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Novel monitoring and management strategies for hepatic glycogen storage diseases

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Novel monitoring and management strategies for hepatic glycogen storage diseases

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The studies in this thesis were conducted at the University of Groningen, University Medical Centre Groningen, Beatrix Children's Hospital, Section of Metabolic Diseases, Groningen, the Netherlands and University of Naples "Federico II", University Medical Centre Naples, Department of Translational Medical Sciences, Section of Paediatrics, Naples, Italy. Part of the studies in this thesis were funded by grants from Ultragenyx Pharmaceuticals Inc., Associazione Italiana Glicogenosi, Vitaflo Italia and Compagnia di San Paolo.

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General introduction and thesis outline

(Partly adopted from "Alberto Casertano, Alessandro Rossi*, Simona Fecarotta, Francesco Maria Rosanio, Cristina Moracas, Francesca Di Candia, Giancarlo Parenti, Adriana Franzese and Enza Mozzillo. An Overview of Hypoglycemia in Children Including a Comprehensive Practical Diagnostic Flowchart for Clinical Use. Front Endocrinol (Lausanne). 2021 Aug 2;12:684011")*

**Contributed equally*

1.1 Childhood hypoglycaemia

Hypoglycaemia is the result of the defect in one or several metabolic pathways or their regulatory mechanisms that normally guarantee glucose homeostasis during feeding and fasting. These pathways and mechanisms include glycogenolysis, gluconeogenesis, mitochondrial fatty acid oxidation, ketogenesis, and hormonal responses. Thus, hypoglycaemia can have a variety of etiologies[1]. In case of impaired metabolic pathways and/or altered hormonal regulation, glucose release into the circulation is insufficient to satisfy peripheral tissue, and in particular neuronal demand, resulting in the classical symptoms of hypoglycaemia. In children both systemic glucose homeostasis and the clinical presentation of hypoglycaemia deviate as compared to adults. In newborns, the adaptation to extrauterine life is characterised by immature hormonal and metabolic pathways. Combined with a relatively high glucose requirement of the brain, these early prenatal features increase the risk of hypoglycaemia. Additionally, infants and children possess relatively smal glycogen stores and a higher systemic glucose demand[2].

Despite being one of the most common metabolic emergencies, with an estimated incidence of 1-3/1,000 live births, there are still controversies on the definition and management of childhood hypoglycaemia[3-5]. The current approach for clinical management of infants and children presenting with hypoglycaemia is based on the collection of a variety of information, including medical history (e.g., age at onset, relation with food), physical examination, (in vivo/in vitro) biochemical (baseline and/or dynamic) tests, imaging tests, continuous glucose monitoring (CGM) and/or molecular analyses[6]. Although the current approach in most cases results in a working diagnosis, it is prone to several limitations: relevant information on medical history cannot always be retrieved; adequate samples (i.e., the “critical sample”) are not in all cases available and/or appropriate analysis may not always be performed; some tests are complex to arrange and/or potentially harmful and/or it may take months until the results are available.

1.2 Hepatic glycogen storage diseases (GSDs)

Glycogen storage diseases (GSDs) are a group of inherited metabolic disorders of glycogen metabolism that result from mutations in enzymes and transporters involved in glycogen breakdown and synthesis. More than 12 GSDs types are recognised and classified based on enzyme or transporter deficiency and tissue involvement. The GSDs types are numbered with Roman numeral according to the chronological order in which their enzymatic basis has been described. All GSDs are inherited in an autosomal recessive pattern, except type IXa and IXd which are inherited in an X-linked recessive manner.

Clinically, GSDs can be further divided in hepatic and muscle GSDs. GSDIII is the only GSD type presenting with concomitant liver and muscle involvement. The hepatic GSDs include GSD type 0a, I, III, IV, VI, IX, and XI (Table 1). The most important presenting symptoms and signs in patients with hepatic GSDs are hypoglycaemia, hepatomegaly, and failure to thrive. Based on the ability to perform gluconeogenesis and mitochondrial fatty acid oxidation for ketone body production, hepatic GSD are further classified as ketotic (GSD0a, GSDIII, GSDVI, GSDIX, GSDXI) or non-ketotic (GSDI). Elevated transaminases and hyperlipidaemia are common features

of hepatic GSDs. Additional symptoms and signs, biochemical disturbances and long-term complications vary widely between hepatic GSD types and can aid differential diagnosis[7].

GSDI is the hepatic GSDs subtype that presents the most severe fasting intolerance; it is due to a defect of either the catalytic subunit (GSDIa, 80%) or the microsomal glucose 6-phosphate transporter (GSDIb, 20%) of the glucose 6-phosphatase (G6Pase) system[8]. Distinctive biochemical features include elevated blood concentrations of lactate, uric acid, and lipids. Additionally, patients with GSDIb show neutropenia/neutrophil dysfunction and recurrent infections[9]. Long-term complications include liver neoplasms (mostly hepatocellular adenomas)[10], renal disease (evolving to kidney failure)[11], osteoporosis[12], anaemia, and inflammatory bowel disease (IBD)[13].

GSDIII results from glycogen debrancher enzyme deficiency. Two main subtypes are recognised: GSDIIIa (85% of the cases, mixed liver and muscle involvement) and GSDIIIb (15% of the cases, isolated liver involvement). As gluconeogenesis is intact in GSDIII, fasting intolerance and hyperlipidaemia are usually less severe than in GSDI. GSDIII patients show prominent ketosis without lactic acidosis and transaminase levels. Liver fibrosis in GSDIII can develop into cirrhosis and eventually malignancies[15]. Additionally, GSDIIIa patients show osteopenia[16] and (cardio)myopathy worsening with age[17]. Muscle involvement can include both proximal and distal muscle weakness and is likely overshadowed by fasting intolerance in childhood[14]. Polyneuropathy caused by glycogen deposition in axons has also been described in adult GSDIIIa patients and may contribute to the muscle phenotype[18].

GSDIV is caused by glycogen branching enzyme deficiency and shows an extremely heterogeneous clinical presentation[19]. The phenotypic continuum includes different degrees of hepatic involvement (from rapidly progressing liver cirrhosis to non-progressive liver disease), the neuromuscular system (e.g., fetal hydrops, arthrogryposis multiplex, hypotonia, adult polyglucosan body disease) and the heart (variably onset cardiomyopathy). Fasting intolerance and the potential of dietary management have recently been recognised[20]. Transaminase levels are increased in GSDIV patients with hepatic involvement[21].

GSDVI and GSIX occur secondary to liver glycogen phosphorylase and glycogen phosphorylase kinase deficiency, respectively. They are generally mild disorders that improve with age[22]. However, they can also present with symptomatic ketotic hypoglycaemia, hyperlipidaemia, increased transaminases and growth retardation[23].

GSDXI (Fanconi-Bickel syndrome) is caused by deficiency is solute carrier family 2 protein (GLUT-2) that is expressed in hepatocytes, pancreatic beta cells, and proximal renal tubule. Patients typically present at 3-10 months of age with hepatomegaly, failure to thrive, fasting hypoglycaemia and postprandial hyperglycaemia. GSDXI patients develop Fanconi syndrome, which is characterised by severe glycosuria, polyuria, hyperaminoaciduria, hypophosphatemic rickets, acidosis, hypokalaemia, hypochloraemia[24].

GSD0a is caused by deficiency of hepatic glycogen synthase. Patients present with fasting-induced ketotic hypoglycaemia and post-prandial hyperglycaemia, hyperlactatemia and glycosuria. Typically, they do not show hepatomegaly. Improvement in fasting tolerance is usually observed with age. Short stature and osteopenia are commonly observed in untreated children[25].

Type	Name	Locus	OMIM#	Gene	Enzyme/Transporter	Glycogen structure	Guidelines*
0a	--	12p12.2	240600	*GYS2*	Glycogen synthase	Normal, decreased in quantity	No formal recommendations
Ia	Von Gierke	17q21.31	232200	*G6PC*	Glucose 6-phopshatase-α catalytic subunit	Normal	Rake et al., 2002[26] Kishnani et al., 2014[27] Bali et al, 2016 [28]
Ib	Von Gierke	11q23.3	232200	*SLC37A42*	Glucose 6-phosphate transporter	Normal	Visser et al., 2000[29] Rake et al., 2002[26] Kishnani et al., 2014[27] Bali et al., 2016[28]
IIIa/IIIb	Cori/Forbes	1p21.2	232400	*AGL*	Glycogen debranching enzyme	Outer chains missing or very short	Kishnani et al., 2010[30] Dagli et al., 2016[31]
IV	Andersen	3p12.31	232500	*GBE12*	Glycogen branching enzyme	Very long unbranched chains	*Magoulas et al., 2019[32] *Derks et al., 2021[20]
VI	Hers	14q22.1	232700	*PYGL*	Liver glycogen phosphorylase	Normal	Kishnani et al., 2019[33] Labrador et al., 2019[34]
IXa	--	Xp22.13	306000	*PHKA2*	Phosphorylase kinase α-subunit	Normal	Herbert et al., 2018[35] Kishnani et al., 2019[33]
IXb	--	16q12.1	261750	*PHKB*	Phosphorylase kinase β-subunit	Normal	Herbert et al., 2018[35] Kishnani et al., 2019[33]
IXc	--	16p11.2	613027	*PHKG2*	Phosphorylase kinase γ-subunit	Normal	Herbert et al., 2018[35] Kishnani et al., 2019[33]
XI	Fanconi-Bickel	3q26.2	227810	*SLC2A2*	GLUT2	Normal	*Pennisi et al, 2020[36]

Table 1. **Hepatic glycogen storage diseases**.
OMIM: Online Mendelian Inheritance in Man; GLUT: glucose transporter
** in some situations, references contain recommendations*

1.3 Current management and monitoring strategies for hepatic GSDs

The management and monitoring approach for hepatic GSDs patients is summarised in guidelines, review articles and care pathways (Table 1)[26].

The main goals for the management of hepatic GSDs patients include: prevention of acute metabolic decompensation, prevention of acute and long-term complications, achieve a regular psychomotor development, and optimising quality of life[26]. As hepatic GSDs are multisystem disorders, a highly specialised multidisciplinary team is required to achieve the above-mentioned goals[27]. Dietary management, which involves avoidance of fasting, regular uncooked cornstarch intake and/or gastric-drip feeding, is the cornerstone of the treatment[34]. Medical treatment can be employed to correct secondary metabolic disturbances (e.g., lipid-lowering drugs, allopurinol for hyperuricemia) or prevent/delay disease complications (e.g., G-CSF in GSDIb, ACE-inhibitors in GSDI). Radiofrequency ablation is required in patients with hepatic adenomas. Liver transplantation could be considered in patients with persistent poor metabolic control despite other treatments, or in case of diffuse liver neoplasms or liver failure[28]. Patients and families should also be instructed on how to prevent/what to do in case of acute metabolic decompensation[26]. In fact, while a good adherence to recommended treatments can ensure normal psychomotor development

and delay development of long-term complications, patients may still face emergency situations. In acute conditions such as intercurrent illness, heat waves, and prolonged fasting they can become catabolic due to (the combination of) high fever, reduced food intake and/or increased losses (e.g. vomiting, diarrhoea).

Current monitoring strategies rely on a combination of traditional biochemical, clinical and imaging parameters. Biochemical tests play a major role in patient monitoring. Although (pre-prandial) blood glucose (BG) concentrations represent a direct biomarker for hepatic GSDs this parameter has proven insufficient to thoroughly assess patients' metabolic status in clinical practice. In fact, BG can show wide variations between days and fluctuations during day and night. Furthermore, the secondary metabolic disruptions that occur in patients with hepatic GSDs may not be adequately reflected by BG concentrations[26]. Serum triglycerides and cholesterol levels are therefore generally also included as biomarkers for all hepatic GSDs types. Serum biotinidase activity[37] and urine glucose tetrasaccharide[38] can represent helpful diagnostic and dietary monitoring biomarkers, respectively. Additional biomarkers are monitored for specific hepatic GSDs subtypes, such as lactate, uric acid, microalbuminuria in GSDI[26], neutrophil count and faecal calprotectin in GSDIb[29], ketones and creatine kinase in GSDIII[30].

Failure to thrive or changes in growth trajectories may reflect poor metabolic control in hepatic GSDs patients. In this respect height, weight, weight/height ratio, body mass index and head circumference are regularly assessed in patients with hepatic GSDs. Puberty progression is also monitored. Xanthomas can appear in patients with poor metabolic control[28]. Additional signs, symptoms and complications can be observed in specific subtypes, e.g., infections and diarrhoea in GSDIb[26], weakness and/or signs of cardiomyopathy in GSDIIIa[30], and neuromuscular symptoms in GSDIV[20]. Imaging studies are mainly used to investigate the hepatic involvement. In this respect, liver ultrasound is regularly performed in patients with hepatic GSDs. Abdominal Magnetic Resonance Imaging may be performed in patients suspected with hepatocellular adenoma and/or carcinoma[27]. Additional imaging studies may be required for specific hepatic GSDs subtypes, e.g., cardiac/muscle ultrasound in GSDIIIa[31]. The efficacy of existing or novel treatments is currently assessed by the above-mentioned monitoring tools.

1.4 Thesis outline

While refined diagnostic methods have significantly improved the identification of (most) patients suffering from hepatic GSDs, the need to develop new methods for patients' monitoring (including long-term complications) and to standardise patients' management (including emergency situations) are among the research priorities defined by patients, carers, and healthcare professionals. More specifically, improving the strategies to prevent and/or treat intestinal and muscle problems were listed as top priorities for GSDIb and GSDIII, respectively[39].

On the one hand, current monitoring strategies are not always sufficiently accurate to stratify the phenotypic heterogeneity or to adequately assess the efficacy of novel treatments in a safe and minimally invasive manner. On the other hand, wide differences in patients' management still exist. Some recommendations are based on so-called best practice or expert opinion while controversies on specific topics are found. Also, long-term management is more extensively covered compared to

the acute treatment in current guidelines. Furthermore, as novel treatment options for hepatic GSDs are becoming available, improved methodology to assess their safety/efficacy is required.

Therefore, the **main aim of this thesis** is to develop novel monitoring and management strategies for hepatic GSDs patients. The chapters of this thesis are categorised according to these two aspects. As the development of innovative management strategies and novel monitoring tools for hepatic GSDs are closely related topics, various aspects of this work are interrelated.

Part I – Developing novel monitoring strategies

As a result of timely diagnosis and treatment of patients with hepatic GSDs, several long-term complications have emerged over the past years. Currently available biomedical parameters of metabolic control are not always sufficiently reliable to capture phenotypic heterogeneity, predict disease prognosis, and assess the safety/efficacy of novel treatments; in some cases they also require laborious, complex, or potentially dangerous procedures. Therefore, the **first objective** of this thesis is to develop novel reliable, safe, and simple monitoring tools for patients with hepatic GSDs. The following **research questions** will be addressed:

- Can CGM reference values be defined for adult GSDIa patients? (Chapter 2)
- Is adrenal cortex dysfunction a feature of GSDI? (Chapter 3)

Part II – Developing novel management strategies

Despite the progress in dietary and medical treatment of hepatic GSDs over the past years, long-term complications and a life-long strict dietary regimen still heavily impact on patients' prognosis and quality-of-life. Moreover, substantial differences in patients' management exist globally among clinical centres impacting on patients' outcome and access to healthcare services. Therefore, there is a urgent need to standardize patients' management and to develop novel treatment strategies. Therefore, the **second objective** of this thesis is to develop new management options for patients with hepatic GSDs. The following **research questions** will be addressed:

- Can patients with hepatic GSDs benefit from dietary lipid manipulation? (Chapter 4)
- Can management of metabolic emergency in patients with hepatic GSDs be optimised and uniformed? (Chapter 5)
- Is treatment with empagliflozin associated with changes in bowel morphology in GSDIb? (Chapter 6)

The outcomes and future perspectives of this thesis are finally discussed in Chapter 7.

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PART I

Developing novel monitoring strategies

Chapter 2

A prospective observational study on continuous glucose monitoring in adult GSDIa patients: towards reference values

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ABSTRACT

Introduction. Continuous glucose monitoring (CGM) is a recognised monitoring modality for patients with diabetes mellitus. Previous research has shown the potential benefit of CGM also for glycogen storage disease (GSD) patients. The current lack of reference values for CGM-derived outcomes limits the use of this technology in day-to-day care and clinical trials for GSD patients. The aim of the present study was to define CGM reference values for this patient group by comparing CGM parameters between adult GSDIa patients and matched healthy volunteers,

Methods. Prospective CGM data were collected during the ENGLUPRO GSDIa trial (NCT04311307), in which ten adult GSDIa patients and an equal number of age-, gender- and BMI-matched healthy volunteers were enrolled. A Dexcom G6 device was used. Descriptive (median, minimum, maximum, range, outcomes of glycaemic variability (GV), time-below-range, time-in-range, time-above-range) and advanced (i.e., 1st and 2nd order derivatives, Fourier analysis) CGM parameters were derived from CGM data calculated over 24-hours as well as overnight (01:00-05:00 a.m.) intervals. To assess the reliability of CGM in GSDIa patients, capillary blood glucose (CBG) values were concurrently measured during two standardized 2-hour time intervals (every 10 minutes during the first hour and at +75, +90 and +120 minutes after starting the coupled measurements) in pre-prandial/fasted and fed states, respectively.

Results. Bland-Altman analysis showed agreement between CBG and CGM values (p<0.05). Reference values for the descriptive CGM parameters were generated for GSDIa patients. Both mean 24-hour and mean overnight GV were higher in GSDIa patients compared to healthy volunteers (p<0.05). Level 2 hypoglycaemia (glucose value < 3.0 mmol/L) was uncommon in GSDIa patients during the study while the time-in-range (≥ 3.9 ≤ 10.0 mmol/L) was lower and the time-above-range (glucose > 10.0 mmol/L) was higher in GSDIa patients compared to healthy volunteers (p<0.05). Three GSDIa patients showed deviating CGM parameters compared to the calculated reference values. Advanced CGM analysis provided in-depth insight into glucose patterns in these patients allowing for a clear differentiation within the patients group.

Discussion. This is the first study to prospectively compare CGM outcomes between adult GSDIa patients and matched healthy volunteers. The generation of CGM reference values for GSDIa patients will allow for comparison of individual GSDIa patients with a GSDIa population as well as a group of healthy volunteers. The results of this study further support the use of CGM as a monitoring tool for GSDIa both in regular healthcare and clinical research/trials settings. In addition, they illustrate the potential contribution of CGM for personalised medicine in GSDIa. Future studies are warranted to assess the value of CGM outcomes as prognostic tools and to investigate their major determinants in GSDIa patients.

INTRODUCTION

Glycogen storage disease type Ia (GSDIa) (MIM# 232200) is an inherited disorder of glycogen metabolism due to mutations in the *G6PC1* gene, encoding the glucose 6-phosphatase-α (G6Pase-α) enzyme. Impaired glycogenolysis and gluconeogenesis results in fasting intolerance with hypoglycaemia, elevated lactate, metabolic acidosis, and secondary metabolic derangements, including hypertriglyceridemia, hypercholesterolemia and hyperuricemia[1]. A strict (personalised) diet, including frequent feedings, uncooked cornstarch (UCCS) and/or continuous nocturnal gastric drip-feeding (CNGDF) constitutes the cornerstone of the treatment for GSDIa patients and has improved their prognosis in the past decades. Yet, GSDIa patients are still at a risk of developing long-term complications[2].

Dietary compliance and the patients' overall "metabolic control" are currently assessed by a combination of clinical (e.g., height, weight, liver size) and biochemical (blood glucose, lactate, triglycerides, cholesterol, uric acid) markers[3]. Although such biomedical parameters can reflect the degree of the disease (de)compensation, they often constitute a static situation, possibly not adequately reflecting the patients' "everyday" status. In addition, their assessment requires regular (expensive and invasive) in-hospital and/or out-patients' evaluations and their interpretation can be challenging due to considerable phenotypical heterogeneity among patients[4]. Finally, it is unknown whether these "traditional" biomarkers are sufficiently reliable to assess the dynamic effects of emerging treatment strategies for GSDIa, such as gene therapy (NCT03517085) or mRNA therapy[5].

Over the past years, continuous glucose monitoring (CGM) has developed into a valuable monitoring modality[6]. For patients with diabetes mellitus (DM), reference values for CGM-parameters (e.g. time-in-range and time-above-range) have been defined and are currently used as outcome measures[7]. As CGM has previously been shown valuable to unveil unrecognised hypoglycaemia and to monitor individual glycaemic variability (GV)[8,9], its potential benefit is high for hepatic GSD patients. However, the current lack of CGM-derived outcome parameters and reference values limits the use of this technology in the day-to-day care for individual hepatic GSD patients as well as in clinical trials.

We have recently proposed indications for CGM monitoring and CGM outcome parameters in hepatic GSD patients[10]. As a follow-up, the aim of the present study was to define CGM reference values for GSDIa patients, by comparing CGM parameters between adult patients and matched healthy volunteers.

SUBJECTS AND METHODS

Study approval

CGM data were collected during the "Endogenous Glucose Production in Patients With Glycogen Storage Disease Type Ia" (ENGLUPRO GSDIa; NCT04311307) study. The study protocol was approved by the Medical Ethical Committee of the University Medical Centre Groningen (UMCG), Groningen, The Netherlands (ref. no. METc 2020/342). The study was conducted according to the

principles of the Helsinki Declaration of 1975 as revised in 2013. All participants provided written informed consent prior to inclusion in the study.

