Improvement of the nutritional management of glycogen storage disease type I

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“The roots below the earth claim no rewards for making the branches fruitful”

Sir Rabrindranath Tagore

Stray Birds (1917)

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Summary

Patients with hepatic glycogen storage diseases are at risk of hypoglycaemia after short fasts. They require an intensive dietary treatment regimen, including feeds as often as 2 hourly and overnight continuous glucose feeds. The use of uncooked corn-starch helps children and adults to have longer fasting intervals and some to discontinue overnight pump feeds of glucose polymer via a gastric feeding tube.

Unfortunately, some adults still remain dependent on nocturnal pump feeds and, for some, frequent doses of starch are still required during the daytime. Some have symptoms of flatulence, abdominal bloating and diarrhoea which may be caused by starch mal-digestion. This thesis studies the short- and longer-term effects of the introduction of a novel, physically-modified corn-starch into the dietary regimen of patients with two of the more severe forms of hepatic glycogen storage disease. Based on the findings of this thesis, updated dietary recommendations can be made to patients with hepatic glycogenoses and their families.

CHAPTER 1 - INTRODUCTION

1.1 Carbohydrates – properties and metabolism

Glucose

The metabolism of glucose is a fundamental requirement for many energy requiring processes in living organisms. Plants fixate energy from the sun by photosynthesis to create simple sugars such as glucose, polymers of which form the most exported form of energy. [2] At physiological pH, the straight-chain form of glucose forms covalent bonds between the aldehyde group of carbon 1 and carbon 5 forming a glucopyranose ring (figure 1.1). The orientation of the hydrogen and hydroxyl group around carbon 1 determines whether D-glucose is an alpha or beta orientation. This stereochemistry of glucose gives it wide ranging properties, such that it can be stored in several different forms.

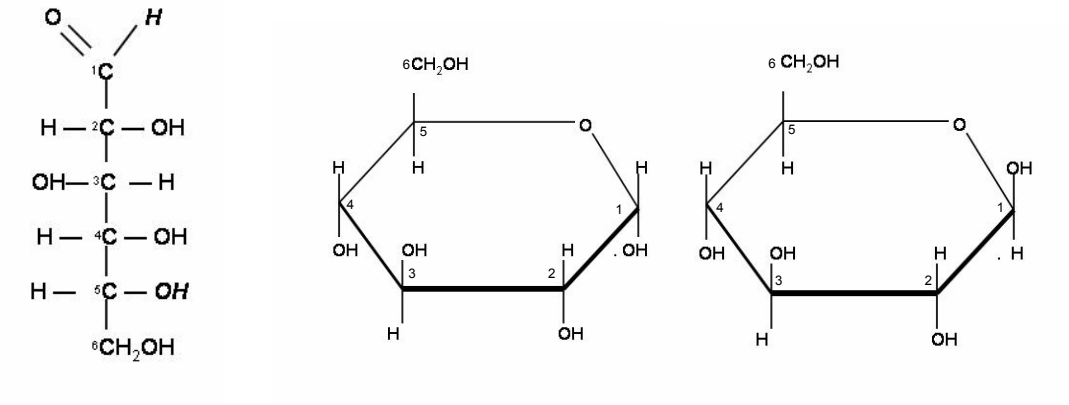


Figure 1.1 – Structure of straight chain glucose (Fisher projection - 1.1a), α-glucopyranose (1.1b) and β-glucopyranose (1.1c)

In animals, under aerobic conditions, the breakdown of each molecule of glucose culminates in the formation of 38 adenosine triphosphate molecules – this process is a fast and efficient means of releasing energy, allowing organisms to respond rapidly to changes in energy requirements.[3] Plants store glucose as glucose polymer in various forms of starch, providing a reservoir of energy to be used according to fluctuating demands.

Starch

Starch is a form of plant glucose polymer. It comprises linear chains of glucose molecules linked by α-1-4-glycosidic bonds, as well as alternative branched chains created by α-1-6 glycosidic bonds (figure 1.2). Amylose is almost exclusively linear 1,4 bonds whereas amylopectin comprises of both linear chains and branched 1,6 glycosidic bonds.

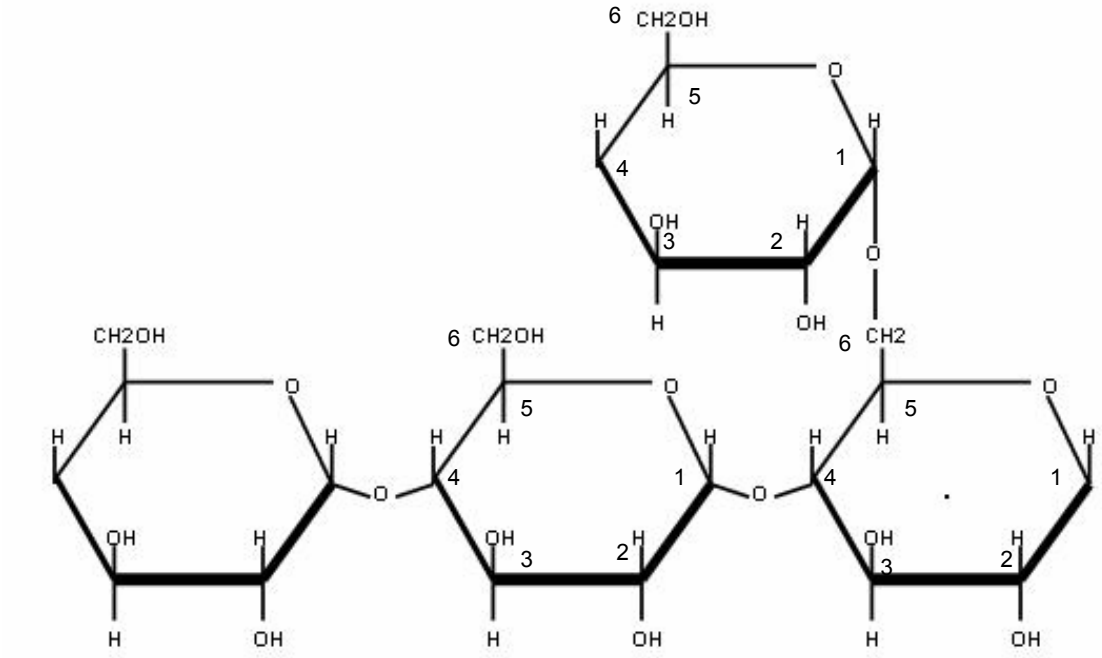


Figure 1.2 – Glucose polymerisation by the formation of linear α1-4 glycosidic bonds and branched α1-6 glycosidic bonds.

Different species of plants have specific proportions of amylopectin and amylose as stored starch, resulting in species specific starch types. The significant effect stereochemistry has on glucose polymeric properties is typified by the structure of cellulose comprising glucose β-1-4-glycosidic bonds. In the β-orientation, the glycosidic bond is further strengthened by hydrogen bonds between adjacent

hydroxyl groups.[2] Like amylose, cellulose is straight chained, but it is tough and fibrous and forms part of cellular architecture. It is also very resistant to digestion.

Starch is organised as granules comprising both amylose and amylopectin chains. Amylopectin chains are often organised to form a crystalline structure with amylose chains filling intermediate space. Variation in granular organisation, determined by relative amounts and molecular interactions of amylose and amylopectin, gives each starch its own unique properties.[4] Starch is not soluble in cold water. However on heating to 55 – 70 ^{o} C starch gelatinises allowing water into its structure – the crystalline amylopectin structure often remains intact but the interstitial amylose chains can leach out into the fluid. The granules swell with water entry forming a gel. At persistent higher heat, the entire gelatinous structure can dissociate to form a liquid. Conversely if allowed to cool, hydrogen bonds reform to create a more solid structure. This process is known as retrogradation. The retrograded starch has different physical properties to both the native, dissociated and the gelatinised starch.[5] The process of gelatinisation and retrogradation can be seen in daily cooking processes such as thickening sauces with cornstarch or making porridge. The physical properties of native starches can also be altered by treatment at high pressure, with chemicals or enzymes. When it comes to digestion of starches, an important determinant of bio-availability of glucose is not only the nature of the native starch, but how it has subsequently been processed.

Release Of Glucose from Dietary Starch

Digestion of starch

The physiology of an individual greatly determines to what extent glucose is liberated from available dietary starch. Starch digestion in humans begins with mastication and the release of salivary α-amylase in the mouth. Amylases cleave 1,4-glycosidic bonds in amylose releasing oligosaccharides and disaccharides such as maltose.

The enzyme is rendered inactive by the low pH in the stomach. On release of a bolus of food from the stomach, salivary α-amylase is reactivated in the more alkaline small intestine and additional pancreatic α-amylase is released. Further digestive oligosaccharidases and disaccharidases are released from the intestinal brush border resulting in the formation of glucose.[6] Of relevance to patients with glycogen storage disease is the fact that pancreatic α-amylase is secreted in only small quantities in infants and hence they may have difficulty digesting complex carbohydrates such as uncooked cornstarch.[7]

Gastrointestinal Transit time

Food enters the stomach and is emptied into the duodenum after a variable period of time. Gastric emptying is controlled locally by release of hormones from the duodenum, or centrally by sympathetic and parasympathetic innervation, and can be influenced by drugs such as metoclopromide. Dietary triacylglycerides delay gastric emptying by stimulating the release of cholecystokinin (CCK) and peptide YY (PYY.)[6] Other hormones such as secretin, gastrin and gastric inhibitory peptide also delay gastric emptying.[8] It has also been shown that dietary constituents such as acetic acid and free fatty acids can specifically delay gastric emptying.[9;10] There is great variation between individuals, and with age, in both gastric emptying and gastrointestinal transit times. Transit times can also be altered by dietary composition with foods such as wheat bran passing through the bowel quicker than other foods such as refined carbohydrate and meat.[11] Hence the composition of the diet has a bearing both on gastric emptying and gastrointestinal transit time and the timing of nutrient absorption after a meal.

Absorption of glucose

The classical model of glucose uptake across the intestinal mucosa is via a sodium dependent glucose / galactose transporter (SGLT1 – figure 1.3). Enterocyte cytoplasmic sodium concentrations are maintained at low levels by Na /K ATPase at

the basolateral membrane. Sodium in the gastrointestinal lumen follows its concentration gradient into the cell via SGLT1.[12] The SGLT1 trans-membrane protein changes its configuration in the presence of sodium allowing the passage of glucose or galactose into the cell. The classical model proposes that glucose leaves the cell and enters the bloodstream via the GLUT2 glucose transporter. There are several studies demonstrating increased expression of GLUT 2 in enterocytes in response to elevations of environmental glucose in tissue cultures.[13-15] However, there is still controversy about the extent of the role of GLUT 2 in glucose transport. Some propose intracellular microsomal trafficking as an alternative means of glucose transport.[16](figure 1.3)

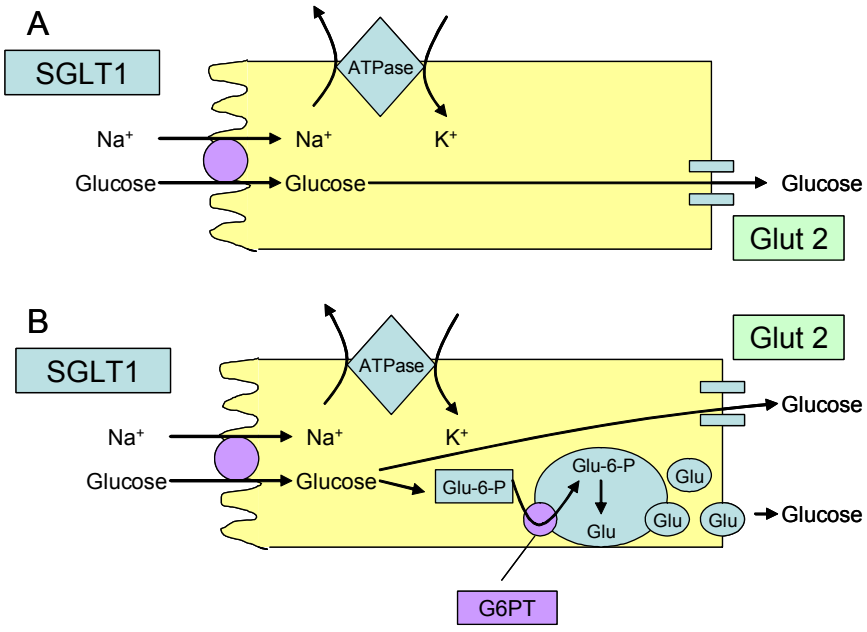


Figure 1.3 A) – the classical model of glucose uptake via SGLT1 at the brush border and GLUT 2 at the basolateral membrane. B) An alternative hypothesis of glucose uptake via microsomal trafficking using G6PT and G6Pase

Figure adapted by kind permission of Elsevier Ltd from Santer et al: Gastoenterology 2003[17]

Santer et al performed a study looking at glucose and galactose uptake in a patient with GLUT 2 deficiency and another with Glycogen Storage Disease type Ib (a known defect in microsomal glucose transport.) On the basis of significant elevations of

breath hydrogen from baseline in the patient with GSD Ib after ingestion of glucose and galactose, compared to the patient with GLUT2 deficiency and a normal control, they suggested there was evidence of impaired glucose uptake in the patient with GSD Ib. They concluded that this was evidence that microsomal trafficking in enterocytes rather than facilitative diffusion by GLUT 2 was the predominant factor determining glucose transport into the blood-stream from enterocytes.[17] This determinant of glucose absorption clearly has particular relevance to the processing of dietary carbohydrate in patients with GSD I and has not yet been conclusively elucidated. The function of expressed glucose-6-phosphatase (G6Pase) and glucose-6-phosphate translocase (G6PT) in enterocytes is yet to be defined.

There is a an increased incidence of inflammatory bowel disease in patients with GSD Ib, which may itself account for the breath hydrogen data in Santer's study.[18;19] In addition, many patients with GSD Ia are also known to have chronic or intermittent diarrhoea.[7;20] The digestion and absorption of starch and glucose respectively can be disrupted if there is evidence of bowel inflammation. This inflammation and disruption of mucosal integrity too could lead to sub-optimal absorption of glucose from dietary starch.

Humoral response to glucose in the blood.

The body maintains plasma glucose within a narrow margin. If the concentration is too low, end organ requirements for glucose as an energy source may not be met. If plasma glucose concentrations are too high, osmotic effects of hyperglycaemia, such as diuresis, may ensue. There are a complex range of regulatory mechanisms that keep the plasma glucose concentration relatively constant in healthy individuals.

Insulin has a principal role in the regulation of the plasma glucose concentration. It is released in response to elevations in the plasma glucose and its actions involve the uptake of glucose by peripheral tissue. When plasma glucose concentrations are low,

counter-regulatory hormones such as glucagon, growth hormone or catecholamines induce glucose release from stores such as glycogen.[3] Over the last 15 years, further layers of complexity have been discovered that overlay this simple model. (figure 1.4) Whilst insulin has a primary role in glucose uptake and decreased lipolysis, other humoral factors can also regulate, and are modified by, the action of insulin. Adipocytes release the hormone leptin with increased amounts in healthy individuals correlating with satiety. Mutations in the leptin gene resulting in leptin deficiency are associated with morbid obesity.[21;22] Rats and humans with either congenital or acquired lipodystrophy also have low plasma leptin concentrations resulting in secondary amenorrhoea, insulin resistance and diabetes.[23] Elevation of plasma insulin can also result in leptin release: insulin release can induce satiety.[24] Conversely ghrelin is a hormone released by the stomach, pancreas, gastrointestinal mucosa as well as the hypothalamus and rises pre-prandially. Acylated ghrelin induces hunger, with the plasma concentrations decreasing post-prandially.[25] In mice, blocking ghrelin release during an oral glucose tolerance test results in lowering fasting glucose concentrations, attenuated glycaemic excursions and enhanced insulin responses with the converse occurring when ghrelin was infused.[8] This suggests that ghrelin antagonises the action of insulin in the short-term. Several studies also suggest that ghrelin concentrations are lower in obese individuals with insulin resistance compared to those who are insulin sensitive.[24;26] Morbidly obese subjects that have bariatric surgery can have remission of type II diabetes mellitus, with those having a Roux-en-Y gastric bypass procedure reducing by upto 84% compared to 48% of those with gastric banding. The former procedure bypasses the stomach with food entering the small bowel distal to the duodenum. This procedure therefore, reduces gastric storage capacity but also bypasses some of the duodenal humoral feedback mechanisms related to feeding. The Roux-en-Y procedure is also associated with increased insulin sensitivity and reduction of leptin release long-term, but is not associated with increased ghrelin release either in the days post-

procedure, or long-term. Given that these subjects have significant demonstrable weight loss, the failure to induce appetite stimulation by ghrelin release appears an important mechanism of sustained insulin sensitivity and weight loss. There is therefore, a complex interaction between humoral mechanisms of glucose control that changes with insulin sensitivity. Understanding of these relationships would also help in the understanding of blood glucose variations in disorders such as the glycogen storage diseases.

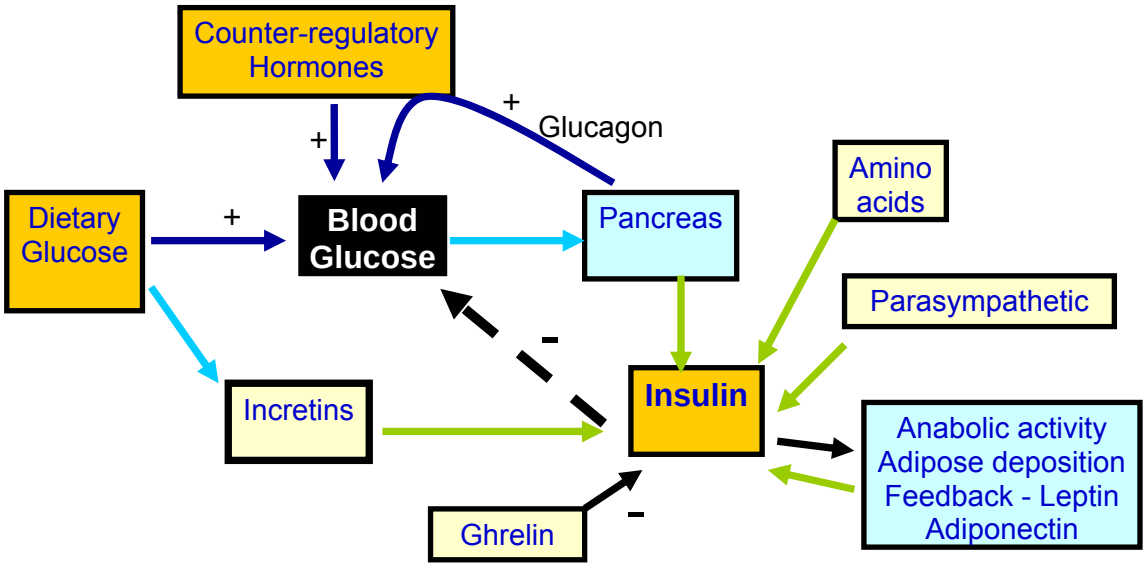


Figure 1.4 – The complexity of glucose homeostasis - inter-action with insulin

The incretins are another group of peptides that have been extensively studied with relation to insulin release. They are gastrointestinal hormones including glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like-peptide-I (GLP I). GIP is secreted in response to eating, especially after fatty food in humans. Its principal action is to enhance glucose dependent insulin release by stimulating pancreatic β-cell exocytosis.[27] It also increases insulin biosynthesis and β-cell proliferation. GLP increases the sensitivity of pancreatic β-cell to glucose by stabilising the β-cell potassium channel KIR6.2 and its expression at higher levels of glucose. As a consequence, it too enhances glucose-dependent insulin release.

Of particular relevance to patients with GSD, a study performed by Wachters-Hagedoom demonstrated sustained increases of GLP-1 and insulin when 7 healthy men ingested 53.5g of uncooked cornstarch compared to similar quantities of glucose and pasta. (figure 1.5)[28]

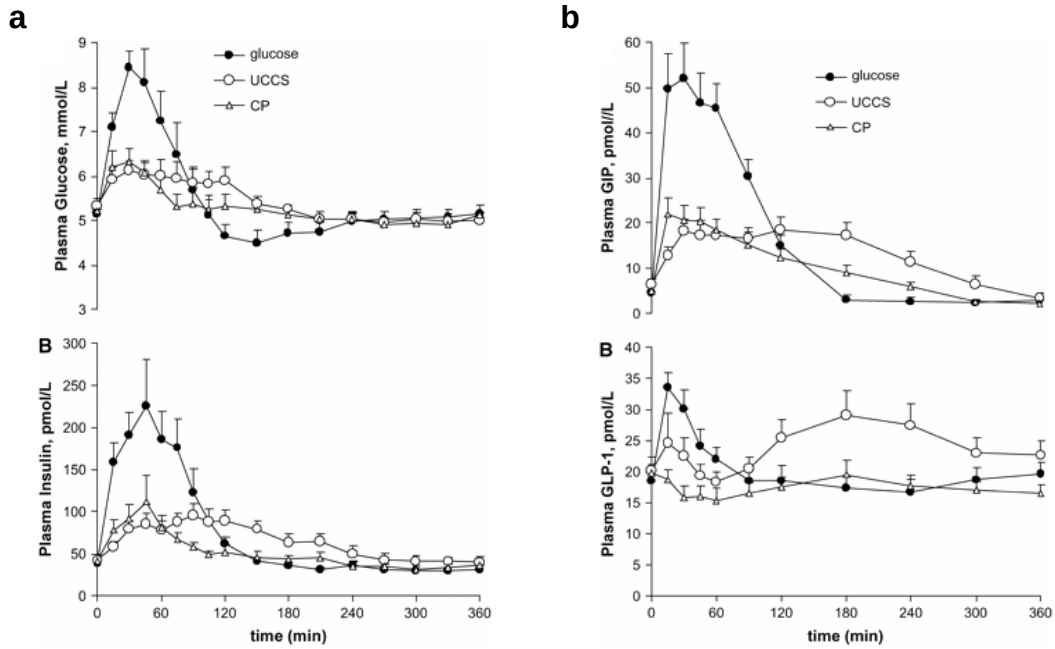


Figure 1.5 - Glucose and insulin response (a) and Incretin response (b) of 7 healthy volunteers after equivalent carbohydrate quantity meals of corn derived glucose, uncooked cornstarch and cooked corn-pasta

Reproduced by kind permission of the American Society of Nutritional Sciences; Wachters- Hagedoorn et al, Journal of Nutrition 2006 [28]

This cross-over study of healthy volunteers attempted to study rate of appearance of glucose from an exogenous carbohydrate source by setting a background infusion of dideuteroglucose and subsequent ingestion of a naturally labelled ^{13}C starch. By examining the $^{13}CO2$ content in the breath for the subsequent 6 hours after carbohydrate ingestion, rate of appearance of glucose from exogenous carbohydrate sources was calculated. The calculated rate of appearance of glucose from ingested carbohydrate closely matched the glucose and insulin profile of figure 1.5a; there was a rapid increase and fall when glucose was ingested with a slower rise in the pasta and uncooked cornstarch profile. However, the uncooked cornstarch profile

continued to be higher than the pasta profile throughout the study. Figure 1.5 demonstrates sustained elevation of insulin, GLP-1 and GIP with specific ingestion of cornstarch compared to the other carbohydrates. The sustained elevations were corroborated by calculations of area under curves (AUC), which were made between 0 – 120 minutes and 120 - 240 minutes for each analyte for each study. As would be expected, the mean 0 - 120 minute AUC for plasma glucose (181.7 mmol/L – 2 hrs) and serum insulin (13450 pmol/L – 2hrs) after ingestion of glucose was much higher than for UCCS (74.7 and 4964 respectively) and cooked pasta (65.3 and 4154 respectively). Between 2 and 4 hours both parameters were very low after ingestion of glucose but remained higher with UCCS compared to cooked pasta; Glucose AUC was 20.2 mmol/L – 2hrs for UCCS compared to 10.6 for cooked pasta and insulin was 3726 pmol/L – 2 hours for UCCS and 976 for cooked pasta. The GIP AUC for UCCS from 2 – 4 hours was also elevated at over twice the value of cooked pasta whilst the GLP – 1 AUC for UCCS was elevated at 10 times the level for this time period. This demonstrates dietary factors have a dramatic role to play in humoral responses. Of the 3 carbohydrates tested, uncooked cornstarch specifically resulted in persistent elevation of incretins and insulin.[28]

1.2 – Hepatic Glycogen Storages Disease

Glycogen

Animals are unable to synthesis starch but have a small amount of protein bound carbohydrate called glycogen, which comprises up to 60,000 glucose molecules. This enables organs to temporarily store glucose in an insoluble form contributing little to cytosolic osmolarity.[29] The highly branched structure of glycogen is a feature of mammalian cells that is absent in plant cells. (figure 1.6)

The "densely" arranged glucose molecules also provide a readily available energy source for cellular respiration that is liberated in response to appropriate humoral triggers, such as the release of catecholamines. Defects preventing the normal flux of glucose molecules in the synthesis and degradation of glycogen can consequently lead to devastating consequences in cellular energy metabolism.

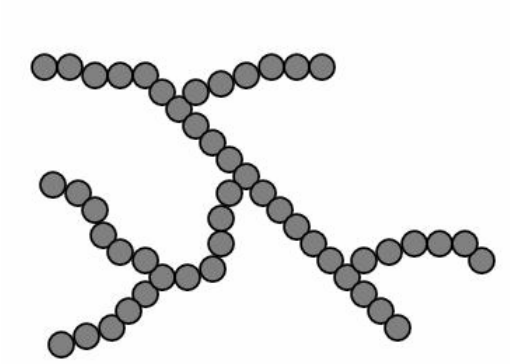


Figure 1.6 Diagramatic representation of structure of glycogen comprising glucose straight chains formed by α 1-4 glycosidic bonds and branch points every 6-10 residues of α 1-6 glycosidic bonds.

Metabolism of Glucose and Glycogen

Post-prandial elevation of blood glucose causes release of insulin which induces uptake of glucose by peripheral tissues. Organs that have a high demand for glucose include the liver, brain and skeletal muscle. Many organs are able to store glucose temporarily as glycogen. In humans, glycogen forms only a small part of total energy

reserves, with fats and proteins being much more abundant sources of energy.[30] However, there is significant turnover of glycogen with relative flux between breakdown and synthesis determined by the concentration of glucose in the blood. If the concentration of glucose is low, glycogenolysis is favoured whereas when blood glucose is high glycogen synthesis is favoured. Glycogen stored in organs such as skeletal muscle is used for glucose production in that particular organ. Glycogen stored within the liver, however, is used to export glucose around the body at times of need.[31] As mentioned in the previous section, these processes are controlled by insulin and the counter-regulatory hormones such as glucagon. In the hepatic glycogen storage diseases, the process of glycogen breakdown is disrupted leading to fasting hypoglycaemia.

Humoral control of glycogen metabolism is exerted through reciprocal action on glycogen synthase in the glycogen synthetic pathway and phosphorylase in the glycogen degradation pathway. An increase in cAMP mediated by counter-regulatory hormones such as glucagon, activates phosphorylase kinase resulting in increased phosphorylase action with corresponding increase in glucose release. Conversely, the enzyme responsible for dephosphorylation, namely phosphoprotein phosphatase is inhibited. Phosphoprotein phosphatase activity is increased by insulin via an inhibitory action of insulin on glycogen synthase kinase 3, leading to increased glycogen synthase activity and decreased phosphorylase activity. [32;33] The reciprocal action of this regulatory cascade is indicated later in figure 1.10.

The glycogen storage diseases (GSD) – Historical aspects

The GSDs comprise a group of rare inherited disorders of glycogen metabolism. Identification of the causes of GSDs has been intertwined with the unravelling of essential biochemical processes of glucose metabolism and intracellular structure. Von Gierke described the pathological findings of glycogen storage within the liver and kidney in "Hepatonephromegalia glykogenica." The American scientists, Carl and Gerti Cori spent most of their academic lives investigating the interaction of glucose and glycogen. They discovered what was known as the Cori ester – glucose-1-phophate in 1936, later demonstrating that this was the first step of glycogen synthesis. They subsequently isolated glycogen phosphorylase and were also able to synthesise glycogen and starch in vitro. Their work on glycogen and glucose interactions led them to describe the process of re-cycling glucose via lactate and pyruvate (Cori cycle). It was for this work that they received the Nobel Prize for Physiology or Medicine in 1947.[34] This work led them to identify glucose-6-phosphatase (G6Pase) as the enzyme responsible for glycogen storage disease type I.[35] This work was the first ever description of a disorder that was directly linked to an enzyme deficiency. At the same time as they published this finding, they noted that 2 of the 10 pathological samples they had examined, showed abnormal glycogen structure – one with "longer inner and outer chains than normal glycogen….resembled amylopectin," and the other "had very short outer branches….resembling a phosphorylase limit dextrin."[36] They were subsequently able to identify amylose 1,6 glucosidase (debrancher) as the enzyme deficient in a 12 year old girl with limit dextrinosis, descrbing the enzymatic deficiency of glycogen storage disease type III.[37]

In the 1960's a Belgian group, led by a student of the Coris', Christian de Duve, attempted to characterize hepatic glucose-6-phosphatase. In the process of their research, they fortuitously discovered the lysosome,[38;39] and subsequently identified lysosomal acid maltase; the enzyme responsible for storage of glycogen within

muscle causing Pompe's disease or glycogen storage disease type II. The first author of this paper, Henri-Geri Hers correctly predicted in his first description that this enzyme deficiency would be the first of many lysosomal storage diseases.[40]

The descriptions of glycogen storage diseases types I, II, III and IV were all possible through meticulous and inspired thought about the biochemical processes of glucose metabolism. Over the subsequent years, several other diseases were identified based on this diligent early work. (figure 1.7)

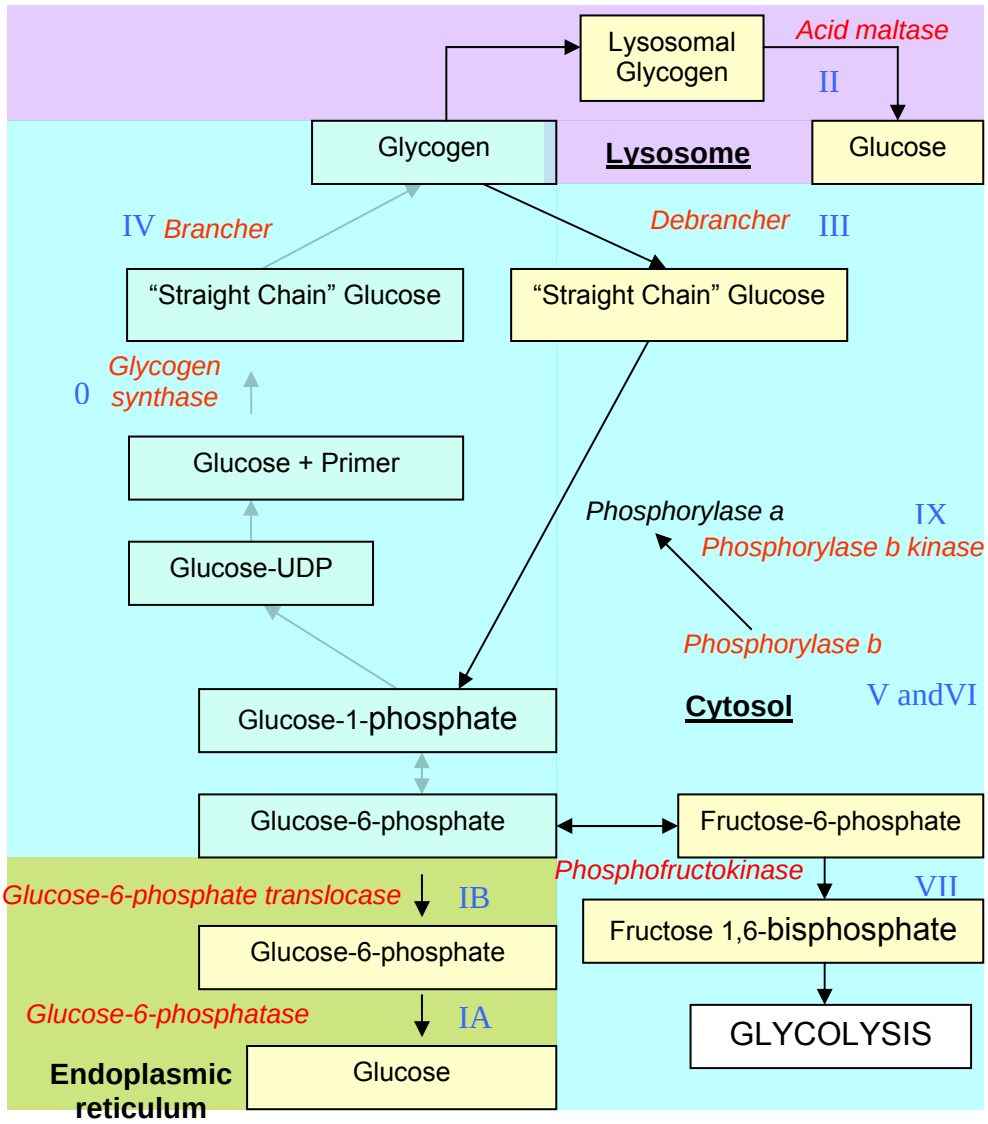


Figure 1.7 – The Glycogen storage diseases

Enzymes indicated in red and GSD disorder number in blue

Glycogen Storage disease Type I

GSD I (McKuisick 232200) is caused by reduced activity of glucose-6-phosphatase: GSD Ia, by deficiency of the hydrolytic enzyme and GSD Ib, by deficiency of the endoplasmic reticulum trans-membrane glucose-6-phosphate transport protein, G-6-P translocase (figure 1.8). The major metabolic consequence of ineffective function of G-6-P-ase is hypoglycaemia, provoked by relatively short fasts.[41]

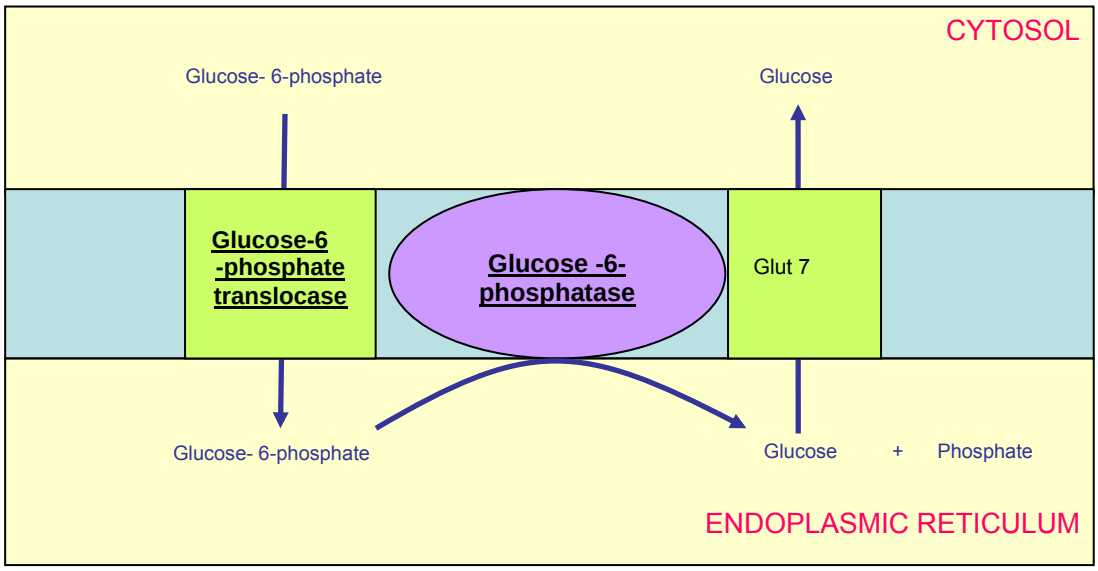


Figure 1.8 – Formation of glucose in the endoplasmic reticulum of cells

The glucose-6-phosphatase enzyme complex comprises 357 amino acids. It is a trans-membrane enzyme with catalytic activity on the luminal surface of the endoplasmic reticulum.[42] It is expressed in the liver, kidney and gastrointestinal mucosa. Its role is the production of glucose from glucose-6-phosphate and is deficient in GSD type Ia. In the liver, glucose-6-phosphatase facilitates export of glucose, controlled by counter-regulatory hormones such as adrenaline or glucagon. The endoplasmic reticular trans-membrane protein, glucose-6-phosphate translocase is deficient in GSD type Ib. Glycogenolytic and gluconeogenetic processes create glucose-6-phosphate, *but this cannot be exported in the form of glucose.* Consequently the blood glucose cannot be raised and there is increased flux of

glucose-6-phosphate through other pathways (figure 1.9).[41] There are several additional features of GSD Ib including neutropenia with recurrent infections and inflammatory bowel disease. The basis of these additional features of GSD Ib has not been fully elucidated.

The secondary metabolic features of GSD type I arise in response to hypoglycaemia. (figure 1.9) This leads to glucose-6-phosphate production from glycogen breakdown or gluconeogenesis. Increased intra-hepatic catabolism of glucose-6-phosphate leads to greater flux along the glycolytic pathway resulting in increased synthesis of pyruvate, lactate and acetyl co A. Accumulation of the latter leads to greater synthesis of triglycerides and cholesterol. In addition, catabolism of glucose-6-phosphate through the pentose phosphate shunt leads to increased production of the 5-carbon sugar ribose, which forms an essential component of nucleotides; the increased turnover of these results in elevations of uric acid. Poor metabolic control comprising high plasma concentrations of uric acid, cholesterol, lactate and triglycerides consequently occur when patients are frequently hypoglycaemic. Experiences gained over a series of studies demonstrate better secondary metabolic control when the tendency toward hypoglycaemic episodes is decreased.[43-45] The principal goal of treatment is therefore to maintain a steady “normal” blood glucose which leads to amelioration of secondary metabolic disturbance.

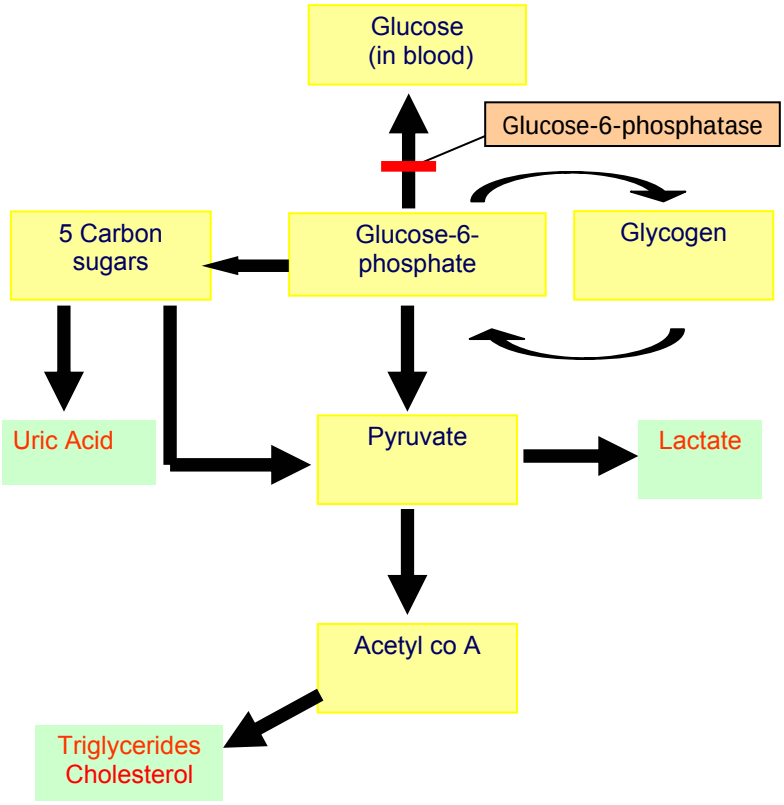


Figure 1.9 – Secondary metabolic effects of glucose-6-phosphatase deficiency

Glycogen storage disease type III

Aetiology. GSD III (McKuisick 232400) is caused by glycogen debrancher deficiency. The AGL gene encoding the enzyme is large, containing 35 exons, spanning 85kb of DNA and translates a monomeric protein product of 1,532 amino acids.[41] This enzyme complex is unusual in that it has two distinct catalytic sites that perform different functions on the same glycogen polymer. Four glucose residues from a branch point, oligo-1,4-1,4-glucantransferase transfers three glucose molecules from the branch chain to an adjacent linear chain, thereby elongating the linear chain. The glucosidase site then cleaves the remaining glucose (1,6 linked.)[46] Deficiency of this enzyme consequently results in accumulation of a short chain form of glycogen – limit dextrin. Glycogen debrancher deficiency differs from GSD I in that some glucose can be released from glycogen by the continued action of glycogen phosphorylase and glucose-6-phosphatase. Despite this, the infantile presentation of this disease can be very similar to GSD I, and it is sometimes difficult to discriminate clinically the

two conditions. Hyperlactataemia and hyperuricaemia are absent, because there is less flux of glucose-6-phosphate through glucose and pentose phosphate compared to GSD I. However, hyperlipidaemia is a feature. Gluconeogenesis is not prevented and consequently lipolysis is not inhibited. Therefore, ketosis is present in GSD III but absent in GSD I. GSD I and III may also be distinguished by the expression of deficiency within skeletal muscle in most patients with GSD III. Those patients that have deficiency only in liver are labelled as GSD IIIb, whilst the majority (85%) that have more widespread deficiency in both skeletal and cardiac muscle are labelled as GSD IIIa.

