J Inherit Metab Dis (2017) 40:703–708
DOI 10.1007/s10545-017-0054-2

ORIGINAL ARTICLE

Tight metabolic control plus ACE inhibitor therapy improves GSD I nephropathy

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Received: 31 January 2017 / Revised: 7 April 2017 / Accepted: 28 April 2017 / Published online: 13 June 2017

Abstract The onset of microalbuminuria (MA) heralds the onset of glomerulopathy in patients with glycogen storage disease (GSD) type I. Unlike tubulopathy, which responds to improved metabolic control, glomerulopathy in GSD I is considered refractory to medical intervention, and it is thought to inexorably progress to overt proteinuria and renal failure. Recent reports of reduced microalbuminuria following strict adherence to therapy counter this view. In contrast to type Ia, little is known regarding the prevalence of kidney disease in GSD Ib, 0, III, VI, and IX. Subjects were evaluated with 24-h urine collections between 2005 and 2014 as part of a longitudinal study of the natural history of GSD. ACE inhibitor therapy (AIT) was commenced after documentation of microalbuminuria. Elevated urine albumin excretion was detected in 23 of 195 GSD Ia patients (11.7%) and six of 45 GSD Ib (13.3%). The median age of onset of microalbuminuria in GSD Ia was 24 years (range 9–56); in GSD Ib it was 25 years (range 20–38). Of 14 with GSD Ia who complied with dietary and AIT during the study period, microalbuminuria decreased in 11, in whom metabolic control improved. All 135 patients with the ketotic forms of GSD (0, III, VI and IX) consistently had normal microalbumin excretion. Strict adherence to dietary therapy and maintenance of optimal metabolic control is necessary to halt the progression of GSD Ia glomerulopathy in patients treated with AIT. With optimal care, protein excretion can be reduced and even normalize.

Communicated by: Bridget Wilcken

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Abbreviations

ACE	Angiotensin converting enzyme
AIT	ACE inhibitor therapy
GSD	Glycogen storage disease
MA	Microalbuminuria

Introduction

The glycogen storage diseases (GSD) are a group of inborn errors of metabolism associated with abnormal storage or utilization of glycogen. The clinical spectrum of GSD depends on the organ involved: liver, kidneys, heart, muscle or intestines. Hypoglycemia is the hallmark of the hepatic GSDs. In GSD I, the kidney is also involved, and deposition of glycogen in the renal cortex leads to nephromegaly (Chen and Van Hove 1995). The epithelial cells of the proximal convoluted tubules have the greatest glucose-6-phosphatase activity, and renal tubular dysfunction occurs (Van Schaftingen and Gerin 2002; Chen et al 1990). While not a site of glucose-6-phosphatase activity, glomerular dysfunction is also observed. The glomerulopathy of GSD type I is preceded by development of hyperfiltration (Chen 1991; Martens et al 2009). Without intervention, this process has been associated with progression to focal segmental glomerulosclerosis, albuminuria, and renal failure (Chen et al 1988; Wolfsdorf et al 1997; Reitsma-Bierens 1993). Other renal abnormalities associated with GSD are nephrolithiasis secondary to hypocitraturia,

hypercalciuria, nephrocalcinosis, and secondary amyloidosis (Chen 1991; Weinstein et al 2001).

In 2002, the European Study on Glycogen Storage Disease Type I (ESGSD I) found that renal disease occurred in almost all adults with GSD Ia (Rake et al 2002). Several studies, however, have demonstrated that improved metabolic control may delay or prevent development of GSD nephropathy (Martens et al 2009; Wolfsdorf et al 1997; Weinstein and Wolfsdorf 2002). Even with advances in treatment, renal disease has continued to develop in some patients with GSD type I. With progression from microalbuminuria to proteinuria, the prognosis worsens, and many patients progress to renal insufficiency and renal failure even with ACE inhibition (Melis et al 2005; Melis et al 2015). Previous investigations have demonstrated that improved metabolic control can prevent development of complications in GSD I (Wang et al 2001; Minarich et al 2012). In addition, adenoma regression has been documented to occur with improved metabolic control (Beegle et al 2015). These investigations were designed to determine if improved metabolic control combined with ACE inhibition can similarly reverse renal disease in GSD I patients. In addition, the natural history of the ketotic forms of glycogen storage disease were evaluated to determine if similar renal complications develop.

Methods

The study was approved by the Institutional Review Board of the University of Florida, Gainesville. Written informed consents were obtained from the patients and from the parent if less than 18 years old, prior to the study. Assent was obtained for all children under 18 years of age.

Retrospective chart review was conducted on patients with the hepatic forms of GSD being seen at the University of Florida GSD Program. Patients have been followed prospectively as part of a natural history protocol investigating whether improved metabolic control can prevent complications in GSD. Study subjects included both children and adults aged 2 to 65 years enrolled between 2005 and 2014. A total of 375 confirmed GSD patients were enrolled in the natural history study (195 GSD Ia, 45 GSD Ib, 59 GSD III, nine GSD VI, 58 GSD IX, nine GSD 0). Patients were admitted at least once per year for metabolic evaluation. Timed 24-h urine collections and biochemical markers of control were collected and documented at the time of admission. Microalbuminuria (MA) was defined as excretion of albumin in the urine greater than 30 mg but less than 300 mg/24 h (approximately 20–200 mcg/min). The 24-h urine specimens were analyzed for albumin concentration by immunoturbidimetric assay with Cobas 8000 series c 702 module (Roche Diagnostics, Indianapolis, USA). Accuracy and precision information is available on line. Participants were only considered to have MA if two or more consecutive collections were found to be abnormal. Exclusion criteria for the longitudinal study included lack of relevant follow up data, non-compliance with prescribed corn starch therapy (missing over 50% of prescribed cornstarch), acute kidney injury from a non-GSD cause, and death. A total of seven GSD Ia patients and six GSD Ib were excluded from the study. Of note, no patient followed by the team has received a kidney transplant or has required dialysis. None of the patients excluded died from anything related to renal disease.

Triglycerides were obtained as a biomarker for metabolic control as has been frequently used in GSD I studies (Wang et al 2001; Beegle et al 2015). Fasting in glycogen storage disease cannot be performed. Therefore, triglycerides were measured on samples obtained in the morning prior to consumption of food as far away from meals as possible.

Table 1 Demographics of GSD populations

	GSD Ia with albuminuria $n = 23$	GSD Ia without albuminuria $n = 172$	GSD Ib with albuminuria $n = 6$	GSD Ib without albuminuria $n = 39$
GSD Ia and Ib populations				
Gender	12 F / 11 M	81F / 91 M	4 F / 2 M	22F / 17 M
Age range (years)	9–56	3–48	20–38	6–45
Age mean ± SD (years)	25.1 ± 10.9	18.9 ± 10.7	27.2 ± 7.6	18.9 ± 11.0
Age median (years)	24	16.5	25	17
	GSD 0 $n = 9$	GSD III $n = 59$	GSD VI $n = 9$	GSD IX $n = 58$
Ketotic GSD population				
Gender	4F / 5 M	28F / 31 M	4F / 5 M	12 F /46 M
Age range (years)	7–25	3–62	6–20	3–56
Age mean ± SD (years)	14.8 ± 5.9	19.7 ± 14.7	11.6 ± 4.8	12.8 ± 8.5
Age median (years)	14	16	12	11

Table 2 Clinical information for GSD patients with albuminuria

Age at time of study	Sex	GSD type	Age at diagnosis of GSD	Age when MA was detected (years)	Mutation 1	Mutation 2
41	F	Ia	3 months	20	R83C	R83C
36	M	Ia	6 months	28	R83C	R295C
32	F	Ia	4 months	17	R83C	R83C
54	M	Ia	birth	unknown	R83C	R83C
61	F	Ia	birth	56	R83C	Q347X
38	F	Ia	18 months	17	R83C	Q347X
35	F	Ia	9 months	29	R83C	R83C
25	M	Ia	6 months	16	R83C	R170Q
33	F	Ia	3 months	19	R83C	R83C
44	F	Ia	11 months	25	R83C	202X
29	M	Ia	2 months	23	R83C	R83C
48	M	Ia	5 months	17	R83C	Q347X
53	M	Ia	3 months	49	R83C	Q347X
30	F	Ia	2 months	21	R83C	R83C
27	M	Ia	5 months	unknown	R83C	R83C
19	F	Ia	13 years	9	Q347X	Q347X
26	M	Ia	9 months	16	979-81delTTC	979-81delTTC
48	M	Ia	No data	31	R83C	R83C
29	F	Ia	7 months	26	R83C	R83C
40	M	Ia	6 weeks	34	R83C	R83C
31	M	Ia	birth	21	Q242X	Q347X
36	F	Ia	10 months	28	Q347X	Q347X
44	F	Ia	3 months	24	R83C	R83C
21	M	Ib	12 months	20	C381 + 1G > T	1042delCT
27	F	Ib	2 months	24	W137X	A373D
27	F	Ib	birth	26	G150R	A356D
30	M	Ib	2 months	20	G68 K	1103ins12
44	F	Ib	4 months	35	G339C	1042delCT
41	F	Ib	12 months	38	G339C	1042delCT

Triglycerides were analyzed by an enzymatic colorimetric test by Cobas 8000 series c 702 module (Roche Diagnostics, Indianapolis, USA).

Statistical analysis In GSD Ia patients followed longitudinally, improvement in microalbuminuria was classified if the urine albumin concentration fell to less than 50% of the maximum value during the study. The differences between initial and final triglyceride levels were analyzed for both the improving microalbuminuria group (n = 11) and the worsening microalbuminuria group (n = 3). The distribution of the differences was tested for normality. Box plot and normal Q-Q plots suggested a normal distribution, as did the Shapiro-Wilk test. The paired T-test (two tail) was used to determine statistical significance at a level of 5%. The null hypothesis, that the mean of these differences was no different than zero, was tested.