Study design

The ENGLUPRO GSDIa study was a single-centre, prospective, observational clinical trial conducted at the UMCG between October 2020 and July 2021. On study day 1, a Dexcom G6 (Dexcom, San Diego, California) CGM sensor was placed on either the upper arm (n=18) or the abdomen (n=2, in participants 014 and 015). Instructions on the appropriate use of the CGM device were provided by an experienced research nurse. Participants were asked to keep the CGM device for 10 days while performing their every-day activities and following their usual diet.
At the end of the study, the CGM device was removed by each participant and the material was sent back to the study site. CGM data of each study participant were collected for further analysis during the entire course of the study.

Study participants

Ten GSDIa patients and an equal number of age-, gender- and BMI-matched healthy volunteers healthy volunteers were included in the study. Inclusion criteria were (a) age > 16 years, (b) stable medical condition before the start of the test procedures and for patients with GSDIa (c) confirmation of GSDIa with enzyme assay and/or *G6PC1* variant analysis. Exclusion criteria included (a) pregnancy, (b) recent (< 1 month) history of hospitalisation due to hypoglycaemia, (c) intercurrent illness (defined as (a combination of) decreased dietary intake, vomiting, diarrhoea and fever (>38.5^{0}C) in the week prior to the study visit), and for healthy volunteers also (d) confirmed diagnosis or history suggestive of diabetes mellitus, (e) first grade family member with a confirmed diagnosis associated with fasting intolerance, (f) symptoms or signs by suggestive of fasting intolerance, metabolic instability, fever or gastrointestinal complaints. *G6PC* mutations were reported according to ClinVar or based on published literature in case a mutation was not deposited on ClinVar.

Continuous glucose monitoring (CGM) system

The Dexcom CGM Systems are approved for children with DM of 2 years of age and older. In this study a Dexcom G6 (Dexcom, San Diego, California) device was used. Dexcom G6 exhibits a relatively high accuracy in the hypoglycaemic range and sensitivity for detecting hypoglycaemia in DM patients[11]. The CGM device consists of a wireless receiver, a transmitter, and a sensor. The sensor is inserted in the subcutaneous tissue in the interstitial space. The sensor coated with glucose oxidase reacts with glucose, producing an electrical current every 5 minutes, resulting in 288 measurements per day. The glucose concentration is derived from the subcutaneous glucose concentration using computer-driven algorithms, where after the measurement is transmitted to the wireless receiver. As the Dexcom G6 is factory calibrated, calibration by the user is not required.

Capillary blood glucose (CBG) measurements

Capillary blood glucose (CBG) measurements were performed at the study site under supervision of a research nurse and a physician using a Freestyle freedom Lite device (Abbott, Chicago, Illinois). CBG values were collected during two 2-hour time frames in which CBG and CGM levels were concurrently measured (every 10 minutes during the first hour and subsequently at +75, +90 and +120

minutes after starting the synchronised measurements) in a pre-prandial/fasted (i.e. before breakfast) and fed (i.e. after lunch) state, respectively. As a result, 20 paired CGM and CBG measurements (i.e. 10 paired measurements in a pre-prandial/fasted state and 10 paired measurements in a fed state), were generated for each study participant.

Outcome parameters and data analysis

During the ENGLUPRO GSDIa study, data on participants' demographic, genotype, diet, and CBG were collected and stored as explorative outcome parameters during the ENGLUPRO GSDIa study. The raw CGM data files were retrieved from the Dexcom CLARITY Clinical Portal (https://clarity.dexcom.eu/professional/patients) and stored anonymously as CSV-files prior to the analysis[12]. The Dexcom CGM System has been validated for glucose concentrations above 2.2 mmol/L (>40 mg/dL). In case the CGM sensor indicated a low value, the lowest possible CGM value of 2.2 mmol/L was used, as omitting these values would introduce bias in the descriptive statistical analyses.

CGM derived outcome parameters were defined as previously described[10] and included:

1. descriptive CGM outcomes:

- median, minimum, maximum, range;

- outcomes of glycaemic variability (GV): standard deviation (SD), variance, coefficient of variation (CV, calculated as SD divided by the mean);

- outcomes of glycaemic control[7]: time-below-range [TBR, defined as glucose values either ≥3.0 mmol/L and < 3.9 mmol/L (i.e., level 1 hypoglycaemia) or <3.0 mmol/L (i.e., level 2 hypoglycaemia)], time-in-range [TIR, defined as glucose values either ≥3.9 and ≤7.8 mmol/L or ≥ 3.9 and ≤10.0 mmol/L], and time-above-range [TAR defined as glucose values either >7.8 mmol/L or >10.0 mmol/L]. The occurrence of level 3 hypoglycaemia (i.e. glucose levels that are so low that mental or physical functioning is impaired) was also recorded.

To minimise the effect of diurnal variations in the dietary intake and physical activity on glucose values, descriptive CGM outcomes were calculated on 24-hour CGM data as well as the CGM data collected between 1:00 and 5:00 a.m. (i.e., 'overnight').

2. Advanced CGM outcomes:

- The first order derivative (change over time) calculated as $glucose' = \frac{dglucose}{dt}$
- The second order derivative (speed of change overtime) calculated $glucose'' = \frac{d^2glucose}{dt^2}$

- Fourier analysis, performed as described previously[13] by mathematically transforming the CGM data with a Fast Fourier Transformation (FFT) and converting the data in one or more sinusoidal curves. Two major parameters define a sinusoidal curve:

1) amplitude, i.e, the peak deviation of the curve from 0);

2) frequency, i.e., the number of oscillations (cycles) that occur within the time unit (a cycle is a complete wave oscillation).

Biologically three parameters were considered in this study: the frequency (i.e., the number of cycles in the glucose curve during the overnight interval), the number of frequencies in the overnight glucose curve (a glucose pattern can consist of one frequency or multiple patterns) and the amplitude of each overnight glucose curve). Adequate glucose control is characterised by a low frequency, a low number of frequencies and a small amplitude.

Statistical analysis

Statistical analysis for descriptive CGM outcomes was performed using Prism 9.2 software (GraphPad Software, Inc. La Jolla, California). Reference values for CGM descriptive outcomes were generated by defining 95 % confidence intervals (95%CI). 95%CI were calculated as $95\%CI = x \pm z_{\alpha/2}\left(\frac{\sigma}{\sqrt{n}}\right)$ (x: sample mean; α: 0.95; σ: standard deviation; n: sample size; z was calculated at a 95% confidence level). For each descriptive CGM parameter 95%CI of 24-hour and overnight CGM-derived outcomes were compared between GSDIa patients and healthy volunteers. In case the 95%CI of patients and healthy volunteers did not overlap, the difference was considered to be statistically significant (p<0.05). Agreement between the paired measurements obtained by CBG and CGM was assessed using the Bland–Altman analysis.

RESULTS

Study participants

Information on the study participants is presented in Table 1. Ten GSDIa patients (5 females, 5 males) with a median age of 22.2 years (range: 17.8-53.1) and a median BMI of 26.1 kg/m2 (range 22.4-29.8) were enrolled. Nine patients were using frequent feedings and uncooked cornstarch (UCCS), of whom two patients (participants 007 and 017) also received CNGDF. One patient (participant 009) was on frequent feedings without UCCS, but with CNGDF. Ten age-, gender- and BMI-matched healthy volunteers healthy volunteers were also enrolled.

Comparison between CBG vs CGM

Bland-Altman analysis indicated agreement between CBG and CGM values collected under both pre-prandial/fasted and fed states. CGM showed a non-significant overestimation of glucose values as compared to CBG values (mean glucose difference: -0.85 ± 0.87 mmol/L, with 95% limits of agreement, from −2.6 mmol/L to 0.9 mmol/L) (Figure 1A). Although a significant trend for larger differences between CBG and CGM values at higher glucose concentrations was observed, a similar trend was not observed when fractional changes in CBG and CGM values were compared (mean difference: 14.5 ± 14.7%, with 95% limits of agreement, from – 43.2% to 14.2%) (Figure 1B)

Participant	Age (years)	Gender	BMI (kg/m^2)	Genotype (*G6PC* mutations)		Dietary information
				Nucleotide change	*Protein change*	
001	44.1	F	25.3	c.809G>T c.1039C>T	p.Gly270Val p.Gln347Ter	Frequent feedings Glycosade (3.2 g/kg/day)
002	21.6	M	29.8	c.189G>A c.189G>A	p.Trp63Ter p.Trp63Ter	Frequent feedings Glycosade (2.2 g/kg/day)
004	17.8	F	22.4	c.1039C>T c.1039C>T	p.Gln347Ter p.Gln347Ter	Frequent feedings Glycosade (1.6 g/kg/day)
006	53.1	F	27.3	c.1039C>T c.247C>T	p.Gln347Ter p.Arg83Cys	Frequent feedings Glycosade (2.4 g/kg/day)
007	22.7	M	29.5	c.1039C>T c.247C>T	p.Gln347Ter P.Arg83Cys	Frequent feedings Glycosade (2.1 g/kg/day) CNGDF
009	18.0	F	24.5	c.562G>A c.508C>T	p.Gly188Arg p.Arg170Ter	Frequent feedings CNGDF
014	26.9	F	25.6	c.247C>T c.187T>C	p.Arg83Cys p.Trp63Arg	Frequent feedings Glycosade (3.2 g/kg/day)
015	19.3	M	23.0	c.247C>T c.187T>C	p.Arg83Cys p.Trp63Arg	Frequent feedings Glycosade (3.1 g/kg/day)
017	18.3	M	26.6	c.247C>T c.866G>A	p.Arg83Cys p.Ser289Asn	Frequent feedings UCCS (1.9 g/kg/day) CNGDF
020	48.3	M	26.9	c.809G>T c.1039C>T	p.Gly270Val p.Gln347Ter	Frequent feedings UCCS+Glycosade (2.3 g/kg/day)
Healthy volunteers	22.4 (17.1-50.8)[1]	5 M 5 F	23.2 (19.8-28.9)[1]	------	-------	----------

Table 1. Clinical and molecular characteristics of the study participants.
UCCS: uncooked cornstarch; CNGDF: continuous nocturnal gastric drip-feeding; N.A.: not available
[1]median and range are shown

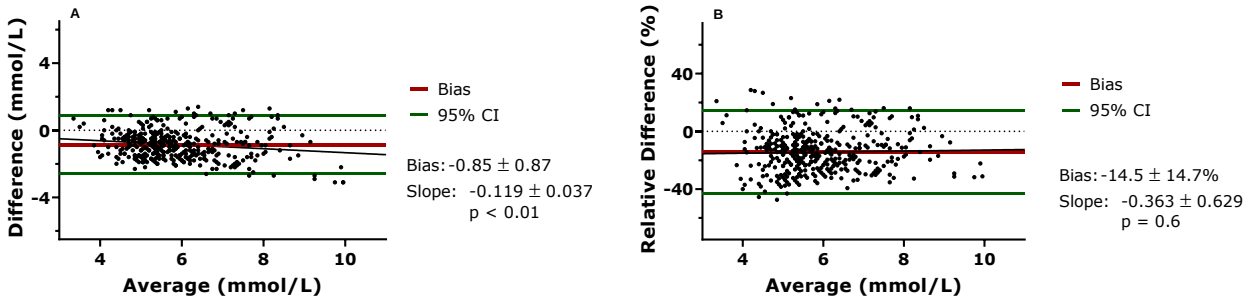


Figure 1. Bland-Altman plots. Difference between CGM values and CBG measured by the Freestyle freedom Lite (Abbott, Chicago, Illinois) device, expressed as absolute (A, in mmol/L) and relative (B, in %) values. Data were collected under pre-prandial/fasted [n=200 (i.e. 10 time points x 20 participants)] and fed [(n=200 (i.e. 10 time points x 20 participants)] conditions. Y-axis shows the absolute (A) or relative (B) difference between CBG and CGM values at each study time point. X-axis shows the average glucose value at each study time point.

Descriptive measures

Descriptive outcomes are presented in Table 2. Mean 24-hour CGM values for GSDIa patients and healthy volunteers were 6.1 mmol/L (95% CI: 5.2 – 7.1; n = 25,504) and 5.9 mmol/L (95% CI: 5.2 – 6.6; n = 27,153), respectively. Mean 24-hour glucose SD, glucose variance and glucose CV were significantly higher in GSDIa patients compared to healthy volunteers. Between 01:00-05:00 a.m. (i.e. "overnight"), mean values for maximum CGM values, glucose SD and glucose CV were significantly higher in GSDIa patients compared to healthy volunteers.

TBR, TIR and TAR are presented in Table 3. Lever 3 hypoglycaemia was not observed, while level 2 hypoglycaemia (i.e., glucose values ≥ 3.0 < 3.9 mmol/L) occurred in 6/10 GSDIa patients (of whom 5/6 patients for <1% of the recorded time) during the 24-hour and in 1/10 GSDIa patients during the overnight timeframe. Mean 24-hour TBR (glucose values ≥3.0 mmol/L and < 3.9 mmol/L) and 24-hour TAR (glucose values > 10.0 mmol/L) were higher while the mean 24-hour TIR (glucose values ≥ 3.9 and ≤10.0 mmol/L) was lower in GSDIa patients compared to healthy volunteers. Overnight TIR (glucose values ≥ 3.9 and ≤10.0 mmol/L) was lower, and TAR (glucose values > 10.0 mmol/L) was higher in GSDIa patients compared to healthy volunteers.

CGM glucose course

Overnight (01.00-05.00 a.m.) CGM time courses are shown in Figure 2. During two night-time intervals (i.e. 02.15-03.00 a.m. and 04.00-04.30 a.m.), CGM values were significantly higher in GSDIa patients compared to healthy volunteers.

Participant	Time frame	Time points	Days/ nights	Descriptive outcomes			Glycaemic variability (GV)		
				Median	Min	Max	SD	Variance	CV
		n	n	mmol/L	mmol/L	mmol/L	mmol/L	mmol²/L²	%
001	24-hour	2,845	10	6.2	2.8	11.0	1.4	1.9	21.3
	01:00-05:00 a.m.	480	10	7.2	4.9	11.0	1.1	1.3	15.7
002	24-hour	1,206	4	5.4	2.2	9.3	1.2	1.4	21.5
	01:00-05:00 a.m.	192	4	5.0	3.3	7.7	0.8	0.7	16.6
004	24-hour	2,802	10	5.4	2.4	9.2	1.0	1.0	17.9
	01:00-05:00 a.m.	480	10	5.4	3.7	8.2	0.7	0.5	13.1
006	24-hour	2,612	9	6.7	2.8	17.0	1.7	2.8	24.7
	01:00-05:00 a.m.	437	9	6.8	3.7	10.0	1.2	1.3	16.8
007[#]	24-hour	2,207	8	7.6	3.7	13.0	1.5	2.2	19.2
	01:00-05:00 a.m.	384	8	8.0	4.6	12.4	1.7	2.8	20.6
009[#]	24-hour	2,851	10	5.4	3.0	10.4	1.3	1.6	22.2
	01:00-05:00 a.m.	480	10	5.1	3.7	7.0	0.6	0.3	11.5
014	24-hour	2,480	9	5.6	2.7	13.8	1.7	2.9	28.5
	01:00-05:00 a.m.	432	9	5.0	3.2	10.0	1.4	1.9	25.3
015	24-hour	2,856	10	5.7	2.2	13.9	1.9	3.5	32.0
	01:00-05:00 a.m.	490	10	4.9	2.4	8.9	1.6	2.6	30.6
017[#]	24-hour	2,713	10	5.7	2.2	10.0	1.1	1.2	19.0
	01:00-05:00 a.m.	480	10	5.9	3.2	8.3	0.9	0.8	15.0
020	24-hour	2,932	10	5.7	3.2	9.7	1.0	0.9	16.7
	01:00-05:00 a.m.	461	10	5.9	3.4	8.2	0.8	0.7	14.0
GSD Ia[1]	24-hour	25,504	90	5.9 (5.5-6.4)	2.2 (2.4-3.0)	17.0 (10.1-13.3)	1.6 (1.2-1.6)*	2.5 (1.4-2.5)*	25.8 (20.0-24.6)*
	01:00-05 a.m.	4,264	90	5.8 (5.3-6.6)	2.4 (3.2-4.1)	12.4 (8.1-10.3)*	1.5 (0.8-1.3)*	2.2 (0.7-1.9)	24.4 (14.2-21.6)*
Healthy volunteers[1]	24-hour	27,153	96	5.7 (5.4-6.0)	2.2 (2.4-3.2)	12.0 (9.9-10.9)	1.1 (0.9-1.1)	1.2 (0.8-1.1)	18.9 (15.8-17.2)
	01:00-05:00 a.m.	4,616	96	5.5 (5.3-6.0)	2.4 (3.1-4.3)	10.1 (6.6-8.0)	0.9 (0.4-0.8)	0.8 (0.1-0.8)	16.0 (8.2-13.2)

Table 2. Continuous glucose monitoring (CGM) median, minimum, maximum glucose values and outcomes of glycaemic variability in the study participants.

*SD: standard deviation; CV: coefficient of variation; [1] For descriptive and GV measures mean and 95%CI (in brackets) are shown. *Significant difference between GSDIa patients and healthy volunteers. [#]GSDIa patients receiving CNGDF.*

Participant	Time frame	Time points (n)	Days/ nights (n)	TBR (%)		TIR (%)		TAR (%)	
				< 3.0 mmol/L	≥ 3.0 < 3.9 mmol/L	≥ 3.9 ≤ 7.8 mmol/L	≥ 3.9 ≤ 10.0 mmol/L	> 7.8 mmol/L	> 10.0 mmol/L
001	24-hour	2,845	10	0.1	1.1	82.1	97.9	16.7	1.0
	01:00-05:00 a.m.	480	10	0.0	0.0	76.9	96.9	23.1	3.1
002	24-hour	1,206	4	0.6	5.1	89.6	94.3	4.7	0.0
	01:00-05:00 a.m.	192	4	0.0	4.2	94.8	94.8	0.0	0.0
004	24-hour	2,802	10	0.4	2.1	95.8	97.5	1.7	0.0
	01:00-05:00 a.m.	480	10	0.0	0.4	98.1	98.8	0.6	0.0
006	24-hour	2,612	9	0.0	1.0	80.9	95.6	17.8	3.2
	01:00-05:00 a.m.	437	9	0.0	0.5	83.1	98.2	15.1	0.0
007[#]	24-hour	2,207	8	0.0	0.1	56.8	92.5	42.7	7.1
	01:00-05:00 a.m.	384	8	0.0	0.0	44.3	86.2	53.6	11.7
009[#]	24-hour	2,851	10	0.0	3.8	89.3	95.7	6.6	0.2
	01:00-05:00 a.m.	480	10	0.0	1.0	96.9	96.9	0.0	0.0
014	24-hour	2,480	9	0.2	4.4	79.9	92.3	15.0	2.6
	01:00-05:00 a.m.	432	9	0.0	2.8	88.4	94.4	6.0	0.0
015	24-hour	2,856	10	1.8	10.7	73.6	84.1	13.3	2.8
	01:00-05:00 a.m.	490	10	8.8	12.2	72.9	76.1	3.3	0.0
017[#]	24-hour	2,713	10	0.9	1.9	92.8	96.6	3.9	0.0
	01:00-05:00 a.m.	480	10	0.0	1.0	93.8	95.6	1.9	0.0
020	24-hour	2,932	10	0.0	1.0	96.1	99.0	2.9	0.0
	01:00-05:00 a.m.	461	10	0.0	0.4	98.3	99.6	1.3	0.0
GSD Ia[1]	24-hour	25,504	90	0.5 (0.0-0.8)	3.4 (1.2-5.0)*	82.6 (76.3-91.1)	94.2 (91.8-97.2)*	13.5 (4.5-20.1)	1.9 (0.3-3.1)*
	01:00-05 a.m.	4,264	90	1.0 (0.0-2.6)	2.2 (0.0-4.7)	86.1 (74.4-95.2)	95.4 (89.3-98.3)*	10.7 (0.0-21.0)	1.4 (0.9-6.1)*
Healthy volunteers[1]	24-hour	27,153	96	0.2 (0.0-0.2)	0.7 (0.3-1.1)	92.6 (89.4-96.6)	98.8 (98.6-99.4)	6.4 (2.2-10.2)	0.2 (0.1-0.3)
	01:00-05:00 a.m.	4,616	96	0.1 (0.0-0.1)	0.6 (0.0-1.4)	95.9 (90.5-100)	99.3 (98.5-100)	3.3 (0.0-8.8)	0.0 (0.0-0.0)

Table 3. Outcomes of glycaemic control in the study participants.