Clinical features Patients generally present in infancy with fasting hypoglycaemia, failure to thrive and hepatomegaly. Motor developmental delay is not uncommon. Typically, by adult life the hypoglycaemic tendency diminishes, but may become more apparent at times of stress such as pregnancy or peri-operatively. The myopathy of GSD IIIa is distal and slowly progressive in adulthood and some patients also develop a hypertrophic cardiomyopathy. Pathogenesis of these complications is unknown. Reduced bone density is seen in patients with both GSD IIIa and b. Polycystic ovarian disease and insulin resistance have been noted in glycogen storage disease type III.[47;48] There are a few reports of cirrhosis and subsequent hepatocellular carcinoma occurring in adult patients.[49;49;50]

Glycogen Storage Disease Type VI

Hers described hepatic phosphorylase deficiency (McKuisick 232700) from liver biopsies of patients that had similar symptoms to patients with GSD Ia and III, but did not have these enzymatic deficiencies.[46] Hepatic phosphorylase deficiency is very rare and all cases so far reported have not had muscle, cardiac or brain involvement. Hepatomegaly, hypoglycaemia and growth delay are features of this disease. Typically patients present after the first year of life and hypoglycaemia is less pronounced in childhood than either GSD I or III and resolves by adult life.

Hyperlactataemia and hyperuricaemia are not features of this disease but there may be mild hyperlipidaemia, with ketosis during hypoglycaemic episodes. Hypoglycaemia can be prevented with uncooked cornstarch if necessary, which can also stimulate catch-up growth.[51]

Glycogen Storage Disease type IX

Defects of the phosphorylase system have been subject to some confusion in nomenclature. With the benefit of hindsight, it is now possible to describe the genetic and enzymatic basis of disease and hence clarify the numerical classification. In the past, some patients may have been thought to have had deficiency of hepatic phosphorylase and possible involvement of the brain, skeletal muscle or heart. It transpires that many of these patients have defects of the phosphorylase kinase system, which is currently classified as GSD IX.[29;41] The sex-linked variant of GSD IX (McKuisick 306000), had alternatively been classified as GSD VIII by some, whilst others had classified the neurological form of phosphorylase kinase deficiency as GSD VIII.

Aetilogy Phosphorylase kinase is the final important step of the regulatory cascade controlling glycogen metabolism as indicated in figure 1.10. The active enzyme is a complex tetramer comprising four different monomers: α, β, γ, and δ. The α-subunit is encoded for by 2 different genes which confer expression in a particular organ: PHKA1 in muscle and PHKA2 in liver.[52] Similarly the γ-subunit is encoded by PHKG1 for expression in the muscle and PHKG2 for the liver and testis. The β and δ subunits are coded for by single genes. The α and β-subunits are large enabling regulation of the enzyme. The γ-subunit contains the active site and δ-subunit is part of the calmodulin group of proteins that bind and respond to calcium activation. The calmodulin proteins have widespread regulatory functions and are not implicated in the pathogenesis of GSD IX. There is considerable phenotypic variability in GSD IX,

which is not surprising as the corresponding mutations could be in any one of a number of genes which may or may not be expressed in a particular tissue.[53-55]

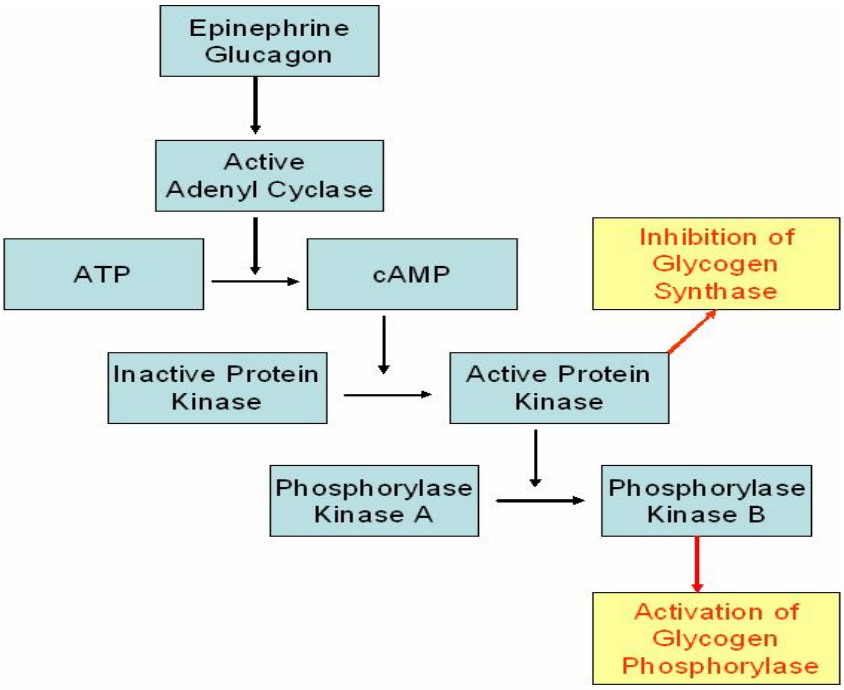


Figure 1.10 – The activation cascade regulating the function of glycogen phosphrylase and glycogen synthase

GSD IX is the most common of the glycogen storage diseases, but because of the phenotypic variability, difficulties with enzymatic and genotypic diagnosis as well as some relatively mild phenotypes, it is probably still under-diagnosed. The commonest clinical presentation is as “X-linked hepatic GSD IX “identified by Hug et al in 1966. Patients present in infancy or early childhood with similar manifestations to GSD type Ia, but the hypoglycaemic episodes may be less severe, and is not a feature of disease in some. There may be mild hyperlipidaemia and ketosis with fasting but hyperuricaemia is not a feature. Most symptoms resolve by adult life. Typical to an X-linked pattern of inheritance, most affected individuals are boys, but there are also mildly affected females reported.

Historical treatment of glycogen storage disease type I

In the early 20th century, glycogen storage disease type I was associated with significant mortality and morbidity. In response to some of the vital early work on glucose metabolism performed by Carl and Gerti Cori and Henry-Geri Hers, it was realised that patients' symptoms improved with the use of frequent meals. Porto-caval shunts in the 1960's aimed to reduce hepatic first pass metabolism of dietary glucose.[56] The use of parenteral nutrition appeared a more effective strategy.[57] This was simplified further by the introduction of continuous nocturnal enteral feeds, in conjunction with frequent daytime meals.[43;44] The latter strategy demonstrated very clear improvement in growth and overall metabolic control. This treatment appears effective, but it is onerous and ongoing concerns with mechanical pump failure and tube dislodgement leading to dangerous hypoglycaemia remain.[58;59] Daytime meals may need to be frequent (<2 hours interval) and consequently several studies looked at dietary starches that release glucose slowly, to extend this period of euglycaemia.[4;7;60;61] Some of these studies are considered further in section 1.3.

The introduction of uncooked cornstarch (UCCS) into the dietary regimen in the 1980s improved daytime management and allowed some patients to replace nocturnal pump feeds of glucose polymer with a dose of cornstarch. An important consequence of the introduction of these dietary therapies was an improved quality of life and prognosis.

Complications of glycogen storage disease related to diet

Whilst many patients clearly benefit from UCCS, some patients do not have sustained normoglycaemia and many have symptoms of bloating, flatulence and gastrointestinal disturbance.[19] In some patients these symptoms may be related to incomplete digestion of starch.[62] Bodamer et al studied 8 patients with GSD Ia compared to 15 healthy controls examining $^{13}CO_2$ breath enrichment and H_2 excretion after a cornstarch load. The study aimed to quantitate glucose oxidation

from ingested cornstarch. The authors' data demonstrated that glucose oxidation was comparable to controls for the first 2.5 hours. Thereafter, glucose oxidation was significantly diminished in subjects with GSD Ia compared to control, (cumulative mean utilization 11.5% versus 18.4%.) Oxidation was significantly lower between 4.5 hours and 6 hours. Based on previous studies, the authors comment that comparable oxidation for the first 2.5 hours indicates that gastrointestinal factors are similar between the 2 populations; ie gastric emptying, digestion and absorption of glucose. Differences in glucose oxidation observed after 4.5 hours is indicative of differences in post-absorptive metabolism. They comment that absorbed glucose is incorporated into glycogen with a slower turnover. This gives some explanation of the variable efficacy of cornstarch reported by Lee et al in 1996.[63] The baseline hydrogen excretion of the GSD Ia population was significantly greater than controls and 2 of the 8 GSD Ia subjects fulfilled the criteria for carbohydrate malabsorption 5 hours into the study. Breath hydrogen is a measure of colonic fermentation (as discussed later) and the results including the elevation at baseline are indicative of increased colonic fermentation of carbohydrate in the GSD Ia population compared to controls. This could lead to symptoms of abdominal bloating, flatulence and diarrhoea. Prior to the introduction of uncooked cornstarch, into the dietary regimen, diarrhoea was a well recognised feature of both GSD Ia and Ib. In 1978 a series of in-vitro and in vivo studies were performed by Milla et al.[64] They demonstrated impaired glucose absorption in patients with GSD Ia and Ib compared to controls and concluded that glucose malabsorption contributes to diarrhoea. However, the management of patients of GSD in the 1970's does appear to have significantly improved since the introduction of nocturnal continuous glucose based feeds. [44;65]This improved outcome seems in contrast to a hypothesis that significant glucose malabsorption occurs.[17] However, several patients do complain of diarrhoea, and whether simple or complex carbohydrate malabsorption is causative, is yet to be determined.

It is recommended that patients with GSD take 65% of their dietary intake as carbohydrate. This is greater than that taken by the general population.[66-68] Carbohydrate ingestion inevitably leads to insulin release which has widespread metabolic consequences as discussed in section 1.1. Long-term effects of persistent elevations of insulin in the general population are subject to a huge volume of research world wide.[25;27;69-71] Hyperinsulinism is associated with the metabolic syndrome including hypertension and obesity, non-insulin dependent diabetes mellitus (NIDDM), increased cardiovascular mortality and polycystic ovarian disease. It is somewhat surprising that neither a significantly increased incidence of cardiovascular events nor NIDDM are reported in patients with GSD. There are case reports only of NIDDM in patients with GSD.[72;73] This may be because the majority of patients are young. Exaggerated insulin responses by some obese GSD I patients were reported by Mundy et al after oral glucose tolerance tests.[74] Lee et al reported a higher incidence of polycystic ovaries (PCOD) in adults and children with GSD Ia and III than controls. Adults with both GSD Ia and III showed evidence of insulin resistance after a glucose tolerance test but the children did not.[47] Insulin resistance is implicated in the patho-physiology of PCOD in the general population, and may cause this complication in the hepatic glycogen storage diseases.[75;76]

There are a number of long-term morbidities associated with the hepatic glycogenoses, including the development of hepatic adenoma and potentially hepatocellular carcinoma, renal tubular and glomerular disease and fractures related to osteopenia.[44;77-80;80-82] Whilst the pathophysiology of these morbidities is not known, it is clear that those with better metabolic control are less likely to develop significant complications. [45;59;83]There is evidence, therefore, of appropriate dietary treatment improving short-term glycaemic control, secondary metabolic features and long-term complications.[60]

1.3 Carbohydrates – Dietary treatment in GSD and in healthy individuals

Specific Dietary Treatment of GSD – As discussed, the principal problem of GSD I is impairment of endogenous hepatic glucose production. After the publication of endogenous glucose production rates by assessing incorporation of 6,6-dideuteroglucose label into blood glucose in a wide range of children, it was possible to calculate predicted requirements[84]. Since there is significant impairment of endogenous production in GSD I, glucose needs to be provided from exogenous sources. The study by Bier et al in 1977 calculated that requirements correlated linearly with estimated brain size implying that the endogenous glucose production was principally for the brain. The calculated requirement mean values were: 7.1 mg/kg/min for children under the age of six, 5.4 mg/kg/min for older children and 2.3 mg/kg/min for adults. Schwenk and Hammond specifically assessed six GSD I children aged between 1 and 7 years using similar methodology with incremental doses of labelled glucose.[1] It was noted that rates of 8-9 mg/kg/minute of glucose infusion completely suppressed lactate production and partially suppressed endogenous glucose production, without excessive insulin rise. Extrapolating from this basal requirement means that 65% of total dietary energy in young children with GSD needs to be from carbohydrate. Assuming the adult basal requirement is 2.5mg/kg/hr, and the total calorie intake is 2500 calories, a 70 Kg adult would need 40% of their energy requirement in the form of carbohydrate. The UK average contribution of carbohydrate to total energy requirements is 50%, with intakes of 40-50 in other Western European populations.[85;86] The World Health Organisation has recommended carbohydrates from various sources provide 55% of energy intake in healthy adults.[87]

The aim of dietary treatment in children is to provide carbohydrate, sufficient protein to maintain adequate growth and to maintain satisfactory micronutrient intake. Uncooked cornstarch has little nutritional value compared to other carbohydrates

such as rice and pasta and consequently there is potential for nutritional deficiencies if this forms the bulk of the 65% carbohydrate intake.[88] There are examples of low concentrations of essential fatty acids and Vitamin B12 deficiency being recorded in GSD I.[89;90] For adults, relative carbohydrate requirements are less and consequently nutritional management should be more straightforward.

Lactose and Fructose restriction In a series of informative studies, John Fernandes demonstrated the clear effect that fructose, sucrose and lactose had on blood glucose in a small number of patients with GSD I and III. Quite large doses (1 – 2 g/kg/hr) of glucose and fructose were continuously infused for 3 hours and the effect on lactate and glucose was observed.[91] Plasma glucose concentrations increased further in the GSD I patients given glucose compared to fructose; lactate concentrations were higher in the fructose group. In control individuals and patients with GSD III, fructose is converted to glucose via glucose-6-phosphate as an intermediary. Fernandes hypothesised that gluconeogenesis is impaired in GSD I due to ineffective function of glucose-6-phosphatase. Fructose is obligatorily metabolised via the glycolytic pathway producing lactate as a consequence. He showed similar findings with the disaccharides sucrose (glucose-fructose,) and lactose (glucose-galactose) compared to maltose (glucose dimer.)[92] He proposed restriction of lactose, fructose and sucrose as they contribute to lactic acidosis and do not contribute effectively to gluconeogenesis. Theoretically this hypothesis has great credibility, but in practice such restriction could lead to multiple dietary deficiencies. The foods associated with lactose intake (milk) and fructose (fruits) and sucrose (table sugar), are in a wide range of processed foods and contribute significantly to overall dietary quality providing protein, calcium, essential fatty acids, vitamin C and fibre. This restriction coupled with a high carbohydrate intake could lead to significant dietary imbalances.

There are therefore, divergent attitudes world-wide about the management of these patients with some opting to restrict these sugars and supplementing the diet, whilst others including the author's group currently favouring no restriction. Long-term studies have tried to address complication rate between these two management strategies but to date have failed to find a difference.[59] Preliminary data presented on behalf of The International Study of Glycogen Storage Disease type I (ISGSD I) registry appeared to demonstrate comparable long-term outcome with severe restriction and no restriction, but less favourable outcome with moderate restriction (awaiting publication – oral presentation Hamburg SSIEM 2007.)

Patients with hepatic GSDs can have an intensive dietary regimen with substantial carbohydrate intake. Some also have a fructose and lactose restricted diet that could compromise nutrition further. Such dietary management may have a significant impact on the completeness of the diet and itself lead to clinical problems such as obesity, diabetes, micronutrient deficiency and osteopenia.

The role of the physical properties of a starch on metabolic control

Uncooked cornstarch has been used in the dietary treatment of glycogen storage disease for over 20 years. It is a starch that is resistant to digestion and consequently releases glucose slowly as digestion progresses. In the cooked form, cornstarch gelatinises and the glucose polymer hydrolyses such that the starch becomes much less resistant to digestion.

Our research group have undertaken the development of a new starch for glycogen storage disease in a number of ways. We have tried various synthetic starches with different ratios of amylose/amylopectin, but have not found the desired metabolic profile. High amylose content, for example in high amylose maize, is difficult to digest. The use of products with delayed gastrointestinal time and the use of pancreatic enzyme supplements have also not appeared effective. Much of the work

of Mundy, Lee and Tester in the development of an appropriate starch is unpublished. However, of the several single case studies performed, the biochemical profile of one starch named WMHM20 appeared desirable. This starch is treated with heat under pressure with controlled moisture content and thus is a physically modified cornstarch. Englyst et al have described in-vitro studies of carbohydrate digestion that provide a quantitative classification of carbohydrate composition based on how resistant the starch is to digestion.[93] The method involves assay of simple sugars at various time points from a food and digestive enzyme incubation, reflecting in-vivo physiology. The Englyst method demonstrated that physical modifications render uncooked WMHM20 more resistant to digestion than uncooked cornstarch (67% and 60% respectively,) which could result in a better metabolic profile.[93]

Studies in the general population – glycaemic index and insulin sensitivity

In 1981, the group of David Jenkins introduced a landmark concept into nutritional management using carbohydrates.[94] They coined the term “Glycemic Index” or GI to represent how substantial the rise of plasma glucose was after a standardized carbohydrate meal. Prior to this publication, the authors commented that instructions for diabetic patients were based on the carbohydrate content of food irrespective of the properties of the carbohydrate. Setting glucose as the control substance, the incremental area under the glucose curve for the first 120 minutes was compared using identical amounts of different carbohydrates in healthy individuals. The results were expressed as a ratio to the glucose curve. Their results indicated that substances such as Lucozade and honey had incremental areas similar to glucose but legumes such as kidney beans and lentils had incremental areas 30% that of glucose. Whilst not explicitly mentioned in this first publication, the implication was that plasma insulin concentrations would be positively correlated to the glycemic index.

Regular consumption of high GI meals compared to isoenergetic low GI meals leads to higher 24 hour average glucose, insulin and glycosylated haemoglobin levels in both diabetic and non-diabetic individuals.[95] The greater relative release of insulin after a high GI meal as opposed to a low GI meal favours greater anabolic responses such as lipogenesis and glycogenesis. This is illustrated by significant elevations of the glucose, insulin and triacyglycerol responses in a test meal with rapidly available glucose (RAG) compared to slowly available glucose (SAG).[96] This study by Harbis et al examined insulin resistant individuals. They showed sustained elevation of the triacylglycerol for 6 hours when the RAG meal was taken with a concomitant rapid rise of apo B-100 and sustained elevation of apo B-48 for 6 hours when compared to a SAG meal. This association of elevations of glucose and insulin resulting in hyperlipidaemia is part of a "vicious cycle" whereby hyperlipidaemia also results in insulin resistance and hyperglycaemia.

Hyperlipidaemia is associated with increased adiposity and the adipose tissue plays an important regulatory role in fatty acid metabolism. This is mediated through the hormone adiponectin that is highly expressed specifically in adipose tissue.[69;97] It has an insulin sensitising role. Adiponectin deficient mice are prone to insulin-resistance and diabetes. In humans, a certain T to G polymorphism in exon 2 of the adiponectin gene can result in increased circulating concentrations of adiponectin even though this polymorphism confers no change in the amino acid structure.[69] Family studies of the silent G polymorphism showed an increased incidence of diabetes and increased weight.

These type of studies have important implications for patients with glycogen storage disease:

- Because patients take large amounts of carbohydrate, they are prone to hyperglycaemia and hyperinsulism.

- As discussed above, this may lead to hyperlipidaemia and increased adipose tissue.

- Patient also have elevations of plasma lipids for independent reasons to the general population - metabolism of surplus glucose-6-phosphate via acetyl coA to tryglycerides.

- Increased fat deposition may lead to insulin resistance independently of recurrent hyperglycaemia.

- Insulin resistance leads to complications such as poly-cystic ovarian disease and may result in other morbidities including obesity, non-insulin-dependent diabetes mellitus and cardiovascular disease.

1.4 Outline of thesis

The nutritional management of glycogen storage disease has often been called "the intensive regimen." The intensive regimen may not be without consequence. This thesis aims to characterise the intensive regimen and implement changes. Methods and participant characteristics are discussed in chapter 2. The studies performed were as follows:

Chapter 3 examines this nutritional management as a cross-sectional dietary survey of children and adults with GSD.

Chapter 4 looks at the short-term effect a new carbohydrate therapy has on biochemical indices of metabolic control focusing on glucose, lactate and insulin profiles.

Chapter 5 studies further short-term metabolic effects of starch by examining hydrogen breath test data and enrichment of $^{13}CO_2$ in breath.

Chapter 6 examines the implementation of the new dietary starch into subjects' long-term dietary regimen.

Chapter 7 brings together these various studies drawing conclusions and suggestions for further study.

Aim of Thesis

The objective of this thesis is to improve the dietary macronutrient and micronutrient balance of patients with .the hepatic glycogen storage diseases.with the adjunct of a novel starch created specifically for their treatment

Hypothesis – The blood glucose profile and secondary biochemical parameters are improved when modified cornstarch (WMHM20) is used compared to uncooked cornstarch. Dietary treatment also improves when WMHM20 is integrated into the dietary regimen of patients with the hepatic glycogen storage diseases.

CHAPTER 2 – METHODS AND PATIENTS

2.1 Background to methods used

Cornstarch Loads

The cornstarch load is widely used in the practice of metabolic medicine to determine the optimal dose and frequency of cornstarch. Whilst it has been widely used, it has never been validated as an investigative tool. This situation has arisen because it has been used as an intuitive profiling study, charting physiological responses to administered cornstarch. Biochemical profiles of patients with glycogen storage disease have been a feature since the enzyme defect was identified. In the 1960's and 70's, John Fernandes performed a series of informative studies, observing glucose and lactate responses to a variety of interventions[98;99] - his methods of investigation not only formed the foundation of subsequent starch load protocols, but influenced management of patients for decades to come.

By the time that Sidbury, Chen and colleagues reported their findings in trials using cornstarch in 1984, the methods that they used were established practice.[7] It is not clear from this publication what specific profiling protocol was used, but the paper reported that glucose and lactate were measured pre-intervention and after various doses of uncooked cornstarch were given. Measurement of insulin was not part of the protocol. The paper did make some pertinent comments about starch loads that are useful in the design of such studies. Firstly, they comment that if the baseline glucose is low, duration of normoglycaemia is reduced compared to higher baseline glucose. They also noted that heating starch and adding lemonade led to a rapid rise [100]and fall of the glucose. Finally, they noted that the 8 month old infant that they studied showed little response to uncooked cornstarch. The optimum dosing regimen and precise effect on metabolic control cannot be gauged from this first paper, but this information becomes clearer from subsequent publications. Hourly glucose, lactate and insulin profiles were performed in the study by Fernades' group in 1984

[101]. They also measured hydrogen breath excretion. This study replicated the benefit of uncooked cornstarch in terms of adequate glucose and lactate profiles, also demonstrating that insulin response was not excessive and that there was no evidence of malabsorption from the hydrogen breath test. The same group performed carbohydrate profiles using several substrates including glucose, barley groats, couscous, macaroni and cornstarch, which they published in 1988.[4] Patient data and pre-test management were not indicated in this paper. Blood glucose was measured at 30 minute intervals after administration of carbohydrate and lactate, insulin and hydrogen were measured at hourly intervals. The test was terminated if the blood glucose fell to less than 2.0 mmol/L or there was evidence of symptomatic hypoglycaemia. Of the sources of carbohydrate tested, uncooked barley groats had the best profile including a similar duration of "normoglycaemia," to uncooked cornstarch but better lactate suppression and less insulin rise. The authors justified a cut-off definition of hypoglycaemia of 2.0 mmol/L, by stating that subjects with GSD were less sensitive to hypoglycaemia if it occurred gradually. However, it would not now be considered ethically viable to have a definition of hypoglycaemia set well below higher evidence-based definitions of hypoglycaemia (2.6 mmol/L and above).[102-105] Subsequent studies also demonstrated satisfactory growth and metabolic control using uncooked cornstarch for several years.[20;45]

Cornstarch loads formed part of the protocol to determine the efficacy of cornstarch in a study published by Lee et al in 1996.[63] This study was a retrospective analysis of clinical practice. 14 patients' data were presented and all were managed by frequent feeds and overnight pump feeds. In the four to six weeks prior to the starch load, cornstarch was gradually introduced into the diet so that participants would become accustomed to the treatment. Patients were admitted to hospital the night before the starch load and given their nocturnal nasogastric feed. The following morning an additional 10g of carbohydrate was given as a "bolus" prior to termination of the feed

and uncooked cornstarch was administered within 1 hour of this. The dose of uncooked cornstarch was individualised. Blood glucose alone was reported as being measured hourly after the baseline samples were taken and the test ended when the blood glucose was below 3.0mmol/L. Using this protocol, the median duration of normoglycaemia was 4.25 hours.

As a method, the uncooked cornstarch load has arisen from the history of metabolic profiling in glycogen storage disease. There has been no set protocol or consistency between protocols. It has often been applied using whatever local resources are available, with investigators taking certain steps, such as controlling baseline data, to avoid inconsistent results.

Glucose – There has been much debate in the literature about the best means of measuring glucose. The first issue is which type of sample is being assessed – whole blood, plasma or intracellular. Plasma has relatively more water content with dissolved glucose than erythrocytes and consequently exhibits a higher glucose value than whole blood, depending upon the haematocrit. The World Health Organisation recommends a correction factor of 1.12 converting whole blood glucose to plasma values and assumes a haematocrit of 45%. This assumption is less appropriate if there is over-hydration or dehydration, anaemia or altered osmolarity of the blood. The intracellular compartment, rather than the plasma or whole blood is the physiological site where glucose action might appear most relevant. However this site is less accessible to measurement than blood or plasma.

There are 3 enzymatic methods of measuring glucose – glucose oxidase, glucose dehydrogenase and hexokinase.[106] The glucose oxidase method is the most common method to be used especially for point-of-care machines. However, it can be unreliable. The enzymatic reaction produces hydrogen peroxide and glucuronic acid – the measurement of hydrogen peroxide is often made by indirect means on

point-of care machines and this is more susceptible to error. Hydrogen peroxide or its oxidizing potential cause ionic or colour changes that are measured indirectly by point-of-care machines. These machines also use test strips that separate blood from the enzymatic surface. As a consequence, such machines are less accurate especially at lower concentrations of blood glucose, when there is limited production of hydrogen peroxide. It should be noted that this point-of-care machines have been designed to detect high blood glucose concentrations in diabetic patients. The YSI 2300 uses standard YSI direct glucose oxidase measurements. The main difference between it and other point-of-care glucose oxidase machines is that its membrane, impregnated with glucose oxidase is in contact with the sample.[107] The hydrogen peroxide is assayed directly by oxidation at the platinum sensing electrodes. This method has been validated against several others and is known to be reliable even at lower blood glucose concentrations. There are many advocates of the Haemocue™ system that measures haemolysed blood glucose using glucose dehydrogenase as an enzyme. It therefore provides a measure of intracellular glucose and whole blood. Intuitively, one would expect this method to be reliable as it incorporates haematocrit into its measurement. There are several favourable reports of good correlations with measurements of plasma glucose concentrations when the appropriate correction is applied.[108] However, there are some reports of less reliable correlations especially for hypoglycaemia.[109] The hexokinase method is considered by many as the gold standard method and is used by many laboratories. However, equipment is expensive, requires regular maintenance and is not portable.

For the protocols used in chapter 4.1 and 4.2, 2 mls of blood were collected into a sodium fluoride bottle with the sample subsequently being analysed by a hexokinase method (Vitros Fusion 5.1, Ortho-Clinical Diagnostic, High Wycombe, UK.). The fluoride in these bottles inhibits glycolysis thereby allowing stability of the sample for several hours.[110] A drop of blood from this sample was placed on a point-of-care machine (Advantage II, Roche diagnostics, Mannheim Germany)) as a screening tool

for hypoglycaemia. This gave an estimate of blood glucose within 2 minutes of sampling and several hours before a reliable result was obtained. The protocol of chapter 4.3 used the YSI 2300 (Yellow Springs, Ohio, USA) at the bedside. A reliable plasma glucose result was therefore available within 10 minutes of sampling.

Lactate Lactate is usually produced from pyruvate; it is a product of glycolysis in anaerobic conditions. When lactate is sampled from whole blood, glycolytic activity continues within erythrocytes , resulting in increased lactate production with time.. Consequently lactate either needs to be measured directly after sample collection, or intracellular anaerobic respiration needs to be arrested. Several laboratories therefore rapidly "deproteinise" samples to curtail respiration. The first study in this thesis used a lactate dehydrogenase enzymatic method. The sample was rapidly deproteinised using perchloric acid. The action of lactate dehydrogenase on the plasma formed NADH, which was quantified spectrophotometrically at 340 nm. Similarly to the measurement of glucose, there are discrepancies when whole blood lactate is compared to plasma – lactate is produced from peripheral tissues and diffuses with time into erythrocytes. Therefore, plasma lactate is often more elevated than haemolysed blood and is likewise dependent on haematocrit.

The YSI 2300 uses similar methodology for lactate measurement as it does for glucose measurement. The immobilised enzyme, lactate (L-2-hydroxyacid) oxidase produces hydrogen peroxide, which is measured directly by the sensor. This method has been validated against standard de-proteinising techniques and was used in the protocol of chapter 4.3 of this thesis.[111]

Stable isotopes - Natural enrichment of $^{13}CO_2$ in the breath – Many elements exist partly as "stable isotopes" which have extra mass due to additional neutron or neutrons within the nucleus of the atom. Carbon is present in 3 forms ^{12}C, ^{13}C and ^{14}C with 6, 7 and 8 neutrons within each atom respectively. By far the most abundant

is ^{12}C, which forms almost 99% of atmospheric carbon dioxide. 1.1% of atmospheric carbon dioxide has ^{13}C and there is only a trace amount of the radioactive ^{14}C. Some plants such as potatoes preferentially incorporate ^{12}C into their carbon skeleton. These plants fix CO_2 using ribulose-bisphosphate carboxylase forming a 3-carbon compound product via the Calvin pathway.[112] In warmer climates, plants such as maize have evolved a more energy efficient process of CO_2 fixation in which a 4-carbon product results from the C_4 or Hatch-Slack pathway.[112] These plants are more able to utilise ^{13}C within atmospheric CO_2 and hence incorporate more ^{13}C within their carbon structure.[113] The variation in the proportion of ^{13}C and ^{12}C within plants lends itself to research applications studying the fate of such ingested substances. In this context the substance ingested acts as a natural tracer.[114;115]

The appearance of $^{13}CO_2$ in the breath following the ingestion of a particular substrate labelled with ^{13}C depends upon the production of labelled CO_2 as a metabolic end-product. The ratio of $^{13}CO_2$:$^{12}CO_2$ in breath can be measured by isotope ratio mass spectrometry (IRMS) and, assuming constant or complete oxidation of the products of digestion and constant production of CO_2, the rate and extent of substrate digestion and absorption can be quantified.

The methodology of Isotope ratio mass spectrometry (IRMS) introduces cleaned and dried CO_2 into a vacuum system. It is then ionised, accelerated and deflected in an arc which is proportional to the ionic mass. The two commonest masses of CO_2 reflect the incorporation of stable isotopes, $^{12}C^{16}O_2$ with a mass of 44 and $^{13}C^{16}O_2$ with a mass of 45. The ions of different mass are separated and collected individually. They are converted into electrical currents and measured. The ratio of ^{13}C:^{12}C can then be calculated. The technique is suitable for detecting small differences in enrichment, but requires a relatively large sample (of the order of micromoles of CO_2).[115]

Continuous-flow isotope ratio mass spectrometry (CF-IRMS) was developed around 20 years ago for $^{13}CO_2$ analysis in expired air. It provides rapid automatic sample gas preparation using a smaller sample size than conventional IRMS but with better precision than gas chromatography / mass spectrometry (GC/MS) and precision comparable to IRMS.

^{13}C enrichment is commonly measured by reference to an international standard. That chosen is PeeDee Belemnite (PDB), a limestone of well-defined isotopic abundance. The relative difference is then expressed as delta per mil (‰). The ^{13}C:^{12}C ratio for PDB is 0.0112372. The natural abundance of ^{13}C relative to PDB within uncooked cornstarch, WMHM20 and Maxijul (SHS Ltd, Liverpool, UK) was quantified by elemental analyser isotope ratio mass spectrometry after complete combustion (Isoanalytical, Sandbach, UK). These were δ-‰ -11.13, -10.75 and -11.32 respectively. In a breath test experiment baseline ^{13}C is quantified in the breath and subsequent results are expressed as enrichment or delta over baseline (DOB).[115]

Most healthy individuals in Western Europe consume a diet low in C_4 derived carbon which is therefore low in ^{13}C, with a δ-‰ of approximately -24. As cornstarch has a δ-‰ of -11, oxidation of cornstarch can usually reliably be detected against a normal Western European diet, because CF-IRMS can detect differences as small as 0.1 – 0.2 ‰. This does not necessarily hold true for all individuals, some of whom may have different baseline ^{13}C enrichments.

The oxidation or utilisation of glucose derived from treatment starch is calculated from the ^{13}C enrichment in expired CO_2 using the formula on the following page:

Starch utilisation (mg/kg/min)[62] =

[δ ‰CO_2(t)- δ ‰CO_2(t_0) / δ ‰ starch- δ ‰CO_2(t_0)] X [VCO_2(t)X 180/(22.4X6XWt)]

δ ‰CO_2(t) – delta value of expired CO_2 at given time-point

δ ‰CO_2(t_0) – delta value of expired CO_2 at baseline

δ ‰ starch – delta value of starch after complete combustion

VCO_2 (t)- Mean rate of exhaled CO_2 (ml/min)

Molecular weight of glucose = 180g

1/22.4 – conversion to moles of CO_2

1/6 – conversion to moles of glucose

Wt– weight

It was not the aim of this study to accurately determine starch utilisation, but rather to measure the difference in utilisation between the two starches in the same individual. Therefore, a number of additional assumptions were made compared to previous studies. VCO_2 was not measured but derived from a previous publication at 227 ml/kg/min.[62] Actual body mass was used as opposed to lean body mass. In both instances, these were constants that applied to the calculation of utilisation for each of the starches used. Therefore, these assumed values did not influence the trend of utilisation in any direction.

The study described in chapter 4.4 used naturally enriched cornstarch and WMHM20 as a substrate with $^{13}CO_2$ / $^{12}CO_2$ enrichment being assessed by CF-IRMS.

<u>*Hydrogen Breath Test*</u> Hydrogen is a product of bacterial fermentation and cannot be produced or utilised by mammalian cells. It is absorbed into the bloodstream and excreted in the breath. Administration of a non-absorbable carbohydrate produces a rise in hydrogen production after a period of time and this is detectable in end tidal breath measurements. This is the basis of the hydrogen breath test and it has been

used as a measure of both small intestinal carbohydrate malabsorption and oro-caecal transit time.[116;117] It was first described for carbohydrate malabsorption 35 years ago and has been used to estimate the degree of malabsorption by the amount of substrate being fermented. It has also been used in young infants to measure the fermentation of lactose.

Breath hydrogen was assessed using a portable hydrogen device (micro-H_2 , Medisense, Rochester, UK) in the studies described in chapter 4.3 and 4.4. The sensor comprises a heated palladium silicon dioxide interface that dissociates hydrogen on contact leading to a decrease in potential which is registered by the monitor. The relative change in potential is compared to a known calibrated standard.[100] This method appears to be a reliable method of analysis of hydrogen compared to other methods with sensitivity of 1 ppm of hydrogen in the range 0 – 500 ppm.

Dietary Assessment The impact of diet on disease has been of substantial interest over the last 30 years with associations being made, for example, between high glycaemic index diets and diabetes and high fat diets with ischaemic heart disease and cancer susceptibility.[118-120] Despite these assertions, the valid quantification of dietary intake is difficult to ascertain. There are numerous methods of dietary assessment but each has inherent errors as a tool.[121;122] Methods that can be used include: direct observation of intake by a researcher, 24 hour retrospective recall, prospective diaries completed by research subjects for between 1 and 7 days, weighed records of intake and food frequency questionnaires. Errors can be divided into 3 categories – the assessment is not representative of the overall diet, inaccurate quantification of actual intake or assessment leads to change in eating behaviour.

The 24 hour retrospective recall involves a detailed interview with a specialist dietician. With appropriate time and examples of food portion measures, this can be an accurate assessment of what the subject took for 24 hours, but 24 hours may not represent what a person normally takes. Diet diaries can be variable, dependent on the intellectual ability of the subject completing the form. They may not be accurate if the subject forgets to enter data into the diary and are regarded as semi-quantitative if the subject uses household measures as an estimate. The longer a subject can accurately record what they take, the more representative the record is of the long-term diet, but compliance decreases with longer assessments. For this reason, many opt for a 3 or 5 day record as opposed to a 7 day record. A 3 day record should include a weekend day and 2 working days for those working full-time in order to represent what is normally taken in the week. There is potential for a diary to alter eating behaviour if for example a subject opts for an easy to record “ready meal” in preference to recording an intricate recipe. Direct observation may seem the most reliable method, but is labour intensive and prone to subjects altering behaviour to what the subject feels the observer expects. A food frequency questionnaire has a list of foods (sometimes greater than 100,) and asks subjects to complete how often and how much of each food subjects have. This method can be more representative of long-term diet because it considers longitudinal intake over months.[123] However at best, it is semi-quantitative. It has a use for large epidemiological studies where large amounts of data can be assessed at relatively low cost, but is less appropriate for smaller studies, especially from an ethnically diverse population such as London, where the questionnaire may not represent the dietary intake from different cultures.

The methods used in this study are written diet diaries for 3 days in adults and 5 days for children, which were examined immediately on receipt by a state registered dietitian. After review, inconsistencies or clarification was made either with the patient or carer by face-to-face questions or by telephone. In this manner, data collected

were a composite of prospective diet diaries and recall. Data were entered onto specialist software (Dietplan 6, Forestfield Software, Ltd. Horsham, UK. – referenced to McCance and Widdowson's The Composition of Foods.)[88] Output from diet diaries were collated and comparisons made with both UK dietary reference values and UK national dietary surveys.[66;67;124]

Dietary Reference Values

In the United Kingdom, the panel on dietary reference values was set up in 1987 by the Committee on Medical Aspects of Food Policy (COMA) to review recommendations for food energy and nutrient intake. The panel created four working groups examining requirements for energy and protein, fat and carbohydrate, vitamins and minerals. The working groups reported back to the panel culminating in the publication in July 1991 of the COMA report on Health and social subjects 41.[124] The panel departed from previous reviews that instructed "recommended daily amounts" (RDA) which was a specific quantity of recommended intake, to a range of dietary values that people should take in order to avoid problems. COMA suggested that dietary reference values (DRV) would comprise "reference nutrient intakes" (RNI) which has been set as 2 standard deviations above the estimated average requirement (EAR) and is deemed sufficient to prevent problems in most people. Using a Gaussian curve, COMA also suggested that there should be a lower reference intake (LRNI.) The panel felt that if intake is habitually below this value then untoward problems were likely to ensue in the majority of people. The problems that could occur are specific to the nutrient concerned but categories of requirement include:

a) the nutrient intake required to maintain a circulating level or degree of enzyme saturation or tissue concentration;

b) the intakes of a nutrient which are associated with the absence of deficiency diseases;

c) the intake of nutrient associated with an appropriate biological marker of nutritional adequacy.