Table 3 Incidence of microalbuminuria by age in patients with GSD type Ia

Age (years)	% GSD Ia with microalbuminuria	% GSD Ib with microalbuminuria	% GSD Ia + Ib with microalbuminuria
0–5	0/21 (0%)	0/6 (0%)	0/27 (0%)
6–10	1/33 (3%)	0/9 (0%)	1/42 (2%)
11–15	0/36 (0%)	0/7 (0%)	0/43 (0%)
16–20	7/27 (26%)	2/5 (40%)	9/32 (28%)
21–25	5/23 (22%)	1/4 (25%)	6/27 (22%)
26–30	4/14 (29%)	1/6 (17%)	5/20 (25%)
31–35	2/17 (12%)	1/2 (50%)	3/19 (16%)
36–40	0/10 (0%)	1/4 (25%)	1/14 (7%)
41–60	2/14 (14%)	0/2 (0%)	2/16 (12%)

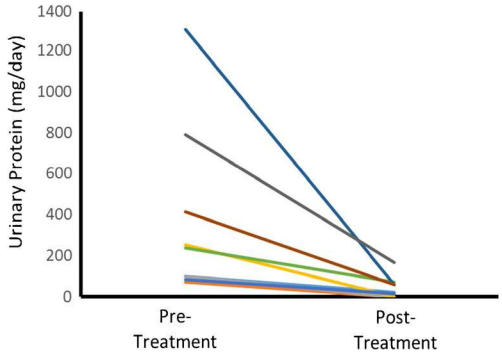

Fig. 1 Decreasing microalbuminuria in GSD Ia population

Results

Prevalence and incidence of microalbuminuria and proteinuria

Microalbuminuria was not detected in any of the 135 patients with the ketotic forms of GSD (types 0, III, VI, IX), but was detected in 23 of 195 GSD Ia patients (11.7%) and six of 45 GSD Ib patients (13.3%). The clinical characteristics of the GSD populations with and without albuminuria are summarized in Tables 1 and 2. The incidence of microalbuminuria is shown in Table 3.

Longitudinal study of metabolic control and progression of presumed glomerulopathy

Longitudinal data were available for 14 of the 23 participants with GSD Ia. The reasons for excluding participants were as follows: four patients lacked longitudinal data due to failure to comply with study visits, three patients died (two drug overdoses and one hepatocellular carcinoma), one developed leukemia and chemotherapy induced acute renal injury, and one discontinued ACE inhibition against medical advice. The small number of GSD Ib patients precluded longitudinal analysis of this cohort.

A decrease by over 50% in the daily protein excretion occurred in 11 of the 14 participants (Fig. 1). The patients with decreased protein excretion all experienced marked improvement in their metabolic control with a 55% decrease in their triglycerides ($p < 0.05$) (Table 4). In contrast, the three participants who worsened while on ACE inhibition had no change in their metabolic control from baseline. None of the 14 patients with abnormal albumin excretion developed chronic kidney disease stage III or greater.

Discussion

Over the past two decades, the care of patients with GSD Ia has improved through restriction of non-utilizable sugars, intense dietary counseling, more frequent cornstarch dosing, and use of an extended release overnight cornstarch preparation (Burda and Hochuli 2015; Chen and Weinstein 2016; Ross et al 2016). There is increasing evidence that the complication rate in GSD has decreased with improved metabolic control (Wang et al 2001; Minarich et al 2012; Beegle et al 2015). This study adds to the growing literature which demonstrates that development of renal disease can be delayed and possibly prevented with good metabolic control (Martens et al 2009; Wolfsdorf et al 1997; Weinstein and Wolfsdorf 2002).

The ESGSD I study found that 100% of GSD Ia patients had developed microalbuminuria or proteinuria by 24 years of age (Rake et al 2002). In contrast, the prevalence of MA in our cohort was 11.7%, and the median age of its onset was 24 years. Surprisingly, we did not find the incidence of MA to increase with age. There are several factors which may explain this unexpected finding. The incidence of MA in GSD types Ia and Ib was greatest between ages 16 to 20 years (Table 3). This late teenage group is recognized to have the poorest compliance with medical therapy for many chronic medical conditions (Taddeo et al 2008; Storch et al 2009). Taken together, these finding suggest that compliance with dietary therapy may be an important factor that contributes to the development of MA. The older population may also be phenotypically milder with partial enzyme activity. This not only allowed the older patients to survive before modern care was initiated, but it may have helped protect against development of the complication. A genotype-phenotype correlation can only be hypothesized, however, since this study is much too small to assess this correlation.

Table 4 Triglyceride vs microalbuminuria status at start and end of treatment

	Triglyceride concentration in patients with decreased microalbuminuria (mg/dL)	Triglyceride concentration in patients with increased microalbuminuria (mg/dL)
Initial	667 ± 353	802 ± 119
Final	370 ± 196	800 ± 342
Initial minus final (Δ)	280 ± 337	2.3 ± 224
Δ, *p* value	0.038	0.99

AIT has been shown to slow progression of kidney disease of diverse etiologies, and is particularly effective in proteinuric glomerular disorders associated with hyperfiltration, including diabetic nephropathy, glomerulosclerosis, and conditions associated with reduced nephron mass (Giatras et al 1997; Remuzzi et al 2006; Neild et al 2004; Yang and Fogo 2014). Prior studies in GSD, however, have demonstrated that pharmacologic therapy alone does not prevent progression of the renal disease. The main objective in this study was to evaluate longitudinally whether intense dietary therapy combined with ACE inhibition therapy (AIT) would improve the outcome in a cohort who had already developed increased albumin excretion. As recommended in the North American consensus guidelines for GSD I management, all patients received AIT once MA was documented (Kishnani et al 2014). Of the 14 patients followed longitudinally, all but three had improved MA excretion at the end of the study period. All of the patients who had decreased protein excretion experienced improvement in their metabolic control. Compliance with diet, medication, and return visits, however, was much poorer in the three subjects who experienced worsening of their proteinuria based on self-reporting. These results support that maximizing metabolic control is needed in addition to pharmacologic therapy.

There are several important questions, however, that still have not been addressed. First, use of prophylactic AIT prior to development of MA has been proposed, but long-term studies to assess the efficacy of prophylaxis still need to be performed (personal communication, Professor Peter Smit, Groningen, Netherlands). Second, while the combination of improved metabolic control and ACE inhibition appears to be beneficial over AIT alone, the study does not address whether good metabolic control without AIT can ameliorate proteinuria as was seen for hepatic adenomas. A genotype-phenotype correlation may also exist, and there may be a subset of patients who benefit more from the interventions.

Screening for renal disease is recommended for the other types of GSD, but there is a paucity of literature on this topic (Kishnani et al 2010). There has been concern about the risk of renal disease in the ketotic forms of GSD in particular since a diet which is very high in protein has been recommended (Kishnani et al 2010; Derks and Smit 2015; Tsilianidis et al 2013). High protein diets have been associated with hyperfiltration and worsening renal function in diabetic nephropathy, conditions with reduced nephron mass, and with advanced chronic kidney disease (Friedman 2004; Ko et al 2017). However, we failed to detect MA in any patient with the ketotic type of GSD who have been treated with a high protein diet (3–4.5 g/kg/day). Of note, a diet with over 3 g/kg/day of protein is traditionally consumed by the general population on the Faroe Islands, and no kidney disease has been documented in this population (personal communication, Dr. Ulrike Steuerwald). The absence of renal disease in these populations with extremely high protein intake is notable, and further studies are warranted to better understand how protein intake impacts renal disease. It is important to note that the population studied in our study still is relatively young. Complications in GSD usually develop during or after puberty, and many of the participants in our population are at or below the age where complications typically develop. Additional studies assessing the natural history of renal disease in the ketotic forms of GSD are needed, and patients should continue to be followed carefully until more definitive studies are performed.

A major limitation of this study is its observational nature and use of historical controls. Another is the possibility of orthostatic proteinuria contributing to a higher proportion of MA detected in the late teenage group. The extent that this would alter MA results in 24 h urine collections is questionable. The exclusion of several study subjects due to other health problems, lack of data, and insufficient compliance with therapy is also a concern.

In conclusion, strict metabolic control that results from adherence to appropriate dietary counseling appears to lower the incidence and prevalence of GSD I renal disease. Our findings add to the growing literature which supports that development of GSD nephropathy can be delayed. Once established, maximizing medical management in addition to pharmacologic therapy is recommended.

Compliance with ethical standards

Conflict of interest G. O. Okechuku, L. R. Shoemaker, M. Dambska, L. M. Brown, J. Mathew and D. A. Weinstein declare that they have no conflict of interest.

Funding This research was made possible by philanthropic support provided by the Global Center for GSD and the following funds managed through the University of Florida Office of Development: Scott Miller GSD Program Fund, the GSD Dream Fund, and the Johnny Damon Fund. The work was also supported in part by the NIH/NCATS Clinical and Translational Science Award UL1 TR000064 granted to the University of Florida.

Ethical approval These investigations were approved by the IRB at the University of Florida, and they were carried out in accordance with the Declaration of Helsinki.

Informed consent Consent (and assent when appropriate) was obtained from all participants.

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Received: 1 May 2019 | Revised: 5 July 2019 | Accepted: 13 August 2019

DOI: 10.1002/jimd.12160

ORIGINAL ARTICLE

JIMD SSIEM WILEY

Cornstarch requirements of the adult glycogen storage disease Ia population: A retrospective review

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Funding information
Global Center for Glycogen Storage Disease, Grant/Award Number: NA

Abstract

Cornstarch has been the primary treatment for glycogen storage disease type Ia (GSD Ia) for over 35 years. When cornstarch was first described as a treatment, few people survived beyond early childhood. As the prognosis for this population has improved, the need to ensure appropriate cornstarch dosing for different age groups has become imperative. Records from 115 patients (10-62 years of age) with GSD Ia evaluated at our center between 2015 and 2017 were reviewed. Data collected included weight, age, genetic mutation, amount and frequency of cornstarch doses, body mass index, gender, 24-hour glucose and lactate concentrations, and biochemical markers of metabolic control. The data demonstrate that adult treatment needs vary greatly from younger age groups, and the required cornstarch support decreases with age ($P < .001$). The required number of doses, however, did not change with a mean of six doses (range 4-8) daily in all age groups. General laboratory findings across time demonstrate that significantly reducing the amount of starch required to maintain euglycemia with aging can be done without sacrificing metabolic control. Carbohydrate requirements decrease with aging, and older patients were found to require less cornstarch. Failure to lower the cornstarch doses contributes to over-treatment in adults with GSD Ia. Not only does this lead to worsening hepatomegaly and excessive weight gain, but over-treatment contributes to relative hyperinsulinism and rebound hypoglycemia. This knowledge is essential in designing nutritional therapies for the aging GSD population.

KEYWORDS

adults, cornstarch, glycogen storage disease type Ia, treatment

1 | INTRODUCTION

Glycogen storage disease, type Ia (GSD Ia) (OMIM # 232200) was first described in 1929, and is caused by a deficiency or absence of the enzyme glucose 6-phosphatase (G6PC) (EC 3.1.3.9). Characteristics of this autosomal recessive disorder have been well-described and classically include hepatomegaly, hypoglycemia, lactic acidosis, hyperuricemia, and hyperlipidemia.[1] When optimal metabolic control is not attained, long-term complications include hepatic adenomas, hepatocellular carcinoma, focal segmental glomerulosclerosis, and osteoporosis.[2-4]

The goal of treatment in GSD Ia is to maintain euglycemia and prevent metabolic abnormalities. Since the impaired

G6PC activity prevents stored glycogen from being released as glucose, patients require a continuous supply of exogenous glucose. While continuous glucose infusions were initially utilized to provide required energy, uncooked cornstarch has been the primary treatment for GSD Ia in the United States for over 35 years.[5] In other parts of the world, daytime cornstarch combined with nocturnal enteral drip feedings are common. Before cornstarch was first described as a treatment for this disorder, few people survived to adulthood.[6] Treatment during early life is well-described; there is a paucity of literature, however, regarding how treatment needs may change throughout adult stages of life.

