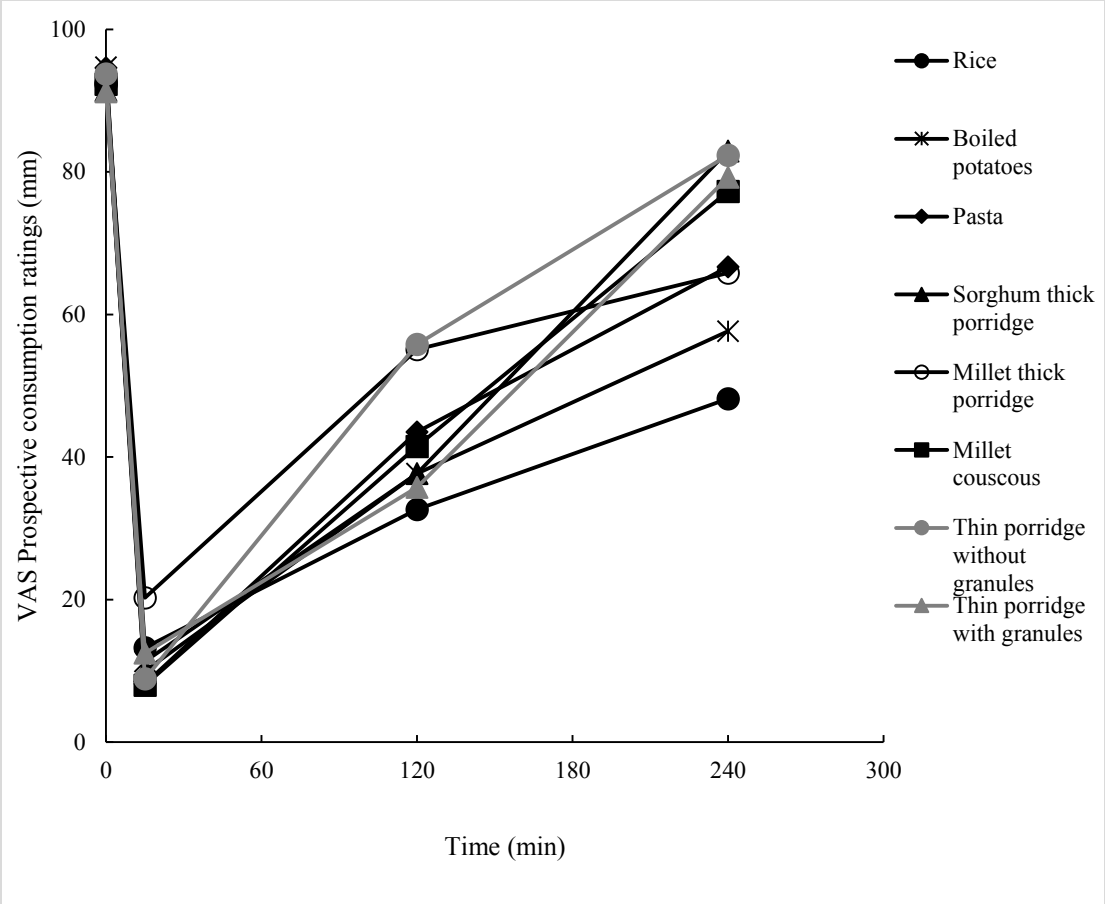

Figure 2.12 Subjective "prospective consumption" ratings after ingestion of the different test meals, n = 6.

Comparisons are based on repeated-measures ANOVA with post hoc Bonferroni multiple comparison tests. No Significant main effects of treatment (P=0.251) and significant effect treatment-by-time interactions were observed (P=0.002).

CHAPTER 3. PRE-INGESTION OF SLOWLY DIGESTIBLE STARCH-ENTRAPPED MICROSPHERES AFFECTS GASTRIC EMPTYING RATE OF A NON-NUTRITIVE PASTE

3.1 Abstract

In the past, infusion of different carbohydrates solutions (glucose, hydrolyzed starch) in the distal part of the small intestine showed reduction in gastric emptying time, the ileal brake. However, the effect of distal glucose delivery on gastric emptying rate using a dietary carbohydrate approach has not been studied in humans. We hypothesized that slowly digestible carbohydrates given in a pre-meal load can be used to moderate nutrient delivery rate to the body through delayed gastric emptying. Thus, the purpose of this study was to show how slowly digestible carbohydrates can be used to moderate gastric emptying rate. Institutional Review Board (IRB) Purdue University approval was obtained. Ten subjects [mean ± SD age: 28 ± 5.77 y; body mass index (in kg/m^2): 22.32 ± 1.86] participated in the study at four occasions separated by one week washout periods using a non-invasive ^{13}C-labelled octanoic acid breath test method to evaluate the emptying rate of a non-nutritive viscous paste made from sodium alginate and agar. Breath samples were collected and analyzed. Polycose® solution, representing a rapidly digestible starch (maltodextrin) and slowly digestible, cooked and washed alginate-based waxy starch-entrapped microspheres (of variable digestion rates: 0.5 and 1.5% initial solution of alginate from which the beads were fabricated) were consumed as a preload, followed 20 minutes later by the ^{13}C-labelled paste mixture. Paste alone was used as the control.

The same thick paste presented different emptying rates depending on the preload. A comparatively fast emptying rate was observed when the paste was consumed alone [T (Lag) = 0.87 ± 0.08 h, $T_{1/2}$ = 1.72 ± 0.10 h], whereas the slowest digesting starch microsphere (1.5% alginate) microspheres preload [T (Lag) = 1.64 ± 0.14 h, $T_{1/2}$ = 2.82 ±

0.20 h] resulted in the slowest paste emptying rate as measured by the lag phase and the half emptying time parameters of the gastric emptying test ($P < 0.05$). Slowly digestible carbohydrate fabricated microspheres cause a slower gastric emptying rate and this may exist in slowly digestible carbohydrate-containing foods, and this property potentially could be incorporated into ingredient form. Slow gastric emptying has some potential benefits of controlling nutrient delivery rate after ingestion of a meal, moderating glucose response, and perhaps affecting appetitive response.

3.2 Introduction

The rate at which nutrients are digested and absorbed in the small intestine is at least somewhat dependent on food emptying rate from the stomach. Gastric emptying also plays a role in postprandial glucose response. Though not consistent in the literature, it has been shown to be directly proportional to satiety and hunger (Bergmann et al., 1992), and is controlled by certain gut hormones [glucagon-like peptide 1 (GLP-1), peptide YY (PYY), and cholecystokinin (CKK)] related to appetite. Regulation of gastric emptying rate by macronutrients is known as the ileal brake mechanism which retards stomach emptying rate and overall gut motility through the release of gut hormones. Therefore, dietary factors that regulate gastric emptying of ingested food is of interest, since their understanding may help in controlling overall food intake.

The role of macronutrients on small intestine gastric functions (secretions and emptying) has been studied in the past using intubation and perfusion techniques. The digestion products of the different macronutrients perfused in the ileum of the small intestine have been shown to have regulatory effect on gastric function by slowing gastric emptying rate (Miller et al., 1981; Apiroz et al., 1985; Layer et al., 1990; Siegle et al., 1990). The intestinal phase is a major regulator of the movement of chyme to the duodenum (gastric emptying) to make nutrient digestion and absorption more efficient. Siegle et al. (1990) showed that when isoenergetic amounts of lipid (oleate), protein (amino acid), and carbohydrates (glucose) were perfused, glucose exerted the most potent effect in slowing small intestine motility. The effect of starch and its digestion products have been studied further and it has been shown that their moderating effect on gastric emptying is more potent when they are perfused in the ileum. Accordingly, the perfusion of carbohydrate solution (75% rice starch + 25% glucose) showed a reduction in gastric emptying of a homogenized mixed meal (60% carbohydrate, 20% protein, 20% fat) and this effect was more pronounced when an amylase inhibitor was added (Jain et al., 1989). The inhibition of intestinal α-amylase in humans showed a reduction in gastric emptying time of rice starch and an 85% decrease in peak rise of postprandial plasma glucose (Layer et al., 1986).

Slowly digested and absorbed carbohydrates have been shown to reduce the rise of postprandial glucose response (Jenkins et al., 1978) as well as influence the gastric emptying rate of foods. From our group, Venkatachalam et al. (2009) showed that when starch is entrapped in an alginate matrix in the form of microspheres, it exerts slowly digesting properties that lower glycemic and insulinemic response. Lower glycemic responses are directly associated with slow gastric emptying rates (Torsdottir et al., 1984; Mourot 1988), as well as higher satiating properties (Santangelo et al., 1998; Marciani et al., 2001). Thus, the consumption of slowly digestible carbohydrates within a meal or as a preload may help to regulate the gastric emptying rate of ingested foods resulting in controlled postprandial glucose response and energy delivery to the body. Such an approach has the potential of helping to prevent and manage chronic diseases related to metabolic syndrome (i.e. diabetes, cardiovascular disease, and obesity).

The present study was designed to show how our fabricated microsphere-based slowly digestible carbohydrates affect gastric emptying rate. We hypothesized that slowly digestible microspheres given as a preload delay gastric emptying.

3.3 Subjects and Methods

This study protocol was approved by the Purdue University Institutional Review Board (IRB).

3.3.1 Preliminary study

A preliminary study was done to study the effect of the timing between ingestion of the preload and the ^{13}C-labelled test meal on gastric emptying parameters (lag phase and half emptying time). The alginate (0.5%) based starch entrapped microsphere (SDS1 preload) was tested at 10, 20, 30 minutes, and 1 hour before the ^{13}C-labelled test meal. Polycose® as source of rapidly digested starch, and absorbed glucose, (polycose preload) was tested at 20 minutes. The ^{13}C-labelled paste alone was used as the control. The preliminary study was conducted using the test meal and procedure described below in the respective sections.

3.3.2 Subject eligibility

Eligibility criteria were: males or females aged 20 – 50 years, normal body mass index (18 $kg/m^2 \leq BMI \leq 25\ kg/m^2$), not under any medication, no history of any gastrointestinal disease or surgery, and no diabetes. A written consent form with IRB approval was obtained from each subject before his or her participation in the study.

3.3.3 Test meals

The test meal consisted of a non-nutritive viscous paste made up of sodium alginate (8 g, FMC Biopolymer Manugel GHB MGLGHB) and agar (4 g, Now Foods, Bloomingdale, IL 60108, USA). A 6% solution made with these two carbohydrates and 200 mL of distilled water was cooked for 10 minutes. After that, 100 mg of ^{13}C-octanoic acid (Sigma-Aldrich, St. Louis, MO, USA) was mixed into each subject's weighed test meal portion (151.6 ± 1.1 g) as a tracer and the paste was cooled at room temperature. A preload material with different degrees of glucose release, as shown in Table 1, was given 20 minutes before consumption of the labelled paste. A solution of Polycose® (Abbott Nutrition, Abbott Laboratories, Columbus, Ohio, USA) (Polycose preload), as a source of rapidly absorbed glucose, was prepared by mixing 25 g of powder in 200 mL of purified water. Two slow digesting, cooked, and washed alginate-based waxy starch-entrapped microspheres (SDS1 preload and SDS2 preload) with different starch digestion rates were used as sources of slowly absorbed glucose. The difference between SDS1 preload and SDS2 preload was the amount of the alginate forming the matrix around the starch (SDS1 preload and SDS2 preload were processed with 0.5 and 1.5% alginate solutions). The processed, dried microspheres were cooked in water prior to using for 5 (SDS1 preload) and 20 minutes (SDS2 preload) in a pressure cooker (Nesco Digital Cooker, model PC6 – 25, The Metal Ware Corporation Two Rivers, WI) in order to gelatinize the starch. After cooking, the microspheres were washed several times with distilled water to remove any free surface starch. The total starch content of the microspheres, after powdering with a ball mill, was determined using the Megazyme Total Starch kit (Megazyme International Ireland Ltd, Wicklow, Ireland). SDS1 preload and SDS2 preload material amount was 25 g starch (dry weight basis).

The microspheres were subject to microbiological testing for *Escherichia coli* count, and total *Coliforms* and *Salmonella* (Covance Laboratories, Battle Creek, MI).

3.3.4 Design

Each subject was tested for four treatments (Polycose, 2 microspheres, control) on four different days, each separated by a washout period (**Table 3.1, Figure 3.1**). For the microspheres, subjects ingested the preload and 20 minutes later they consumed the ^{13}C-labelled paste. The same was done for the rapidly absorbed glucose Polycose treatment. The order of these conditions was randomly assigned.

3.3.5 Procedure

Gastric emptying was assessed using the ^{13}C-octanoic acid breath test technique (Ghoos et al., 1993; Choi et al., 1997; Clegg et al., 2010). Subjects visited on four occasions, with a 7 day washout period. On each day, subjects were asked to come to the Food Science Department at 8:00 AM andwere provided the same test meal and preload. They were asked to refrain the day before from physical activity, alcohol, and natural ^{13}C enriched food products such as corn-based products and cane sugar. They were instructed to fast overnight between 10:00 PM to 8:00 AM (~ 10 hours) prior to the test.

Upon arrival, the preload with 100 ml water was given to subjects whom were instructed to consume it within 10 minutes. Twenty minutes later the test meal (^{13}C-labelled paste) was given with 100 ml of water and eaten within 10 minutes. Breath samples were taken in duplicate before the preload (used as a baseline value) and during 4 h after consuming the test meal (the ^{13}C-labelled paste mixture) in 15 min intervals. Breath samples were analyzed using a ^{13}C breath analyzer (POCone, Otsuka Co., Japan), an infrared spectrophotometer that determines the ratio of $^{13}CO_2$ to $^{12}CO_2$ (Schadewaldt et al., 1997; Braden et al., 1999; Sanaka et al., 2007).

Calculation of gastric emptying parameters

The breath analyzer provides data in terms of the change in the $^{13}CO_2$ DOB (delta over baseline, ‰), where the $^{13}CO_2/^{12}CO_2$ ratio of a sample gas is compared to the corresponding ratio of a reference gas (i.e. baseline value). Using this value, CO_2 production, percent dose ^{13}C recovery per hour (PDR), and cumulative percentage dose

recovery over time (CPDR) were calculated (Ghoos et al., 1993; Braden et al., 2007; Haycock et al., 1978). CO_2 production was assumed to be 300 mmol / (m^2 body surface area x hour), with body surface area calculated using the formula developed by Haycock et al. (1978). After calculating PDR and CPDR values from the obtained data set, these functions were modeled using the following equations to discern parameters related to the gastric emptying.

y = $at^b c^{-ct}$ where y = percentage dose recovery per hour, t = time in hours, a, b, and c = constants.

y = $m(1 - e^{-kt})^{\beta}$ where y = cumulative percentage dose recovery over time, t = time in hours, m, k, and β = constants and m = total cumulative dose recovery when time is infinite.

Modeling of data was achieved by nonlinear regression using SAS statistical analysis software (v.9.2, SAS Institute Inc., Cary, NC) and was confirmed using a Macro program in Excel (Microsoft Corp., Redmond, WA). Gastric emptying parameters were then calculated using the following formulas:

Lag phase (i.e. time required for the $^{13}CO_2$ excretion rate to attend its maximal level) (Sanaka et al., 2010).

T (Lag) = $(ln\beta)/k$

Half emptying time (i.e. time necessary for half of the ^{13}C dose to be metabolized) (Sanaka et al., 2010).

($T_{1/2}$) = $\left(-\frac{1}{k}\right) \times \ln(1 - 2^{-\frac{1}{\beta}})$

Statistical analyses

Multiple comparisons of lag phase, half-emptying times, and values across treatments were performed using a randomized complete block design (RCBD) with post-hoc Tukey tests to form statistical groupings (α=0.05).

3.4 Results

3.4.1 Preliminary study

In a preliminary study, two healthy volunteers (2 women) were used with a mean ± SD age of 32.0 ± 11.3, weight of 54.4 ± 12.0 kg, height of 142.5 ± 24.8 cm. **Table 3.2**

shows the mean values of gastric emptying parameters [lag phase, T (Lag) and half-emptying time, $T_{1/2}$] of the different time points tested. **Figure 3.2** represents T (Lag) and $T_{1/2}$ of the different time points tested. Paste alone [T (Lag) = 0.87 ± 0.02 h, $T_{1/2}$ = 1.37 ± 0.08 h], and the paste given 20 minutes after preload 1 [T (Lag) = 1.01 ± 0.04 h, $T_{1/2}$ = 1.46 ± 0.09 h], followed by the paste given 1 hour after preload 2 [T (Lag) = 1.10 ± 0.03 h, $T_{1/2}$ = 1.73 ± 0.006 h] had the fastest emptying rate compare to paste given 10, 20, and 30 minutes after preload 2. Ten minutes [T (Lag) = 1.53 ± 0.61 h, $T_{1/2}$ = 2.49 ± 1.22 h], 20 minutes [T (Lag) = 1.52 ± 0.009 h, $T_{1/2}$ = 2.42 ± 0.10 h], and 30 minutes [T (Lag) = 1.42 ± 0.53 h, $T_{1/2}$ = 2.48 ± 1.09 h] after preload 2 presented slow gastric emptying rate. These preliminary results showed that 10, 20, and 30 minutes between the Preload 2 and the test meal exerted a greater effect on reducing gastric emptying of the ingested food. In the subsequent study, 20 minutes was chosen as the time between the preload and the test meal.

3.4.2 Subject characteristics

The 10 healthy volunteers (5 men and 5 women) were aged between 24 – 33 years old (28 ± 5.77 mean ± SD) with a mean (± SD) body mass indices (BMI) of 22.32 ± 1.86 kg/m^2. Their heights and weights were, respectively, 169.76 ± 13.71 cm, and 65.17 ± 13.87 kg.

3.4.3 Gastric emptying

Figure 3.3 shows the mean rate of recovery of ^{13}C in breath after ingestion of the ^{13}C-labelled octanoic acid infused test meal. The paste alone was characterized by a rapid increase in the ^{13}C recovery followed by a quick decrease in recovery after 75 minutes. The paste consumed after Polycose preload had maximum recovery at 1 hour after ingestion followed by a somewhat slow decrease in recovery in comparison to paste alone. When SDS1 preload was given, the ^{13}C-labelled test meal presented a moderate increase of the ^{13}C recovery followed by a decrease after one and half hours compared to paste alone and preload 1. However, when SDS2 preload was given, the labelled test meal was characterized by a slow increase of recovery which peaked at 1.75 hours followed by a slow decrease after 2 hours in comparison to other treatments. **Figure 3.4** displays the cumulative percentage dose of $^{13}CO_2$ recovered curves after ingestion of the

labelled test meal with octanoic acid. **Table 3.3** shows the mean values of the gastric emptying parameters [lag phase, T (Lag) and half-emptying time, $T_{1/2}$] of the different treatments. **Figure 3.5** shows the T (Lag) and $T_{1/2}$ of the different treatments. Paste alone [T (Lag) = 0.87 ± 0.08 h, $T_{1/2}$ = 1.72 ± 0.10 h] had a fast emptying rate compared to the labelled paste with SDS1 and SDS2 preloads, but did not differ significantly in $T_{1/2}$ from the paste with the Polycose preload. Polycose preload + paste [T (Lag) = 1.30 ± 0.08 h, $T_{1/2}$ = 2.02 ± 0.11 h] did not differ significantly from the paste with SDS1preload, but had an emptying time faster than the paste with SDS2 preload. SDS1 preload [T (Lag) = 1.44 ± 0.05 h, $T_{1/2}$ = 2.29 ± 0.05 h], and SDS2 preload (1.5% alginate microspheres) [T (Lag) = 1.64 ± 0.14 h, $T_{1/2}$ = 2.82 ± 0.20 h] had similar T (Lag), but their $T_{1/2}$ were significantly different from each other. SDS2 preload had the highest $T_{1/2}$.

3.5 Discussion

Gastric emptying rate is influenced by post-gastric feedback (intestinal feedback) and gastric contribution (gastric distension), especially when a preload is given. However, it is unclear whether the gastric or post-gastric phase has a more predominant effect. A study using liquid preloads containing fat given 20 minutes before a test meal, suggest that stimulation of intestinal receptors delays emptying rate of the solid meal (Cunningham et al., 1989), whereas another study suggests that the volume of the preload remaining in the stomach is also a contributor to a slower emptying rate (Collins et al., 1991). However, the timing between the preload and test meal itself is not the only factor to consider, as some have also not reported slower emptying when a solid meal is fed 20 minutes after a soup preload (Spiegel et al., 1994). According to the explanation in the last two studies, in our study one might expect that slower gastric emptying will be seen when the preload (microspheres) is given 10 minutes before the test meal, since a high amount of preload might still be in the stomach when the test meal is ingested. However, at 20 and 30 minutes before the test meal, when more of the preload is assumed to have emptied from the stomach, there were similar slow emptying rates of the test meal to 10 minutes. Slow gastric emptying at these time points is speculated to be due to glucose release triggering the ileal brake mechanism, rather than the gastric distension caused by the preload.

In the preliminary study, similar emptying rates were seen when the paste was consumed 10, 20, and 30 minutes after the SDS1 preload (microspheres). A significant difference was seen between the gastric emptying parameters of the paste alone (control) and the paste with the SDS1 preload at these time intervals. Past 30 minutes, the half-emptying time and lag phase began to decrease and were more closely related to the behavior seen when the paste was consumed alone. We used this as an indication that the starch microsphere preload may have already been digested into glucose and absorbed, and thus by the time the labeled paste was consumed, there was not enough stimuli to see a delay in gastric emptying rate. Twenty minutes was chosen as the timing between the preload consumption and the ^{13}C-labelled paste as being an average point between 20 and 20 minutes. This timing was also used by Cunningham et al. (1989) and Spiegel et al. (1994). Levels of GLP-1 and PYY, gut hormones that regulate gastric emptying, have also been shown to rise and peak within 20 minutes after meal consumption (Kim et al., 2005).

Previous studies showed that ileal infusion of glucose (Siegle et al., 1990), glucose and hydrolyzed starch (Lin et al., 1992), and a mixture of starch and maltose (Layer et al., 1990; Layer et al., 1995) retarded gastric emptying, decreased small intestine motility, and increased GLP-1 levels. The presence of nutrients in the distal small intestine triggers the secretion of gut hormones into the blood which in turn are involved in the ileal brake mechanism (Konturek et al., 2004; Leibowitz et al., 2004; Murphy and Bloom 2006). In this study, the preloads were given in order to stimulate the ileal brake mechanism through distal glucose release. The alginate coated starch microspheres were designed to have different glucose release rates and to test the effect of the location of nutrient delivery on gastric emptying through a dietary approach, rather than an infusion method.

The results of this study show that the same non-nutritive thick paste presented different emptying rates depending on the type of preload. A fast emptying rate was observed when the paste was consumed alone and with the rapidly absorbed glucose (Polycose) preload, whereas the SDS1 and SDS2 microsphere preloads, both with slowly digestible starch, presented slower emptying rates of the paste as measured by the lag

phase and the half emptying time parameters of the gastric emptying test. The SDS2 microspheres emptied the paste at the slowest rate. These results suggest that slowly digestible carbohydrates delivering glucose more distally into the small intestine have a moderating effect on gastric emptying rate by eliciting the ileal brake mechanism.

We considered also the potential role of the physical form of the microspheres on gastric emptying rate, as form, size, and density of particles or particular food structures have been shown to influence gastric emptying rate. Nondigestible shapes such as a ring (3.6 cm of diameter) and a tetrahedron (2 cm each leg) were shown to be retained in beagle dogs for 24 hours (Cargill et al., 1988); though Fix et al. (1993) failed to show the same effect in humans or even larger dogs. When nondigestible particles with different size and density were ingested in different viscous solutions, it was only in the low viscosity fluid that particles segregated out with the small particles, with density similar to the meal, leaving the stomach with the first portion of the meal (Sirois et al., 1990). On the other hand, at higher viscosity, almost all particles emptied independently from their size and density. Furthermore, here too the smallest particles emptied when their density was close to that of the meal; but for the intermediate viscosity fluid, a fixed particle size with different densities showed different emptying rate. Podczeck et al. (2007) found that nondigestible dense tablets remain longer in the stomach than light ones; moreover, independent from density, the gastric emptying time of a 6.6 mm diameter tablet was longer than 12.0 mm diameter tablet. They explained that the small diameter tablet may stick to the folds of the empty stomach, whereas the larger ones on the surface of the folds. Tablet size (3-7 mm diameter) was not found to influence gastric emptying rate or small intestine transit in fed volunteers (Khosla et al., 1989), and in the same group showed that a nondigestible tablet has to be larger than the hole of the resting pylorus in order to be retained in the stomach longer. Important to our study, Kosha and David (1990) found only particles with 13 mm diameter and greater stay longer in the stomach. Microsphere size in the current study was about 300-800 μm in diameter. Taken together, and relevant to our study, small size particles with similar density to the meal will empty with the meal. We expect the microspheres used in this study may be emptied from the stomach at the same time with the non-nutritive paste.

Our findings show that when glucose is delivered distally in the small intestine, it slows gastric emptying rate of an ingested food assessed using a ^{13}C-octanoic acid breath test. This supports the idea that slowly digestible carbohydrates cause slow gastric emptying rate properties that may have beneficial effect of control of nutrient delivery including extending energy delivery, and to moderate the glycemic response profile.

3.6 References

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Table 3.1 Preloads of varying glucose release rates fed to human subjects for gastric emptying rate measurements

Preload #	**Preload**	**Function**
-	None	Paste control
1	Polycose© solution	Rapid glucose release
2	0.5% alginate-coated starch microspheres	Slower glucose release
3	1.5% alginate-coated starch microspheres	Slowest glucose release

Table 3.2 Gastric emptying parameters for the preliminary study

Preload	-	Glucose	0.5% SM	0.5% SM	0.5% SM	0.5% SM
Test meal serving time	Paste alone	20 min	10 min	20 min	30 min	1 hour
Lag phase, hours	0.87 ± 0.02	1.01 ± 0.04	1.53 ± 0.61	1.52 ± 0.009	1.42 ± 0.53	1.10 ± 0.03
Half emptying time, hours	1.37 ± 0.08	1.46 ± 0.09	2.49 ± 1.22	2.42 ± 0.10	2.48 ± 1.09	1.73 ± 0.006

Values are means (± SD) of lag phase and half emptying time of the different test meals (n = 2). Glucose and 0.5% SM (0.5% alginate coated starch microspheres) are the preloads. 10, 20, 30 minutes and 1 hour are the different timing tested.

Table 3.3 Gastric emptying parameters

	Paste alone	**Preload 1**	**Preload 2**	**Preload 3**
Lag phase, T(Lag), hours	0.87 ± 0.08	1.30 ± 0.08	1.44 ± 0.05	1.64 ± 0.14
Half emptying time, $T_{1/2}$, hours	1.72 ± 0.10	2.02 ± 0.11	2.29 ± 0.05	2.82 ± 0.20

Values are means (± SEM) of lag phase and half emptying time of the different test meals (n = 10).