The basis of the DRV for different nutrients consequently varies depending on the nutrient; this thesis compares values obtained in patients with published DRVs. In most instances either the RNI or EAR are published and used. In some instances, the LRNI is quoted too. Age and gender related EAR for energy intakes are given in appendix 1.

National Diet and Nutrition Surveys

The DRVs are values that an expert panel recommend subjects take. These expert considered guidelines are not necessarily based on rigorously derived evidence. The national diet and nutrition surveys are assessments made to see what nutrition people actually take. The first surveys commissioned by the UK Department of Health and Ministry of Agriculture, Fisheries and Food were carried out in 1986 /7 and were performed on adults. This was published in 1990 and due to its success; a complete programme of surveys was instituted as part of the UK Office for National Statistics. The first of these was performed in children aged 1½ to 4½ performed in 1991 and published in 1995. After feasibility studies, the next survey, on children aged 4 to 18 years, was carried out in 1997 and published in 2000. A further survey was performed on adults in 2001 and published in 2004.

The programme is a well resourced national survey. The methods of these surveys are very rigorous with great attention paid to detail. Each survey had greater than 2000 participants and subjects were stratified in sub-analyses by age, gender, social class and region. Selection processes of participants were diversified across regions and once a target region was identified, selection of individual participants was random. Dietary assessments were made by specialised teams of field workers and a weighed intake for 7 days was performed. Each family was issued with

standardised calibrated weighing scales and educated in the use of completing diaries with weighed foods. Field workers visited households 24 hrs after the diary commenced offering support and advice. They visited several other times in the 7 day period if further support was required. If a subject ate out, portion sizes and ingredients were ascertained directly from the retailer.

The dietary survey indicates normal eating behaviour in the UK. For the purposes of this thesis, this is a control population against which patients with GSD have been compared. The 2 surveys used are the children survey of 1997 published in 2000 and the adult survey performed in 2001 and published in 2004.[66;67] Details of data used are indicated in appendix 1.

2.2 Patients

A number of studies are described in this thesis. All patients were recruited from the 3 tertiary metabolic units in London: children were from Great Ormond Street Hospital and The Evelina Children's Hospital and adults from the National Hospital for Neurology and Neurosurgery, Queen Square. All adults recruited gave voluntary informed written consent to the studies after being issued with participant information sheets. The carers of children under the age of 16 gave written informed consent, having first been issued with a carer's information sheet. Children between the age of 10 and 16 were issued with an age appropriate participant information sheet and were asked to give consent if deemed to be competent enough to do so by the researcher. This written consent was taken in addition to parental consent. Children under the age of 10 had a full verbal explanation of the study by the researcher and gave verbal consent to the studies.All patients that took part in these studies had evidence of fasting hypoglycaemia, with either enzymatic demonstration of glycogen storage disease from liver or from leucocytes (GSD III,) or recognised mutations in the appropriate genes. All patients took uncooked cornstarch in their dietary treatment regimen. Characteristics of patients are described on table 2.

ID	Age yrs	SEX (M/F)	GSD Type	Diagnosis (Gene / Biopsy)	Presentation & age of diagnosis	Normal Carbohydrate use	Medications	Complications
A	3	F	IA	c.79delC/N G188S/N	Hypoglycaemic fits age 3 days, hyperlipidaemia	D - 30g UCCS TDS N - 100g polymer	Allopurinol	Nephrocalcinosis Died post studies
B	4	F	IA	Homozygous C150-151delGT	At birth – sibling of C	D –25g UCCS QDS N – 90g polymer	Multivitamins	
C	5	M	IA	Homozygous C150-151delGT	FTT, large abdomen, recurrent infections	D - 35g UCCS TDS N 105g polymer	Multivitamins	
D	5	M	IA	R83C C347X	Haematuria, FTT, lactic acidosis	D – 40g UCCS TDS N – 105g polymer	Allopurinol Multivitamins	
E	7	M	IA	Biopsy	Sweaty, FTT, large abdomen age 2.5 yrs	D – 50g UCCS TDS N – 150g polymer	Allopurinol Multivitamins	Obese
F	12	M	IA	Biopsy	Large abdomen 3 weeks Resp. distress 7 months	D – 60g UCCS TDS N – 125g polymer	none	Behavioural problems
G	21	M	IA	Homozygous C150-151delGT	Diarrhoea and FTT at 6 months. Seizure disorder	D – UCCS 60g BD N – 125g polymer	Allopurinol,Ramipril , Clonazepam Carbamazepine	Intellectual impairment, Seizures
H	22	F	IA	Homozygous C150-151delGT	Irritability at 8 months	D – 30g UCCS BD N – 55g UCCS	Allopurinol Ramipril. Vit B12	PCOD Renal impairment
I	22	M	IA	Homozygous R83C	Hypoglycaemia – 6 months FTT	D – UCCS – 65g BD N – 160g polymer	Allopurinol Salazopyrin	Inflammatory bowel disease
J	23	F	IA	Homozygous C150-151delGT	FTT, hypoglycaemic fits, large abdomen at 7 months.	D – UCCS 40g QDS N – UCCS 80g	Allopurinol 300mg Multivitamins	Short stature Chronic fatigue Renal calculi
V	25	F	IA	Homozygous R83C	Hypoglycaemic seizures – 2 months	D – UCCS 40g QDS N - 120g Polymer	Allopurinol 300g Multivitamins	PCOD

Table 2.1 – Patient characteristics

ID	Age yrs	SEX (M/F)	GSD Type	Diagnosis (Gene / Biopsy)	Presentation & age of diagnosis	Normal Carbohydrate use	Medications	Complications
K	33	M	IA	Biopsy	18 months – irritability and hepatomegaly	D –UCCS 50g QDS N – UCCS 50g X2	Allopurinol 300mg, simvastatin, codeine, lansoprazole	Hepatic adenoma (resected) Chronic scar pain Heartburn
W	41	F	IA	Biopsy	Hepatomegaly Short Stature age 1	N – UCCS 30g	None	
X	42	M	IA	Biopsy	Hepatomegaly FTT – 18 months	N – UCCS 40g	None	Short Stature
L	34	M	IA	Biopsy	Enlarged liver, FTT at 6 months	N – UCCS 60g	None	
M	47	M	IA	Biopsy	FTT age 6 months	N – UCCS 60g	Allopurinol 300 mg Enalapril, Ezetimibe Atorvastatin,	Hepatic adenoma Renal impairment TIA - ? cause Hyperlipidaemia
N	13	M	IB	Biopsy	Age 3 yrs - Lethargy, Developmental delay	D – 80g UCCS BD N – 175g polymer	Allopurinol 50mg	
O	14	F	IB	1042delCT 480delA	Large abdomen, listless Resp infection from birth - 3 months (biopsy)	D – 60g UCCS TDS N – 60g UCCS X2	Allopurinol 150 mg Septrin, GCSF Thalidomide	Crohns Disease Bronchiectasis
P	15	F	IB	496G>A 1393G>A	Hepatomegaly 18 months	D – 25g UCCS BD N – 45 g UCCS X2	Allopurinol 150mg GCSF	
Y	17	M	IB	Homozygous IVS7+2delAAGT	Hepatomegaly Family history (Z & Z1)	D – 40g UCCS TDS N – 140g polymer	Allopurinol	Obese
Z	19	F	IB	Homozygous IVS7+2delAAGT	Hepatomegaly (Family History (Z)	D – 30g UCCS TDS N – 120g Polymer	Allopurinol GCSF	Anaemia Recurrent infections

Table 2.1 continued

ID	Age yrs	SEX (M/F)	GSD Type	Diagnosis (Gene / Biopsy)	Presentation & age of diagnosis	Normal Carbohydrate use	Medications	Complications
Z1	25	M	IB	Homozygous IVS7+2delAAGT	Hepatomegaly 6 months Recurrent infections	D – Poor compliance N -60g UCCS	Allopurinol GCSF	Anaemia, fatigue Crohns Disease Transplant list
Q	27	M	IB	Homozygous IVS7+2delAAGT	Age 3 years	D – 40g UCCS QDS N – 40g UCCS BD	Allopurinol 200 mg GCSF	Short stature Diarrhoea
R	35	M	IB	R415X 2nd unknown	Large abdomen at 2.5 yrs Sibling of S	D- N- 30g	Allopurinol – 300mg	Hepatic adenoma Chronic anaemia
S	38	F	IB	R415X 2nd unknown	5 yrs - Large abdomen, sweating, irritability	D – 20g UCCS BD N – 40g	Ferrous sulphate	Chronic anaemia
T	3	F	IIIa	Liver biopsy	Recurrent Hypoglycaemia – 2 months	D – 30g UCCS TDS N – 75g polymer	Multivitamins Protein supplement	Myopathy
U	12	M	IIIa	Liver Biopsy	Lethargy, sweatiness at 5 months.	D – 45g UCCS BD N – 70g polymer & milk	Multivitamins	Overweight

Table 2.1 Recruited subjects age, disorder and treatment characteristics at time of participation at first study.
ID – identity- referred to in subsequent chapters

D = Day; N = night;

UCCS – Uncooked cornstarch;

FTT – failure to thrive; PCOD – Poly-cystic ovarian disease.

GCSF – Granulocyte Colony Stimulating Factor

** Details in chapter 3.1*

CHAPTER 3 – DIETARY TREATMENT OF GSD

Introduction

This chapter assesses the dietary management of patients with glycogen storage disease. There are specific targets of carbohydrate intake for patients with GSD which originated in studies performed in the 1960s and were confirmed by calculations from endogenous glucose production studies performed predominantly in children. It is recommended that 65% of dietary calories should be in the form of carbohydrate. This quantity of carbohydrate intake is greater than that recommended or taken by the general population. In our clinical experience, adults have a limited pattern of eating learnt from childhood, that may not necessarily be appropriate in adult life. Excessive carbohydrate intake, especially of low glycaemic index foods are well recognised to have adverse health outcomes and it is therefore important to characterise the GSD diet in order to see whether improvements can be made. [71;95;119] Children in particular can often require 2 hourly feeds of glucose polymer, which has a short duration of action. Adults may take uncooked corn-flour regularly. Both glucose polymer and uncooked corn-flour have little additional nutritional value apart from providing carbohydrate. Therefore, the potential for dietary insufficiencies exist if the remainder of the diet is not complete.

There are several dietary targets of management that have been established for individuals from the general population as a whole as well as specific targets for patients with GSD. In the UK, population targets are referred to as dietary reference values comprising the estimated average requirement (EAR) and “reference nutrient intakes” or RNI which has been set as 2 standard deviations above the EAR and is deemed sufficient to prevent problems. (chapter 2.1)[124]

The basis of the RNI for different nutrients varies depending upon the nutrient; it is a nutritional target that it is projected will prevent nutritional diseases due to

insufficiency. However, the converse of nutritional disease due to excess is not addressed by RNI. Because these recommendations developed in 1991, do not address surplus intake, this chapter also examines patients' nutritional intake compared to what is taken on average within the UK by comparison with national dietary surveys.[66;67;124] Further details on dietary reference values and the national dietary surveys are in chapter 2.1. Biochemical and clinical indices of nutrition are also presented.

This chapter is divided into four sections. Chapter 3.1 describes several short case histories demonstrating how patients integrate dietary treatment into their lifestyle. Several different cases are selected, demonstrating how people can be affected very differently with GSD. They show that death can still occur, that morbidities such as end-stage renal failure or poor metabolic control leading to hepatic transplantation may still occur. Even with good compliance, if a fixed insult occurred early in life, neurological complications can ensue and that compliance means intensive therapy even in adult life. The first case demonstrates the therapy before implementation of currently accepted treatments of naso-gastric feeds and uncooked cornstarch Chapter 3.2 describes a cross-sectional survey of the diets of children and adults with GSD I and III. Chapter 3.3 discusses the results. Chapter 3.4 summarises the findings and conclusions.

3.1 – Dietary Case Studies

Subject K – Subject K is a 40 year old factory worker with GSD Ia, born in 1969 He is single and lives at home with his parents. He was the fifth of six children with no other child being affected. Birth was uneventful. At the time of weaning his mother noted that he had sweaty episodes with periods of eyes rolling. A protuberant abdomen was also noted. After several admissions to a local hospital, the diagnosis of GSD Ia was made by liver biopsy performed at a tertiary hepatology unit at the age

of 18 months. In the early 1970's he was placed on sucrose and fructose restricted diet in early childhood, but was never treated with overnight continuous pump feeds. His parents recall waking him up for glucose feeds overnight when he was at school; he only started taking uncooked cornstarch when he started work at the age of 18. He recalls always needing to eat carbohydrates regularly as part of treatment. At the age of 34 years, several adenomata were seen within the liver and these were surgically removed during a partial hepatectomy. Since this time, he has had chronic pain at the site of the scar requiring treatment with low dose opiates. He also has symptoms of heartburn and intermittent diarrhoea and constipation. He is treated with lansoprazole and periodically takes laxatives. He was referred to the tertiary metabolic unit at the age of 34, after the adenomata had been resected. He now takes UCCS five times a day. An example of a daily dietary record, taken from an actual diet diary is given below:

- 05:00 – 50g starch
- 6:15 – 1 weetabix with milk & tea
- 09:00 – 50g starch – Fish-paste sandwich
- 13:00 – 50g starch
- 16:30 – 2 slices of toast and 1 egg
- 17:00 – 50g starch
- 20:00 – Chicken and chips 50g starch
- 23:00 - 100g starch and half bottle of lucozade.

Subject Z

Subject Z is a 27 year old male, born in 1982, being the second of four children. Both he and a younger sister have GSD Ib, reported in a previous publication.[125] He is single and lives at home with his parents. He developed hepatomegaly and irritability at the age of 9 months and had recurrent episodes of infection. He was treated initially with nocturnal nasogastric pump feeds of glucose polymer and frequent daytime carbohydrate. As a child he was very reluctant to insert nasogastric tubes

and as a teenager completely refused to do so. Due to recurrent infections, recombinant GCSF therapy was commenced at the age of 10. At the age of 12, he had frequent loose stools – colonoscopy revealed inflammatory bowel disease and sulphasalazine was commenced. By the age of 14, both daytime and nocturnal uncooked cornstarch were prescribed, but adherence to dietary and medical therapy was sub-optimal. Z preferred taking commercially available high glucose drinks rather than prescribed therapy. Despite frequent hospitalisations for recurrent infections, Z was academically competent and commenced a degree in Computer Science at University at the age of 19 years. However, complications became increasingly more prominent as a young adult as he became chronically fatigued, under-weight with frequent episodes of infection and severe diarrhoea. Assessments demonstrated chronic anaemia (haemoglobin 7g/dl – range 13 – 15). At the age of 22 he took a temporary break from his studies in order to regain health but after 2 years, he continued not to be able to return back to university. Due to his chronic ill health, he was placed on the liver transplant programme at the age of 24 and subsequently received an orthotopic liver at the age of 25. An example from his dietary record, before his liver transplant, is given below:

- 04:00 – 200mls lucozade (– 18% sugar)
- 08:00 – 90g cornstarch with 10mls ribena (13% sugar) and water
- 10:30 – Full plate of grilled chicken and plain rice & 200 mls lucozade
- 16:00 – Milkshake 220 mls (14% sugar) - small portion rice and chicken
- 19:30 – 1 banana & 220mls of milkshake
- 22:00 – Full plate of rice and chicken. 200mls mixed fruit juice (8% sugar)
- 24:00 - 90g cornstarch with 10mls ribena (13% sugar) and water

Subject V –Subject V is a 28 year old woman, born in 1981 with GSD type Ia. She is single and lives at home with her parents. She presented at 6 months of age with irritability and hypoglycaemic seizures. She was treated from this age with nocturnal glucose pump feeds and frequent daytime meals. She was very compliant with

therapy; by the age of 3 years she was able to pass her own naso-gastric feeding tube. In childhood, she had frequent episodes of symptomatic hypoglycaemia if therapy was discontinued or during inter-current illness. Such episodes still occur periodically in adult life. By the age of 7, she commenced regular therapy with uncooked cornstarch but has never discontinued nocturnal pump feeds. She was academically gifted at school and completed her degree and masters and is completing a doctorate in her chosen field. Her medical complications include poly-cystic ovarian disease. An example of her dietary record is given.

- 08:30 – discontinue nocturnal feed 20mls of 25% glucose polymer.
- 08:45 – 20g rolled oats with 150 mls semi skimmed milk
- 09:00 – 50g cornstarch and 200 mls semi skimmed milk
- 11:00 – 40g fruit yoghurt
- 13:00– 4 slices roast pork, 75g cooked pasta with ½ teaspoon of tomato sauce. 50g cornstarch with 200mls semi-skimmed milk
- 16:30 – banana
- 17:00 - 50g cornstarch and 200 mls semi skimmed milk
- 18:30 – 2 lamb chops, mashed potato and ½ boiled carrot.
- 21:00 - 50g cornstarch and 200 mls semi skimmed milk
- 23:30 – 25 g cornstarch and 200 mls semi skimmed milk
- 00:30 – 40mls/hr of 25% glucose polymer via nasogastric tube until 08:30 with sachet of multi-vitamin preparation.

Subject H Subject H is a 26 year old woman, born in 1983 with GSD Ia. She is married and lives with her husband and an extended family. She presented at 9 months of age with a protruberant abdomen and failure to thrive. She was treated with nocturnal glucose pump feeds and frequent daytime meals. Uncooked cornstarch was used in the dietary regimen from the age of 10 years. As a child and young teenager H was very compliant with therapy. In the peri-pubertal years, she tried to limit her calorie intake, being conscious of the effect large amounts of

carbohydrate were having on weight. She discontinued nocturnal pump feeds and took some cornstarch, but therapy was inconsistent. Despite poor adherence to therapy, she attended university, qualifying with a degree in Genetics. After qualification, she commenced full-time employment and recently married. Over her adult life, weight has fluctuated considerably. She has had proteinuria and hypertension for several years and over the course of the last year, there has been a dramatic decline in the glomerular filtration rate to 24 mls/min/1.73 m^2. This has been accompanied by consistent elevation of the plasma creatinine. She takes maximal reno-protective medications. An example of her dietary record is given. She is a vegetarian.

- 06:30 – 25g cornstarch with 100 mls water
- 07:15 – ½ bowl of fruit and fibre cereal with 100mls semi-skimmed milk
- 11:15 – 1 chocolate glazed, custard filled doughnut ; 250 mls water
- 13:45 – 4 strawberries; 3 slices of French bread – 2 with hummus and 1 with cheese dip. 5 potato crisps.
- 14:45 – 25g of cornstarch with 100mls water.
- 16:30 – 50g of pasta, 3 spoons of pesto sauce, small portion of mixed salad, 1 banana and 1 tangerine.
- 20:30 – 20g of potato crisps
- 21:00 – 1 Indian style fried dumpling made from mixed milled grains, (millet, rice, wheat and barley) and chick peas (12g). Small bowl of boiled rice and 20mls of low fat natural yoghurt
- 21:45 – 7 chocolate finger biscuits. No nocturnal cornstarch

Subject G Patient G is a 24 year old man with GSD 1a. He is single and lives at home with his parents. He was diagnosed at the age of 6 months having had a history of irritability and seizures. In early childhood he had several life threatening problems with hypoglycemia and seizures. He had a tendency to severe diarrhoea and vomiting resulting in admissions with hypernatremic dehydration. At the age of

three, he had one particularly severe episode of encephalopathy presumed to be related to protracted hypoglycaemia. On this occasion, persistent elevation of intracranial pressure was measured by invasive intra-cranial monitoring. Residual learning difficulties and seizures ensued. He continued to have refractory epilepsy throughout much of childhood and adult life. He has moderate learning difficulties. As an adult he has supervised training for work within restaurants organized by a learning disability team, but has not developed skills sufficient to live independently. He has limited understanding of his underlying condition and periodically fails to adhere to prescribed therapy. He continues to take a nocturnal pump feeds and daily doses of uncooked cornstarch. An example of his dietary record is given.

- 07:00 – Off nocturnal feed50g cornstarch & 250mls of milkshake (10 % sugar)
- 11:50 – 2 wheat wholemeal chapatis with butter. 1 dessert spoon of black bean curry with 1 tablespoon of mango and carrot sweet chutney.
- 13:15 – 50g cornstarch with 250 mls of milkshake
- 13:30 Indian condensed milk ice cream (Kulfi) – 80g and 200mls whloemilk with 8 fresh strawberry milkshake.
- 19:30 – Vegetable quarter pounder – (from packet)
- 23:30– Vegetable burger (from packet) and 500 mls lucozade sport (3.5% sugar; 6.4% carbohydrate.)
- 23:45- 80g Kulfi
- 24:00 35% glucose polymer solution at 40ms/hr until 07:00

Subject A Patient A was a three year old girl at the time of this study. She presented at the age of 3 days with hypoglycaemic seizures and hyperlipidaemia. Diagnosis was made on the basis of the identification of pathogenic mutations in G6PC gene. She remained in hospital for the first 4 months of life in order to establish a safe enteral feedng regimen without episodes of hypoglycaemia. She remained on two hourly feeds with overnight continuous feeds of glucose polymer until the age of 2 years. Prior to this, uncooked cornstarch was introduced but was poorly tolerated due

to abdominal pain. Fasting tolerance improved to 4 hours without hypoglycaemia at the age of 3 years when she participated in these studies. 6 months later at the age of 4 years she became comatose overnight at home. This episode was presumed to be related to hypoglycaemia, possibly due to failure of the overnight pump feed. She was urgently admitted to hospital but unfortunately died shortly thereafter. An example of the dietary record provided for this study is shown below:

- 08:00 – Off Nocturnal pump – 10g bolus of glucose polymer
- 0815 – Three table spoons of tinned custard. 5g of crisps, 100 mls of blackcurrant and apple juice.
- 09:00 – 30g of uncooked cornstarch mixed in 100mls of semi-skimmed milk
- 10:30 – 1 hash brown, 1 tablespoon of baked beans, 50 mls of diluted blackcurrant cordial.
- 13:00 – 120g of full cream fruit yoghurt. 10 grapes. 50 mls of orange and mango smoothie.
- 14:00 – 30g of uncooked cornstarch mixed in 100mls of semi-skimmed milk
- 17:30 – "Take away" Fish and chips – 10 chips, 1/3 small battered cod.
- 18:00 – 30g of uncooked cornstarch mixed in 100mls of semi-skimmed milk
- 20:00 - 100g glucose polymer in 500mls water at 40 mls/hr for 12 hrs.

The dietary management of glycogen storage disease as indicated by these dietary diaries is intensive. In childhood, parents are concerned about the real risk of hypoglycaemia related morbidity and mortality, which persists to some extent in adult life. Chapter 3.2 attempts to collate the analyses of these data for the population of patients with GSD that were recruited to the cross-sectional survey for this thesis.

Chapter 3.2 A cross sectional survey of the diets of children and adults with hepatic glycogen storage disease

Aim To document current nutritional intake of patients with GSD and make recommendations to improve the macronutrient and micronutrient balance of the diet in line with peers and current guidelines.

Methods

This study was part of other studies investigating nutritional management of the hepatic glycogen storage diseases and was performed in 2-stages – the assessment of diet in children aged 3- 15 years old with hepatic GSDs and the assessment of diet in adults with GSD type 1. All patients took uncooked cornstarch as part of their dietary regimen. There was ethical approval for these studies from the joint ethics committee of Institute of Child Health & Great Ormond Street Hospital and the joint ethics committee of The National Hospital for Neurology & Neurosurgery & Institute of Neurology. Written informed consent was taken prior to collection of data. Carers of children were instructed on how to complete a diet diary by a specialist nutritional nurse when they attended the hospital and were issued with a 5-day diet diary. Participants were encouraged to document all recipes of home prepared foods with estimates of quantity ingested, and retain, for inspection, nutritional information on packaging from processed foods. Diaries were performed on 2 weekend days and 3 week days. Blood was drawn at this visit for the following tests:

- Full blood count
- Iron
- Vitamin B12
- Red cell Folate
- Thiamin
- Vitamin D
- Zinc
- Copper

-Manganese

- Selenium

- Calcium

- Albumin

- Phosphorus

-Liver Function Tests

Patients and carers re-attended the hospital between 7 and 28 days after issue of the diet diary in which time the diary was completed. At the second visit, the diary was discussed between the nurse and carer to ensure data were complete. All diary data were anonymised and entered onto specialist software (Dietplan 6, Forestfield Software, Ltd. Horsham, UK. – referenced to McCance and Widdowson's The Composition of Foods.)[88], by an experienced state-registered dietician. Data were expressed as an output giving mean daily intake for the five days.

Adults were asked to complete a 3-day diet diary incorporating one weekend day and 2 week days. They attended the hospital in a similar manner to the children and blood was taken for the same tests, as described above. Participants were instructed on diary completion by a state-registered dietician who also examined diaries returned by post. If any clarification was needed on written information, participants were contacted by telephone and queries were discussed. Data were analysed using Dietplan 6 and expressed as mean daily intake.

Results - *Patients*

Demographic data are presented in table 3.1. The identity of the patient is referred to in subsequent tables.

Table 3.1 – Patient characteristics

Identity	AGE (yrs)	Weight (kg)	Height z-score	BMI z - score	Gender	GSD Type
A	3	16.3	0.1	-1.6	F	1a
B	4	20	2.1	0.79	F	1a
C	5	21.3	0.44	1.23	M	1a
D	5	31.3	0.35	> 3.0	M	1a
E	7	37	-0.60	> 3.0	M	1a
G	24	58	-0.12	-0.05	M	1a
I	25	87	1.88	0.92	M	1a
H	25	63.2	-1.28	0.57	F	1a
V	25	76.4	0.88	0.68	F	1a
K	38	55	-3.00	-1.00	M	1a
W	41	72	0.05	0.44	F	1a
X	42	74.2	- 0.27	-0.43	M	1a
M	13	60.9	-1.29	1.64	M	1b
O	14	56	-1.77	0.34	F	1b
P	15	52.2	-0.56	0.20	F	1b
Y	17	86.3	-2.85	2.69	M	1b
Z	19	61.7	-1.12	0.60	F	1b
Z1	25	49.1	-3.0	-1.48	M	1b
Q	27	50.4	-2.55	-1.59	M	1b
R	35	55.3	-2.85	-1.04	M	1b
T	3	18	0.03	0.15	F	3

The following tables indicate both absolute amounts quantified per day from diet diaries as well as relative contributions. Data are also presented relative to dietary reference values from data prepared by the Committee on Medical Aspects of Food Policy (within the UK), as well as relative to UK national diet and nutrition Surveys.[66;67] None of the patients participated in occupations or recreational activities that classified them as being physically active. Hence, no correction for increased

energy expenditure was necessary. Data from the national diet and Nutrition surveys are age and sex matched – data taken from these surveys and used in comparisons in the following tables are presented in appendix 1. The data presented in the following tables therefore reflects what nutritional deficiencies are possible in these patients and the extent to which the diet differs from the rest of the population.

Results of Dietary Analyses

Table 3.2 Energy Intake estimated from diet diaries compared to estimated average requirements and stratified into contributions of macronutrients.

ID	*Age*	Total Energy Intake	*Estimated Average Require-ment*	*Percent Intake* relative to EAR	*Percentage contribution to total energy by:*		
					CHO	Protein	Fat
A	3	1597	1165	137	74	6	19.9
B	4	1398	1165	120	74.6	8.5	16.9
C	5	1695	1715	99	73.9	7.4	18.8
D	5	2574	1715	150	64.3	10.8	24.6
E	7	2075	1715	121	70.4	8.7	20.9
G	24	3032	2914	104	59.5	9.5	31
I	25	3000	3020	99	75.7	14.1	10.2
H	25	1570	2079	76	59.2	8.2	32.5
V	25	2946	2294	128	69.2	17.8	13
K	38	2415	2599	93	77.4	7.6	15
W	41	1861	2079	90	68.7	12.6	18.7
X	42	2551	2914	88	66.1	12.9	21
M	13	2940	2220	132	68.2	12.8	18.9
O	14	2186	1845	118	64.7	9.8	25.5
P	15	2944	2110	140	66.8	12. 2	21.2
Y	17	3502	2755	127	58.8	11.4	29.7
Z	19	2147	2217	97	70	8.7	21.4
Z1	25	2942	2561	115	71.1	18.1	11.3
Q	27	3044	2561	119	59	14.2	26.8
R	35	1870	2599	72	55.9	14.9	29.2
T	3	1075	1165	92	76.6	8.6	14.8
			Mean	**110.3**	**67.8**	**11.1**	**21.0**
			St Dev	**21.5**	**6.5**	**3.4**	**6.4**

Table 3.2 Indicates increased energy intake compared to estimated average requirements, with carbohydrates providing most of total energy intake.

Figure 3.1 – Relative contribution to total energy intake of macronutrients assessed by this study of GSD patients and the wider population.

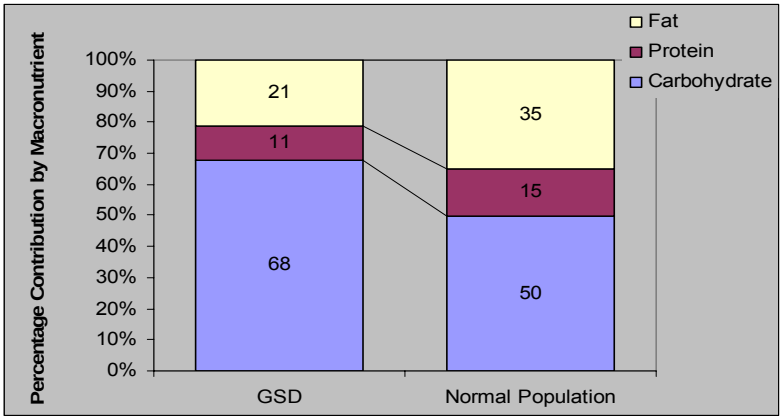


Table 3.3 Carbohydrate intake relative to national diet and nutrition survey and estimated average requirements

ID	*Age*	*CHO Intake (g)*	*Mean survey Intake (g)*	*Percent Relative To survey*	*CHO EAR (g)*	*Percent Relative To EAR*
A	3	298	191	156	187	159
B	4	275	191	144	187	147
C	5	321	209	154	276	116
D	5	425	209	203	276	154
E	7	388	248	156	276	141
G	24	481	273	176	446	108
I	25	598	273	219	464	129
H	25	250	196	128	312	80
V	25	537	196	274	350	153
K	38	500	279	179	391	128
W	41	340	206	165	312	109
X	42	450	279	161	446	101
M	13	529	271	195	340	156
O	14	377	228	165	276	137
P	15	524	214	245	317	165
Y	17	545	301	181	419	130
Z	19	393	206	191	336	117
Z1	25	542	277	196	385	141
Q	27	478	277	173	385	124
R	35	278	279	100	391	71
T	3	214	191	112	187	114
			Mean	**174.9**		**127.6**
			Std Dev	**40.6**		**25.2**

Table 3.3 Indicates dramatically increased carbohydrate intake compared to the general population and estimated average requirements.

Table 3.4 Protein intake relative to national diet and nutrition survey and estimated average requirements.

ID	*Age*	*Protein Intake (G)*	*Mean Survey Intake*	*Percent Relative To Survey*	*Protein RNI (g)*	*Percent Relative To RNI*
A	3	22.7	44.5	51	14.5	157
B	4	28.9	49	59	14.5	199
C	5	30.7	49	63	19.7	156
D	5	68.1	49	139	19.7	346
E	7	44.2	54.8	81	19.7	224
G	24	71.9	77.8	92	55.5	130
I	25	104	77.8	134	55.5	187
H	25	32.6	58.7	56	45	72
V	25	129.3	58.7	220	45	287
K	38	46.1	90.1	51	55.5	83
W	41	58.2	65.1	89	45	129
X	42	82.6	90.1	92	55.5	149
M	13	93.4	64	146	42.1	222
O	14	53.7	52.9	102	41.2	130
P	15	89.7	54.8	164	45.4	198
Y	17	99.4	76.5	130	55.5	179
Z	19	45.6	59.9	76	45	101
Z1	25	130	90.6	143	55.5	234
Q	27	108	90.6	119	55.5	195
R	35	69.3	90.1	77	55.5	125
T	3	22.4	44.5	50	14.5	154
			Mean	**101.6**		**174.1**
			Std Dev	**44.9**		**65.8**

Table 3.4 Indicates that patients broadly take similar quantities of protein to the general population, which is much greater than estimated average requirements. However there are 2 individuals (H and K) that fail to meet these requirements (see discussion).

Table 3.5 Fat intake relative to national diet and nutrition survey and estimated average requirements.

ID	*Age*	*Fat Intake (g)*	*Mean Survey Intake*	*Percent Relative To Survey*	*Fat RNI (g)*	*Percent Relative To RNI*
A	3	33.3	56	60	45.3	74
B	4	25.3	56	45	45.3	56
C	5	34.9	60	58	66.7	52
D	5	68.7	60	114	66.7	103
E	7	47.2	70	68	66.7	71
G	24	104	86	121	113	92
I	25	33.7	86	39	117.4	29
H	25	57.3	60	96	80.8	71
V	25	42.1	60	70	89.2	47
K	38	40.2	87	46	101	40
W	41	38.5	61	63	80.8	48
X	42	59.5	88	67	113	53
M	13	61.2	77	79	86.3	71
O	14	62	67	92	71.8	86
P	15	68.8	51	136	82.1	84
Y	17	114.8	89	129	101.1	114
Z	19	50	64	78	86.2	58
Z1	25	36.2	87	42	99.6	36
Q	27	90.6	87	104	101	90
R	35	60.6	88	69	101	60
T	3	17.3	56	31	45.3	38
			Mean	**76.5**		**65.4**
			Std Dev	**30.6**		**23.2**

Table 3.5 Indicates that patients take much less fat than the general population. They also take much less fat than the estimated average requirement. At low levels of fat intake (such as patient I,) the quality and type of fat taken, may be important (see discussion).

Table 3.6 Absolute carbohydrate intake of patients with GSD indicating proportions from treatment (Rx) relative to total energy intake and compared to target delivery with reference to Schwenk & Hammond[1] *Final column assumes highest glucose delivery rate ie 9mg/kg/min for children aged 7 years and below.*

ID	AGE	BMI	Type	% Rx CHO Energy Relative to Total Energy	%CHO energy relative to Total Energy	Rx glucose Delivery mg/kg/min	CHO glucose delivery mg/kg/min	Target Glucose delivery	% glucose delivery relative to target
A	*3*	*13.5*	*1a*	45	74	7.8	12.7	7- 9	**141**
B	*4*	*16.5*	*1a*	43	74	5.4	8.5	7- 9	**94**
C	*5*	*17*	*1a*	45	74	6.2	10.6	7- 9	**118**
D	*5*	*25.9*	*1a*	38	64	5.6	8.6	7- 9	**96**
E	*7*	*30.6*	*1a*	55	70	5.4	7.3	7- 9	**81**
G	*24*	*21.8*	*1a*	31	59	2.8	5.9	3	**197**
I	*25*	*28.4*	*1a*	37	76	2.2	4.6	3	**153**
H	*25*	*26*	*1a*	19	59	0.8	3.6	3	**120**
V	*25*	*26.7*	*1a*	49	69	3.4	5.0	3	**167**
K	*38*	*22.3*	*1a*	54	77	4.3	6.1	3	**203**
W	*41*	*26.8*	*1a*	40	68	1.9	3.1	3	**103**
X	*42*	*24.2*	*1a*	35	66	2.2	4.2	3	**140**
M	*13*	*27.1*	*1b*	46	68	4.0	6.0	3	**200**
O	*14*	*21.2*	*1b*	23	65	1.7	5.0	3	**167**
P	*15*	*24.9*	*1b*	35	67	3.4	6.4	3	**213**
Y	*17*	*34.6*	*1b*	34	59	2.4	4.4	3	**147**
Z	*19*	*25*	*1b*	36	70	2.1	4.1	3	**137**
Z1	*25*	*19.9*	*1b*	24	71	1.9	6.3	3	**210**
Q	*26*	*19.7*	*1b*	35	59	3.8	6.6	3	**220**
R	*35*	*22.2*	*1b*	9	56	1.7	3.0	3	**100**
T	*3*	*14.9*	*3*	61	77	6.1	7.0	7 - 9	**78**
		Mean		**37.8**	**67.7**	**3.6**	**6.1**		**146.9**
		Std Dev		**12.5**	**6.5**	**1.9**	**2.4**		**46.6**

Table 3.6 shows the proportion of total energy derived from treatment carbohydrates (uncooked cornstarch and glucose polymer) as well as that from the diet. The absolute carbohydrate intakes are expressed as glucose delivery rates. In most cases, delivery exceeds target.

Table 3.7 Mineral intake and RNI by dietary assessment

(patient taking complete micronutrient supplement – supplement composition not included in analysis)*
Blue indicates intake above dietary reference ranges and red indicates intake below the range.

	Calcium	RNI	Magnesium	RNI	Phosphorus	RNI	Iron	RNI	Copper	RNI	Zinc	RNI	Manganese	Selenium	RNI
A*	243	350	88	85	420	270	3.6	6.9	0.41	0.4	2.2	5	0.74	9	15
B*	340	350	148	85	531	270	7.6	6.9	0.66	0.4	3.9		2.15	15.9	15
C*	558	450	141	120	640	350	6.9	6.1	0.66	0.6	4.4	6.5	2.58	15.8	20
D*	335	450	163	120	845	350	7.1	6.1	1.21	0.6	9.1	6.5	1.92	60.9	20
E*	548	450	114	120	729	350	6.3	6.1	1.31	0.6	6	6.5	0.79	31.7	20
G	1783	700	300	300	1772	550	8.9	8.7	0.85	1.2	8.6	9.5	2.22	24.7	75
I*	1054	700	359	300	1545	550	18	8.7	1.66	1.2	12	9.5	3.04	109.6	75
H	589	700	216	270	728	550	9.4	15	1.39	1.2	4.8	7	2.11	25	60
V*	1658	700	302	270	2004	550	7.5	15	0.67	1.2	12	7	1.17	72.4	60
K	618	700	183	300	810	550	15	8.7	1.2	1.2	6.2	9.5	1.73	19	75
W	672	700	267	270	1074	550	23	15	1.58	1.2	15	7	5.83	113	60
X	665	700	306	300	1330	550	13	8.7	1.25	1.2	10	9.5	2.92	54.5	75
M*	976	1000	312	280	1614	775	14	11	1.24	0.8	14	9	3.92	51	45
O*	556	800	219	280	906	625	11	15	1.11	0.8	5.5	9	2.31	33.2	45
P	1479	800	333	300	1600	625	16	15	1.89	1	10	7	2.63	84.3	60
Y*	704	1000	304	300	1474	775	13	11	1.35	1	10	9.5	3.5	62.8	70
Z	353	700	164	270	727	550	4.9	15	1.09	1.2	4.9	7	1.59	49.4	60
Z1	1232	700	370	300	1666	550	14	8.7	2.53	1.2	14	9.5	2.65	119	75
Q	446	700	251	300	1673	550	22	8.7	1.74	1.2	13	9.5	2.56	44	75
R	434	700	153	300	779	550	7.7	8.7	0.62	1.2	5.2	9.5	1.43	33.2	75
T*	308	350	60	85	322	270	4.6	6.9	0.5	0.4	3.5	5	0.61	8.7	15

Table 3.8 Dietary Assessment of Vitamin compared to RNI

(patient taking complete micronutrient supplement – not included in analysis) (Underlined values below reference range)*

	Thiamin	RNI	Ribo-flavin	RNI	Niacin	RNI	B12	RNI	Folate	RNI	Panto-thenate	Vit C	RNI
A*	0.5	0.5	0.5	0.6	6.9	7.7	1.7	0.5	73	70	1.6	58	30
B*	1.4	0.5	0.4	0.6	5.04	7.7	0.3	0.5	117	70	1.1	18	30
C*	0.7	0.7	0.7	0.8	5.33	11	1	0.8	76	100	1.6	21	30
D*	1.2	0.7	1.1	0.8	18.25	11	2.6	0.8	227	100	2.5	35	30
E*	0.7	0.7	0.7	0.8	10.1	11	3.5	0.8	81	100	2.1	23	30
G	1.4	1.2	3.4	1.3	9.16	19	11	1.5	202	200	7.5	63	40
I*	2.4	1.2	2.2	1.3	37.7	20	6.2	1.5	529	200	5.8	500	40
H	1.1	0.8	0.8	1.1	10	14	0.7	1.5	194	200	3	197	40
V*	2	0.9	3.2	1.1	31.9	15	12	1.5	226	200	11	37	40
K	1.1	1	1	1.3	15.77	17	1.8	1.5	1243	200	7.6	109	40
W	5	0.8	5.7	1.1	52.3	14	21	1.5	556	200	12	157	40
X	1.9	1.2	1.4	1.3	19.4	19	4.2	1.5	319	200	5.3	130	40
M*	1.5	0.9	z2.3	1.2	25.3	15	5.9	1.2	212	200	5.5	84	35
O*	1.2	0.7	0.7	1.1	15.9	12	1.4	1.2	170	200	3.1	137	35
P	1.4	0.8	2.4	1.1	21	14	5.8	1.5	261	200	5.1	101	40
Y*	1.6	1.1	1.1	1.3	26.54	18	4.2	1.5	256	200	6.2	133	40
Z	0.9	0.8	0.5	1.1	11.9	14	2.4	1.5	180	200	2.7	271	40
Z1	4	1.3	4.2	1.3	85.2	17	7.3	1.5	630	200	11	40	
Q	1	1	1.3	1.3	21.5	17	9.7	1.5	154	200	3.5	48	40
R	1.2	1	1	1.3	15.87	17	2.3	1.5	166	200	3.7	77	40
T*	0.4	0.5	0.5	0.6	2.84	7.7	0.6	0.5	67	70	0.8	34	30

Table 3.9 – Biochemical measurements of nutritional status in 17 subjects with GSD

(Underlined values below reference range)

ID	Folate	B12	B1	Ferritin	Copper	Zinc	Selenium	Vitamin D	Phosphate	Calcium	Alk phos
A*	6.6	679	129		22.2	12.1	1.04	50	1.33	2.31	
B*	20.6	731	253	19	20	13.8	0.84	46	1.46	2.34	
C*	24	871	210	24	21.4	16.2	0.92	47	1.27	2.41	
D*	15.4	962	171	44	20	15.1	0.94	51	1.43	2.44	
E*	7.2	451	256	22	17.7	11.9	0.96	64	1.61	2.22	
G	226	180	184	40	20.8	14.6	0.88	28	1.38	2.55	231
H	5.8	163	112	48	17.7	13.9	1.16	20		2.18	85
V*	937	387	149	103	15.9	17.4	1.28	67	1.14	2.39	72
K	368	163	153	36	11.3	14.7	0.9	74		2.37	
M*			221		21.3	13.7	1.0	61	0.77	2.34	
O*	7.2	>1200	328	21	13.6	14	0.77	41	1.57	2.36	
P	3.9	793	145	41	16.4	12.9	0.9	40	1.23	2.27	
Y*	147	1345	110	38	25	15.7	0.71	28	1.51	2.45	104
Z	280	911									
Q	6.7	500	63		24.4	15.5	0.84	23	0.9	2.41	175
R	388	240	52	67	24.4	10.4	1.04	32	1.36	2.32	395
T*	10	786	272	22	23.5	10.7	1.15	40	1.69	2.4	
Normal Range	3.0 – 20.0 ug/L	170 – 900 ng/L	66 – 200 nmol/L	7 – 150 ug/L	11– 22 umol/L	11 –24 umol/L	0.9– 1.7 umol/L	15 – 100 nmol/L	0.81- 1.80 mmol/L	2.22 – 2.51 mmol//L	30 - 126 U/L

Chapter 3.3 Discussion

The diet diaries form a record of dietary intake of this group of patients from the age of 3 to 38. This is a very broad age range but several patterns of intake of nutrients are observed. This discussion first looks at the macronutrient intake concentrating on total energy and carbohydrate. The derivation of carbohydrate target intake is then discussed. Following this the intake of fat is examined, followed by micronutrient intake and then the social impact of the diet. Finally, methods used are appraised.