*TBR: time below range; TIR: time in range; TAR; time above range. [1]For TBR, TIR and TAR mean and 95% CI (in brackets) are shown. *Significant difference between GSDIa patients and healthy volunteers. [#]GSDIa patients receiving CNGDF.*

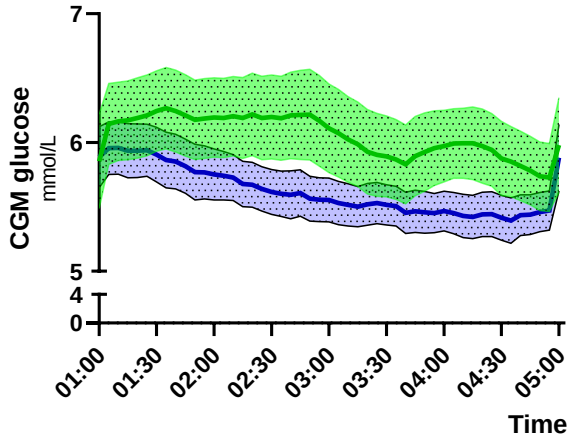


Figure 2. Overnight (01:00 a.m.-05:00 a.m.) CGM values in all GSDIa patients (green) and healthy volunteers (blue). *Mean (thick line) and 95%CI (shaded area) are shown.*

Single patient analysis

After comparing the CGM parameters between GSDIa patients and healthy volunteers we questioned whether extreme, individual GSDIa patients' CGM outcomes would be associated with extremes in the traditional biomedical markers of metabolic control. Based on descriptive measures and outcomes of glycaemic control, three GSDIa patients were identified who remarkably deviated within the patients' cohort (Table 2 and 3). Participant 007 (compound heterozygote for the c.1039C>T and c.247C>T variants) showed higher median and minimum glucose levels, lower TIR and higher TAR. Participant 015 (compound heterozygote for the c.247C>T and c.187T>C variants) showed higher GV and TBR and lower TIR. Participant 020 (compound heterozygote for the c.809G>T and c.1039C>T variants) showed lower GV, TBR and TAR and higher TIR. The targets for traditional biomedical markers and diet characteristics were met in these 3 patients (table 4). Comparison of the overnight CGM curves and advanced CGM outcome parameters with those from the other GSDIa patients revealed a remarkable divergence of participant 007 from the 95%CI of the other GSDIa patients (Figure 3), while the CGM curves from participant 015 and participant 020 showed large overlap with those from the other GSDIa patients (data not shown).

Participant	Preprandial capillary glucose (mmol/L)	BMI (SDS)	TG (mmol/L)	UA (mmol/L) (Ref 0.20-0.45)	UCCS (g/kg/6 hours)	Interval between UCCS doses (hours)/24h	CNGDF (%daily TEI)
Reference target *Rake et al 2002*[14] *Kishnani et al. 2014*[3]	> 3.5-4.0 > 4.0	0.0/+2.0	< 6.0	High normal range	1.5-2.0 1.7-2.5	4-6	25-30
007	3.7-6.3	+1.5	3.4	0.52	1.0 Glycosade	3-4 during the day	33 (*CH: 25 g/hour= 4 mg/kg/min)*
015	3.6-5.7	+0.1	3.3	0.37	1.0 Glycosade	6-7 during the day and night	n.a
020	3.8-4.7	+1.0	4.2	0.18	1.1 UCCS Glycosade	3 hours during the day 7 hours in the night	n.a.

Table 4. Traditional biomarkers and dietary information for participants 007, 015 and 020.
SDS: standard deviation score, TG: triglycerides, UA: uric acid, UCCS: uncooked cornstarch; CNGDF: continuous nocturnal gastric drip feeding; TEI: total energy intake; CH: carbohydrates; n.a.: not applicable

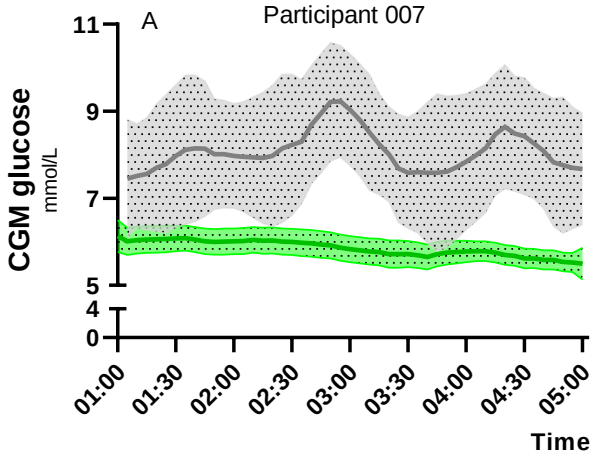
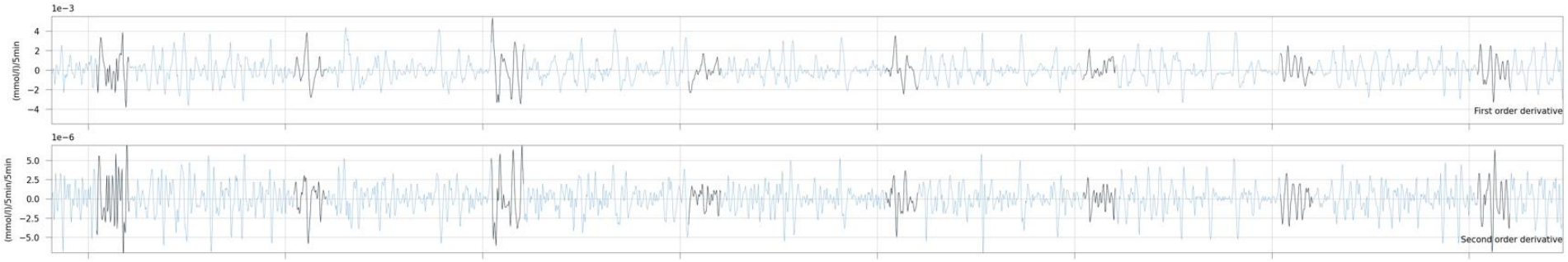


Figure 3. Overnight continuous glucose monitoring (CGM) course in participant 007 (grey), compared to the average CGM values of the remaining nine GSDIa patients (green). Mean (thick line) and 95%CI (shaded area) are shown.

Assessment of the first and second order derivatives curves showed that the fluctuations in CGM values (1st order derivative) and the speed of such fluctuations (2nd order derivative) were higher in participants 007 and 015, compared to their matched healthy volunteers. Conversely, both curves showed overlapping trends when comparing participant 020 (GSDIa patient) and 018 (matched healthy volunteer) (Figure 4). The Fourier analysis showed overall comparable number of frequencies (number of cycles per day) but higher amplitude in all 3 GSDIa patients compared to their matched healthy volunteer. Remarkably deviant patterns on specific days (compared to the other days) were noted in healthy volunteer, while figures appeared more standardised in each single patient. Still, wide differences among patients could be noted. A pattern with limited number of frequencies (indicating more stable glucose concentrations) was noted on 5/10 days in participant 020, 3/10 days in participant 015 and 1/8 days in participant 007 (Figure 5).

007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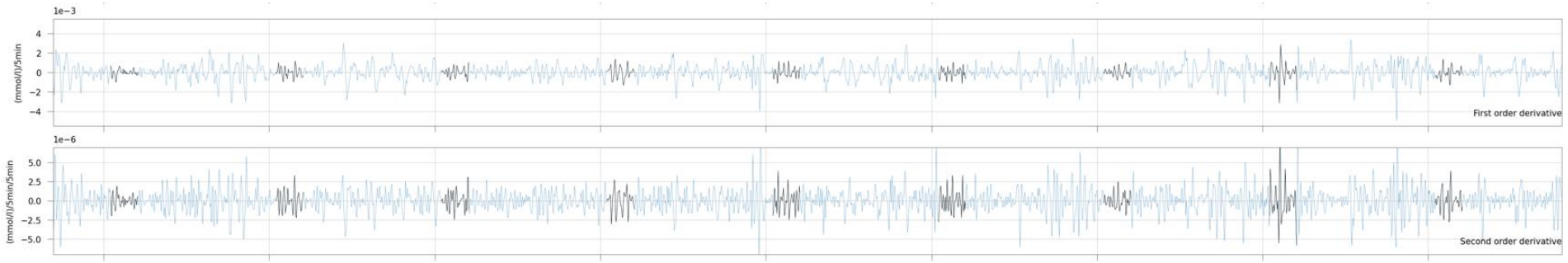
Second order derivative

012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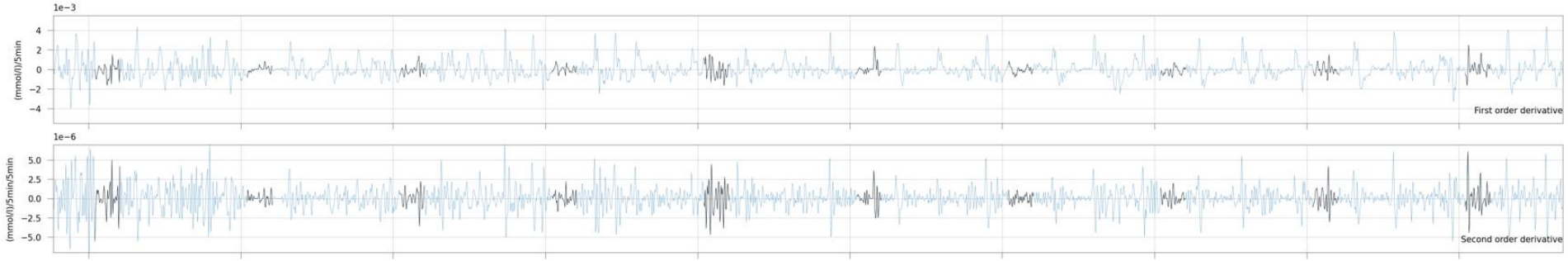
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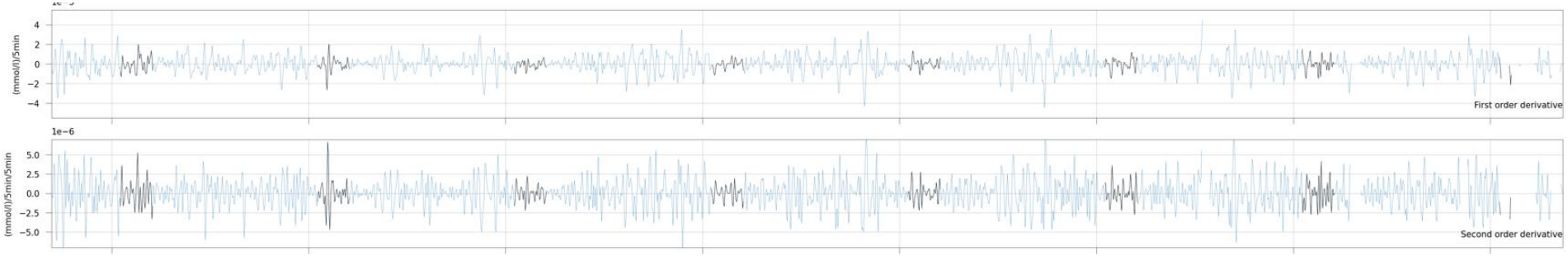
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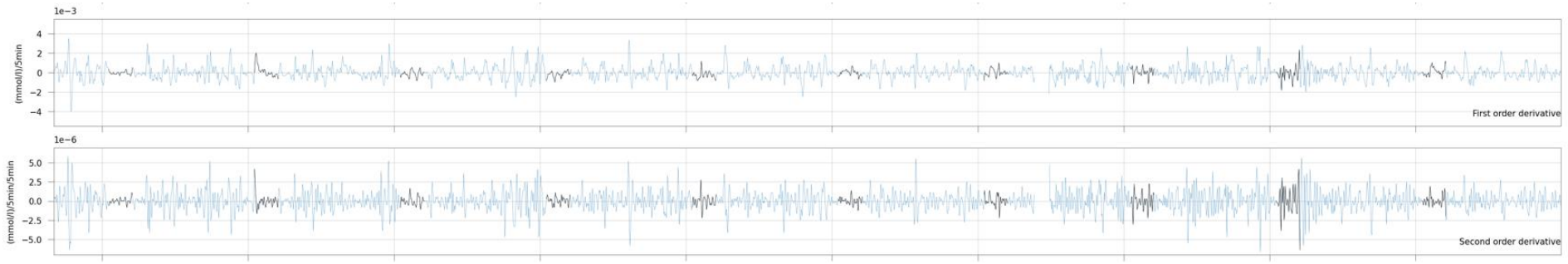
020



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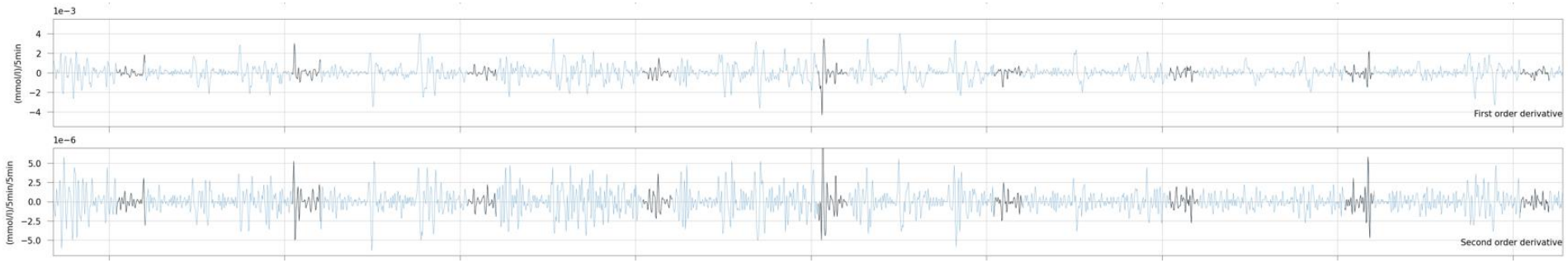
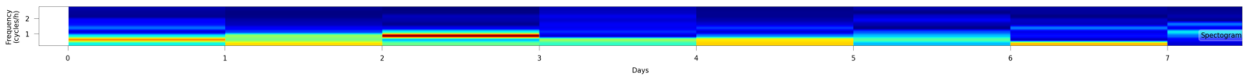


Figure 4. First and second order derivatives calculated on the 24-hour continuous glucose monitoring (CGM) values in participants 007, 015 and 020 and their matched healthy volunteers (012, 016 and 018, respectively). The y-axis shows the change of CGM values (mmol/l) within the time unit (5 minutes) (1st order derivative) or the speed of the change of CGM values (mmol/l/5minutes) within the time unit (5 minutes) (2nd order derivative). Data collected between 01:00 and 05:00 am are shown in dark blue.

007



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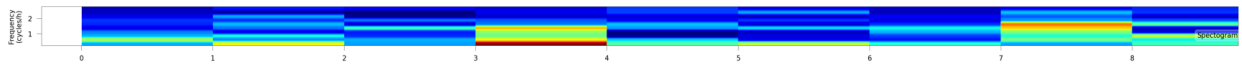

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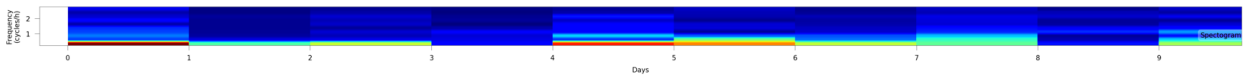

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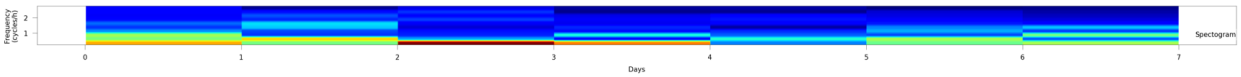

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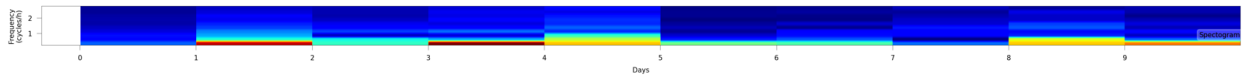

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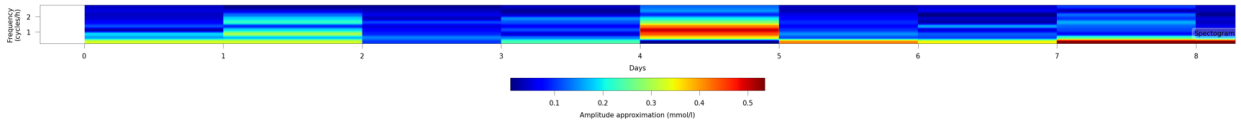

Figure 5. Fourier analysis spectrogram derived from the 24-hour continuous glucose monitoring (CGM) profiles in participants 007, 015 and 020 and their matched healthy volunteers (012, 016 and 018, respectively). The y-axis shows the frequency of the sinusoidal CGM pattern in cycles per hour. The colour shows the amplitude of the frequency (waterfall plot JOT colour scheme).

DISCUSSION

This is the first prospective study to provide CGM reference data by comparing adult GSDIa patients and matched healthy volunteers. No GSDIa patient showed level 3 hypoglycaemia in the present study. Compared to healthy volunteers, the 24-hour CGM profiles of GSDIa patients revealed significantly more time spent in level 1 hypoglycaemia, more time spent in "above-range (> 10.0 mmol/L) glucose and less time "in-range" (glucose values ≥ 3.9 and ≤10.0 mmol/L), whereas glycaemic variability was increased. Overnight CGM values of GSDIa patients were higher compared to healthy controls and, in contrast to the 24-hour measurement, 9/10 GSDIa patients did not demonstrate level 2 hypoglycaemia between 01.00-05.00 a.m.

CGM-derived outcome parameters are increasingly recognised for monitoring of DM patients[15,16] and can also guide dietary changes and/or medication adjustments[17]. Previous studies demonstrated the feasibility of CGM in GSD patients, and evidence on the benefit of CGM use in patients with hepatic GSD is accumulating[8-10,18-20]. Monitoring glucose levels at home, alarming tools, and real-time CGM data sharing with caregivers and healthcare professionals significantly improves hypoglycaemia awareness and allows for treatment optimisation and definition of reference values in specific hepatic GSD cohorts[10,19,20]. However, the definition of CGM outcome parameters (such as the time spent in the "low-range"), the type of devices used, the duration of monitoring, and the study population characteristics varied among the previous studies in hepatic GSD patients. Those studies showed that historical CGM results can be used as a reference for monitoring individual hepatic GSD patients, before and after therapeutic interventions[9,10,18,19]. The current study adds to this by demonstrating that individual GSDIa patients' CGM outcomes can be compared to reference values obtained from a GSDIa reference population as well as a to a group of matched healthy volunteers. It should be taken into account that factors such as dietary intake, exercise, and medication, may influence glucose homeostasis. To ensure proper interpretation of CGM results, we therefore suggest to separately analyse and compare CGM data collected during day- and night-time.

Circulating glucose concentrations are important biomarkers to monitor the hepatic GSD disease course and (over)treatment of GSDIa[21]. Glycaemic variability is an independent risk factor for macro- and micro-vascular complications in DM[22]. A wide glycaemic variability correlates with the risk of developing cardiovascular and microvascular complications[23]. The latter includes DM-related kidney disease[24], which shares a common pathogenesis with GSDIa-related renal disease[25]. By means of CGM, Kasapkara and co-workers demonstrated that the decreased number of hypoglycaemic events following a dietary intervention in GSDI patients was associated with a reduction in liver size and improvement of multiple disease biomarkers[9]. In a cohort of 14 GSDI patients monitored by CGM, Kaiser and co-workers showed that an increased number of time spent below 4 mmol/L (CGM levels) and more daily hypoglycaemic events were associated with the presence of liver adenomas or microalbuminuria in GSDI patients[20], suggesting a prognostic role for CGM-derived parameters. Yet, the predictive value of CGM monitoring in GSDIa patients has not been fully established, and future studies are therefore warranted.

TBR, TIR and TAR are established CGM-derived prognostic markers in DM[7]. Higher TIR is associated with better outcomes in patients with DM[26]. TAR directly correlates with glycosylated haemoglobin (HbA1c) levels while TBR inversely correlates with HbA1c variability[27]. In the present study GSDIa patients demonstrated lower TIR and higher TBR and TAR compared to healthy

volunteers. On the one hand these parameters may reflect the higher glycaemic variability. On the other hand, they may be attributed to other factors, such as counterregulatory response to hypoglycaemia, disproportionate carbohydrate intake and/or imbalanced meal/UCCS schedule). The correlation between CGM results and dietary intake was not an objective of present study. To validate the role of CGM-derived outcomes in optimising the dietary management in GSD patients follow up studies investigating the major determinants of CGM-derived parameters are warranted

Analysis of descriptive CGM outcome parameters allowed to identify three GSDIa patients who remarkably differed from the patient and healthy volunteer reference populations. The Fourier analysis also revealed clearly different patterns when comparing each of the three participants with his/her matched healthy volunteers. To assess the reliability of novel CGM-derived parameters, information on the traditional biochemical and dietary targets was collected. In this small sample size study, most of the traditional biomedical targets were met in these three GSDIa patients, not allowing any major differentiation among them. It can be questioned whether longer duration of CGM would enable further assessment of the correlation between CGM outcome parameters and traditional biomedical outcomes.

The present study showed an overall agreement between CBG and CGM values. Previous work has shown satisfactory agreement between CBG and CGM values in hepatic GSD patients, in whom MiniMed (Medtronic) and Dexcom G4 Platinum (Dexcom) devices have been used, respectively[9,19]. However, smaller mean differences between the CBG and the CGM values were found in those previous studies compared to the present study (0.20-0.23 mmol/L vs 0.85 mmol/L). We hypothesise that the larger differences in CBG and CGM are related to the postprandial sampling in the current study.