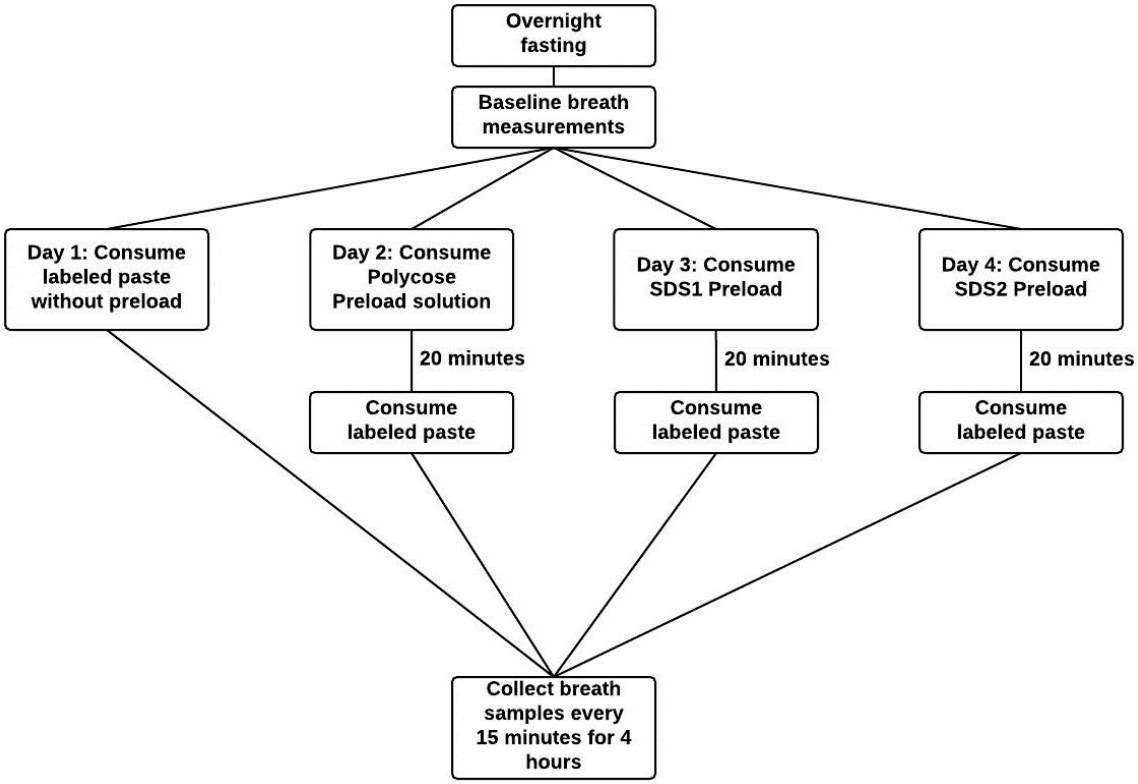

Figure 3.1 Design for assessing gastric emptying rates of human subjects using starch-entrapped microspheres and alginate paste.

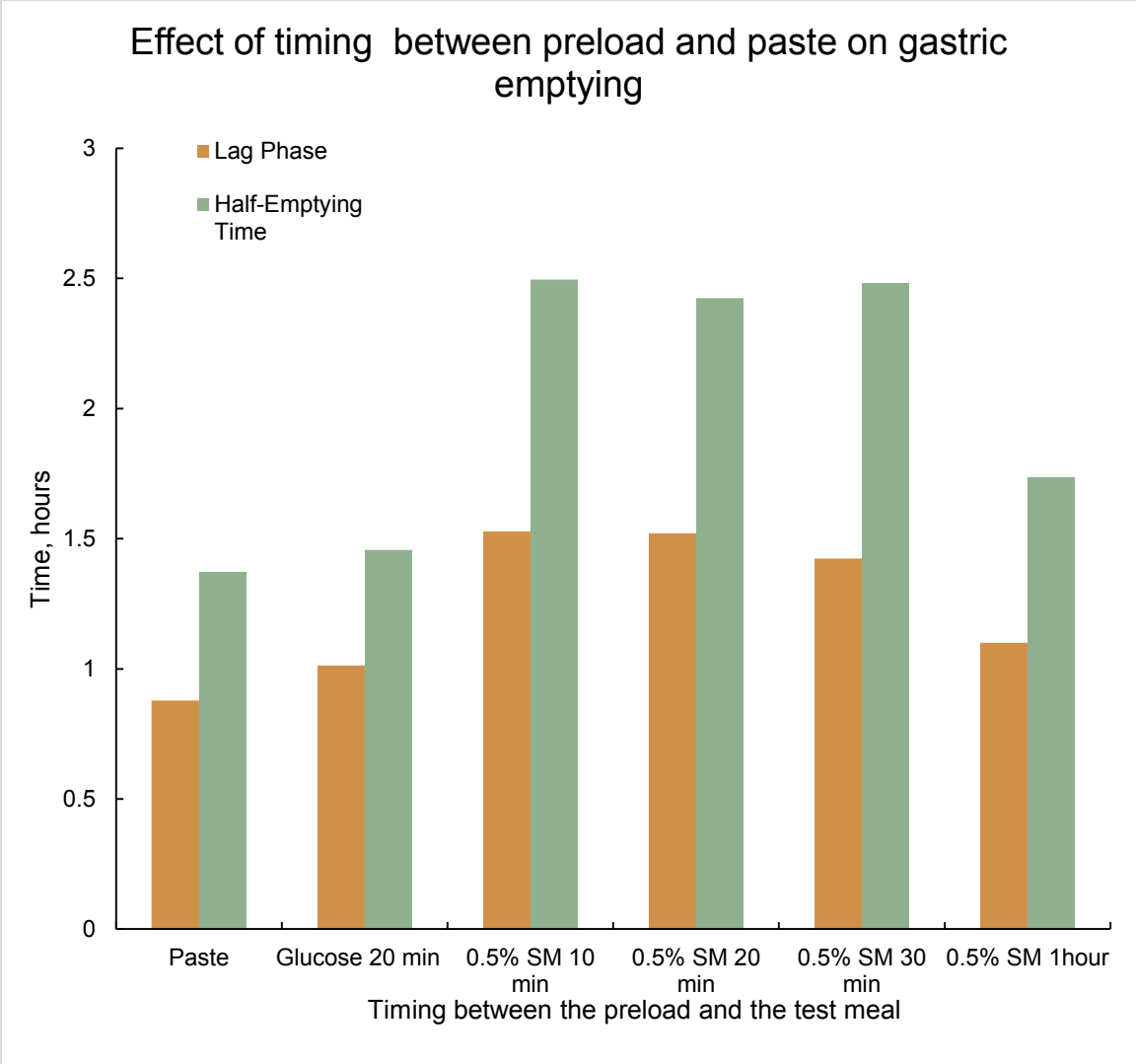

Figure 3.2 Gastric emptying parameters.
Mean of lag phase (gray bars) and half emptying time (black bars) of the different timing tested (n=2). Glucose and 0.5% SM (0.5% alginate coated starch microspheres) are the preloads. 10, 20, 30 minutes and 1 hour are the different timing tested.

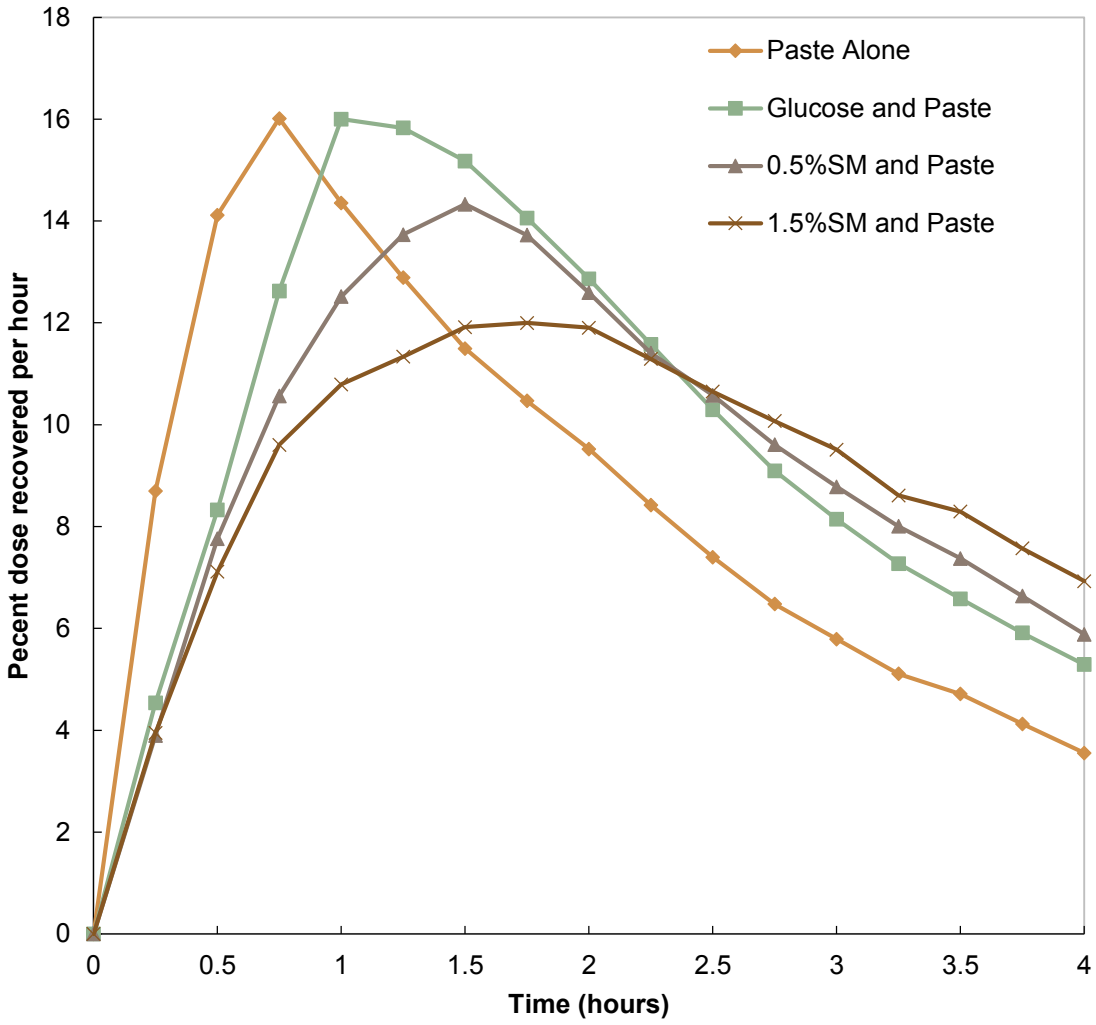

Figure 3.3 Curves of $^{13}CO_2$ excretion in breath (%dose/h) after ingestion of the different test meals.
Values are mean of excretion measured in 10 subjects.

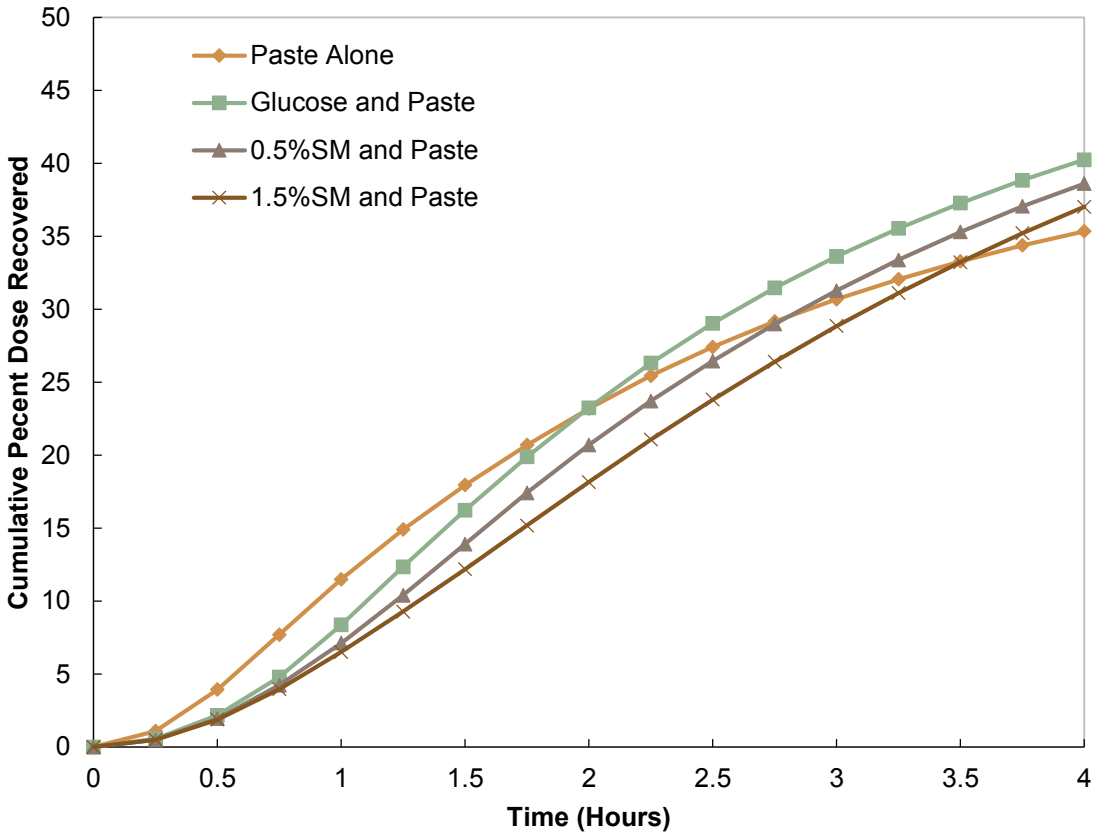

Figure 3.4 Curves of the cumulative breath $^{13}CO_2$ excretion over time after ingestion of the different test meals.
Values are mean of excretion measured in 10 subjects.

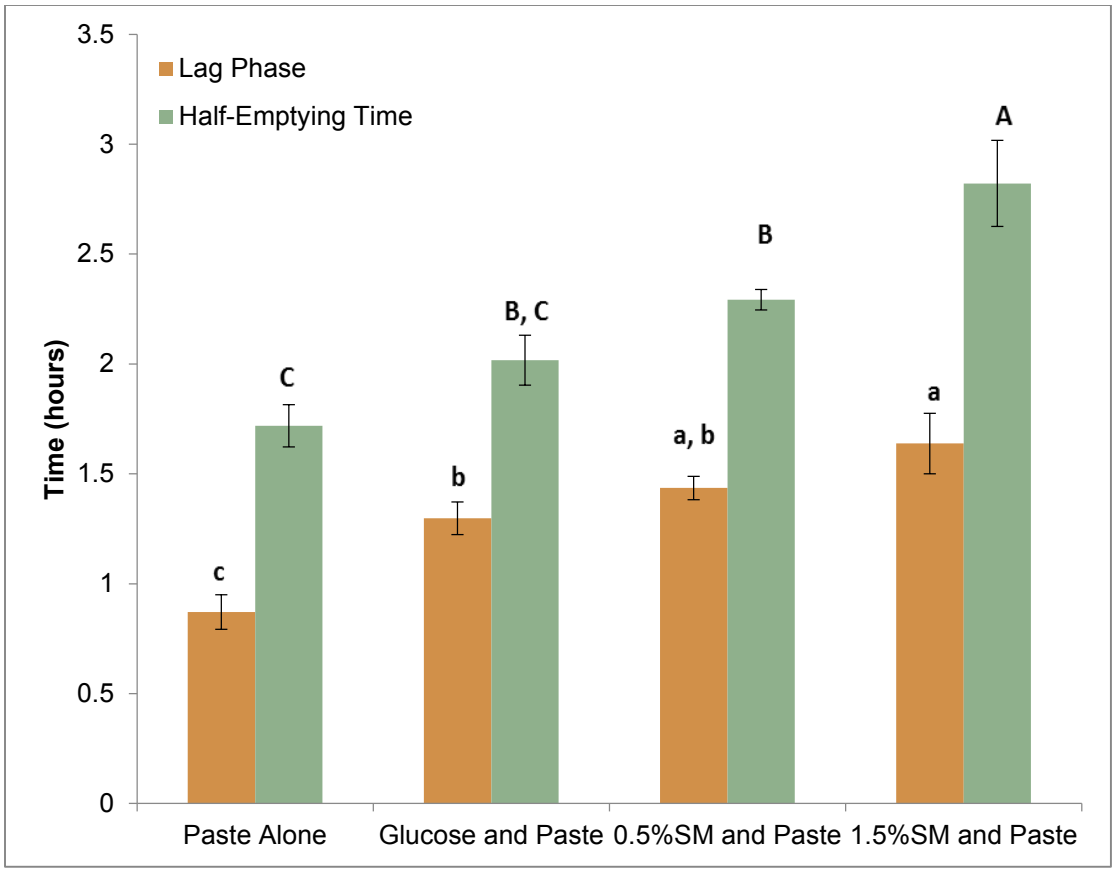

Figure 3.5 Gastric emptying parameters.
Mean (± SEM) of lag phase (gray bars) and half emptying time (black bars) of the different test meals. Comparisons are based on complete randomized block design (RCBD) with post-hoc Tukey's multiple comparison test ($\alpha = 0.05$). Different letters indicate statistically significance differences between treatments.

CHAPTER 4. ASSESSMENT OF STARCH DIGESTION IN MODERATELY MALNOURISHED MALIAN TODDLERS AND IDENTIFICATION OF BETTER ENERGY PROVIDING STAPLE FOODS

4.1 Abstract

Starches are a dominant source of dietary energy in complimentary feeding of growing toddlers. Glucose is the only carbohydrate oxidized by the brain, and in toddlers, 40% of the glucose needed for brain metabolism comes from the diet with the remainder produced by endogenous gluconeogenesis from amino acids, lactate, glycerol, and fatty acids. After weaning, dietary glucose is generated primarily from starch which is the main component of most complimentary foods. Young infants lack secreted α-amylase until weaning and after this period malnourished toddlers have been shown to continue to have reduced α-amylase activity. We hypothesized that malnourished (stunted) weaned toddlers have impaired ability to digest starch due to developmental and/or nutritional pancreatic α-amylase insufficiency which impedes normal growth when fed with the sorghum porridges used at home. A new, non-invasive modified ^{13}C-breath test (BT) was used to assess α-amylase activity, the ability to digest sorghum starch, and to evaluate the gastric emptying of sorghum porridge in healthy (n=16) and moderately stunted toddlers (n=32) from 18 – 30 months of age in Bamako, Mali. Four different ^{13}C-labelled substrates (uniformly labeled algal starch, uniformly labeled algal limit dextrins, partially enriched sorghum, and uniformly labeled octanoic acid) were fed on separate days. Serial breath samples (every 15 min for 3 hours) were collected and analyzed using a $^{13}CO_2$ infrared spectrophotometer. We found that α-amylase insufficiency was present in both Malian healthy and stunted toddlers. However, children with α-amylase insufficiency digested, absorbed, and oxidized the released glucose from normal sorghum porridge as well, and in some cases even better than, as the healthy group; indicating that the α-glucosidases compensate for the α-amylase insufficiency, and particularly well in the

stunted group. A thicker porridge and its α-amylase thinned counterpart were still digested well by the stunted group. There was no difference in the gastric emptying rate parameters between the healthy and stunted groups. We conclude that: 1. α-amylase sufficiency can be tested by a non-invasive breath test method using ^{13}C-starch substrate, 2. the digestion of sorghum porridge starch to glucose was unrelated to α-amylase sufficiency, 3. sorghum porridge, even in a thick form, is a starch-containing complimentary food that is well digested because of sufficient α-glucosidase activities in Malian weaned children, 4. the stunting of Malian children was unrelated to sorghum feeding and starch digestion to glucose because these children digested the sorghum porridge to glucose as well as the healthy group. This work suggests that thick energy dense porridges supply digestible carbohydrates to stunted children, and suggest that they could be used in supplemental feeding programs.

4.2 Introduction

Digestible carbohydrates, which are mainly from starchy foods, are the main source of dietary energy in growing children after weaning; the period when non-breast milk food is introduced to the child until the stoppage of breastfeeding. Weaning foods in Sahelian West Africa are cereal-based and starch is their major component. Glucose is needed for a developing weaned child, not only as the principal dietary energy source, but also for brain development. Glucose is the only energy molecule utilized by the brain, and in children, 40% of the glucose needed for brain development comes from the diet (Chugani, 1998; McCall, 2004). After weaning, glucose mainly originates from starch in complementary foods. There are different types of enzymes which are responsible for the digestion of starch. They are salivary α-amylase, pancreatic α-amylase, and the brush border or mucosal α-glucosidases (Dewit et al., 1990). There also is α-amylase in breast milk, which may help children digest starch from weaning foods (Lindberg and Skude, 1982; Dewitt et al., 1990). Salivary α-amylase is present in the early stage of gestation (by 20 weeks) and is present, but with low activity, at birth. After birth, its activity increases to reach a high level by the third month (McClean and Weaver, 1993; Christian et al., 1999). Pancreatic α-amylase, the more important of the two α-amylases for starch digestion, is not detectable during the gestation period and its activity starts to increase after 1 month after birth, reaching a high level after 24 months of age (weaning age) (McClean and Weaver, 1993; Christian et al., 1999). The brush border α-glucosidases are present in infants and they have full activity at birth (Auricchio et al., 1965; Raul et al., 1986).

The weaning process, with the introduction of non-milk foods, influences the development and the quantity of pancreatic α-amylase secreted. For example, the consumption of a carbohydrate-rich diet increased α-amylase activity in rats (Snook et al., 1971). Zoppi et al., (1972) showed that addition of starch or protein to the milk of preterm children resulted in increased secretion of α-amylase, and trypsin and lipase in their duodenal aspirates.

Since the 1950's, it has been known that malnutrition causes insufficiency of pancreatic enzyme production, including α-amylase that digests starch (Thompson and

Trowell, 1952). Watson et al. (1977) showed suppressed α-amylase activity in tears, saliva, and serum of marginally and severe malnourished Colombian children. α-Amylase activity was more diminished in older toddlers with severe malnutrition. Later studies provided additional evidence of α-amylase insufficiency in children with moderate malnutrition. Pancreatic function insufficiency was found which included markedly lower amylase activity in duodenal aspirations of Abidjan children with kwashiorkor (73.9 U/ml) compared to healthy control African children (186.9 U/ml) and French children (263.5 U/ml); and in Dakar children both clinically malnourished and control (moderately malnourished) patients showed low amylase activity of 50.2 and 109.7 U/ml, respectively (Sauniere et al., 1988). In a portion of the children with kwashiorkor in Abidjan, the alteration of the pancreatic function disappeared after refeeding (Sauniere et al., 1986).

The low luminal pancreatic α-amylase concentration in young children resulted in high amount of resistant starch which may have contributed to diarrhea through high osmotic load (Christian et al., 1999; Teitelbaum et al., 2003).

An extension of the above studies is that marginally and severe malnourished children with low pancreatic function may have starch digestion problems which interfere with their development and proper growth. Bandsma et al. (2011) reported altered glucose absorption in severely malnourished children. The malnourished state most often occurs during the period when solid food (weaning food) is introduced into a children's diet, usually occurring after 6 months of breastfeeding (Weaver, 1994).

In developing countries, these weaning foods are generally local cereal-based gruels that are in essence thinned porridges. An inherent problem with these foods is that they are relatively low in flour content and are thus deficient in energy and nutrients. Various approaches have been used to increase energy density to achieve the same thin porridge consistency with higher energy content; such as addition of malted grain flour or through fermentation, both of which introduce amylases to break down the large starch molecules that create viscous flow (Weaver et al., 1995; Tou et al., 2007a, b). For example, addition of oil (Hellstrom et al., 1981), and increase in flour amount with use of malt or commercial α-amylase to reduce the viscosity of the bulky foods by increasing the energy density (Weaver et al., 1995; Moursi et al., 2002; Onyango et al., 2004) have

been used. Songre-Ouattara et al. (2009, 2010) used fermentation with amylolytic lactic acid bacteria to improve porridge energy density for children using African local pearl millet mixed with groundnut or soybean. However, findings from different studies gave different results regarding how the increase of the energy density and/or lowering the viscosity of the weaning foods affected overall energy intake. For example, Weaver et al. (1995) showed that partial digestion of a weaning food using amylase-rich flour (decreasing the viscosity) resulted in an increase in digestion and absorption. On the other hand, other studies showed that the total daily energy intake from an amylase-treated thick energy-dense porridge was not significantly different from the non-treated one (Stephenson et al., 1994; Moursi et al., 2002).

Non-invasive breath tests have been used to assess the effect of diet on different processes and metabolic pathways in different organs such as the pancreas, the small intestine; as well the emptying rate of the stomach (Hiele et al., 1995; Weaver et al., 1995; Pelton et al., 2004; Parra et al., 2006; Robayo-Torres et al., 2009; Van Den Driessche et al., 1999). The principle of this method is based on the fact that after ingestion of a ^{13}C-labelled substrate, ^{13}C will appear in the breath after metabolism and oxidation of the substrate. It has been used for starch digestion assessment, as was demonstrated by Dewit et al. (1992) and Amarri et al. (1998) who showed impaired starch digestion using a ^{13}C breath test technique in children 25-48 months and 4-15 years, respectively, with cystic fibrosis. In the case of gastric emptying assessment, the rate limiting step of the presence of ^{13}C in the breath is the passage of the substrate from the stomach to the duodenum. The breath test can also be used as an indicator of starch digestion and absorption of glucose. The principle is based on ^{13}C-rich starch being hydrolyzed to glucose which is then transported to the liver and its oxidation leads to the presence of $^{13}CO_2$ in the breath. This is measured and provides information on the digestion of the starch. The rate limiting step this time is starch hydrolysis by the different enzymes.

Different invasive techniques have been used to measure exocrine pancreatic functions, including pancreatic α-amylase activity. Those techniques, such as duodenal aspirations (Sauniere et al., 1986; Sauniere et al., 1988), serum, urine, and tears collection

(Ceska et al., 1969; Watson et al., 1977) can be difficult to perform, expensive, and require a specialized medical operator and facilities. In this study, through work with Dr. Buford Nichols, Baylor College of Medicine, a novel non-invasive ^{13}C breath test method was used to assess the pancreatic α-amylase insufficiency in healthy and malnourished stunted infants in Mali.

The purpose of this study was: 1) to assess and compare the pancreatic α-amylase activity among stunted and healthy children between 18 – 30 months using a novel non-invasive ^{13}C-breath test technique, 2) to evaluate and compare the digestibility of common and modified starch-based sorghum porridges in stunted and healthy children, and 3) to evaluate the gastric emptying rate of sorghum porridge in stunted and healthy children. Simple, safe, and noninvasive ^{13}C-breath test methods were used to diagnose α-amylase insufficiency and to determine the relative efficiency of starch digestibility.

4.3 Subjects/Materials and methods

This study design was developed and the study was conducted in collaboration with Dr. Buford Nichols, USDA Children's Nutrition Research Center, Baylor College of Medicine and Dr. Atossa Rahmanifar, nutrition consultant. At the study site, Centre Hospitalier et Universitaire Gabriel Toure in Bamako, Mali, collaborators were Drs. Toumani Sidibe, Mariam Sylla, and Hawa Diall. All field work in Bamako was performed by Fatimata Cisse with assistance of Dr. Hawa Diall . Funding was provided by the Bill and Melinda Gates Foundation.

4.3.1 Subject eligibility

A written consent form was obtained from the parents or caretakers for the participation of their child to the study, which was approved by the National Ethical Committee for Health and Life Sciences in Mali and the Institutional Review Board of Purdue University. Demographic characteristics were collected from parents including mother's age, education level, and occupation. Eighty children were screened for their eligibility based on whether they were weaned or not, and the birth date on their issued Malian health card. Fifty-five children, between 18 to 30 months (at the recruitment time), were selected and recruited from the above eighty based on their availability for the duration of the study and whose parents had signed an informed consent. Their

weight and height were measured and used to determine their Z-score. Seven children had to be withdrawn from the study, because were not able to perform the breath test. Thus, sixteen healthy (control group) and thirty-two stunted moderately malnourished (treatment group) children participated and completed the study. They were aged 18 – 30 months (25 ± 3.24 mean ± SD) with a mean (± SD) height of 82 ± 4.32 cm, and weight of 10.14 ± 1.1 Kg. The entry criteria for the treatment group were stunted weaned children in the age range 18 – 30 months with height-for-age Z-score (HAZ) below -2.0 (HAZ<-2.0). The entry criteria for the control group were healthy weaned children in the same age range as the stunted group. Children were excluded who were younger than 18 months and older than 30 months, wasted with weight-for-height Z-scores lower than -2.0 [wasting refers to a short-term response to insufficient intake and when the weight for height Z-score is lower than -2 the child can be moderately (-2 > Z-score ≥ -3) or severely (Z-score < -3) wasted], acutely ill, under any medication, presented any gastrointestinal, cardiovascular or respiratory disorders, and had any other medical problem (health issue) rather than their malnutrition status.