Total Energy and carbohydrate

The mean energy intake of this population from table 3.2 is 110 % of EAR. The age matched control means from national surveys vary between 77% and 93% of EAR (appendix 1,) with girls aged 15 to 18 taking the lowest amount of total energy relative to EAR. The fact that GSD patients take more energy than estimated requirements, as opposed to the general population that takes less, means that energy intake for this group differs from their peers. The mean proportion of macronutrients contributing to total energy intake are shown graphically on figure 3.1. Whilst mean carbohydrate intake of 67.8% of total energy is slightly above the target of 65% for patients with GSD, the quality of diets that have carbohydrate intake between 70 and 78% of total energy could well be poor; it is unlikely that the remainder of the diet could be sufficiently broad in range to be nutritionally complete. It is also of concern that some individuals with GSD have energy intake of the order of 150 % of EAR. The mean contribution (+/- SD) of the macronutrients, carbohydrates, protein and fat are 67.8% (+/- 6.5), 11.2 % (+/- 3.6) and 21.0 (+/- 6.4%) respectively. The percentage contribution to dietary energy are misleading if the absolute intake is either very high or very low. For instance, subject H has an energy contribution from protein of 8.2%, which is not out of keeping with the rest of the GSD population (mean 11.1% +/- 3.6), but has reduced energy intake. The absolute protein intake of H is 56 % of what the average from age and sex matched controls take and 72% of the estimated average

requirement. This patient also has vitamin B12 deficiency (biochemically demonstrated as well as estimated from food diary,) in keeping with a low protein diet of this scale. Typical of the general population, most other subjects have protein intakes greater than the EAR.

The target contribution to energy requirements by carbohydrates is 65%. Some subjects clearly take more than this. In some instances this proportion of carbohydrate is within a diet that itself has far greater energy content than average. For instance subject V has an energy intake of 128% greater than EAR, of which 69.2% is from carbohydrates. This proportion results in a carbohydrate intake of 274% compared to controls and 153% compared to EAR.

The significant amount of carbohydrate taken and the requirement to ensure adequate protein intake inevitably results in decreased intake of dietary fat in most cases. As long as essential fatty acid intakes are adequate, a low fat diet itself does not result in harm and may provide some health benefits.

The data presented clearly demonstrate the differences in the GSD population from age and sex matched controls. The hepatic glycogen storage diseases are life-threatening diseases associated with mortality and serious morbidity if the acute and repetitive tendency towards hypoglycaemia is not addressed. It is not surprising, therefore that patients, and clinicians alike, take extra care to prevent these episodes and in some instances this could result in carbohydrate intake greater than necessary. However, these data also suggest that some individuals meet the carbohydrate intake requirements at the expense of protein, essential fat, vitamin and mineral intakes, which themselves can lead to clinical sequelae.

There is evidence from these data of different approaches taken by patients to accommodate the dietary regimen of GSD:

1 To maintain total energy intake similar to the general population and incorporate treatment starch and dietary starch within the 65% target, thereby limiting fat or protein intake. (eg subjects G and O)

2 To take a similar diet to peers but with treatment starch as extra leading to greater energy intake and risk of obesity. (eg subjects D, Y and V)

3 To take less energy than is required – some patients lose their appetite with larger starch intakes or have concerns about obesity – starch is a much less efficient energy source than fat. These subjects are at particular risk of micronutrient deficiencies. (eg subjects H and K)

4 Patients with GSD Ib – several have inflammatory bowel disease and diarrhoea, consequently taking greater amounts of food but due to mal-absorption do not gain weight. (eg subject Z1 and Q).

Endogenous glucose production studies

The premise of 65% carbohydrate intake as a requirement is based on rates of endogenous glucose production studies in both control "normal" individuals and patients with glycogen storage disease specifically. The studies in GSD have predominantly been performed in children and may not be applicable to adult treatment.

In 1977, Bier et al published endogenous glucose production rates in children.[68] These were calculated from studies on 54 children in the form of an isotope dilution study. This physiological assessment has been used since the 1950's and there have been refinements to protocols since the early studies. [98] The method involved the infusion of dideuteroglucose followed by subsequent quantification of the proportion of labelled and unlabelled glucose. By making serial measurements of this proportion after

infusion is discontinued, the endogenous rate of appearance of glucose can be calculated. Bier calculated that endogenous production was 7.1 mg/kg/min for children under the age of six, 5.4 mg/kg/min for older children and 2.3 mg/kg/min for adults. An absolute requirement of this study methodology is to ensure that research subjects are in a steady state of equilibrium between infusion and production – a subsequent report suggested that unless the priming infusion was for 5 hours, steady state was not attained and errors were likely with over-estimation of endogenous production.[99] In this study, endogenous production in adults was 2.16 mg/kg/min with a 2.5 hour priming infusion and 1.76 mg/kg/min using a 5 hour priming infusion in the same adults, which replicated magnetic resonance imaging studies of glycogen turnover in fasted subjects[100] - the Bier Study priming infusion was between 3 and 4 hours.

In the hepatic glycogen storage diseases, the primary aim is to prevent episodes of hypoglycaemia and to minimise the secondary biochemical disturbances of lactate, triglycerides and uric acid. It has been postulated that suppressing endogenous glucose production by providing sufficient exogenous glucose can also prevent these biochemical disturbances.[2] Hypoglycaemia provides the stimulus for both endogenous glucose production and secondary metabolic disturbance, whilst elevated blood glucose concentrations can potentially suppress both processes. The study of 6 children aged 1 to 7 years, by Schwenk and Hammond demonstrated that an infusion rate of nasogastric glucose of 8.6 mg/kg/minute reduced blood lactate levels but that endogenous glucose production was completely suppressed at a higher infusion rate of 10.5 mg/kg/minute. As described earlier, this methodology may be prone to the same over-estimation of endogenous glucose production that applied to the study of Bier et al that used short priming infusions.

There are several reports and some data in these studies, presented later, showing that fasting intervals in patients with glycogen storage disease increase as patients grow older.[48;103;104] This should mean that treatment carbohydrate can be taken less often. Collins et al studied endogenous glucose production rates in adults and children

with GSD I and III using isotope dilution techniques. Of the 4 patients under the age of 5 that were studied, endogenous glucose production rates were about 50% of that predicted from the normal population (normal population defined as those from the study by Bier et al.)[68] Of the 6 patients who were aged 15 and older, 2 similarly had production rates at about 50% of that expected from the normal population. The remaining 4 adults not only had endogenous production between 60 and 100% of predicted levels but also showed up to 100% variation between identically managed studies. This suggests that adults with GSD can have more between individual variation and intra-subject variation in endogenous glucose production rates than children.

It has traditionally been assumed that knowledge of endogenous glucose production rates is important to derive theoretical glucose requirements of individuals with GSD. It has been these studies that have been used to derive the target figure of 60 – 70% of total food energy from carbohydrates.[101] Fernandes and Pikaar suggested a broader intake of carbohydrate between 50 – 70% before either nasogastric feeds or cornstarch became established therapies.[102] Clearly if endogenous glucose production is normal, as has been demonstrated in some adults, then the total daily carbohydrate intake need be no higher than normal. Children and adults differ in terms of the insulin response to a glucose load but, in addition, adults are usually less sensitive to insulin than children. On the bases of endogenous production and insulin resistance, the tendency towards hypoglycaemia is decreased in adults, yet most still have intake from carbohydrates greater than 65%. A target of glucose delivery may seem appropriate in childhood, when data from endogenous glucose production studies and insulin sensitivity appear consistent, but in adults with GSD, these factors vary more and indeed have not been studied to the same extent. The level of variability between patients means that management should be individualised rather than generalised from published data. It is of more practical relevance to gauge how much carbohydrate

is needed to maintain euglycaemia and suppress lactate in an individual rather than estimate glucose production. To some extent this does happen, but over and above a physician's desired target for the patient, is the patient's expectation of their disease and this can guide their treatment too. From the long-term studies discussed in chapter 6, we describe significant reduction in overall glucose delivery in some adults, without deterioration of metabolic control; part of this can be attributed to features of the new starch but part of this is undoubtedly patients' willingness to accept changes to preconceived ideas on treatment with a new therapy.

It may be for some patients with GSD that treatment has become a way of life, that has preserved their life, and it has become difficult for them to alter their routine.

Intake of Fat

The intake of protein by the general population is substantially greater than the estimated average requirement. For most individuals with glycogen storage disease, therefore some reduction in protein intake will not cause substantial problems. However, the most efficient method of accommodating extra carbohydrate is by reducing fat intake. Each gram of triacylglycerol releases more than twice the energy than a gram of carbohydrate upon complete oxidation.[105] Consequently every gram reduction of fat intake permits more than 2g intake of carbohydrate with the same total energy intake. It is for this reason that dietary recommendations suggest restriction of fat in preference to protein. The dietary reference values of fat by the COMA report in the UK are predominantly derived from population data and social eating habits in the UK rather than actual deficiencies of fat.[1] The only known absolute requirements for fats are those for the essential fatty acids, linoleic and α-linolenic acid, which collectively are recommended to contribute a minimum of 1.2% to total energy intake. It is also acknowledged that if this requirement is not met the main functional derivatives of these two fatty acids, 20:4 cis n-6 Eicosatetraenoic (arachidonic) acid and 22:6 cis n-3 Docosahexaenoic acid (DHA) respectively, become essential. DHA is

an important component of neural and retinal tissue and dietary sources include fish products. It is often regarded as a desirable, if not essential nutrient within the diet. The n-3 and n-6 long chain fatty acid biosynthetic pathways use the same enzymes for elongation and desaturation within the endoplasmic reticulum as well as partial oxidation within peroxisomes. Because of this, the 2 pathways compete with each other for substrate and the ratio of intake of the two polyunsaturated fats is important.[106] (figure 3.2) The ratio of intake of α-linolenic acid to linoleic acid that is recommended, is 1 to 5.

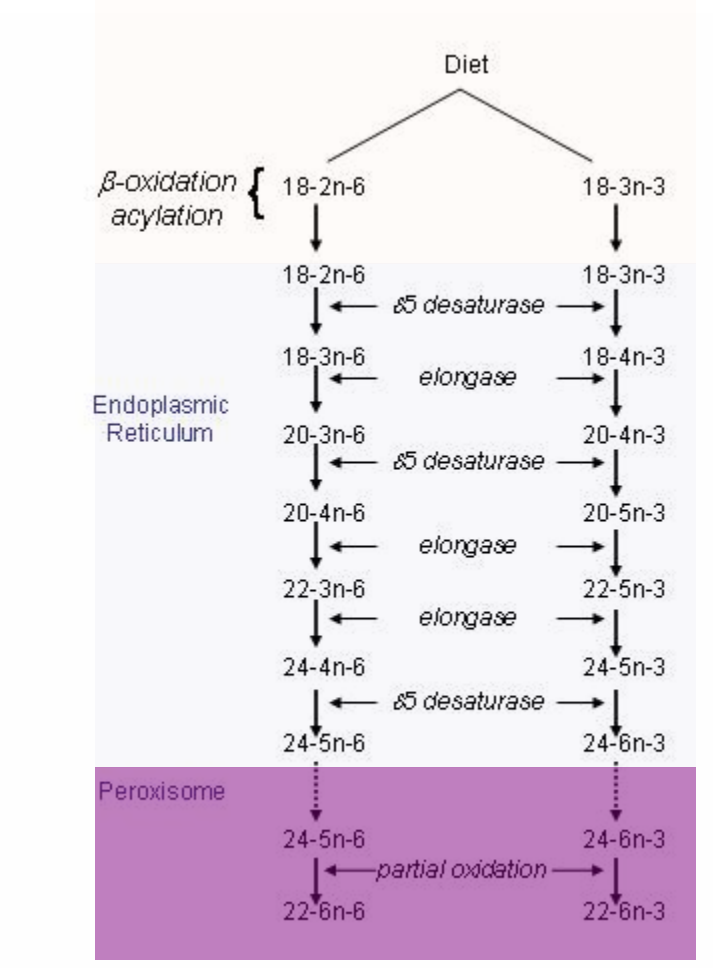


Figure 3.2 – Metabolism of linoleic, C18:2 ω6 (left) and α-linolenic acid, C18:3 ω 3 (right).[126]

The diet diaries demonstrated low intakes of all fats especially of mono-and poly-unsaturated fats. The software used was not able to generate reliable outcome data for the poly-unsaturated fat species. The database used and its references have not been

updated to include many currently available foods and neither do food labels provide sufficient data to allow determination of each essential fatty acid. Calculations of essential fat from these references were made but the author was confident that they were unreliable for the reasons mentioned and the data has not been included in this thesis. Therefore, no definitive comment can be made from these assessments. Neither were the essential fatty acids measured, so no deficiencies were noted. The low fat intake does provide a risk of deficiency of essential fatty acids but this has not been quantified. In children and pregnant women, such deficiencies may lead to sub-optimal maturation of growing neonatal and infantile brains. The further potential health benefits of these fatty acids for adults may also be compromised.[127;128]

From published literature, there is no uniform consensus about the level of intake of essential fatty acids that are necessary. COMA report 41 gave a very low estimate of requirements compared to other studies. There have also been several studies published subsequent to the COMA report from 1991, that have recommended much higher dietary intakes. The COMA report stated that its recommendations were based on requirements sufficient to prevent diseases of EFA deficiency. However, there is a difference between what is essential to prevent disease and what is desirable to enhance health. There have been several studies looking at the beneficial effects of mono and poly-unsaturated fats on heart disease, intellectual ability and neoplastic diseases but deficiency states of these fats are rarely reported.[1;107-110] As far as heart disease and neoplastic diseases are concerned, evidence based on epidemiological studies and some animal models that has so far failed to prove conclusive benefit in humans. Intellectual ability of children at the age of 4 was improved in one double-blind study of mothers taking supplements of α-linolenic acid during pregnancy.[110] Essential fatty acids have received considerable public attention as desirable food supplements. It is still difficult to define how much of each is required for optimal health. The COMA DRV for the essential fatty acids linoleic acid (18:2 cis n-6 Octadecadienoic acid) and

α-linolenic acid (18:3 cis n-3 Octadecatrienoic acid) is that they should contribute 1% and 0.2% to total energy intake respectively. Dietary intake of fat in patients with GSD is meant to be lower than the general population so it is more likely that patients will not meet these requirements. Part of the problem, is that these patients have a deliberate skewed increase in energy intake of carbohydrate so proportionate contribution to total energy intake may not be entirely representative. However, despite this, the absolute intake is still lower than other populations quoted in national dietary surveys and other published literature. In summary, essential fatty acid intake is projected to be low from diet diaries.

Plasma triglyceride concentrations and to a lesser extent cholesterol are well known to be elevated in GSD I. These can also be mildly elevated in GSD III, VI and IX.[41;102] Hyperlipidaemia in the general population is associated with the formation of atheroma, ischaemic heart and cerebrovascular disease. Over the last 40 years, several investigators have considered whether hyperlipidaemia may specifically cause clinical problems in GSD and whether lipid lowering treatment is indicated. The vascular complications of hyperlipidaemia do not appear prevalent in patients with GSD although there are relatively few patients who are over the age of 50 years, the age when these hyperlipidaemic diseases become more common. Hyperlipidaemia is, however, associated with other complications in GSD such as pancreatitis. There have been several studies trying to characterise and intervene with the hyperlipidaemia. Levy et al described essential fatty acid deficiency in patients with GSD I and went on to supplement patients with fish oil and demonstrate reduction of the total plasma tryglycerides as well as plasma total cholesterol and LDL, with a concomitant increase in HDL.[72] If atherogenesis is a serious risk, this form of therapy would be of benefit. Another recent Japanese study used MCT milk as a treatment and this led to a reduction of total plasma triglycerides in 3 patients.[111] They postulated that the shorter chain fatty acids are metabolised to acetyl co-A in contrast to long-chain fatty acids

whose transport into mitochondria via CPT I is inhibited by malonyl coA. The acetyl Co A generated inhibits glycolysis as well as enhancing ketone body production. These data are in stark contrast to Fernandes and Pikaar's published nearly 40 years earlier[102]. This detailed study demonstrated that MCT resulted in elevation of triglycerides – they postulated that acetyl CoA was used to synthesize palmitate as opposed to ketone bodies and glucose. It is likely that the patients studied have different types of disease – the Japanese group indicate that the common GSD Ia mutation in Japan, G747T causes a milder phenotype than classically observed elsewhere.

Hyperlipidaemia in the hepatic glycogenoses is likely to be due to increased de novo synthesis of lipid due to increased substrate. There is some evidence of decreased turnover.[129] Biochemical elevations may respond in selected populations to therapy with MCT or essential fatty acid supplements. However, it remains to be seen whether such interventions have any relevant long-term clinical benefit. There does not appear to be any increase in morbidity or mortality in this group related to cardiovascular events; indeed vascular endothelial studies show that these patients should be relatively protected against such episodes.[130-132]There are, however, insufficient longitudinal outcome data to dismiss the potential risk of long-term hyperlipidaemia in this group of patients and further studies and clinical follow up are required.

Micronutrient intake

The intake of carbohydrate is substantial in patients with GSD. There is consequently potential for micronutrient deficiencies if there is insufficient attention to the completeness of the diet. Surprisingly there are few reports of micronutrient deficiencies in the literature. Kishnani et al report the case of one individual with long-term incomplete adherence to nutritional therapy[73]. At the age of 16 ½, he presented with weight loss, depression, vomiting and malaise. He had no evidence of inflammatory bowel disease. At presentation he was pancytopenic with a haemoglobin

concentration of 4.9 g/dl. The authors demonstrated low plasma concentrations of folate, iron and vitamin B12. Symptoms and biochemistry resolved after therapy. This case highlighted how individuals can focus on carbohydrate therapy to the exclusion of the remainder of the diet and makes the case for micronutrient supplementation.

Most of the patients from the cohort studied in this thesis that take overnight pump feeds take a multivitamin preparation as part of their overnight recipe. This includes 7 of 8 children and 2 of 3 adults on pump feeds. Conversely most adults that take uncooked cornstarch as part of their normal dietary regimen at night-time do not take a complete nutritional supplement. Dietary insufficiency from diet diary was noted in 4 of the 21 subjects for calcium and 8 for magnesium. Of the four patients with projected calcium deficiency, 3 were on a multivitamin preparation that corrected the deficiency but only 3 of the 8 with projected magnesium deficiency had this corrected with a multivitamin. Biochemical measurements of magnesium were only performed in children and in all cases were normal. Similarly the plasma calcium was normal in all 17 patients that had this measured as part of this study. However, plasma calcium concentrations do not accurately reflect total body calcium deficiency. In order to assess calcium balance effectively, factors such as bone mineral deposition, enzymes of calcium turnover (eg alkaline phosphatase), humoral regulation (eg parathyroid hormone) and renal function need to be considered.[133] In glycogen storage disease all these factors may be affected, resulting in a greater effect on calcium balance than diet alone.[78;80]

Several subjects had other projected mineral deficiencies. Because most of the children were on dietary supplements, there were no instances of deficiencies being measured biochemically. However, for adults plasma zinc concentrations were just below the reference range in 2 individuals and selenium was low in 5 individuals. In all these cases of selenium deficiency, the diet assessment indicated that intake was well below the RNI. A further 7 patients had projected intake less than RNI for selenium.

2 subjects had serum vitamin B12 concentrations below the reference range, with only one of these being estimated to be below the RNI from the dietary diary. This subject (H) is a vegetarian and is known to have had vitamin B12 deficiency in the past. Functional assessments of B12 deficiency namely measurements of methylmalonic acid and total homocystine were not made. Several subjects were assessed not to achieve RNI for niacin, riboflavin and folate. Of these, only folate was measured (some from serum and some from red blood cells,) but deficiency was not noted.

Social impact of the diet

The case histories show that a number of adults continue to have an intensive, repetitive pattern of eating, with a limited range of foods taken. It is striking that several have the same meal several times a day; the 3-day diet diaries show that this pattern can continue for several days. Several also use high glucose drinks as an alternative to treatment carbohydrate. This may appear to some as a more socially acceptable alternative to mixing and drinking cornstarch. However, high glycaemic index drinks are a less desirable method of treatment as they promote greater insulin release than lower glycaemic index alternatives; in the long-term insulin resistance would also be more likely if this strategy of treatment is applied.

From a broader social perspective, it is notable that a significant proportion of adults with GSD continue to live with their parents. This pattern is present in the wider group with only 5 of the 12 adults aged 17-42 living independently and only 2 of the 12 being with long-term partners. Parents have an immense burden of social and medical care placed on them, when they learn how to look after a child with GSD. Similar to several other chronic illnesses, parents have to adjust their lives to accommodate the illness. This modification to life plans can persist for many years, even when the children have become adults. Unlike several other diseases, the management of these children does

require constant vigilance, both day and night, with the potential for devastating consequences if this is relaxed inappropriately. It can therefore, be difficult for parents to step back from this intense level of care and likewise it can be difficult for the children to adjust to become independent, both in their own medical management and forming relationships with others. The psychosocial needs of children and parents living with chronic diseases have been addressed for several diseases such as cystic fibrosis and diabetes mellitus.[134-136] This has only recently been systematically studied in GSD.[137] The study from Storch et al surveyed a cohort of patients managed in Florida. This study showed that parents perceived great difficulties for their children over a range of activities, whereas the children themselves felt more similar to their peers. The authors comment that this is probably because the children try to adapt to or deny the differences that are present, such that they feel they are similar to peers.

The potential of patients with the hepatic glycogen storage diseases to have diets similar to their peers seems very limited. The dietary treatment is intensive, especially in childhood, but the disrupted pattern of eating is maintained in adult life. Quite apart from what is medically justified, patients have patterns of eating that can lead to problems. The difference from peers can lead to both physical and psychological disturbance. Our data does not reveal the torment that some individuals report in a clinical setting about obesity, low self esteem related to large body shape or slight frames in GSD Ib.

Appraisal of Methods

This study attempts to assess the quantity and quality of foods taken by patients with glycogen storage disease. If the survey were published in peer review literature, it would be the largest detailed survey of dietary records to have taken place. 21 patients were analysed of which 17 had biochemical investigations relating to nutrition. The survey relies on diet diary records. These can often be difficult for patients to complete

accurately. They rely on meticulous record keeping and reliable estimations of quantity. Our research team anticipated problems and direct face-to-face teaching about completion of such records were performed directly to individual participants by either a nutrition nurse or state registered dietician. The same person received the form from the research subject and entered data onto the database. If there were issues that needed clarification, the researcher contacted the person completing the form directly and on occasions visited the home to clarify recipes and measures used. Within the resources available, this was the best possible ascertainment of dietary intake possible. However, it may still not have been accurate enough. This method cannot legislate for items such as snacks and drinks that may have been forgotten on the dietary record. These are quite often forgotten even upon direct questioning.[122;138;139]

The dietary records were then summarised into total quantities of each food taken and entered onto a software programme. The programme then provided an output of food group constituents from the dietary record, which was then entered onto database software (Microsoft Excel, Illinois, USA) and group descriptive data were generated. At several points in this process there is potential for manual error and mis-entering of data. Each output and input was checked several times by different personnel but errors were still possible. The diet analysis software programme is referenced to standard texts including “McCance and Widdowson's The Composition of Foods.” However the output is generated without detailed knowledge of the internal workings of the software programme. Recipes of cooked foods in particular could be misrepresented – details of exact ingredients and methods of processing food can be assessed incorrectly by the programme. Sample outputs were re-assessed manually to see if they appeared plausible. Some differences were noted, notably a discrepancy between “McCance and Widdowson's The Composition of Foods” and the manufacturer’s data of an individual cereal the reference book claimed to cite. Also significant discrepancies were noted in the calculation of essential fatty acid

composition in 2 diet diaries analyzed manually and hence the derived values have not been used in this thesis. It was not the object of this thesis to validate the software being used – it is currently extensively used in clinical practice. Errors were, however, detected in some results obtained from the programme and these need further investigation.

Data from research participants were compared with both nationally recommended dietary reference values and data from National Diet and Nutrition Surveys. Dietary reference values are recommendations made by government appointed expert panels. The expert panel have reviewed literature in order to derive recommendations. This does not, however mean that there is validated evidence for their use. Firstly, the principal aim of the reference values is to address insufficiency and intake may be adjusted disproportionately high in order to guarantee that insufficiency does not occur. The report on health and social subjects 41 in which DRVs are defined states the following: “Current surveys in the UK indicate energy by the population below the prevailing RDA (recommended daily amount.)… As in most developed countries, problems relating to insufficient energy intakes are uncommon in the UK. In contrast, however, many of the diseases which are characteristic of developed countries are related to overweight and obesity in the population which result from a chronic excess of dietary energy intake over expenditure.” Therefore, the recommendations are set higher than survey values of energy intake, yet it is known that the general population is becoming more obese.

For this reason, a comparison was also made with dietary surveys. These surveys provide a comprehensive summary of UK diets with approximately 2000 individuals studied for each survey. They are regionally representative and great care has been taken to maintain the quality of the data. Our sample of patients is presumed to be representative of this wider population. However, the genetic basis of glycogen storage

disease means that certain populations are at greater risk of developing disease than others. In particular the research participants in this study had 4 patients with GSD Ia from a Gujarati Indian background, each of whom were homozygous for the C150-151delGT frame-shift mutation.[140] Similarly, there were 4 Pakistani patients with GSD Ib who were homozygous for a splice site mutation at the exon 8 and intron 8 junction and 2 Jamaican siblings who unusually have had only one mutation identified (R415X), despite complete sequencing of the gene as part of a research study.[141] [125] The recruitment of 4 Gujarati and 4 Pakistani from a total of 21 subjects in particular skews the research population from the "normal population." Culturally, Gujarati Indians are vegetarian and all 4 subjects recruited have a vegetarian diet. Culturally, Pakistani individuals eat only halal meat, so their choices for meat products can be altered if halal preparations are unavailable. From the Gujarati and Pakistani patients studied, there may be an over-representation of a vegetarian Asian diet compared to the reference population. The reference data has been stratified for age and sex and further regional and social stratification could have been used; however, the greatest influence on intake in our recruits is ethnicity, and associated cultural eating patterns, rather than social or regional background.

2 subjects aged 3 yrs and 5 months and 3yrs and 11 months had their data compared to the reference data of subjects aged 4 to 6 years old in the the national dietary surveys. They could have been compared to age matched populations from the survey carried out on subjects aged 1 ½ to 4. However this survey, conducted in 1992, was one of the earlier surveys by this group. The presentations of results are less detailed in this survey compared to later ones and it is probable that available foods may have changed in the intervening years between this national survey and our study. The later studies in older children conducted in 1997 and in adults in 2001 have been used for our comparisons.

Further comparisons of patients were made compared to biochemical reference values. In general the reference ranges are derived from a standard population and

values below the reference range are usually in the lowest 5% of the population and could represent deficiency. However, in this study, no functional assessments of deficiency were made, either in terms of corroborative biochemistry such as methylmalonic acid and homocysteine in B12 deficiency or in terms of clinical assessments such as an echocardiogram for selenium deficiency or bone mineral density for calcium deficiency. For some minerals or vitamins such as vitamin B12, the diet diary and serum measurement is a useful tool in screening for functional deficiency. However for others such as calcium, the functional test of bone mineral density is more representative of total body calcium homeostasis.

Chapter 3.4 - Conclusion

There are undoubtedly regional, social and cultural differences in dietary intake. The cross-sectional survey performed in this thesis cannot legislate for these differences. However, the nature of the intensive dietary treatment of glycogen storage disease does mean that there are patterns of nutrient intake and eating behaviour that differentiate this group of individuals from the general population, irrespective of their background. This difference may not necessarily cause physical harm, but certain characteristics could pre-dispose to disease states or result in social differences leading to physical or mental health problems.

The dietary analyses have found that patients with GSD have substantial intake of carbohydrate. For some adults in particular, it seems difficult to justify the amount and quality of carbohydrate being taken. The large amounts of this macronutrient in the diet does compromise intake of essential fats, minerals and vitamins in some individuals. In most cases micronutrients and essential fatty acid supplements are required. In others, excess overall nutritional intake may also lead to obesity. Further study and modification of these patients' diets are indicated in order to optimise their health and long-term outcome.

CHAPTER 4 – Short-term metabolic effect of heat-modified waxy maize starch on patients with hepatic glycogenosis.

Introduction

Uncooked cornstarch forms the basis of treatment for many patients with the hepatic glycogen storage diseases. As discussed in chapter 1.3, there are many problems with its use and some adults remain on treatment with nocturnal overnight pump feeds of glucose polymer and some still need to administer corn-starch at 4 hourly intervals. The quest for an efficacious starch has pre-occupied many research groups, including our own, for over 30 years. After a series of studies, the metabolic profile of a heat modified waxy maize starch appeared favourable. This chapter describes accumulated experience of the use of this starch in GSD from a pilot study (chapter 4.1) in one individual to a larger study involving children and adults (chapter 4.2) and a further study in adults only (chapter 4.3). This chapter will focus on the effect the starch has on short-term biochemical markers: glucose, lactate and insulin with chapters 5 and 6 concentrating on different aspects of these trials. The hypothesis for each study is the same, but the investigations are different.

Hypothesis Heat modified waxy maize starch (WMHM20) has a longer duration of normoglycaemia compared to equivalent amounts of uncooked cornstarch in patients with hepatic glycogen storage disease.

4.1 Pilot Study

Aim To compare duration of normoglycaemia using equivalent amounts of WMHM20 and uncooked cornstarch in 1 patient with glycogen storage disease type 1.

Method

A 22 year old female subject with glycogen storage disease type Ia (further details on this subject – subject V in chapter 2.2) attended the ward on 2 separate occasions, 2

weeks apart for an unblinded starch load. Written informed consent was taken prior to the starch being administered. The subject's normal treatment comprised of 40g uncooked cornstarch taken four times a day with an overnight pump feed of 25% glucose polymer infused at 40mls/hr between midnight and 07:30. At 07:30 the subject had a bolus of 20g of glucose polymer drink. The night before each occasion the subject had a starch-load, she stayed locally to the hospital. The following morning, the 20g bolus of glucose was given was given and the subject attended the ward to take the 60g of the nominated starch, mixed in cold water, within 60 minutes. Prior to ingestion of the starch, clinical observations and enquiry were made to ensure that the patient was well, an intravenous cannula was placed and baseline glucose and lactate were taken. Blood was collected into sodium fluoride bottles. The samples were deproteinised with potassium perchlorate within 30 minutes of collection and separated. Plasma glucose and lactate were measured by glucose oxidase and lactate dehydrogenase methods respectively as described in chapter 2. Glucose was also measured at the bedside using 1 drop of whole blood on a point-of-care glucometer (Advantage II, Roche diagnostics, Mannheim Germany) to guide time of test termination. The test was terminated when the blood glucose was less than 2.6 mmol/L. After the test, 200mls of 25% glucose polymer was administered followed by 40g of uncooked cornstarch, The plasma glucose and lactate measured in the laboratory are presented in the results.

The results from the paired starch loads in the pilot subject are shown below in table and figure 4.1.

	Glucocse (mmols /L)		Lactate (mmols/L)	
Time (Hrs)	**UCCS**	**WMHM20**	**UCCS**	**WMHM20**
0	5.3	5.7	2.66	2.20
1	6.5	7.2	3.16	3.43
2	6.0	5.7	2.53	2.43
3	6.1	5.4	2.50	2.10
4	4.5	4.8	2.54	2.63
5	2.9	3.9	3.94	3.63
6	1.7	3.2	6.28	6.34
7		2.4		7.63

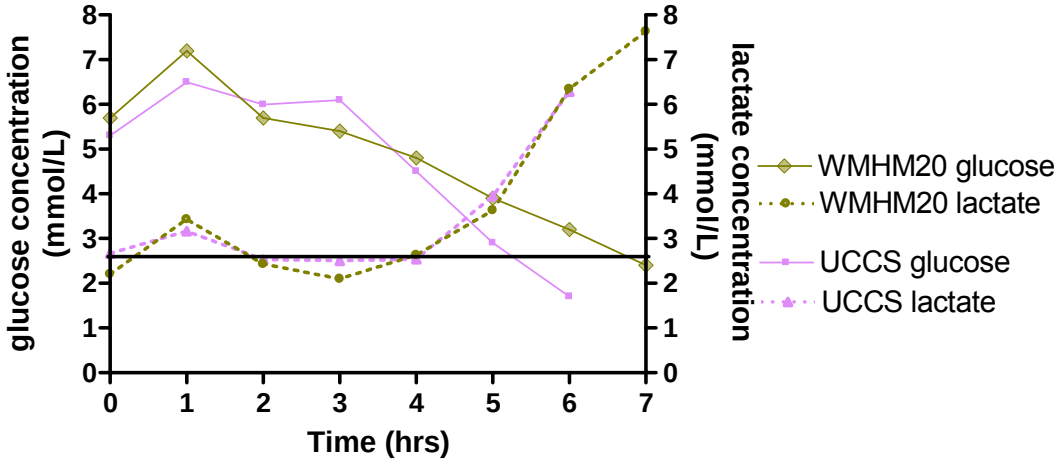


Table and figure 4.1 – glucose and lactate profile after 60g of starch ingested in pilot subject.

The patient starts with equivalent glucose and lactate concentrations, has a higher peak glucose with WMHM20 at 1 hour which decreases below the UCCS plasma glucose at 2 hours. From this point the glucose decreases at a rate of 0.66 mmols/hr for the WMHM20 curve whereas the UCCS curve decreases at a rate of 1.1 mmols/L/hr. For the last 3 hours of the UCCS test, the rate of decline is 1.5 mmols/L / hour wheras the WMHM20 decline remains steady between 0.6 – 0.8 mmols/l/hr. At 4 hours the glucose concentrations are comparable but thereafter, the glucose concentration is higher for WMHM20 compared to UCCS. The lactate curves are

closely matched, but because the WMHM20 test lasted longer, the lactate at the end of the test is higher.

The pilot study in one individual appeared to show that WMHM20 resulted in a longer duration of normoglycaemia with a slower rate of decline of glucose compared to UCCS; on this basis further studies were performed.

4.2 – Short –term biochemical outcome comparing 2g/kg (maximum 120g) of WMHM20 with UCCS.

Introduction Having shown that WMHM20 appeared favourable in one patient, this protocol was designed to assess the effect in a wide range of patients with GSD. The largest recommended dose of UCCS was used in this study to try and maximise any potential differences, with an equivalent amount of WMHM20 being used as comparison.

Aim To evaluate duration of normoglycaemia using an equivalent large amount of WMHM20 compared to uncooked cornstarch in patients with glycogen storage disease type I and III.

Method This study formed part of a broader study – data from breath samples collected are presented in chapter 5. The study protocol was approved by the Joint Ethics Committee of The National Hospital for Neurology & Neurosurgery and Institute of Neurology, London U.K., and the Institute of Child Health / Great Ormond Street Hospital Ethics committee, London, U.K. GSD I and III patients were recruited from adult and paediatric tertiary referral metabolic units in London. Written informed consent was taken from all adults above 16 years and a legal guardian of children under 16 years. The diagnosis of GSD I and III was based on a liver biopsy showing reduced activity of the appropriate enzyme, a mutation in the appropriate gene or white blood cell glycogen debrancher enzyme activity indicative of GSD III. All had evidence from their medical history of fasting hypoglycaemia and were taking UCCS.

The study had a randomised double-blind cross over design. Patients anonymised by reference number were randomly allocated to receive either UCCS or WMHM20. Each starch was manufactured using food-grade techniques and packaged in identical containers bearing a reference number. The patient reference numbers and container reference numbers were paired by Glycologic Ltd, (Glasgow, Scotland) and the supervising physician was blinded to this pairing. Research participants were asked to re-attend for the second starch load, using the alternative starch 3 days to 28 days afterwards. The supervising physician devised a safe pre-test personalised fasting period for each patient based on previous cornstarch loads and medical history. Instructions were given to the research subject, or their carer, for the participant to have the same diet the day before, and fast interval immediately before, each starch load.

Starch Load Test Initially, an intravenous cannula was placed in the patient's arm and baseline blood and breath samples were collected. Then, 2g of the nominated starch per kg body weight (maximum 120g) was mixed in cold water and ingested. Breath and blood samples were performed hourly after the starch administration. No further intake, apart from drinking water, was allowed. The starch load test ended when the patient had fasted for 10 hours, the blood glucose was ≤3.0 mmol/L on the bedside glucose monitor or the patient wished to end the test. When the blood glucose was ≤4.0 mmol/L in children aged 3 to 16 years, blood tests were performed at 30 minutes intervals, until the test end.