Several limitations of this study need to be addressed. First, a relatively small number of GSDIa patients were studied. Therefore, it is unclear whether the sample size can adequately reflect the large clinical and biochemical heterogeneity observed in GSDIa patients[4]. Second the number of measurements which are minimally required to obtain a reliable CGM profile in GSDIa patients remains to be established. In DM patients fourteen-day data collection is recommended to adequately predict glycaemic variability over a 3-month period[28]. The setup of the current study did not allow to establish a similar timeframe for GSDIa patients. Third, additional factors (e.g., dietary intake, physical activity, emotional stress) that are known to affect glucose concentrations were not systematically recorded in the present study. Fourth per manufacturer instructions, the Dexcom G6 does not require calibration[29]. Yet, it is possible that the instrument's accuracy varies within the initial days after sensor insertion. Fifth, although participants with a BMI>30 were excluded from the present study, the amount of subcutaneous fat may impact on the equilibrium between interstitial glucose concentrations and the blood compartment, affecting the CGM accuracy in participants with relatively large subcutaneous fat depots[30]. Finally, it should be considered that not all CGM parameters can be directly derived from the Dexcom CLARITY Clinical Portal but require additional data processing, potentially limiting their immediate use.

Clinical studies in hepatic GSD patients have traditionally employed multiple outcome parameters to assess clinical efficacy, such as blood glucose levels such as glycaemic responses during invasive *in vivo* starch load tests (NCT02318966) or controlled fasting challenges (NCT03517085). The development of CGM-derived outcome parameters is particularly relevant as clinical trials with novel

medical[5] (NCT03517085) and dietary treatments (NCT02318966) are currently being performed. The results of the present study further support the application of CGM as a (additional) monitoring tool in both regular healthcare and clinical research/trials settings. Future studies may address the application of CGM as an educational tool to detect hypoglycaemia unawareness, for example by integrating the CGM data with a diary on disease symptoms. CGM-derived algorithms allow for hypoglycaemia prediction, detection and prevention of (medical/dietary) under-/over-treatment and unrecognised hypoglycaemia in DM patients[31]. The development of GSD-specific algorithms through machine learning approaches offers opportunities to increase GSDIa patients' safety by early warning, and further improvement of (self)management.

In summary, availability of CGM reference data from GSDIa patients and healthy volunteers can improve the individual GSDIa patient's monitoring. Ideally, individual GSDIa patients' CGM parameters could be compared with (1) the patient's historical CGM data as well as CGM data from both (2) a matched patients' cohort and (3) matched healthy volunteers. For a detailed interpretation of CGM results, CGM data from daytime and night-time should be analysed separately.

FUNDING

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ACKNOWLEDGEMENTS

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Chapter 3.

Imbalanced cortisol concentrations in glycogen storage disease type I: evidence for a possible link between endocrine regulation and metabolic derangement

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ABSTRACT

Background: Glycogen storage disease type I (GSDI) is an inborn error of carbohydrate metabolism caused by mutations of either the G6PC gene (GSDIa) or the SLC37A4 gene (GSDIb). Glucose 6-phosphate (G6P) availability has been shown to modulate 11β-hydroxysteroid dehydrogenase type 1 (11βHSD1), an ER-bound enzyme catalyzing the local conversion of inactive cortisone into active cortisol. Adrenal cortex assessment has never been performed in GSDI. The aim of the current study was to evaluate the adrenal cortex hormones levels in GSDI patients.

Methods: Seventeen GSDI (10 GSDIa and 7 GSDIb) patients and thirty-four age and sex-matched controls were enrolled. Baseline adrenal cortex hormones and biochemical markers of metabolic control serum levels were analyzed. Low dose ACTH stimulation test was also performed.

Results: Baseline cortisol serum levels were higher in GSDIa patients ($p = 0.042$) and lower in GSDIb patients ($p = 0.041$) than controls. GSDIa patients also showed higher peak cortisol response ($p = 0.000$) and Cortisol AUC ($p = 0.029$). In GSDIa patients, serum cholesterol ($p = 0.000$), triglycerides ($p = 0.000$), lactate ($p = 0.000$) and uric acid ($p = 0.008$) levels were higher and bicarbonate ($p = 0.000$) levels were lower than controls. In GSDIb patients, serum cholesterol levels ($p = 0.016$) were lower and lactate ($p = 0.000$) and uric acid ($p = 0.000$) levels were higher than controls. Baseline cortisol serum levels directly correlated with cholesterol ($\rho = 0.65$, $p = 0.005$) and triglycerides ($\rho = 0.60$, $p = 0.012$) serum levels in GSDI patients.

Conclusions: The present study showed impaired cortisol levels in GSDI patients, with opposite trend between GSDIa and GSDIb. The otherwise preserved adrenal cortex function suggests that this finding might be secondary to local deregulation rather than hypothalamo-pituitary-adrenal axis dysfunction in GSDI patients. We hypothesize that 11βHSD1 might represent the link between endocrine regulation and metabolic derangement in GSDI, constituting new potential therapeutic target in GSDI patients.

BACKGROUND

Glycogen storage disease type I (GSDI) is an inborn disorder of carbohydrate metabolism caused by the deficiency of microsomal glucose-6-phosphatase (G6Pase) system. It is characterized by accumulation of glycogen and fat in the liver and kidneys. Two major subtypes of GSDI have been identified: GSDIa, which is caused by mutations in the gene encoding the G6Pase alpha (G6Paseα), and GSDIb, caused by mutations in the gene encoding the glucose 6-phosphate (G6P) translocase (G6PT), which transports G6P from cytoplasm to microsomes. G6Paseα is expressed in the liver, kidney and intestine, whereas G6PT is ubiquitous. The clinical and biochemical phenotype of GSDI includes fasting hypoglycaemia, hepatomegaly, lactic acidosis, hypertriglyceridemia, hypercholesterolemia and hyperuricemia; GSDIb is also associated with neutropenia and neutrophil dysfunction, resulting in recurrent infections and predisposition to inflammatory bowel disease (IBD)[1]. G6P availability has been shown to modulate 11βhydroxysteroid dehydrogenase type 1 (11βHSD1) activity. In GSDIa, the G6P excess in the endoplasmic reticulum (ER) (due to G6Paseα deficiency) has been associated to increased 11βHSD1 activity, while in GSDIb the lack of G6P in ER (due to G6PT deficiency) has been associated to decreased 11βHSD1 activity[2]. 11βHSD1 is an ER-bound enzyme catalyzing the conversion of inactive cortisone in active cortisol. It is typically expressed in glucocorticoid receptor-rich tissues, such as the liver, adipose tissue, lung and brain[3]. 11βHSD1 requires NADPH as a cofactor generated by the hexose-6-phosphate dehydrogenase (H6PDH)-mediated conversion of G6P to 6-phosphogluconactone (6PGL)[4]. The accumulation of G6P in ER fuels the G6PT-H6PDH-11βHSD1 system, leading to increased pre-receptorial activation of glucocorticoids[5]. Therefore, the G6PT-H6PDH-11βHSD1 system is crucial in the coupling between glucose metabolism and glucocorticoid response (see Fig. 1). Interestingly, in H6PDH knock-out mice decreased negative feedback on the hypothalamo-pituitary-adrenal (HPA) axis has been observed[6]. Although an inverse correlation between serum cortisol concentrations and weight SDS has been demonstrated[7,8], adrenal cortex assessment has never been performed in GSDI patients. The aim of the current study was to evaluate adrenal cortex function in GSDI patients unveiling possible differences between GSDIa and GSDIb patients.

METHODS

Subjects

The study protocol was in accordance with the Italian regulations on privacy protection and with the Helsinki Doctrine for Human Experimentation. All studies were performed after informed consent was obtained from adult subjects or the infants' parents. Patients were recruited over a 12 months period. Seventeen GSDI patients (6 males and 11 females) were enrolled. Ten GSDIa patients (4 males and 6 females, mean age 12.11 ± 1.52, range 6–20 years) were compared to 20 age and sex matched controls. Seven GSDIb patients (2 males and 5 females, median age 14.90 ± 2.25, range 8–23 years) were compared to 14 age and sex matched controls. The diagnosis of GSDIa and GSDIb was based on mutation analysis of the G6PC and SLC37A4 gene, respectively. All patients were on dietary treatment. Each patient received uncooked cornstarch (UCCS), nocturnal gastric drip feeding (CNGF) or a combination of the two. Dietary regimens varied among different patients according to their families' requests and attitudes. Thirty-four subjects with normal random blood glucose and no

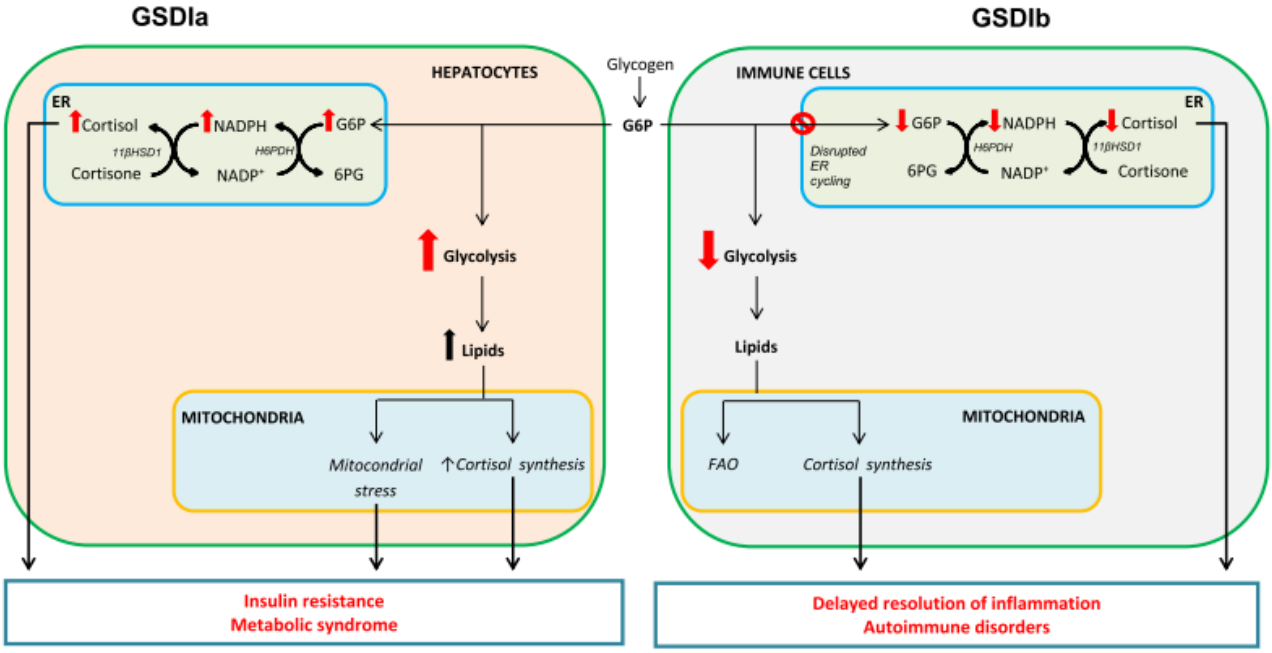

Figure 1. Proposed pathomechanism linking endocrine regulation and metabolic imbalance in GSDI. In GSDIa G6P accumulates in both cytosol and ER within the hepatocytes. Increased G6P availability in the ER upregulates 11βHSD1 activity resulting in increased cortisol regeneration. Increased G6P in the cytosol enhances glycolysis and lipid load to mitochondria resulting in mitochondrial stress and increased cortisol synthesis (secondary to increased substrate availability). Together, these secondary metabolic disturbances lead to increased risk of insulin-resistance and metabolic syndrome. In GSDIb G6PT defect results in disrupted ER cycling in immune cells (e.g., neutrophils, lymphocytes) and subsequently decreased cortisol regeneration with the ER and potentially reduced substrates to mitochondria for cortisol synthesis. Reduced cortisol availability might contribute to chronic inflammation and higher risk for autoimmune disorders. *G6P: glucose 6-phosphate, 6PG:6-phosphogluconactone, 11βHSD1:11β-hydroxysteroid dehydrogenase type 1, H6PDH: hexose-6-phosphate dehydrogenase, FAO: fatty acid oxidation*

history of hypoglycemia were included as healthy control participants. Each GSDIa or GSDIb patient was compared to two age and sex-matched controls.

Clinical and biochemical parameters

The following clinical parameters were recorded: height, weight, body mass index (BMI), systolic and diastolic blood pressure (BP). Blood samples were obtained at 8 a.m. Fasting time ranged between 4 and 9 h. This was calculated according to patients' usual fasting tolerance. 16/17 patients showed fasting tolerance between 4 and 6 h. One adult patient showed fasting tolerance of 9 h. To overcome the bias due to patients' short fasting time the control subjects were asked to have blood and urine sampling after the same fasting time of his/her age and sex matched patient. Serum glucose, cholesterol, triglycerides (TG), lactate, uric acid and bicarbonate were assessed as markers of metabolic control. In order to control for possible interaction of cholesterol with triglycerides, Corrected Cholesterol (CChol) was also calculated as following: Cholesterol – (TG/5)[9].

Hormonal studies

Fasting blood samples were obtained at 8 a.m. HPA axis function was assessed by evaluating adrenocorticotropic hormone (ACTH), cortisol, androstenedione, 17- hydroxyprogesterone (17OHP), dehydroepiandrosterone sulphate (DHEAS), renin, aldosterone serum levels as well as and 24-h Urinary Free Cortisol (UFC) levels using routine assays with commercially available kits. Cortisol, DEHAS, androstenedione, 17OHP were evaluated at baseline and after a low dose ACTH stimulation test using 1 μg Synacthen® (synthetic ACTH analogue). The timing of the ACTH stimulation test was arranged in order not to exceed patients fasting tolerance.

Statistical analysis

"Peak cortisol" was defined as the maximum observed cortisol value measured following ACTH administration regardless of when it occurred. Area under the curve (AUC) was calculated by trapezoid formula. All data in the text or shown in the figures are expressed as mean ± SE. Statistical analysis was performed using Statistical Package for Social Science (SPSS 10 for Windows Update; SPSS Inc., Chicago, Illinois, USA). The comparisons between numerical variables were performed by Student's t-test corrected for Fisher's exact test. The normality of the distribution was checked by the Shapiro–Wilk test. One-way ANCOVA with Bonferroni adjusted post hoc tests analysis was performed to control cortisol concentrations for covariates (cholesterol, triglycerides and CChol). Correlation study was performed by Spearman's rank correlation. Cholesterol, TG and CChol were further assessed in multivariable linear regression analysis. The predictive capability of the multivariable regression model was checked by the F-test. Statistical significance was set at $p < 0.05$.

RESULTS

Clinical and biochemical parameters (Table 1 and Additional file 1)

GSDIa patients showed increased cholesterol ($p = 0.000$), TG ($p = 0.000$), lactate ($p = 0.000$) and uric acid ($p = 0.008$) serum levels and reduced bicarbonate serum levels ($p = 0.000$) compared to controls. GSDIb patients showed reduced cholesterol ($p = 0.016$), CChol ($p = 0.010$) and bicarbonate ($p = 0.002$) serum levels and increased lactate ($p = 0.000$) and uric acid ($p = 0.000$) serum levels ($p = 0.002$) compared to controls. GSDIb patients showed lower height ($p = 0.040$) and height centile ($p = 0.002$) and weight centile ($p = 0.030$) than controls. Glucose concentrations ranged 4.4–7.8 mmol/L in GSDIa patients and 4.0–8.1 mmol/L in GSDIb patients (Additional file 1A). No significant difference in the remaining parameters was observed between GSDIa and GSDIb patients and controls.

Hormonal studies

Baseline serum hormone levels and UFC are shown in Table 2 and Additional file 1. Serum cortisol levels were higher in GSDIa patients ($p = 0.042$, Fig. 2a) and lower in GSDIb patients ($p = 0.041$, Fig. 2b) than controls. GSDIa patients showed higher 60 min ($p = 0.019$, Fig. 2a) and 90 min ($p = 0.000$, Fig. 2a) cortisol levels after ACTH stimulation and higher peak cortisol response ($p = 0.000$, Fig. 2c) as well as cortisol area under the curve (AUC) (21,536 ± 884 vs 18,716 ± 764, $p = 0.029$) than controls. No significant difference in the remaining serum hormone levels, AUC and UFC were

observed between GSDIa or GSDIb patients and controls. After controlling for covariates, no significant difference in 30 min and 60 min cortisol levels was observed between patients and controls (GSDIa: $p = 0.645$, GSDIb: $p = 0.850$); 90 min cortisol levels were significantly higher in GSDIa patients than controls ($p = 0.007$). Correlation study Baseline cortisol serum levels directly correlated with cholesterol ($\rho = 0.65$, $p = 0.005$) and TG ($\rho = 0.60$, $p = 0.007$).

	GSDIa		**Controls**		**GSDIb**		**Controls**			
	Mean	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Ia vs C**	**Ib vs C**
Age (years)	12.10	1.52	11.90	1.00	14.90	2.25	15.18	1.59	0.909	0.922
Fasting time (hours)	5.20	0.46	5.65	0.27	5.29	0.20	5.79	0.24	0.418	0.200
Height (cm)	139.00	5.80	144.70	4.00	143.00	4.00	155.00	3.40	0.420	**0.040**
Height (centile)	20.10	9.00	40.80	4.90	20.70	7.50	56.80	4.40	0.080	**0.002**
Weight (Kg)	46.80	6.60	49.70	4.40	54.90	7.40	61.70	5.20	0.710	0.460
Weight (centile)	68.00	7.70	72.00	4.10	75.70	6.60	87.80	1.20	0.610	**0.030**
BMI (Kg/m2)	22.93	1.30	23.05	10.80	25.90	2.12	25.00	1.44	0.947	0.734
BMI (centile)	88.80	3.20	88.80	2.20	92.00	2.40	91.90	2.03	0.811	0.520
Systolic BP (mmHg)	104.50	3.11	98.00	2.25	103.30	3.14	112.50	3.66	0.104	0.121
Diastolic BP (mmHg)	69.00	1.94	65.00	1.80	64.71	1.78	66.79	1.45	0.132	0.400
Glucose (mmol/L)	5.14	0.32	4.76	0.07	5.91	0.56	5.09	0.14	0.113	0.080
Cholesterol (mmol/L)	4.95	0.29	3.86	0.13	2.70	0.15	0.22	8.62	**0.000**	**0.016**
Triglycerides (TG) (mmol/L)	4.28	0.63	1.00	0.09	1.31	0.32	1.22	0.12	**0.000**	0.757
CChol (mmol/L)	4.09	0.20	3.66	0.12	2.44	0.11	3.33	0.21	0.090	**0.010**
Lactate (mmol/L)	2.16	0.15	1.33	0.05	3.26	0.67	1.35	0.06	**0.000**	**0.000**
Uric acid (µmol/L)	303.37	17.62	227.23	16.64	367.11	33.54	225.19	14.00	**0.008**	**0.000**
Bicarbonate (mmol/L)	22.40	0.71	26.31	0.43	20.77	1.14	24.57	0.48	**0.000**	**0.002**

Table 1. Clinical and biochemical markers of metabolic control in GSDI patients and control subjects.
CChol: corrected Cholesterol

	GSDIa		Controls		GSDIb		Controls		Significance (p)		Reference range
	Mean	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Ia vs C**	**Ib vs C**	
ACTH (pmol/L)	6.28	1.73	5.40	0.55	7.15	2.24	5.31	0.42	0.545	0.282	2.2-11.0
Cortisol (nmol/L)	455.44	41.74	352.27	19.30	230.22	59.37	372.56	35.47	**0.042**	**0.041**	< 15 years: 83-580 > 15 years: 220-525
Androstenedione (nmol/L)	1.16	0.33	1.44	0.23	2.24	0.28	2.28	0.35	0.493	0.944	Depending on Tanner stage
17OHP (nmol/L)	1.37	0.25	1.09	0.13	2.07	0.53	1.38	0.11	0.276	0.108	Depending on Tanner stage
DHEAS (nmol/L)	3392	1255	3496	272	4195	1755	3068	286	0.938	0.549	Depending on Tanner stage
Renin[1] (pmol/L)	0.14	0.04	0.18	0.01	0.20	0.05	0.16	0.01	0.611	0.478	< 5 years: 0.07-0.21 > 5 years: 0.06-0.08
Aldosterone[1] (pmol/L)	25.42	6.53	25.53	1.11	17.64	5.46	24.12	1.27	0.750	0.432	< 15 years: 1.80-28.80 > 15 years: 2.50-11.00
UFC (µg/24h)[2]	55.83	8.02	65.30	5.72	81.29	47.94	62.67	10.26	0.360	0.610	1-10 years: 2-27 11-20 years:5-55 > 20 years: 20-90

Table 2. Baseline hormone serum levels in GSDI patients and control subjects.
[a]7 GSDIa and 6 GSDIb patients; [b]5 GSDIa and 3 GSDIb patients
ACTH: adrenocorticotropic hormone, 17OHP: 17-hydroxyprogesterone, DHEAS: dehydroepiandrosterone sulphate, UFC: 24-hour urinary free cortisol

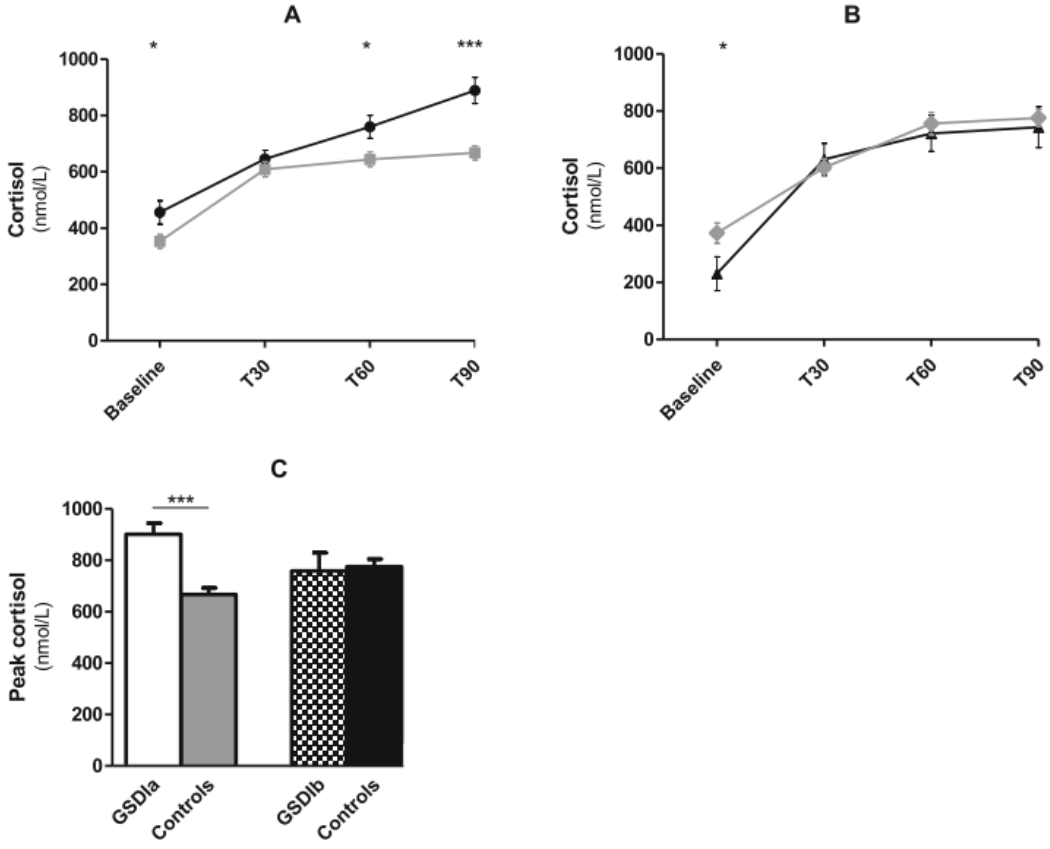

Figure 2. A. Baseline and ACTH-stimulated cortisol levels in GSDIa patients (●) and controls (■). B. Baseline and ACTH-stimulated cortisol levels in GSDIb patients (▲) and controls (◆). C. Peak ACTH-stimulated cortisol levels in GSDIa and GSDIb patients and controls.
* $p < 0.05$, *** $p < 0.001$.*T30: 30 min after ACTH analogue administration, T60: 60 min after ACTH analogue administration, T90: 90 min after ACTH analogue administration*

Correlation study

Baseline cortisol serum levels directly correlated with cholesterol (ρ = 0.65, *p* = 0.005) and TG (ρ = 0.60, *p* =0.012) serum levels in GSDI patients (Fig. 3). A direct correlation between cholesterol and triglycerides was found (ρ = 0.77, *p* = 0.000). Multivariate analysis (F-test, *p* = 0.031) showed no significance for cholesterol (β = 0.50, *p* = 0.149), TG (β = 0.32, *p* = 0.640) and CChol (β = 0.39, *p* = 0.150).