4.3.2 Materials

A Malian sorghum variety with double usage (high grain quality and feed value) "DARREL KEN" was selected for making the different porridges. This variety was derived from the crossing of an improved Guinea-type variety (N'Tenimissa) and a local Guinea type variety (Seguetana) for grain quality and yield. ^{13}C-labelled algal starch (UL-algal starch-13C) was purchased from Isotec, Inc. (Sigma-Aldrich, Miamisburg, OH) and UL-algal limit dextrins (LDx) was made in our laboratory by hydrolyzing ^{13}C algal starch with α-amylase for 120 minutes to produce ^{13}C α-limit dextrins (with assistance by Dr. A. Lin). ^{13}C greenhouse-enriched brown sorghum (CSC3XR28 – F1 hybrid) (Awika et al., 2003) was obtained from collaborator Dr. M. A. Grusak at the USDA-ARS Children's Nutrition Research Center (Baylor College of Medicine, Houston, TX). Labelled sorghum flour was obtained by decorticating and milling the enriched sorghum grains. Briefly, the grains were decorticated to remove the bran layers using a tangential abrasive dehulling device (TADD) (model 4E-110/230, Venables Machine Works LTD, Saskatoon, Canada) for 3 minutes. Decorticated grains were

collected using a sample collector attached to a vacuum and milled using a coffee grinder with a Chamber Maid Cleaning System (Mr. COFFEE, model IDS75/IDS76/IDS77, Boca Raton, FL). Total starch and tannin contents of the labelled sorghum were determined using the Megazyme Total Starch Assay kit (Megazyme, Bray, Co. Wicklow, Ireland) and the vanillin test (vanillin and vanillin/HCL tannin determination) procedure described by Earp et al., (1981). A food-grade thermostable α-amylase was obtained (Food Enzymes, Dupont™ Genencor® Science). ^{13}C-octanoic acid was purchased from Sigma-Aldrich (St. Louis, MO).

4.3.3 Test meals

A cursory survey was conducted to inventory the different types and composition of the weaning foods generally given to children in Bamako (Mali). Sorghum flour was chosen to prepare the porridges, because sorghum is one of the most important staple cereal crops in Mali and was found to be typically used to prepare weaning porridge. All porridges were prepared at the time of each day's test based on typical preparation method of the weaning food in Mali. Briefly a slurry was made of the flour and 1/3 of the water amount (200 mL), and added to the remaining 2/3 boiling water. Lemon juice was added for taste, and the mixture was cooked for 6 minutes. As ^{13}C was used as a tracer in the studies, background ^{13}C was kept to a minimum. For this reason, sugar from beet, as a C3 low ^{13}C plant, was used instead of cane sugar, as cane is a C4 plant higher in endogenous ^{13}C (Schoeller et al., 1980). Porridges were fed at around 37 ºC. Preliminary testing on four children and two nurses was to determine the appropriate sweetness level and to determine the amount of porridge that can be consumed by the targeted age range children (18 – 30 months). An objective was that the whole amount of labelled substrate was consumed by each child in each test period. The composition of the porridges is given in **Table 4.1**.

4.3.4 Procedure

4.3.4.1 Study design

The study was done in Bamako, Mali at the Nutrition Center of the Centre Hospitalier et Universitaire Gabriel Toure. Children enrollment was done over a 6

months of period, and their time of participating was dependent on their availability. A maximum of four children were tested at one time. Upon enrollment, children were scheduled for the following Monday for six consecutive days of testing, or any a future week suitable for the mothers or caretakers. Children were tested on each day in the presence of one of the parents or a caretaker, if the parent was not available. The porridges were prepared as indicated in **Table 4.1** and the child was monitored to consume all the amount prepared. The recruited children and their parent or caretaker were instructed to come to the Center on the test days with the child beginning the test after at least 3 hours of fasting. The test meal was given orally within a time limit of 15 minutes and breath samples were collected in breath bags before (two breath samples at basal condition – 1.5 L each, aluminum lined) (Cambridge Isotope Laboratories, INC Andover, MA) and after test meal ingestion every 15 minutes for 3 hours. The breath collection consisted of blowing into a small breath bag (300 mL, aluminum lined QCH-1524) (Cambridge Isotope Laboratories, INC Andover, MA). The collected breath samples for each child and at each time point were analyzed for the presence of ^{13}C in the expired CO_2 using a non-dispersive infrared spectrophotometer (POCone, Ostuka Co, Japan) (Robayo-Torres et al 2009). The measured ^{13}C enrichment (DOB delta over baseline) in the expired CO_2 is the difference of the CO_2 abundance of a measured breath sample from the reference $^{13}CO_2$ abundance (the baseline breath sample). Appropriate age matched toys were available for play during the testing period. All recruited children were successfully tested for all the treatments and at all-time points; there were no missing data points. Since the length of each test day was 3 hours, and to avoid the children getting hungry, a sweetened rice porridge was served midway into the test period ($1^{1/2}$ hours after the test meal was ingested). Rice was chosen for this porridge, since it is a C3 crop low in ^{13}C (Schoeller et al., 1980); beet sugar was used as a sweetener.

4.3.4.2 Determination of the prevalence of pancreatic α-amylase deficiency

4.3.4.2.1 Principle and method

The method, proposed by B. Nichols, used to assess pancreatic α-amylase insufficiency in this study is a novel non-invasive ^{13}C-breath test technique which uses a

starch substrate fully enriched with a non-radioactive ^{13}C stable isotope. The method requires the use of ^{13}C-labelled algal starch and ^{13}C-labelled LDx and involves the measurement of the ratio of ^{13}C isotopes present in breath CO_2 after ingestion of meal containing the above substrates on two separate days (Day 1 and Day 2). The principle is that LDx, which is already predigested by α-amylase, by-passes pancreatic α-amylase digestion and goes directly to the mucosal α-glucosidases for digestion to glucose; while the algal starch must be digested by pancreatic α-amylase prior to glucogenesis. Thus, the test is a measure of sufficiency of pancreatic α-amylase to digest starch. Expired $^{13}CO_2$ in the breath which is the end-product of the oxidative metabolism is collected and its ^{13}C enrichment measured.

4.3.4.2.2 Procedure

To test for α-amylase insufficiency, sorghum porridge was prepared with 16 g of sorghum flour mixed with one of two different ^{13}C labelled substrates (25 mg of UL-algal starch and 25 mg of UL-algal LDx), and were fed on two separate days respectively (Day 1 and Day 2). The ability to digest the starch was assessed according to the procedure outlined in the study design section of Christian et al. (2002).

4.3.4.3 Evaluation of the digestibility of starchy foods

Three different sorghum porridges with a portion (500 mg) of ^{13}C greenhouse-enriched sorghum flour were fed in three separate days: common sorghum porridge (Day 3), shear modified and thickened sorghum porridge (Day 4), and α-amylase pre-treated Day 4 modified sorghum porridge (Day 5).

4.3.4.3.1 Common sorghum porridge (Day 3)

To test for the starch digestibility of a common Malian weaning food in healthy and malnourished stunted children, sorghum porridge was prepared as described above (Sec. 4.3.3) with 16 g of sorghum flour mixed with 500 mg of ^{13}C greenhouse-enriched sorghum flour and was fed on Day 3.

4.3.4.3.2 Shear modified and thickened sorghum porridge (Day 4) and the same porridge pre-treated with α-amylase (Day 5)

For Days 4 and 5, porridges were prepared with 20 g of sorghum flour plus 10 g of waxy corn starch (almost double of the dry matter (flour) quantity used in Day 3), mixed with 500 mg of ^{13}C greenhouse-enriched sorghum flour, and shear was applied to the cooked porridge for 3 minutes. This created a thicker porridge for Day 4 treatment. For Day 5, the porridge was pre-treated with food-grade thermostable α-amylase (Food Enzymes, Dupont™ Genencor® Science) before consumption. The aim was to compare the digestibility of a shear modified thicker sorghum porridge before and after α-amylase pretreatment in healthy and malnourished stunted children. The children were tested with the same breath testing procedure as described above.

4.3.4.4 Determination of the viscosity of the porridges in Days 3, 4, and 5

Viscosity is one of the most important flow properties related to the quality of thin porridges, and is defined as a liquid substance's resistance to flow [shear stress (force/area) over shear rate (velocity/distance)]. Viscosity of the porridge samples was determined by a flow curve measurement method using a dynamic mechanical rheometer (AR-G2 Rheometer, TA Instruments, New Castle, DE). A cone-plate assembly was used to assess the viscosity of the porridge samples using steady shear measurement with controlled stress. After warming the instrument, a small portion of porridge sample was loaded onto the plate and the viscosity was determined up to a shear rate of 300/s at 37ºC. The experiment was carried out at constant stress and the same stress was used for all samples. Analyses were performed in duplicate.

4.3.4.5 Evaluation of the gastric emptying rate of the modified thick sorghum porridge (Day4)

In this case, the same ^{13}C breath test was used, but the ^{13}C source was octanoic acid (UL-octanoic acid), instead of the ^{13}C-labeled sorghum starch (Veereman-Wauters et al 1996; Van Den Driessche et al., 1999). The ^{13}-labelled octanoic acid was mixed with the modified sorghum porridge used on Day 4. The procedure used is described in detail in Chapter 2.

Calculation

The raw data collected from the ^{13}C spectrophotometer analysis of the breath bags does not imply the actual CO_2 production from each individual. Age, gender, weight, and height are parameters that influence CO_2 production. For example, adults produce more CO_2 than children; likewise males typically produce more than females. Therefore, normalization of the raw data was necessary to avoid bias in the estimation of the enrichment level of the breath $^{13}CO_2$ by the internally produced CO_2. CO_2 production was estimated by using the method adopted by Klein et al. (1999). The obtained adjusted $^{13}CO_2$ breath enrichment values represent an indication of the degree of digestion of the different substrates. The higher the values signifies that more substrate has been digested.

The adjusted $^{13}CO_2$ breath enrichments for all time points (from 0 to 180 min) were summed for each child and for each substrate. Healthy control subjects were used to define the horizontal (Y-axis) and the vertical (X-axis) lower reference levels (LL) defined as mean – 1SD for each substrate. This concept is used in the medical field as a basis to interpret the results for the treatment groups. Therefore, the lower reference level from the healthy group was used to compare the children's ability to digest the different ^{13}C enriched substrates on the different testing days, thus in indicating their degree of starchy food digestion.

Calculations used to determine pancreatic amylase sufficiency were performed as follows. The sum of the $^{13}CO_2$ breath enrichment of ^{13}C-limit dextrin (Day 2) was divided by the sum of $^{13}CO_2$ breath enrichments of the ^{13}C-algal starch (Day 1), and this ratio was termed "α-amylase sufficiency" [α-amylase sufficiency (D2/D1) = Σ 13C-limit dextrin / Σ 13C-algal starch]. A histogram implies that when D2/D1 is lower than 1, then full pancreatic maturity has occurred. The amylase sufficiency ratio was used to classify the children based on their pancreatic maturity:

- Sufficient α-amylase amplification (ratio < 1.0, thus full pancreatic maturity has occurred)
- Moderate α-amylase insufficiency (ratio is between 1 to 2)
- Severe α-amylase insufficiency (ratio > 2)

Statistical analysis

Comparisons of lag phase, half-emptying times and values across foods were analyzed by one-way repeated-measure ANOVA with post-hoc Tukey tests used to form statistical groupings (α=0.05). For each comparison made, $P < 0.05$ was considered significant.

4.4 Results

4.4.1 Pre-test questionnaire on weaning food practices

Thirty women were interviewed on weaning practices in Bamako, Mali. The results showed that all were providing weaning food to their child or children in the form of porridge made from local cereal crops (sorghum, millet, maize, rice), plus sometimes wheat and soy bean. Eleven women were providing only one type of weaning food [porridge made with sorghum or millet or composite flour (millet, sorghum, maize, rice, soy bean, pea)], five mothers were feeding their children with 2 types of weaning foods (the porridge plus a soup made with fish or meat), and 14 were giving multiple types of weaning foods such as porridge, soup, and/or fruit juice, egg, and vegetable puree. These weaning foods were given in different frequencies; eight women were serving their children these foods once a day, seven were providing them twice a day. Seven mothers fed their children 3 times/day, whereas the rest of the women fed their children it was more than 4 times/day. Among the children (18 girls and 12 boys); four were under than 12 months, 16 were between 12 – 18 months, and 10 more between 18 – 30 months. Fifteen children were breastfed and the rest had been weaned.

4.4.2 Household demographic characteristics

Table 4.2 shows the demographic characteristics of the children's mothers. There were 46 interviewed mothers and 98% of them were married. The mean age of the mothers was about 28 years and none of them had a university education level. Thirty-seven percent of the mothers had attended school and only 13% were still in school. Overall, 63% were illiterate and 74% were housewives.

4.4.3 Subject characteristics

Table 4.3 reports the anthropometric characteristics of the recruited children. The 16 healthy children (healthy group: 8 boys and 8 girls) were of a mean age of 25 ± 3.4

months a mean height of 86.2 cm, and a mean weight of 11.1 kg. The 32 stunted, moderately malnourished children (treatment group, 12 boys and 20 girls), who finished the study, were of a mean age of 25 months, a mean height of 79.9 cm in height, and a mean weight of 9.6 kg . There was a significant statistical difference in height and weight between the healthy stunted groups at ($P < 0.001$). The healthy group had a significantly greater height and weight than the stunted group. As a whole, the children had a mean age of 25.0 months, a mean height of 82.0 cm, and a mean weight of 10.1 kg. The individual characteristics of the children in the study are given in **Table 4.4**.

4.4.4 Pancreatic α-amylase insufficiency

4.4.4.1 Healthy group

The $^{13}CO_2$ breath enrichment values which indicate the digestion rate of the ^{13}C-labelled algal starch in Day1 and ^{13}C-labelled LDx in Day 2 for the individuals in the healthy group are shown in **Figure 4.1** and **4.2**, respectively. **Figure 4.2** shows that the ^{13}C-labelled LDx (predigested algal starch) was well digested by all children compared to the undigested algal starch (**Figure 4.1**), with the exception of children #22, 23, and 39. This is illustrated better in **Figure 4.3** which reveals that the mean-derived digestion rate profile of Day 2 is significantly greater than the rate profile of Day1 (p-value=0.0045). The mean $^{13}CO_2$ enrichment level for Day 2 peaked at 105 minutes (151.6‰), while the one for Day 1 reached its highest digestion point at 120 minutes (101.6‰),

4.4.4.2 Stunted group

The ^{13}C-labelled algal starch (Day 1) and the ^{13}C-labelled LDx (Day 2) digestion rate profiles for the individuals in the stunted group are presented in **Figures 4.4** and **4.5**, respectively. These figures show that the stunted group follows the same trend as the healthy group for both days, although this group had a somewhat higher number of children with low digestion rates for Day 2. As in the healthy group, the average ^{13}C enrichment level for Day 1 in the stunted group peaked at 120 minutes (106.2‰) and Day 2 at 105 minutes (137.9‰) (**Figure 4.6**). Overall, in both groups the porridge in Day 2, containing the ^{13}C-labelled LDx was digested better than in Day 1, where there was the

undigested algal starch and indicates that pancreatic α-amylase was insufficient to reduce it adequately to LDx for rapid α-glucosidase digestion to glucose.

In both groups (healthy and stunted), individual children sometimes digested the two substrates quite differently and sometimes unexpectedly (^{13}C-labelled algal starch and predigested ^{13}C-labelled algal starch), with examples illustrated in **Figures 4.7**, **4.8**, **4.9**, **4.10**, and **4.11**. **Figure 4.7** shows a low algal starch digestion rate (Day 1) and high LDx digestion rate (Day 2) in a healthy child (#25), therefore indicating insufficient pancreatic α-amylase. Contrarily, child #39 (also healthy) was able to digest the algal starch, but not the predigested algal starch (**Figure 4.8**). **Figure 4.9** displays the digestion rate profile of a healthy child (#23) who was not able to digest either of the labelled substrates (algal starch and predigested algal starch). **Figures 4.10** & **4.11** show two stunted children (#18 & 16) with different digestion profiles as we saw in the healthy group. **Figure 4.10** shows a stunted child having low algal starch digestion (Day 1), but high LDx digestion rate (Day 2). On the other hand, **Figure 4.11** displays the digestion profiles of a stunted child who was not able to digest either of the substrates on both days. In the following figures, presentations are made both including these children and considering them as outliers.

It is seen in **Figures 4.12** and **4.13** that both healthy and stunted groups digested the ^{13}C-labelled algal starch and the ^{13}C-labelled LDx (predigested ^{13}C-labelled algal) similarly as seen in ^{13}C breath enrichment profiles (p-value=0.26 for both groups). This is supported by the plot of the sum of ^{13}C breath enrichments for Day 1 vs. Day 2 for all children in **Figure 4.14** which shows that most, whether healthy or stunted, are clustered above the horizontal line (87.5%) and to the right of the vertical line (89.6%). Children that are below the horizontal line (12.5%) have a poor starch digestion rate, as well as the ones that are on the left side of the vertical line (10%). The latter have either a mucosal α-glucosidase problem or any other associated digestion or absorption problems, because were not able to digest further an already predigested algal starch. The histograms in **Figure 4.15** displays the distribution of the children (healthy on the left and stunted on the right) based on their pancreatic α-amylase amplification (insufficiency) ratio which is an indicator for their pancreatic α-amylase maturity. When this ratio is lower than 1 that

means pancreatic α-amylase maturity occurs, therefore it is sufficient; whereas when the ratio is greater than 1, pancreatic α-amylase is insufficient. Most of the children were between 1 and 2 (moderate α-amylase insufficiency); and relatively few of them were amylase sufficient (around 20 % for each group). The plot of the α-amylase amplification ratio (D2/D1) by age in all children shows that α-amylase insufficiency is seen in all tested age groups and is somewhat more pronounced after 20 months (**Figure 4.16**). The results in **Figure 4.16** show that most subjects in the study, whether stunted or healthy, have α-amylase insufficiency as estimated by α-amylase amplification (D2/D1) [their α-amylase amplification ratio is higher than 1(> 1.0)]. Thus, at 18 – 30 months, α-amylase insufficiency was common in both the healthy and stunted children studied in Bamako, Mali. The α-amylase amplification (D2/D1) ratios are given in **Tables 4.5** and **4.6** for healthy and malnourished stunted groups.

4.4.5 Measurement of the digestibility of starchy foods

4.4.5.1 Digestion of the common sorghum porridge in Day 3

Figures 4.17 and **4.18** show the $^{13}CO_2$ breath enrichment levels for the healthy and the stunted groups, respectively, after ingestion of the common sorghum porridge (Day 3), demonstrating their digestion rate profiles of the porridge. Healthy and malnourished stunted children digested the common sorghum porridge at the same rate (*p*-value=0.16) (**Figure 4.19**). The plot of the $^{13}CO_2$ breath enrichment in Day 3 vs Day 1 reveals that most of the children who digested the algal starch (right hand of the vertical line) were able to digest the common sorghum porridge, with the exception of few children (~ 10 % - children below the horizontal line) (**Figure 4.20**). **Figures 4.21**, **4.22**, and **4.23** show the relationship between digestion rate of the common sorghum porridge (Day 3) and the amylase insufficiency ratio for healthy and malnourished stunted children. The results show that almost all of the children (79.2 %) digested the starch in the common sorghum porridge (above the horizontal lower reference limit for the healthy group), even though nearly all were pancreatic α-amylase insufficient (amylase insufficiency > 1, right hand of the vertical line). In **Figure 4.22**, child # 39 was excluded and, in **Figure 4.23,** two children (#39 and #3) were excluded; both were considered

possibly as outliers. Child #39 was considered to be excluded, because as a healthy child it was able to digest the algal starch (**Figure 4.8**), but not able to digest the ^{13}C-labelled LDx (predigested algal starch) as well as the common sorghum porridge and similarly child #3 was considered to be another outlier.

4.4.5.2 Digestion of the shear modified thickened sorghum porridge in Day 4

The digestion rate profiles of the shear modified thickened sorghum porridge (Day 4) for the healthy and malnourished stunted groups showed approximately the same trends and values. For the healthy group (**Figure 4.24)**, three subjects (#1, 2, and 4) had a high porridge digestion rate compared to the other children; with a similar finding in the malnourished stunted group (**Figure 4.25**) for subject #3. When compared, the digestion of the shear modified thickened sorghum porridge for the healthy and malnourished stunted groups were essentially the same (**Figure 4.26**). **Figure 4.27** shows how shear modified thickened sorghum porridge digestion of Day 4 is related to the algal starch digestion of Day 1. The general correlative relationship indicates that children digested both the algal starch and the shear modified thickened sorghum porridge similarly. **Figure 4.28** elucidates the relationship between the test subject's developmental pancreatic α-amylase insufficiency and the shear modified thickened sorghum porridge digestion of Day 4. In both healthy and malnourished stunted groups, it is seen that almost all children (89.6 %) digested the shear modified thickened sorghum porridge starch despite their moderate amylase insufficiency. The correlation between the two parameters was not strong (R^2 = 0.0595 and 0.216 for healthy and malnourished stunted, respectively). However, when outliers (children #1, 2, 4, and 39) were excluded from the healthy group, as well as child #3 from the malnourished stunted group, a difference in the trends for stunted versus healthy children is noted (**Figures 4.29** and **4.30**). In normal children, increase in degree of α-amylase insufficiency correlated to lower shear modified thickened sorghum porridge starch digestion (R^2 = 0.52); however, in malnourished stunted children, there was no correlation between α-amylase insufficiency and starch digestion (R^2 = 0.00008).

4.4.5.3 Digestion of the α-amylase pre-treated shear modified thinned sorghum porridge of Day 5

Figures 4.31 and **4.32** depict the individual subject digestion profiles for the α-amylase pre-treated shear modified thinned sorghum porridge of the healthy and malnourished stunted groups given in Day 5. Overall, the Malian children in the study digested well the α-amylase pre-treated shear modified thinned sorghum porridge. Moreover, both groups digested the porridge similarly **(Figure 4.33)**. **Figure 4.34** shows that the digestion of the α-amylase pre-treated shear modified thinned sorghum porridge in Day 5 is positively related to the algal starch digestion in Day 1. **Figures 4.35, 4.36**, and **4.37** show the relationship between the α-amylase pre-treated shear modified thinned sorghum porridge digestion and pancreatic α-amylase insufficiency in children. In **Figure 4.35**, which includes healthy and malnourished stunted children, it is seen that almost all of subjects digested the α-amylase pre-treated shear modified thinned sorghum porridge in Day 5. There was no relationship between α-amylase insufficiency and the α-amylase pre-treated shear modified thinned sorghum porridge starch digestion in either group (healthy – R^2 = 0.0013, stunted – R^2 = 2E-06), even when outlier subjects #1, 2 (healthy) and 3 (stunted) were excluded **(Figure 4.36)**. When subject #39 is excluded, a similar trend is observed between digestion of α-amylase insufficiency and α-amylase pre-treated shear modified thinned sorghum porridge (**Figure 4.37**), to that shown in Figure 4.30 for the untreated shear modified thickened sorghum porridge. There was no relationship in stunted children between pancreatic α-amylase insufficiency and α-amylase pre-treated shear modified thinned sorghum porridge digestion (R^2 = 0.0013).

4.4.5.4 Comparison of digestion results for the untreated and α-amylase pre-treated shear modified sorghum-based porridges

Despite the subject group (healthy or malnourished stunted), similar digestion responses were found among the children consuming the untreated and α-amylase pre-treated shear modified sorghum-based porridges. **Figures 4.38** and **4.39** show the averaged digestion profiles of the two sorghum porridges consumed in Days 4 and 5 in the healthy and stunted groups. In both charts, the untreated and the α-amylase pre-treated porridges in Days 4 and 5 were digested almost at the same rate by the two

healthy and malnourished stunted groups. This is seen in **Figures 4.40**, **4.41**, and **4.42** which illustrate the distribution of the $^{13}CO_2$ breath enrichment peaks for the untreated and the α-amylase pre-treated porridges in healthy, malnourished stunted, and in the whole group, respectively (without the outliers #1, 2, 4, 3, 19).

4.4.5.5 Viscosity of the different porridges of Days 3, 4, and 5

Figure 4.43 shows the viscosity of the common sorghum porridge in day 3. **Figure 4.48** represents the viscosity of the untreated and α-amylase pre-treated shear modified porridges consumed by the children in Days 4, and 5. The viscosity of the untreated shear modified sorghum porridge in Days 4 was greater than that of the α-amylase pre-treated one in Day 5 (**Table 4.7**) As shear rate increased, the viscosity of the untreated shear modified sorghum porridge of Day 4 became significantly higher than that of the α-amylase pre-treated shear modified sorghum porridge of Day 5.

4.4.6 Gastric emptying rate of the modified sorghum porridge

Figures 4.45 and **4.46** show the ^{13}C-breath enrichment recovery levels after emptying of the ^{13}C-labelled octanoic acid thick modified sorghum porridge of Day 6 (same porridge as used on Day 4) by the healthy and malnourished stunted groups. In both groups, breath enrichment levels peaked early (15 – 30 min) and decreased over time. Average ^{13}C-labelled breath enrichment levels for healthy and malnourished stunted children groups are shown in **Figure 4.47**. The two groups appeared to empty the porridge in a very similar manner with gastric emptying evaluation parameters showing no difference in rate between healthy and malnourished stunted children (**Figure 4.48**). Similar gastric emptying rates were based on parameters that characterize gastric emptying rate (lag phase, and half-emptying time).

4.5 Discussion

4.5.1 Pancreatic α-amylase insufficiency assessment using a novel ^{13}C breath test

Malnutrition in children under 5 years is a persistent public health problem in the developing world with a broad range of implications such as increased risk of death, immune function impairment, increased infectious diseases, loss of appetite, and exocrine pancreatic insufficiency (Black et al., 2013; Rytter et al., 2014). Stunting (low height for

age), which is the result of chronic undernourishment based in low caloric intake, affects not only a child's physical but also developmental growth, particularly during the first 2 years of life (Shrimpton et al., 2000; Victoria et al., 2010). One of the aims of this study was to assess developmental pancreatic insufficiency in weaned healthy and stunted moderately malnourished children using an innovative non-invasive breath test designed by one of our investigators (B. Nichols).

Duodenal aspirations have been used to assess pancreatic function including α-amylase insufficiency in healthy and malnourished children (Thompson and Trowell, 1952; Sauniere et al., 1986 and 1988). This method is invasive, needs specialized medical staff and appropriate facility, is expensive, and is -somewhat limited as it represents only one time point (α-amylase secretion from the pancreas varies over time relative to food ingested and other factors). Serum, urine, and tears collections used by Ceska et al. (1969) and Watson et al. (1977) are less invasive than the previous method, but involve blood drawing and need special facilities and specialized medical staff.

In our study, a novel innovative non-invasive breath test technique was used to assess and compare the developmental pancreatic insufficiency in healthy and stunted children. Based on a simple principle, this method does not need a special facility or medical staff (though one was used in the present study); and it is safe, simple, user-friendly, and inexpensive. The test can be performed in the home, in a non-medical setting, and non-medical personnel can be easily trained to conduct the test.

Here, it was demonstrated that using this novel breath test method, pancreatic α-amylase insufficiency could be measured in healthy and malnourished stunted children. However, in order to validate these results and to be able to compare insufficiency our study groups (Malian healthy and stunted children), a US healthy control group is needed and this has not been done in this study.