Biochemical data The blood samples were analysed: bedside whole blood glucose (Advantage II, Roche diagnostics, Mannheim Germany), laboratory plasma glucose and lactate (Vitros Fusion 5.1, Ortho-Clinical Diagnostic, High Wycombe, UK) and serum insulin was performed by a solid-phase, two-site chemiluminescent immunometric assay (Immulite 2000, Diagnostic Products Corporation, Los Angeles, USA). The laboratory glucose and lactate sample were collected into lithium fluoride, transported on ice and separated within 30 minutes of sampling. The insulin samples were collected as a clotted sample and also separated within 30 minutes. The bedside glucose monitor was used as

a screening tool to identify hypoglycaemia (blood glucose of ≤3.0 mmol/L) and consequently determine when to end the test. Statistical analyses were performed on laboratory plasma glucose data and not the bedside test results. Further details about glucose and lactate measurements used are presented in chapter 2.2

Results - Patients Patient demographics are shown in Table 4.2

Biochemical Outcomes If the patient ended the test with a laboratory glucose ≤3.0 mmol/L, the duration of normoglycemia is indicated by the last glucose > 3.0 mmol/L – usually 1 hour (occasionally 30 minutes) previous to the low value. Median test duration for WMHM20 was 9 hours (IQ range 6.0 – 10.0) and for UCCS was 7 hours (IQ range 5.0 – 9.0)(p = 0.0935.) . Comparative test duration is indicated in table 4.3 and illustrated as a Kaplan – Meier survival plot on figure 4.1. The patients ended the tests for various reasons: for WMHM20, 6 had genuine hypoglycaemia, 2 patients ended their trial because the bedside glucose monitor under-read the laboratory glucose, 6 for personal reasons unrelated to hypoglycaemia and 7 lasted the full test duration of 10 hours. For UCCS, 9 patients ended the test with genuine hypoglycaemia, 4 because the bedside glucose monitor under-read the laboratory glucose, 3 for personal reasons unrelated to hypoglycaemia and 5 patients lasted for the full test duration of 10 hours. Patients as a whole had a longer period of euglycemia using WMHM20, but 8/21 from the WMHM20 group and 7/21 from the UCCS group terminated the study prematurely. With time-to-event analyses, censoring data that did not finish with a genuine hypoglycaemia, there was no statistical difference between the 2 treatments (figure 4.2).

Age (Yrs)	ID	SEX (M/F)	GSD Type	Normal Carbohydrate use	Medications	Complications
3	A	F	IA	D - 30g UCCS TDS N - 100g polymer	Allopurinol	Nephrocalcinosis
4	B	F	IA	D –25g UCCS QDS N – 90g polymer	Multivitamins	
5	C	M	IA	D - 35g UCCS TDS N 105g polymer	Multivitamins	
5	D	M	IA	D – 40g UCCS TDS N – 105g polymer	Allopurinol; Multivitamins	
7	E	M	IA	D – 50g UCCS TDS N – 150g polymer	Allopurinol; Multivitamins	
12	F	M	IA	D – 60g UCCS TDS N – 125g polymer	none	
21	G	M	IA	D – UCCS 60g BD N – 125g polymer	Allopurinol,Ramipril, Clonazepam, Carbamazepine	Intellectual impairment.
22	H	F	IA	D – 30g UCCS BD N – 55g UCCS	Allopurinol, Ramipril. Vit B12	PCOD Renal impairment
22	I	M	IA	D – UCCS – 65g B N – 160g polymer	Allopurinol, Salazopyrin	Inflammatory bowel disease
23	J	F	IA	D – UCCS 40g QDS N – UCCS 80g	Allopurinol, Multivitamins	Short stature Chronic fatigue
33	K	M	IA	D –UCCS 50g QDS N – UCCS 50g X2	Allopurinol. simvastatin, codeine, lansoprazole	Hepatic adenoma Chronic scar pain
34	L	M	IA	D – N – UCCS 60g	None	
47	M	M	IA	D – N – UCCS 60g	Allopurinol Enalapril, Ezetimibe Atorvastatin, Aspirin, Amlodipine	Hepatic adenoma Renal impairment
13	N	M	IB	D – 80g UCCS BD N – 175g polymer	Allopurinol 50mg	
14	O	F	IB	D – 60g UCCS TDS N – 60g UCCS X2	Allopurinol 150 mg Septrin, GCSF Thalidomide	Crohns Disease Bronchiectasis
15	P	F	IB	D – 25g UCCS BD N – 45 g UCCS X2	Allopurinol GCSF	
24	Q	M	IB	D – 40g UCCS QDS N – 40g UCCS BD	Allopurinol GCSF	Short stature
35	R	M	IB	D- N- 30g	Allopurinol, Ferrous sulphate	Hepatic adenoma Chronic anaemia
38	S	F	IB	D – 20g UCCS BD N – 40g	Ferrous sulphate	Chronic anaemia
3	T	F	IIIa	D – 30g UCCS TDS N – 75g polymer	Multivitamins Protein supplement	Myopathy
12	U	M	IIIa	D – 45g UCCS BD N – 70g polymer & milk	Multivitamins	

Table 4.2 – Patients recruited to study – *For further diagnostic details, please refer to chapter 2.2 using ID from this table*

D = day; N=Night

Table 4.3 – Pre-test management, trial duration and conditions for trial termination for both WMHM20 and Cornstarch

ID	Pre-load fast (hrs)	Nocturnal regimen	WMHM20 (hrs)	T*	UCCS (hrs)	T*
A	<0.5	CNPF	3	h	3	h
B	<0.5	CNPF	4	h	4	h
C	<0.5	CNPF	3	h	4	h
D	<0.5	CNPF	7	u	4	u
E	<0.5	CNPF	9	p	6	u
F	1	CNPF	9	p	7	p*
G	2	CNPF	10	m	10	m
H	4	UCCS	10	m	9	h
I	1	CNPF	6	h	5	u
J	2	UCCS	6	h	8	h
K	2	UCCS	10	m	6	h
L	10	UCCS	10	m	10	m
M	12	UCCS	7	p	10	m
N	1	CNPF	9	p	9	p
O	5.5	UCCS	10	m	8	h
P	3	UCCS	9	p	10	m
Q	4	UCCS	10	m	7	u
R	2	UCCS	8	h	10	m
S	12	UCCS	10	m	5	h
T	<0.5	CNPF	5.5	u	2	h
U	0.5	CNPF	7	p	8	p
		Median	**9**		**7**	

T* Conditions for test end:

h = hypoglycaemia (glucose < 3.0 $mmolL^{-1}$,) confirmed on laboratory glucose.

u = under-reading: bedside glucose (< 3.0 $mmolL^{-1}$), resulting in test end; lab glucose > 3.0 $mmolL^{-1}$.

p = personal reasons unrelated to hypoglycaemia at test end.

m= maximum test duration = 10 hours.

CNPF – Continuous nocturnal pump feed. UCCS – Uncooked cornstarch

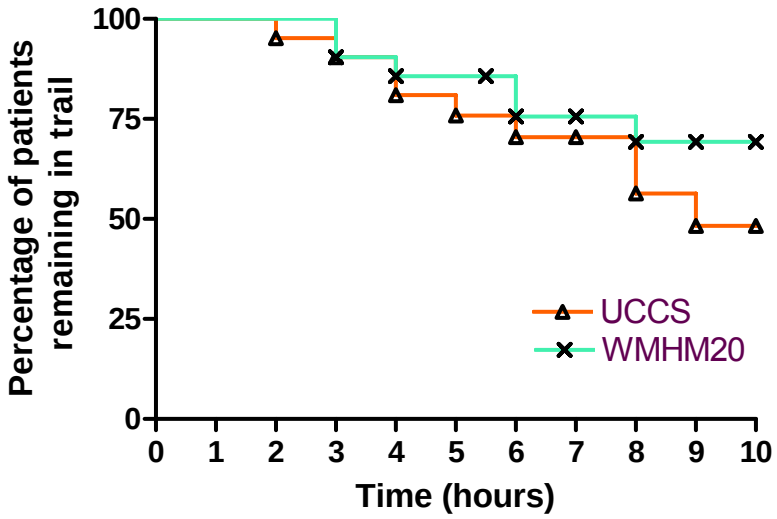


Figure 4.2 Kaplan-Meier survival plot of test duration – time to event analysis censoring patients that failed to finish with glucose <3.0: p = 0.30

Figure 4.3 shows the mean glucose profile. There was no statistical difference between the 2 profiles but the baseline glucose concentrations are not comparable.

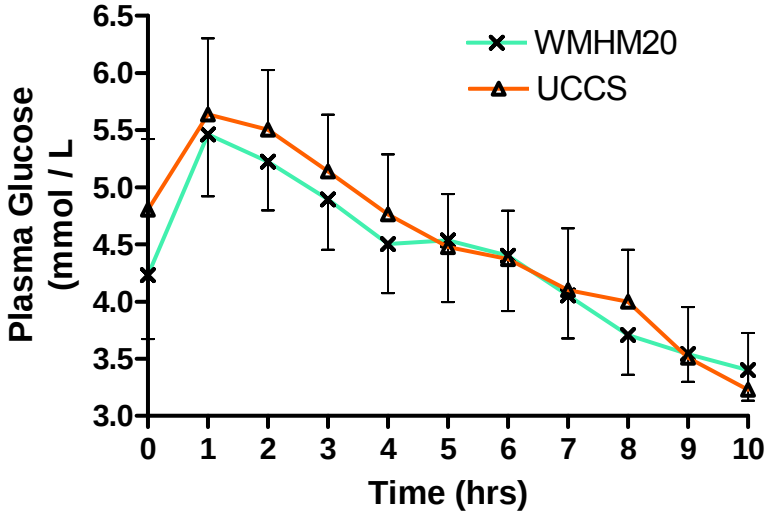
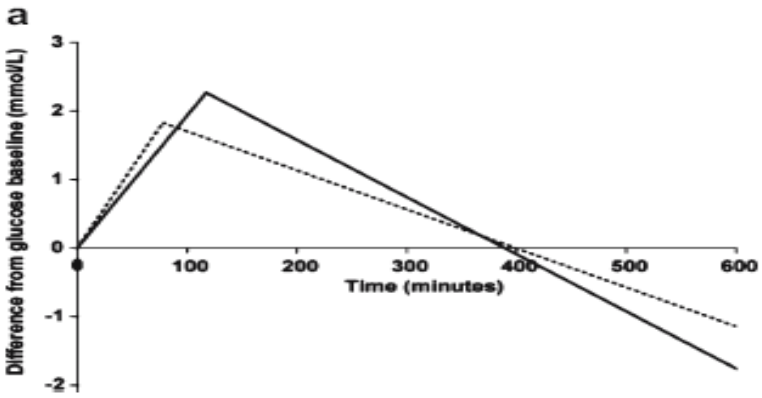


Figure 4.3 Mean Glucose profile (+/- 95% confidence interval for 21 patients)

There was no significant difference in the mean area under the curve for the glucose (p=0.47) profiles. However, the area under the curve does not necessarily represent the primary outcome of duration of normoglycemia as discussed later. Consequently the gradients for each glucose and lactate profile from baseline to peak values and

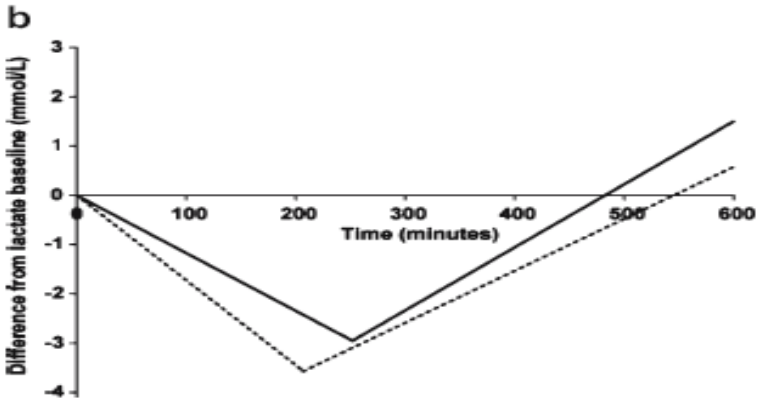
from peak to trough were taken as described in methods. There was no statistical difference for the gradient of increase in glucose but WMHM20 had a slower glucose decline than cornstarch (p=0.05,) in the whole cohort. There were no statistical differences in the lactate profile but the mean lactate decreased faster in all GSD I patients (p=0.17) for WMHM20 compared to UCCS. The mean gradients for each sector are demonstrated in Figure 4.4 to illustrate how the difference applies to the glucose and lactate profiles.



	Baseline to peak	*Peak to trough*
ALL	0.563	**0.0464**
GSD I	0.394	0.0820
GSD IA	0.571	0.114

····· WMHM20

—— UCCS



	Baseline to trough	*Trough to peak*
GSD I	0.173	0.466
GSD IA	0.381	0.380

Figure 4.4 – Extrapolated profile based on mean gradients of rise and fall of glucose (4.4a) and lactate (4.4b).

Lactate Similar to the glucose profiles, there were no significant differences in the lactate profiles. The same parameters of assessing gradients from baseline to trough and from trough to peak lactate were performed for patients with GSD I as indicated in figure 4.4b. GSD III is not associated with hyperlactataemia and these patients lactate profiles are consequently not included.

Insulin The mean insulin profile is shown on figure 4.5. Comparison of paired mean values throughout the profile by t-test shows that the WMHM20 curve is lower than the UCCS curve (p = 0.0007). However, similar to the glucose profile, the baselines are different.

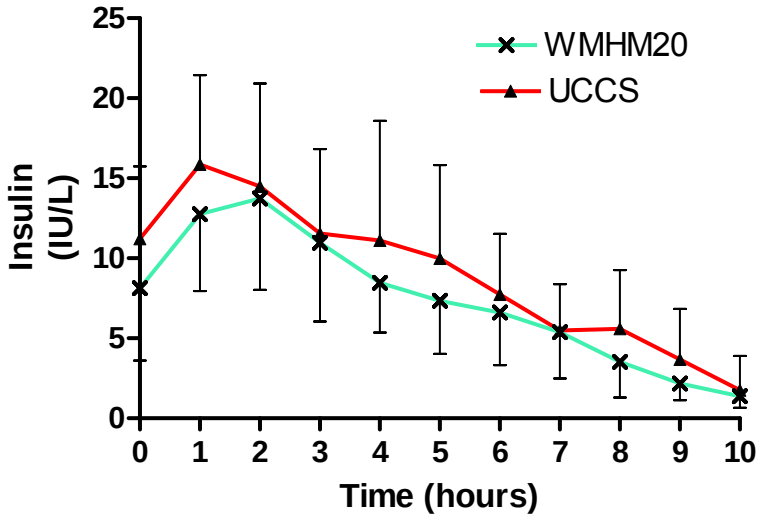


Figure 4.5 – Mean Insulin profile (+/- 95% confidence interval) for 21 patients that took equivalent amounts of UCCS compared to WMHM20. p < 0.0007 for mean profile.

The area under the insulin curves were compared for each individual by non-parametric analyses. The areas were not normally distributed with the range of areas between 2 IU/L hrs and 295 IU/L hrs. The median areas and inter-quartile ranges were 40.4 IU/L hrs (12.1 - 79.7) for WMHM20 and 54.1 IU/L hrs (18.1 – 86.1) for UCCS. Comparison by Wilcoxon paired rank test showed that there was a trend towards the

WMHM20 areas being less (p=0.073.) This changed slightly when 5 subjects with non comparable baseline insulin concentrations were excluded (p=0.088.)

Conclusion

This study demonstrated that WMHM20 could be better than UCCS.. There was a trend for median duration of normoglycaemia to be longer although this finding was not statistically significant. The rate of decline of glucose extrapolated from gradients of decline from individual profiles was less (p=0.05.) The mean insulin profile was lower ($p < 0.001$) although baseline values differed and there was a trend towards a lower area under the insulin curve. On the basis of these findings, which were all compatible with one another, it was concluded that further studies were indicated and that the product should be developed.

Chapter 4.3 – The short-term effect of 50g of Vitastarch^{tm} on the metabolic profile of patients with Glycogen storage disease type I.

Introduction Having demonstrated that large doses of WMHM20 appeared to show a benefit greater than UCCS, the WMHM20 product was developed by a nutrition company (Vitaflo Ltd, Liverpool, UK.) The addition of a small amount of emulsifying fats made the starch more miscible in water and potentially more palatable as a consequence.

This study protocol formed part of a larger study examining the long-term use of Vitastarch in patients with GSD. The effect starch treatment has on breath tests is discussed in chapter 5 and on long-term management goals in chapter 6. The lower dose of 50g was used in this study of adults as this is a common amount used in everyday management. This means that this dose could be used in a long-term clinical study and should translate readily into clinical practice if proven to be efficacious.

Aim To evaluate duration of normoglycaemia when 50g Vitastarch compared to 50g uncooked cornstarch is ingested in adults with GSD I.

Method The study protocol was approved by the Joint Ethics Committee of The National Hospital for Neurology & Neurosurgery and Institute of Neurology, London U.K. The study had a randomised double-blind cross over design. Patients anonymised by reference number were randomly allocated to receive either UCCS or Vitastarch. Each starch was manufactured using food-grade techniques and packaged in identical sachets bearing a reference number. The patient reference numbers and sachet reference numbers were paired by Vitaflo Ltd and the supervising physician was blinded to this pairing. The supervising physician devised a safe personalised fasting period for each patient based on previous cornstarch loads and medical history.

Starch Load Test Initially, an intravenous cannula was placed in the patient's arm and baseline blood and breath samples were collected. When the participant's blood glucose was under 4.5 mmol/L, they mixed 50g of the nominated starch in cold water and drank the fluid. Breath and blood samples were performed at 30 minute intervals for the next 2 hours and hourly thereafter. No further intake, apart from drinking water, was allowed. The starch load test ended when the patient had fasted for 10 hours, the plasma glucose was ≤3.0 mmol/L on the bedside glucose analyser (YSI 2300) or the patient wished to end the test. When the blood glucose was ≤ 3.5 mmol/L, blood tests were performed at 30 minutes intervals, until the test end.

Biochemical data Plasma was extracted from whole blood samples immediately at the bedside. Plasma glucose and lactate were analysed immediately (YSI 2300 - Yellow Springs, Ohio, USA). Serum insulin was frozen within 30 minutes of collection and subsequently thawed and analysed by a solid-phase, two-site chemiluminescent immunometric assay (Immulite 2000, Diagnostic Products Corporation, Los Angeles, USA).

Repeat Starch Load Data from the first starch load was used to guide management using the particular starch for the subsequent 16 weeks. After a further washout period of

2 – 4 weeks on normal treatment, participants re-attended for a further starch load with the alternative starch.

Results - *Patients*

10 patients aged 16 – 38 were recruited to the study. A total of 4 patients withdrew from the trial protocol. One patient completed 14 weeks of trial protocol on Vitastarch but failed to attend the end of phase outpatient assessment or any other clinical follow up. This participant felt the trial protocol was too onerous. Two participants completed the first phase – one having taken Vitastarch and the other UCCS, but did not attend for the second phase because they too felt the protocol was too onerous. The fourth patient completed the first phase on uncooked cornstarch but withdrew early in the second phase taking Vitastarch as they found the taste unacceptable. Six patients completed the entire study and seven patients had paired biochemical data for starch loads on UCCS and Vitastarch.

Starch Loads

The duration of these starch loads are indicated on table 4.4 and figure 4.6

Age (Yrs)	Sex (M/F)	GSD Type	UCCS (Hrs)	Vitastarch (Hrs)
23	M	GSD IA	7	7.5
24	F	GSD IA	4.5	6
25	F	GSD IA	6	6.5
38	M	GSD IA	5	10
16	M	GSD IB	7	7.5
18	F	GSD IB	5	7.5
24	M	GSD IB	4	5.5

Table 4.4 – test duration for patients taking 50g of Vitastarch compared to UCCS.

The median duration of action of UCCS was 5 hrs (range 4- 7) and Vitastarch 7.5 hrs (5.5 – 10.) On a Kaplan-Meier style survival curve, time-to-event analysis was performed using the log-rank test. The one patient that failed to become hypoglycaemic after fasting 10 hours was right-censored at 10 hours. Using this analysis, median test duration was significantly greater for Vitastarch compared to UCCS (p = 0.023.)

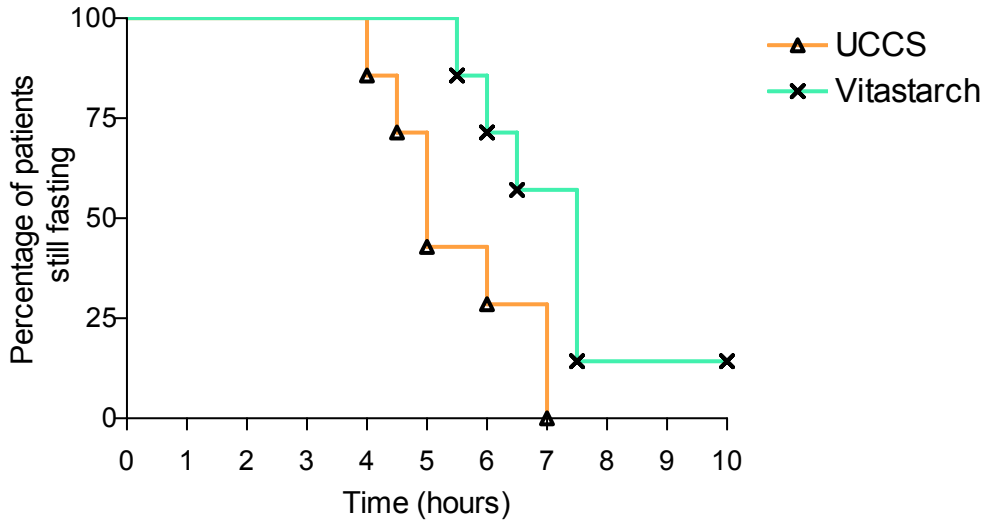


Figure 4.6 - Kaplan-Meier survival plot of 7 patients completing paired starch loads. P = 0.023 by time-to-event analysis.

The mean glucose profiles for the 7 paired starch loads are shown on figure 4.7. The baseline glucose concentrations are very similar for UCCS 3.82 and one SD (+/- 1.07) versus 3.81 (+/- 0.89) for Vitastarch. The graph indicates that for the first four hours of the starch load the plasma glucose is always higher with UCCS. At four hours the mean glucose is the same value – 3.98 mmol/L and subsequently the mean glucose for UCCS is always lower than Vitastarch. The higher peak glucose is reflected with 6 from 7 patients having higher peak glucose with UCCS compared to Vitastarch (p = 0.043 using Wilcoxon signed rank test.)

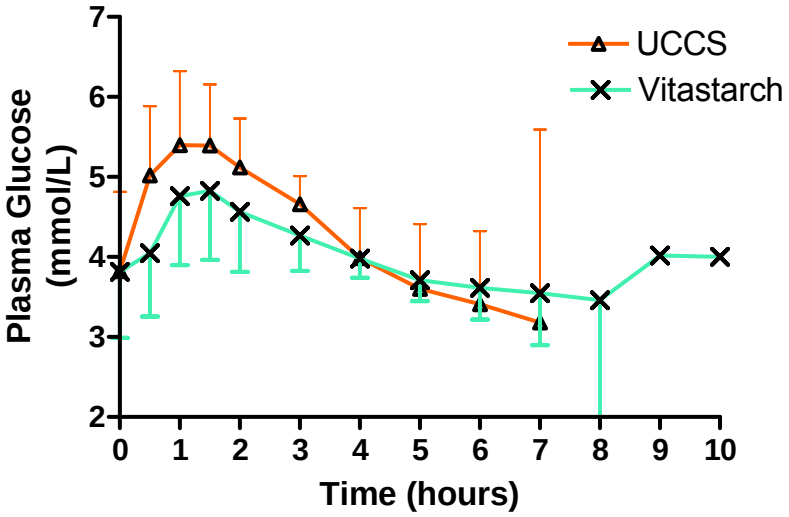


Figure 4.7 - Mean glucose profile (+ / - 95% confidence interval) for 7 patients starch loads

Similar to the previous study (Bhattacharya et al, 2007)[142], the glucose rises more slowly to it's peak with Vitastarch compared to UCCS with a mean rise of 0.63 mmol/hr compared to 1.61 mmol/L, although this finding is not statistically significant. The gradient of decline is also less, decreasing at a mean of 0.357 mmol/hr with Vitastarch compared to 0.632 mmol/hr with UCCS (p = 0.028 using non parametric analysis).

In all cases, peak glucose concentrations occur in the first four hours of the starch load with the mean and median profile for UCCS being greater than Vitastarch. After four hours the profiles are reversed with Vitastarch being higher than UCCS. Simple analyses of areas under the curve and median curves for the entire profile are consequently unrepresentative of the data. This coupled with the fact that some subjects withdraw from the study after 4 hours due to hypoglycaemia, mean that there is incomplete data after 4 hours for comparisons. For this reason, a series of analyses have been performed examining the profiles in the first 4 hours, when there is complete data from 7 pairs of starch loads (figure 4.8). Firstly the median glucose profile of UCCS was significantly greater for the first four hours ($p<0.02$.) There was a trend towards the

Vitastarch area under the glucose curve (above the cut-off of 3.0 mmol/L), being less than the UCCS area under the curve (p=0.11 – figure 4.9)

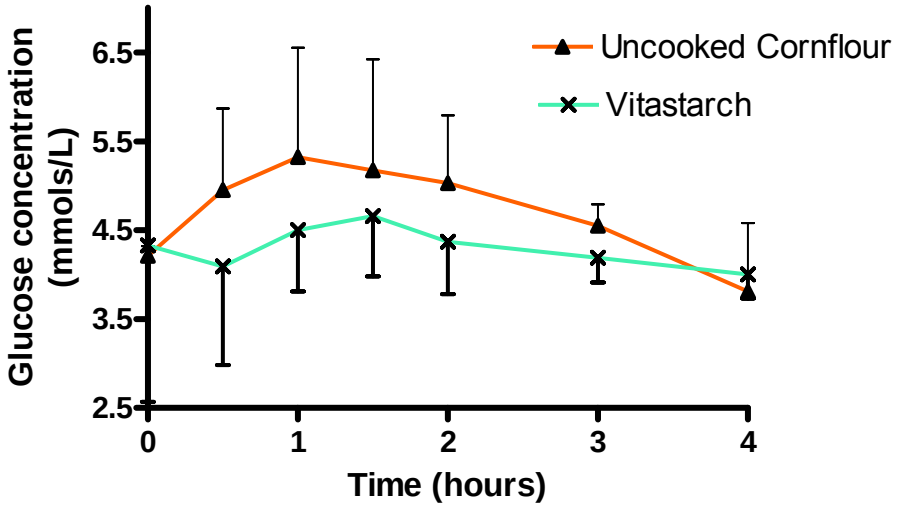


Figure 4.8 – Median glucose profile for first 4 hours (+/- interquartile range) for 7 patients that took 50g of UCCS and Vitastarch . p < 0.02 (Wilcoxon signed rank).

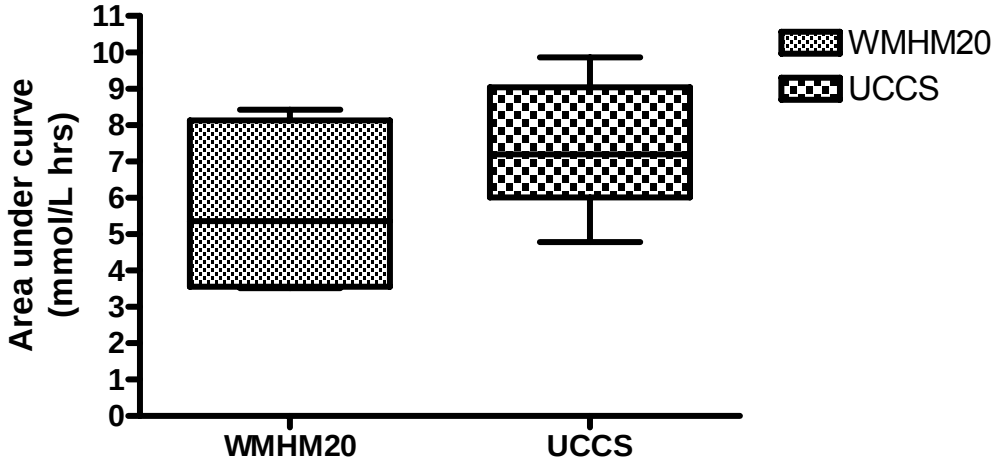
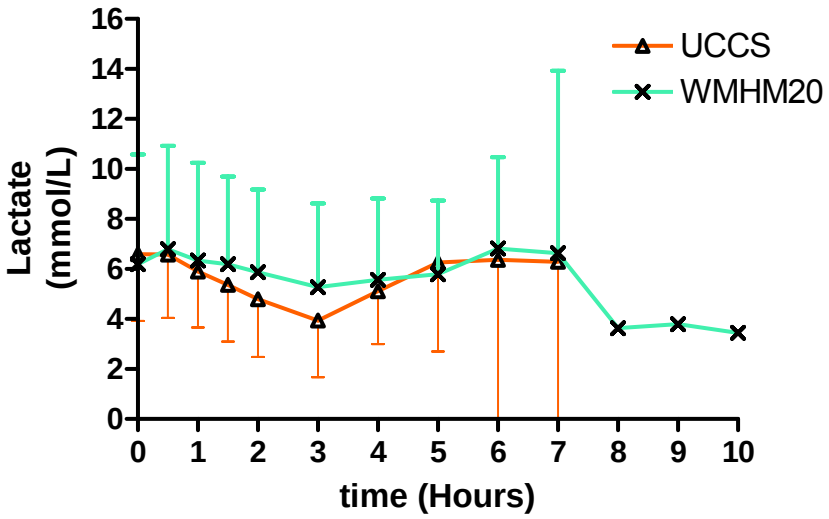


Figure 4.9 Area under the glucose curve for 7 patients for the first 4 hours of each starch load p = 0.11

Lactate

The mean lactate profiles were very closely matched throughout the starch loads. There was no statistical difference between the mean profiles. Also comparison of paired area under curves for the first four hours were similar (p = 0.47; figure 4.10)



Figue 4.10 – Mean lactate profile (+/- 95% confidence interval)

Insulin Similarly to the glucose data, there was a rise in the insulin profile in the first 2 hours after the starch load. The mean and median insulin concentrations are similar at baseline and at 7 hours when insulin measurements were discontinued. At all other times throughout the profile, both the median and mean insulin profile is greater for UCCS than for Vitastarch. Comparison of the mean profile was statistically significant by a two tailed paired t-test (p =0.0098.) Comparison of the paired area under the insulin curve for the first 4 hours of the starch loads, for which there is complete data also demonstrates that the Vitastarch median area of 8.48 IU/ml hrs is less than the UCCS median area of 47.6 IU/ml hrs as indicated in figure 4.12 (p = 0.03).

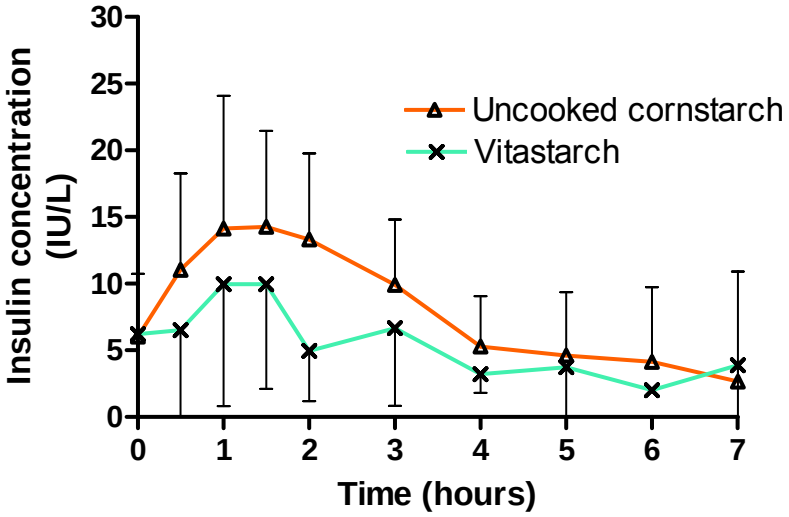


Figure 4.11 – Mean Insulin profile (+/- 95% confidence interval) for 7 patients that took 50g of UCCS and Vitastarch. p = 0.0098 (paired t-test).

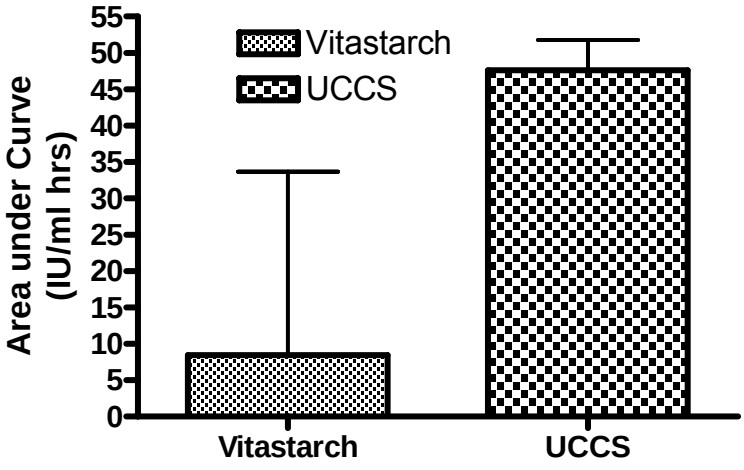


Figure 4.12 Area under the Insulin curve for 7 patients for the first 4 hours of each starch load p = 0.03 (Wilcoxon signed rank test). Column shows median and interquartile range.

Discussion

Data from these studies demonstrate that Vitastarch comprising emulsified WMHM20 has a beneficial effect on the metabolic profile compared to UCCS. At equivalent doses there is a longer duration of action, lower glucose peak, slower glucose decline and lesser insulin release compared to uncooked cornstarch.

Many of these differences are seen more clearly in the second clinical trial, which ironically had fewer subjects participating. The pilot study recruited a compliant subject whose management is meticulous such that baseline criteria were reproducible. This pilot study demonstrated a longer duration of action that warranted further investigation.

Control of Baseline Characteristics - The first study (chapter 4.2) recruited a wide range of patients aged between 3 an 47 years of age, with 3 different diseases, namely GSD Ia, GSD Ib and GSD III. The age ranges and disease differences alone had an effect on the variability of data. However, over and above this, there was evidence of variability within individuals; some of which could be accounted for difference in pre-test preparation for the two tests; other individuals prepared in the same way for the 2 starch loads but still had widely varied baseline biochemistry. In GSD type I, a patient that has fasted would have a low glucose, high lactate and low insulin. The pre starch-load preparation was individualised, but advised the same evening meal the night before each starch load, and a safe fasting period prior to starch administration. This was determined by the last cornstarch load and their normal dietary routine. For patients that were not on overnight continuous pump feeds of glucose polymer, cornstarch was administered between 2 and 12 hours before the starch load. Pre –test instruction for these individuals was relatively straightforward as it was usually close to normal treatment. Most patients on an overnight continuous feed knew that within 1 hour of discontinuing the feed, they would become hypoglycaemic. These patients

would often have a “bolus” of glucose polymer just before they stopped the pump. The effect of this bolus of glucose polymer would be to cause an initial rise in glucose and insulin and suppression of lactate. However, the insulin rise would lead rapidly to hypoglycaemia, low insulin and raised lactate. This shift was seen in some of the subjects, most notably young children, on pump feeds. For example 1 patient at baseline had a plasma glucose of 2.5 mmol/L, lactate of 5.8 mmol/L and insulin of <2 IU/L prior to one starch load. Prior to the other starch load, baseline biochemistry was glucose 5.4 mmol/L, lactate 5.4 mmol/L and insulin 9.9 IU/L, yet there was only 10 minutes difference in timing of these samples. The rapid changes of glucose in these types of circumstances are indicative of greater insulin sensitivity in younger children compared to adults.

Ideally, starch would be administered for a corn-starch load when the glucose concentration is normal, lactate is suppressed and when insulin release is minimal. It has been reported that cornstarch efficacy is sub-optimal if these conditions are not met[7;61;143]. In practice, controlling all 3 inter-dependent variables is very difficult. To fulfil this ideal, the first requirement would be real-time glucose, insulin and lactate measurements. The next requirement would be for the researchers to be able to control the subjects’ energy intake in order to achieve the desired baseline characteristics. The best way to do this would be by administration of intravenous glucose titrated to endogenous requirements as shown in the protocol by Schwenk and Hammond. [1] Such ideal pre-test management has resource implications and still may not be perfect. From a resource perspective, patients would need to be admitted the night before the starch load in order to adjust doses accordingly – the cost of overnight admission alone was double the entire cost of the first study. The disadvantage of controlling study variables to such a degree is that the findings could translate poorly into clinical practice; in reality, patients eat a variety of meals, not just corn-starch, and have fluctuating biochemistry and activity levels that are ignored in tightly controlled studies.

The second study (chapter 4.3) benefited from reliable glucose and lactate measurement at the bedside; whole blood glucose and lactate were measured within 2 minutes and plasma glucose and lactate within 10 minutes of sample collection. Bearing in mind the example of the child above, even this has a "Heisenburg" type uncertainty about it – by the time the glucose and lactate at a given time-point is defined, the metabolic circumstances have changed and has to be re-defined.

Definition of test-end. – The first study, chapter 4.2, used a bed-side glucometer to screen for hypoglycaemia and guide test management. These are known to be unreliable but are readily available, quick and relatively cheap. Analyses of results of glucose and lactate data were performed from plasma samples measured by accurate laboratory glucose dehydrogenase and lactate dehydrogenase methods respectively. However, complete results of these were often not available until the day after the starch load and could not guide management during the studies. Six of the 42 (14%) starch loads terminated prematurely because the bed-side glucometer under-read the "true" glucose. A further nine of the 42 (21%) starch loads finished prematurely when the patient was not hypoglycaemic. Eight of these were children aged between 7 and 13 years. In four out of five of these cases, the children had fasted for 9 hours using the new starch WMHM20 and one had fasted for 9 hours using UCCS. Patients with GSD, particularly children cannot fast for protracted periods – the artificial circumstance of a starch load testing maximum possible fast is contrary to their daily treatment routine. The perception of hypoglycaemia is a subjective feeling and can manifest as tiredness, irritability and sweating in the early stages with perception varying between individuals. Many patients avoid this perception by eating regularly; some consequently are not used to perceiving either hypoglycaemia or hunger. It is possible that some associate hunger with hypoglycaemia and consequently finished the test feeling hungry rather than hypoglycaemic. The plasma glucose concentration at the end for the six patients finishing prematurely taking WMHM20 ranged between

3.2 – 5.7 mmol/L, and for UCCS between 3.2 - 4.6 mmol/L. It is conceivable that subjects perceived hypoglycaemia when the plasma glucose was nearer 3.0 mmol/L but it seems unlikely when the blood glucose is greater than 4.0 mmol/L that genuine hypoglycaemia was imminent or perceived correctly. However, because the bed-side glucometer was the only device available to guide management and is known to be inaccurate,[106] for safety reasons, tests ended when the patient perceived hypoglycaemia irrespective of the bed-side reading.

Improvements made to Vitastarch study (chapter 4.3) - Several modifications to the protocol were made to the second study, in attempts to overcome some of the difficulties of the first study. Additional resources were available but there was still not enough to arrange overnight admissions for patients. A reliable point-of care glucose machine that measured both whole blood glucose and lactate within 2 minutes of sample collection and plasma concentrations within 10 minutes was used (YSI 2300.) It was possible to be confident about the measurement of glucose and lactate and relay this information to the patient. For this reason, most tests ended appropriately. Only adults were recruited meaning that the rapid changes in glucose seen in the first study were less evident. The second study also used a lower dose (50g versus 120g) so fasting times were in general shorter and patients were less likely to end the test because they were hungry. An attempt was made to control baseline characteristics more effectively by trying to administer the starch when the glucose concentration was between 4 and 4.5 mmols/L. After an intravenous cannula was placed, the blood glucose was taken and if it was greater than 4.5, no starch was given and the blood glucose was repeated 30 minutes later. Starch was administered when the glucose fell into the desired range. However of the 14 starch loads assessed, there were still 5 exceptions to this management (35%). In four cases, the plasma glucose was below the level of 4.0 mmol/L at baseline. The researcher has 3 choices in this scenario: a) to continue to administer the starch, b) to administer glucose or glucose polymer to

elevate plasma glucose to target baseline or c) terminate the test and repeat on another day when the glucose is at baseline. The last option was not considered as this was a major inconvenience for the patient and had not been assessed by an ethics committee. Elevation of the glucose to the target glucose baseline may have seemed a reasonable solution but it does not address the potential problem of lactate and insulin variation. It is inevitable that infusion of glucose or glucose polymer to correct hypoglycaemia will cause insulin release and this in itself will prejudice starch-load duration. Despite the flaws, starch was still administered when the plasma glucose was below 4.0 mmol/L. In one case, starch was administered when the baseline glucose was 4.85 mmols/L. This subject had a baseline lactate of 7.29 mmol/L, which is likely to have risen as the glucose fell. In this case too, starch was administered despite not being in the target range. As mentioned earlier, the best way to have controlled baseline would have been to titrate plasma glucose with administered glucose for several hours prior to starch administration.