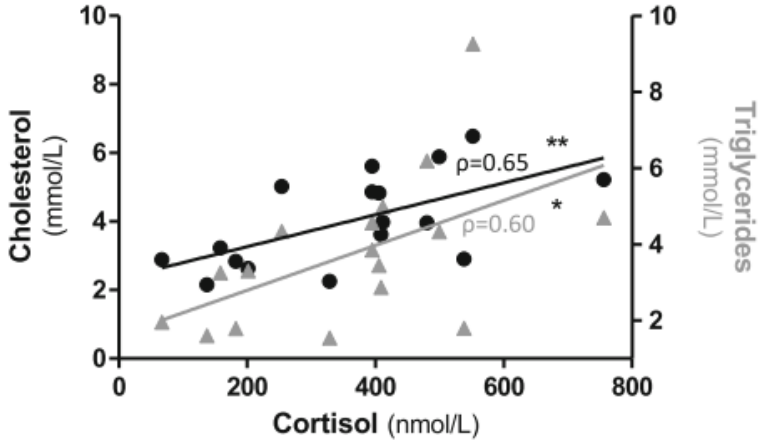

Figure 3. Correlation between baseline cortisol levels and cholesterol (●, ρ = 0.65, *p* < 0.01) **and triglycerides** (▲, ρ = 0.60, *p* < 0.05) **levels in GSDI patients**. * *p* < 0.05, ***p* < 0.01

DISCUSSION

An endocrine involvement has been extensively reported in GSDI[7,8,10–12]. Interestingly, most of the typical findings in GSDI (short stature, delayed puberty, hypothyroidsm, polycystic ovaries, osteoporosis) are similar to those of Cushing's syndrome, suggesting a possible impairment in glucocorticoid metabolism in GSDI. To the best of our knowledge, systematic adrenal cortex assessment has never been performed in GSDI. In order to gather information on the function of the adrenal cortex, data concerning adrenal cortex hormones (both at baseline and after ACTH challenge) were collected in GSDIa and GSDIb patients. GSDIa patients showed higher baseline and ACTH-stimulated cortisol levels with GSDIb patients showing decreased baseline cortisol levels. The opposite cortisol profile between GSDIa and GSDIb points to a possible role of the metabolic defect per se in the endocrine imbalance. The results of the current study suggest that

imbalanced cortisol levels in GSDI might be due to local deregulation rather than HPA axis activation. Cortisol role as a counter-regulatory hormone in glucose homeostasis should also be taken into account. No patient showed low blood glucose concentrations in the present study. Two GSDIb patients showed glucose concentration slightly above 4.0 mmol/L (Additional file 1A). Notably, GSDIb patients showed lower cortisol levels than controls in the present study. Glucose concentrations were not routinely measured at the end of the ACTH stimulation test based on the following considerations:1) the timing of the ACTH stimulation test was arranged in order not to exceed patients fasting tolerance and 2) the administration of ACTH stimulates the release of cortisol

from the adrenal cortex and no glucose lowering effect was expected. Indeed, data on glucose concentration at the end of the ACTH stimulation test available in four patients showed a relatively stable trend (Additional file 2). No correlation was found between glucose concentrations and cortisol levels at the end of the ACTH stimulation test in those patients (p = 0.800) suggesting that glucose concentration likely did not affect cortisol levels in the present study. The regulation of adrenal cortex function is under control of HPA axis[13]. Nonetheless, 11βHSD1 has recently emerged as a local regulator mechanism[4]. An important biological function of liver 11βHSD1 (different from tissue-specific pre-receptoral metabolism) is a systemic shift of the cortisol:cortisone equilibrium towards active cortisol promoting the crucial metabolic and circulatory effects of cortisol [14]. Glucocorticoid excess is known to cause obesity and diabetes[15]. The considerable similarities between Cushing's syndrome and metabolic syndrome (MS) have driven investigations on possible pathogenic role of glucocorticoids. Among all possible determinants (e.g. HPA axis, intracellular receptors density, prereceptorial metabolism), 11βHSD1 has emerged as the most plausible mechanism[16,17]. The hepatic 11βHSD1 plays a key role in the development of MS[18,19]. Conversely, 11βHSD1 knock-out mice are resistant to the development of MS[20,21]. 11βHSD1 is nowadays a promising therapeutic target and a number of 11βHSD1 inhibitors are in development as potentially effective in the treatment of MS and diabetes[22,23]. Interestingly, the G6P excess in the liver ER has been associated to increased 11βHSD1 activity in GSDIa[2]. The increased 11βHSD1 activity might play a role in the increased prevalence of insulin-resistance (IR) and MS reported in GSDIa patients[24].

Biochemically, glucocorticoid synthesis involves the shuttling of precursors between mitochondria and the ER, with cholesterol entering the mitochondria as first step[25]. Most steroidogenic cholesterol is derived from circulating lipoproteins, but it may be also produced de novo within the ER[26]. Interestingly, increased G6P levels in ER[27] and mitochondrial dysfunction[28] have been suggested to be the cause and the effect of hypercholesterolemia in GSDIa, respectively. Notably, G6Pase activity has been shown in zona reticularis and zona fasciculata that are actively involved in cortisol synthesis[29]. The increase of cortisol synthesis might in principle represent a mechanism to divert cholesterol excess within the mitochondria in GSDIa. Correlation data support this hypothesis. Despite not statistically significant, these data suggest that the combination of cholesterol and TG would best explain the cortisol levels in GSDI patients. The lack of significance at multivariate analysis might be due to small sample size and high correlation between the two independent variables.

GSDIb is typically associated with neutropenia, neutrophil dysfunction and predisposition to inflammatory bowel disease (IBD)[1]. Increased prevalence of autoimmune disorders has been reported[10,30]. In GSDIb the lack of G6P in ER has been associated to decreased 11βHSD1 activity[2]. 11βHSD1 is widely expressed in immune cells[31]. 11βHSD1 expression has been associated with a switch in energy metabolism suggesting that 11βHSD1 deficiency might worsen tissue damage in the case of chronic inflammation[32,33]. Indeed, 11βHSD1-deficient mice showed delayed resolution of the inflammation[34]. Glucocorticoids are also essential regulators of T-cells development[35]. The engagement of glucocorticoid receptor has been recently shown as crucial determinant conferring protection from autoimmunity during pregnancy in mice[36]. Regulatory T cells (Tregs) are particularly responsive to glucocorticoid signals[37] and impairment of Tregs has been described in a number of autoimmune diseases[38]. Interestingly, disrupted Tregs function has been reported in GSDIb patients[39]. We hypothesize that reduced 11βHSD1 activity in GSDIb patients' immune cells could impair energy

metabolism and cell function and play a role in delayed resolution of inflammation and development of autoimmune disorders.

CONCLUSIONS

Opposite cortisol levels were found in GSDIa (increased) and GSDIb (decreased) patients. The findings of the current study suggest that imbalanced cortisol concentrations might be due to local deregulation rather than HPA axis activation in GSDI. 11βHSD1 activity modulation by G6P availability could explain the opposite cortisol profile in GSDIa and GSDIb patients. We speculate that glucocorticoid deregulation might play a role in the development of the emerging complications in GSDIa (namely IR and MS) and GSDIb (delayed inflammation, autoimmune disorders) patients (Fig. 1). The results of the current study suggest that adrenal evaluation should be considered to define the pathophysiology of complications in GSDI and possibly provide additional disease biomarker. It is noteworthy that the dysregulation of cortisol secretion is opposite in GSDIa and GSDIb. Future studies dissecting the connection between G6Pase system and 11βHSD1 are warranted in order to identify new potential therapeutic targets in GSDI patients.

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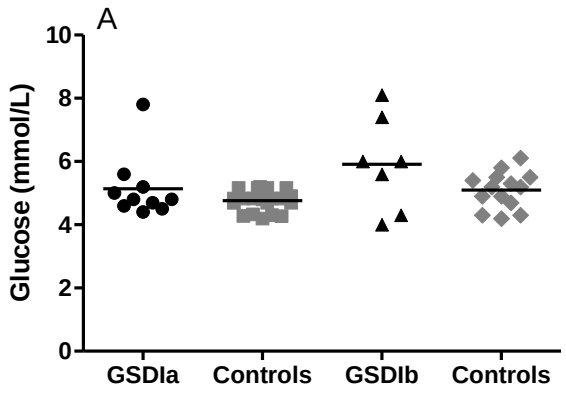
SUPPLEMENTARY MATERIAL

Additional file 1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Glucose (mmol/L)
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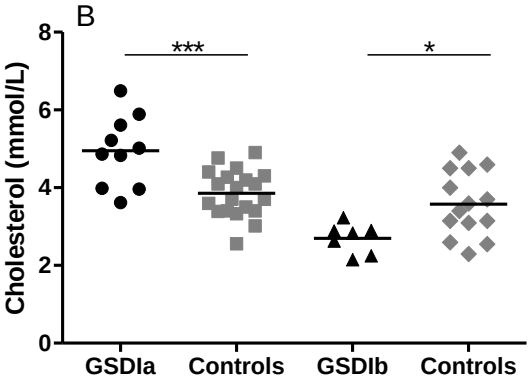
2
4
6
8
10
GSDIa
Controls
GSDIb
Controls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Cholesterol (mmol/L)
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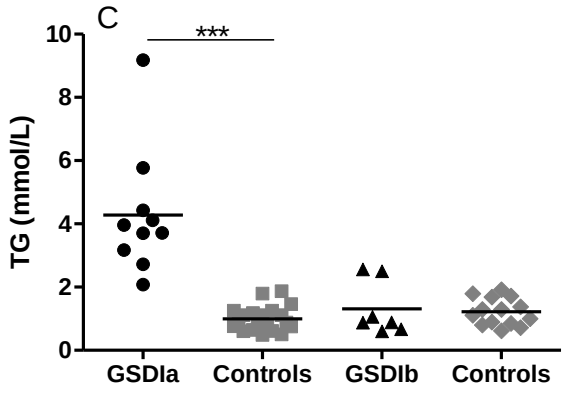
2
4
6
8
***
*
GSDIa
Controls
GSDIb
Controls


C
TG (mmol/L)
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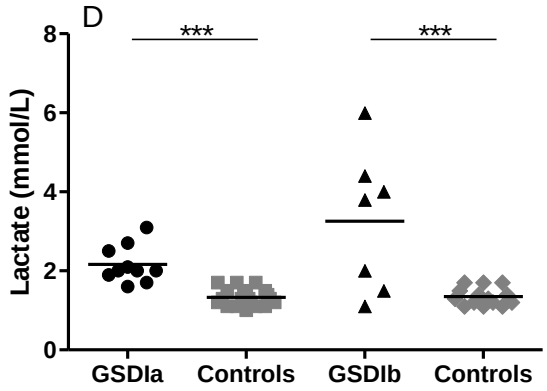
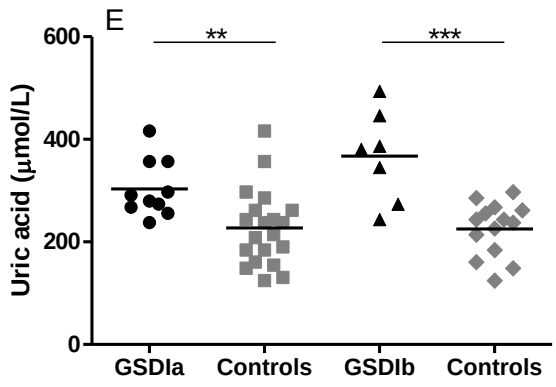
2
4
6
8
10
***
GSDIa
Controls
GSDIb
Controls


D
Lactate (mmol/L)
0
2
4
6
8
***
***
GSDIa
Controls
GSDIb
Controls


E
Uric acid (µmol/L)
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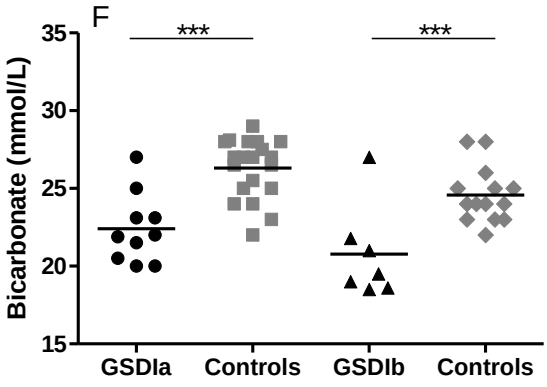
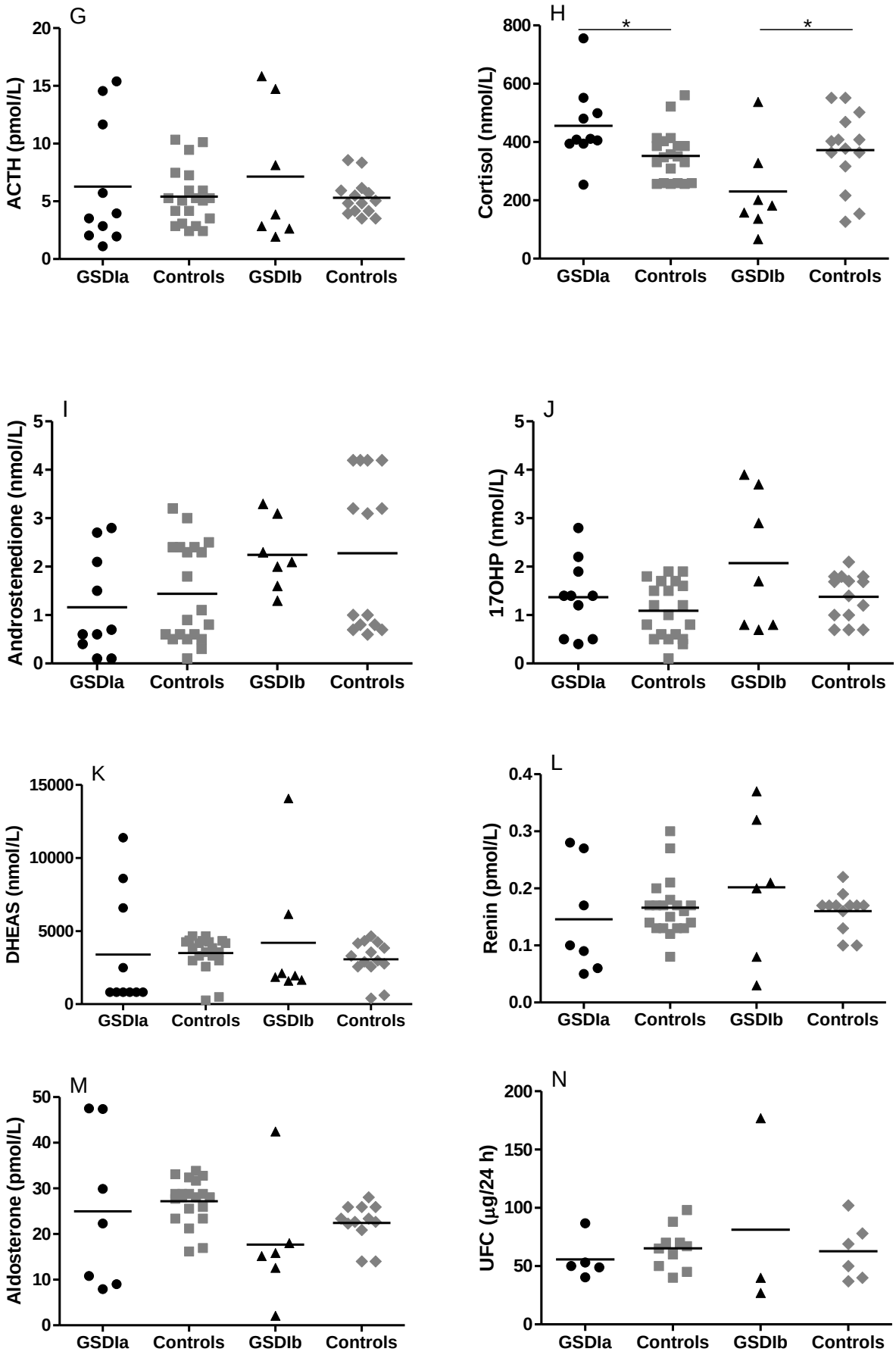
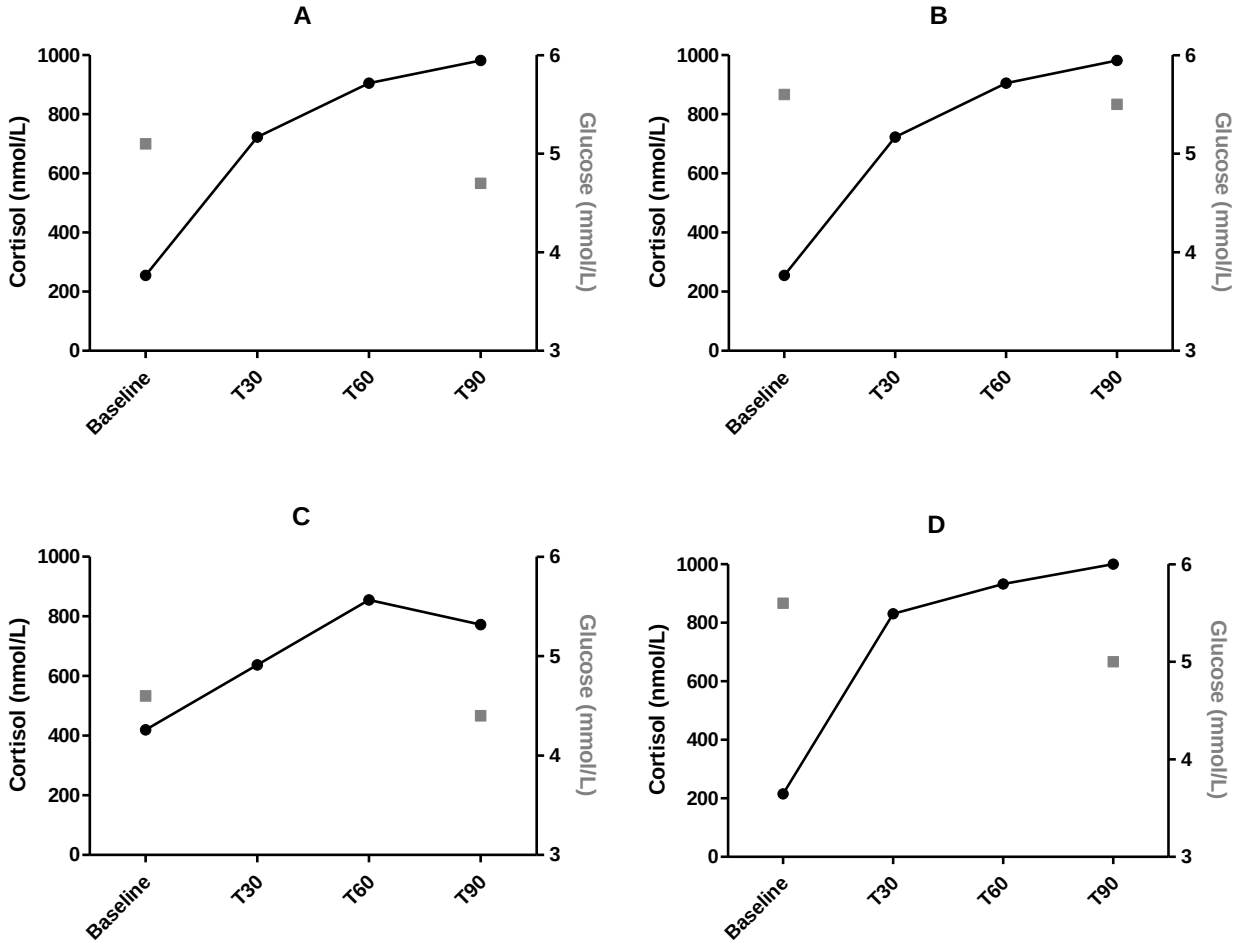
200
400
600
**
***
GSDIa
Controls
GSDIb
Controls


F
Bicarbonate (mmol/L)
15
20
25
30
35
***
***
GSDIa
Controls
GSDIb
Controls


G
ACTH (pmol/L)
20
15
10
5
0
GSDIa
Controls
GSDIb
Controls
H
Cortisol (nmol/L)
800
600
400
200
0
*
*
GSDIa
Controls
GSDIb
Controls
I
Androstenedione (nmol/L)
5
4
3
2
1
0
GSDIa
Controls
GSDIb
Controls
J
17OHP (nmol/L)
5
4
3
2
1
0
GSDIa
Controls
GSDIb
Controls
K
DHEAS (nmol/L)
15000
10000
5000
0
GSDIa
Controls
GSDIb
Controls
L
Renin (pmol/L)
0.4
0.3
0.2
0.1
0.0
GSDIa
Controls
GSDIb
Controls
M
Aldosterone (pmol/L)
50
40
30
20
10
0
GSDIa
Controls
GSDIb
Controls
N
UFC (µg/24 h)
200
150
100
50
0
GSDIa
Controls
GSDIb
Controls

Additional file 1. Biochemical parameters (A-F), baseline adrenal cortex hormones (G-M) and 24-hour urine free cortisol in GSDIa patients (●), GSDIa-related controls (■), GSDIb patients (▲) and GSDIb-related controls (◆).**p< 0.05, **p<0.01, ***p<0.001*

Additional file 2



Additional file 2. Cortisol (●) and glucose (■) concentrations at the beginning and at the end of the ACTH stimulation test in GSDIa (A,B,C) and GSDIb (D) patients.