As the longevity of the GSD Ia population improved, the question of appropriate treatment in adulthood arose. Rake et al[7] recognized that glucose requirements would decrease with age, and decreased therapy is required following adolescence. The authors emphasized that over-treatment will result in glucose lability, and it would lead to rebound hypoglycemia and increased peripheral body fat storage.[7] In the general population, it is accepted that energy needs change throughout the life cycle.[8] It stands to reason that we would see comparable changes in the GSD population. Since the primary energy source in GSD Ia comes from frequent doses of uncooked cornstarch, investigations were performed to assess alterations in dosing of cornstarch in an older cohort of GSD Ia patients.

2 | METHODS

This single center study was done under the auspices of Institutional Review Board approved GSD Natural History Studies performed at the University of Florida and Connecticut Children′s Medical Center. Consent was obtained from all patients 18 years and above. Patients 7-17 years of age assented to the study, and consent was also obtained from their legal guardian.

The American College of Medical Genetics certified consensus guidelines recommends that patients be screened annually for complications of their glycogen storage disease.[3] Biochemical evaluations including assessment of hepatic transaminases, lipids, and uric acid occur every 3-6 months. Our center′s annual evaluation consists of an abdominal ultrasound, 24-hour urine collection and a 24-hour observation for titration of the cornstarch therapy. Cornstarch is weighed to the 100th of a gram on a Sartorius ENRIS 2202-1S scale, which is calibrated annually by the hospital engineers. Upon admission, hourly samples of glucose and lactates are drawn through an indwelling intravenous catheter. The samples are analyzed on a YSI 2300 STAT plus glucose lactate analyzer. Using the data from the laboratory results, cornstarch doses are titrated to maintain blood glucose concentrations between 75 mg/dL (4.2 mmol/L) and 100 mg/dL (5.6 mmol/L). Lactate concentrations are targeted at 2.0 mmol/L or below, as described in the consensus guidelines. During the 24-observation period, patients are encouraged to maintain normal levels of physical activity. It is very likely, however, that they are a bit more sedentary than usual due to the fact that they are in a hospital setting. During the observation, patients receive meals that are chosen from the hospital′s GSD Diet menu. This menu has limited carbohydrate options, and restricts simple sugars, fructose, galactose, and sucrose. Meals contain 10-15 g of carbohydrates, and snacks contain 5-10 g of carbohydrates.

A retrospective review of clinical records was conducted for all GSD Ia patients greater than 10 years of age who were seen at our center for evaluation and re-titration of treatment between 2015 and 2017. Only patients who were being treated with cornstarch or a combination of cornstarch and an extended release starch (Glycosade) were included in the study. The potential study population contained 115 subjects (63 males/52 females) between the ages of 10 and 62 years. Three patients were excluded due to treatment with alternative therapies. The remaining 112 subjects were divided into four predefined groups based on age: adolescence/secondary school (10-17 years, n = 43), emerging adulthood/college age (18-25 years, n = 30), young adulthood/early career (26-35 years, n = 22), and adults/established career (36 years and above, n = 17). These age categories were selected prior to any data analysis being performed. The population was initially divided at 18 years of age to separate pre and post adolescent populations. Another transition point for young adults in the United States with chronic medical conditions is age 25, when they are transitioning from being covered by their parents′ insurance to their own. The remaining adults were separated by those who started cornstarch in early childhood and those who commenced therapy later since cornstarch therapy began in 1982.

Data collected for each subject included the following: gender, age, anthropomorphic measurements, genotype, amounts of prescribed cornstarch, frequency of doses in a 24-hour period, 24-hour glucose and lactate concentrations, and biochemical markers of metabolic control. A summary of subject genotypes appears as Appendix A.

3 | STATISTICAL ANALYSIS

Each variable was descriptively summarized with proper summary statistics, for example, mean and SD for continuous variables. The difference between genders and age groups as well as the trend across age groups in total starch on discharge were tested using linear regression models, with adjustment for other covariates. A P value $< .05$ was deemed to be statistically significant. All the statistical

TABLE 1 Starch requirements per day in grams by age group

Age group	N	Mean prescribed starch (g/d)	SD
10-17	43	337	39
18-25	30	330	46
26-35	22	321	52
36+	17	272*	75

*Unadjusted: $P < .001$.
Adjusted for gender, treatment type, and weight: $P = 0.006$; Adjusted for gender, treatment type, and BMI: $P = .017$.

analyses were performed by a professional statistician using R 3.5.0.

4 | RESULTS

Total starch on discharge (grams/day) significantly differed among age groups (P value $< .001$), with mean and SD for each group provided in Table 1. On average, the 36+ age group required lower total starch per day than other age groups. The differences between the 36+ age group and the other age groups remained statistically significant after adjusting for gender, treatment type, and weight or BMI (P values $< .05$). Despite decreasing grams of prescribed daily cornstarch doses with age, we found no change in the interval of dosing required with the mean number of doses being six for each age group (range of 4-8 per day). The use of Glycosade, an extended release cornstarch, allows some subjects to extend the time between their nighttime doses.[9] The number of doses per day for all subjects is included in Table A1 in Appendix A. Total starch by body weight was not statistically significant, which supports the assertion that cornstarch should not be dosed by body weight, but rather by the carbohydrate requirement and central nervous system demands of each patient.[10]

Females were found to require 7.5% less total starch compared with males (333.4 ± 49.6 in males vs 308.1 ± 55.9 in females, $P = .014$). The gender difference remained statistically significant after adjusting for age groups, treatment type, and BMI or body weight. The statistical difference between genders mirrors the difference in carbohydrate requirements by gender in the general population.[8] There was no evidence shown that the gender difference varied with age groups, regardless of adjustment for other covariates (P values $< .05$).

To ascertain if the decreased starch doses for the subjects in the 36+ age group were effective in maintaining metabolic stability over time, laboratory values from the identified baseline visit were compared with subsequent laboratory values. Where follow-up data were available from multiple lab visits, the most recent biochemical studies were utilized. The mean amount of time between the baseline visit

TABLE 2 Follow-up laboratory studies for patients 36+ years of age

Subject #	AST at observation (U/L)	AST at follow-up (U/L)	ALT at observation (U/L)	ALT at follow-up (U/L)	Triglycerides at observation (mg/dL)	Triglycerides at follow-up (mg/dL)	Uric acid at observation (mg/dL)	Uric acid at follow-up (mg/dL)
96	32	N/A	26	N/A	565	N/A	4.8	4.5
97	39	24	33	28	374	301	4.5	7.3
98	28	17	25	17	330	325	7.7	7.4
99	13	17	13	10	243	118	5.9	5.1
100	50	35	46	34	1206	803	8.0	4.7
101	49	23	96	30	396	254	7.2	6.9
102	23	24	18	28	173	212	5.2	5.3
103	35	22	39	32	504	439	6.3	6.4
104	21	32	16	22	473	702	7.3	6.8
105	36	26	21	18	521	251	5.6	4.4
106	58	41	65	38	272	335	6.8	9.2
107	28	20	16	17	169	121	8.4	6.7
108	41	26	37	35	523	376	4.6	6.2
109	35	31	39	52	847	599	8.6	7.4
110	19	42	17	29	307	545	6.3	4.3
111	32	35	31	40	593	617	5.9	6.1
112	20	26	19	20	282	402	6.7	6.5

Reference ranges: AST: 10-55 U/L; ALT: 10-55 U/L; Triglyceride: <200 mg/dL; Uric acid: 4.0-8.0 mg/dL.

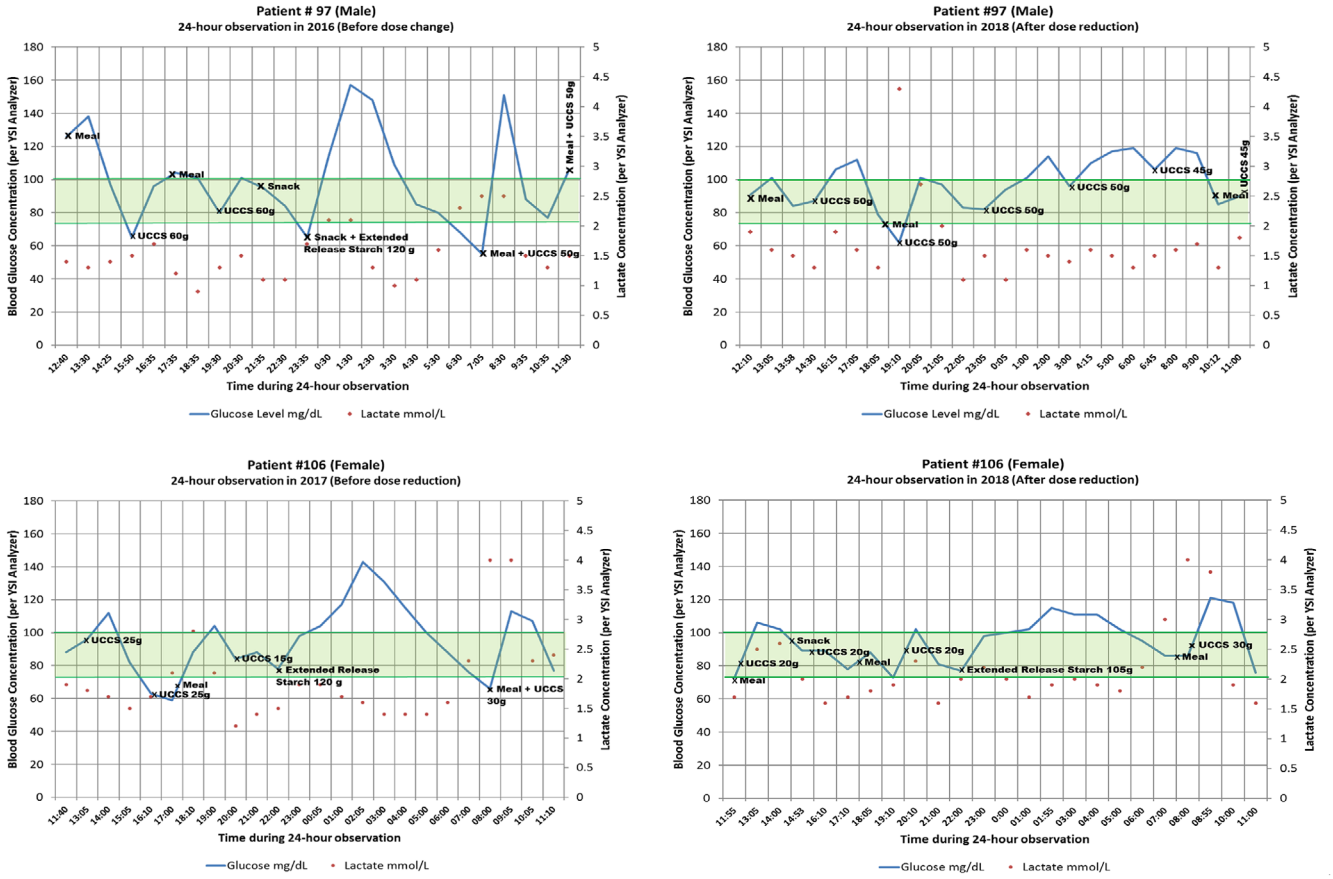

FIGURE 1 Glycemic control at baseline and follow-up appointments for Patient #97, and Patient #106

and the most recent follow-up was 14.25 months, with a range of 4 to 26 months. Even with substantially smaller doses of cornstarch, the majority of the patients over 36 years maintained metabolic stability, as evidenced by their normal hepatic transaminase levels (Table 2). The 36+ year group continued to demonstrate mild hypertriglyceridemia, but 10 of 17 were found with improved triglycerides at follow-up while on lower doses of cornstarch (Table 2). Only subject #10 was taking a lipid-lowering medication during this time (fenofibrate), but this therapy was used throughout the study period. Other improvements at follow-up included normal uric acid concentrations for 16 of the 17 subjects (10 of whom were taking Allopurinol throughout), more stable 24-hour glucose profiles (Figure 1), and a mean weight reduction of 1.6 kg (range +4.0 kg to −10.7 kg) between the baseline discharge and their subsequent evaluation 12 to 24 months hence.