Insulin

The mean insulin profile from the first study (figure 4.5) demonstrated less insulin release form patients taking WMHM20 compared to UCCS. However it was difficult to be certain of this finding because of the difference at baseline.

Insulin sensitivity - One very striking feature of the insulin data gathered in this study was the huge range of values of area under curve insulin values (above a lower measurable limit of 2 IU / L) ranging from 2 IU/L hrs to 295 IU/L hrs. This range of values clearly reflects insulin sensitivity with small amounts of insulin having a great effect on plasma glucose in young children, whilst large amounts of insulin have relatively little effect in some adults. This range of insulin release demonstrates that it is difficult to be confident of differences between the starches over such an age range. There is a greater difference in insulin release related to the age of the patient rather than the starch. It was considered, whether adjustment of data for each starch to

incorporate individual insulin sensitivity, was a possibility and these enquiries are detailed below:

The difficulty in defining insulin sensitivity by simple ratios is indicated in figure 4.13. The baseline "fasting" glucose / insulin ratio has been used by some as an index of insulin resistance.[71;76;144]

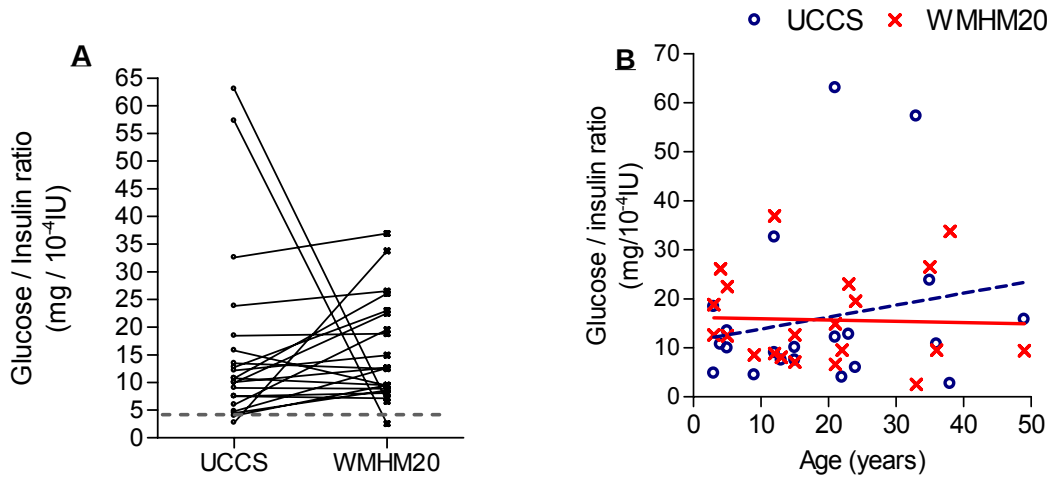


Figure 4.13 – Apparent Insulin sensitivity determined by baseline glucose / insulin ratio before each starch load from subjects recruited in study of chapter 4.2. A) Insulin sensitivity from each starch load with insulin resistance defined as <4.5 mg / 10^{-4}IU indicated by dotted line.[76] B) Baseline glucose /insulin ratios correlated with age for each starch load (best fit lines indicated).

The variation in baseline characteristics is exemplified by figure 4.13. An insulin sensitive subject releases small amounts of insulin to effect change in glucose compared to an insulin resistant subject. Consequently fasting glucose – insulin ratios have been used to indicate insulin resistance with lower values indicating greater resistance to insulin action.[76;145] These patients with glycogen storage disease have fasted for as long as they can prior to a starch load and have had baseline "fasting glucose and insulin" concentrations measured. If this was a reliable measure for them, all lines on 4.13 A would be horizontal, and all crosses and noughts, for individual patients, on 4.13 B would be super-imposed upon another. This is clearly not the case;

indeed the best-fit lines indicate a positive correlation for the baseline values with age for the UCCS starch load and a negative correlation for the WMHM20 baselines.

Patients with GSD have a different physiology to the normal population – for most a fasting glucose concentration is well below the lower reference range and a true fasting glucose to insulin ratio would not indicate the degree of insulin sensitivity – both are likely to be very low. For this study, most patients did not have a true fasting glucose because of the clinical risk this kind of assessment would pose. It seems that the compromise position of baseline glucose and insulin was not sufficient to help define insulin sensitivity either. Some patients did have much greater insulin excursions than others, as discussed earlier. An alternative model such as the area under the insulin curve from a glucose tolerance test cannot be applied to define sensitivity from these data either. This is because the starch given in this test was not glucose and this would have a different pattern of response as a consequence. There are models that incorporate mixed meals but these also rely on rebound hypoglycaemia and correction to steady state as part of their mathematical modelling[146]. Correction of rebound hypoglycaemia is not a feature of GSD. Finally, as mentioned in the next section, there is not enough resolution of the rise and fall of either glucose or insulin in the first 3 hours of each study, to be confident of capturing the full humoral response to the starch. The best methods of defining insulin sensitivity would be either an oral glucose tolerance test or a euglycaemic clamp study.

Improvements made to Vitastarch study (chapter 4.3) - Most of the studies investigating insulin release after carbohydrate ingestion detect the insulin and glucose response frequently after ingestion, because there are rapid changes in both. Most studies sample at intervals of between 5 and 15 minutes for 120 minutes after ingestion and many looking at glycaemic load, rather than index, would follow the profile for as much as 6 hours[94;119;147]. These studies give a clear idea of the nature of the rise and fall of glucose and insulin after carbohydrate ingestion. The first cross-over study sampled glucose and insulin at 60 minute intervals. It was possible to define a

slower decrease of plasma glucose using WHM20 but little else could be concluded with confidence. The difference between this study and glycaemic index studies is that our primary endpoint was duration of action rather than early responses. However, there was some evidence from this study, that the early response to glycaemic load had a bearing on duration of starch action. For this reason, study 2 measured glucose and insulin at 30 minute intervals for the first 2 hours to provide better resolution of the rise and fall of these parameters. This, coupled with the fact that the patients were a more homogenous adult population, led to more consistent results: with increased duration of action, slower glucose decline and lesser insulin release being demonstrated. . The glucose profile for Vitastarch was significantly lower for the first fours than the UCCS glucose response.

Conclusion These studies in chapter 4.2 were performed in 21 heterogenous subjects with inconclusive results whereas the study in chapter 4.3 demonstrated more striking results in a much small number of more homogenous adults with GSD I. Compared to equivalent amounts of UCCS, 50g of Vitastarch had a longer median duration of action (6.5 hours versus 5 hours; p= 0.059 – figure 4.6), a slower decrease in the glucose (0.357 mmol/hr versus 0.632 mmol/hr p = 0.028,) a lower glucose curve in the first 4 hours (figure 4.8, $p<0.02$) and lower insulin and area under insulin curves (figure 4.11, $p<0.02$ and figure 4.12, $p=0.03$ respectively.) All these findings are compatible with one another and provide compelling evidence that Vitastarch does last longer than UCCS for the patients investigated with a lesser insulin profile and glucose excursion.

Chapter 5 Gastrointestinal processing of Uncooked Cornstarch and Heat Modified Waxy Maize Starch – Evidence from Breath Tests

5.1 Introduction

As discussed in chapter I, there are a number of gastrointestinal processes involved in the successful extraction and utilisation of glucose from dietary starch. One method of assessing the entire process from ingestion to complete oxidation of dietary starch involves the assay of enriched carbon dioxide in the breath after ingestion of tracer (chaper 2). In this regard, uncooked cornstarch can act as a naturally enriched tracer.[114;148] This form of assessment attempts to quantify the proportion of dietary carbohydrate actually utilised. By contrast, the quantification of breath hydrogen assesses the amount of carbohydrate that remains undigested, fermented by colonic flora and so, not being utilised to synthesize glucose.[17;116;117] The two investigations can often be complimentary in assessing starch digestion and utilisation.

This chapter examines the short-term effect on breath enrichment with $^{13}CO_2$, after ingestion of uncooked cornstarch compared to the new dietary starch WMHM20 in children and adults with GSD I and III. Similar to cornstarch, waxy maize starch is also derived from North America and has similar enrichment of $^{13}CO_2$. The precise amount was quantified by complete combustion as discussed in the method below. Breath hydrogen was also measured to assess the degree of fermentation of each starch. This study was performed at the same time as other biochemical measurements for the studies discussed in chapter 4. There are 2 studies described in this chapter: the first examines both hydrogen and $^{13}CO_2$ excretion in children and adults with GSD I and III after ingestion of 2g/kg (max 120g) of each starch. In the second study only hydrogen excretion is measured in adults with GSD I after ingestion of 50g of

emulsified WMHM20 (Vitastarch.) Heat modified waxy maize starch has a longer duration of action than uncooked cornstarch and it may be anticipated that this would be reflected in data from utilisation and fermentation studies.

Hypothesis Because WMHM20 has a longer duration of action, there would be evidence of increased utilisation and decreased fermentation when similar quantities of UCCS are taken.

5.2 Short-term study of the utilisation and fermentation of 2g/kg (maximum 120g) of uncooked cornstarch compared to WMHM20 in children and adults with GSD I and III.

Introduction A pilot study (chapter 4.1) demonstrated that WMHM20 had a longer duration of action than uncooked cornstarch in 1 individual. A study protocol was designed to see if this benefit was observed in a larger sample of patients with GSD. Breath tests were collected as part of these studies to assess utilisation and fermentation.

Method The study protocol was approved by the Joint Ethics Committee of The National Hospital for Neurology & Neurosurgery and Institute of Neurology, London U.K., and the Institute of Child Health / Great Ormond Street Hospital Ethics committee, London, U.K. GSD I and III patients were recruited from adult and paediatric tertiary referral metabolic units in London. Written informed consent was taken from all adults above 16 years and a legal guardian of children under 16 years. The diagnosis of GSD I and III was based on a liver biopsy showing reduced activity of the appropriate enzyme, a mutation in the appropriate gene or white blood cell glycogen debrancher enzyme activity indicative of GSD III. All had evidence from their medical history of fasting hypoglycaemia and were taking UCCS.

The study had a randomised double-blind cross over design. Patients anonymised by reference number were randomly allocated to receive either UCCS or WMHM20. Each starch was manufactured using food-grade techniques and packaged in identical containers bearing a reference number. The patient reference numbers and container reference numbers were paired by Glycologic Ltd and the supervising physician was blinded to this pairing. Research participants were asked to re-attend for the second starch load, using the alternative starch 3 days to 28 days afterwards. The supervising physician devised a safe personalised fasting period for each patient based on previous cornstarch loads and medical history. Instructions were given to the research subject, or their carer, for the participant to have the same diet the day before and fast interval immediately before each starch load. They were instructed to abstain from taking certain foods such as pulses that were likely to ferment for the day prior to the starch load.

Starch Load Test An intravenous cannula was placed in the patient's arm and baseline blood and breath samples were collected. Then, 2g of the nominated starch per kg body weight (maximum 120g) was mixed in cold water and ingested. Breath and blood samples were performed hourly after the starch administration. No further intake, apart from water, was allowed. The starch load test ended when the patient had fasted for 10 hours, the blood glucose was ≤3.0 mmol/L on the bedside glucose monitor or the patient wished to end the test.

Breath data Breath hydrogen was measured immediately at the bedside using an appropriately calibrated portable hydrogen measuring device (Micro H_2, Micro Medical, Rochester, UK) while $^{13}CO_2$ breath samples were collected into a gas sampling system (Micro Medical, Rochester UK) and gas transferred using a gas-tight syringe to a gas-tight 10ml vacuum tube (Labco Ltd., High Wycombe, UK). Breath CO_2 was analysed for $^{13}CO_2/^{12}CO_2$ enrichment by gas chromatography on a CP-Poraplot-Q column

(Varian Inc., Oxford, UK) followed by isotope ratio mass spectrometry on a Thermo Finnigan Delta-XP (Thermo Finnigan, Bremen, Germany). Sample $^{13}CO_2/^{12}CO_2$ enrichment was standardised against a CO_2 cylinder (5.0 grade, BOC Special Gases, Guildford, UK,) calibrated against the international standard Pee Dee Belemnite [PDB] (Iso-Analytical, Sandbach, Cheshire UK). The WMHM20 and UCCS $^{13}C/^{12}C$ ratios were analysed by elemental analyser isotope ratio mass spectrometry (Iso-Analytical, Sandbach, UK). The enrichment of UCCS, WMHM20 and Maxijul glucose polymer (SHS Ltd, Liverpool, UK) after complete combustion were δ ‰ = -11.13, -10.75 and –11.32 respectively. UCCS and WMHM20 utilisation were calculated from the $^{13}CO_2/^{12}CO_2$ ratios as described in chapter 2.

The same study protocol was performed using the alternative starch between 5 and 28 days later.

Statistics

Mean glucose oxidation breath values for each starch load, were compared at 60 minute intervals using a two-tailed paired t-test. However it was noted, using an unpaired two tailed t-test that there was a statistical difference (p=0.0035) in the baseline values of $^{13}CO_2$ breath enrichment of those participants who had overnight Maxijul glucose polymer pump feeds prior to the starch loads, with the mean +/- 1 standard deviation being (-18.4 +/- 2.70 ‰ vs. PDB) compared to those just taking UCCS (-21.6 +/- 2.97.) The ^{13}C content of Maxijul by complete combustion was ascertained due to this difference in baseline and is indicated in chapter 2. Subsequent analyses excluded patients taking Maxijul, and were therefore performed on those patients that were not managed with continuous nocturnal pump feeds and that were fasted for 2 hours or greater, before the starch load as indicated. The results of these analyses are shown in Figure 5.1, in which hourly and cumulative starch oxidation is indicated.

The mean hourly hydrogen excretion for each starch was compared using a two tailed paired t-test as indicated in figure 5.2. The area under the graph for each profile was also calculated; the mean area for each cohort and comparison by paired t-tests is indicated in table 5.2. However, the area under the curve may not be entirely representative of hydrogen excretion as shorter trials have less area than longer trials with similar excretion. Consequently the mean hydrogen excretion per starch load is also indicated in table **5.2.**

Results – patients

Patient characteristics are indicated on table 5.1

						Test Duration (hours)	
ID	***Age (Yrs)***	***SEX (M/F)***	***Type***	***Pre-load fast (hrs)***	***Nocturnal regimen***	***WMHM20***	***UCCS .***
B	4	F	IA	<0.5	CNPF	4	4
C	5	M	IA	<0.5	CNPF	3	4
D	5	M	IA	<0.5	CNPF	7	4
E	7	M	IA	<0.5	CNPF	9	6
G	21	M	IA	2	CNPF	10	10
H	22	F	IA	4	UCCS	10	9
I	22	M	IA	1	CNPF	6	5
J	23	F	IA	2	UCCS	6	8
K	33	M	IA	2	UCCS	10	6
L	34	M	IA	10	UCCS	10	10
M	47	M	IA	12	UCCS	7	10
O	14	F	IB	5.5	UCCS	10	8
P	15	F	IB	3	UCCS	9	10
Q	24	M	IB	4	UCCS	10	7
R	35	M	IB	2	UCCS	8	10
S	38	F	IB	12	UCCS	10	5
U	12	M	III	0.5	CNPF	7	8

Table 5.1 – Characteristics of recruited participants and starch load duration. Nocturnal treatment regimen prior to starch load is indicated. *(Further details of participants by subject ID in chapter 2)*

CNPF – Continuous nocturnal pump feeds *UCCS – Uncooked Cornstarch*

All ten adults and 7 of the 11 children were able to perform the breath tests. However, as discussed above, there was a statistical difference in baseline oxidation between those subjects that had been given glucose polymer prior to the starch load, and those that had fasted having taken uncooked cornstarch. Therefore, further analyses were confined to patients that had had uncooked cornstarch as part of their dietetic regimen.

Results

When compared on an hourly basis, there was no statistically significant increase in utilisation of UCCS compared to WMHM20, (figure 5.1A), due to the wide confidence intervals. However the hourly mean oxidation of UCCS appeared greater than WMHM20 from 3 to 10 hours.

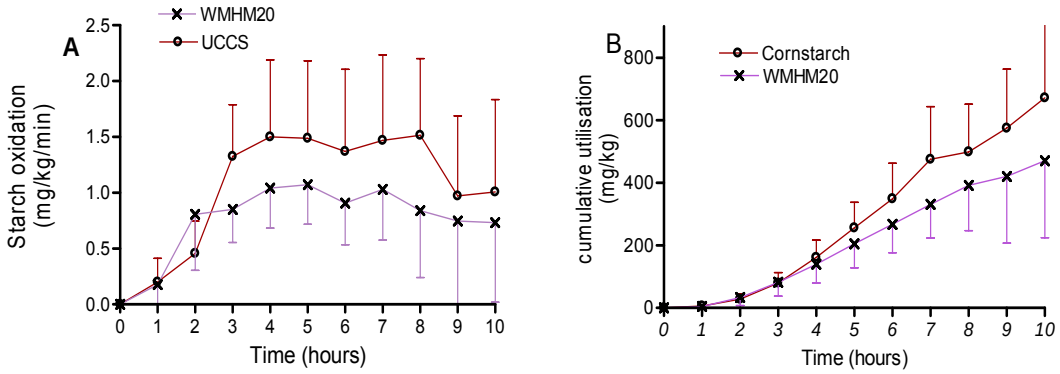


Figure 5.1 – A) Mean Starch oxidation (+/- 95% confidence interval) derived enrichment of breath $^{13}CO_2$ for 10 subjects that were able to perform breath tests (and not taking glucose polymer) B) Cumulative oxidation

The cumulative oxidation of uncooked cornstarch was greater than WMHM20 over the full time period of 10 hours ($p < 0.02$ – figure 5.1B)

The mean hydrogen breath data are shown in Figure 5.2 There was a statistically significant difference at 6 hours indicating that at this peak point there is greater fermentation of uncooked cornstarch. In addition the mean profiles were also different when compared by non parametric methods. (p=0.001).

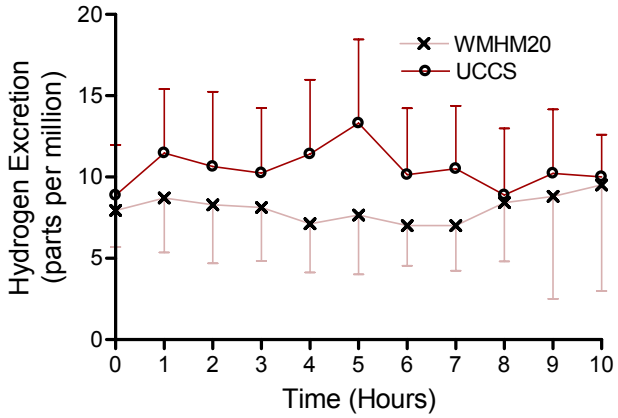


Figure 5.2 –Mean Hydrogen excretion (+/- SD) for 18 patients able to perform breath tests

		UCCS		WMHM20	
Age	**TYPE**	**Area**	**Area/ time**	**Area**	**Area/ time**
4	1a	540	1.80	600	2.50
5	1a	1530	4.25	690	2.88
5	1a	540	2.25	660	1.57
9	1a	1230	3.42	2460	4.56
21	1a	5880	9.80	5850	9.75
21	1a	11610	19.35	4650	7.75
22	1a	5250	12.50	1830	3.81
23	1a	9630	17.83	4050	8.44
34	1a	4140	9.86	11760	19.60
35	1a	4800	8.00	8130	13.55
49	1a	6990	11.65	2610	6.21
14	1b	2430	4.50	1410	2.35
15	1b	11250	18.75	2550	4.72
24	1b	6900	19.17	4530	7.55
33	1b	3990	6.65	9300	15.50
38	1b	6750	11.25	1740	2.90
12	3	1470	3.06	3030	7.21
	Mean	**5216**	**10.06**	**3926**	**7.10**

Table 5.2 – Area under the hydrogen curve – (ppm minutes) and mean area for subjects taking starch loads with UCCS and WMHM20

Table 5.2 shows the calculated area under the hydrogen curve. There were no statistical differences between the areas under the hydrogen curve when these were compared for the 2 starches by paired t-test (p=0.26.) Because starch load duration

differed between starch loads, this total figure was unrepresentative of the data; therefore, the mean hydrogen excretion was calculated from area /time. Comparing the 2 starches, this mean hydrogen excretion showed more difference (p= 0.14). It may be anticipated that hydrogen excretion is greater in GSD Ib than Ia; therefore, mean hydrogen excretion was compared by unpaired t-tests between the two populations for cornstarch (p = 0.44) and for WMHM20 (p=0.81.)

Relationship between Age and Hydrogen Excretion

For the 6 children in our study that were 14 years and under that were able to perform breath tests adequately, the mean hydrogen excretion (+/- 1 SD) for the duration of the studies were 3.8 ppm (+/- 2.6) for WMHM20 and 3.5 ppm (+/- 2.1) for UCCS. For the 12 patients 15 years and over, these values were 9.8 ppm (+/- 6.1) for WHMH20 and 13.4 ppm (+/- 6.8) for UCCS. In addition, there was statistical significance (p <0.00001) using a two tailed unpaired t-test comparing the mean hourly excretion between the 2 age ranges for each starch (figure 5.3).

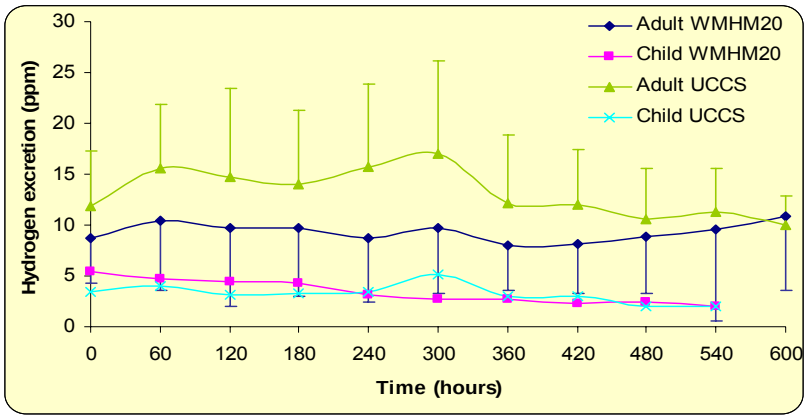


Figure 5.3 – The effect of age on hydrogen excretion in the breath

The relationship between the age and mean hydrogen excretion for an individual was investigated by curve fitting. There was a positive linear correlation (r=0.498; p= 0.042)

between the mean hydrogen excretion throughout the profile for cornstarch loads and the research subjects age. (figure 5.4A), as well as an exponential correlation (r=0.624; p= 0.0074).

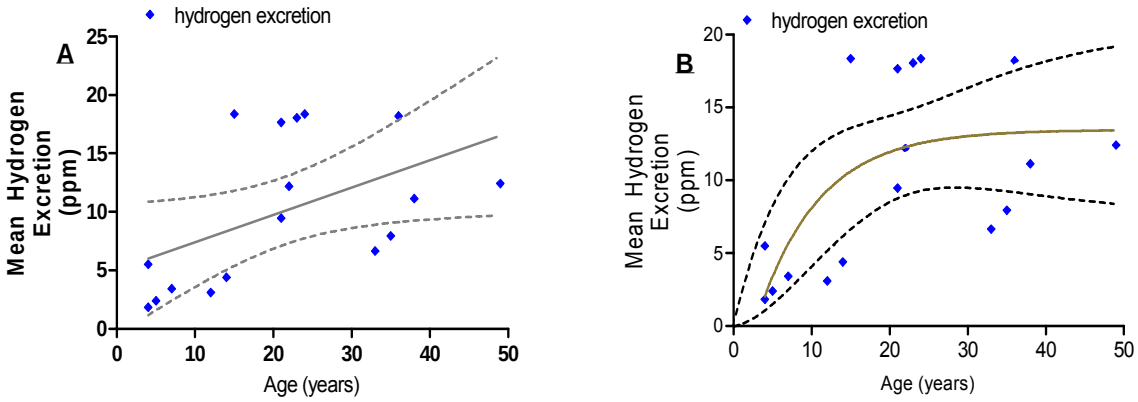


Figure 5.4 – Sample curves fitting describing the effect of age on mean hydrogen excretion throughout profile of a cornstarch load (Best fit lines and 95% confidence intervals indicated) – A) Linear association B) non-linear association

5.3 Short-term study of the fermentation 50g of uncooked cornstarch compared to Vitastarch adults with GSD I.

Introduction Data from the study performed in chapter 5.1 appeared to show greater fermentation of uncooked cornstarch compared to WMHM20. Since fermentation of starch is correlated with malabsorption, further studies were indicated. However, the enrichment of $^{13}CO_2$ in the breath was confounded by the ^{13}C content of the glucose polymer, independent of the starch ingested. Therefore, the measurement of hydrogen excretion alone was performed in this study. These measurements were made during the study described in chapter 4.3 and were part of the longer term study of the treatment using Vitastarch described in chapter 6.

Aim To assess the degree of malabsorption of uncooked cornstarch compared with Vitastarch using the hydrogen breath test.

Method The study protocol was approved by the Joint Ethics Committee of The National Hospital for Neurology & Neurosurgery and Institute of Neurology, London U.K. The study had a randomised double-blind cross over design. Patients anonymised by reference number were randomly allocated to receive either UCCS or Vitastarch. Each starch was manufactured using food-grade techniques and packaged in identical sachets bearing a reference number. The patient reference numbers and sachet reference numbers were paired by Vitaflo Ltd and the supervising physician was blinded to this pairing. The supervising physician devised a safe personalised fasting period prior to each starch load for each patient based on previous cornstarch loads and medical history.

Starch Load Test An intravenous cannula was placed in the patient's arm and baseline blood and breath samples were collected. When the participant's blood glucose was under 4.5 mmol/L, they mixed 50g of the nominated starch in cold water and drank the fluid. Breath and blood samples were performed at 30 minute intervals for the next 2 hours and hourly thereafter. No further intake, apart from drinking water, was allowed. The starch load test ended when the patient had fasted for 10 hours, the plasma glucose was ≤3.0 mmol/L on the bedside glucose analyser (YSI 2300) or the patient wished to end the test. When the blood glucose was ≤ 3.5 mmol/L, blood tests were performed at 30 minutes intervals, until the test end, but breath continued to be sampled hourly.

Breath Tests Breath hydrogen was measured immediately at the bedside using an appropriately calibrated portable hydrogen measuring device (Micro H_2, Micro Medical, Rochester, UK). Breath measurements were taken at hourly intervals until the test end.

Repeat Starch Load Data from the first starch load was used to guide management using the particular starch for the subsequent 16 weeks. After a further washout period of 2 – 4 weeks on normal treatment, participants re-attended for a further starch load with the alternative starch.

Results

Patients

						Test Duration (hours)	
ID	**Age (Yrs)**	**SEX (M/F)**	**Type**	**Pre-load fast (hrs)**	**Nocturnal regimen**	**UCCS**	**WMHM20**
G	23	M	IA	2	UCCS*	7	7.5
H	24	F	IA	4.5	UCCS	6	6.5
V	25	F	IA	1.5	UCCS*	4.5	6
K	38	M	IA	8	UCCS	5	10
O	16	M	IB	1.5	CNPF	7	7.5
Y	17	M	IB	4.5	UCCS*	4.5	X
P	17	F	IB	8	UCCS	5	7.5
Z	19	F	IB	4.5	UCCS*	X	5.5
Q	26	M	IB	4.5	UCCS	4	5.5
R	35	M	IB	3	UCCS	X	8

Table 5.3 – Participants recruited to study 4.2. Test terminated when patient felt symptomatic hypoglycaemia or a measurement of plasma glucose was < 3.0 mmol/L.

** Participant normally managed with CNPF but took UCCS for this study.*

X – Failed to attend for second part of study.

(Further participant details in appendix under ID code.)

CNPF – Continuous nocturnal polymer feed. UCCS – uncooked cornstarch

Patients that failed to complete the trial

Three participants failed to complete the trial, all of whom had GSD type Ib. One of these patients had protracted hospital admissions in the first phase of the 16 week long-term trial for medical problems unrelated to the trial. One of the remaining cited academic commitments as a reason for not re-attending for the second starch load and the final participant had work commitments preventing them from re-attending.

The study described in chapter 5.1 demonstrated that there is significant variation in excretion of hydrogen between individuals, and that this is partly age-dependent. For this reason data from the 3 individuals that failed to complete both starch loads is not included in the subsequent analyses but are in the appendix. Data from 7 paired starch loads were consequently analysed:

The median hydrogen excretion is indicated in figure 5.6. There was no significant difference between the median profiles (p = 0.137).

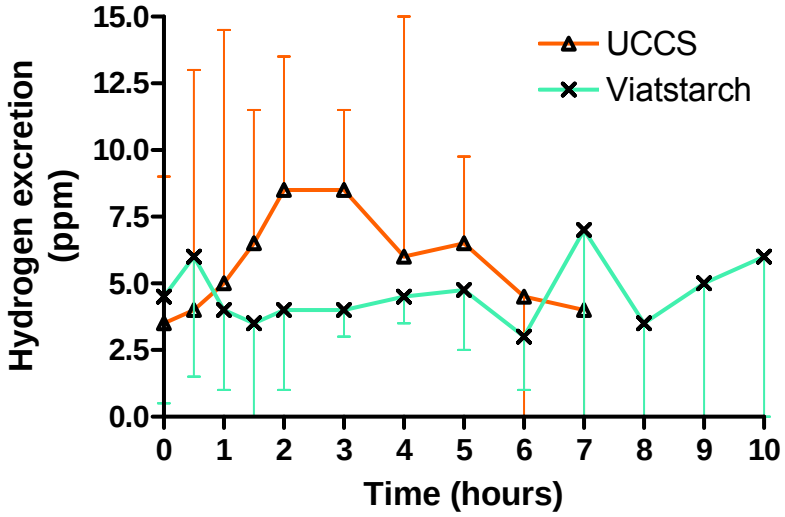


Figure 5.6 – Median and quartile range of hydrogen excretion from 7 subjects after ingesting 50g of starch.

		UCCS		Vitastarch	
Age	**Type**	**Area**	**Area/ time**	**Area**	**Area/ time**
21	1a	2550	10.63	2235	7.45
25	1a	893	3.72	1298	7.20
25	1a	2520	6.00	1320	3.15
38	1a	5400	18.00	4575	7.63
16	1b	1215	2.90	2003	4.70
17	1b	2798	9.33	953	2.64
24	1b	1995	8.31	1793	5.98
	Median	**2520**	**8.31**	**1793**	**5.98**

Table 5.4 Area under the hydrogen graph (ppm minutes) and mean area (over time) for 7 subjects that took 50g of Vitastarch.

Table 5.4 shows the calculated area under the hydrogen curve. There was no statistical difference between the areas under the hydrogen curve when these were compared for the 2 starches by non-parametric methods (p=0.30.) Because starch load duration differed between starch loads, the mean hydrogen excretion was calculated from area /time (p= 0.22).

Discussion

Combined Results

The first study performed on both adult and children appears to demonstrate reduced $^{13}CO_2$ enrichment in the breath of the novel starch compared to uncooked cornstarch consistent with decreased utilisation, whereas the hydrogen data demonstrates greater fermentation of uncooked cornstarch consistent with greater malabsorption of uncooked cornstarch. These findings would appear to be contradictory because it would be expected that the least absorbed starch would exhibit greater fermentation.

However, there were many problems with research methodology that meant that the circumstances of study were far from ideal due to the intensive nature of the patients' treatment.

Ideally, starch load tests and hydrogen / $^{13}CO_2$ breath tests should be performed after a substantial fast in order to discriminate interference from other ingested substances, but this is rarely possible in patients with GSD. In addition there should also ideally be a pre-test "washout" of ^{13}C containing food. This again is not possible in this study population who are dependent on regular cornstarch with high ^{13}C content leading to a statistically different baseline breath $^{13}CO_2$ when compared to the normal population (Bodamer et al 2002.)[62] The best compromise was to recommend that patient's pre-test management was identical for each load, with patients acting as their own control. It is likely that the previous cornstarch use also contributed to background and baseline fermentation as discussed later. We assumed that day to day variation was minimal on the two test days, yet this was not always the case, despite similar pre-test management.

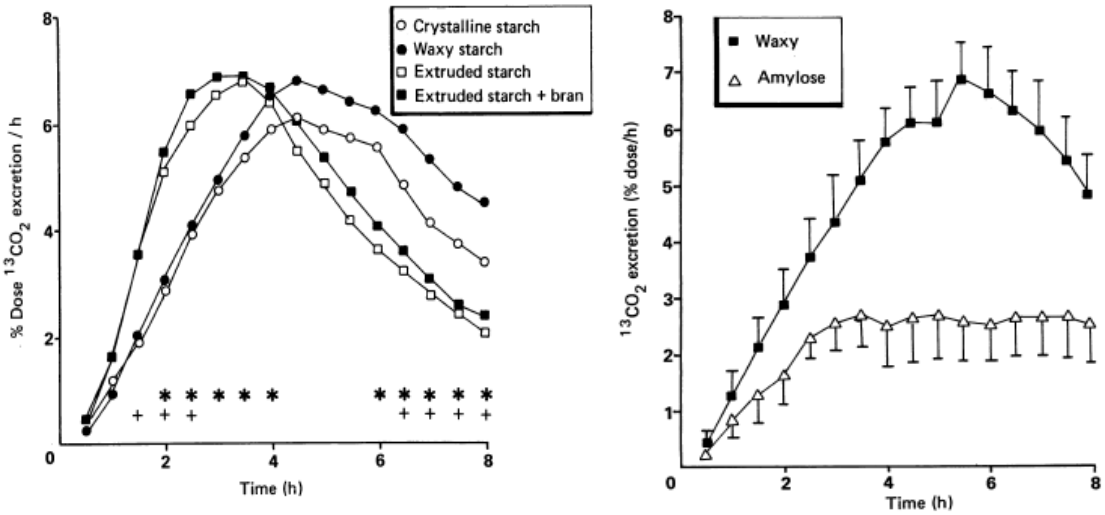
Enrichment of $^{13}CO_2$ in the breath

The central premise for stable isotope studies is that an ingested tracer is metabolised and that any increase in breath of the specific isotope reflects the extent of this metabolic process. The workings and constraints of this metabolic process cannot be gauged by these tests; the method has been likened to a "black box," where only the beginning and end results are known and little is known about mechanism.[149] The review by Rating et al gives the example of children studied by stable isotope studies suffering from diabetic coma or Crohn's disease. It is presumed the former subjects would have lower breath $^{13}CO_2$ enrichment because of decreased exogenous glucose metabolism due to an increased endogenous glucose pool, whereas the latter would have decreased enrichment due to decreased absorption. The end result is the same but the mechanism is completely different.

In the glycogen storage diseases both processes may be affected – both impaired absorption and disordered glucose metabolism are features of type I GSD. By using a

cross-over study, it was hoped to ameliorate some of the disease specific differences but each variable can fluctuate in the same individual.[19;150]

The enrichment data appears to show greater enrichment of cornstarch compared to heat modified waxy maize starch. However, it important to assess some of the detail of the enrichment curve. It has been reported that the first 180 minutes of breath enrichment reflects gastric emptying, and subsequent enrichment reflects post-absorptive metabolism.[62;151] Figure 5.1 indicates that the first 60 minutes of the studies are similar; at 120 minutes there is greater enrichment of WMHM20 but at 180 minutes and beyond, there is greater enrichment of uncooked cornstarch. The diminished WMHM20 curve could therefore represent delayed gastric emptying. Further evidence of this type of effect has been demonstrated by Hiele and colleagues (1990). This team investigated breath enrichment after ingestion of several different ^{13}C rich corn substrates. The four starches differed in their physical properties and were described as “normal crystalline starch, waxy starch, starch treated by extrusion cooking and high amylose starch.” The characteristics of the starches differed such that the waxy starch had the highest amylopectin content (98%) followed by the crystalline starch with the high amylose starch being 30% amylopectin. The extruded starch had a high amylopectin content (74%) but was the only starch to be highly gelatinised (79%.) Five subjects had paired starch loads with 50g of carbohydrate comparing high amylose starch to waxy maize starch (5.5b). Six subjects had breath enrichment studied after ingesting 50g carbohydrate of waxy maize, crystalline, extruded starch and extruded starch with bran. Result are indicated in figure 5.5a.



* P = 0.05 comparing extruded starch with crystalline starch
+ P = 0.05 comparing waxy starch with extruded starch

Figure 5.5 – Breath $^{13}CO_2$ enrichment after ingestion of 50g of starch by healthy adult volunteers. (pre-test management identical with natural enrichment quantified by combustion – ratios of excretion to natural ^{13}C abundance)

By kind permission of BMJ publishing group Ltd – From Hiele et al GUT 1990.[152]

The data indicated that the extruded starch had peak enrichment at 2.5 hours and rapidly decreased whereas the waxy starch peaked at 4 hours and had sustained elevations above the other preparations 8 hours after ingestion of the starch. It is probable that the WMHM20 ie heat modified waxy maize starch with a high amylopectin content (99.5%) is more likely to behave similar to its native starch than any of the other corn derivatives. Delayed peak enrichment would represent delayed gastric emptying with sustained elevation compatible with slower total gastrointestinal transit time.

From a methodological perspective, it should be noted that the study by Hiele et al collected breath at 30 minute intervals for 8 hours in healthy individuals. The study presented in chapter 5.1 assayed breath at hourly intervals. Therefore much of the resolution of the peak breath enrichment is lost. There are several other differences between the Hiele study and the one described in this chapter:

- There are a broad age range of subjects studied in this thesis and there may well be a difference in enrichment due to age – because the numbers are small, this cannot be tested. (n= 10 after those taking glucose polymer are excluded.)

- The dose of carbohydrate ingestion is larger in this study than most other studies (2g/ kg of starch to a maximum of 120g = 1.84g/kg to a maximum 110g of carbohydrate.) The study of glycaemic index by Jenkins et al found greater differences when 50g of carbohydrate was ingested compared to 100g.[94]

- The duration of the starch loads are variable. Studies on healthy volunteers in general have fixed duration of study but studies in patients with glycogen storage disease are terminated when the subject becomes hypoglycaemic. Some of these subjects terminated this study as early as 6 hours due to hypoglycaemia. Peak enrichment was demonstrated with the waxy starch of Hiele et al at 4 hours.[152]

- Conversely, 13 of the 20 starch loads lasted for 10 hours without hypoglycaemia. This implies that glucose oxidation from dietary carbohydrate is still occurring at the time the tests are terminated and that glucose oxidation has not been fully quantified.

The method is confounded further in patients with GSD by the assumption that breath enrichment exclusively represents ingested substrate metabolism. The ^{13}C pool within patients with GSD is likely to be high given that subjects have ingested enriched cornstarch for much of their lives. The difference to the normal population was observed by Bodamer et al.[62] Within the context of a fasting study, however metabolism and oxidation of endogenous ^{13}C within fat, protein or glycogen to $^{13}CO_2$ may occur and prejudice results.