T30: 30 minutes after ACTH analogue administration, T60: 60 minutes after ACTH analogue administration, T90: 90 minutes after ACTH analogue administration

PART II

Developing novel management strategies

Chapter 4

Dietary lipids in glycogen storage disease type III: a systematic literature study, case studies, and future recommendations

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ABSTRACT

A potential role of dietary lipids in the management of hepatic glycogen storage diseases (GSDs) has been proposed, but no consensus on management guidelines exists. The aim of this study was to describe current experiences with dietary lipid manipulations in hepatic GSD patients. An international study was set up to identify published and unpublished cases describing hepatic GSD patients with a dietary lipid manipulation. A literature search was performed according to the Cochrane Collaboration methodology through PubMed and EMBASE (up to December 2018). All delegates who attended the dietetics session at the IGSD2017, Groningen were invited to share unpublished cases. Due to multiple biases, only data on GSDIII were presented. A total of 28 cases with GSDIII and a dietary lipid manipulation were identified. Main indications were cardiomyopathy and/or myopathy. A high fat diet was the most common dietary lipid manipulation. A decline in creatine kinase concentrations ($n = 19$, $P < .001$) and a decrease in cardiac hypertrophy in paediatric GSDIIIa patients ($n = 7$, $P < .01$) were observed after the introduction with a high fat diet. This study presents an international cohort of GSDIII patients with different dietary lipid manipulations. High fat diet may be beneficial in paediatric GSDIIIa patients with cardiac hypertrophy, but careful long-term monitoring for potential complications is warranted, such as growth restriction, liver inflammation, and hepatocellular carcinoma development.

INTRODUCTION

Glycogen storage diseases (GSD) are inborn errors of glycogen synthesis or degradation. Although a wide spectrum of clinical and biochemical presentation is observed, GSD are usually classified into hepatic and muscle GSD. Primary manifestations of the hepatic GSD subtypes 0, I, III, VI, IX, and XI are fasting intolerance associated hypoglycaemia, hepatomegaly and failure to thrive. In addition, GSDIII patients also show a myopathic phenotype with skeletal muscle involvement and/or cardiomyopathy[1].
Management guidelines have been published for GSD subtypes Ia[2,3], Ib[4], III[5], and VI and IX together[6] Dietary management is the cornerstone of treatment for hepatic GSD patients to maintain normoglycaemia, prevent secondary metabolic derangements and long-term complications. Strict dietary management and compliance has significantly improved the outcomes for many GSD patients[7,8]. Traditionally, dietary carbohydrates and protein have received most interest, whereas lipids usually have been restricted. Several case reports have described beneficial effects of dietary lipid manipulations in hepatic GSD patients, including (modified) ketogenic diets and medium-chain triglyceride (MCT) enrichment[9-13]. However, the role of dietary lipids as a third macronutrient in dietary management is still controversial[14].

The aim of this study was to describe current experiences with dietary lipid manipulations in hepatic GSD patients. We performed a systematic literature study of all published cases describing hepatic GSD patients with a dietary lipid manipulation. Thereafter, an international, observational, retrospective study was performed to include unpublished cases. The subsequent discussion provides recommendations for future patient care and research.

METHODS

Systematic literature study

Published cases were retrieved by a systematic literature search conducted according to the Cochrane Collaboration methodology on December 31, 2018. PubMed and EMBASE were searched using both MeSH terms and free text. A flowchart of the detailed search strategy can be found in Supplementary File S1. Initially, all hepatic GSD patients with a dietary lipid manipulation were identified. However, the majority of cases describing GSD type I and VI patients were published before the introduction of management guidelines and lacked important clinical information[15-18]. Therefore, these data were not included, and further data analysis was solely focused on GSDIII. All reports about GSDIII patients receiving dietary lipid manipulation were included. Inclusion criteria were GSDIII diagnosis based on biochemical or molecular evaluation and English language. Exclusion criteria were no individual data presentation and/or absence of follow-up data. Two independent reviewers (I.J.H., V.B.B.) performed title, abstract screening and subsequently full-text assessment. After selection of eligible full-text papers and conference abstracts, case information was collected in a data table specifically designed for the purpose of this study, including patient's age at start dietary intervention, gender, GSDIII subtype, indication to start dietary intervention, specifications of diet, duration of the intervention and follow-up, and outcome measures (laboratory results, imaging tests, and clinical picture).

Case studies

Unpublished cases were retrieved via the International GSD Conference 2017, organised in Groningen, The Netherlands on June 15 to 17, 2017. All metabolic dieticians were invited to join a networking session on the role of MCT in hepatic GSD. In October 2017, after the IGSD2017, all delegates who had attended the networking session received an invitation by email to share unpublished data of hepatic GSD patients with a dietary lipid manipulation. Data were collected through the same table used for published cases.

Data synthesis and analysis

Data on macronutrients were presented as energy percentage (E-%) of total caloric intake, or if otherwise noted in the legend. MCT supplementation was defined as regular GSD diet enriched in MCT. MCT replacement was defined as long-chain triglycerides substituted with MCT. High fat diet was defined as a diet in which lipids were the main macronutrient based on E-% values. Ketogenic diets were also categorised as high fat even in the absence of E-% values. Standard deviations of BMI were calculated using standard growth charts established by the CDC/2000. Age specific outcomes were presented as Z-scores or in subgroups (i.e, child and adult). The cut-off value for adulthood was set at 16 years of age. Laboratory parameters were presented as range (minimum-maximum value) before and after the dietary intervention, respectively. For each parameter, individual differences (Δ) were presented as percentage difference between mean values before and after the dietary intervention, respectively. Concentrations were considered increased when $\Delta > +10\%$, decreased when $\Delta < -10\%$ and stable if Δ between -10% and $+10\%$. Z-scores were calculated for interventricular septum dimensions (IVSd) to normalise for the body surface area. For Z-score calculation, the regression equation by Pettersen was used[19]. The Haycock formula was used for BSA calculation[20].

Statistical analysis

Data were analysed using Prism 7 software (GraphPad Software, Inc. La Jolla, California) and Statistical Package for Social Sciences, version 23.0 (SPSS, IBM Corp., Armonk, New York). Differences in outcome measures before and after dietary lipid manipulation were analysed with a paired t test if data were normally distributed (assessed by the Shapiro-Wilk test). Data were analysed with Wilcoxon signed ranks test in case of non-normally distributed data after log-transformation. Pearson's or Spearman's correlations tests were used to define relationships between dietary parameters and changes in laboratory outcomes. Statistical significance was defined as $P < .05$

RESULTS

Cases

Literature search revealed four full text articles and five conference abstracts describing 14 GSDIII patients (Supplementary File S2), whereas 14 unpublished cases were collected from six metabolic centres from three different countries (Supplementary File S3). Therefore, a total of 28 cases with GSDIII and a dietary lipid manipulation were collected.

Patients features, indication to start the diet and compliance

Main features of GSDIII patients receiving a dietary lipid manipulation are presented in Table 1. The main indication to start the dietary intervention was cardiomyopathy and/or myopathy. Four patients (cases 9, 19, 26, 27) did not follow the modified diet regimen regularly: either poor compliance was reported, or the diet was discontinued several times.

Cases, n			
	Published		14
	Unpublished		14
	Total		28
Gender, n (%)			
	Male		11 (39%)
	Female		15 (54%)
	Unknown		2 (7%)
Age[a], years			
	Median [range]		7 [0-41]
Indication, n (%)			
	Hyperlipidemia		2 (7%)
	Poor metabolic control		7 (25%)
	Muscle involvement		19 (68%)
		-Skeletal muscle weakness	*3*
		-Cardiomyopathy	*6*
		-Skeletal and cardiac muscle involvement	*9*
		-Hypotonia	*1*
Intervention, n (%)			
	High fat diet		26[b] (93%)
	MCT supplementation/replacement		6 (21%)
	Atkins, ketogenic diet		5 (18%)
	Corn oil supplementation		1 (4%)
Months of dietary intervention			
	Median [range]		18 [1-60]

Table 1. Features of published and unpublished cases with GSDIII and a dietary lipid manipulation (n = 28).

[a]Age at start dietary intervention.

[b]Four patients received both MCT and a high fat diet (cases 15, 16, 20, and 21), five patients received a ketogenic diet which was also categorised as high fat diet (cases 2 and 8-11), one patient received a high fat diet with corn oil substitution (case 14)[17], and one patient received a high fat diet supplemented with D,L-3-hydroxybutyrate (case 12)[13].

Abbreviation: MCT, medium-chain triglyceride.

Diet composition

Most common lipid manipulation was high fat diet (Table 1). Figure 1A presents the diet composition before and after dietary intervention in GSDIII patients receiving a high fat diet. Lipid intake ranged from 0.9 to 8.0 g/kg/day (2.9-8.0 g/kg/day in children, 0.9-2.7 g/kg/day in adults) (Figure 1B). Less common interventions included corn oil supplementation together with high fat diet (case 14)[17], and MCT supplementation alone (cases 6 and 7)[21] (Supplementary File S2).

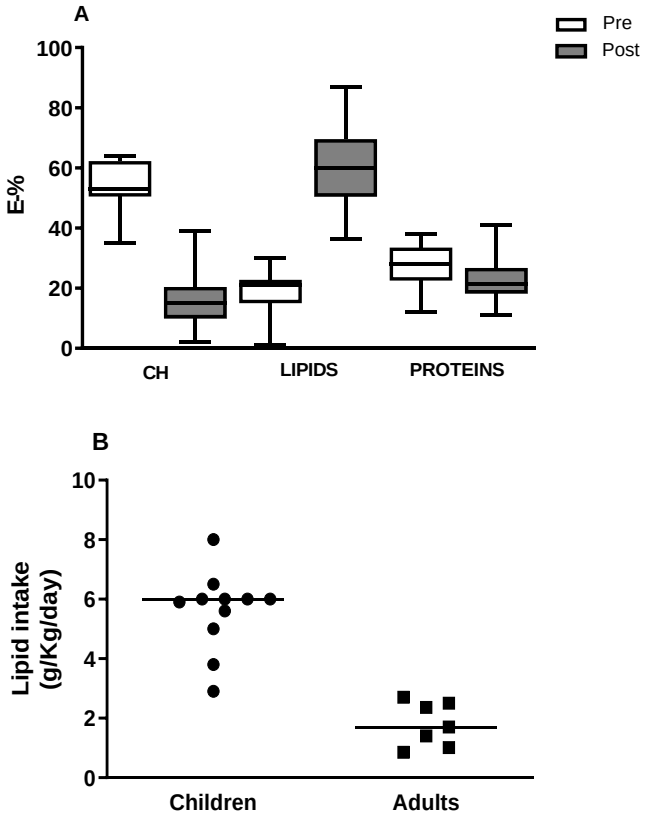

Figure 1. Dietary features of GSDIII patients. A, Diet composition in GSDIII patients before (*n* = 10) and after (*n* = 24) high fat diet. B, Lipid intake in GSDIII patients receiving high fat diet (*n* = 18, patients on high fat diet also receiving MCT supplementation were included). Data are presented as median [range]. CH, carbohydrates

Laboratory results

The changes in laboratory parameters in GSDIII patients receiving high fat diet are presented in Figure 2 and Supplementary File S4.

Creatine kinase (CK) concentrations were available in 73% (19/26) of GSDIII patients receiving high fat diet (Figure 2A). Mean CK concentrations were lower after receiving high fat diet in 89% (17/19) of GSDIII patients (2070 U/L ± 1634 vs 1078 U/L ± 1148, $P < .001$). One previously unreported patient showed an increase in CK concentrations (case 25); however, CK concentrations remained

within the reference range[22]. Another patient showed stable CK concentrations (case 26). No correlations between ΔCK and changes in macronutrients were found.

Liver transaminases (ALT/AST) were documented in 58% (15/26) of GSDIII patients on a high fat diet (Figure 2B,C). In adult GSDIII patients, ALT concentrations decreased in all cases (*n* = 6); AST concentrations decreased in five patients (83%) and were stable in the sixth patient. In paediatric GSDIII patients, ALT concentrations increased in four patients (44%), decreased in one patient (11%) and were stable in four patients (44%); AST concentrations increased in five patients (56%), decreased in two patients (22%), and were stable in two patients (22%).

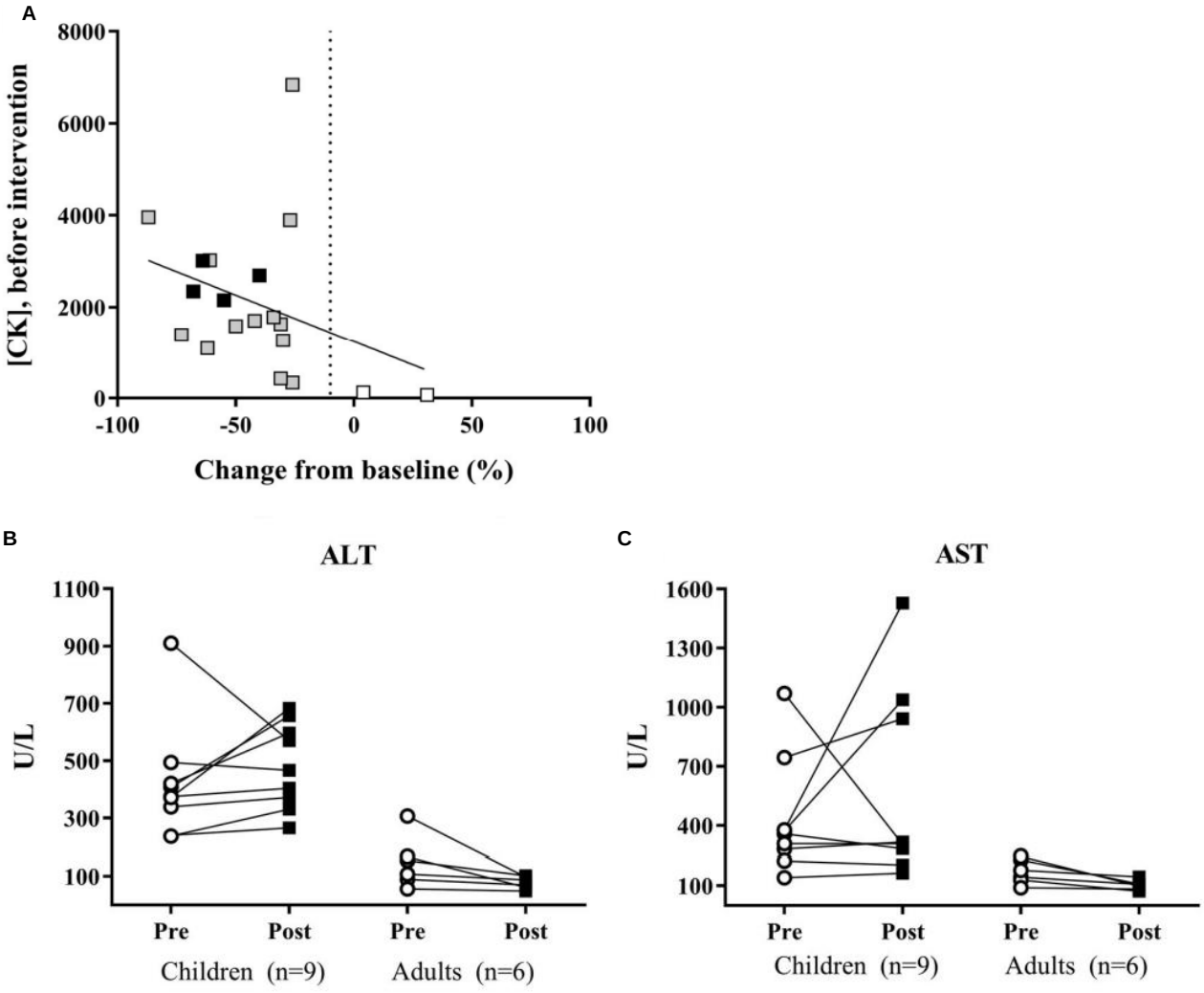

FIGURE 2. Changes in laboratory parameters by dietary lipid manipulation in GSDIII. A, Relation between CK concentrations before intervention and change in CK concentration of 19 individual patients with GSDIII with high fat diet, including patients with combined high fat diet and MCT supplementation (*n* = 4). Spearman's rho correlation coefficient = −0.40, *P* > .05. Grey square; GSDIII patient, black square; GSDIII patient receiving combined high fat diet and MCT supplementation, white square; GSDIII patient showing CK concentrations within age-related reference values before and after dietary lipid manipulation[22]. B, Measured blood ALT concentrations in GSDIII patients before (circle) and after (square) the introduction of a high fat diet. C, Measured blood AST concentrations in GSDIII patients before (circle) and after (square) the introduction of a high fat diet.

Imaging and clinical outcomes

IVSd Z-scores decreased in paediatric GSDIII patients with a high fat diet (n = 7, $P < .01$; Figure 3), but not in adult GSDIII patients ($n = 4$, Supplementary File S3). There were no correlations between the change in IVSd Z-scores and changes in macronutrients. Data on muscle ultrasound and muscle function tests were available in two adult GSDIIIa patients on a high fat diet with MCT replacement (cases 15 and 16). There was no effect on muscle density. Muscle strength as assessed with dynamometry improved only for case 15. Subjective improvements of exercise tolerance and/or muscle strength were reported in 78% (14/18) of paediatric GSDIII patients and 50% (4/8) of adult GSDIII patients on high fat diet. Among paediatric GSDIII patients receiving a high fat diet 18% (2/11) showed improved height SDS, 64% (7/11) showed stable height SDS and 18% (2/11) showed decreased height SDS. All paediatric patients showed normal BMI (60% stable, 40% normalised). BMI was stable in all adult GSDIII patients.