5 | DISCUSSION

Cornstarch was introduced as a therapy for patients with glycogen storage disease in 1982. At that time, long-term survival was uncommon, and the majority of early publications described treatment in children.[6,11-13] Over the years, the prognosis for patients with GSD Ia has improved, and the vast majority of patients are now surviving to adulthood. Specific guidelines for managing the older population (over 35 years of age) have not been published, and general recommendations for treating adults were not addressed in the consensus guidelines for GSD Ia management.[3]

One might expect, given the poor prognosis for people with GSD Ia in the pre-cornstarch era, that fewer of the severe (p.R83C) cases would have survived. Surprisingly, we find that 71% of our patients above the age of 35 have at least one p.R83C allele, compared to 49% of the 10 to 17 year old patients (Table 3). One possible explanation for this disparity in the prevalence of p.R83C lies in the declining number of Ashkenazi Jewish patients presenting with this mutation. With a GSD Ia disease prevalence five times higher than the general Caucasian population, a founder effect was confirmed in the Ashkenazi Jewish population.[14] It is important to note that the Dor Yeshorim Jewish genetic screening program included screening for p.R83C in 1998. A resulting decline in the occurrence of GSD Ia among younger Ashkenazi Jewish children has perhaps led to an overall decline in the number of cases of GSD Ia caused by this mutation. Notably, the decrease in cornstarch found in

TABLE 3 Summary of genotype by age group (G6PC homozygosity/heterozygosity for p.R83C and p.Q347X)

	10-17 years (n = 43)	18-25 years (n = 30)	26-35 years (n = 22)	36+ years (n = 17)
Homozygous p.R83C	12 (28%)	9 (30%)	8 (36%)	4 (24%)
Heterozygous, with at least 1 allele of p.R83C	10 (23%)	7 (23%)	6 (27%)	8 (47%)
Total p.R83C	22 (51%)	16 (53%)	14 (64%)	12 (71%)
Homozygous p.Q347X	2 (5%)	1 (3%)	2 (9%)	1 (6%)
Heterozygous, with at least 1 allele of p.Q347X	14 (33%)	6 (20%)	1 (5%)	5 (29%)
Total p.Q347X	16 (37%)	7 (23%)	3 (14%)	6 (35%)

our older cohort therefore cannot be explained by a less severe genotype.

Aging is associated with decreases in the metabolic rate in the general population.[15,16] It is therefore not surprising that treatment requirements were found to decrease with age in the GSD population. Failure to reduce cornstarch with age leads to over-treatment, which in turn, results in obesity, worsening hepatomegaly, and contributes to relative hyperinsulinism and rebound hypoglycemia. Higher cornstarch doses during the baseline visit resulted in glucose lability, as illustrated in Figure 1. The green band in each chart of Figure 1 represents the target glucose range of 75 to 100 mg/dL. The red dots on the secondary horizontal axis represent the lactate concentration at each time point, with a target lactate of ≤2.0 mmol/L. When adult patients with GSD become hyperglycemic following cornstarch and/or nutritional intake, a corresponding low occurs shortly thereafter, due to the associated insulin response. Since insulin suppresses the breakdown of glycogen, and hence lactate production in GSD Ia, it follows that relatively normal lactates in the setting of hypoglycemia are indicative of insulin sensitivity. This pattern of rapidly falling glucose concentrations supports that patients are insulin sensitive and not insulin resistant as is common with aging in the generation population. It also supports that the obesity in the older cohort of GSD Ia patients is due to over-treatment and not metabolic syndrome. When admitted for follow-up on the reduced cornstarch doses, the glucose profiles for these patients were more stable. To illustrate this, the 24-hour glucose profiles of two patients (one male, one female), are included in Figure 1.

The use of continuous glucose monitoring (CGM) has been reported to be an increasingly accurate and powerful tool in managing the daily fluctuations in blood glucose concentrations. Patients are able to better identify their individual responses to foods, cornstarch and activity level, leading to improved metabolic control. CGM is also extremely useful for demonstrating large fluctuations in glucose concentrations which can occur with over-treatment and for picking up severe hypoglycemia.[17] While there is no doubt that there is a role for this technology, CGM still has several limitations. It is estimated that 6% of hypoglycemia is not detected using this technology.[18] In addition, CGM does not provide information on lactate concentrations which is critical when determining whether people are over or under treated.

Another consideration in optimizing the health of adult patients with GSD Ia is managing body weight. These 17 patients had a mean BMI of 31.6 at baseline. The mean weight loss at follow-up was 1.6 kg, which is primarily attributed to the daily reduction in cornstarch of 47 g per day. Just this small change in cornstarch results in a savings of over 62 000 cal per year. It is interesting to note that the 14.6% drop in cornstarch support is similar to the 11%-12% decrease in basal energy requirements seen in the non-GSD population from 20 to 40 years of age. This decrease in cornstarch support was seen not only when comparing the cohorts, but it was also demonstrated when examining the individuals in the older cohort (Figure 2 and Table B1 in Appendix B).

While the present study provides information regarding dosing in adults with GSD Ia, additional studies are warranted to more precisely define how adjustments should be performed. This will be important as the population continues age. In order to ensure that all treatment and populations are included, future analysis with worldwide

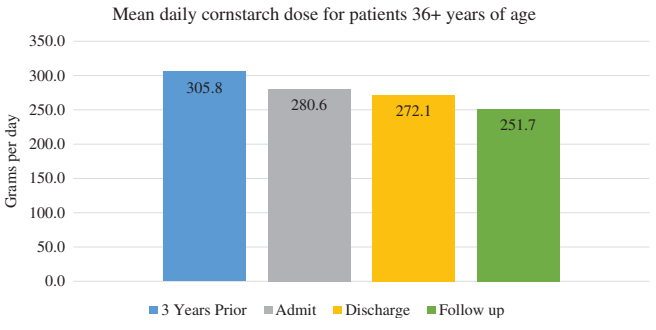

FIGURE 2 Mean daily cornstarch dosing over time for patients 36+ years of age

databases like the International Study for GSD would be beneficial.

ACKNOWLEDGEMENTS

This work was supported by the Global Center for Glycogen Storage Disease (West Hartford, CT).

CONFLICT OF INTEREST

K.D., I.F., T.R., G.B., C.-L.K., and P.R. declare that they have no financial conflict of interest. M.D., K.R., and D.W. are all part of the GLYDE study which is an international trial assessing the efficacy of Glycosade extended release cornstarch for daytime use in GSD. D.W. receives grant support from the following companies, but the work is unrelated to this study: Ultragenyx, Generation Bio, and Logic Bio. None of the authors have intellectual property related to glycogen storage disease.

AUTHOR CONTRIBUTIONS

K.D. served as co-primary investigator for the study, helped to design the study, directed data collection, assisted with data analysis and interpretation, co-created first draft of manuscript, edited manuscript, approved manuscript. I.F. served as co-investigator for the study, collected data, assisted with data analysis and interpretation, co-created first draft of manuscript, edited manuscript, approved manuscript. M.D. served as co-investigator for the study, collected data, assisted with data analysis and interpretation, co-created first draft of manuscript, edited manuscript, approved manuscript. T.R. collected data, assisted with data interpretation, edited manuscript, approved manuscript. K.R. collected data, edited manuscript, approved manuscript. G.B. collected data, approved manuscript. C.-L.K. provided biostatistical analysis, edited manuscript, approved manuscript. P.R. edited manuscript, approved manuscript. D.W. senior author, created the project, provided oversight for all aspects of project, provided care to GSD patients, coordinated data collection, edited manuscript, approved manuscript.

ETHICS APPROVAL

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study.

This study was conducted in accordance with the GSD Natural History Study, approved and reviewed by both the University of Florida, and the Connecticut Children′s Medical Center Institutional Review Boards.