Excretion of Hydrogen in Breath

Hydrogen is produced in the human gastrointestinal tract by the action of bacteria on carbohydrate. Symbiotic bacteria are usually present within the colon and use

undigested or unabsorbed carbohydrate as their own source of energy. Hydrogen produced as a by-product of this process is absorbed into the blood and excreted from the lungs. Increased breath hydrogen production has been shown to correlate with the increased delivery of non-absorbed substrate to the colon in the case of lactose intolerance, and pancreatic insufficiency as well as the increases in the population of bacteria in the case of bacterial overgrowth syndrome.[153-155] To differentiate these processes and increase specificity, studies have selected appropriate carbohydrate substrates to test the appropriate disease such as lactose for lactose intolerance and glucose for bacterial overgrowth. Fermentation of complex carbohydrates reflect the culmination of several physiological processes namely digestion, absorption of glucose and bacterial fermentation, each of which may be altered resulting in increased breath hydrogen.[116]

The study performed in section 5.1 demonstrated increased fermentation of uncooked cornstarch compared to WMHM20. By paired t-tests, this difference was statistically significant at 5 hours. This increased hydrogen excretion is compatible with increased colonic delivery and fermentation of UCCS compared to WMHM20, suggesting greater malabsorption of UCCS. If the criterion for malabsorption of peak hydrogen excretion > 20 ppm were used, eight UCCS subjects and four WMHM20 met this criterion.[156] The area under the hydrogen curves were also assessed with both the mean area and the mean area over time being increased in the uncooked cornstarch group, but these findings were not statistically significant (table 5.2.). The results from the study presented in chapter 5.2 are similar with the median profile for Vitastarch being lower than the profile for uncooked cornstarch – this finding is not statistically significant p= 0.137.) The median area and area over time are also lower during the Vitastarch trial but these too are not statistically significant. The mean baseline hydrogen excretion was approximately 8 ppm for each starch, which is much higher than the general population. This reflects the fact that these subjects have high carbohydrate intake

throughout the day and night, which is being fermented at the start of the study. Ideally all patients would be fasting for 12 hours prior to starting the study to eliminate background fermentation, but this is not possible in this disorder.

All of the results, therefore, appear to demonstrate greater fermentation of uncooked cornstarch compared to WMHM20 / Vitastarch. These findings could indicate there is greater malabsorption of UCCS compared to Vitastarch.

However, as discussed increased fermentation does not necessarily mean that there is more mal-digestion or mal-absorption of cornstarch. One possibility is that colonic flora has positively been selected by chronic use of uncooked cornstarch resulting in greater fermentation. This hypothesis is actively being tested by a research group elsewhere by characterising colonic flora in subjects with GSD compared to controls.[157] Another possibility continues from the hypothesis generated from the breath enrichment data. If there is increased gastrointestinal transit time, fermentation of WMHM20 starch may be delayed rather than prevented. Both figure 5.2 and figure 5.6 demonstrate that fermentation of WMHM20 increases in the latter 3 hours of both studies. This supports, but does not prove, the hypothesis that there is an increased transit time of WMHM20 compared to UCCS.

From a methodological perspective the problems with breath enrichment apply to hydrogen breath tests too:

- Peak fermentation of complex carbohydrates occurred after 5 hours of study. Several patients had terminated the test prior to or soon after this time and consequently data were not collected when fermentation was greatest. The fact that 7 of the 17 patients used uncooked cornstarch in the 6 hours prior to the study, and all had UCCS within the preceding 12 hours, meant that some fermentation of non-studied starch would have occurred during the study.

- Several subjects did not have hypoglycaemia even after 10 hours of fasting and these subjects would continue to be utilising dietary carbohydrate, consequently colonic fermentation would continue for some period of time after absorption of glucose was completed.

- The dose in this study was greater than most other studies.

- The age range studied was very varied in this study and we were able to demonstrate a statistically significant difference in hydrogen excretion between children and adults as well as positively correlating age with hydrogen excretion. This means that age is likely to have an effect on hydrogen excretion in this group of patients as discussed below.

- In addition, only seven participants contributed data to the study presented in chapter 5.2. From 4 hours into the study some of these seven failed to contribute further data as they became hypoglycaemic and needed to eat. After 7 hours, no patient taking UCCS and only 3 subjects taking Vitastarch contribute data on hydrogen excretion. Since fermentation is a function of colonic delivery of starch, typically taking 5 hours or longer, there are not sufficient data at the latter stages of study 5.2 to be confident of its findings.[116;154]

Significantly increased breath hydrogen excretion was not demonstrated in 2 previous studies of patients under the age of 22 with glycogen storage disease type I,[19;101] but was demonstrated in another study in which older adults were studied.[62] The first of these studies by Smit et al published in 1984 by a group based in Groningen was one of the first publications of the use of uncooked cornstarch in the treatment of glycogen storage disease. They report an increase in breath hydrogen excretion in the 9 subjects tested, but this was not in the diagnostic range for carbohydrate malabsorption.[101] No further data are presented in this study than this statement. The subsequent study published by the same Dutch group performed specific investigations of the gastrointestinal manifestations of glycogen storage disease in a

study published in 2002.[19] This publication reports studies performed on18 subjects with GSD Ia and 4 with GSD Ib, aged between 3 and 22 years with a median age of 16.3. Six of the GSD Ia patients and all the GSD Ib patients had a history of diarrhoea. In addition to other investigations, hydrogen excretion was measured at baseline and hourly for 14-16 hours after ingestion of 1.5g/ kg of uncooked cornstarch in some of these research subjects. Patients were allowed normal diet after 6 hours investigation without further starch intake. The publication reports that breath tests were performed on 9 of the 18 patients with GSD Ia and none of the subjects with GSD Ib. It is not clear what the ages, of those that were investigated, are. For these 9 subjects with GSD Ia, breath hydrogen never increased above 20 ppm.

Bodamer et al studied 8 subjects with GSD Ia aged between 16 and 42 years, mean age 28.3, after ingestion of 1g/kg of uncooked cornstarch and compared them to age matched control healthy volunteer subjects.[62] Breath enrichment and hydrogen excretion was measured at 30 minute intervals for the subsequent 6 hours as tolerated. Five of the eight subjects with GSD Ia maintained normoglycaemia for 6 hours with 2 studies lasting 5 hours and one being terminated after one hour – the data for the studies that lasted greater than 5 hours are presented on table 5.4. From 3 hours into the study, the subjects with GSD Ia excreted significantly greater hydrogen than controls (table 5.5)

Time	Control		GSD Ia		p value
(hours)	Mean	SD	Mean	SD	unpaired t-test
3	3	3.87	17.3	20	< 0.04
4	2.6	2.7	18	12.2	< 0.001
5	2.09	1.87	13	9.14	< 0.002
6	1.82	2.09	16.33	13.65	< 0.03

Table 5.5 – Mean hydrogen excretion (ppm +/- SD) after ingestion of 1g/Kg of UCCS in subjects with GSD and controls.

Adapted from Bodamer et al Eur J Gastro Hepat 2002[62]

The authors conclude that 2 of the 7 subjects fulfilled the criteria for malabsorption from published evidence.

The studies presented in chapter 5.1 and 5.2 indicate a statistical difference in mean hydrogen excretion for the duration of the cornstarch load for those patients aged 14 and under compared to those 15 and over (figure 5.4). Furthermore figure 5.5 correlates mean hydrogen excretion positively with age, implying that there is acquired increased fermentation in this patient group. The author of this thesis has not been able to find any studies documenting an increase in hydrogen excretion with age. The control population from the above study by Bodamer et al were age matched with much lower hydrogen excretion. The acquired increased fermentation could be due to gastrointestinal mucosal factors such as inflammation resulting in decreased absorption of glucose, impaired digestion of complex carbohydrate or bacterial overgrowth. We cannot, however, exclude that the difference between this study and those published previously are due to differences in dose or regional variations in management; both studies showing no increased hydrogen excretion were Dutch and both positive studies were from London. The centres also used different definitions of starch malabsorption, without raw data being presented; consequently it is difficult to ascertain whether there are differences between the populations.

Conclusion

Breath data demonstrated non-statistically significant increase in utilisation of uncooked cornstarch compared to WMHM20, whilst there was significant increase in fermentation of uncooked cornstarch too. These results appear inconsistent. However, there is weak evidence to suggest that WMHM20 has an increased gastrointestinal transit time on the basis of reduced early breath enrichment of $^{13}CO_2$ and increased fermentation in the last 3 hours of each 10 hour study. The validity of the results were compromised by some of the study methodology, which is well validated in healthy

controls but difficult to implement in patients with glycogen storage disease due to their tendency to develop hypoglycaemia. Further work would need to address:

- The baseline characteristics that are influenced by treatment with both glucose polymer and uncooked cornstarch.

- Early enrichment of $^{13}CO_2$ in the breath to reflect gastric emptying.

- Longer assessments of both starch oxidation and fermentation to fully assess these variables independently of hypoglycaemia occurring.

Such further studies are considered in chapter 7.

CHAPTER 6 – THE LONGTERM USE OF VITASTARCH IN GSD

Introduction

The intensive dietary regimens of patients with hepatic glycogen storage diseases, have a major impact on the lives of the patients and their families. Chapter 2 examined this intensive regimen and showed that carbohydrate intake could be large and that deficiencies of micronutrients were possible. The effect of the introduction of a novel starch on short-term biochemical parameters was assessed in chapter 3, with preliminary evidence of benefit. Some of the mechanisms of how these benefits may occur were examined in chapter 4.

Vitaflo Ltd created a new starch called Vitastarch by the addition of emulsifying fats to the raw starch WMHM20 (heat-modified waxy maize starch). In addition to this starch, there was an alternative preparation enriched with vitamins and minerals, to supplement dietary treatment called Vitastarch plus. In the UK, both Vitastarch and Vitastarch plus have been classified as “borderline” foods and not medicines by the UK regulatory body: “The Medicines and Healthcare products Regulatory Agency” (MHRA). They are consequently exempt from regulation by the UK Medicines for Humans Act (2000). This chapter describes a long-term study of the introduction of Vitastarch and Vitastarch plus into the dietary regimen of patients with glycogen storage disease type I, by comparing their use with uncooked cornstarch. The starch loads performed as part of this trial have been presented in chapter 4.3 and the breath tests in chapter 5.2.

Hypothesis Because WMHM20 has a longer duration of action, over a long-term period, patients with glycogen storage disease take less Vitastarch and Vitastarch plus than uncooked cornstarch.

Method

The study protocol was approved by the Joint Ethics Committee of The National Hospital for Neurology & Neurosurgery and Institute of Neurology, London U.K. The study had a

randomised double-blind cross over design. Patients anonymised by reference number were randomly allocated to receive either UCCS or Vitastarch. Each starch was manufactured using food-grade techniques and packaged in identical sachets bearing a reference number. The patient reference numbers and sachet reference numbers were paired by Vitaflo Ltd and the supervising physician was blinded to this pairing. The supervising physician devised a safe personalised fasting period for each patient based on previous cornstarch loads and medical history.

Assessments performed at each hospital visit (starch load admission & outpatient):

Weight and blood pressure.

The following blood tests:

- Fasting triglycerides and cholesterol,
- Uric acid
- Iron
- Vitamin B12
- Red cell Folate
- Thiamin
- Vitamin D
- Zinc
- Copper
- Selenium
- Calcium
- Albumin

Starch Load Test An intravenous cannula was placed in the patient's arm and baseline blood and breath samples were collected. When the participant's blood glucose was under 4.5 mmol/L, 50g of the nominated starch was mixed in cold water and the participant drank the fluid. Breath and blood samples were performed at 30 minute

intervals for the next 2 hours and hourly thereafter. No further intake, apart from drinking water, was allowed. The starch load test ended when the patient had fasted for 10 hours, the plasma glucose was ≤3.0 mmol/L on the bedside glucose analyser (YSI 2300) or the patient wished to end the test. When the blood glucose was ≤ 3.5 mmol/L, blood tests were performed at 30 minutes intervals, until the test end.

Biochemical data Whole blood was centrifuged at the bedside and plasma glucose and lactate were analysed immediately (YSI 2300 - Yellow Springs, Ohio, USA).

Prescription of starch The glucose and lactate data from the load test were interpreted by two investigators who have experience in interpreting these results. They were blinded to the identity of both the participant and the starch. They were given some background information of normal starch dosing and the last available cornstarch load data for that participant (for examples see appendix) On the basis of this information a safe frequency of daytime dosing for the given starch was discussed, agreed and prescribed. The participants were asked to take the nominated starch for 16 weeks.

Delivery of Starch With prior consent, the investigators arranged a projected 2 month supply of allocated starch to the participants' home, based on the blind prescription. The starch was delivered in boxes with individual sachets bearing a reference code identifying the type of starch only to representatives from Vitaflo Ltd. The participants were asked to take the nominated starch as recommended by the blinded prescribers. However, it is recognised that some patients take less or more starch doses in the home environment – the participant was asked to note what they actually took. Any subject that had been advised in their clinical management to take a vitamin or mineral supplement was also supplied with Vitastarch plus. Vitastarch was delivered in sachets of 25g and Vitastarch plus was delivered in sachets of 57g with 50g of starch and 7g of minerals and Vitamins. If patients were taking Vitastarch plus, this replaced an equivalent dose of

Vitastarch. Prescriptions were all given in multiples of 25g to conveniently accommodate the size of sachets.

Monitoring of starch use and symptoms. For the 16 week study period, participants were asked to keep a record of subjective episodes of hypoglycaemia. Whilst measurement of blood glucose was not recommended, participants were also asked to keep a record of any blood glucose measurement that was taken. One member of the research team contacted the participant to note these data as well as general information about symptoms and appetite over the preceding week (example of record in appendix) The participant was asked to complete a diet diary after they had taken the nominated starch for at least 6 weeks. They were asked to write down everything they ate for a 3 day period and the nutritional content of the diet including the starch taken were analysed by an experienced state registered dietician using specialised software (Dietplan 6, Forestfield Software, Ltd. Horsham, UK.)

End of study period. At the end of the 16 week study period, the research volunteer was asked to attend an outpatient clinic. At this visit, in addition to the tests mentioned at each visit, a patient acceptability questionnaire was completed asking the patient's opinion of the product that they had been taking. This was followed by a washout period of 2 – 4 weeks and the patient re-attended for a starch load with the alternative starch with identical assessments.

Table 6.1 below summarises the trial protocol:

Table 6.1 – Trial timeline for the use of Vitastarch in Adults with GSD I

Baseline	Week 1 - 16	Between Week 6- 16	Week16	washout	Baseline 2	Week 1b – 16b	Between Week 6b- 16b	Week16b
Starch 1 Load Test Breath Test	Start starch 1 in diet	3 Day Diet Diary	Acceptability questionnaire	Normal Regimen for 2 – 4 weeks	Starch 2 Load Test Breath Test	Start starch 2 in diet	3 Day Diet Diary	Acceptability questionnaire
Biochemistry	Monitor Use of starch and symptoms		Biochemistry		Biochemistry	Monitor Use of starch and symptoms		Biochemistry
Anthropometry			Anthropometry		Anthropometry			Anthropometry

Results – monitoring throughout the trial

Patients that failed to complete the trial – all four participants that failed to complete the trial completed most if not all of one 16 week phase of the trial. Two participants were randomised first to Vitastarch. Neither of these noted greater symptoms or problems with Vitastarch. One of these failed to attend the termination of phase assessment, but opted to continue Vitastarch supply after the trial was completed, citing fewer episodes of diarrhoea for his reason to opt for Vitastarch. The other patient attended the end of phase assessment with equivalent clinical and biochemical monitoring parameters but failed to attend the UCCS load. This participant cited study commitments as the reason for not re-attending. This participant had also been admitted into hospital during the first phase of the trial with significant renal impairment due to post-streptococcal glomerulonephritis. This complication was unrelated to therapy.

Two patients were randomised to take UCCS first – one patient reported that he felt clinically worse than normal but the end of phase assessment was equivalent to baseline. This patient failed to attend for the second phase and consequently took no Vitastarch. This participant repeatedly cancelled scheduled repeat attendances for the second phase, citing academic commitments as the reason for not to re-attending. After four months in the wash-out phase, it was felt that this subject had significantly breached the trial protocol and was withdrawn from the trial. The fourth patient felt similar to normal during the first phase, apart from during a hospitalisation for an unrelated problem. This participant attended for the starch load with Vitastarch but did not like the taste when this starch was used in the home environment. Because this patient failed to establish a routine of taking Vitastarch without UCCS, they were withdrawn from the trial.

Results from 6 patients that completed both 16 week phases of trial

Starch use over trial period

The amount of starch used per week by individual patients is indicated in table 6.2. If a patient was unwell leading to alteration of treatment, the entire week's data were omitted.

	V		K		G		M		Q		H	
Week	**VS**	**uccs**	**VS**	**uccs**	**VS**	**uccs**	**VS**	**uccs**	**VS**	**uccs**	**VS**	**uccs**
1	1475	1450	1050	1950	1050	-	-	725	-	2100	1225	850
2	1400	1500	1050	2500	1050	1050	700	700	875	2100	1225	700
3	1500	-	1050	2600	1050	1050	700	700	675	2100	1225	-
4	1500	1400	975	2500	1050	950	700	700	625	2100	1225	700
5	1525	1450	1000	2750	1000	1050	700	700	1050	2100	1225	700
6	1525	1450	875	2675	1050	1050	700	700	1050	2100	1225	675
7	1475	1475	875	2800	1050	1050	750	700	1050	2100	1225	875
8	1400	1425	875	2850	1050	-	700	700	1050	2100	1225	700
9	1525	1525	875	2480	1050	-	700	700	1050	2100	1075	525
10	1400	1525	875	2550	1050	1050	700	700	1050	2100	875	550
11	1500	1525	875	2850	1050	1050	700	700	1050	2100	875	875
12	1400	1425	875	2550	1050	1050	700	700	1050		775	700
13	1575	1550	850	2700	1050	1050	700	700	1025		700	700
14	1625	1450	875	2925	1050	975	700	700	1050	2100	700	700
15	1400	1525	875	3000	1050	1000	700	700	1050	2100	700	860
16	1425	1525	875	2700	1050	1050	700	700	1050	2100	790	675
Mean	**1478**	**1480**	**920**	**2648**	**1047**	**1033**	**703**	**702**	**983**	**2100**	**1018**	**688**
T Test	**0.559**		**1.49851E-11**		**0.378**		**0.337**		**5.0527E-10**		**0.00023**	

Table 6.2 – Amount of each starch used by each patient weekly (grams per week.)

There was little difference in the amount of starch used by participants V, G and M. There was a very significant reduction in the amount of starch used by K and Q. Both patients have short fasting intervals (5 and 4 hours respectively) with UCCS, meaning that the reduction in use of starch had a large impact on their dependence on regular dosing. Participant H took significantly more Vitastarch. This patient was randomised to taking UCCS first and had to be informed of significant deterioration in long-term renal function at the end of the first 16 week period. This was unrelated to the trial. Subsequently, there was a dramatic improvement in adherence to prescribed medical therapy including the use of starch. This is substantiated by an increase in weight over the washout period as indicated in table 6.3. The adherence to prescribed treatment persisted through the 2nd phase of the trial on Vitastarch. This patient returned to their normal starch use between weeks 9 and 16 of the Vitastarch phase as indicated in table 6.2.

Weight change during trial

The change in weight of patients is shown on table 6.3. The product that the participant was randomised to take first is indicated in the first column. Patient Q was randomised to take Vitastarch first and took substantially less starch than they normally do, and lost 2.8 Kg weight. Q took more UCCS than Vitastarch in the second phase and regained 2 Kg weight. Patient H was randomised to take UCCS first and lost 2.4 kg. As mentioned above, information was given at the end of this phase that lead to strict adherence to therapy leading to a 4.3 Kg increase in weight in the washout phase. A further 7.1 kg was gained in the second phase on Vitastarch. Patient M had little weight change on UCCS but increased 6.9 kg on Vitastarch. This participant did not like the taste of Vitastarch plus and added chocolate powder to the product which added a further 10% to energy intake. This, combined with a reduction in exercise during school summer holidays, led to an increase in weight.

There was no significant weight change for G and V. K was randomised to UCCS first and increased 4.6 kg of weight concomitant with a change in employment. They took substantially less starch on the Vitastarch phase and finished the trial at baseline weight.

ID	First starch	Pre UCCS	Post UCCS	Post - pre	Pre VS	Post VS	Post - pre
Q	**V**	50.3	52.3	2.0	53.2	50.4	-2.8
H	**U**	63.2	60.8	-2.4	65.1	72.2	7.1
M	**U**	73.1	72.8	-0.3	73.6	80.5	6.9
G	**V**	57.8	58.0	0.2	56.7	57.7	1.0
V	**U**	77.6	76.4	-1.2	76.5	76.0	-0.5
K	**U**	55.0	59.6	4.6	59.5	55.0	-4.5

Table 6.3 – Weight change during trial (kg) – *First column indicates participant identity and second column the product which they were randomised to take first. V = Vitastarch; U = Uncooked cornstarch.*

Biochemistry – Metabolic Control

The biochemical parameters, triglyceride, cholesterol and uric acid reflect metabolic control over days and weeks for patients with GSD I. The data are shown below in table 6.4. Elevations in all parameters are common. Hyperuricaemia can lead to gout or renal stones and is effectively treated with Allopurinol. Patient Q was randomized to Vitastarch first and had gross elevation of the plasma uric acid concentration at baseline. The participant was urged to comply with therapy in the first instance and had an increased dose of Allopurinol at the end of the first phase through to the end of trial. There was also some increase in the plasma triglyceride concentration over the Vitastarch phase. There was no significant alteration in metabolic control for all other participants. In summary, metabolic control as measured by these three parameters appears equivalent.

Trial ID	Biochemical Test	Pre VS	Post VS	Pre UCCS	Post UCCS	Reference Range
H	*Triglyceride*	3.62	5.1	4.52	5.92	0.42 - 2.00mmol/L
	Cholesterol	5.4	6.2	6.2	6	3.35 - 6.2mmol/L
	Uric acid	267	238	435	244	149 - 369umol/L
K	*Triglyceride*	1.92	2.14	5.12	2.76	0.42 - 2.00mmol/L
	Cholesterol	2.6	2.8	4.8	3.4	3.35 - 6.2mmol/L
	Uric acid	235	194	304	237	149 - 369umol/L
G	*Triglyceride*	10.7	7.71	9.0	5.5	0.42 - 2.00mmol/L
	Cholesterol	8.1	6.9	6.5	6.4	3.35 - 6.2mmol/L
	Uric acid	326	325	393	335	149 - 369umol/L
V	*Triglyceride*	8.99	5.64	7.4	5.83	0.42 - 2.00mmol/L
	Cholesterol	6.8	5.6	6.9	5.7	3.35 - 6.2mmol/L
	Uric acid	406	269	309	264	149 - 369umol/L
Q	*Triglyceride*	2.91	5.44	5.03	2.24	0.42 - 2.00mmol/L
	Cholesterol	4.1	4.5	4.1	3.2	3.35 - 6.2mmol/L
	Uric acid	872	753	364	345	149 - 369umol/L
M	*Triglyceride*	3.6	3.95	1.46	3.45	0.42 - 2.00mmol/L
	Cholesterol	1.48	4.2	3.6	4.3	3.35 - 6.2mmol/L
	Uric acid	402	458	379	389	149 - 369umol/L

Table 6.4 –Biochemical indices of metabolic control during trial.

Assessments from Diet diaries – macronutrients

Comparing the diet diaries between the two phases, the relative contribution of starch to the total calorie intake of the diet was reduced by 50% with Vitastarch in 2 individuals, increased in one individual and remained the same for the others (table 6.5)

Trial ID	% energy from UCCS	Starch Glucose Delivery mg/kg/min	Total Glucose Delivery mg/kg/min	% energy from Vitastarch	Starch Glucose Delivery mg/kg/min	Total Glucose Delivery mg/kg/min
G	17	1.7	5.9	14	1.7	7.0
H	19	0.8	3.6	30	1.3*	2.6*
V	26	1.8	5	23	1.7	4.4
K	54	4.3	6.1	24	1.6	3.3
M	16	0.9	3.7	13	0.8	3.2
Q	35	3.8	6.6	17	1.8	3.5

Table 6.5 – Energy contribution of treatment starch as percentage of total energy intake and absolute glucose delivery rate from treatment starch and diet calculated from diet diaries.

** This subject had a 15% increase in weight between assessments. If the baseline weight from the UCCS assessment were used as reference, the treatment starch delivery would have been 1.5 mg/kg/min and total carbohydrate intake 3.0 mg/kg/min.*

Calculation of glucose intakes, derived from total daily carbohydrate intake were also made from the diet diary. The glucose delivery from the treatment starch was also calculated. Mean glucose delivery from UCCS was 2.21 mg/kg/min, being 1.38 from Vitastarch. There was a trend to reduction of treatment starch glucose delivery, $p = 0.0625$ (by Wilcoxon signed rank test.) The reduction is indicated on figure 6.1. There are 2 subjects who appear to take more starch than others, reducing intake when taking Vitastarch such that they are taking similar quantities of starch to the rest of the group.

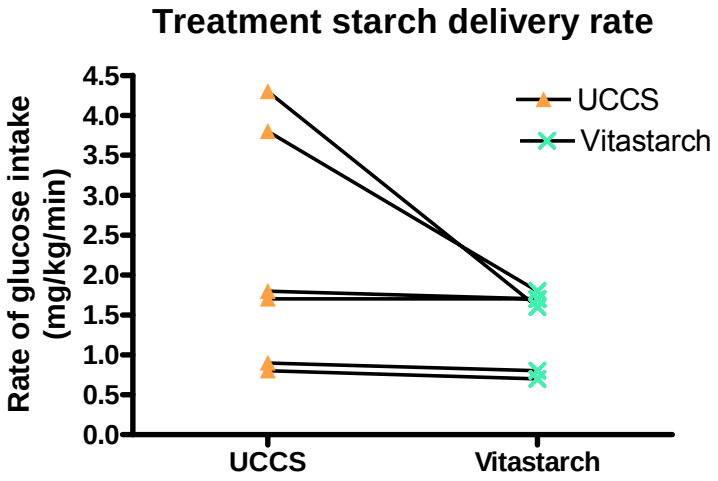


Figure 6.1 – Glucose intakes rates calculated from dietary intake of UCCS and Vitastarch from diet diaries (p = 0.0625.)

	UCCS					Vitastarch				
ID	Total Energy Intake	Percent Intake relative to EAR	Percentage Contribution to total Energy by			Total Energy Intake	Percent Intake Relative to EAR	Percentage Contribution to total Energy by		
			CHO	Prot	Fat			CHO	Prot	Fat
G	3032	104	59.5	9.5	31	3711	128	58.2	8.2	33.6
H	1570	76	59.2	8.2	32.5	1645	79	69.1	7.3	23.6
V	2946	128	69.2	17.8	13	2943	128	70.1	15.4	14.5
K	2415	93	77.4	7.6	15	1876	72	69.3	8.6	22.1
M	2238	81	65	12.3	22.8	2343	85	63.2	14.8	22
Q	3044	119	59	14.2	26.8	2454	96	49.7	17.9	32.4

Table 6.6 – Total energy intake relative to estimated average requirements and energy contribution by macronutrients whilst taking UCCS compared to Vitastarch.

	UCCS			Vitastarch		
ID	CHO	Prot	Fat	CHO	Prot	Fat
G	108	130	92	133	141	126
H	80	72	71	97	66	53
V	153	287	47	150	240	50
K	128	83	40	87	71	44
M	91	123	52	92	153	53
Q	124	195	91	81	189	85

Table 6.7 – Percentage intake of macronutrients relative to age and sex matched estimated average requirements.

CHO – Carbohydrate *Prot – Protein* *UCCS – Uncooked Cornstarch*

Tables 6.5 – 6.7 and figure 6.1 show different aspects of dietary treatment. Carbohydrate intake, both from treatment and diet are indicated in table 6.5. Absolute energy intake and percentage contribution by constituent macro nutrients are shown in table 6.6, and the intake of these relative to requirements are shown on table 6.7.

A summary of each subject's intake follows for each limb of the study:

Subject G – G took the same amount of treatment starch on both limbs of the study. However, the participant had increased total energy intake compatible with increased appetite when taking Vitastarch (from 104% EAR to 128% - table 6.6). The participant increased dietary fat and carbohydrate intake in particular but the proportions of each remained broadly similar in both dietary assessments.

Subject H – This person took more Vitastarch than UCCS in this trial. Although treatment starch intake increased, dietary carbohydrate intake reduced when taking Vitastarch leading to only a slight increase in absolute energy intake. There was an absolute reduction of fat intake whilst taking Vitastarch, leading to a proportionate increase in contribution from carbohydrate.

Subject V - There was little difference between the 2 limbs of the study.

Subject K – This person took a substantial amount of treatment starch when taking uncooked cornstarch, which reduced from 54% of total energy intake to 24% when taking Vitastarch. The substantial reduction in treatment carbohydrate intake did not lead to a change in the diet with absolute fat and protein intake remaining similar but becoming proportionally greater within a lower total energy and carbohydrate diet.

Subject M - Apart from a 10% increase in energy intake due to the use of chocolate powder with Vitastarch plus, there was little difference in the dietary analyses.

Subject Q – This person reduced total treatment starch intake when taking Vitastarch Substantially from 34% of total energy intake to 17%. Similar to subject K, there was no dietary compensation for this reduction. Consequently there was a reduction in total energy intake with concomitant proportionate increase of fat and protein.

Micronutrients – Vitastarch plus and biochemical monitoring during trial

Bold and underlined below reference range.

VS + NOT TAKEN *ID*: **G**	**Pre Vitastarch**	**Post Vitastarch**	**Pre Cornstarch**	**Post Cornstarch**	**Reference Range**
Iron		18.5	18.6		5.0 - 25.0umol/L
Vitamin B12	160		180	**141**	150 - 900ng/L
Redcell Folate	244		226	297	150 - 650ug/L
Thiamine		194	184		66 - 200nmol/L
Vitamin D		24	28		15 - 100nmol/L
Zinc		16.8	14.6		11 - 18umol/L
Copper		18.5	20.8		11 - 22umol/l
Selenium		**0.87**	**0.88**		0.89 - 1.65
Calcium		2.42	2.55	2.51	2.10 - 2.55
Albumin			53	52	35 -50

VS + taken *ID*: **H**	**Pre Vitastarch**	**Post Vitastarch**	**Pre Cornstarch**	**Post Cornstarch**	**Reference Range**
Iron		11.1	6.5	24.3	5.0 - 25.0umol/L
Vitamin B12	152 (serum)	**130**	163	186	150 - 900ng/L
Redcell Folate	4.6	332	(serun) 5.8	(serum) 5.7	3.0 - 20ug/L
Thiamine	180		112	148	66 - 200nmol/L
Vitamin D	23		20	21	15 - 100nmol/L
Zinc	11.1	17.2	13.9	11	11 - 18umol/L
Copper	16.88	12.73	17.7	18	11 - 22umol/l
Selenium	1.1		1.16	0.18	0.89 - 1.65
Calcium	2.24	2.22	2.18		2.10 - 2.55
Albumin	41	39	41	41	35 - 50

VS + taken *ID*: **V**	**Pre Vitastarch**	**Post Vitastarch**	**Pre Cornstarch**	**Post Cornstarch**	**Reference Range**
Iron	13.1	10.5	15.6	10.2	5.0 - 25.0umol/L
Vitamin B12	477	493	387	602	150 - 900ng/L
Redcell Folate	1226	779	937	1057	150 - 650ug/L
Thiamine	164	142	149	153	66 - 200nmol/L
Vitamin D	96	46	67	55	15 - 100nmol/L
Zinc	13	14.6	17.4	13.9	11 - 18umol/L
Copper	19.9	17.85	15.9	17.2	11 - 22umol/l
Selenium	1.66	1.69	1.28	1.49	0.89 - 1.65
Calcium	2.4	2.36	2.39		2.10 - 2.55
Albumin	49	48	48	48	35 - 50

VS + taken *ID*: **K**	**Pre Vitastarch**	**Post Vitastarch**	**Pre Cornstarch**	**Post Cornstarch**	**Reference Range**
Iron	8.8	12.6	7.6	7.3	5.0 - 25.0umol/L
Vitamin B12	288	214	163	224	150 - 900ng/L
Redcell Folate	361	673	414	368	150 - 650ug/L
Thiamine	148	172	156	153	66 - 200nmol/L
Vitamin D	35	63	74	37	15 - 100nmol/L
Zinc	11.2	11.8	14.7	12	11 - 18umol/L
Copper	14	14.96	11.3	16.1	11 - 22umol/l
Selenium	***0.58***	1.07	0.9	**0.68**	0.89 - 1.65
Calcium	2.12	2.21	2.37	2.29	2.10 - 2.55
Albumin	38	41	43	43	35 - 50

VS + taken *ID*: **M**	**Pre Vitastarch**	**Post Vitastarch**	**Pre Cornstarch**	**Post Cornstarch**	**Reference Range**
Iron	7.8	6.9	8.4	**2**	5.0 - 25.0umol/L
Vitamin B12	1500	1500	1185	1372	150 - 900ng/L
Redcell Folate	13	738	849	750	150 - 650ug/L
Thiamine	188	153	175	144	66 - 200nmol/L
Vitamin D	41	48	45	43	15 - 100nmol/L
Zinc	14	13.7	15.2	15	11 - 18umol/L
Copper	16	18.1	20.3	18	11 - 22umol/l
Selenium	**0.71**	1.05	0.99	**0.88**	0.89 - 1.65
Calcium	2.27	2.41	2.37	2.34	2.10 - 2.55
Albumin	42	46	42	46	35 - 50

VS + taken *ID*: **Q**	**Pre Vitastarch**	**Post Vitastarch**	**Pre Cornstarch**	**Post Cornstarch**	**Reference Range**
Iron	10	**1.9**	**5**	**5.2**	5.0 - 25.0umol/L
Vitamin B12	886	>1500	500	758	150 - 900ng/L
Redcell Folate	253	489	(serum) 6.7	619	150 - 650ug/L (3 - 20.0ug/L)
Thiamine	78	93	**63**		66 - 200nmol/L
Vitamin D	23	17	23		15 - 100nmol/L
Zinc	16.9	16	15.5	17.4	11 - 18umol/L
Copper	14.5	22	24.43	14.47	11 - 22umol/l
Selenium	**0.76**	1.41	**0.84**		0.89 - 1.65
Calcium	2.48	2.54	2.54	2.41	2.10 - 2.55
Albumin	48	48	44	43	35 - 50

Table 6.8 – Biochemical tests monitoring nutrition in individual subjects

	UCCS						Vitastarch						
	G	**H^**	**V***	**K^**	**M***	**Q^**	**G ~**	**H #**	**V #**	**K #**	**M #**	**Q #**	**RNI**
Calcium	1783	**589**	2023	618	1931	**446**	2001	816	1942	955	982	1655	700 - 1000 mg
Copper	**0.85**	1.39	1.46	1.2	2.47	1.74	1.39	1.31	1.79	1.37	1.5	2.06	1.2 mg
Zinc	**8.6**	**4.83**	16.6	**6.23**	19.8	12.6	9.76	9.71	19.11	10.08	15.6	17.03	7 - 9.5 mg
Selenium	**24.7**	**25**	97.4	**19.3**	105.8	**44.1**	**42.6**	**35.7**	65.5	**39.2**	**52.2**	68.5	60 - 75 µg
Thiamin	1.35	1.06	2.42	1.05	2.68	**1.01**	1.69	1.32	1.64	1.59	1.72	1.45	0.83 -1.02 mg
Riboflavin	3.36	**0.82**	3.62	**0.96**	3.51	1.3	2.92	**0.98**	3.66	1.41	1.97	3.34	1.10 - 1.30 mg
Niacin	**9.16**	**10.03**	38.6	**15.8**	38.9	21.54	**12.79**	**6.53**	16.8	**12.48**	28.3	19.2	13.7 - 18.9 mg
B12	10.7	**0.7**	13.9	1.56	9.1	9.7	9.4	1.7	11.7	2.2	5.9	16.1	1.5 µg
Folate	202	**194**	459	243	883	**154**	**179**	234	316	260	286	272	200 µg
Vitamin C	63	197	54	109	73	48	69	61	66	136	42	58	40 mg

Table 6.9 – Micronutrient intake estimated from diet diaries for subjects taking UCCS and Vitastarch

*^ inclusive of selective mineral supplement; * inclusive of multivitamin preparation, ~ Not taking Vitastarch plus; # taking Vitastarch plus*
Bold and underlined below RNI

Table 6.8 and 6.9 show individual participant's nutritional biochemistry investigations, at each phase of the trial, and nutritional assessments from diet diaries respectively. Biochemical selenium deficiency is noted in four of the subjects which resolved in all 3 that used Vitastarch plus. Dietary assessment also noted intake below the reference nutrient intake (RNI) in four patients taking UCCS and Vitastarch, with the median value being higher in those taking Vitastarch plus (24.9 and 39.2 μg / day respectively.) 2 vegetarian subjects were noted to have biochemical evidence of B12 deficiency, although functional assessments were not made; only one of these was evident from the dietary assessment. The only other nutritional deficiency measured by biochemical assessment was subject Q who had a borderline low plasma thiamine. Thiamine has an essential role in the production of energy being a co-factor in the pyruvate dehydrogenase complex. Excessive flux in the glycolytic pathway with decreased co-factor leads to clinical and biochemical sequelae. Cases of Beri-Beri are well recognised in populations with high carbohydrate and low thiamine intake.[158] Both Q and M had low serum Iron, but these were the only subjects from this cohort that have GSD Ib and this is a well recognised feature of GSD Ib.

Niacin intake was below the reference nutrient intake in 3 dietary assessments, when the subjects were taking both UCCS and Vitastarch plus. Three subjects had an estimated low Zinc intake from dietary assessment taking UCCS that improved taking Vitastarch plus.

All subjects had a normal plasma calcium. However, there was substantial variation in dietary intake with 2 subjects having intakes below the RNI taking UCCS and 2 subjects having more than twice the RNI intake of calcium.

As discussed in chapter 3, subject H had multiple projected deficiencies by dietary assessment including calcium, zinc, selenium, riboflavin, niacin, vitamin B12 and

folate. This subject also had a low total energy intake with macronutrient insufficiencies.

Patient Preference Questionnaire

At the end of each phase of the trial, the participant was asked to complete a structured questionnaire asking them their opinion of the taste, texture convenience and ease of use of the product they had been taking over the preceding 16 weeks. The scores are indicated in table 5.10. The questionnaire and scoring system is shown in appendix 3. The higher scores indicate greater preference. M showed a clear preference for UCCS, whilst Q favoured Vitastarch. The remaining patients expressed similar preferences on the basis of these questionnaires. However when patients were formally unblinded, all 6 opted for use of Vitastarch. Two of these objectively took less Vitastarch than UCCS, one cited fewer gastrointestinal disturbances and another that they had increased appetite. The remaining 2 patients gave no reason for opting for the new starch apart from the fact that it is "new".

	K		M		G		V		H		Q	
	UCCS	VS	UCCS	VS	UCCS	VS	UCCS	VS	UCCS	VS	UCCS	VS
1	2	3	3	2	3	3	1	3	3	2	2	3
2	2	2	3	1	2	3	3	3	2	2	2	3
3	2	2	3	1	3	2	3	3	2	2	2	2
4	2	2	3	1	3	2	3	3	2	2	2	2
5	2	2	1	1	1	2	2	2	1	2	2	2
6	2	2	3	2	3	3	3	3	3	2	2	2
7	1	3	3	3	2	3	3	3	2	3	1	3
8	1	1	3	2	3	3	3	3	3	3	2	3
9	2	2	3	3	3	3	3	3	2	2	2	3
Total	16	19	25	16	23	24	24	26	20	20	17	23

Table 5.10 – patient preference scores – (refer to appendix)

Symptom monitoring during trial

The participants were telephoned on a weekly basis and asked about various symptoms that were entered onto a case report form. Tables 6a – 6f (appendix) indicate reported symptoms that were noted by patients on a weekly basis during the trial. The scoring system is indicated for each table. The lower the score, the less troublesome the symptom being assessed.

Summary of symptom monitoring:

- There was no difference in either subjective episodes or measurement of hypoglycaemia.
- One patient was more bloated with UCCS but there was no difference with other patients.
- Several patients reported marginally improved appetites on Vitastarch, whilst one patient reported an improved appetite.

Discussion

Two individuals appeared to use significantly less Vitastarch than uncooked cornstarch over the course of the trial – subject K's mean intake of Vitastarch was 35% that of UCCS whilst on average subject Q's Vitastarch intake was 47% that of UCCS. The change of intake of these two participants was reflected in the diet diary assessment of macronutrient intakes with the K and Q reducing their starch intake nearer to the mean value for the group. In contrast, subject H's mean UCCS intake was 68% of the Vitastarch intake. As mentioned earlier, however, subject H was given some bad prognostic data at the end of the first period of the trial, when she had UCCS and this led

to adherence to prescribed therapy. This resulted in a gain of 4.3 Kg weight, in the washout period and subsequent continued increase starch intake. This increased adherence to therapy diminished in the last 4 weeks of the second trial period, when the participant was taking Vitastarch

The absolute macronutrient intake of the diet did not alter dramatically indicating that those that did alter carbohydrate intake did not alter their diet to accommodate the change. This aspect of the trial was observational with data being noted. Consequently, patients were not instructed to alter their diets and there was no observed change in the diet assessments. If however, there is a substantial decrease in starch intake with concomitant increased appetite; appropriate dietetic advice could lead to a more nutritionally complete diet.