In this study, the non-invasive ^{13}C breath test technique has been used not only to assess pancreatic α-amylase insufficiency, but also for starch digestion and gastric emptying rate in healthy and stunted children using different ^{13}C labeled substrates (^{13}C-labeled sorghum flour starch, ^{13}C-labeled octanoic acid). ^{13}C breath test method was used previously to assess starch digestion in healthy children (Weaver et al., 1995) and

children with cystic fibrosis (Dewit et al., 1992), as well as gastric emptying rate in breast-fed and formula-fed (Van Den Driessche et al., 1999) and in preterm (Veereman-Wauters et al. 1996) infants.

4.5.2 Pancreatic α-amylase insufficiency and porridge starch digestion

The findings of this study demonstrate that pancreatic α-amylase insufficiency is present in the majority of weaned healthy and malnourished stunted children in Bamako, Mali. α-Amylase insufficiency previously was shown in a kwashiorkor population, using duodenal aspirates (Thompson and Trowell, 1952), and in undernourished Colombian children using serum, tears, and urine (Watson et al. 1977). In this thesis study, in both healthy and malnourished stunted groups, comparatively low ^{13}C-labelled algal starch digestion was observed to the predigested ^{13}C-labelled algal limit dextrin (LDx), indicating some degree of α-amylase insufficiency. Most of the children had an α-amylase insufficiency ratio greater than 1 indicating α-amylase insufficiency. Exocrine pancreatic function of malnourished children in West Africa (Dakar, Senegal and Abidjan, Cote d'Ivoire) was assessed duodenal aspirates (Sauniere et al., 1986; Sauniere et al., 1988). Perhaps in agreement with our findings, they found that even normal (healthy) African children had decreased pancreatic secretion with lower activity compared to normal, healthy French children. In our study, even though the anthropometric characteristics (height and weight) of the healthy group were significantly better than the stunted group, they all were almost α-amylase insufficient (81%). The high proportion of subjects with low pancreatic α-amylase insufficiency may be explained by factors such as pre-birth nutritional status of the mothers (Martin-Gronert et al., 2006; Abu-Saad et al., 2010), and chronic consumption of a protein deficient diet (Dahri et al., 1991) which result in low synthesis of α-amylase (Watson et al., 1977). Refeeding of a well-balanced diet has been shown to promote recovery from protein malnutrition and a restoring of the pancreatic enzyme secretion (Veghelyi et al., 1950), as well as the brush border enzymes (Rossi et al., 1986).

Interestingly, the stunted children group in the study digested the sorghum-based porridges as well as the healthy group, and even appeared to digest the porridges, whether thick or thin, better when α-amylase insufficiency was more pronounced. It would appear

that the stunted group had enhanced activity of the mucosal α-glucosidases and/or the glucose transporter SGLT-1 compared to the healthy group. Due to the importance of securing glucose from the diet for the body, particularly in stunted children, it seems plausible that there could be an up-regulation or enhanced processing of the α-glucosidases/SGLT-1. In diabetics, where there is a similar condition of glucose-starving, it has been shown that there are higher enzyme and transporter levels (Tandon et al., 1975; Olsen et al., 1985). The finding that stunted children digest well the starch from thick porridges may have a practical implication in that thick cereal-based porridges could be given in supplementary feeding programs, and furthermore that thickness could be optimized so that energy-density of the porridges would be sufficiently high for adequate energy intake within a meal.

Starch is normally digested first by salivary and pancreatic α-amylases to small maltooligosaccharides (linear α-glucan oligomers and α-LDx), and then to glucose by the mucosal α-glucosidases. It is known that in children these small intestine brush border enzymes have comparable activity to that of adults very early at birth (Auricchio et al., 1965; Raul et al., 1986); and they may help in starch digestion even if the other enzymes (salivary and pancreatic amylases) are insufficient. A previous work from our lab showed that one of the four α-glucosidases, commonly known as glucoamylase (or Ct-maltase-glucoamylase, Ct-MGAM) has the ability to digest native molecular starch at a level approaching α-amylase (Lin et al., 2012; Lin et al., 2012). Dhital et al. (2013) and Lee et al. (2014) additionally showed that Ct-MGAM likely assists pancreatic α-amylase in early digestion of starch. Thus, it seems reasonable that the mucosal α-glucosidases could compensate for lower pancreatic α-amylase activity in the stunted children to still efficiently digest starch.

As mentioned, when children who had higher degree of α-amylase insufficiency consumed the thick porridges, the stunted children digested starch better than the healthy. There was no relationship between α-amylase insufficiency and sorghum porridge digestion ($R^2 = 0.00008$), indicating that mucosal α-glucosidase activity remained the same as α-amylase insufficiency for individual children increased, whereas in healthy children who had higher α-amylase insufficiency there was lower starch digestion ($R^2 =$

0.5218). This suggests that stunted children have higher mucosal α-glucosidase levels than normal children. Thus, stunted children seem to be able to digest even comparably thick porridges independently from their developmental pancreatic α-amylase insufficiency.

In a supportive way, Pinheiro et al. (2013) showed that rats born from protein restricted mothers presented up-regulated disaccharidases (lactase, sucrase, and maltase). This increase in enzyme activity could help in optimizing digestion and nutrient absorption to secure survival (Armitage et al., 2004).

In 10% of the studied children (2 healthy and 3 stunted), there was poor digestion of the sorghum porridge in Day 3 (**Figures 4.20** and **4.21**, the children that are below the horizontal line). This was likely due to inherent metabolic problems related to malnutrition such as deficiency in mucosal α-glucosidases activities, failure in glucose transporters, and/or deficient in brain glucose oxidation, and/or environmentally induced enteric dysfunction (Keusch et al., 2013 and 2014). Bandsma et al (2011) showed altered glucose absorption in children with severe malnutrition.

4.5.3 Thickened and thinned porridge starch digestion

Pre-treatment of starch-based porridges with α-amylase for thinning or liquefying purpose of weaning foods has been done to increase energy density so that higher energy consumption can be achieved in malnourished children, and also because thinned porridges are considered to have improved digestibility Watson et al. (1995) and Gopaldas et al., (1992). Contrary to this conventional view, thinning (α-amylase pre-treated porridge of Day 5), in the present study, did not show an improvement in starch digestion compare to the untreated thick porridge of Day 4 in the healthy or stunted children. The common and conventional view is that undernourished children better digest thin, rather than thick, food preparations; and a well-known approach is to treat thick, gelatinized starch-based pastes with an α-amylase (e.g., in malted grain) to reduce viscosity or liquefy preparations for both better digestion and to increase energy density. However, viscosity reduction with increased energy density has not always led to increased energy intake. Stephenson et al. (1994) reported that consumption of a semi-solid high density porridge resulted in elevated daily energy intake, but addition of α-

amylase to the same porridge did not add any supplementary energy intake. Owino et al. (2007) studied treated and non-treated fortified blends (maize, kidney bean, Bambara nuts and peanuts) prepared using extrusion cooking that were given to nine month old Zambian children. Results showed that both groups had the same improvements in percentage fat mass and hemoglobin concentration, and that there were no differences in their weight and height Z-scores. Moreover, the use of malted flour (amylase-rich flour) is associated with some drawbacks, such as long preparation time of the malted grains, safety problems (contamination with microorganisms as the result of unhygienic or unsanitary practice during preparation). Hence, the results are contradictory on the use of malt or industrial α-amylase-thinned porridges to increase energy intake or improve nutritional status of beneficiaries. On the other hand, bearing in mind that starch digestion of thick porridges when consumed by stunted children was good in the present study, a reconsideration of using energy-dense thick porridges in supplementary feeding programs of marginally malnourished children seems in order. Novel strategies should be considered that would produce high energy density thick porridges that are safe and nutritious and digestible. This could be an affordable alternative for populations in developing countries. Mixed local product formulations (cereals, legumes, and fruits) to meet energy, protein, and micronutrients requirements (Amuna et al., 2000), optimization of the energy density of the traditional cereal-based porridges, as well as ensuring high digestion rate could be developed. Fermentation additionally might be used to reduce the bacterial contamination of complementary foods and further improve nutritional quality (Mensah et al., 1990; Songre-Ouattara et al., 2009 & 2010).

Apart from the fortified and thinned high density energy complementary foods prepared locally, there are ready to use therapeutic foods supplied by feeding programs to help in the treatment of children with moderate and severe acute malnutrition. Plumpy-nut™ is a well-known example. These therapeutic foods are lipid-based products with high energy density that do not require any cooking; and that have low moisture content making them less susceptible to bacterial contamination (Imdad et al., 2011). Of note, though, is that these therapeutic foods are lacking or do not contain starch (glucose) which is thought to be necessary for proper brain development in children, and is

important in other glucose dependent developmental pathways. Glucose ingestion has been shown to enhance memory performance (Smith et al., 2011; Smith and Scholey, 2014). Gibson et al. (2013) showed high intake of saturated fat was associated with learning and memory deficits in women. Therefore, one might ask how a long term feeding of these therapeutic lipid-based foods affects overall health and developmental status of malnourished children. Additionally, these therapeutic foods may not be available to local populations and they are more costly than the locally made complementary foods (Hendricks, 2010).

There was no significant difference in gastric emptying rate between healthy and stunted children (mean half emptying time was around 100 min in both groups). Relatively fast half emptying time may imply good starch digestion in both healthy and stunted children. This could well be related to the similar digestion rates found in both healthy and stunted groups. A comparison between our results and the previous published studies cannot be done because children's age range and test meal composition are different. The ^{13}C-labelled octanoic acid breath test was used by Van Den Driessche et al. (1999) to compare emptying rates in formula fed and breast fed newborns, and by Veereman-Wauters et al. (1996) to assess gastric emptying in preterm infants. The first study showed that gastric emptying is faster in breast-fed infants (half emptying time = 47 min) than in formula fed ones (half emptying time = 65 min). The authors suggested that the fast emptying rate of human milk may be related to a quick transport or digestion of the gastric content. In the second study, the mean half emptying time of preterm infants was 57 min (17 to 100 min).

4.6 Conclusions

Our innovative noninvasive breath test technique using ^{13}C-labelled algal starch and ^{13}C-labelled pre-digested algal starch identified developmental α-amylase insufficiency in weaned healthy and stunted moderately malnourished children in Mali. This method is safe, simple, can be performed at home, the breath samples can be stored for more than a week and analyzed later, and it is relatively inexpensive. This method is concluded to be an appropriate technique to assess pancreatic α-amylase insufficiency in children. Our findings notably revealed that, despite their developmental pancreatic α-

amylase insufficiency, healthy and stunted Malian children were able to adequately digest the different sorghum porridges tested. Moreover, α-amylase insufficiency was correlated to sorghum porridge digestion in the healthy group, meaning that children with greater α-amylase insufficiency were less able to digest porridge starch, whereas even the more α-amylase insufficient children in the stunted group were able to digest well the starch. This implies that weaned stunted children have higher mucosal α-glucosidase activity which allows them to digest the sorghum porridge independently from their developmental pancreatic α-amylase insufficiency.

The untreated shear modified thickened sorghum porridge was digested as well as the α-amylase pre-treated thinned porridge by both healthy and stunted groups. This suggests that thick local porridges with an optimized energy density and maximum starch digestion may be as beneficial to stunted moderately malnourished children as a thinned α-amylase treated energy dense porridge, and would be easier to prepare and give in supplementary feeding programs.

The knowledge generated from this study, and with further investigation, could form the basis of a change in supplementary feeding and nutrition education programs. It holds the promise of more simply, cheaply, and effective digestible and energy dense complementary food that could bring stunted, moderately malnourished children back to nutritional health.

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Table 4.1 Composition of porridges

Designation	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
	Normal porridge	Normal porridge	Normal porridge	Modified porridge	Modified thinned porridge	Modified porridge
Sorghum flour (g)	16	16	16	20	20	20
Water (mL)	200	200	200	200	200	200
Beet sugar (g)	14	14	14	20	20	20
Waxy corn starch (g)	-	-	-	10	10	10
^{13}C substrate (mg)	25 of AS[1]	25 of LDx[2]	500 of LSF[3]	500 of LSF	500 of LSF	50 of OA[4]
Mixing time in a blender (min)	-	-	-	3	3	3
α-amylase (μL)	-	-	-	-	40	-

[1] AS – algal starch; [2] LDx – limit dextrins; [3] LSF – labelled sorghum flour; [4] OA – octanoic acid

Table 4.2 Household demographic characteristics

	Healthy group (n = 15)	Stunted group (n = 31)	Overall (n = 46)
Maternal age	26.7 ± 5.2	28.2 ± 5.1	27.7 ± 5.1
Maternal marital status [n (%)]			
Married	14 (93)	31 (100)	45 (97.8)
Single	1 (6.7)	-	1 (2.17)
Divorced	-	-	-
Widowed	-	-	-
Maternal education [n (%)]			
Illiterate	8 (53.3)	21 (67.7)	29 (63)
Primary	4 (26.7)	7 (22.6)	11 (23.9)
Secondary	1 (6.7)	2 (6.5)	3 (6.5)
College	2 (13.3)	1 (3.23)	3 (6.5)
University	-	-	-
Maternal occupation [n (%)]			
Salaried	2 (13.3)	4 (12.9)	6 (13)
Self-employed	-	-	-
Housewife	10 (66.7)	24 (77.4)	34 (73.9)
student	3 (20)	3 (9.7)	6 (13)

Table 4.3 Subjects' characteristics

	Healthy group	Stunted group	Overall
Number	16	32	48
Age, months	25 ± 3.4	24.3 ± 3.3	25 ± 3.2
Weight, Kg	11.1 ± 0.9	9.6 ± 0.9	10.1 ± 1.1
Height, Cm	86.2 ± 3.4	79.9 ± 2.9	82.0 ± 4.3

Table 4.4 Individual characteristics of all subjects

Subject number	Gender	DOB[1]	Age, months	Height, Cm	Weight, Kg	HAZ[2]	WHZ[3]
1	G[4]	2/6/2011	26	84	10	< 0	< -1.5
2	B[5]	1/4/2011	27	88	12.2	< 1	< 0
3	B	1/18/2011	27	83	9.3	< -2	< -1.5
4	B	4/28/2011	24	84	9.9	< 0	< -1.5
5	G	12/8/2010	28	84.5	10.8	< -2	< 0
6	G	9/11/2010	30	90	11.4	< 0	< -1.5
8	G	3/29/2011	26	81	9.5	< -2	< -1.5
9	B	4/27/2011	25	82	10	< -2	< 0
10	G	5/11/2011	24	78	9.2	<-2	< -1
11	M	1/5/2011	24	88	11.3	< 0	<-1
13	G	5/23/2011	24	83	10.9	< 1	< 0
14	B	3/31/2011	26	86.5	12	< 0	> 0
15	G	8/17/2011	21	76	8.7	< -2	< -1
16	G	8/31/2011	21	75	8.6	< -2	< -1
17	B	12/2/2010	29	90	11	< -1	< -1.5
18	G	11/27/10	30	82	10.3	< -2	< 0
19	G	6/21/2011	25	81	9.4	< -2	< -1
20	B	6/20/2011	24	80	9.8	< -2	< 0
22	G	12/30/10	30	85	11.3	< -1	0
23	G	9/6/2011	21	86.5	11.9	< -1	0
24	B	1/13/2011	29	95	12.7	< 1	< 1
25	B	4/22/2011	26	85.5	10.4	< -1	> 1
26	G	8/1/2011	22	82	10	< 0	< 0
27	G	7/3/2011	23	79.5	9	< -2	< -1.5
28	G	12/25/10	30	82.5	9.7	< -2	< -1
29	B	6/6/2011	25	81	9.8	< -2	< 0
30	G	1/22/2011	29	84	9.5	< -2	-1.5
31	B	1/1/2011	30	85	10.9	-2	0
32	G	10/4/2011	21	85	10.2	< 0	< -1
33	G	8/12/2011	23	77	7.9	< -2	-2
34	B	5/10/2011	26	82	9.3	< -2	< -1.5
35	G	8/30/2011	23	79	10.2	-2	> 0
37	B	1/11/2011	30	83.5	9.3	< -2	-2
38	G	2/28/2011	29	79.5	8.8	< -3	< -1.5
39	B	10/31/11	21	81.5	11.8	< -1	> 0
42	B	8/11/2011	24	82	10.5	< -2	< 0

Table 4.4

43	G	11/15/11	21	77	10.3	< 2	> 0
44	G	12/24/11	19	74	9.1	-3	< 0
45	B	19/03/11	28	82	11.4	< -2	> 0
46	G	11/3/2011	20	85	10.7	< 1	< 0
47	B	2/11/2011	18	76.5	8.8	< -2	< -1
48	G	8/20/2011	24	79	8.5	< -2	-2
49	G	8/16/2011	24	77.5	9.9	< -2	>0
51	B	10/6/2011	22	79	9.1	< -2	<-1
52	G	6/10/2011	26	82.5	10.5	< -2	< 0
53	G	8/22/2011	24	78	10	< -2	>0
54	B	7/5/2011	26	80	11.3	-3	>0
55	G	5/22/2011	25	74	9.3	< -3	>0

[1] DOB – date of birth; [2] HAZ – height for age z-score; [3] WHZ – weight for height z-score; [4] G – Girl; [5] B – Boy. Black color – healthy group; Blue color – stunted group

Table 4.5 Amylase amplification ration for the healthy group

HEALTHY GROUP		
Subject number	Age	Amylase amplification ratio
2	27	1.58
4	24	1.85
11	24	1.20
14	26	2.16
17	29	1.62
24	29	1.35
25	26	1.39
39	21	0.33
1	26	1.95
6	30	1.53
13	24	0.87
22	30	3.17
23	21	0.71
26	22	1.27
32	21	1.24
46	20	2.17

- Sufficient α-amylase amplification (ratio < 1.0, thus full pancreatic maturity has occurred)
- Moderate α-amylase insufficiency (ratio is between 1 to 2)
- Severe α-amylase insufficiency (ratio > 2)

Table 4.6 Amylase amplification ratio for stunted group

STUNTED GROUP		
Subject number	Age	Amylase amplification ratio
3	27	4.62
9	25	1.72
20	24	1.54
29	25	1.77
31	30	1.33
34	26	1.27
37	30	0.82
42	24	0.88
45	28	1.01
47	18	1.26
51	22	1.42
54	26	1.14
5	28	1.27
8	26	0.96
10	24	1.65
15	21	1.96
16	21	0.65
18	30	2.04
19	25	1.72
27	23	0.84
28	30	1.14
30	29	1.51
33	23	1.38
35	23	1.18
38	29	0.91
43	21	1.37
44	19	1.30
48	24	2.78
49	24	1.43
52	26	1.05
53	24	3.27
55	25	1.01

Table 4.7 Viscosity of the untreated and α-amylase pre-treated shear modified sorghum porridges in Days 4 and 5

Shear rate, 1/s	Viscosity, Pa.s	
	Porridge D4	Porridge D5
0.10	226.13	152.68
0.16	161.07	96.22
0.25	110.79	61.34
0.40	76.83	41.48
0.63	54.24	27.80
1.00	38.35	18.20
1.58	27.68	11.90
2.51	20.19	7.91
3.98	14.56	4.57
6.31	10.58	2.67
10.00	7.73	1.56
15.85	5.70	0.82
25.12	4.26	0.38
39.81	3.19	0.21
63.10	2.48	0.11
100.00	2.07	0.06
158.49	1.81	0.04
251.19	1.55	0.03
300.00	1.44	0.03

Porridge D4 = untreated shear modified sorghum porridge in Day 4

Porridge D5 = α-amylase pre-treated shear modified sorghum porridge in Day 5

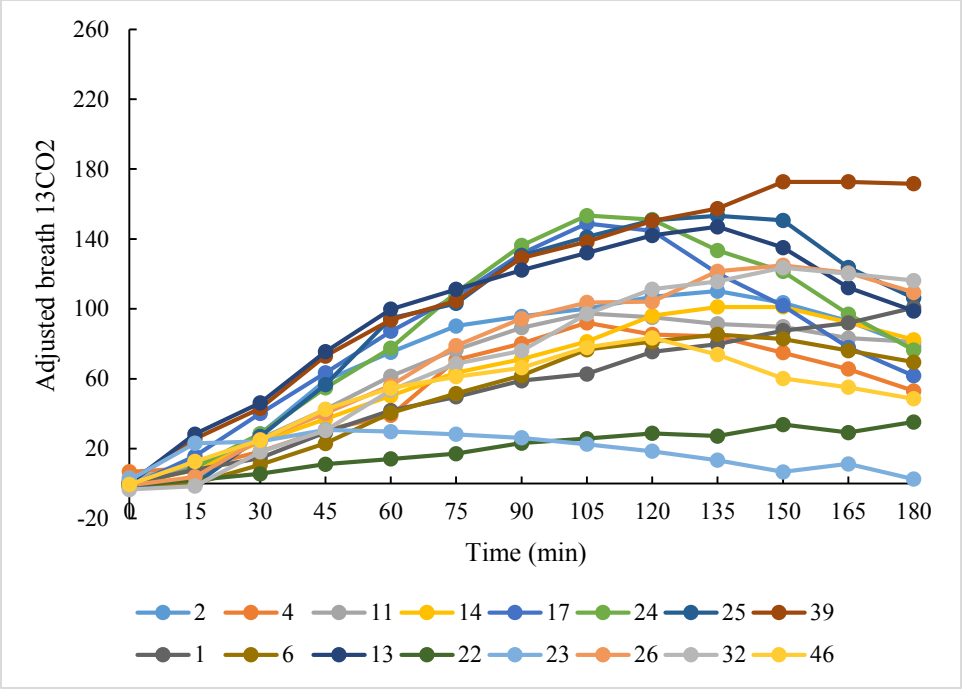

Figure 4.1 Adjusted breath $^{13}CO_2$ levels after digestion of ^{13}C-labelled algal starch in day 1 in 16 healthy children

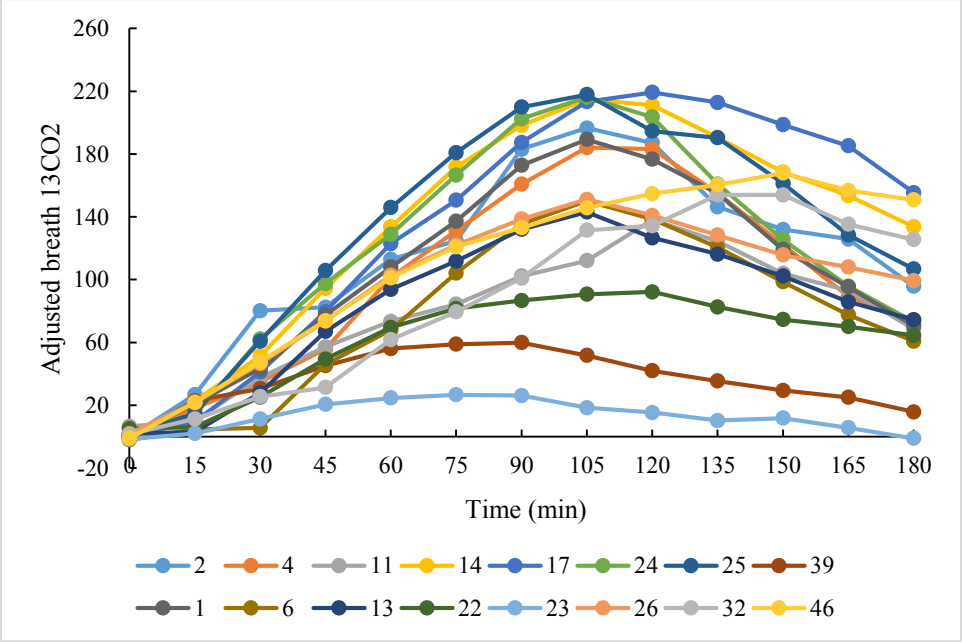

Figure 4.2 Adjusted breath $^{13}CO_2$ levels after digestion of pre-digested ^{13}C-labelled algal starch (LDx) in day 2 in 16 healthy children.

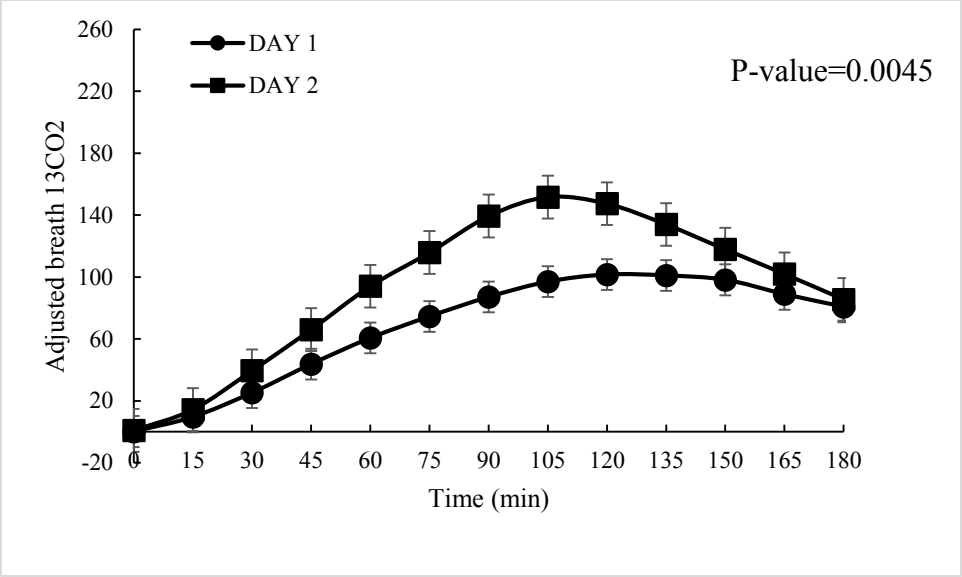

Figure 4.3 Plots of adjusted breath $^{13}CO_2$ levels after digestion of the ^{13}C-labelled algal starch (day 1) and the pre-digested ^{13}C-labelled algal starch (LDx) (day 2) in 16 healthy children. Values are average of the adjusted breath $^{13}CO_2$ levels.

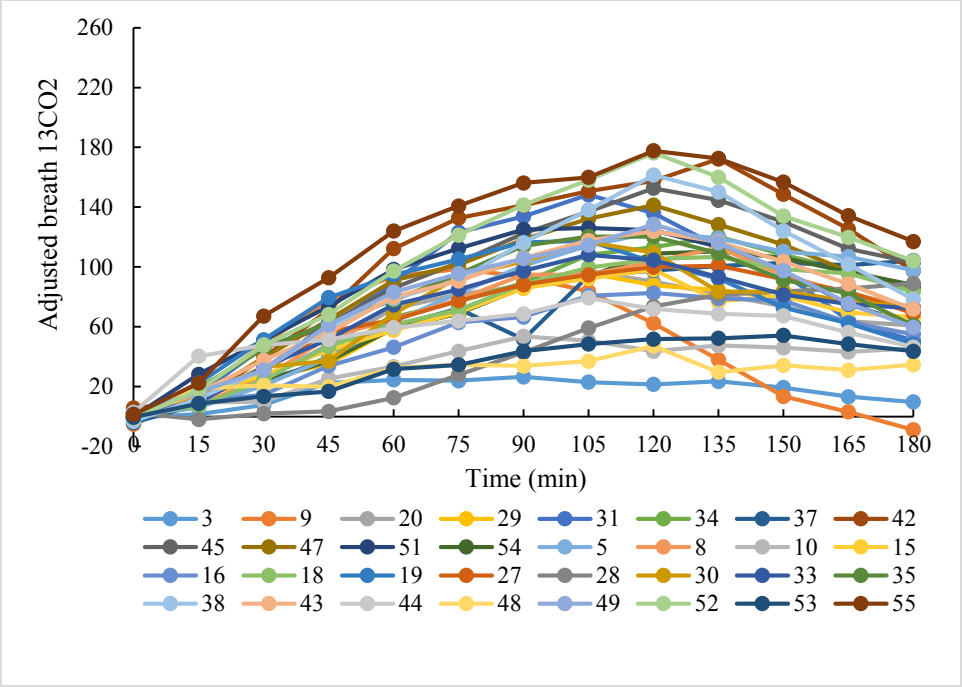

Figure 4.4 Adjusted breath $^{13}CO_2$ levels after digestion of ^{13}C-labelled algal starch in day 1 in 32 stunted children.