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How to cite this article: Dahlberg KR, Ferrecchia IA, Dambska-Williams M, et al. Cornstarch requirements of the adult glycogen storage disease Ia population: A retrospective review. *J Inherit Metab Dis*. 2019;1–10. https://doi.org/10.1002/jimd.12160

APPENDIX A

TABLE A1 Study participants: Genotype, gender, age, height, weight, BMI, and starch doses

Subject	Mutation 1	Mutation 2	Gender	Age at appointment	Height (cm)	Weight (kg)	BMI (kg/m^2)	# of starch doses/d
1	p.35X	c.230+1G>C	M	10	155.5	51.6	21.3	6
2	p.G188S	p.V338F	M	10	147	52.3	24.2	7
3	p.Q347X	p.Q347X	F	10	146.3	60.1	28.1	7
4	p.35X	p.130X	M	10	141.2	48.2	24.2	6
5	p.130X	p.130X	M	10	131.7	36.0	20.7	5
6	p.R170Q	p.Q347X	M	10	140.8	45.5	23.0	5
7	p.R83C	p.Q347X	F	11	138.9	40.2	20.8	7
8	p.R83C	p.Q347X	M	11	145.6	68.7	32.4	7
9	p.R170Q	p.Q347X	M	11	140.5	45.0	22.8	5
10	p.Q347X	p.Q347X	F	12	157.2	80.3	32.5	5
11	p.R83C	p.130X	F	12	155.3	61.8	25.6	7
12	p.G188R	p.I339D	F	12	162.3	58.7	22.3	6
13	p.E110K	UNK	M	13	161.6	88.9	34.0	6
14	p.V166G	Q347X	F	13	163.1	70.9	26.7	6
15	p.R83C	p.R83C	F	13	150.5	62.7	27.7	5
16	p.Q347X	p.G188R	M	13	160.5	53.2	20.7	7
17	p.G188S	p.Q347X	F	13	144.1	47.1	22.7	5
18	p.130X	p.Q347X	F	13	150.0	54.7	24.3	5
19	p.R83C	p.R83C	M	14	156.2	53.0	21.8	5
20	p.R83C	p.R83C	F	14	164.2	63.2	23.4	7
21	p.R83C	p.E110K	M	14	155.6	48.1	19.9	6
22	p.R170Q	p.Q347X	M	14	165.2	74.8	27.4	5
23	p.R83C	p.R83C	M	15	154.8	50.1	20.9	7
24	p.R83C	p.R83C	M	15	159.5	61.0	24.0	6
25	p.R83C	p.R83C	F	15	143.5	51.1	21.4	5
26	p.W156L	p.W156L	M	15	173.3	64.5	21.5	4
27	p.R170Q	p.Q347X	M	15	161.9	46.9	17.9	5
28	p.R83C	p.G188R	M	15	172.2	103.2	34.9	6
29	p.E110K	UNK	F	16	166.4	83.6	30.2	5
30	p.R83C	p.R83C	M	16	168.9	66.9	23.4	8
31	p.Q347X	p.G188R	M	16	171.1	78.0	26.6	6
32	p.R83C	p.R83C	M	16	165.4	93.6	34.2	6
33	p.R83C	p.G188R	F	16	157.5	64.0	25.8	6

(Continues)

TABLE A1 (Continued)

Subject	Mutation 1	Mutation 2	Gender	Age at appointment	Height (cm)	Weight (kg)	BMI (kg/m^2)	# of starch doses/d
34	p.R83C	p.Q347X	F	16	158.2	66.0	26.3	6
35	p.W77G	p.Q347X	M	16	157.0	55.1	26.5	6
36	p.R83C	c.79delC	M	16	176.9	86.8	27.7	7
37	p.R83C	p.Q347X	M	17	174.0	66.3	22.3	6
38	p.Q347X	c.447-1G>A	F	17	159.3	68.6	25.5	7
39	p.R83C	p.R83C	M	17	169.5	62.8	21.9	6
40	p.R83C	p.R83C	M	17	168.2	64.8	22.9	6
41	p.R83C	p.R83C	M	17	148.4	66.5	30.2	6
42	p.R83C	p.R83C	F	17	157.2	43.1	17.4	8
43	p.R83C	p.E110K	M	17	172.4	80.4	27.0	6
44	p.G188R	p.I341N	M	18	175.0	71.2	23.2	6
45	p.R83C	p.R83C	F	18	154.5	64.4	27.0	7
46	p.R83C	p.G270V	M	18	170.4	84.5	26.4	6
47	p.35X	p.R170X	M	18	162.3	66.7	25.3	6
48	p.G188R	p.Q347X	F	19	174.3	97.8	32.2	6
49	p.R83C	p.R83C	F	19	150.5	64.2	28.3	6
50	p.R83C	p.R83C	M	19	162.6	70.0	26.5	6
51	p.Q347X	p.Q347X	F	19	167.0	90.4	32.4	6
52	p.W77R	UNK	F	20	163.2	76.0	28.6	6
53	p.R83C	c.230+1G>C	M	20	179.5	97.3	30.2	7
54	p.G188S	p.W156L	F	20	154.9	79.7	33.2	6
55	p.T108I	p.T108I	F	20	155.9	102.5	42.1	6
56	p.R83C	p.R83C	F	20	159.2	73.6	29.0	5
57	p.R83C	p.R83C	M	20	177.8	90.7	28.7	6
58	p.R83C	p.Q242X	M	21	158.6	51.6	20.5	6
59	p.R83C	p.Q347X	M	21	172.5	99.5	33.4	6
60	p.R83C	p.130X	M	21	175.8	70.7	22.9	6
61	UNK	UNK	F	21	154.5	61.1	25.6	6
62	p.R83C	p.Q347X	F	22	170.8	74.3	25.5	5
63	p.R83C	p.R83C	F	22	154.5	60.8	25.5	5
64	p.Q27Rfs	p.Q27Rfs	M	22	177.8	84.8	26.8	6
65	p.R83C	p.R83C	F	22	163.6	76.5	28.6	6
66	p.G188R	p.Q347X	F	22	169.3	90.4	31.5	6
67	p.R83C	UNK	M	23	182.1	88.9	26.8	5
68	p.R83C	p.R83C	F	23	158.6	61.1	24.3	5
69	p.35X	p.Q347X	M	23	176.8	76.9	24.6	5
70	p.W77R	p.G118S	M	24	156.4	47.6	19.5	6
71	p.F177C	p.Q347X	M	24	188.8	96.2	27.0	8
72	p.R83C	UNK	M	25	165.4	59.5	21.8	6
73	p.R83C	p.R83C	F	25	154.3	62.7	26.3	6
74	p.130X	p.T2551	F	26	157.6	57.1	23.0	7

(Continues)

TABLE A1 (Continued)

Subject	Mutation 1	Mutation 2	Gender	Age at appointment	Height (cm)	Weight (kg)	BMI (kg/m^2)	# of starch doses/d
75	p.R83C	p.W63X	M	26	169.6	88.5	30.8	6
76	p.R83C	p.R170Q	M	26	158.2	67.7	27.0	8
77	p.R83C	p.G118S	M	27	177.7	87.2	27.6	6
78	p.35X	p.Q347X	M	27	156.0	56.8	23.3	5
79	p.R83C	p.C167Y	F	28	153.4	54.1	23.0	6
80	p.W70X	c.757delA	M	28	179.5	79.7	24.7	6
81	p.R83C	p.R83C	M	28	157.0	66.1	26.9	5
82	p.R83C	p.R83C	F	29	150.0	75.7	33.6	6
83	p.R83C	p.R83C	M	29	159.3	66.2	26.1	6
84	p.Q347X	p.Q347X	M	29	172.4	82.3	27.7	5
85	p.R83C	p.R83C	M	30	159.2	64.7	25.5	6
86	p.W70X	p.W70X	F	31	161.8	71.7	27.4	6
87	p.R83C	p.E110K	F	32	169.1	79.0	27.6	7
88	p.R83C	p.R83C	M	32	177.5	83.5	26.5	6
89	p.R83C	p.R83C	M	33	168.0	74.8	26.6	5
90	p.R83C	p.R83C	F	33	149.2	58.5	26.3	6
91	p.Q347X	p.Q347X	F	33	159.4	63.1	24.8	5
92	p.R83C	p.R295C	F	33	147.9	62.7	28.7	5
93	p.R83C	p.G188S	F	34	152.0	67.4	29.2	7
94	p.W77R	UNK	M	35	168.5	57.8	20.4	6
95	p.R83C	p.R83C	F	35	155.5	70.4	29.2	7
96	p.Q27Rfs	p.W63X	M	36	181.5	106.0	32.2	6
97	p.R83C	p.E110K	M	36	170.9	88.3	30.2	5
98	p.R83C	p.R83C	F	37	148.8	64.5	29.1	5
99	p.Q242X	p.Q347X	M	37	172.2	80.7	27.2	8
100	p.R83C	p.Q347X	M	38	169.4	65.3	22.8	6
101	p.R83C	p.R295C	M	38	168.8	71.0	25.0	6
102	p.Q347X	p.Q347X	F	38	161.1	55.3	21.3	8
103	p.R83C	p.R83C	F	39	148.8	62.5	28.2	6
104	p.R83C	p.Q347X	F	39	168.4	119.1	42.0	7
105	p.R83C	UNK	F	42	156.2	104.6	42.9	5
106	p.R83C	p.R83C	F	43	155.4	87.5	36.2	5
107	p.R83C	p.R83C	F	44	154.8	79.7	33.3	7
108	p.Q347X	p.A124T	M	44	175.1	90.2	29.4	6
109	p.W156 L	p.G188S	F	45	159.6	97.1	38.2	6
110	p.R83C	p.G188S	M	45	168.1	82.7	29.3	4
111	p.R83C	p.Q347X	M	50	164.0	81.5	30.3	7
112	p.R83C	p.Q347X	F	62	143.1	81.3	39.7	5

APPENDIX B

TABLE B1 Cornstarch dosing by individuals in the 36+ year old cohort

Patient #	3 years prior (dose in g)	Admit (dose in g)	Discharge (dose in g)	Follow-up 12-24 months′ post (dose in g)
96	NA	360	325	335.0
97	403	360	325	270.0
98	275	245	240	260.0
99	280	400	360	280.0
100	464	365	345	NA
101	300	300	310	285.0
102	310	224	210	200.0
103	360	355	315	297.0
104	275	280	295	258.0
105	265	239	320	300.0
106	285	220	195	180.0
107	NA	280	295	280.0
108	340	330	315	305.0
109	275	210	220	NA
110	180	105	105	90.0
111	365	326	305	285.0
112	210	171	145	150.0

Orphanet Journal of Rare Diseases

RESEARCH Open Access

A triple-blinded crossover study to evaluate the short-term safety of sweet manioc starch for the treatment of glycogen storage disease type Ia

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Abstract

Background: Glycogen storage disease type 1a (GSD Ia) is characterized by severe fasting hypoglycemia. The clinical management includes the administration of uncooked cornstarch (UCCS). Although such a diet approach is effective in achieving euglycemia, its impact on the quality of life of patients should be considered. In vitro analyses suggest a longer release of glucose when using sweet manioc starch (SMS).

Methods: We compared the efficacy and safety of the administration of SMS and UCCS during a short-fasting challenge in patients with GSD Ia in a randomized, triple-blind, phase I/II, cross-over study. GSD Ia patients aged ≥ 16 years and treated with UCCS were enrolled. Participants were hospitalized for two consecutive nights, receiving UCCS or SMS in each night. After the administration of the starches, glucose, lactate and insulin levels were measured in 1-h interval throughout the hospitalization period. The procedures were interrupted after 10 h of fasting or in a hypoglycemic episode (< 3.88 mmol/L).

Results: Eleven individuals (mean age: 21.6 ± 4.3 years; all presenting body mass index > 25 kg/m^2) participated in the study. The average fasting period was 8.2 ± 2.0 h for SMS and 7.7 ± 2.3 h for UCCS ($p = 0.04$). SMS maintained euglycemia for a greater period over UCCS. Increased lactate concentrations were detected even in absence of hypoglycemia, not being influenced by the different starches investigated ($p = 0.17$). No significant difference was found in total cholesterol, HDL, triglycerides and uric acid levels in both arms. None of the patients showed severe adverse events.