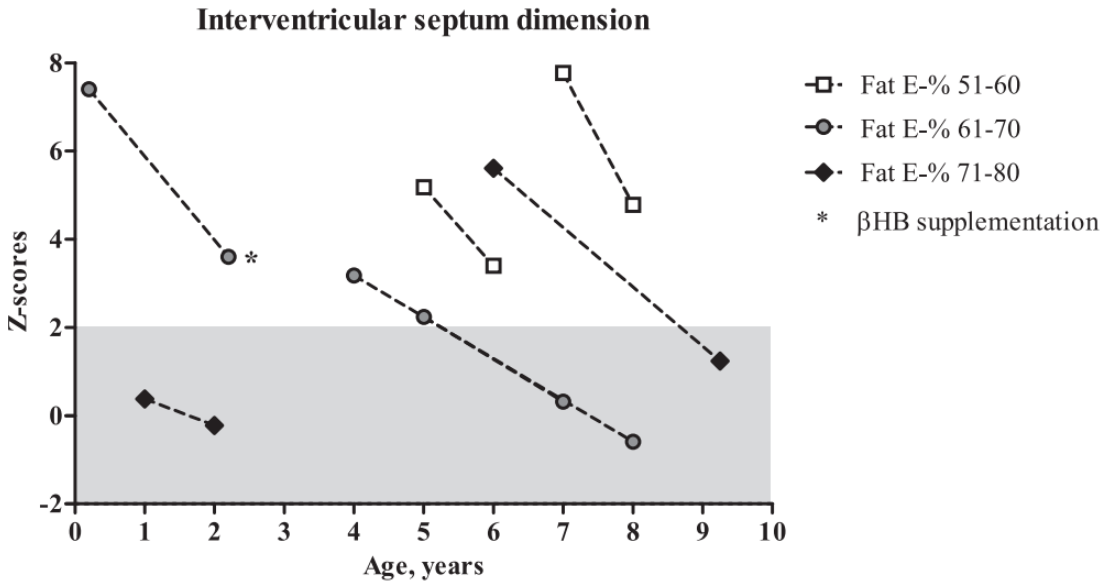

FIGURE 3 Effect of high fat diet on interventricular septum dimension in paediatric GSDIIIa patients (*n* = 7). Measurements are displayed as Z-scores. GSDIIIa subjects are noted with symbols according to E-% of fat. Grey column represents range of normal Z-scores.

Side effects

Side effects were reported in two patients. Hypoglycaemia is an intrinsic symptom of hepatic GSD and was reported in two GSDIII patients on a high fat diet. Specifically, one paediatric GSDIIIa patient (case 18) reported isolated hypoglycaemia 3 years after the start of a high fat diet, and one paediatric GSDIIIa patient (case 19) presented with an isolated hypoglycaemia 1 year before and 2 years after starting with a high fat diet.

DISCUSSION

Complex carbohydrates and, for ketotic GSD patients, protein enrichment are the cornerstones of dietary management in hepatic GSD. The role of lipids has not been systematically assessed and the current guidelines do not provide clear indications for their use[2-6]. This systematic literature study and retrospective international multicentre cohort study presents that a high fat diet could be considered in paediatric GSDIII patients with cardiomyopathy. The significant reduction in blood CK concentrations and subjective improvement in muscle strength reported in GSDIII patients necessitates further quantification of the effect of a high fat diet on muscle quality and function. Also, liver function, morphology, and growth should be carefully monitored under a high fat regimen given the potential impact on underlying liver disease.

Before discussing the results, some methodological issues need to be addressed. The analysis and interpretation of the data were hampered by large variation in age, dietary intervention (eg, lipid amount, high fat diet alone or together with lipid supplementation), duration of intervention, and outcome parameters. Initially, this study was set up to describe all hepatic GSD types. Most of the data on GSDI and GSDVI were limited and/or historical[10,12,15,16,18,23], whereas metabolic control has improved with increasing knowledge on dietary management/glycaemic control and the introduction of management guidelines, as demonstrated for GSDIa patients[24]. Therefore, in this article, we only included data from GSDIII patients. The published cases presented in this study (n = 14) were retrieved from case reports or small cohort studies (describing less than five patients); these data were potentially affected by selection and publication bias. Also, the possible beneficial role of a more compliant dietary scheme during dietary intervention should be considered. Finally, ascertainment bias extends to healthcare professionals attending a GSD conference.

The main indications to start with a dietary lipid manipulation in GSDIII patients were cardiomyopathy, skeletal myopathy or a combination of both. Lipids became the main macronutrient in GSDIII patients at the expense of carbohydrates. Interestingly, cardiac hypertrophy, as quantified by IVSd Z-scores, decreased only in paediatric GSDIIIa patients. We hypothesize that an early switch to high fat diet can reverse—or at least decrease— the cardiac glycogen storage. Moreover, results showed decreased CK concentrations in 89% of GSDIII patients in accordance with literature[9,11,13] and improved subjective strength in most of the patients. Increased blood CK concentrations reflect muscle damage which may partially be influenced by exercise. Whether the beneficial effect of a high fat diet on CK concentrations is caused by a lower carbohydrate intake—and thus less accumulation of abnormal glycogen in muscle tissue—or due to the properties of fat to supply alternative energy substrate for muscle remains to be investigated. Notably, most of the GSDIII patients included in the present study received a combination of a high fat and high protein diet. Therefore, these changes in macronutrient composition could also partly account for the beneficial effect on cardiomyopathy and CK concentrations. Nevertheless, protein intake was comparable before and after intervention in GSDIII patients in the present study (Figure 1A).

The development of chronic liver disease is an important concern in ageing GSDIII patients. Although the prevalence of hepatocellular carcinoma was low in the International Study on GSDIII[25], severe and progressive liver fibrosis has been described at early ages[26]. Only one publication describing high fat diet in two GSDIIIa patients documented data on liver transaminases (cases 4 and 5[9]). Interestingly, we found that ALT concentrations increased in 44% (4/9) of paediatric GSDIII

patients but decreased in all adult GSDIII patients. After dietary lipid manipulation, the concomitant decrease in carbohydrate intake would theoretically lead to less glycogen accumulation in the liver. It remains speculative if these age-specific effects are part of the natural history or influenced by dietary lipid manipulations. However, under these circumstances, careful monitoring and follow-up is warranted for liver complications such as hepatosteatosis, liver inflammation, and hepatocellular carcinoma[27].

Side effects were reported in two patients, consisting in isolated (and mostly mild) hypoglycaemia, an intrinsic symptom in GSD patients[28]. 'Side effects' were not a specific parameter in our data table, and therefore the side effects reported in this study could be an underrepresentation. Previously mentioned concerns regarding MCT in GSD patients are the unknown consequence towards the elongation of fatty acids or gluconeogenesis pathway[14]. Increased triglycerides concentrations after introduction of MCT have been reported in GSDIII patients[29]. However, in the present study, the majority of GSDIII patients received a high fat diet rather than MCT supplementation or replacement. As high fat diets have been associated with an increased risk of osteoporosis[30] combined with the reduced bone mineral density in GSDIII patients[31] the long-term effect of dietary lipid manipulations on bone status should be carefully monitored.

Recommendations for future dietary intervention studies and follow-up of GSDIII patients who start with a high fat diet are summarised in Supplementary File S5. The present study also provides insight in important outcome parameters when assessing the effect of a dietary intervention in hepatic GSD patients. Several additional outcome measures are proposed including muscle[32-34], bone[31], mitochondrial[12,35], and enzymatic[36] markers. Prospective, long-term follow-up studies are warranted to confirm efficacy and safety of dietary lipid manipulations in the international GSDIII and further hepatic GSD cohort.

FUNDING

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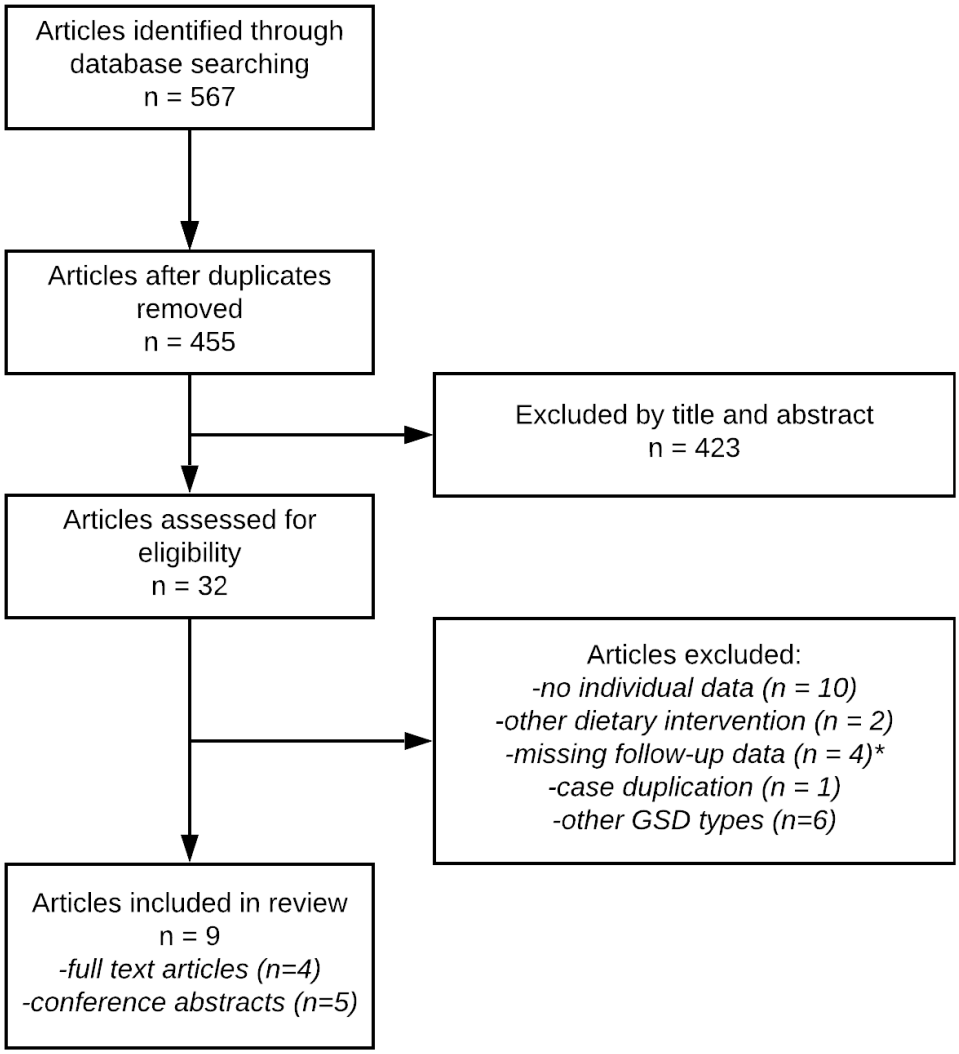
SUPPLEMENTARY MATERIAL

Supplementary File S1

Supplementary File S1



Supplementary File S1. Prisma flowchart of search strategy. PubMed and Embase were searched using both MeSH terms and free text: a. PubMed search: '("Glycogen Storage Disease"[Mesh] OR glycogen storage[tiab] OR glycogenos*[tiab]) AND ("Ketogenic Diet"[Mesh] OR "Diet, Carbohydrate-Restricted"[Mesh] OR ((fat[tiab] OR fatty*[tiab] OR oil*[tiab] OR atkins[tiab] OR ketogen*[tiab]) AND (diet[tiab] OR diets[tiab] OR dietary[tiab] OR dieting[tiab])) OR "triheptanoin" [Supplementary Concept] OR "Triglycerides"[Mesh] OR "Dietary Fats"[Mesh] OR "Fish Oils"[Mesh] OR medium chain triglycerid*[tiab] OR MCT[tiab] OR triheptanoin*[tiab] OR omega-3-fatty acid*[tiab] OR fish oil*[tiab]) NOT (("Animals"[Mesh] NOT "Humans"[Mesh]) OR animal*[ti] OR rat[ti] OR rats[ti] OR mouse[ti] OR mice[ti]); b. Embase search: ('glycogen storage disease'/exp OR ('glycogen storage' OR glycogenos*):ab,ti) AND ('ketogenic diet'/exp OR 'low carbohydrate diet'/exp OR ((fat OR fatty* OR oil* OR atkins OR ketogen*) AND (diet OR diets OR dietary OR dieting)):ab,ti OR 'triheptanoin'/exp OR 'triacylglycerol'/exp OR 'fat intake'/de OR 'fish oil'/exp OR ('medium chain triglycerid*' OR MCT OR triheptanoin* OR 'omega-3-fatty acid*' OR 'fish oil*'):ab,ti) NOT ((('animal'/exp OR 'nonhuman'/exp) NOT 'human'/exp) OR (animal* OR rat OR rats OR mouse OR mice):ti). The search was conducted on the 31th of December 2018. The PubMed search revealed 179 articles whereas the Embase search resulted in 388 articles. After the duplicate check a total of 455 articles could be included for the search strategy. *From one of these cases missing data were collected during the retrospective study part; this case was included as unpublished case (case 21) in Supplementary File S3.

Supplementary File S2

Patient number	**Reference**	**Age at start** (years), **gender** (M/F) **and GSD type**	**Indication to start the dietary intervention**	**Dietary intervention and Diet composition**	**Duration of intervention** (months)	**Outcome parameters: laboratory results** (glucose/lactate/Ketones/ acetoacetate/BOHB/TC/TG/ HDL/LDL: mmol/L, insulin: mU/L, uric acid: mg/dL, AST/ALT/CK: U/L,FFA: µmol/L,TnT/NT-proBNP: ng/L, Mb: µmol/L)	**Outcome parameters: diagnostic imaging**	**Outcome parameters Clinical picture,side effects** Weight:Kg, Height: Cm, BMI: Kg/m2
1	White et al, J Inherit Metab Dis. 2018 ABSTRACT	0.42 F IIIa	High glucose demand, seizure	High-fat, high protein diet 20% carbohydrates, 60% lipids, 20% protein	7	Glucose: >2.8 mmol/L Ketones: 0.5 - 2.4 mmol/L Insulin, TC, TG, CK: n/a Other: n/a	Cardiac US: hypertrophic cardiomyopathy fully resolved.	Increased fasting tolerance.
2	Groselj et al, J Inborn Errors Metab Screen 2017 ABSTRACT	12 F IIIa	Severe hypertrophic cardiomyopathy, hepatomegaly, myopathy.	Ketogenic diet. Ketogenic ratios of meals were from 2.5:1 to 4:1. 2% carbohydrates, 87% lipids, 11% protein	18	Glucose: no hypoglycemia Ketones, insulin, TC, TG, CK: n/a Other: lipid levels improved significantly.	Liver US: significant improvement of hepatomegaly Cardiac MRI: normalization of left ventricular parameters and mass (from 70 g to 35 g), without residual outflow obstruction.	Exertion dyspnea disappeared. Capacity for oxygen consumption almost doubled
3	Kumru et al, J Inherit Metab Dis. 2016 ABSTRACT	6 M IIIa	Hypertrophic cardiomyopathy Fatigue	High-fat, high protein diet. 30% carbohydrates, 50% lipids,20% protein.	18	Glucose, ketones, insulin, TC, TG: n/a CK: from 1628 to 1125 Other: n/a	Cardiac US: left ventricular outflow gradient reduced from 35 to 20 mmHg; IVS thickness reduced from 21 to 10 mm; posterior wall thickness reduced from 18 to 11 mm	Fatigue resolved

4	Brambilla et al,J Inherit Metab Dis. Rep. 2014	7 F IIIa	Severe cardiomyopathy, muscle weakness	High-fat high protein diet 1120 Kcal/day, 15% carbohydrates, 59% lipids, 26% proteins UCCS progressively withdrawn Polyunsaturated fatty acids preferred Only extra-virgin olive oil as relish Additional protein powders to increase protein intake	12	Glucose, lactate: no significant difference (normal) Insulin: n/a Ketones: n/a TC, TG: no significant difference (normal) CK: significant decrease Other: NT-proBNP, Mb, ALT: significant decrease; AST: slight decrease; TnT: no significant difference (normal)	Cardiac US: IVS thickness, posterior wall thickness and outflow tract obstruction significantly reduced	Increased strenght and reduced exertion dyspnea. No significant impact on growth (nomal) and liver size (increased)
5	Brambilla et al,J Inherit Metab Dis. Rep. 2014	5 M IIIa	Severe cardiomyopathy, muscle weakness	High-fat high protein diet 1050 Kcal/day, 15% carbohydrates, 60% lipids, 25% proteins UCCS progressively withdrawn Polyunsaturated fatty acids preferred Only extra-virgin olive oil as relish Additional protein powders to increase protein intake	12	Glucose, lactate: no significant difference (normal) Insulin: n/a Ketones: n/a TC: no significant difference (normal) TG: slight increase CK: significant decrease Other: NT-proBNP, Myoglobin, ALT, AST: significant decrease; TnT: no significant difference (normal)	Cardiac US: IVS thickness, posterior wall thickness and outflow tract obstruction significantly reduced	Increased strenght No significant impact on growth (nomal) and liver size (increased)

6	El-Gharbawy et al, Mol Genet Metab. 2014 ABSTRACT	3.5 n/a IIIa	Poor metabolic control	MCT supplementation UCCS progressively withdrawn	1	Glucose, insulin, TC, TG: no significant difference Ketones: no evidence of ketosis CK: significant decrease Other: ALT, AST: modest decrease	n/a	Improved energy levels
7	El-Gharbawy et al, Mol Genet Metab. 2014 ABSTRACT	2 n/a IIIa	Poor metabolic control	MCT supplementation UCCS progressively withdrawn	1	Glucose, insulin, TC, TG: no significant difference Ketones: no evidence of ketosis CK: significant decrease Other: ALT, AST: modest decrease	n/a	Improved energy levels
8	Mayorandan et al, Orphanet J Rare Dis. 2014	9 M IIIa	Severe cardiomyopathy, muscle weakness	High-fat high protein diet UCCS progressively withdrawn Modified Atkins diet 0.4 g/Kg/day carbohydrates, 8 g/Kg/day lipids, 7 g/Kg/day proteins	32	Glucose, insulin: n/a; occasional hypoglycemia during the first weeks Ketones: increased TC: n/a TG: slight increase CK: significant decrease Other: NT-proBNP: significant decrease; LDL: no significant difference (normal)	Cardiac US: IVS thickness and left ventricular outflow tract-gradient significantly reduced	Increased stamina No significant impact on growth (nomal)
9	Mayorandan et al, Orphanet J Rare Dis. 2014	11 M IIIa	cardiomyopathy, muscle weakness, chest pain, nausea after exercise	High-fat high protein diet UCCS progressively withdrawn Modified Atkins diet 0.5 g/Kg/day carbohydrates, 6 g/Kg/day lipids, 5 g/Kg/day lipids	3 Discontinued for several months, then resumed	Glucose, insulin: n/a Ketones: increased TC: n/a TG: no significant difference (normal) CK: significant decrease Other: LDL: no significant difference (normal) Increase in CK levels and lost ketosis upon diet discontinuation. CK levels fell again and ketosis was re-established when the diet resumed	Cardiac US: Hypertrophic cardiomyopathy disappeared	Chest pain, nausea and weakness disappeared Increased stamina Chest pain and weakness reappeared upon diet discontinuation and reverted again when the diet was resumed

10	Meyer et al, J Inherit Metab Dis. 2013 ABSTRACT	9 M IIIa	Poor metabolic control	High-fat high protein diet Atkins diet	12	Glucose, insulin: n/a Ketones, TC, TG: n/a CK: significantly decreased	Cardiac function stabilised	Improved physical strenght
11	Meyer et al, J Inherit Metab Dis. 2013 ABSTRACT	11 M IIIa	Poor metabolic control	High-fat high protein diet Atkins diet	12	Glucose, insulin: n/a Ketones, TC, TG: n/a CK: significantly decreased Increase in CK levels upon diet discontinuation; CK levels fell again when the diet was resumed	Cardiac function stabilised	Improved physical strenght Chest pain and reduced physical strenght upon diet discontinuation
12	Valayannopoulos et al, Pediatr Res. 2011	0.17 M III	Severe cardiomyopathy	High-fat high protein diet 20% carbohydrates, 65% lipids, 15% proteins + BHB (400-800 mg/Kg/day)	24	Glucose, insulin: significant decrease (normal) Ketones: significant increase TC: no significant difference (normal) TG: no significant difference (elevated) CK: significant decrease Other: FFA: significant increase; AST, ALT: no significant difference (elevated)	Cardiac US: IVS thickness significantly decreased	Normal muscle tone and strength, growth and devlopment Liver size increased within the first 6 months and then remained stable Diet and BHB treatment well tolerated; no further hypoglycemia

13	Fernandes et Pikaar, Am J Clin Nutr. 1969	1 F III	Hyperlipidemia	High fat low carbohydrate diet Period 1. 39% carbohydrates, 50% lipids (32% corn oil, 18% milk fat), 11% proteins Period 2. 39% carbohydrates, 50% lipids (32% olive oil, 18% milk fat), 11% proteins Period 3. 39% carbohydrates, 50% lipids (32% coconut oil, 18% milk fat), 11% proteins Period 4. 39% carbohydrates, 50% lipids (MCT), 11% proteins	5 Period 1: 1.5 Period 2: 0.75 Period 3: 1.25 Period 4: 1.5	Glucose, insulin, ketones, TG, CK: n/a Period 1.TC no significant difference (high) FFA: significant decrease Period 2. TC, FFA: no significant difference Period 3. TC no significant difference, FFA: high fluctuation Period 4. TC, FFA: significant increase	n/a	n/a
14	Fernandes et Pikaar, Am J Clin Nutr. 1969	5 F III	Hyperlipidemia	High fat low carbohydrate diet 35% carbohydrates, 48% lipids (corn oil), 17% proteins	1.4	Glucose, insulin, ketones, TG, CK: n/a Marked fluctuations in TC and FFA levels	n/a	n/a

Supplementary file S2. Table published cases.