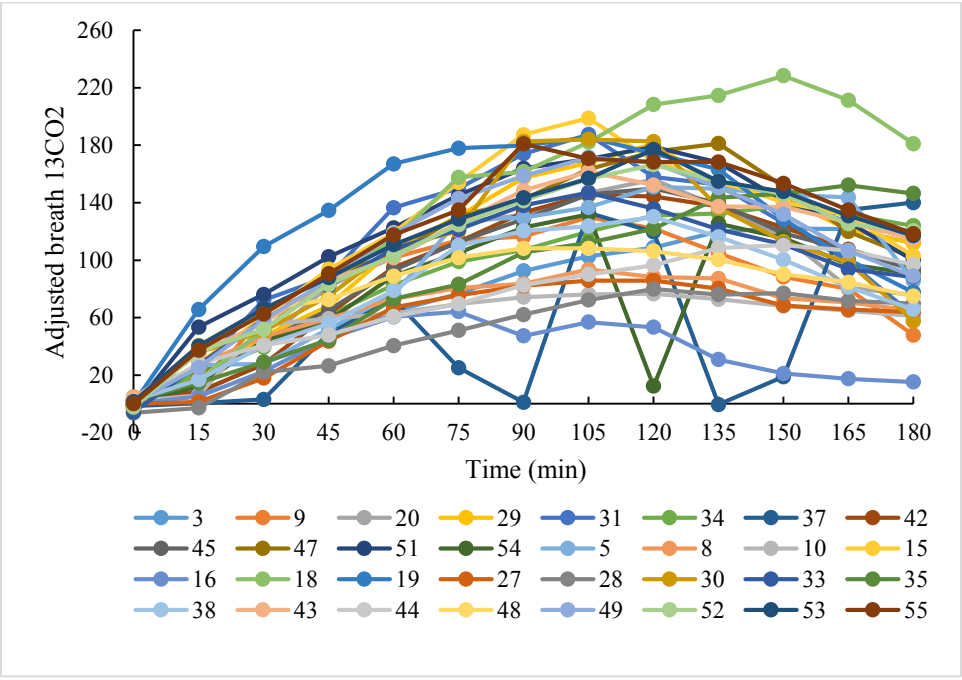

Figure 4.5 Adjusted breath $^{13}CO_2$ levels after digestion of pre-digested ^{13}C-labelled algal starch (LDx) in day 2 in 32 stunted children.

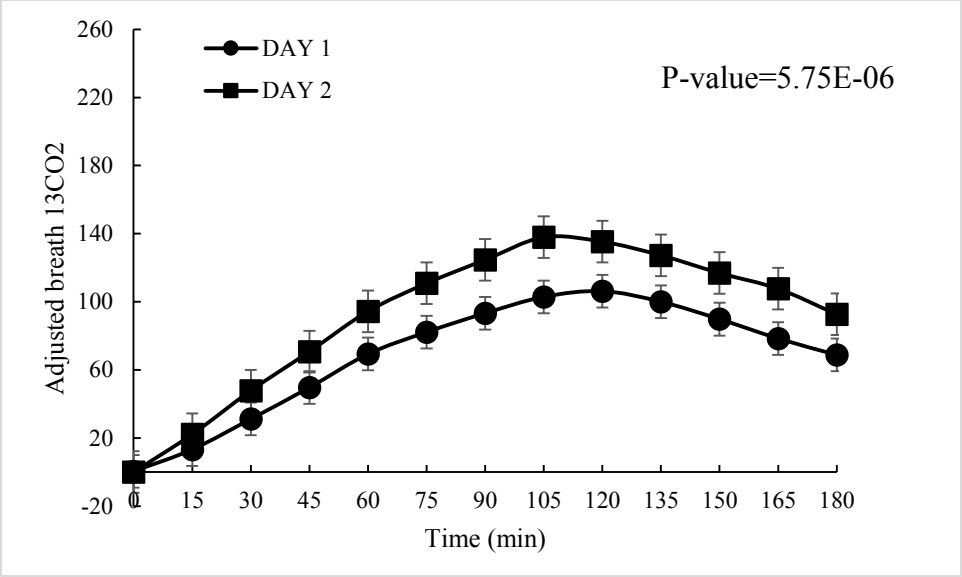

Figure 4.6 Plots of adjusted breath $^{13}CO_2$ levels after digestion of the ^{13}C-labelled algal starch (day 1) and the pre-digested ^{13}C-labelled algal starch (LDx) (day 2) in 32 stunted children. The values are average of the adjusted breath $^{13}CO_2$ levels.

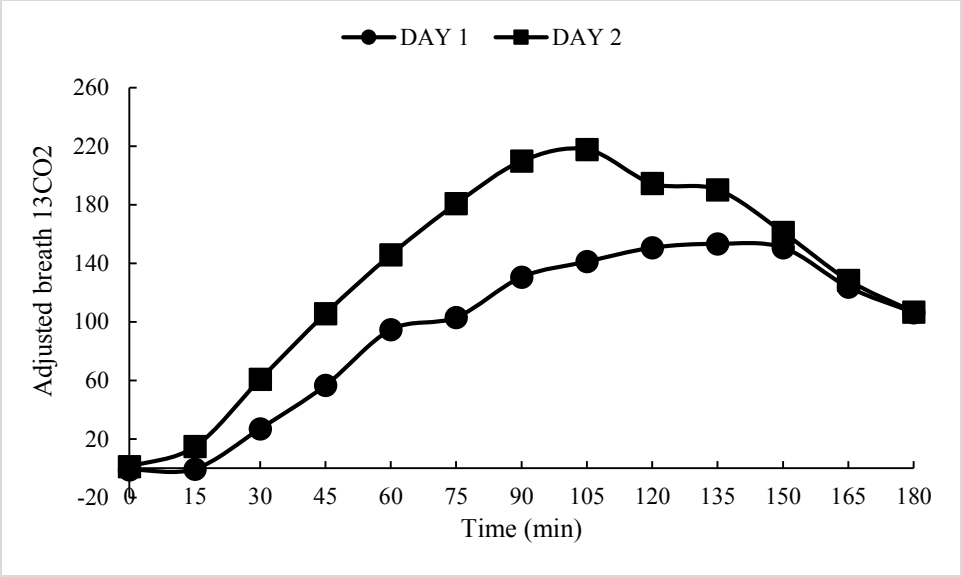

Figure 4.7 Plots of the adjusted breath $^{13}CO_2$ levels after digestion of the ^{13}C-labelled algal starch (day 1) and the pre-digested ^{13}C-labelled algal starch (LDx) (day 2) in healthy child #25.

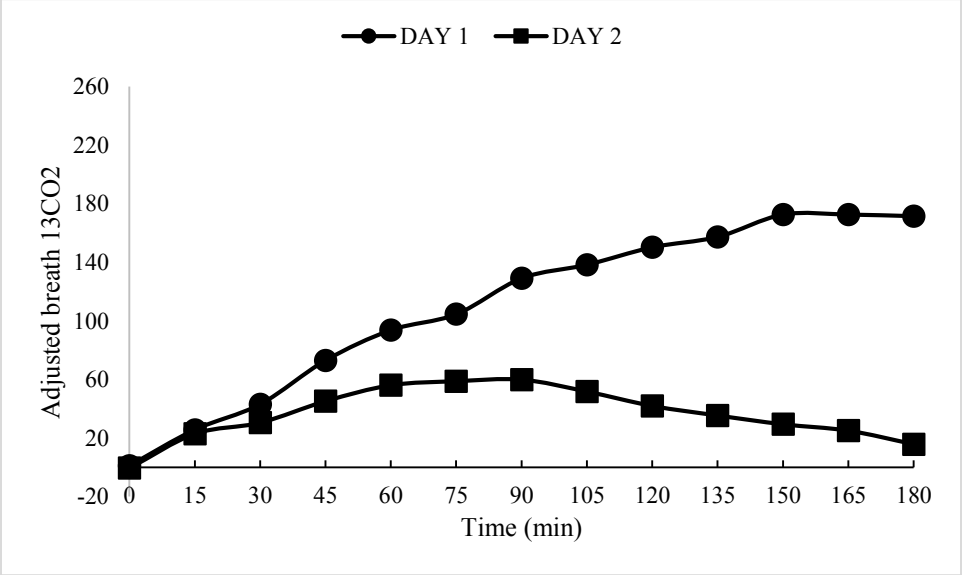

Figure 4.8 Plots of the adjusted breath $^{13}CO_2$ levels after digestion of the ^{13}C-labelled algal starch (day 1) and the pre-digested ^{13}C-labelled algal starch (LDx) (day 2) in healthy child #39.

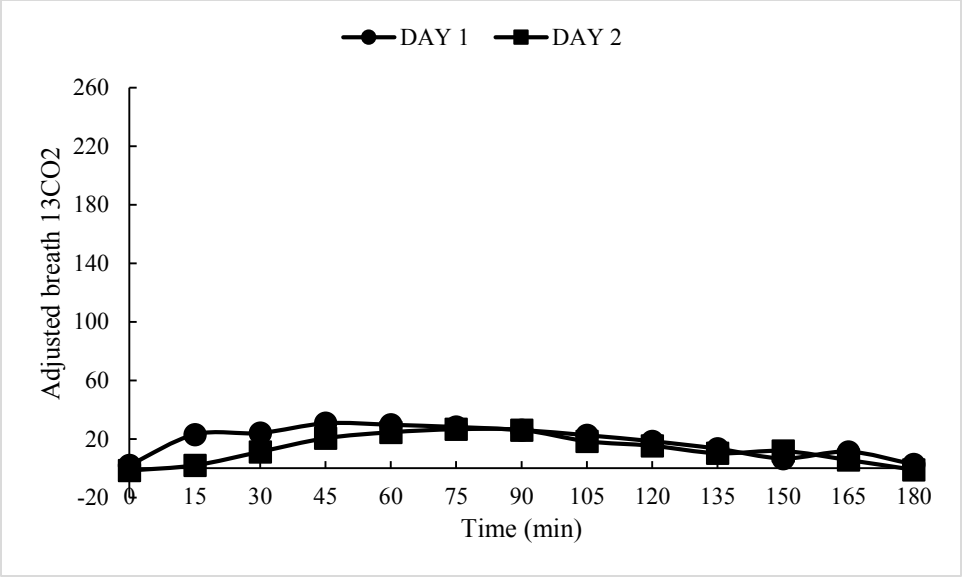

Figure 4.9 Plots of the adjusted breath $^{13}CO_2$ levels after digestion of the ^{13}C-labelled algal starch (day 1) and the pre-digested ^{13}C-labelled algal starch (LDx) (day 2) in healthy child #23.

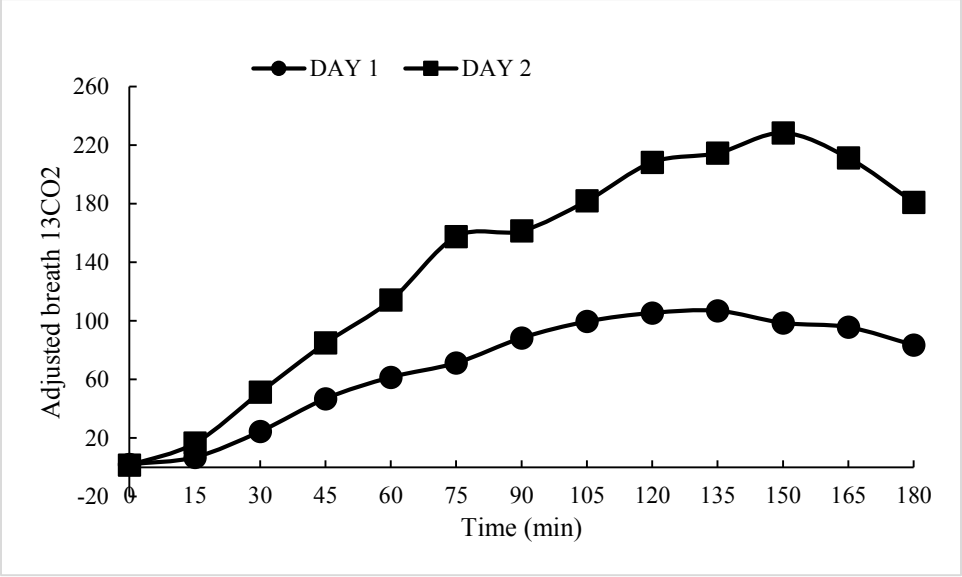

Figure 4.10 Plots the adjusted breath $^{13}CO_2$ levels after digestion of the ^{13}C-labelled algal starch (day 1) and the pre-digested ^{13}C-labelled algal starch (LDx) (day 2) in stunted child #18.

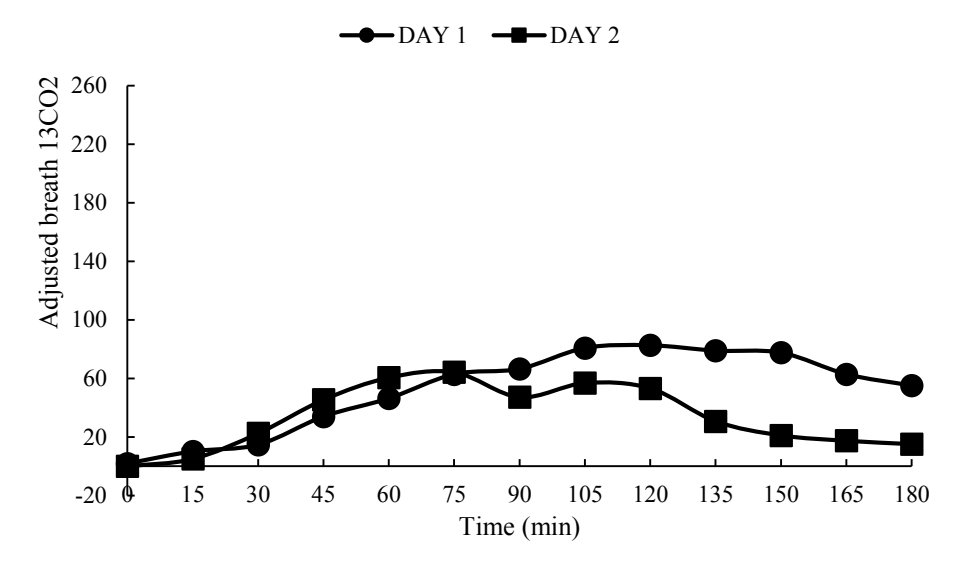

Figure 4.11 Plots of the adjusted breath $^{13}CO_2$ levels after digestion of the ^{13}C-labelled algal starch (day 1) and the pre-digested ^{13}C-labelled algal starch (LDx) (day 2) in stunted child #16.

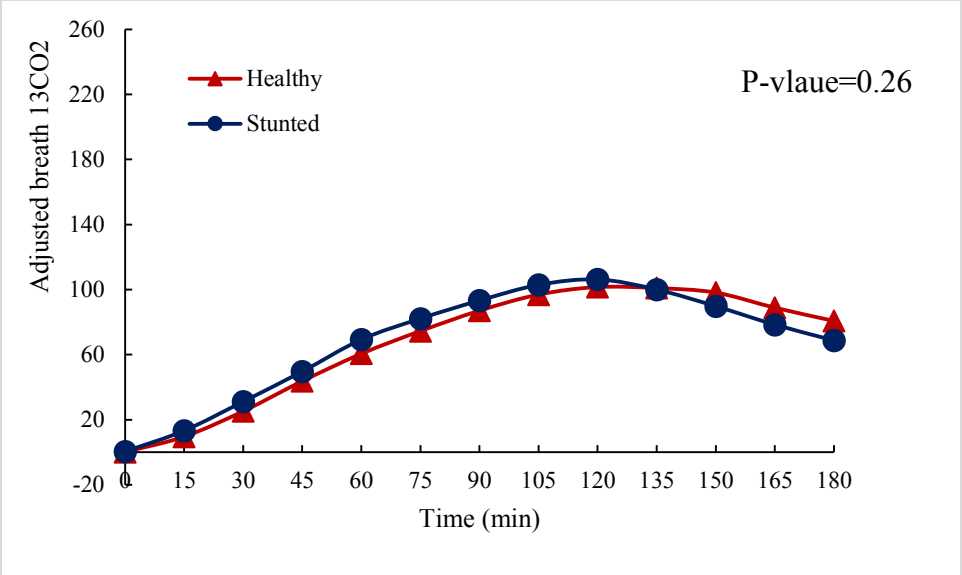

Figure 4.12 Average of adjusted breath $^{13}CO_2$ levels after digestion of ^{13}C-labelled algal starch in day 1 in 48 children (16 healthy – red line and 32 stunted – blue line)

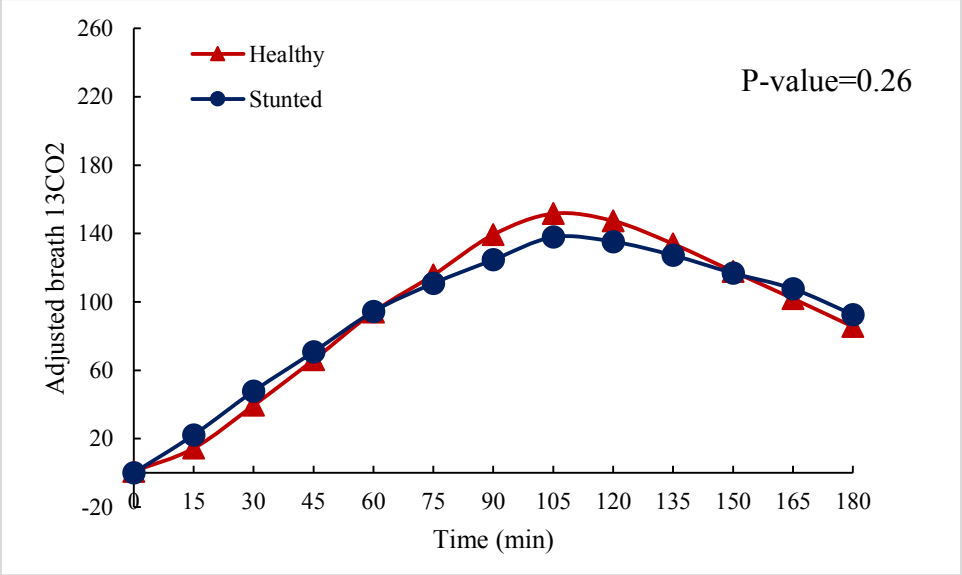

Figure 4.13 Average of adjusted breath $^{13}CO_2$ enrichment levels after digestion of pre-digested ^{13}C-labelled algal starch (LDx) in day 2 in 48 children (16 healthy – red line and 32 stunted – blue line)

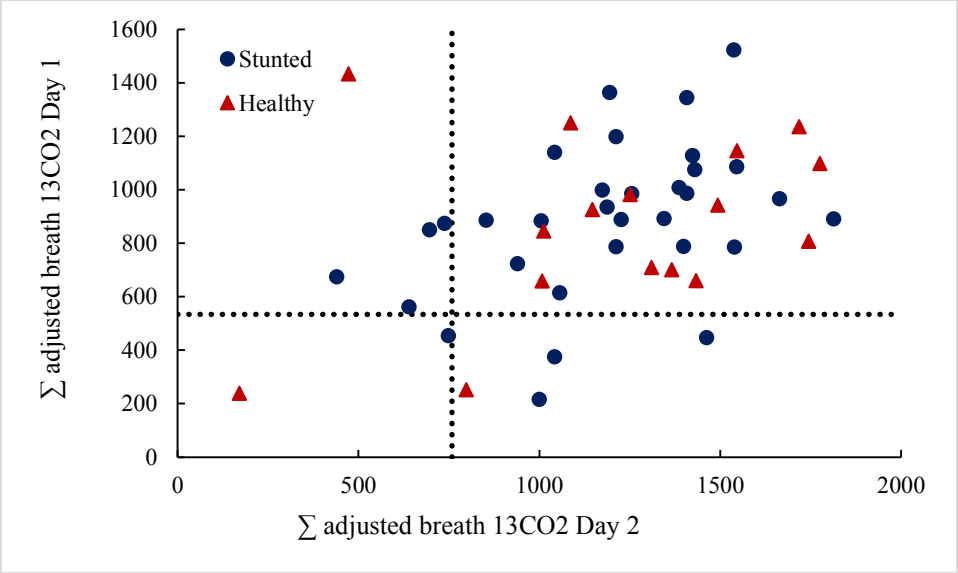

Figure 4.14 Plot of the sums of the adjusted breath $^{13}CO_2$ levels after digestion of pre-digested ^{13}C-labelled algal starch (LDx) and ^{13}C-labelled algal starch in 48 children (16 healthy – red triangles and 32 stunted – blue dots).
Values above the dotted horizontal line digested the ^{13}C-labelled algal starch; values right of the dotted vertical line digested well the pre-digested ^{13}C-labelled algal starch (LDx). The healthy group was used to define the lower reference levels defined as mean – one standard deviation. The dotted lines represent the lower reference lines.

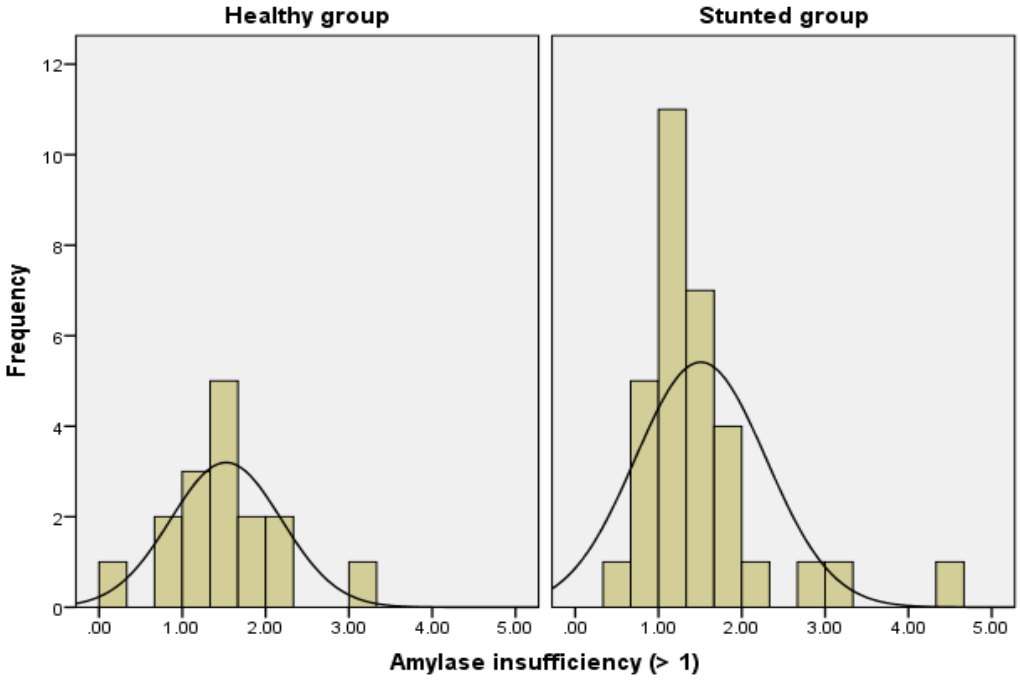

Figure 4.15 Histogram of amylase amplification (D2/D1 > 1 indicates amylase insufficiency)

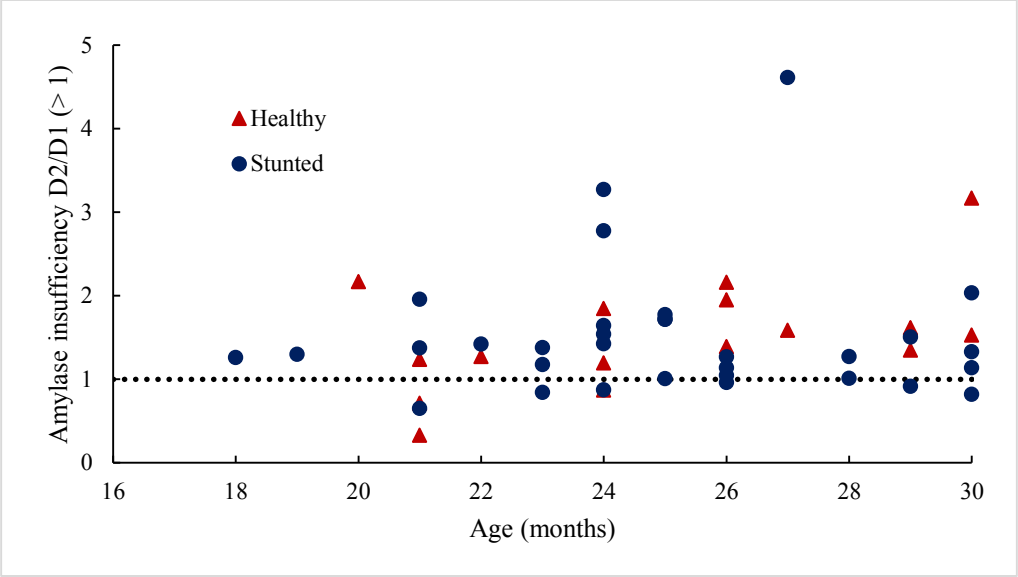

Figure 4.16 Plot of amylase insufficiency vs. age for all children. Values above the dotted horizontal line are α-amylase insufficient.

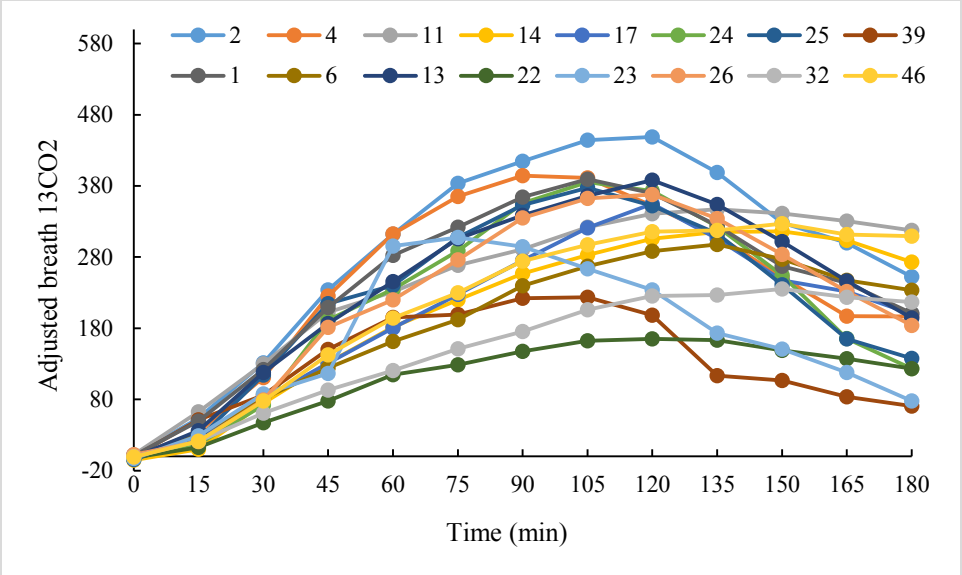

Figure 4.17 Plot of the adjusted breath $^{13}CO_2$ levels after digestion of ^{13}C-labelled sorghum starch in the common sorghum porridge in day 3 in 16 healthy children.