Conclusions: SMS appears to be non-inferior to UCCS in the maintenance of euglycemia, thus emerging as a promising alternative to the treatment of GSD Ia.

Keywords: Inborn errors of metabolism, Hepatic glycogen storage disease, Treatment strategies, Cornstarch , Sweet manioc starch, Dietary treatment

Background

Glycogen Storage Diseases comprise distinct genetic disorders caused by alterations in the synthesis or degradation of glycogen [1]. Glycogen storage disease type 1a (GSD Ia), typically known as Von Gierke disease (OMIM #232200), is an autosomal recessive metabolic disorder

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caused by deficiency of the enzyme glucose-6-phosphatase (G6Pase) [2], encoded by the *G6PC* gene located in the chromosome 17q21.31 [3]. G6Pase is anchored in the endoplasmic reticulum lumen, being highly expressed in the liver, kidney and small intestine [4, 5]. The estimated prevalence of GSD Ia is about 1 in 100,000 live births [6].

GSD Ia results in dramatic metabolic alterations, especially in fasting periods. Due to the deficient endogenous glucose production, patients showed severe hypoglycemia, hypertriglyceridemia, hyperlipidemia and increased production of lactic and uric acids [6]. The clinical management is based on dietary treatment to maintain euglycemia (blood glucose >4 mmol/L or 70 mg/dL) and prevent secondary metabolic disorders [7].

Dietary treatment strategies intend to provide a continuous source of glucose by nocturnal intragastric infusion of glucose or regular administration of uncooked cornstarch (UCCS) [8]. Other potential strategies involve medium-chain triacilglycerol supplementation [8] and gene therapy [9]. In this regard, UCCS is a polysaccharide with slow degradation and glucose release, therefore constituting an interesting option to maintain euglycemia. The recommended UCCS dosage depends on age, weight and period of the day. As reference the dosage consists of 1.6 to 2.5 g *per* kilogram of body weight every 3–4 h for younger children, and every 4–6 h for older children, adolescents, and adults [1, 8]. Although UCCS therapy has shown successful results, there is no optimal protocol that can broadly attend to all the treatment requirements for patients with GSD Ia. In addition, even though UCCS is supposed to be palatable, practical, to prevent excessive weight gain and maintain normal appetite with scarce adverse effects [10], the overtreatment can induce hyperinsulinemia and obesity [8].

Sweet manioc starch (SMS) is a culinary product extracted from cassava (*Manihot esculenta*). As this root is an important staple food crop in many developing (tropical, intertropical, and sub-Saharan) countries, starch is one of its major components (58.9% of the dry matter), being constituted by approximately 80% of amylopectin [11].

Nalin et al. [12, 13] evaluated the digestion of distinct starches brands from Brazil, United States of America and the Netherlands, including modified starch (Glycosade®, Vitaflo Ltda) and SMS (Fritz and Frida®) in a dynamic gastro-small intestine model (TIM-1). These authors showed that a slower glucose release was obtained from SMS compared to other starches. Moreover, their results also indicated that the digested amount of SMS was reduced compared to the other analyzed starches. Subsequently, the authors [12] have also evaluated the amylose/amylopectin ratio in same starch samples. Interestingly, SMS displayed slightly higher amounts of amylopectin.

Hypothetically, the slower glucose release induced by the digestion of SMS and its widespread availability at a relative low cost could constitute an interesting tool in the arsenal to prevent GSD Ia-induced hypoglycemia during fasting periods. In the light of the demand to develop new therapeutic technologies for GSD management [14], the present study aimed to assess the efficacy and safety of SMS administration in patients with GSD Ia.

Results

Eleven GSD Ia participants (M: 6, F: 5) were enrolled in the study (mean age: 21.6 ± 4.3 years). All participants exhibited body mass index >25 kg/m^2 (mean: 28.2 ± 3.6 kg/m^2). The clinical profile of the participants is summarized in Table 1. At baseline, four patients (A, C, G, and K) presented high lactate (>2.2 mmol/L); six patients, high uric acid; and 10 patients, high triglycerides levels.

Participants consumed an average amount of UCCS of 408.6 ± 86.5 g/day or 0.9 ± 0.2 g/kg/dose before the study. For this trial, all participants were given 100 g (1.3 ± 0.2 g/kg/dose) of carbohydrate starch, either SMS or UCCS.

Efficacy

Fasting time had a mean duration of 7.9 ± 1.8 h (SMS: 8.2 ± 2.0, UCCS: 7.7 ± 2.3, $p = 0.04$) (Fig. 1). The nadir time in euglycemia occurred in a 16-year-old male participant (Participant G), who remained in euglycemia during only 4 h after receiving any of the starches (SMS and UCCS). Four participants (B, D, E, I) remained in euglycemia during all the monitoring period (10 h) irrespective from the starch received. One participant fasted for 10 h after receiving SMS but not UCCS (Participant A). Under use of SMS, two patients (H and J) presented somnolence and fatigue, respectively, and had their tests interrupted at 7 h after the starch loading.

The SMS maintained euglycemia for a longer period (ANOVA, $p = 0.04$) and no carry-over effect was observed. In comparison to SMS, which had a more stable glycemic profile in the first 6 h after the load, the UCCS induced medians greater than 6.0 mmol/L in times T1 and T2, with the identification of glycemic peaks (Fig. 2A). All participants displayed similar lactate concentrations throughout the study evaluation (ANOVA, $p = 0.17$) and no carry-over effect was observed. An increase in lactate concentration was found even in the absence of hypoglycemia (Fig. 2B).

A carry-over effect was observed exclusively for the insulin levels regardless of the starch ($p = 0.03$).

Table 1 Genetic and biochemical profile of the GSD Ia patients enrolled in study (n = 11, baseline)

ID	Genotype	Gender (M/F)	Age (years)	Comorbidities	Body weight (Kg)	Height (cm)	BMI (kg/m2)	Starch-dosage 4/4 h (cm)	Glucose (mmol/L)	Lactic acid (mmol/L)	Insulin (UI/mL)	Uric acid (mg/dL)	TC(mg/dL)	HDL (mg/dL)	TG (mg/dL)
A	c.[247C>T];[820G>A]	F	24	Adenomas, nephrolithiasis	72.1	160	**28.2**	45	5.3	**2.55**	**29.1**	**7.7**	**324**	**25**	**1997**
B	c.[113A>T];[1039C>T]	F	16	–	76.8	163	**28.9**	47	4.0	1.69	4.7	6.2	198	44	**307**
C	c. [247C>T];[247C>T]	F	27	Hepatomegaly, hepatic hypervascular nodule	64.2	149	**28.9**	60	5.6	**2.48**	8.9	6.8	**217**	36	**384**
D	c.[113A>T];[1039C>T]	F	16	–	71.9	166	**26.1**	61	6.1	1.74	**30.8**	**7.8**	156	**23**	**344**
E	c.[247C>T];[247 C>T]	M	23	Nephrolithiasis, hepatic steatosis	78.6	164	**29.2**	65	6.2	1.79	**34.0**	6.9	170	**22**	**383**
F	c.[247C>T];[809G>T]	F	21	Adenomas	59.0	153	**25.2**	68	5.3	1.38	**16.7**	6.5	144	35	122
G	c.[247C>T];[247C>T]	M	16	Nephrolithiasis	82.0	169	**28.7**	73	4.1	**3.10**	5.7	**8.2**	196	**21**	**484**
H	c.[189G>C];[1039C>T]	M	20	–	78.0	176	**25.2**	77	5.6	1.65	**22.3**	**8.5**	**246**	39	**746**
I	c.[1039C>T];[1039C>T]	M	22	–	109.5	170	**37.9**	78	5.9	1.93	**20.3**	**7.8**	**244**	**34**	**448**
J	c.[113A>T];[323C>T]	M	27	–	72.0	167	**25.8**	85	5.1	2.10	**14.9**	6.8	**338**	37	**608**
K	c.[247C>T];[563-3C>G]	M	26	Mild auditive loss	75.2	170	**26.0**	90	5.7	**3.51**	**43.2**	**10.0**	**242**	**30**	**553**

M: male, F: female. Reference values: glucose (> 3.8 mmol/L); lactate (0.5–2.2 mmol/L); insulin (1.4 -14 µUI/mL); total cholesterol (TC, < 200 mg/dL); HDL cholesterol (> 35 mg/dL); triglycerides (TG, < 150 mg/dL) and uric acid (3.4 -7 mg/dL). In bold, abnormal values

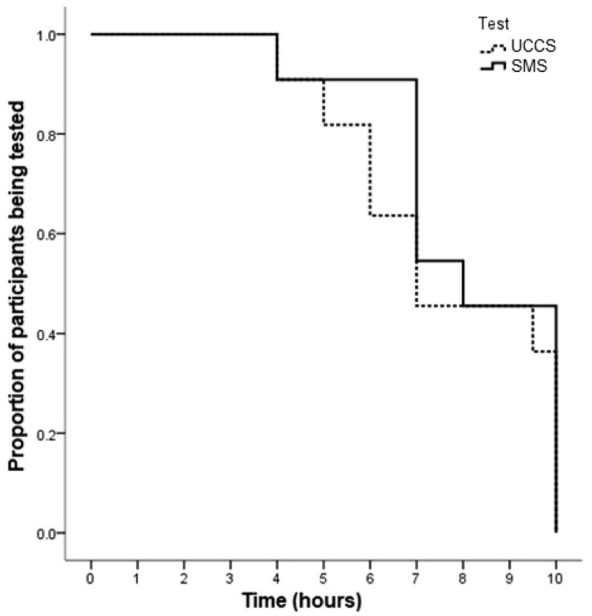

Fig. 1 Kaplan Meier curve indicating test durations for each starch load performed (n = 11). UCCS: uncooked cornstarch and SMS: sweet manioc starch

Biochemical data of the participants (total cholesterol, HDL, triglycerides, and uric acid levels) are summarized in Table 2. No significant difference was found in these values when comparing both starches.

Safety

None of the participants displayed serious adverse events. Mild hypoglycemia-related fatigue was reported in 3/11 participants when treated with UCCS and two under SMS. Two anxiety episodes were also reported in 2 participants treated with UCCS, which were clinically managed without need for medication. One participant displayed anxiety and tachycardia symptoms and stopped the daily protocol because of low levels of capillary blood glucose.

Eight participants (A, C, D, E, G, H, and K) presented high lactate levels (≥ 5 mmol/L) during the protocol. Among these patients, five presented lactate elevation exclusively under SMS, one exclusively under UCCS and two under both starches' ingestion.

No gastrointestinal symptoms were reported and none of the participants discontinued the trial.

Discussion

This randomized, triple-blinded pilot study revealed that SMS maintained blood glucose concentrations within the normal range for a longer period than the UCCS.

The advent of UCCS treatment brought many benefits to hepatic GSD patients. However, similar to all alternative dietary treatment for GSDs, adverse effects were also reported, including interrupted sleep for treatment, anxiety, exhaustion, risk of delayed administration [14] and food intolerance [15].