There were several micronutrient deficiencies observed with selenium deficiency being noted by diet assessment and biochemical monitoring. In all cases that it was used, Vitastarch plus corrected this deficiency. Calcium intake varied greatly, partly because some individuals regularly take their treatment starch in milk. As a consequence some take more than twice the RNI of calcium and may risk bowel disturbance or nephrocalcinosis as a consequence. This practice would be in contrast to many international centres that restrict lactose intake. However, there has hitherto been no clinical observation indicating that this level of intake results in harm in this cohort of patients. By contrast, 2 subjects taking UCCS alone had low calcium intakes. Given that these subjects are at greater risk of osteopenia, this also is a concern.[62] In both instances, Vitastarch plus restored the assessment intake to above the RNI. Several other micronutrient deficiencies were noted particularly in those that had sub-optimal total energy intake. The impact the high carbohydrate diet has on both appetite and actual dietary intake could have an impact on micronutrient deficiencies. Biochemical assessment of fat-soluble- vitamin and essential fatty acid status were not made in this

study. Given the low fat diet that these patients have, this would have been desirable and is something that should be considered in further studies. Detailed discussion on the potential of the GSD diet to result in clinical problems is in chapter 3. This study showed that carbohydrate intake could be reduced in some individuals leaving the potential for a more complete diet.

There are few precedents for the valid introduction of a new dietary treatment in GSD. The major interventions that have had an impact were the introduction of continuous nocturnal pump feeds and the introduction of uncooked cornstarch into the dietary regimen of patients with GSD. The introduction of nocturnal nasogastric feeds was preceded in 1972 by a publication by Folkman et al noting that intravenous hyper-alimentation improved the biochemical parameters of a patient undergoing porta-caval shunt surgery for the treatment of GSD I.[159] This patient was not fit for surgery and improved substantially with intravenous feeding. This led to the publication of a case series of 3 patients having nocturnal feeds continuously via nasogastric feeds by Greene et al.[43] This group had noted improvement in biochemistry within 4 weeks but had beneficial clinical outcome of increased growth velocity observed over a period of 13 months. These studies set the precedent, but more robust evidence of beneficial effect could only be made after therapy had been given for several years.[117 – 119]

Likewise the early publication of Chen et al in 1984 was an “open label,” clinical study of successive patients that were managed with uncooked cornstarch.[7] Complete data obtained from starch loads and clinical follow up were not published. Eleven patients were recruited and starch load data from two of these are presented. The authors comment that they used several doses and “the optimal dose was generally found to be between 1.75 and 2.5g/kg of ideal body weight.” The details of the dosing studies were not presented. This publication was vital but much of the detail of efficacy, dose and metabolic control were much more apparent from subsequent studies.[20,37]

The trial presented in this thesis was a randomized double-blind cross-over study with each treatment phase lasting four months. The short-term effects described in chapter 4 appeared beneficial and consequently a longer-term study was indicated. The primary end-point of this long-term trial was reduction of overall starch use and with this endpoint, results were variable. Of the 10 patients recruited, 4 failed to establish therapy on the cross-over limb of the study. 2 of these never established therapy with Vitastarch. One of the remaining 2 did not show any dramatic improvement with Vitastarch and the other defaulted the end of Vitastarch phase assessment for several months rendering the assessment inadequate. This participant did, however, opt to continue therapy with Vitastarch. Of the remaining 6 patients 2 patients responded well by significantly reducing treatment starch whilst one apparently required more starch – this could be explained by increased compliance. Metabolic control did not deteriorate and the starch was well tolerated. No increase in side-effects were noted and in one subject appetite improved.

Despite these findings, this was a study of a small number of adults from one centre. Individual variability and perception of disease and treatment played a large part in adherence to therapy. Those that reduced their starch intake were more likely to take risks than those that didn't. The three subjects that demonstrated no change of treatment did not take less than the prescribed dose of starch on any occasion. They did however sometimes take extra doses. It is notable that the three subjects that did not reduce their starch doses were the ones that took overnight naso-gastric pump feeds, whereas those that altered therapy took nocturnal corn-starch. In adult life, the former population probably self-selects itself to take fewer risks. It is also true that they have a more limited period of time in the day to alter therapy as they are obliged to take pump feeds for several hours overnight. In this context and with such small study numbers, it can be difficult to make a universal comment about benefit, but Vitastarch could lead to a reduction in starch intake in some individuals.

Nutritional deficiencies were discovered in several of these patients. The GSD diet may have an impact on the overall quality and completeness of nutritional intake. Without age and sex matched control populations, these findings cannot confidently be attributed to GSD, but it seems likely.

Conclusion

The hypothesis that introduction of a new starch into the dietary regimen of patients with GSD would lead to less starch use remains unproven. However, this study demonstrated that Vitastarch was well tolerated in the dietetic regimen of patients with GSD type I. In some subjects, it led to a dramatic decrease in overall starch consumption. However, it did not, spontaneously lead to an improvement in the quality of the diet. Multiple dietary insufficiencies were noted and rigorous dietetic management is needed to prevent clinical problems. In four individuals, Vitastarch plus did show benefit in improving some but not all of the abnormal biochemistry and projected insufficiencies from dietary assessment. Similar to the introduction of nocturnal glucose polymer and uncooked cornstarch, it is likely that firm evidence of long-term benefit will not be available until Vitastarch has been incorporated into the diet for several years.

CHAPTER 7 - DISCUSSION AND FURTHER STUDIES

Chapter 7.1 - Summary of studies performed

This thesis has attempted to characterize the diet of patients with hepatic glycogen storage diseases, implement a change and study the short-term and long-term effects of this change.

The dietary analyses performed in chapter 3 confirmed that the dietary management of such patients is indeed intensive with some individuals taking greater than expected amounts of carbohydrate, leading to nutritional deficiencies. On the whole, these patients and their carers need to be constantly vigilant about their diet; if they are not, patients can rapidly become unwell. This can lead to large amounts of carbohydrates being ingested at the expense of other macro- and micronutrients. Details about the pattern of eating, the nature of the foods consumed and the impact the different eating patterns have on psychological wellbeing have not been examined in detail but is something that should be studied in greater depth.

In a series of studies, chapter 4 examined the impact the use of equivalent amounts of uncooked cornstarch and heat modified waxy maize starch had on short-term concentrations of plasma glucose, plasma lactate and serum insulin. The study presented in chapter 4.2 using native WMHM20 in adults and children appeared to demonstrate benefit on the glucose profile in some but not all individuals , but there was no difference in the lactate and insulin profile. By contrast, the more homogenous group of adults with GSD I studied in chapter 4.3 showed both a significant difference in the glucose and insulin profile as well as in the area under the curves analyzed. If translated into long-term management, this difference could well reduce long-term morbidities.

Chapter 5 attempted to quantify utilization of ingested starch by examining the enrichment of $^{13}CO_2$ in the breath as well as mal-digestion of non-utilized starch by assessing fermentation by measuring hydrogen in the breath. The study presented in chapter 5.1 did demonstrate greater fermentation of uncooked cornstarch but also greater utilisation. This finding appeared inconsistent but there were a number of methodological difficulties that made the findings uncertain. The greatest problem was the duration of assessment of the studies; the durations of studies were very variable, being determined by when hypoglycaemia occurred rather than when utilisation or fermentation of ingested starch had been fully completed. One surprising finding of this study was the positive correlation between mean hydrogen excretion and ages, implying that greater fermentation is acquired as patients grow older.

Chapter 6 examined the introduction of nutritional products Vitastarch and Vitastarch plus into the dietary regimen of adults with GSD I. A small number of patients were recruited of which several failed to complete the trial. It is difficult therefore, to be confident on the outcomes of this trial. However, 2 of the 10 subjects demonstrate dramatic reduction in the amount of starch they took without an impact on monitoring biochemistry. Three subjects showed little change and one increased their starch use during the trial. All 3 adults that did not alter their carbohydrate intake during the trial continue to take nocturnal glucose polymer feeds and consequently have a more conservative approach to treatment than the other 3 subjects that were managed exclusively with dietary starch. However, after completion of the trial all opted to take Vitastarch and one has subsequently discontinued nocturnal glucose polymer after a further clinical starch load with Vitastarch. This subject has consequently decreased the amount of intake of treatment carbohydrate. Vitastarch plus rectified biochemical evidence of Selenium deficiency in 3 subjects and improved nutritional assessments of other micronutrients.

7.2 Limitations of Studies

Limited sites: The patients recruited for the studies performed in this thesis were all managed in 3 centres in London. The 3 centres work closely with each other and have similar management protocols and draw patients from similar ethnic backgrounds. There are widespread ethnic and genetic differences in the hepatic glycogen storage diseases and regional differences in treatment that may mean that the findings from this study will not be applicable to other patient populations. This can only be tested further by other centres replicating these data. To some extent, this has occurred as discussed in chapter 7.5.

Recruitment and retention: Recruitment to studies is rightly by informed consent of adult participants and parents of child participants. For the short-term studies in chapter 4, recruitment of children and adults from the cohort of patients managed at our centre was successful. However, recruiting and retaining patients for the long-term study of chapter 6 was more problematic. Several were not prepared to make frequent trips to the research centre; others found it difficult to integrate the trial protocol into their daily regimen. People with certain personalities may be more likely to recruit to a clinical trial than others. Several of the older patients that had not been managed by our centre since childhood did not participate. This may have been because they were not familiar with researchers. Conversely, those managed at our centre since childhood may have felt obliged to participate, as their treating doctors were also the researchers. Research in rare diseases is well recognised to be problematic as individuals can exert a large effect on outcomes[160]

Most of the patients with GSD Ib did recruit to this study, possibly because they have a higher burden of disease than those with GSD Ia – mostly unrelated to hypoglycaemia. Recruitment to this study was therefore subject to a selection bias, which was dependent on several complex variables.

When dealing with patients with a high burden of disease, it is not surprising that several have disease related complications over the course of an eight month clinical trial. Four of the 10 patients recruited to this study had hospital admissions unrelated to the study. 2 of these patients had simple gastroenteritis and completed the study. The other 2 patients had more complex problems – one is likely to have had a renal stone associated with dysuria, abdominal pain and haematuria and the other had 2 admissions – the first with post streptococcal glomerulonephritis and the second with pneumonia. These complications in these 2 individuals were severe but not trial related. They may have played a part in both subsequently not completing the full trial protocol. Given the significant morbidities of this patient group, it would have been desirable for many more recruits to this study.

Breath data – The studies looking at breath enrichment of $^{13}CO_2$ and hydrogen excretion were designed to help understand some of the mechanism of starch digestion and glucose absorption. However, the studies were confounded by interference with collected data by the use of glucose polymer and treatment cornstarch. Furthermore, data collection was curtailed prematurely in several instances when patients became hypoglycaemic. This, therefore, meant that data was very incomplete.

Chapter 7.3 FURTHER STUDIES

Dietary therapy

Assessment of current dietary therapy. Any modification of current therapy relies on accurate assessment of current therapeutic goals and actual therapy.

A much cited therapeutic target is that 65% of dietary energy should be in the form of carbohydrate. It is not entirely clear where this figure originated from. In 1969 Fernandes and Pikaar, indicated a desirable range of carbohydrate intake of between 50 – 70% for GSD I and 40 – 50% for the other hepatic glycogenoses.[161] This

recommendation was mainly based on dietary manipulations, mainly of fat intake, performed over a 15 week period on 7 children aged between 1 and 4 years of age, two of whom had GSD type I. The main biochemical outcomes of this study were the serum concentrations of cholesterol, phospholipids and total fatty acids. In this short study of 2 children with GSD I, the researchers were unable to demonstrate significant difference in outcome with an iso-caloric diet of 47% carbohydrate compared to 67%. However, in one subject aged one year, the researchers were able to demonstrate significant elevation of the fatty acids, cholesterol and phospholipids in a low carbohydrate (42%) high fat diet specifically when the fat was medium-chain triglycerides (MCT.) For the 5 other patients with GSD type III and IX, high carbohydrate diets (70%) were associated with higher serum concentrations of fatty acids than a low carbohydrate diet (39%) but became more elevated when MCT was used. Other oils such as corn oil, olive oil and milk fat were better tolerated than MCT. This study, similar to several others performed by Fernandes in the 1960s is an important assessment of the physiological milieu of patients with glycogen storage disease. They have broadened the understanding of biochemical consequences of disease and therapy; they have an important role to play for targeting research, even today. They should not however be used to guide therapy in every individual with glycogen storage disease as they are case studies performed in young children looking at specific biochemical outcomes. Clinical correlates are not indicated and other important biochemical variables such as glucose and lactate are not examined in this particular study, although reference is made to the preceding hexose study that does measure these.[162] This study is a short-term physiological intervention study rather than long-term dietary one. The dose of administration of glucose in the hexose study was 2g/kg as a bolus or 1.5g/kg/hour (25mg/kg/min.) Because this dose is so large, it would be also be difficult to gauge what glucose requirements are.

In the 1970s and 1980s a series of studies looking at endogenous glucose production rates were performed. These are discussed in detail in chapter 3. The method involved the infusion of dideuteroglucose followed by subsequent quantification of the proportion of labelled and unlabelled glucose. By making serial measurements of this proportion after infusion is discontinued, the endogenous rate of appearance of glucose can be calculated. Bier calculated, in healthy controls, that endogenous production was 7.1 mg/kg/min for children under the age of six, 5.4 mg/kg/min for older children and 2.3 mg/kg/min for adults.[84] Schwenk and Hammond used a different approach by administering glucose via a nasogastric tube in children under the age of 7, noting changes in insulin, glucose and lactate profiles of patients with GSD type I.[1] They concluded that elevations in lactate were suppressed at infusion rates of 8.6 mg/kg/min. Collins et al studied endogenous glucose production rates in adults and children with GSD I, finding that in adults endogenous production was variable both between individuals and within an individual.[150]In some instances, glucose production was the same as healthy volunteers. One limitation of all these studies is that all are performed at rest. Mundy et al showed that failure of glucose production in patients with GSD led to decrease in glucose within 6 minutes of starting exercise, compared to controls.[163] The dietary analyses performed in chapter 3 also show that total energy intake can be very variable in this group of patients with some taking 150% the energy intake recommended. In adults, burden of disease is very variable and consequently relative carbohydrate proportion should be adjusted according to total dietary energy and needs rather than being based on theoretical glucose production rates. Further evidence of a more pragmatic approach than a theoretical approach is provided by a more recent Japanese study using MCT treatment.[164] In contrast to the Fernandes and Pikaar study there was a slight adjustment to carbohydrate proportion of total energy from 70% to 65% with the balance contributed by MCT in an iso-caloric diet. This was an open intervention for a period of time between 1 and 3 months that could be criticised for bias; the MCT intervention being second in each case without a return to

original treatment, in growing children. Despite these shortcomings, they report an improvement in mean glucose, lactate and triglycerides with an increase in ketone body production. Several previous reports have shown decreased ketogenesis in GSD I; the postulated mechanism being that malonyl-CoA produced from acetyl CoA generated by both glycolysis and β-oxidation inhibits carnitine palmitoyl transferase-I. This inhibits further β-oxidation and consequent ketogenesis. Therefore, the finding that ketosis and presumably gluconeogenesis could be increased is encouraging. However, it has been previously reported that the mutation that these 3 Japanese patients have namely G747T, is associated with a milder than classic phenotype.[165] It is possible therefore in milder cases that carbohydrate intake need not be as great as estimated from previous studies if gluconeogenesis can be enhanced and ketones can be synthesised effectively as an alternative fuel.

For patients with GSD I, it has been proposed that dietary treatment should provide 65% of calories from carbohydrate in children with classic disease taking the reference energy intake. However, increased requirements will be necessary during exercise and decreased requirements are likely in adults and those with milder disease. The range of glucose requirements, in adults with GSD I and patients with “non-classical” disease, have not been sufficiently defined, to make reliable recommendations of intake. Further studies of these are indicated.

Glycogen storage disease type III Dietary treatment of patients with GSD III is subject to more debate and controversy. Because gluconeogenesis remains intact in GSD III compared to GSD I, there are several advocates of diets including gluconeogenic pre-cursors. A high protein diet has been advocated for the musculoskeletal forms of glycogen storage disease, GSD II,III and V by Slonim in several publications.[51;166-169] The rationale for this form of therapy is that the myopathy is caused in part by skeletal

muscle catabolism, which fuels gluconeogenesis. Exogenous protein would, in theory, reduce this catabolic tendency and provide a source of a gluconeogenic substrate. Again there is some evidence of this effect from physiological studies of protein turnover in patients with GSD II and III.[170-172] In the short-term, the amino acid alanine has been shown to reverse these physiological effects. However, a systematic clinical trial has yet to be performed to categorically prove benefit on clinical parameters of either a high protein diet or specific amino acids in the treatment of the musculo-skeletal glycogenoses. Despite this, either or both could play a role in therapy. Clearly determining the role of protein in gluconeogenesis and in the reduction of myopathy is an important factor that needs to be clarified in clinical studies before recommendations can be made on the macronutrient composition of the diet of patients with GSD III.

Patient's understanding of dietary treatment - Reliable determination of carbohydrate requirements from clinical studies can guide clinicians in making dietary recommendations for individual patients. However, what the patient actually takes can be quite different to what is recommended. The carbohydrate intakes demonstrated in chapter 3 showed considerable variation. Patients and carers have been educated and have experience of managing hypoglycaemia and incorporate a recommended treatment into their dietary regimen.

Most clearly understand that simple sugars and complex carbohydrates relieve and prevent hypoglycaemia respectively. With this knowledge, each makes individual dietary choices. However, the consequences of excess carbohydrate in the diet, both in terms of complications related to excess and compromises in macronutrient and micronutrient dietary composition are less well understood because they are not immediate risks for the individual. It is in this area of education that treatment can be improved. Patients should be aware that burden of disease can change and nutritional advice is consequently a dynamic process that should accommodate change.

For the clinician, it therefore becomes imperative not only to be able to assess requirements and monitor changes in these, but respond to the patient's complete dietary management. From the literature, the only detailed assessments of requirements of adults with GSD I is the study by Collins et al[150], but this demonstrates a dramatic variation in endogenous glucose production in a small numbers of adults. The European Study of Glycogen Storage Disease type I recommend serial profiling of patients as an in-patient on an annual basis.[173] This method has short-comings because patients are sedentary and eat differently in a hospital environment compared to a home or work environment. The practice of performing cornstarch loads alone is even less adequate as it does not incorporate dietary intake into the management of patients. Both have an effect on glucose homeostasis. Monitoring glucose and lactate within the home environment would seem the best means of gauging dynamic metabolic control. Saunders et al evaluated a portable finger-prick lactate monitor which appeared to be reliable.[174] Our research group is evaluating sub-cutaneous continuous glucose monitors in patients with GSD I. The unpublished data shows that glucose concentrations measured by one of these devices are unreliable in the hypoglycaemia range.

Essential Fat and Micronutrient Intake – There is great potential for significant dietary imbalance in this patient group. Micronutrient deficiencies have been noted in this study. Essential fatty acid intake may be sub-optimal to enhance health and it would be prudent to measure their biochemical concentrations. Quite apart from deficient intakes, surplus micronutrients – in particular calcium may be problematic in some. In a group of patients that have renal tubular disease, that are at risk of end stage renal failure and nephrocalcinosis, calcium intakes of more than twice the recommended nutrient intake (set 2 standard deviations above the estimated average requirement) is of great concern. This amount of intake occurs as many patients in the UK mix their

cornstarch in milk, and this can be taken several times a day. It is therefore important to perform routine surveillance of intake and complications in these patients.

Outcome data should be gathered in the form of a disease registry. This is already being done by the International Study of Glycogen Storage Disease Type I (ISGSD 1), to which our centres contribute. However regional differences in patient disease burden, accurately recording treatments and dietary management, investigation of complications and outcomes of these, make meaningful analyses very difficult. The findings of the ISGSD I study first series of analyses have been presented, and was confounded by these regional variations of study population, treatment variations and data collection.

Efficacy Of Vitastarch

The Kaplan Meier curves in chapter 4.2 and 4.3 appear to show a longer duration of action of Vitastarch compared to uncooked cornstarch. As previously mentioned, the population that was studied may well be different than other populations world wide. Our research group have encouraged other groups to perform similar starch load data and to this end, the GSD research group based in Florida have recently performed starch loads of 100g of each starch on 12 patients with GSD I aged between 15 and 34 years, demonstrating similar findings in terms of increased duration of action.[175] Median test duration was 7 hours using traditional uncooked cornstarch (Argo^{TM}), and 9 hours with Vitastarch. The difference between test durations for individual subjects by paired non-parametric analyses were statistically significant – p= 0.013. The findings in our studies have therefore now been replicated in a different population.

There have been several assumptions in the design of our studies. Whilst Vitastarch is an uncooked waxy maize cornstarch that may have similar chemical properties to standard cornstarch, it is physically different. From a research perspective, it should be considered to be a new substance and simple trials such as dosing studies need to be

performed. Chen et al reported that 2g/kg was the optimum dose for uncooked cornstarch but this may not apply for Vitastarch.[7] The optimum dose from our studies has not been elucidated – we have used a dose of 50g and 120g in adults and a maximum of 2g/kg in children. Higher doses may result in longer durations of action but this is unknown.

As mentioned earlier, it is envisaged that this carbohydrate therapy will result in less release of insulin and that this may confer health benefits over several years. In particular complications related to chronic hyperinsulinism such as polycystic ovarian syndrome and features of insulin resistance as part of the "metabolic syndrome" may improve.[176] However, such changes cannot be demonstrated in small-scale studies and may only be seen in children treated with this carbohydrate through to adult life. The pathogenesis of both complications is presumed to take several years. Disease registries of large series of patients may detect such differences.

Having demonstrated efficacy in glycogen storage disease, consideration should be made of other diseases and other situations where they may be benefit of the new starch. From a metabolic perspective, uncooked cornstarch is used in some disorders of beta-oxidation such as long-chain acyl-CoA dehydrogenase deficiency (LCHAD). One group has apparently used the native WMHM20 in some children with this disorder but the data have only been presented as a poster without full publication. The data is not presented on the related abstract.[177;178] They report that glucose control was adequate, but that the children still became unwell. It is difficult to comment on this finding without detailed analysis of the data. However, It is possible that in fat oxidation defects, the relatively high level of insulin conferred by cornstarch use, inhibits lipolysis. Glucose homeostasis is maintained but toxic long-chain fatty acid accumulation may not be suppressed by a carbohydrate that releases little insulin. In this sense, insulin exerts a protective effect in beta-oxidation defects. If studies are performed in beta-

oxidation defects, long chain fatty acid metabolites should be measured in addition to glucose in order to gauge whether they are exerting a toxic effect.

For diseases where insulin release does need to be restricted, this carbohydrate may be of use. Clearly patients at risk of diabetes mellitus and older children with congenital hyperinsulinism may benefit from this product. Carefully controlled studies should be considered for such conditions.

Mechanism of action of heat modified waxy maize starch

As discussed in chapter 5, there were several methodological difficulties using breath enrichment of $^{13}CO_2$ and hydrogen breath tests in patients with glycogen storage disease. Critically these were not the principal endpoints of each study so were not evaluated fully. Despite these shortcomings, one hypothesis was suggested, namely that Vitastarch confers delayed gastric emptying compared to uncooked cornstarch. This hypothesis should be tested on its own in healthy volunteers first that are not prone to complicating hypoglycaemia. If research subjects are given a labelled sodium 1-^{13}C-acetate tracer and a lactose ^{13}C-ureide tracer, these are metabolised when food enters the duodenum and the large bowel respectively. If they are administered with food, they therefore measure gastric transit time and coecal transit time repectively.[179] A study comparing this carbohydrate with other reference starches including uncooked cornstarch would clarify whether delayed transit time is the mechanism of action of Vitastarch. The advantage of using healthy volunteers is that the same study could also examine the time to complete oxidation of the naturally enriched ^{13}C carbohydrate. These participants could exclude ^{13}C products for several weeks prior to the study, substantially reducing background release unlike patients with GSD who are dependent on enriched cornstarch and glucose polymer. If patients with GSD are studied, one way of reducing this background $^{13}CO_2$ excretion is by using a ^{12}C source of glucose polymer, essentially derived from potato starch.[180]

Mundy, Lee and Tester performed several unpublished studies using different starches. Of the starch loads performed, WMHM20 conferred the best profile, having the longest duration of normoglycaemia. This starch performed better than native waxy maize starch.[181] The observation that heat and chemically modified waxy maize starch confers greater in vitro slowly digested starch content and lower in vivo glycaemic index has subsequently been independently noted by a Chinese research group.[182] They propose as others have before that there is greater cross-linking between starch molecules rendering the starch less digestible.[183] However, He et al go further stating that their esterified and heat-modified waxy maize starch promotes cross-linking and a relative hydrophobic environment, being less accessible to digestive enzymes. They also suggest from in-vitro kinetic studies that there is non-competitive inhibition of the digestive enzymes by the esterified heat modified waxy maize starch fragments. This hypothesis is therefore one of reduced digestion of starch rather than delay in transit time leading to a lower glycaemic response. It would be anticipated that less digestion of starch would lead to greater fermentation and this was not observed in our limited studies. This hypothesis does need to be studied further in the context of the starch that was used in this thesis. Again, healthy volunteers should be studied first to gauge hydrogen and short-chain fatty acid products of fermentation for longer than we were able to do in our studies.[184]

Conclusion

This thesis has identified nutritional deficits in patients with hepatic glycogen storage diseases. Some take excessive carbohydrate and have micronutrient deficiencies. A new physically modified waxy maize starch confers a better metabolic profile in terms of lesser glucose excursions and decreased insulin release. In a few individuals it has a longer duration of action. This could offer the prospect of less therapeutic carbohydrate intake, allowing patients to integrate their diet with their peers. As a consequence, it is hoped that some long-term complications related to insulin resistance will be diminished and that patients can experience a better quality of life. Various aspects of the implementation of such a dietary strategy require monitoring to ensure long-term benefit. Further studies are required to better understand the mechanism of action and optimal treatment strategy of the new heat modified waxy maize starch.

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Appendix 1 Control Data for chapter 3

The following tables are data from UK national diet and nutrition surveys performed in children aged 4 – 18 years in 1997 and adults in 2000/01.[66;67] These population dietary intakes form the control group with which data collected from this thesis are compared.

MALES					
	4 – 6 yrs (n=184)	**7-10 yrs (n=256)**	**11-14 yrs (n=237)**	**15-18 yrs (n=179)**	**TOTAL (n=856)**
Total calories	1520 (303)	1777 (354)	1968 (435)	2285 (561)	
% of EAR	89	91	89	93	
Total Protein (g)	49 (13.5)	54.8 (12.4)	64 (15.4)	76.5 (13.9)	
Protein as % of food energy	12.9 (1.76)	12.4 (1.85)	13.1 (2.2)	13.9 (2.5)	**13.1 (2.18)**
Total Carbohydrate (g)	209 (42)	248 (51)	271 (64)	301 (84)	
Carbohydrate as % of food energy	51.6 (4.33)	52.4 (4.1)	51.7 (4.6)	50.5 (5.41)	**51.6 (4.68)**
Total Fat (g)	60.1 (14.5)	69.8 (17.0)	77.2 (20.9)	89 (24.2)	
Fat as % of food energy	35.5 (3.9)	35.2 (3.7)	35.2 (4.27)	35.9 (4.68)	**35.4 (4.17)**
FEMALES					
	4 – 6 yrs (n = 171)	**7-10 yrs (n = 226)**	**11-14 yrs (n = 238)**	**15-18 yrs (n = 210)**	**TOTAL (n = 845)**
Total calories	1397 (275)	1598 (281)	1672 (371)	1622 (417)	
% of EAR	91	92	89	77	
Total Protein (g)	44.5 (11.1)	51.2 (11.1)	52.9 (13.2)	54.8 (15.2)	
Protein as % of food energy	12.7 (1.99)	12.8 (1.9)	12.7 (2.17)	13.9 (2.48)	**13.1 (2.2)**
Total Carbohydrate(g)	191 (40)	218 (42)	228 (56)	214 (58)	
Carbohydrate as % of food energy	51.4 (5.01)	51.3 (4.32)	51.2 (5.24)	50.6 (5.62)	**51.1 (5.07)**
Total Fat(g)	55.9 (13.8)	63.8 (13.8)	67.2 (17.6)	64 (20.1)	
Fat as % of food energy	35.9 (4.4)	35.9 (4.1)	36.1 (4.98)	35.9 (5.37)	**35.9 (4.76)**

Table A1a – Mean macronutrient intake of control children (and 1 standard deviation), stratified into 4 age groups and gender.[66]

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MALES				
	19 - 24 yrs (n=108)	**25-34yrs (n=219)**	**35-49yrs (n=237)**	**Total (833)**
Total calories	2247 (525)	2337 (587)	2361 (614)	
% of EAR	89	93	94	**92**
Total Protein (g)	77.8 (18.8)	90.6 (51.0)	90.1 (23.3)	**88.2**
Protein as % of food energy	14.9 (2.6)	16.5 (4.7)	16.7 (2.9)	**16.5 (3.63)**
Total Carbohydrate (g)	273 (62)	277 (75)	279 (86)	**275 (79)**
Carbohydrate as % of food energy	49.0 (6.3)	47.7 (5.8)	47.5 (5.9)	**47.7 (6.0)**
Total Fat (g)	85.8 (29.2)	87.1 (28.0)	88.3 (28.9)	**86.5 (28.2)**
Fat as % of food energy	36.0 (6.0)	35.8 (5.4)	35.9 (5.6)	**35.8 (5.63)**
FEMALES				
	19 - 24 yrs (n=104)	**25-34yrs (n=210)**	**35-49yrs (n=318)**	**Total (891)**
Total calories	1665 (459)	1570 (390)	1654 (424)	
% of EAR	86	82	86	**85**
Total Protein (g)	59.9 (16.3)	58.7 (15.7)	65.1 (15.9)	**63.7 (16.6)**
Protein as % of food energy	15.4 (3.55)	15.9 (3.6)	16.7 (3.49)	**16.6 (3.5)**
Total Carbohydrate(g)	206 (61)	196 (53)	206 (61)	**203 (59)**
Carbohydrate as % of food energy	49.1 (8.3)	48.7 (5.8)	48.6 (6.8)	**48.5 (6.7)**
Total Fat(g)	63.9 (26.4)	59.8 (19.7)	61.9 (21.5)	**61.4 (21.7)**
Fat as % of food energy	35.5 (7.58)	35.4 (5.9)	34.7 (6.3)	**34.9 (6.5)**

Table A1b – Mean macronutrient intake of control adults (and standard deviation), stratified into 3 age groups and gender *(No trial patients were in the age group 50 – 64 and hence these reference data are not shown but they are included in the total.)*[66;67]

MALES					
	4 – 6 yrs (n=184)	**7-10 yrs (n=256)**	**11-14 yrs (n=237)**	**15-18 yrs (n=179)**	**TOTAL (n=856)**
Cis monounsaturated	19.4 (4.87)	22.8 (6.08)	25.6 (7.21)	29.6 (8.35)	24.6 (7.72)
Total cis-polyunsaturated	9.3 (2.91)	11.3 (3.64)	13.3 (4.31)	15.5 (5.21)	12.5 (4.72)
Total cis n-3 polyunsaturated	1.29 (0.570)	1.58 (0.692)	1.95 (1.239)	2.19 (0.862)	1.77 (0.949)
Total n-6 cis polyunsaturated	4.8 (1.20)	4.9 (1.370	5.2 (1.23)	5.3 (1.40)	5.1 (1.33)
FEMALES					
	4 – 6 yrs (n = 171)	**7-10 yrs (n = 226)**	**11-14 yrs (n = 238)**	**15-18 yrs (n = 210)**	**TOTAL (n = 845)**
Cis monounsaturated	17.8 (4.38)	20.9 (4.89)	22.4 (6.1)	20.9 (7.5)	20.6 (5.9)
Total cis-polyunsaturated	8.4 (3.01)	10.5 (3.31)	11.8 (3.85)	11.8 (4.40)	10.7 (3.94)
Total n-3 cis polyunsaturated	1.15 (0.499)	1.44 (0.548)	1.65 (0.716)	1.61 (0.640)	1.48 (0.639)
Total n-6 cis polyunsaturated	4.6 (1.24)	5.1 (1.41)	5.5 (1.46)	5.7 (1.68)	5.3 (1.52)

MALES				
	19 - 24 yrs (n=108)	**25-34yrs (n=219)**	**35-49yrs (n=237)**	**Total (833)**
Cis monounsaturated	29.6 (10.46)	29.9 (9.50)	27.9 (9.23)	29.1 (9.76)
Total cis-polyunsaturated				
Total n-3 cis polyunsaturated	2.1 (0.750)	2.30 (1.100)	2.31 (0.920)	**2.27 (0.960)**
Total n-6 cis polyunsaturated	12.6 (5.35)	13.1 (4.59)	13.1 (4.91)	**12.9 (5.27)**
FEMALES				
	19 - 24 yrs (n=108)	**25-34yrs (n=219)**	**35-49yrs (n=237)**	**Total (833)**
Cis monounsaturated	21.8 (8.77)	19.9 (6.88)	20.2 (7.30)	**20.2 (7.39)**
Total cis-polyunsaturated				
Total n-3 cis polyunsaturated	1.69 (0.750)	1.60 (0.630)	1.68 (0.760)	**1.71 (0.770)**
Total n-6 cis polyunsaturated	10.1 (4.95)	9.4 (3.95)	9.5 (3.91)	**9.4 (3.33)**

Table A1C – Mean unsaturated fat intake of control adults (and standard deviation), stratified into 3 age groups and gender.

Appendix 2 – Symptom monitoring scores – chapter 6

	Y3		W8		Y2		X7		X2		Y7	
Week	**VS**	**uccs**	**VS**	**uccs**	**VS**	**uccs**	**VS**	**uccs**	**VS**	**uccs**	**VS**	**uccs**
1	0	0	0	1	0			0		0	0	0
2	0	3	1	1	0	0	0	0	0	0	0	0
3	0		2	0	0	0	0	0	0	0	0	
4	0		1	1	0	0	0	0	0	0	0	0
5	0	0	0	3	0	0	0	0	0	0	0	0
6	0	0	2	1	0	0	0	0	0	0	0	0
7	0	0	1	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0		0	0	0	0	0	0
9	0	0	0	1	0		0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	1
11	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0		0	0
13	0	0	0	0	0	0	0	0	0		0	0
14	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	
16	0	0	0	2	0	0	0	0	0	0	0	0
Total	0	3	7	10	0	0	0	0	0	0	0	1

Table A2a – Number of subjective episodes of hypoglycaemia per week

	Y3		W8		Y2		X7		X2		Y7	
Week	**VS**	**uccs**	**VS**	**uccs**	**VS**	**uccs**	**VS**	**uccs**	**VS**	**uccs**	**VS**	**uccs**
1	0	0	0	0	0			0		0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0		0	0	0	0	0	0	0	0	0	
4	0		0	0	0	0	0	0	0	0	0	0
5	0	0	0	1	0	0	0	0	0	0	0	0
6	0	0	1	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0		0	0	0	0	0	0
9	0	0	0	0	0		0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	1
11	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0		0	0
13	0	0	0	0	0	0	0	0	0		0	0
14	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	
16	0	0	0	1	0	0	0	0	0	0	0	0
Total	0	0	1	2	0	0	0	0	0	0	0	1

Table A2b – Number of measured episodes of hypoglycaemia (<3.0 mmol/L) per week

	Y3		W8		Y2		X7		X2		Y7	
Week	**VS**	**uccs**	**VS**	**uccs**	**VS**	**uccs**	**VS**	**uccs**	**VS**	**Uccs**	**VS**	**Uccs**
1	0	0	0	2	0			0		0	0	0
2	0	0	0	2	0	0	1	0	0	0	0	0
3	0		1	2	0	0	1	0	0	0	0	
4	0		2	2	0	0	0	0	0	0	1	0
5	0	0	2	2	0	0	0	0	0	0	1	0
6	0	0	3	2	0	0	0	0	0	0	2	0
7	0	0	2	2	0	0	0	0	0	0	0	0
8	0	0	2	2	0		0	0	0	0	1	0
9	0	0	1	2	0		0	0	0	0	1	1
10	0	0	2	2	0	0	0	0	0	0	1	0
11	0	0	1	2	0	0	0	0	0	0	1	2
12	0	0	2	2	0	0	0	0	0		1	0
13	0	0	0	2	0	0	1	0	0		1	0
14	0	0	1	2	0	0	1	0	0	0	0	2
15	0	0	1	2	0	0	1	0	0	0	0	
16	0	0	2	2	0	0	1	0	0	0	0	2
Total	0	0	22	32	0	0	6	0	0	0	10	7

Table A2c– Score for abdominal bloating (*0 =None, 1 =mild, 2=moderate, 3=severe*)

	Y3		W8		Y2		X7		X2		Y7	
Week	**VS**	**uccs**	**VS**	**uccs**	**VS**	**uccs**	**VS**	**uccs**	**VS**	**Uccs**	**VS**	**Uccs**
1	2	0	0	0	0			0		0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0		0	0	0	0	0	0	0	0	1	
4	0		0	0	0	0	0	0	0	0	1	1
5	0	0	0	0	0	0	0	1	0	0	1	0
6	0	0	1	0	0	0	0	1	0	0	0	0
7	0	0	0	0	0	0	0	1	0	0	0	0
8	0	0	0	0	0		0	0	0	0	1	0
9	0	0	0	0	0		0	0	0	0	1	0
10	0	0	0	0	0	0	0	0	0	0	1	0
11	0	0	0	0	0	0	0	0	0	0	1	0
12	0	0	0	0	0	0	0	0	0		1	0
13	0	0	0	0	0	0	0	0	0		1	0
14	0	0	0	0	0	0	0	0	0	0	0	1
15	0	0	0	1	0	0	0	1	0	0	0	
16	0	0	0	0	0	0	0	0	0	0	0	2
Total	2	0	1	1	0	0	0	4	0	0	9	4

Table A2d– Score for diarrhoea (*0 =None, 1 =mild, 2=moderate, 3=severe)*

	Y3		W8		Y2		X7		X2		Y7	
Week	**VS**	**uccs**	**VS**	**uccs**	**VS**	**uccs**	**VS**	**uccs**	**VS**	**Uccs**	**VS**	**Uccs**
1	0	0	1	1	0			0		2	1	2
2	0	0	1	1	0	0	1	1	0	2	1	2
3	0		1	1	2	0	1	0	0	2	1	
4	0		1	1	0	0	1	0	0	2	1	1
5	0	0	0	1	1	2	0	1	0	2	1	1
6	0	0	1	1	2	1	0	0	0	2	1	1
7	0	0	1	1	1	2	0	0	0	2	1	1
8	0	0	1	1	0		0	0	0	2	1	1
9	0	0	1	1	0		0	0	0	2	1	1
10	0	0	1	1	0	1	0	0	0	2	1	1
11	0	0	1	1	0	1	0	0	0	2	1	1
12	0	0	0	1	0	1	0	0	0		1	1
13	0	0	0	1	0	1	0	0	0		1	2
14	0	0	0	1	0	1	0	0	0	0	1	2
15	0	0	0	1	0	0	0	0	0	0	1	
16	0	0	0	1	0	0	0	0	0	0	1	2
Total	0	0	10	16	6	10	3	2	0	22	16	19

Table A2e– Score for appetite (*0 = Good, 1 =Same as normal, 2=Poor)*

	Y3		W8		Y2		X7		X2		Y7	
Week	**VS**	**uccs**	**VS**	**uccs**	**VS**	**uccs**	**VS**	**uccs**	**VS**	**uccs**	**VS**	**uccs**
1	2	0	0	0	0			0		0	0	2
2	0	2	0	0	0	0	0	0	0	0	1	0
3	0		0	0	0	0	0	0	0	0	0	
4	0		0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0		0	0	0	2	0	0
9	0	0	0	0	0		0	0	0	2	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0		0	0
13	0	0	0	0	0	0	0	0	0		0	0
14	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	
16	0	0	0	0	0	0	0	0	0	0	0	0
Total	2	2	0	0	0	0	0	0	0	4	1	2

Table A2f– Score for general health (*0 =same, -2 = better, 2 = worse)*