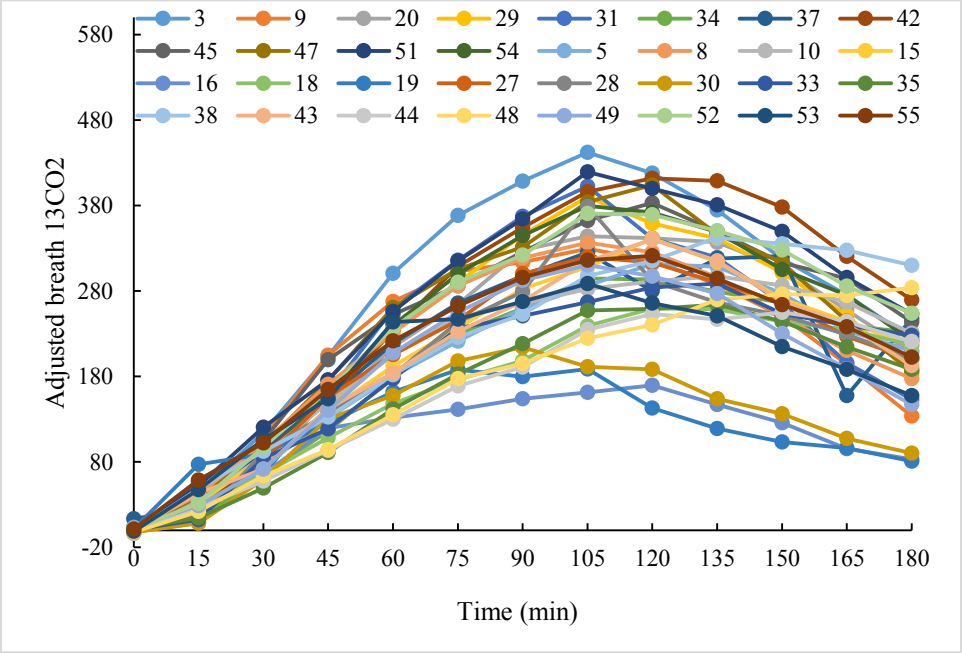

Figure 4.18 Plot of the adjusted breath $^{13}CO_2$ levels after digestion of ^{13}C-labelled sorghum starch in the common sorghum porridge in day 3 in 32 stunted children.

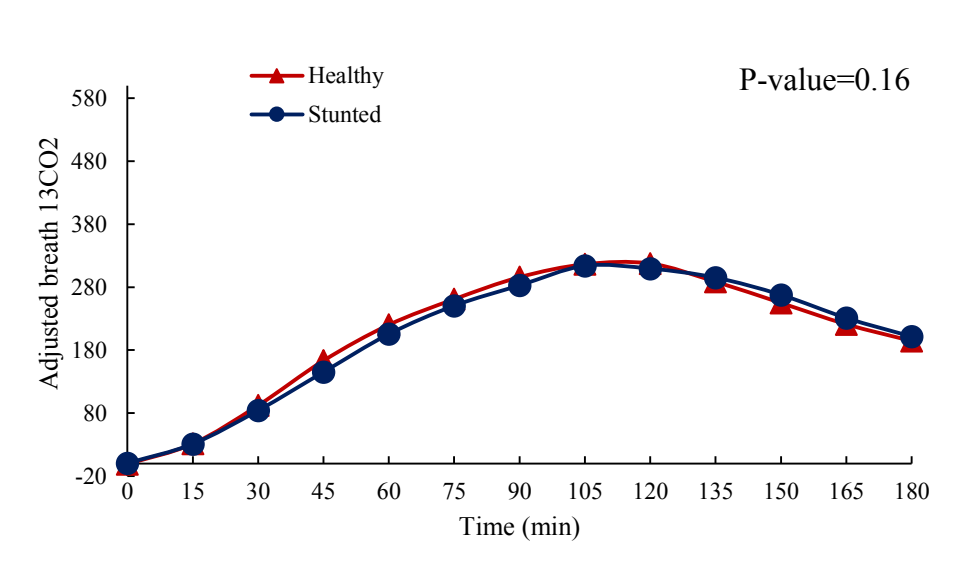

Figure 4.19 Plots of the average adjusted breath $^{13}CO_2$ levels after digestion of ^{13}C-labelled sorghum starch in common sorghum porridge in day 3 in 48 children (16 healthy – red line and 32 stunted – blue line).

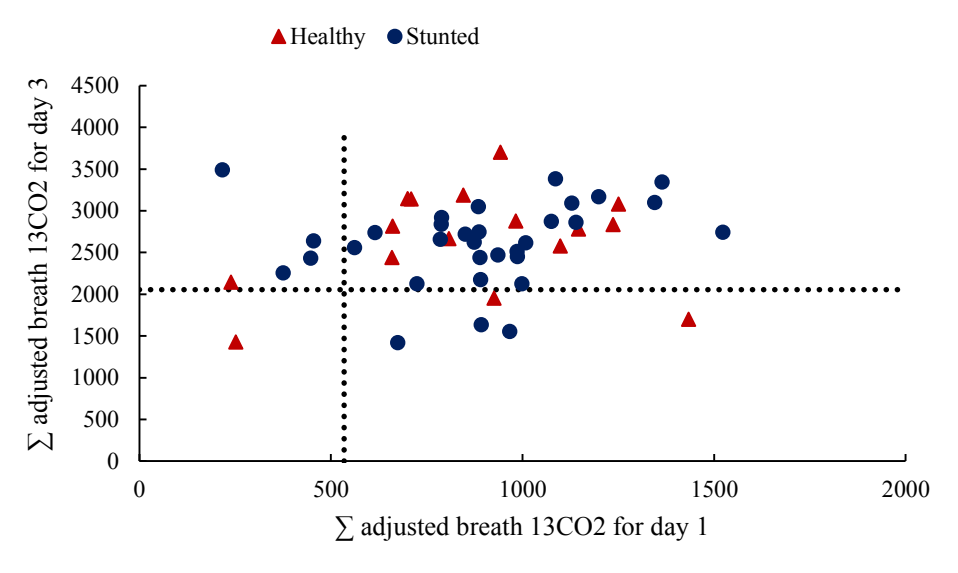

Figure 4.20 Plot of ∑ adjusted breath $^{13}CO_2$ of the ^{13}C-labelled algal starch in Day 1 vs. ∑adjusted breath $^{13}CO_2$ of the common porridge in Day 3 for all children. Values are the sum of the adjusted breath $^{13}CO_2$ levels per infant for Day 1 & 3.
Values above the dotted horizontal line digested the starch from the common porridge; values right of the dotted vertical line indicate digestion of the algal starch. The healthy group was used to define the lower reference levels defined as mean – one standard deviation. The dotted lines represent the lower reference lines.

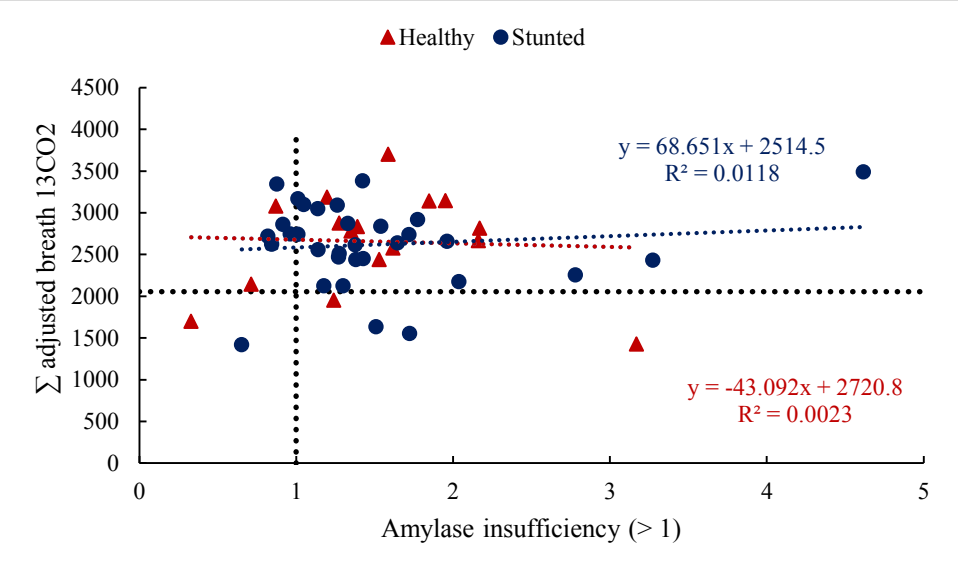

Figure 4.21 Plot of amylase insufficiency (D2/D1) vs. ∑ adjusted breath $^{13}CO_2$ of the common porridge in Day 3 for all children. Values are the sum of the adjusted breath $^{13}CO_2$ levels per infant for Day 3.

Values above the dotted horizontal line digested the starch from the porridge; values right of the dotted vertical line indicate pancreatic α-amylase insufficiency. The healthy group was used to define the lower reference levels defined as mean – one standard deviation. The dotted lines represent the lower reference lines.

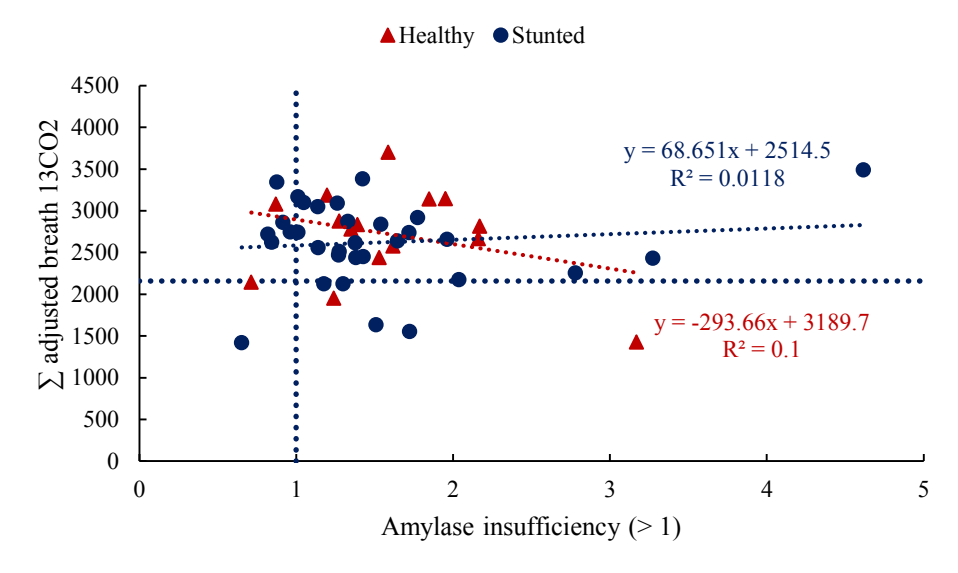

Figure 4.22 Plot of amylase insufficiency (D2/D1) vs. ∑ adjusted breath $^{13}CO_2$ levels of the common porridge in Day 3 for all children without subject #39. Values are the sum of the adjusted breath $^{13}CO_2$ levels per infant for Day 3.
Values above the dotted horizontal line digested the starch from the porridge; values right of the dotted vertical line indicate pancreatic α-amylase insufficiency. The healthy group was used to define the lower reference levels defined as mean – one standard deviation. The dotted lines represent the lower reference lines.

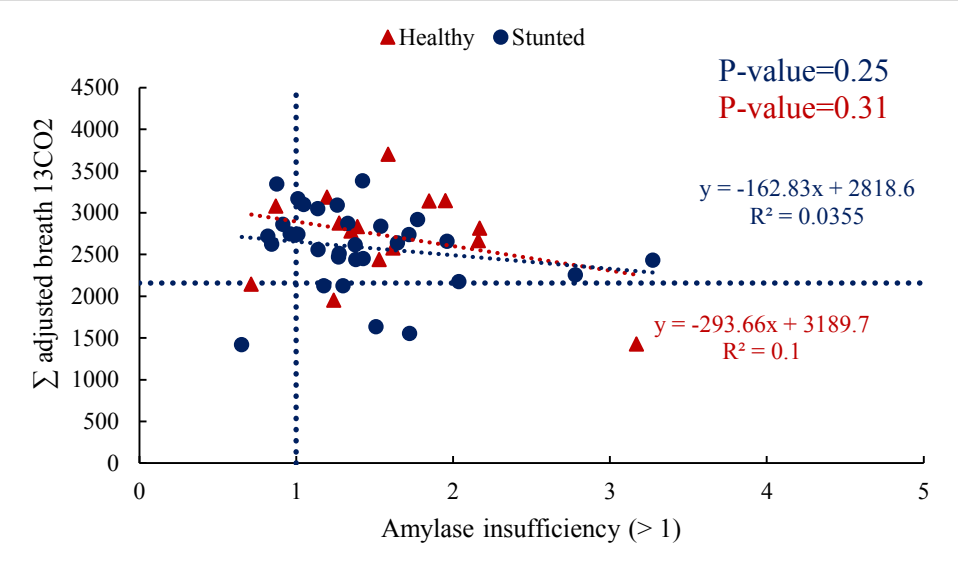

Figure 4.23 Plot of amylase insufficiency (D2/D1) vs. ∑ adjusted breath $^{13}CO_2$ levels of the common porridge in Day 3 for all children without subjects #39 and 3. Values are the sum of the adjusted breath $^{13}CO_2$ levels per infant for Day 3.

Values above the dotted horizontal line digested the starch from the porridge; values right of the dotted vertical line indicate pancreatic α-amylase insufficiency. The healthy group was used to define the lower reference levels defined as mean – one standard deviation. The dotted lines represent the lower reference lines.

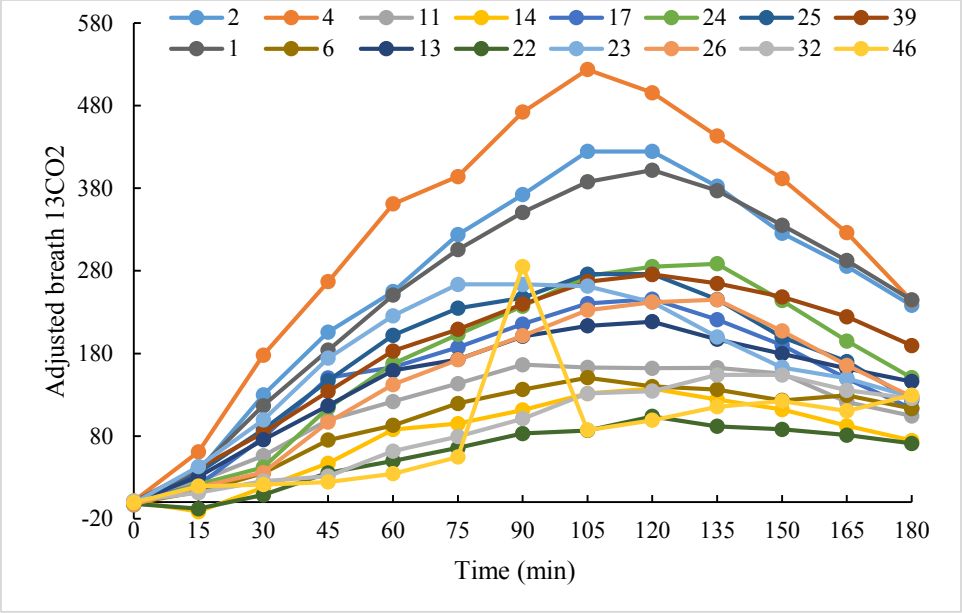

Figure 4.24 Plot of the adjusted breath $^{13}CO_2$ levels after digestion of ^{13}C-labelled sorghum starch in the untreated shear modified sorghum porridge in day 4 in 16 healthy children.

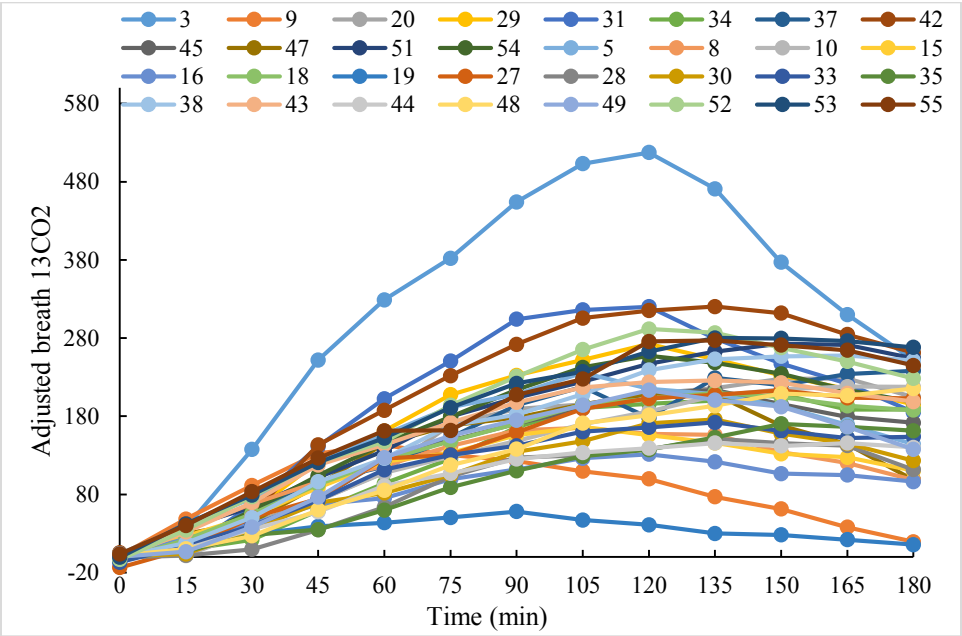

Figure 4.25 : Plot of the adjusted breath $^{13}CO_2$ levels after digestion of ^{13}C-labelled sorghum starch in the untreated shear modified sorghum porridge in day 4 in 32 stunted children.

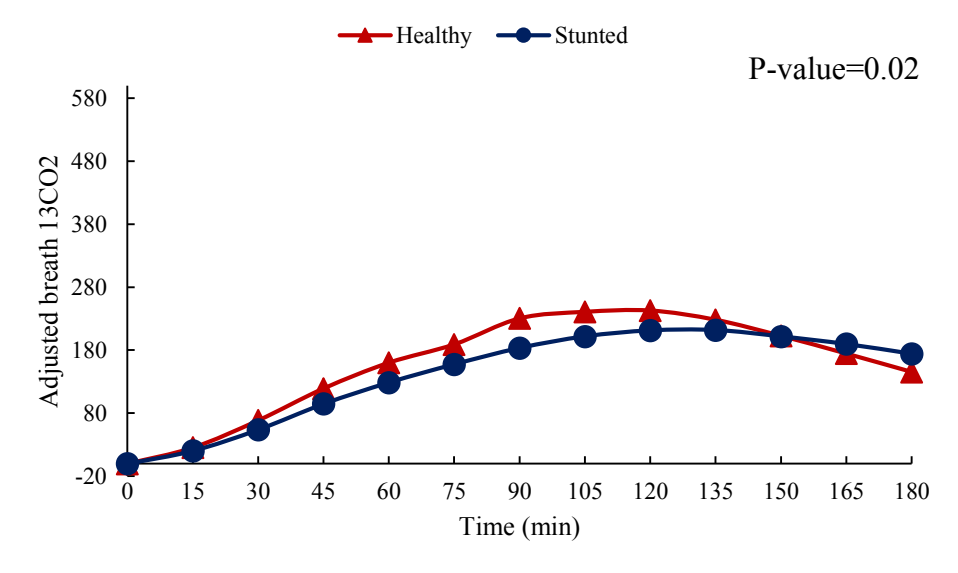

Figure 4.26 Plot of the average adjusted breath $^{13}CO_2$ after digestion of ^{13}C-labelled sorghum starch in the untreated shear modified sorghum porridge in day 4 in 48 children (16 healthy – red line and 32 stunted – blue line).

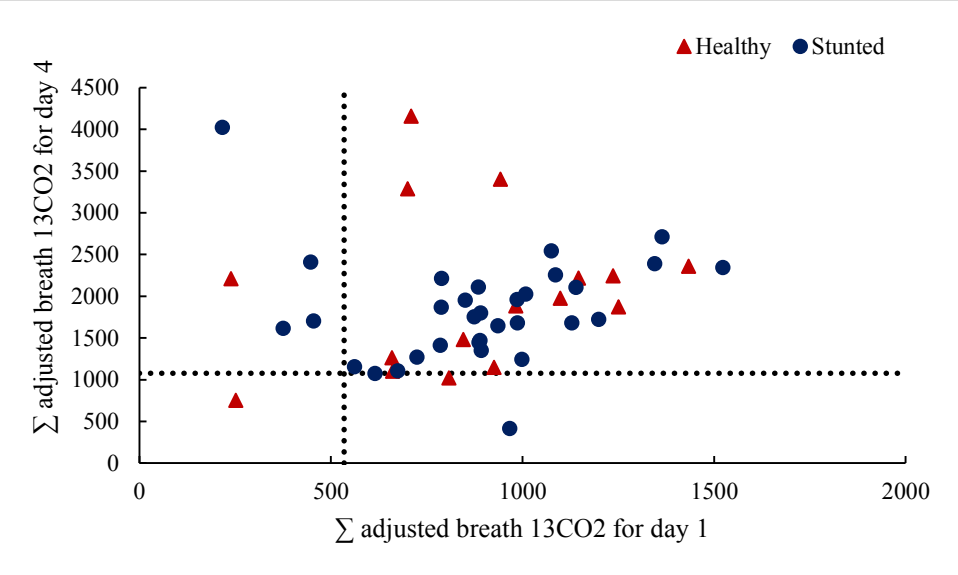

Figure 4.27 Plot of ∑ adjusted breath $^{13}CO_2$ levels of the ^{13}C-labelled algal starch in Day 1 vs. ∑13C breath enrichments of the untreated shear modified sorghum porridge in Day 4 for all children.

Values are the sum of the adjusted breath $^{13}CO_2$ levels per infant for Day 1 & 4. Values above the dotted horizontal line digested the starch from the untreated shear modified porridge; values right of the dotted vertical line indicate digestion of the algal starch. The healthy group was used to define the lower reference levels defined as mean – one standard deviation. The dotted lines represent the lower reference lines.

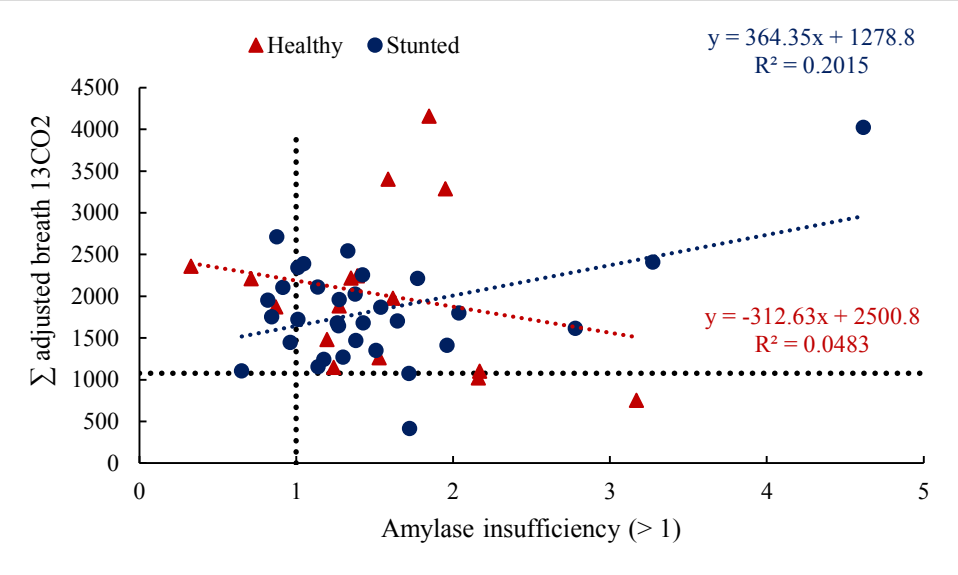

Figure 4.28 : Plot of amylase insufficiency (D2/D1) vs. ∑ adjusted breath $^{13}CO_2$ levels of the untreated shear modified sorghum porridge in Day 4 for all children.

Values are the sum of the adjusted breath $^{13}CO_2$ levels per infant for Day 4. Values above the dotted horizontal line digested the starch from the porridge; values right of the dotted vertical line indicate pancreatic α-amylase insufficiency. The healthy group was used to define the lower reference levels defined as mean – one standard deviation. The dotted lines represent the lower reference lines.

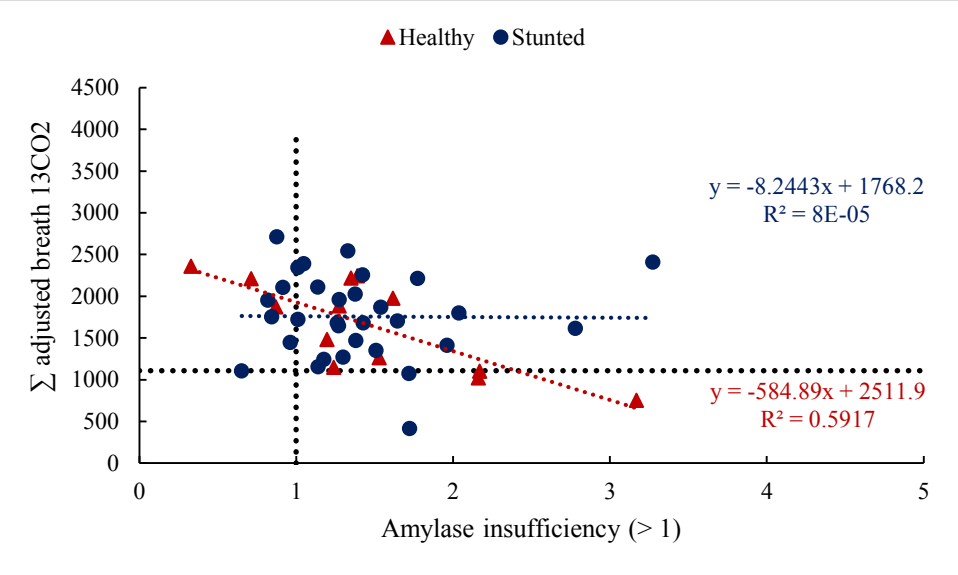

Figure 4.29 Plot of amylase insufficiency (D2/D1) vs. ∑ adjusted breath $^{13}CO_2$ levels of the untreated shear modified sorghum porridge in Day 4 for all children except subjects # 1, 2, 3, 4.

Values are the sum of the adjusted breath $^{13}CO_2$ levels per infant for Day 4. Values above the dotted horizontal line digested the starch from the porridge; values right of the dotted vertical line indicate pancreatic α-amylase insufficiency. The healthy group was used to define the lower reference levels defined as mean – one standard deviation. The dotted lines represent the lower reference lines.

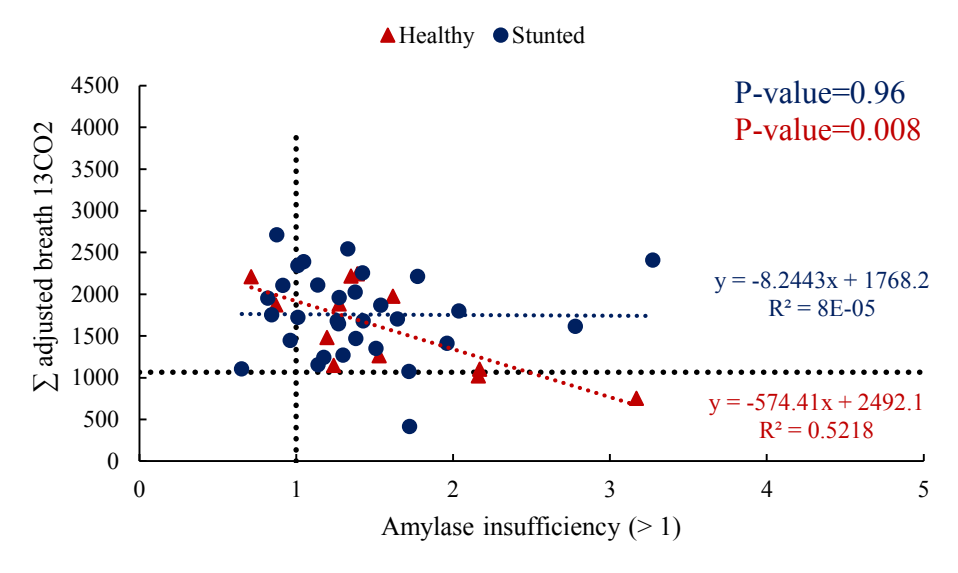

Figure 4.30 Plot of amylase insufficiency (D2/D1) vs. ∑ adjusted breath $^{13}CO_2$ levels of the untreated shear modified sorghum porridge in Day 4 for all children except subjects # 1, 2, 3, 4, 39.