The negative impact of conservative treatment with UCCS has been causing concerns among health care professionals, patients and their families. This became clear with the publication of the consensus on research priorities for hepatic GSD, where one of the 11 cited items was "How can existing cornstarch preparations be modified or alternative treatments be implemented that are easier to administer and/or keep blood sugar levels more stable for patients with liver GSD?" [16]. To avoid these adverse effects, a modified experimental starch was proposed (the modified cornstarch, WMHM20) [10]. The authors concluded that the use of WMHM20 resulted in a longer duration of euglycemia and better short-term metabolic control. Subsequent studies proved its efficacy and safety [17, 18].

New products for assisting the nutritional management of hepatic GSDs have been consistently investigated. In 1986, Sidbury et al. [19] compared the effects of different raw starches, including arrowroot and tapioca, typical roots from South America. The authors have reported distinct patterns of starches absorption. In fact, both arrowroot and tapioca were less hydrolyzed than UCCS. However, UCCS was more efficient in maintaining euglycemia in patients with GSD Ia.

An in vitro study using a dynamic model of the gastrointestinal tract-1 (TIM-1) have demonstrated that the use of SMS resulted in a less rapidly available glucose in the glycemic index method and a higher resistant starch value. In addition, SMS led to a slower glucose release and minimal possible amount of indigestible material compared to UCCS. After 3 h of starches administration, only 55.5% the amount of SMS was digested while nearly 70% of UCCS was already digested [13]. The amylose/amylopectin ratio was also determined, reflecting the starch influence on the rate and the extent digestion. SMS presented a higher amount of amylopectin than UCCS, but not in sufficient amounts to fully explain the difference in digestibility [12].

In the present study, the SMS presented a more stable glycemic profile in the first 6 h and the intervention maintained glycemia within the recommended treatment interval described in guidelines. In patients with GSD, the amount and the quality of the ingested carbohydrates demand to be controlled in order to avoid hypoglycemia during fasting and increased levels of lactate, triglycerides and hepatomegaly. According to experts, the management of GSD should include small and frequent

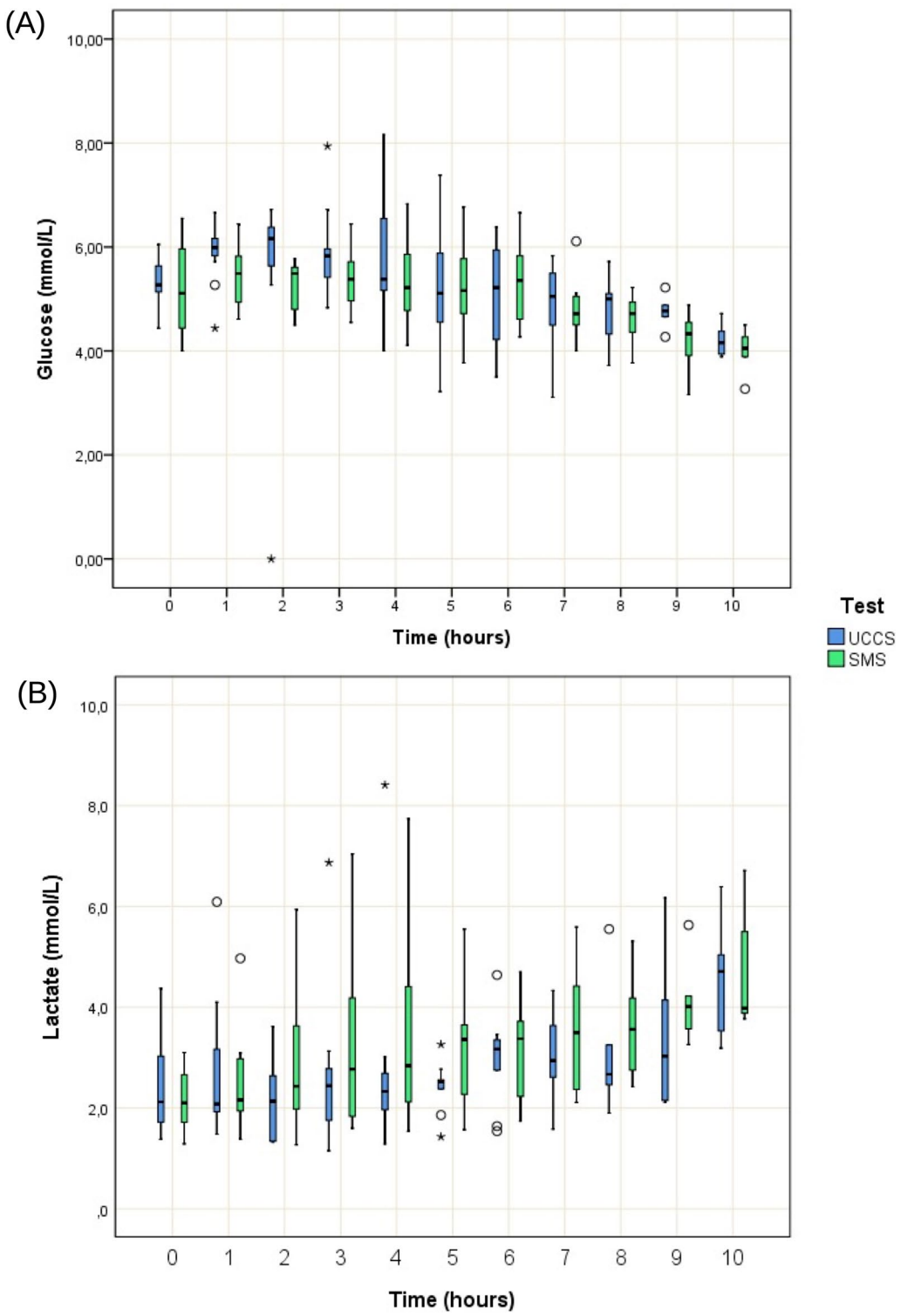

Fig. 2 Blood concentrations of glucose and lactic acid after UCCS or SMS throughout the study period. **A** Glucose level for each starch load performed (n = 11), normal range: > 3.88 mmol/L; **B** Lactic acid levels for each starch load performed (n = 11), normal range: 0.5 to 2.2 mmol/L. UCCS (uncooked cornstarch load) or SMS (sweet manioc starch)

Table 2 Baseline and final levels of total cholesterol and fractions, triglycerides and uric acid(n = 11)

	Baseline (Mean ± SD)		Final (Mean ± SD)		Treatment effect (*p*)
	Before UCCS	Before SMS	After UCCS	After SMS	
TC (mg/dL)	221.7 ± 64.0	219.2 ± 50.0	234.9 ± 135.2	209.5 ± 55.8	0.77
TG (mg/dL)	589.8 ± 505.0	503.6 ± 243.6	456.6 ± 272.9	457.1 ± 216.3	0.14
HDL (mg/dL)	32.0 ± 8.6	31.2 ± 7.0	31.2 ± 9.1	31.1 ± 9.1	0.62
UA (mg/dL)	7.8 ± 1.0	7.3 ± 1.1	8.4 ± 1.3	8.1 ± 1.2	0.98

UCCS: uncooked cornstarch; SMS: sweet manioc starch; SD: standard deviation; TC: total cholesterol (< 200 mg/dL); TG: triglycerides (< 150 mg/dL); HDL: high density lipoprotein (> 35 mg/dL); UA: uric acid (3.4–7 mg/dL)

meals, favoring the complex carbohydrates over the simple carbohydrates [8]. This recommendation is based on the biochemical and nutritional properties of carbohydrates, which can critically determine the rate and extent of digestion and absorption in the small intestine. The greater is the release of glucose in the small intestine, the higher is the glucose blood bioavailability which favors the formation of glycogen. In general, granular starches with higher amylose content are more resistant to digestion, while greater amounts of amylopectin tend to be more easily digested. Other extrinsic aspects may also influence the starches digestion, such as its natural source, the granular structure, the degree of isolation as well as its processing and refinement [20].

Cassava starch consists of 80% amylopectin and 17–20% amylose [11]. Proportional amounts were found by Nalin et al. [11] in three Brazilian batch production. Additionally, SMS is constituted by approximately 170 g/kg of sucrose, trace amounts of fructose [10], with simple carbohydrates representing only 1 to 3% of the product [21]. Further analyses regarding the detailed composition of the studied starches are warranted.

The increased amylose/amylopectin ratio in SMS is associated with slow release of glucose and the maintenance of a prolonged euglycemia, thus constituting a promising alternative in the treatment of glycogen storage disorders, especially in Brazil, where access to slow-release starch is restricted to some patients. However, we also found an increased concentration of lactate irrespective from the starch, likely associated with the duration of fasting. Additional studies are necessary to identify possible starch components involved in hyperlactatemia.

The increased plasma lactate levels (> 2.2 mmol/L) verified in all patients even during euglycemia period deserves further investigation. As G6Pase also catalyzes one important step in gluconeogenesis [4] and this metabolic pathway is underactivated during euglycemia, the increased lactate levels may not be related to the gluconeogenesis. Hypothetically, such increased lactate concentrations could be directly associated with the metabolism of fructose or other sugars. The preparation of SMS using different cassava species, processing techniques of a mixture of brands could be employed in future trials. It also should be highlighted that four participants presented with high lactate levels and ten participants have displayed hypertriglyceridemia at the baseline evaluation, suggesting a previous poor metabolic control.

The main limitations of the present study are its short-term duration and that the evaluated dosages of the starches were distinct from that used in the pre-trial period.

Conclusions

This study demonstrated a longer duration of euglycemia and greater stability of glucose levels in GSD Ia patients who underwent a short-term intervention with SMS, suggesting that this starch is a promising alternative in the treatment of this condition. Additional studies are warranted to understand the long-term effects of the administration of SMS and to identify possible starch components involved in hyperlactatemia.

Methods

Study design

This was a randomized, triple-blinded, phase I/II crossover study designed to evaluate the safety and efficacy of SMS in comparison to UCCS in preventing the hypoglycemia associated with GSD Ia. Participants were randomly assigned to groups receiving distinct starches. The principal investigators, the participants and the statistician were blind to the type of starch received. Only the researcher responsible for randomization and the study dietitian who dispensed study starches were not blinded to the type of starch administered. The study protocol included a hospitalization for two consecutive days and

nights. At the time of hospital admission, anamnesis and physical examination (including weight and height assessment) were performed. All participants remained under their usual dietary treatment for GSD Ia during the day 1. The same dinner meal was served at 6 pm for all participants. At 10 pm, distinct randomized starches were orally administered. Patients remained with a permanent peripheral saline catheter, without any continuous infusion and without mobility restrictions.