Values are the sum of the adjusted breath $^{13}CO_2$ levels per infant for Day 4. Values above the dotted horizontal line digested the starch from the porridge; values right of the dotted vertical line indicate pancreatic α-amylase insufficiency. The healthy group was used to define the lower reference levels defined as mean – one standard deviation. The dotted lines represent the lower reference lines.

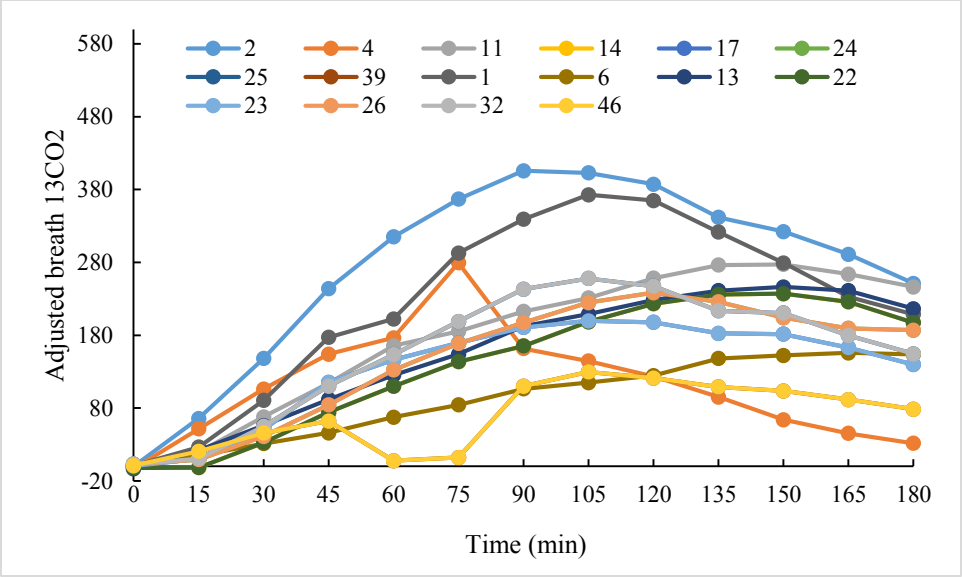

Figure 4.31 Plot of the adjusted breath $^{13}CO_2$ levels after digestion of ^{13}C-labelled sorghum starch in the α-amylase pre-treated shear modified sorghum porridge in day 5 in 16 healthy children.

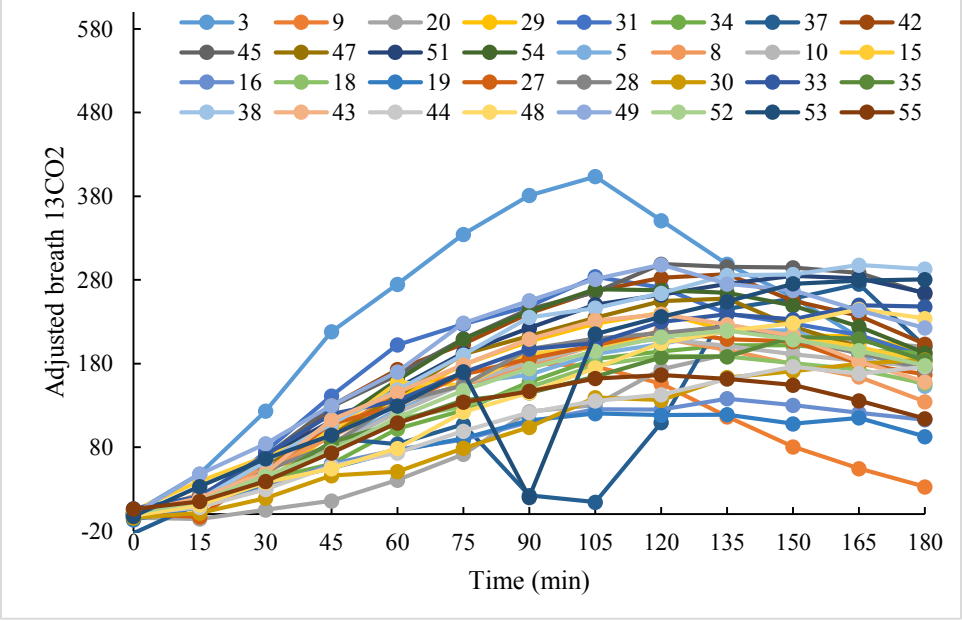

Figure 4.32 Plot of the adjusted breath $^{13}CO_2$ levels after digestion of ^{13}C-labelled sorghum starch in the α-amylase pre-treated shear modified sorghum porridge in day 5 in 32 stunted children.

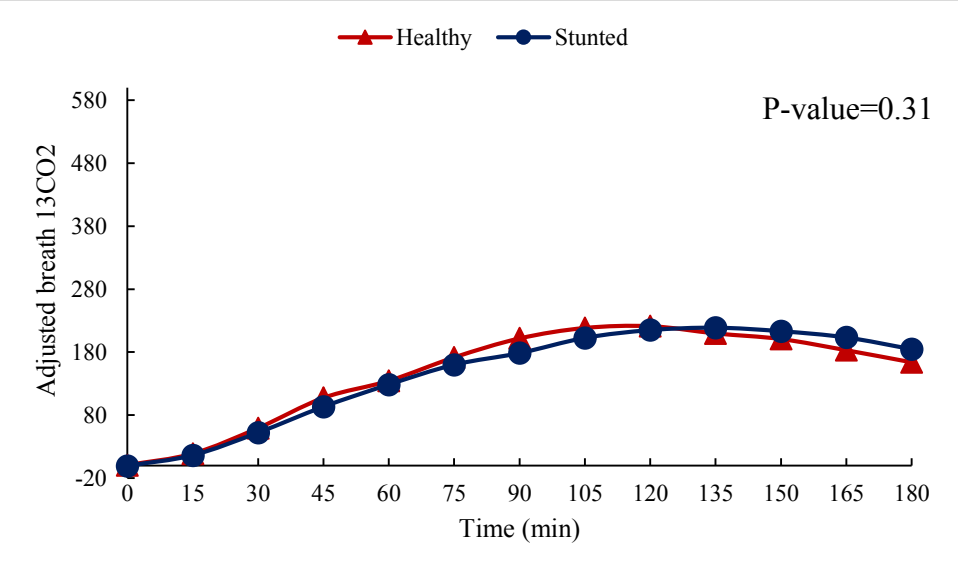

Figure 4.33 Plot of the average adjusted breath $^{13}CO_2$ levels after digestion of ^{13}C-labelled sorghum starch in the α-amylase pre-treated shear modified sorghum porridge in day 5 in 48 children (16 healthy – red line and 32 stunted – blue line).

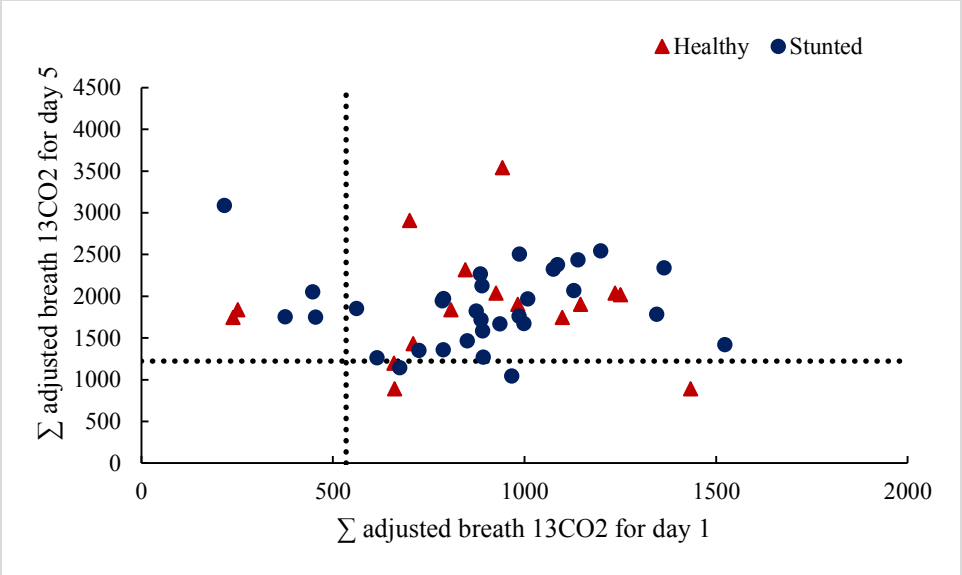

Figure 4.34 Plot of ∑ adjusted breath $^{13}CO_2$ levels of the ^{13}C-labelled algal starch in Day 1 vs. ∑$13CO_2$ breath enrichments of the α-amylase pre-treated shear modified porridge in Day 5 for all children.

Values are the sum of the adjusted breath $^{13}CO_2$ levels per infant for Day 1 & 5. Values above the dotted horizontal line digested the starch from the α-amylase pre-treated shear modified porridge; values right of the dotted vertical line indicate digestion of the algal starch. The healthy group was used to define the lower reference levels defined as mean – one standard deviation. The dotted lines represent the lower reference lines.

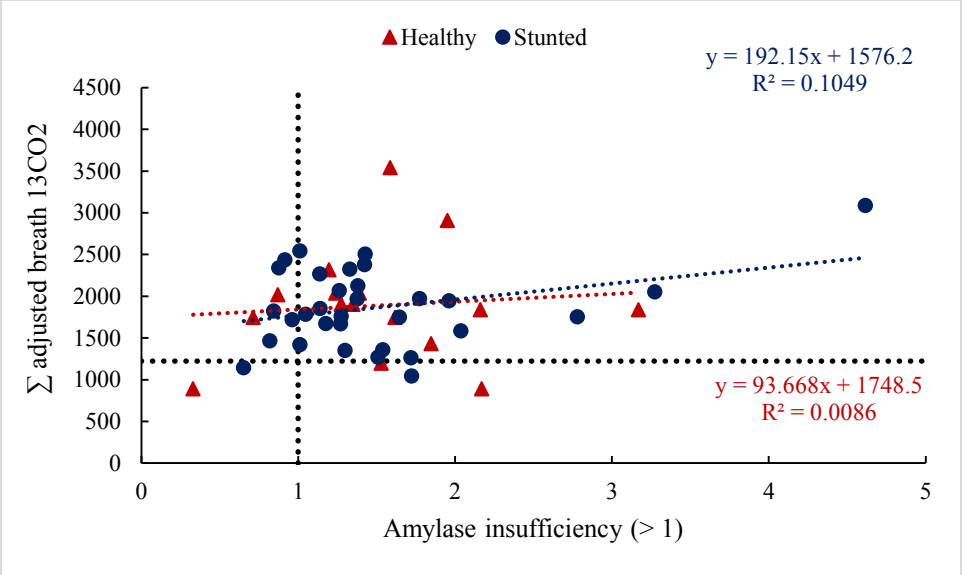

Figure 4.35 Plot of amylase insufficiency (D2/D1) vs. ∑ adjusted breath $^{13}CO_2$ levels of the α-amylase pre-treated shear modified porridge in Day 5 for all children.

Values are the sum of the adjusted breath $^{13}CO_2$ levels per infant for Day 5. Values above the dotted horizontal line digested the starch from the porridge; values right of the dotted vertical line indicate pancreatic α-amylase insufficiency. The healthy group was used to define the lower reference levels defined as mean – one standard deviation. The dotted lines represent the lower reference lines.

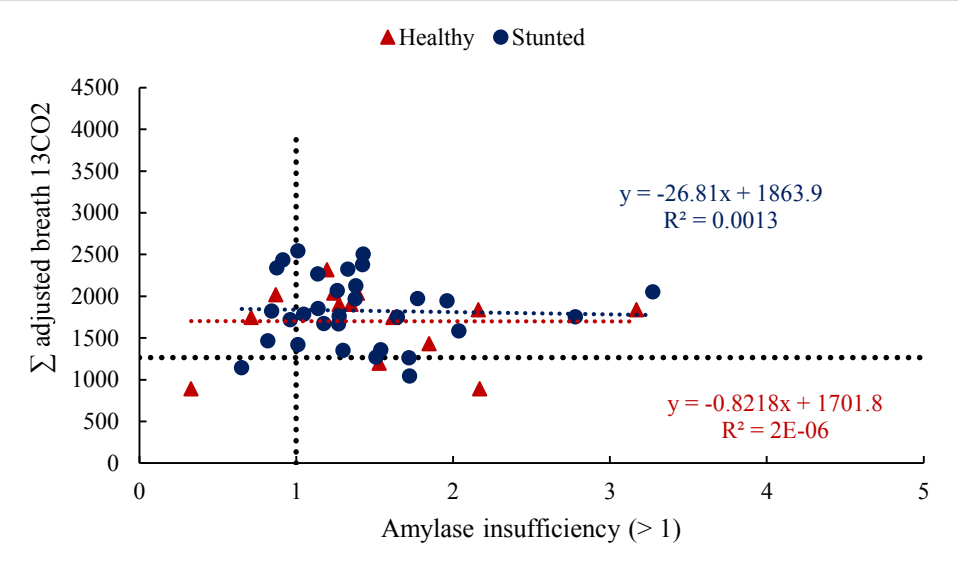

Figure 4.36 Plot of amylase insufficiency (D2/D1) vs. ∑ adjusted breath $^{13}CO_2$ levels of the α-amylase pre-treated shear modified porridge in Day 5 for all children except subjects # 1, 2, 3.

Values are the sum of the adjusted breath $^{13}CO_2$ levels per infant for Day 5. Values above the dotted horizontal line digested the starch from the porridge; values right of the dotted vertical line indicate pancreatic α-amylase insufficiency. The healthy group was used to define the lower reference levels defined as mean – one standard deviation. The dotted lines represent the lower reference lines.

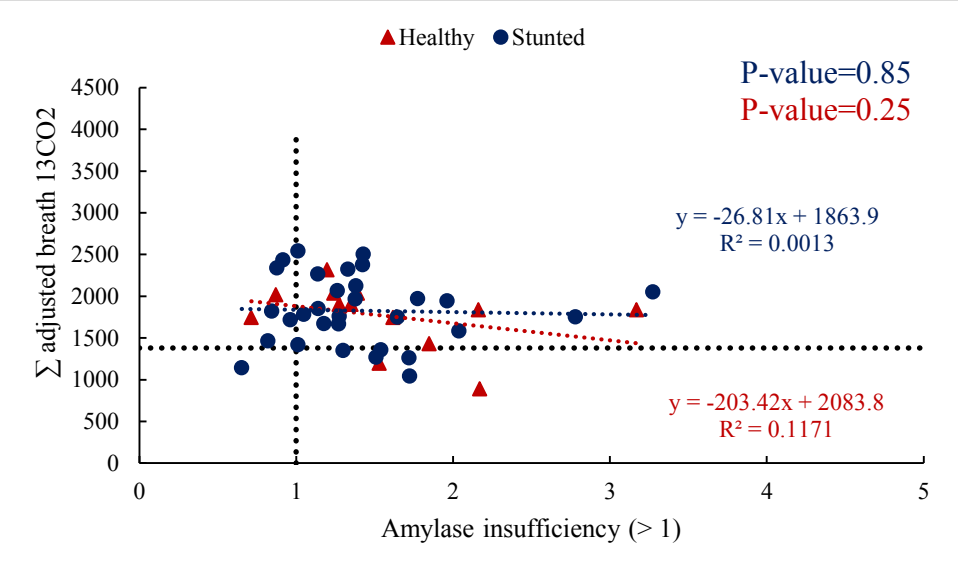

Figure 4.37 Plot of amylase insufficiency (D2/D1) vs. ∑ adjusted breath $^{13}CO_2$ levels of the α-amylase pre-treated shear modified porridge in Day 5 for all children except subjects # 1, 2, 3 & 39.

Values are the sum of the adjusted breath $^{13}CO_2$ levels per infant for Day 5. Values above the dotted horizontal line digested the starch from the porridge; values right of the dotted vertical line indicate pancreatic α-amylase insufficiency. The healthy group was used to define the lower reference levels defined as mean – one standard deviation. The dotted lines represent the lower reference lines.

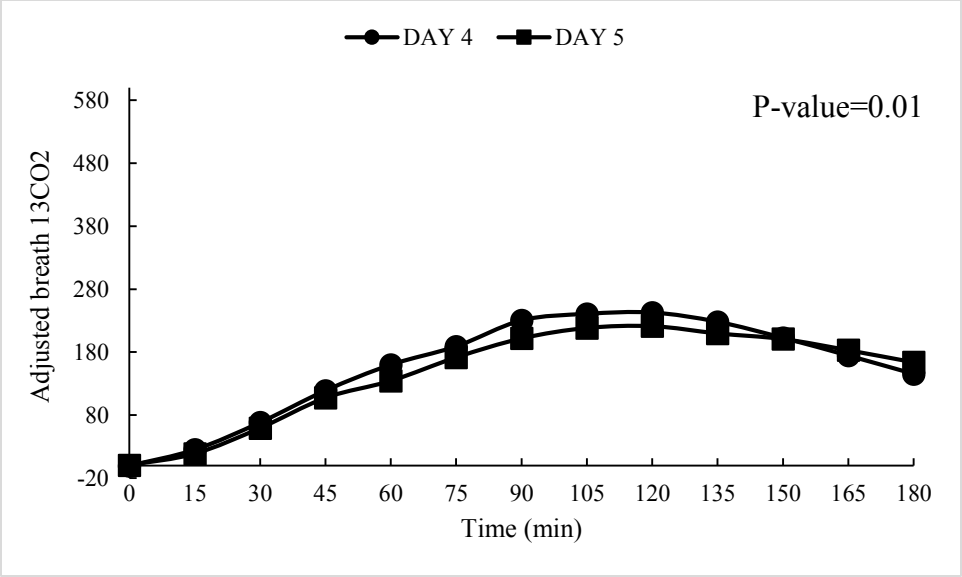

Figure 4.38 Plots of the average adjusted breath $^{13}CO_2$ levels after digestion of the^{13} C labelled sorghum starch in the untreated shear modified sorghum porridge (thick) in day 4, and the α-amylase pre-treated shear modified sorghum porridge (thin) in day 5 in 16 healthy children.

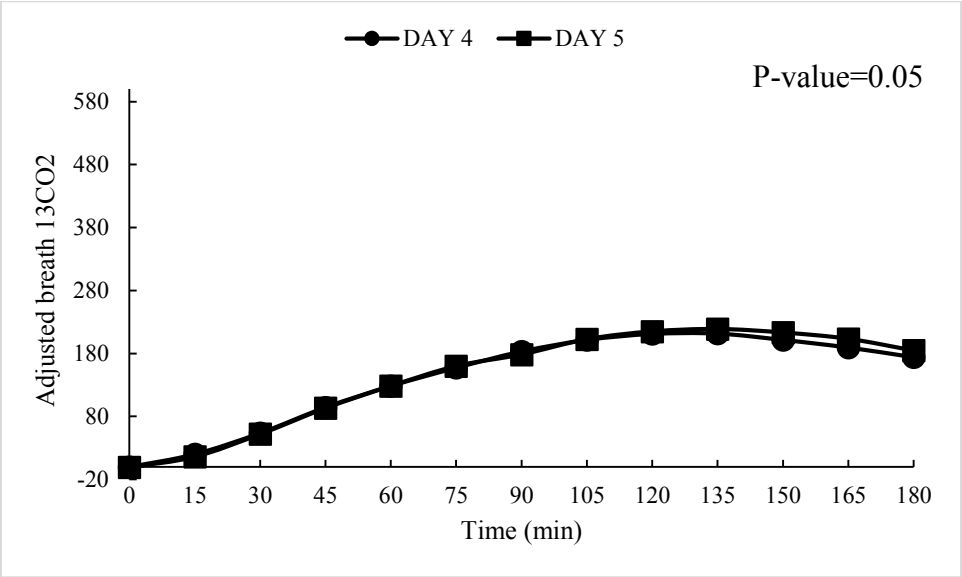

Figure 4.39 Plots of the average adjusted breath $^{13}CO_2$ levels after digestion of the ^{13}C-labelled sorghum starch in the untreated shear modified sorghum porridge (thick) in Day 4, and the α-amylase pre-treated shear modified sorghum porridge (thin) in Day 5 in 32 stunted children.

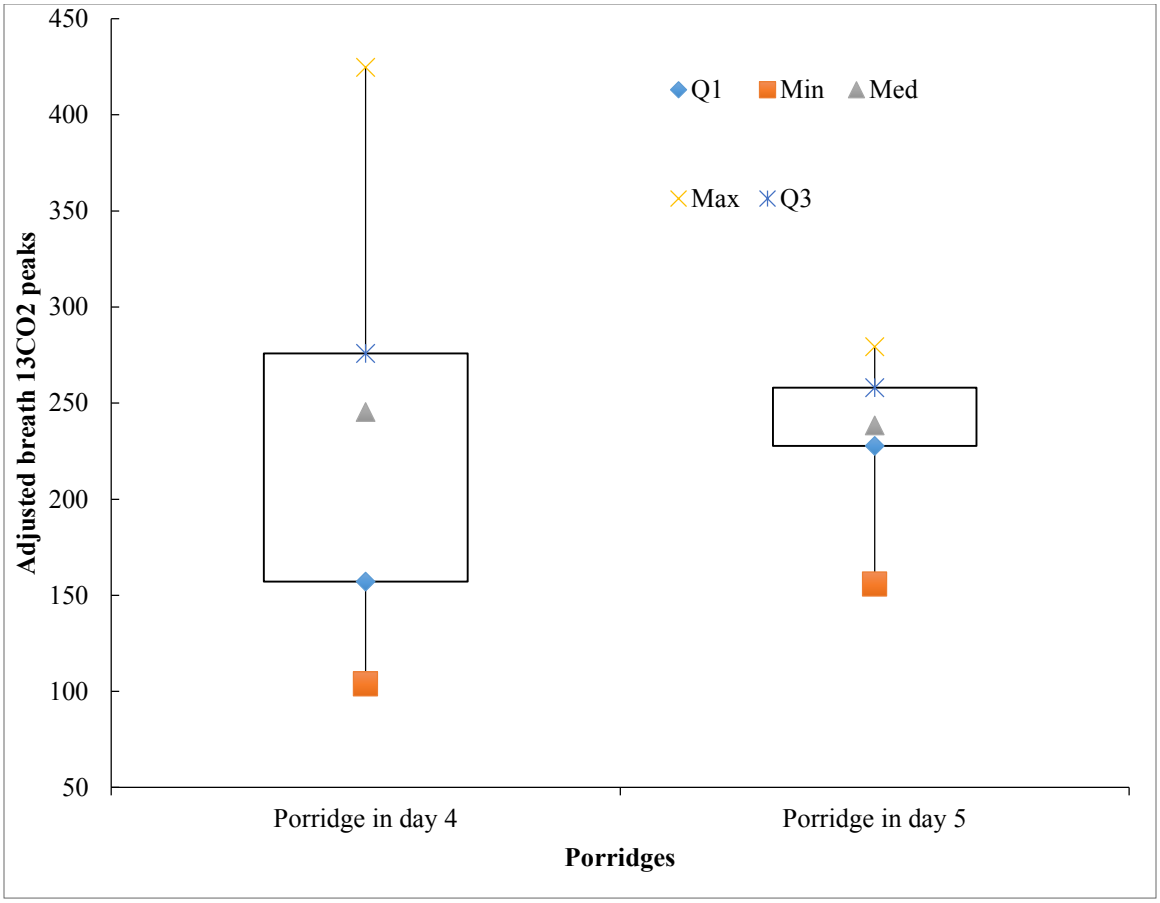

Figure 4.40 Distribution of the adjusted breath $^{13}CO_2$ peaks after ingestion of the untreated shear modified sorghum porridge in D4, and the α-amylase pretreated shear modified sorghum porridge in D5 in the healthy group (y-axis, indicator of starch digestibility, higher values indicate higher digestibility).

Porridge in day 4 = Untreated shear modified sorghum porridge in day 4 n = 14. Porridge in day 5 = α-amylase pretreated shear modified sorghum porridge in day 5 n = 12.

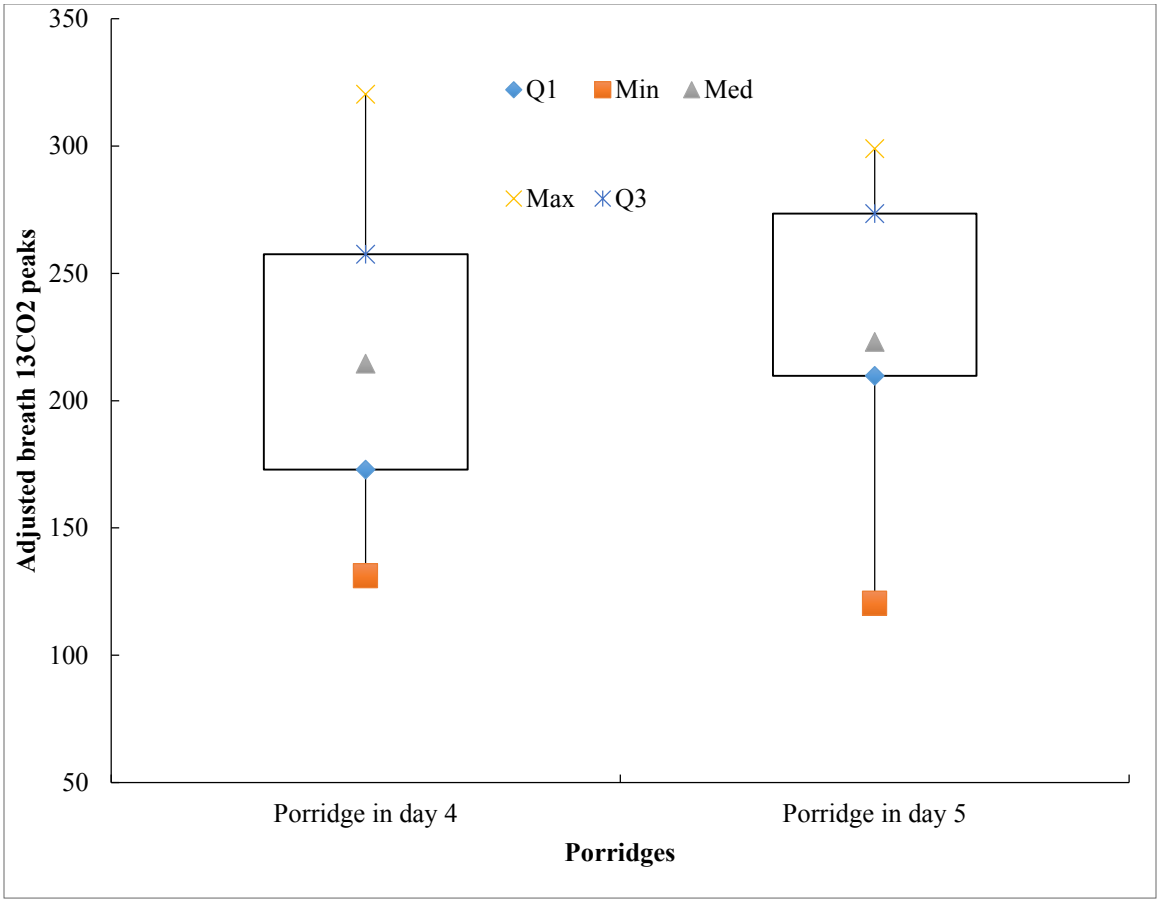

Figure 4.41 Distribution of the adjusted breath $^{13}CO_2$ peaks after ingestion of the untreated shear modified sorghum porridge in D4, and the α-amylase pretreated shear modified sorghum porridge in D5 in the stunted group (y-axis, indicator of starch digestibility, higher values indicate higher digestibility).