Peripheral blood samples were collected at 10 pm (basal evaluation) to determine glucose, lactate, insulin, triglycerides, total cholesterol and HDL fraction and uric acid levels. Subsequently, the participants ingested 100 g of starch (UCCS or SMS) diluted in 200 mL of drinkable water. Blood samples were collected in a 1 h-interval following starch administration and the vital signs were checked. No additional food or beverages (water excepted) ingestion were allowed.

The fasting was interrupted at 8 am. The participants were allowed to follow their usual dietary treatment until 10 pm. After a standardized meal for dinner with an average of 50 g of carbohydrates, the participants received the switched starch, and the same evaluations were performed on the second night. The only modification was the type of starch administered (Fig. 3).

In case of hypoglycemia (blood glucose less than 3.88 mmol/L or 70 mg/dL) or symptoms of hypoglycemia, the fasting was discontinued immediately, and the participant received 10 g of glucose closely with the meal.

Participants

To be eligible, participants should have clinical and genetic diagnosis of GSD Ia, be ≥ 16 years old, and be under UCCS therapy. All participants were seen at the Metabolic Disorders Clinics of Hospital das Clínicas in Porto Alegre, Brazil. Demographic data and clinical variables were retrieved from participants' medical records.

Participants received an anonymous reference number and were randomly assigned to receive SMS or UCCS in the first night of the study (Fig. 3). The starches were manufactured in accordance with the Brazilian standardized techniques for food quality and inspection. Physicians and dietitians planned a safe fasting for each participant before starting the trials.

Tested products

Both starch samples were produced in Brazil (SMS from Fritz & Frida® and UCCS from Maizena®), similarly to the previous study of Nalin et al. [12]. All starch doses were administered as 100 g of raw powder diluted in 200 mL of water at room temperature as preconized for dietary treatment in GSD Ia. The high dose of starch (100 g) was determined in accordance with previous literature [18]. During the experimental procedures, both starches were stored in identical containers numbered in accordance with the randomization sequences by the study dietitian. The starches nutrition information is provided in Table 3.

Randomization

Randomization was performed using an online software (www.randomization.com) by a researcher who was unaware of obtained clinical records. Blind data were maintained to all study personnel until the conclusion of statistical analysis, except for the study dietitian who prepared the starches doses for the participants. The researcher responsible for randomization and the study dietitian who dispensed study starches were not present in the tests and had no contact with the enrolled participants.

Biochemical blood evaluation

Blood analysis was performed as follow: glucose (hexokinase colorimetric assay); lactate (colorimetric assay, normal range values (NRV): 0.5–2.2 mmol/L), insulin (chemiluminescent microparticle immunoassay, NRV: 1.4–14 µUI/mL), total cholesterol (enzymatic colorimetric assay, NRV: <200 mg/dL), HDL cholesterol (homogeneous enzymatic colorimetric method, NRV: >35 mg/dL), triglycerides (enzymatic colorimetric method, NRV: <150 mg/dL) and uric acid (enzymatic colorimetric assay, NRV: 3.4–7 mg/dL). All analyses were performed by using a Cobas c702 analyzer and commercial kits. Insulin evaluation was performed using a Ci4100 analyzer. The plasma was frozen for 15 min after collection and then used in insulin evaluation.

Study outcomes

The maintenance of euglycemia (blood glucose ≥ 3.88 mmol/L) was the primary endpoint of the study. The impact of dietary treatment on plasma lactate was considered a secondary endpoint.

Statistical analysis

Categorical variables were represented as frequencies and percentages and continuous variables were presented as means and/or medians, standard deviation, and percentiles. The main data were analyzed as proposed by Altman (1991), which investigated period effects, treatment-by-period interactions, and treatment effects. The level of significance was established at 5% ($p<0.05$). Analysis of variance (ANOVA) was used to compare the

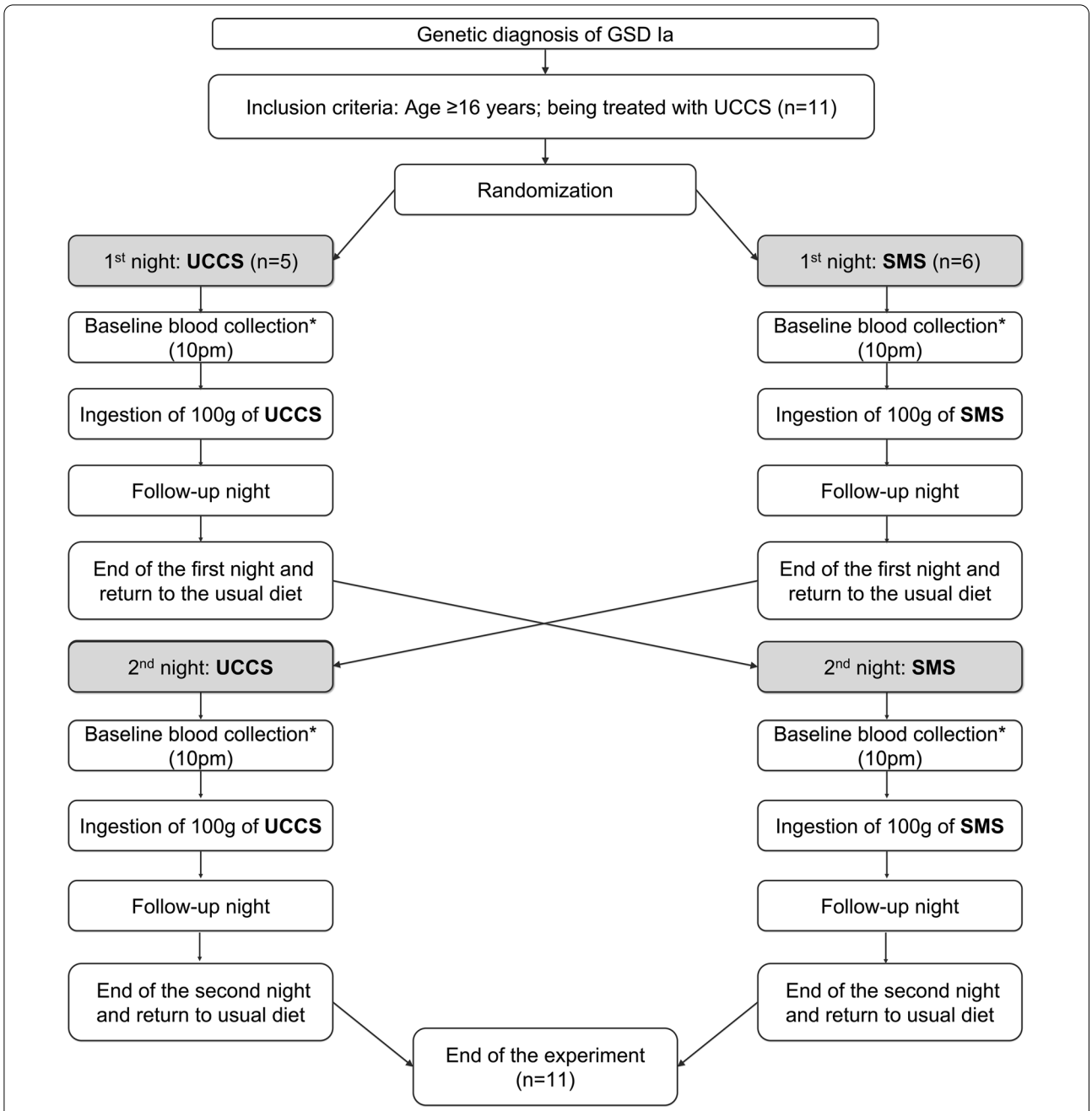

Fig. 3 Study design. *refers to blood collection for the evaluation of glucose, lactic acid, insulin, total cholesterol, triglyceride and uric acid levels. **refers to blood collection for the glucose, lactic acid and insulin levels. GSD Ia: glycogen storage disorder type Ia; UCCS: uncooked cornstarch and SMS: sweet manioc starch

Table 3 Starch nutrition facts

Nutrition Facts	UCCS	SMS
Portion (g)	20	20
Energy (kcal)	70	70
Protein (g)	0	0
Fat (g)	0	0
Carbohydrate (g)	17	17
Fiber (g)	0	0

UCCS uncooked cornstarch, *SMS* sweet manioc starch. Information provided by food brand owners in label data

results and data were analyzed using the software SPSS v.18 and Stata V.

Ethical aspects

The study was approved by the Ethics Board of the Hospital de Clínicas de Porto Alegre, Brazil (protocol #52645116500005327). The study design is registered in ClinicalTrials.gov (NCT03871673). All participants and their legal representatives read and signed the informed consent form before being enrolled in this study. This is an investigator-funded study.

Abbreviations

GSD Ia: Glycogen storage disease type 1a; UCCS: Uncooked cornstarch; SMS: Sweet manioc starch; G6Pase: Glucose-6-phosphatase enzyme; OMIM: Online Mendelian Inheritance in Man; TIM-1: Dynamic gastro-small intestine model; ANOVA: Analysis of variance; HDL: High density lipoprotein; WMHM20: Waxy Maize (Heat Modified) 20; NRV: Normal range values.

Acknowledgements

The authors would like to thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS) and Fundo de Incentivo à Pesquisa e Eventos (FIPE) do Hospital de Clínicas de Porto Alegre for sponsoring the present study. We would like to thank Vania N Hirakata for the statistics analysis, the nurses Caroline Campos and Anna Terra and the nurse team from the Hospital de Clínicas de Porto Alegre. Special thanks to Franciele Trapp, Tássia Tonon, Devora Randon, Rafael Tresbach and Miguel Krigsner. We also thank Camilla Hissamura who assisted in translating this manuscript from Portuguese to English. A very special thanks to all enrolled participants and their families.

Authors' contributions

IVDS was the principal investigator of the study. TN, TGJD, CFMS and IVDS designed the study. FSL performed the genetic analyses. BBS and TN participated in the randomization process. LFR performed the nutritional evaluations and diet prescriptions. VCLM, BMO, TN, CFMS and IVDS performed clinical data collection. BMO and CFMS performed clinical evaluations. VCLM, BMO and BBS performed data analyses. VCLM, BMO and BBS drafted the manuscript. TGJD, CFMS, IVDS critically revised the manuscript. All authors read and approved the final manuscript.

Funding

This study was financially supported by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) and FIPE/HCPA (Hospital de Clínicas de Porto Alegre Research and Event Incentive Fund). The authors are entirely responsible for the study design, data analyses and the scientific content of the manuscript.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study was approved by the HCPA Research Ethics Committee (protocol #52645116500005327) and ClinicalTrials.gov (NCT03871673). All participants or their legal guardians signed an informed consent form.

Consent for publication

"Not applicable".

Competing interests

TN is currently an employee of Ultragenyx Farmacêutica Brasil LTDA and hold stock in Ultragenyx Pharmaceutical Inc. All other authors declare no conflicts of interest.

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Received: 10 February 2021 Accepted: 21 May 2021
Published online: 03 June 2021

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