Porridge in day 4 = Untreated shear modified sorghum porridge in day 4 n = 30. Porridge in day 5 = α-amylase pretreated shear modified sorghum porridge in day 5 n = 30.

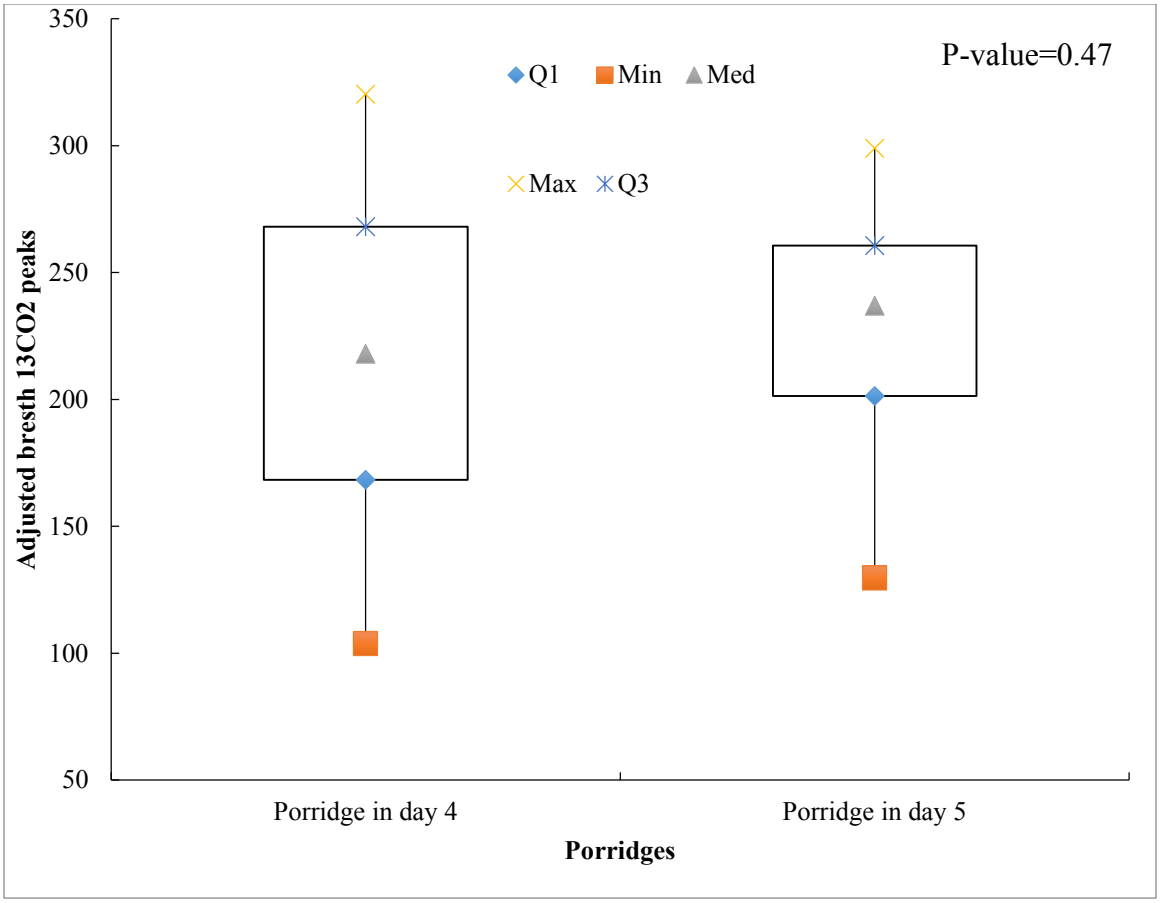

Figure 4.42 Distribution of the adjusted breath $^{13}CO_2$ peaks after ingestion of the untreated shear modified sorghum porridge in D4, and the α-amylase pretreated shear modified sorghum porridge in D5 in all children (y-axis, indicator of starch digestibility, higher values indicate higher digestibility).

Porridge in day 4 = Untreated shear modified sorghum porridge in day 4 n = 43. Porridge in day 5 = α-amylase pretreated shear modified sorghum porridge in day 5 n = 44.

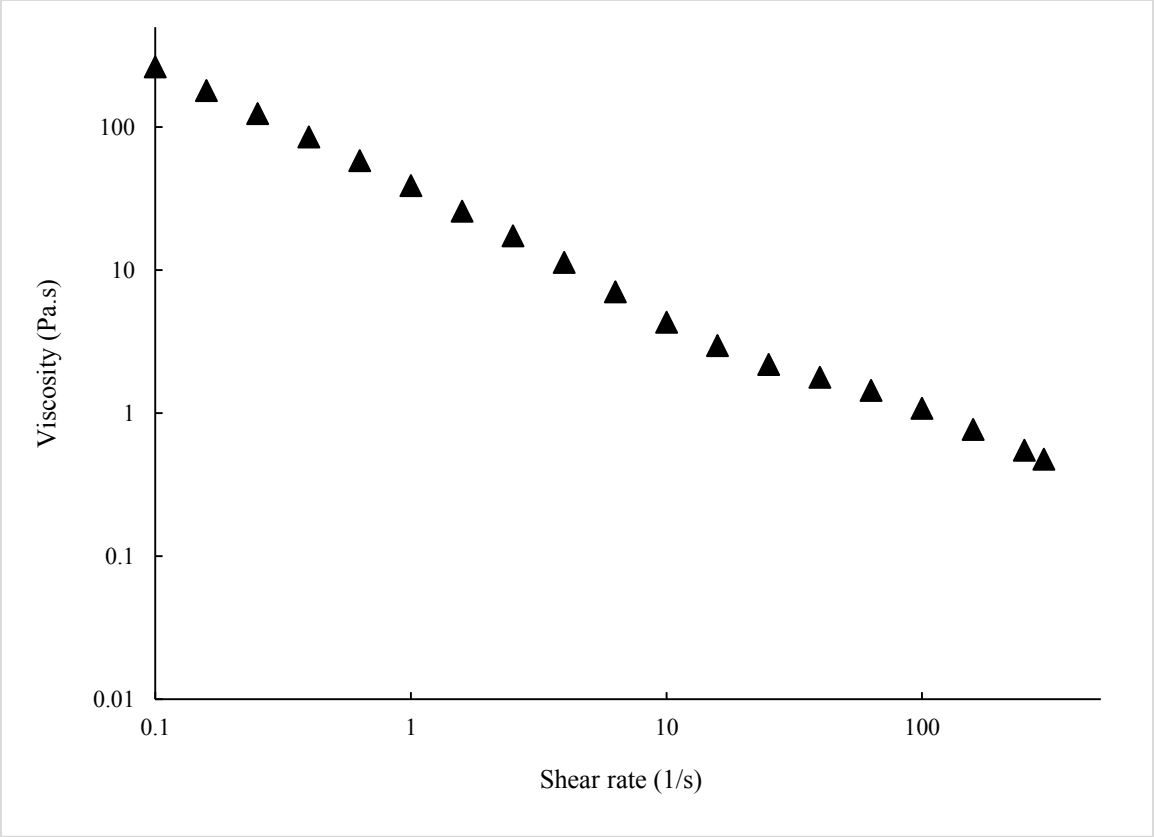

Figure 4.43 Steady shear measurement of the common porridge in Day 3.

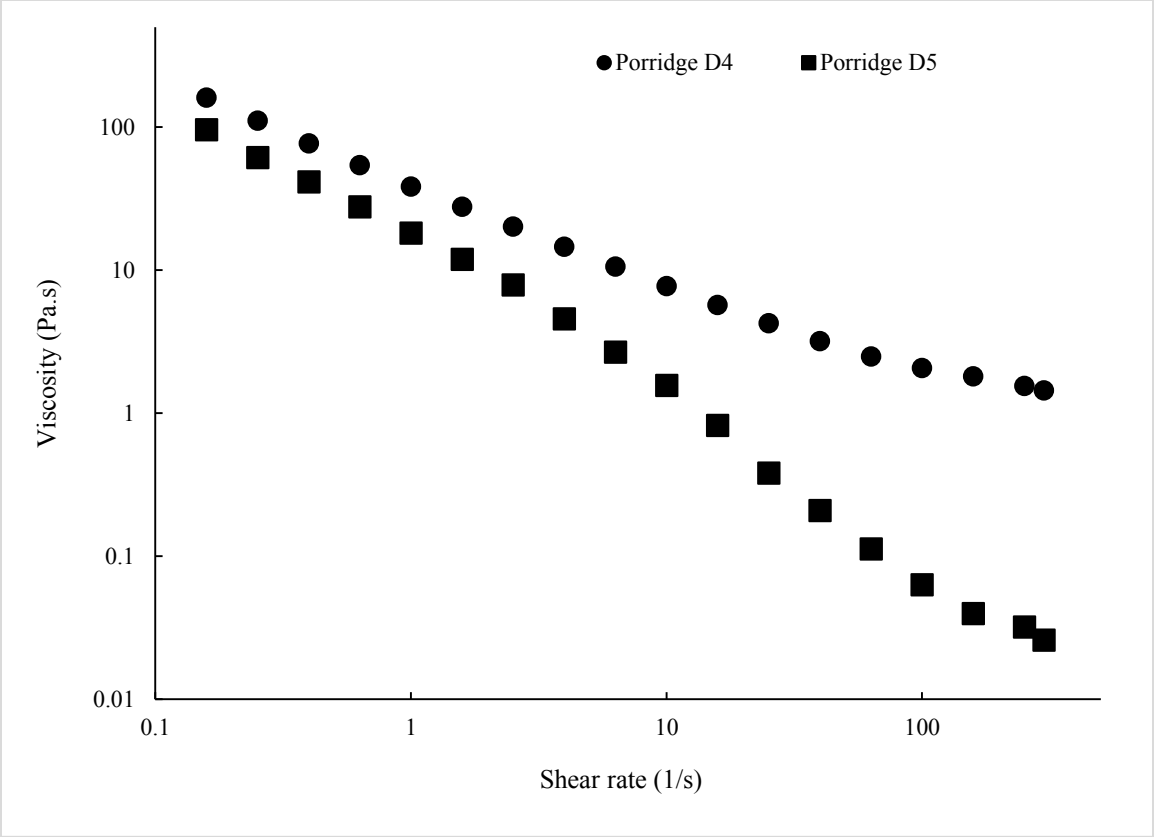

Figure 4.44 Steady shear measurement of the untreated shear modified sorghum porridge in Day 4 (porridge D4), and the α-amylase pretreated shear modified sorghum porridge in Day 5 (porridge D5).

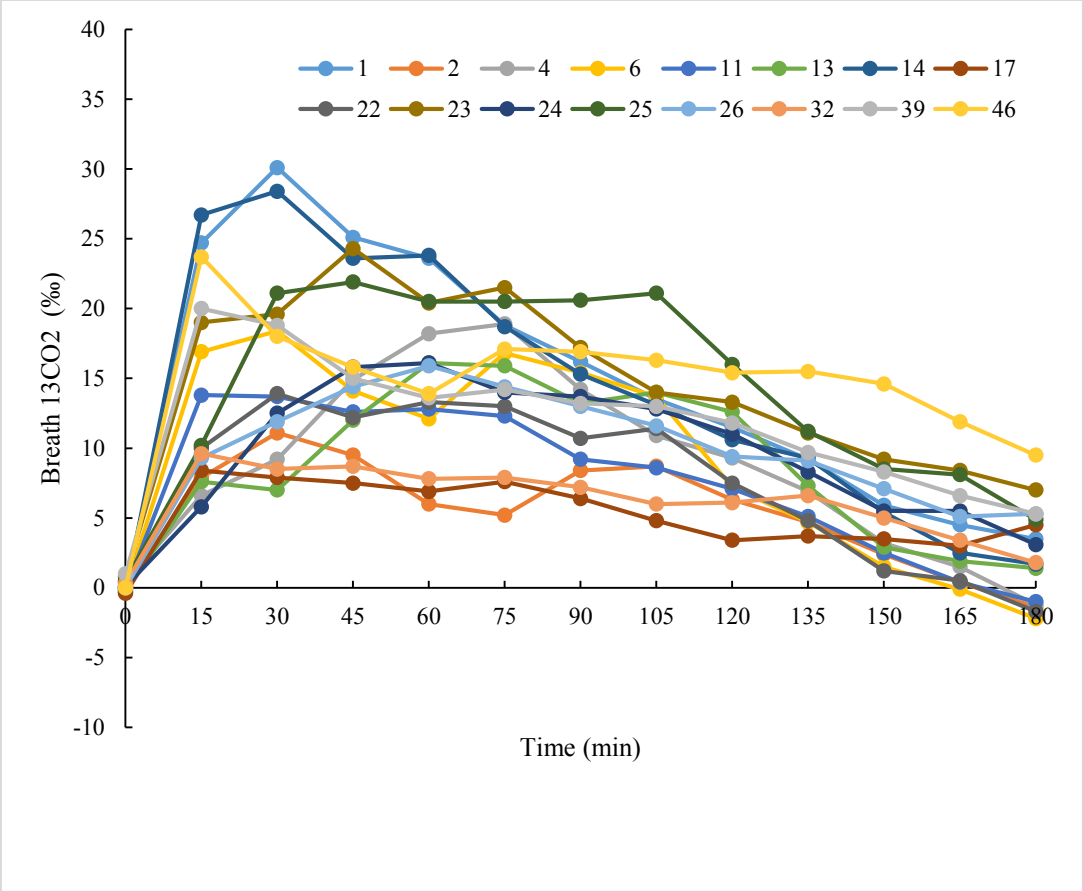

Figure 4.45 Plot of the breath $^{13}CO_2$ levels after emptying of the untreated shear modified sorghum porridge in day 6 in 16 healthy children.

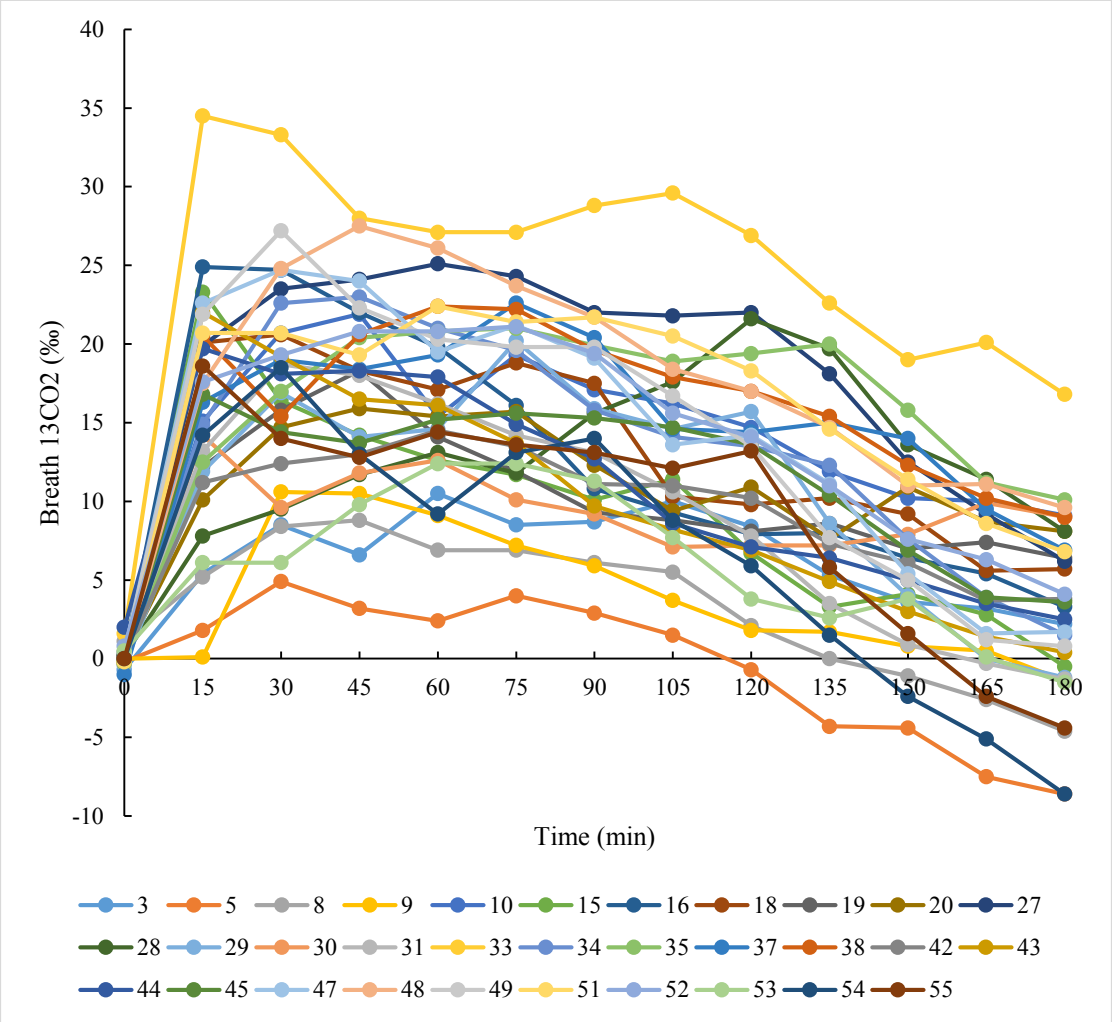

Figure 4.46 Plot of the breath $^{13}CO_2$ levels after emptying of the untreated shear modified sorghum porridge in day 6 in 32 stunted children.

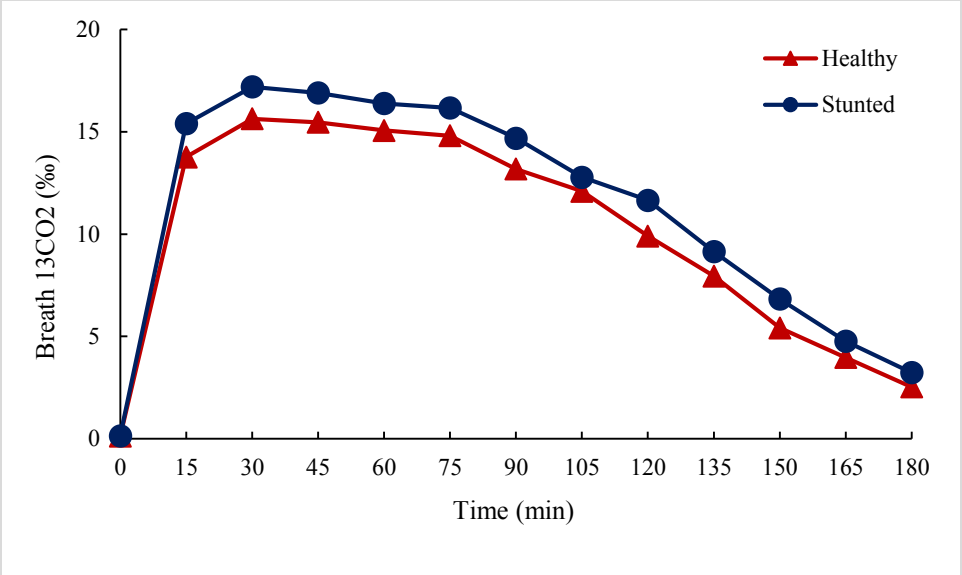

Figure 4.47 Plot of the average breath $^{13}CO_2$ levels after emptying of the untreated shear modified sorghum porridge in day 6 in 48 children (16 healthy – red line and 32 stunted – blue line).

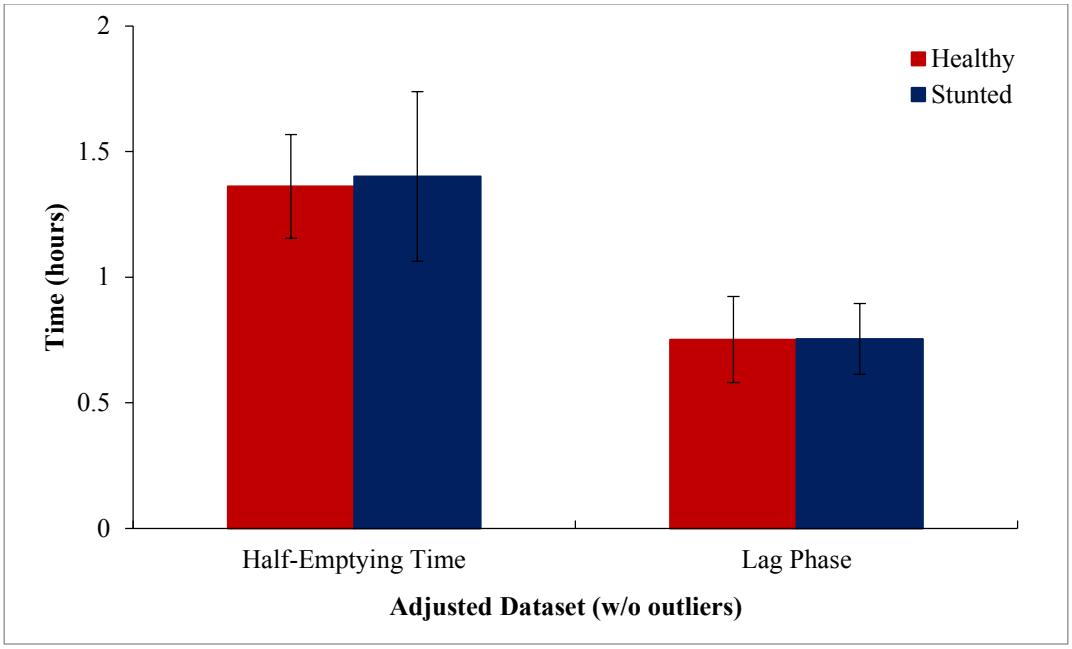

Figure 4.48 Gastric emptying parameters**.** Mean (± SDV) of lag phase and half emptying time of the untreated shear modified sorghum porridge for healthy (red bars) and stunted (blue bars).

CHAPTER 5. CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

The present dissertation has described studies on gastric emptying rate of African starchy foods, the effect of distal glucose release on the emptying rate of a non-nutritive paste, and the issue of starch digestion in marginally malnourished stunted children in Mali. Three variation of a non-invasive ^{13}C breath test technique have been used for all of the studies, in healthy volunteers and stunted children. The first study revealed an emptying rate difference between traditional West African starchy foods (sorghum thick porridge, millet thick porridge, and millet couscous) and the non-traditional "modern" foods (rice, boiled potatoes, and pasta) that are mostly consumed in the urban areas. The African starchy foods were found to have almost double the gastric emptying time compared to the non-traditional ones. The reduced rate of stomach emptying implies that these African starchy foods may provide sustained energy to the body which can be used to enhance their image in urban areas, promote their consumption, and increase their demand; thus to provide better market access for local smallholder farmers.

The second study investigated the effect of pre-ingestion of slowly digestible starch- entrapped microspheres on the emptying rate of a non-nutritive paste. The gastric emptying rate parameters (lag phase and half emptying rate) were evaluated and the results showed that slowly digestible carbohydrate fabricated microspheres led to a slower gastric emptying rate. This finding gives support to the idea that dietary carbohydrates with distal glucose release can modulate gastric emptying, and that this property may either exist in certain slowly digestible carbohydrate-containing foods or could be made into ingredients which in processed foods would have this effect.

The final study investigated first of all pancreatic α-amylase insufficiency in healthy and marginally malnourished stunted Malian children using new non-invasive modified ^{13}C breath test technique. Common and modified starch-based sorghum

porridges (thick and thinned) were prepared and their digestibility was assessed. Furthermore, the gastric emptying rate of the modified sorghum porridge was evaluated. This study revealed that pancreatic α-amylase insufficiency is present in the majority of weaned healthy and marginally malnourished stunted children in Mali. The healthy group presented anthropometric Z-scores (height and weight) significantly better than the stunted group, but still the children were pancreatic α-amylase insufficient. The malnourished stunted group digested the sorghum-based porridges as well as the healthy group. Impressively, children in the malnourished stunted group digested the porridges, whether thick or thin, better than the healthy group when α-amylase insufficiency was more pronounced. Accordingly, there was no correlative relationship between pancreatic α-amylase insufficiency and sorghum porridge digestion in malnourished stunted children, while there was one in the healthy group consuming the modified sorghum porridge. This implies that the malnourished stunted group have enhanced activity of the mucosal α-glucosidases and/or the glucose transporter SGLT-1 compared to the healthy group with higher α-amylase insufficiency that in essence takes over some the duty of digesting and absorbing glucose from the starch. Shear modified thickened sorghum porridge was found to be digested as well as the α-amylase pre-treated thinned porridge by both healthy and stunted groups. The finding that stunted children digest well the starch from thick porridges may have a practical implication in that thick cereal-based porridges could be given in supplementary feeding programs, if children could consume adequate amount. Furthermore, thickness could be optimized so that energy-density of the porridges would be sufficiently high for adequate energy intake within a meal. In 10% of the studied children, poor digestion of common sorghum porridge was observed, which likely was due to inherent metabolic problems related to malnutrition such as deficiency in mucosal α-glucosidase activity or environmentally induced enteric dysfunction. The knowledge generated from this study holds the promise of identifying or developing simple, cheap, and effective foods with good digestibility and energy density that could aid in bringing stunted, moderately malnourished children back to nutritional health.

Simple, safe, and noninvasive ^{13}C-breath test methods were used to assess gastric emptying, to diagnose pancreatic α-amylase insufficiency, and to determine the relative efficiency of starch digestibility. The use of the method to diagnose pancreatic α-amylase insufficiency is novel and innovative, and, particularly with a healthy child comparison group, could be an appropriate technique to assess pancreatic α-amylase insufficiency in children.

In considering the slow emptying rate of African starchy foods and the slowly digestible fabricated starch entrapped microspheres, further investigations are needed related to understanding the mechanism involved. Such study would link distal dietary glucose release and triggering of the feedback control systems to modulate gastric emptying rate and create sustained energy to the body. Studies are needed to evaluate, on the food side, where the carbohydrate digestion and glucose release has to be deposited, and how much is necessary to trigger the desired physiological response. Regarding the use of the starch entrapped microspheres as a preload for triggering the ileal brake mechanism, research has to continue with the aim of finding foods or designing ingredients that will exhibit the same slow digesting properties.

With regard to the study on pancreatic α-amylase insufficiency, it would be valuable and pertinent to study a US healthy control group, which could be used as a benchmark for our Malian children. On the porridge side, further investigations are needed to how thick porridges can be used to improve the nutritional status of marginally or more severely malnourished children. Porridge thickness needs to be optimized for high energy density and maximum starch digestion properties, evaluated for the amount of porridge necessary for one feeding time, the frequency needed to meet the energy density requirements for children, and tolerability and acceptability for the children. In order to evaluate their nutritional impact, the optimized porridges should be tested in a feeding study, and recovery and growth outcomes measured.

VITA

VITA

Fatimata Cisse is a citizen of the Republic of Mali in West Africa. She holds a combined Bachelor and Master Degrees Food Science from Moscow State Academy of Food Productions, Moscow, Russia in 1997. She then worked as full time researcher at the National Agricultural Research Center (Institut d'Economie Rurale du Mali, IER) for 10 years. In 2009, she was granted a USAID-Mali mission scholarship and Purdue University Graduate Research Assistantship to pursue a PhD Degree in Food Science. Her research assistantship focuses on African starchy foods, and starch digestion in malnourished stunted children in Mali. After completing her PhD, she will go back to her home country to IER