ACs: serum acylcarnitines, ALT: alanine aminotransferase, AST: aspartate aminotransferase, BHB: beta -hydroxybutyrate, CK: creatine kinase, CK-MB: creatine kinase isoenzyme MB, FFA: free fatty acids, HDL: High-density lipoprotein, IVS: interventricular septum, LCT: long-chain triglycerides, LD: liver longitudinal diameter, LDL: Low-density lipoprotein, LVW: left ventricular wall, Mb: myoglobin, MCT: medium-chain triglycerides, MRI: magnetic resonance imaging, NT-proBNP: N-terminal prohormone of brain natriuretic peptide, UCCS: uncooked cornstarch, TC: total cholesterol, TG: triglycerides, TnT: Troponin T, US: ultrasound, ω-3FA: omega-3 fatty acids.

Supplementary File S3.

Patient number	**Age at start** (years), **gender** (M/F) **and GSD type**	**Genotype** gene (allele 1/ allele 2) OR **Enzyme test**	**Indication to start dietary intervention**	**Dietary intervention and diet composition** (amount of MCT/fat per day, amount of carbo per day (specify amount of UCCS), amount of protein per day (% of total daily intake, daily g)	**Duration** (months)	**Outcome parameters: laboratory results** (glucose/lactate/Ketones/ acetoacetate/BHB/TC/TG/ HDL/LDL: mmol/L, insulin: mU/L, uric acid: mg/dL, AST/ALT/CK: U/L, CK-MB: ng/mLFFA: µmol/L,TnT/NT-proBNP: ng/L, Mb: µmol/L)		**Outcome parameters: diagnostic imaging** Liver lengths, IVS/LVW thickness: mm	**Outcome parameters Clinical picture,side effects** Weight:Kg, Height: Cm, BMI: Kg/m2
						Before	*After*		
15	37 F IIIa	AGL, c.753_756del (p.Asp251fs)	Exercise intolerance Overweight Cardiomyopathy	MCT replacement UCCS replaced with MCT-emulsion Period 1. 1400 Kcal/day, 12.8% carbohydrates, 63.5% lipids (60% MCT), 24.1% proteins Period 2. 1900 Kcal/day, 11% carbohydrates, 47% lipids (60% MCT), 41% proteins	31	Glucose: 4-5 Insulin: n/a Acetoacetate: 0-0.02 BHB: 0-0.2 TC: 2.3-3.1 TG: 0.51-1.06 CK: 1010-4372 Other: AST:91-196, ALT: 60-119, HDL: 0.8-1.2, LDL: 1.1-1.8, FFA: n/a, CK-MB: 68-118, TnT, NT-proBNP: n/a	Glucose: 5-6.5 Insulin: 4.1-23.8 Acetoacetate: 0.02-0.20 BHB: 0.02-0.25 TC: 3.2-3.9 TG: 0.69-1.23 CK:775-2480 Other: AST:88-126, ALT: 53-86, HDL: 1-1.3, LDL: 1.5-2, FFA:74-807, CK-MB: 35-60, TnT: n/a, NT-proBNP: 3230-4899	*BEFORE* Liver US: hepatomegaly (CC:16 cm), no adenoma Cardiac US: IVS thickness: 14 , LVW thickness: 17.6 , EF: 50%. Mitral insufficiency gr III. Hypertrophic cardiomyopathy with impaired diastolic function. ECG: normal *AFTER* Liver US: hepatomegaly (CC: 16 cm), no adenoma. Cardiac US: IVS thickness: 14, LVW thickness: 18.3, EF: 50%. Mitraclip in situ. Hypertrophic restricted cardiomyopathy. ECG: left axis deviation.	*BEFORE* Weight: 98 Height: 172 BMI: 33 (+ 3.0 SD) *AFTER* Weight: 99 Height: 171 (+0.04 SD) BMI: 33.9 (+3.13 SD). Overall muscle strength improved during period 2 when measured with dynamometry. No difference on muscle ultrasound density.

16	35 F IIIa	AGL, c.4529dup (p.Tyr1510Ter*)	Muscle weakness Not liking UCCS Overweight	MCT replacement UCCS replaced with MCT-emulsion 2614 Kcal/day, 15% carbohydrates, 66% lipids (75% MCT), 19% proteins	36	Glucose: 4-5 Insulin: n/a Acetoacetate: 0-0.02 BHB: 0-0.2 TC:4.20-5.7 TG: 0.76-1.63 CK: 898-3408 Other: AST:123-338, ALT: 94-215, HDL:1.2-2.1, LDL: 2.3-3.3, FFA: n/a, CK-MB, TnT, NT-proBNP: n/a	Glucose: 4.5-6.8 Insulin: 2-48.7 Acetoacetate: 0.02-0.81 BHB: 0.03-2.57 TC: 4.1-4.6 TG: 0.73-1.05 CK:749-1173 Other: AST: 99-118, ALT: 86-118, HDL:1.2-1.6, LDL: 2.5-2.8, FFA: 154-463, CK-MB, TnT: n/a, NT-proBNP: 84-125	*BEFORE* Liver US: hepatomegaly (CC:20 cm), no adenoma. Cardiac US: IVS thickness: 12 , LVW thickness: 13.1 , EF: 60%. Minimal left ventricle hypertrophy. ECG: normal. *AFTER* Liver US: hepatomegaly (CC:18 cm), no adenoma. Cardiac US: IVS thickness: 13 , LVW thickness: 11.2 , EF:55-60% . Minimal left ventricle hypertrophy.	*BEFORE* Weight: 81.3 Height: 178 BMI: 25.7 *AFTER* Weight: 79.5 Height: 178 BMI: 25.1 (+1.21 SD). Muscle dynamometry showed a progressive myopathy affecting especially the proximal muscles. Worsening of muscle weakness when on MCT diet. Muscle ultrasound showed decrease in muscle mass and increase in muscle density.
17	6 M IIIa	AGL, c.3235C>T (p.Gln1079Ter*)	Severe cardiomyopathy	High fat diet UCCS withdrawn 1726 Kcal/day, 9% (37 g/day) carbohydrates, 77% (147 g/day) lipids, 14% (62 g/day) proteins	39	Glucose: 3-5.5 Insulin: n/a Ketones: 0.0 TC: 3.9-5.3 TG: 1.6-4.6 CK: 145-534 Other: AST: 126-437, ALT: 139-539, HDL: 0.5-0.7, LDL: 1.4-4.1, FFA, CK-MB, TnT, NT-proBNP: n/a	Glucose: 3.4-5.5 Insulin:0.5-22.6 Ketones: 0-0.2 TC: 4-4.8 TG: 2-3 CK:58-449 Other: AST: 124-510, ALT: 145-598, HDL: 0.5-0.8, LDL: 2.7-3.7 mg/dL, FFA, CK-MB, TnT, NT-proBNP: n/a	*BEFORE* Liver US: hepatomegaly, fatty liver Cardiac US: IVS 10-19, left ventricular mass +4SD Severe hypertrophic cardiomyopathy with intraventricular and subaortic obstruction, diastolic dysfunction grade III ECG. n/a *AFTER* Liver US: hepatomegaly, fatty liver Cardiac US: IVS 8.2, left ventricular mass +1.5 SD Improvement of cardiomyopathy with	*BEFORE* Weight 25 (1 SD) Height 116 (-0.3 SD) BMI: 18.6 (1.6 SD) Muscle strength: n/a *AFTER* Weight 34.4 (0.7 SD) Height: 144 (1.2 SD) BMI 16.6 (0.1 SD) Muscle strength: n/a

								reduction of ventricular mass and no obstruction ECG: n/a	
18	4 F IIIa	AGL, arr[GRCh37] 1p21.2(100274994_100623922)x1 pat/ c.4202G>A (p.Trp1401Ter*)	Cardiomyopathy	High fat diet UCCS withdrawn 1770 Kcal/day, 13% (57 g/day) carbohydrates, 68% (134 g/day) lipids, 19% (83 g/day) proteins	48 The prescribed ratio was not fully respected (0.8:1 instead of 0.9:1)	Glucose: 4-4.9 Insulin: 1.42-5.48 Ketones: 0-0.1 TC: 6.4-7.9 TG: 4.2-4.4 CK: 878-1305 Other: AST: 179-438, ALT: 226-522, HDL: 0.59-0.60, LDL: 4.4-5.8, FFA, CK-MB, TnT, NT-proBNP: n/a	Glucose: 2.6-5.2 Insulin:0.5-5.25 Ketones: 0.1-0.9 TC: 6.2-9.5 TG: 2.4-9.8 CK:133-711 Other: AST: 197-420, ALT: 248-560, HDL: 0.75-1.11, LDL: 2.7-8.7, FFA, CK-MB, TnT, NT-proBNP: n/a	*BEFORE* Liver US: hepatomegaly, fatty liver, no adenoma Cardiac US: IVS:10, mild hypertrophic cardiomyopathy ECG. n/a *AFTER* Liver US: hepatomegaly, fatty liver, no adenoma. Cardiac US: IVS: 4.8, regression of hypertrophic cardiomyopathy ECG. n/a	*BEFORE* Weight 15.9 (0.3 SD) Height 93 (-1.5 SD) BMI: 18.3 (1.8 SD) Muscle strength: n/a *AFTER* Weight 20.8 (-1.2 SD) Height: 108 (-3.5 SD) BMI 17.8 (0.9 SD) Muscle strength: n/a
19	5 F IIIa	AGL, c.3988G>A/c.4332insAA (p.Trp1330*/p.Gly1445Lysfs*27)	Cardiomyopathy Myopathy	High fat diet UCCS withdrawn 1536 Kcal/day, 12% (43 g/day) carbohydrates, 65% (111 g/day) lipids, 23% (89 g/day) proteins	24 Prescribed ratio was not respected (0,6:1 instead of 0,9:1)	Glucose: 2.4-5.6 Insulin: n/a Ketones: n/a TC: 4-4.9mg/dL TG: 2.3-4.9 CK: 622-2938 Other: AST: 236-509, ALT: 283-531, HDL: 0.6-0.75, LDL: 1.5-3.6, FFA, CK-MB, TnT, NT-proBNP: n/a	Glucose: 2.8-4.2 Insulin:0.5-1.35 Ketones: 0.2-0.3 TC: 4.75-6.8 TG: 2.1-4.3 CK:643-1692 Other: AST: 694-1382, ALT: 489-824, HDL: 0.7-0.9, LDL: 3.3-5.9, FFA, CK-MB, TnT, NT-proBNP: n/a	*BEFORE* Liver US: hepatomegaly, fatty liver Cardiac US: IVS 8.5mm, moderate hypertrophic cardiomyopathy ECG: n/a *AFTER* Liver US: hepatomegaly, fatty liver Cardiac US: IVS 6.1mm, regression of hypertrophic cardiomyopathy ECG: n/a	*BEFORE* Weight 18.5 (0.6 SD) Height 100 (-1 SD) BMI: 18.3 (1.8 SD) Muscle strength: n/a *AFTER* Weight 24.8 (0.8 SD) Height: 116 (-0.5 SD) BMI 18.4 (1.4 SD) Muscle strength: n/a

20	33 F III	Amylo-1,6-glucosidase: 0	Poor metabolic control	MCT supplementation Low carbohydrate diet enriched in MCT. UCCS withdrawn. 2575 Kcal/day, 10% carbohydrates , 62% lipids (11% MCT, 20 g/day), 19% proteins	24	Glucose: 4.2-4.9 Insulin: 1.2-2.3 Ketones: n/a TC: 6.6-7.2 TG: 2-3.3 CK: 2993-3032 Other: AST: 9-111, ALT: 32-195, HDL: 0.8-0.9, LDL: 2.1-2.8, FFA, CK-MB, TnT, NT-proBNP: n/a	Glucose: 4.3-4.6 Insulin:1.2-2.3 Ketones: n/a TC: 6.1-7.4 TG: 2.2-3.2 CK: 998-1143 Other: AST: 82-141, ALT, HDL, LDL, FFA, CK-MB, TnT, NT-proBNP: n/a	*BEFORE* Liver US: hepatomegaly, fatty liver Cardiac US: normal IVS thickness ECG: n/a *AFTER* Liver US: hepatomegaly, fatty liver Cardiac US: normal IVS thickness ECG: n/a	*BEFORE* Weight: 66 (0.7 SD) Height: 171 (1.2 SD) BMI: 22.6 (0.2 SD) *AFTER* Weight: 66 (0.7 SD) Height: 171 (1.2 SD) BMI: 22.6 (0.2 SD) Muscle strength: n/a
21	33 M IIIa	Amylo-1,6-glucosidase: 0	Severe cardiomyopathy Poor metabolic control	MCT supplementation Low carbohydrate diet enriched in MCT. UCCS withdrawn. 2005 Kcal/day, 25% carbohydrates, 50% lipids (20% MCT, 20 g/day), 25% proteins	24	Glucose: 4.2-5.1 Insulin: 0.8-5.8 Ketones: n/a TC: 3.3-4.7 TG: 1.5-2.6 CK: 803-3887 Other: AST: 118-377, ALT: 112-501, HDL: 0.5-0.9, LDL: 2.7-3.3, FFA, CK-MB, TnT: n/a, NT-proBNP: 1260-5850	Glucose: 3.6-5.3 Insulin: 0.5-4.3 Ketones: n/a TC:3.1-5.7 TG:0.8-1.7 CK: 327-1154 Other: AST: 86-110, ALT: 67-128, HDL: 0.6-1.1 mg/dl, LDL: 2.4-4.2, FFA, CK-MB, TnT: n/a , NT-proBNP: 2010-3380	*BEFORE* Liver US: hepatomegaly, fatty liver Cardiac US: IVS thickness: 17, LVW thickness: 12, EF:27% ECG: n/a *AFTER* Liver US: hepatomegaly, fatty liver Cardiac US: IVS thickness: 15, LVW thickness: 10, EF:47% ECG: n/a	*BEFORE* Weight: 68.5 (-0.2 SD) Height: 168 (-1.2 SD) BMI: 24.3 (0.4 SD) *AFTER* Weight: 64.3 (-0.6 SD) Height: 168 (-1.2 SD) BMI: 22.8 (-0.1 SD) Better compliance to the diet Improved muscle strength Previous ischemic stroke
22	23 F IIIa	AGL, c.2147delG /c.3216_3217delGA	Fatigue Exercise intolerance Refusal of night meals and UCCS	High fat diet UCCS withdrawn 1770 Kcal/day, 6% carbohydrates, 76% lipids, 18% proteins	9	Glucose: 3 Insulin: n/a Ketones: n/a TC: 5.4 TG: 1.1 CK: 867-1918 Other: AST: 111-144, ALT: 143-190, HDL: 1.08, LDL: 3.7, FFA, CK-MB, TnT, NT-proBNP: n/a	Glucose: 2.8-3.9 Insulin: n/a Ketones: 0.2-1.4 TC: 4.5 TG:1.1 CK: 244-511 Other: AST: 61-82, ALT: 48-76, HDL: 1.16, LDL: 2.8, FFA, CK-MB, TnT, NT-proBNP: n/a	*BEFORE* Liver ultrasound: hepatomegaly, fatty liver Cardiac US: IVS thickness: 11 ECG: normal *AFTER* Liver ultrasound: hepatomegaly, no fatty liver Cardiac US: IVS thickness 11 ECG: normal	*BEFORE* Weight: 60 (0.2 SD) Height: n/a BMI: n/a *AFTER* Weight: 55 (-0.3 SD) Height: n/a BMI: n/a Muscle strength: n/a

23	7 F IIIa	AGL c.3444T>A/ 4347-1G>T	Cardiomyopathy Excercise intolerance	High fat diet UCCS withdrawn. 1550 Kcal/day, 18% carbohydrates, 54% lipids, 28% proteins	60	Glucose: 3.4-6.2 Insulin: n/a Ketones (plasma): n/a TC: 5.5-5.8 TG: 1.7-4.7 CK: 849-1693 Other: AST: 85-197, ALT: 193-280, HDL: 0.4-0.5, LDL: 3.9-4.1, FFA: n/a, CK-MB: 42-50, TnT: n/a NT-proBNP: n/a, Mb: 139-160	Glucose: 4-6.2 Insulin: n/a Ketones (plasma): n/a TC: 5.5-6.8 TG: 2.4-4.9 CK: 570-1211 Other: AST: 98-227, ALT: 185-474, HDL: 0.5-0.65, LDL: 3.3-3.7, FFA: n/a, CK-MB: 17-19, TnT, NT-proBNP: n/a, Mb: 56-124	*BEFORE* Liver US :hepatomegaly (LD: 110), fatty liver. Cardiac US: : mild ventricular hypertrophy; IVS thickness: 9, LVW thickness: 7 ECG: biventricular hypertrophy *AFTER* Liver US :hepatomegaly (LD:150), fatty liver. Cardiac US: no ventricular hypertrophy; IVS thickness: 6.2, LVW thickness: 6.2 ECG: mild ventricular hypertrophy	*BEFORE* Weight: n/a (-1.3 SD) Height: n/a (-1.3 SD) BMI: n/a *AFTER* Weight: n/a (-1.9 SD) Height: n/a (-1.9 SD) BMI: n/a Mild improvement on physical activity. The family was not able to increase lipids over 54%. Pubertal delay
24	41 M IIIa	AGL, c.2919_2920insTTGG / c.2936delG	Fatigue Exercise intolerance Cardiomyopathy	High fat diet UCCS withdrawn. 1800 Kcal/day, 16% carbohydrates, 60% lipids, 23% proteins	6 Lost to follow-up	Glucose: 4-5.4 Insulin: n/a Ketones : n/a TC: 3.9-5 TG: 1.15-2.4 CK: 4640-9032 Other: AST: 136-221, ALT: 87-129, HDL: 0.85-1.06, LDL:1.97-3.62, FFA: n/a, CK-MB: 60-190, TnT: 49, NT-proBNP: n/a, Mb: 714-1119	Glucose: 3.9-5.6 Insulin: n/a Ketones: n/a TC: 4.34-5.3 TG: 3.2-5.3 CK: 4274-5784 Other: AST: 140-149, ALT: 84-91, HDL: 0.9-1, LDL:3-3.1, FFA: n/a, CK-MB: 75-108, TnT: 27-41, NT-proBNP: n/a, Mb: 482-644	*BEFORE* Liver US: hepatomegaly, severe fatty liver, pericolecistic areas of hypoechogenicity. Cardiac US: mild concentric hypertrophy; IVS thickness: 11, LVW: 11 ECG: left ventricular hypertrophy. *AFTER* Liver US:hepatomegaly, moderate fatty liver, no focal areas of hypoechogenicity. Cardiac US: mild hypertrophy, IVS thickness: 11, LVW thickness: 10	*BEFORE* Weight: 90 (1.3 SD) Height: n/a BMI: n/a *AFTER* Weight: 83.5 (1 SD) Height: n/a BMI: n/a Less fatigue in climbing stairs (subjective)

								ECG: Left ventricular hypertrophy	
25	1 F III	AGL, c.3911dupA (p.Asn1304Lysfs*7)	Severe hypotonia, Delayed motor skills Developmental delay	High fat diet UCCS withdrawn 1600 Kcal/day, 14-25% carbohydrates, 40-50% lipids, 23-30% proteins	60	Glucose: 2.4-4.3 Insulin: n/a Ketones (plasma): n/a TC: 5.1-5.9 TG: 3.5-5.2 CK: 63-72 Other: AST: 222-534, ALT: 246-498, HDL, LDL, FFA, CK-MB, TnT, NT-proBNP: n/a	Glucose: 3-5 Insulin: n/a Ketones (plasma): n/a TC: 5.9-7.5 TG: 4.5-8.1 CK: 86-91 Other: AST: 237-2818, ALT: 334-1030, HDL, LDL, FFA, CK-MB, TnT, NT-proBNP: n/a	*BEFORE* Liver US: hepatomegaly, moderate-severe fatty liver Cardiac US: normal ECG: normal. *AFTER* Liver US: hepatomegaly, moderate-severe fatty liver Cardiac US: normal ECG: normal	*BEFORE* Weight: n/a (-0.7 SD) Height: n/a (-2.1 SD) BMI: n/a *AFTER* Weight: n/a (-0.7 SD) Height: n/a (-2.1 SD) BMI: n/a Mild improvement on physical activity. The family was not able to increase lipids over 50%.
26	4 M III	AGL, c.2590C>T (p.Arg864Ter*)	Poor metabolic control	High fat diet UCCS withdrawn 1300 Kcal/day, 18-20 % carbohydrates, 55-60 % lipids, 25-28% proteins	36 Poor compliance Lost to follow-up	Glucose: 2.6-4.7 Insulin: 2.9-7.9 Ketones: n/a TC: 0.3-7.7 TG: 5.4-9.3 CK: 85-171 Other: AST: 469-1020, ALT: 366-475, HDL: 0.6, LDL: n/a, FFA, CK-MB, TnT, NT-proBNP: n/a	Glucose: 3.2-3.8 Insulin: n/a Ketones: n/a TC: 5.8-6.9 TG: 4.5-9 CK: 133 Other: AST: 439-1446, ALT: 426-766, HDL: 0.5-0.7, LDL: 3.5, FFA, CK-MB, TnT, NT-proBNP: n/a	*BEFORE* Liver US: severe hepatomegaly, fatty liver Cardiac US: normal ECG: normal *AFTER* Liver US: severe hepatomegaly, fatty liver Cardiac US: normal ECG: normal	*BEFORE* Weight: 14.7 (0.3 SD) Height: 84 (-3 SD) BMI: 20.8 (3 SD) *AFTER* Weight: 19.5 (-1.6 SD) Height: 108.5 (-3 SD) BMI: 16.6 (0.5 SD) Muscle strength: n/a
27	36 F IIIa	AGL, c.2681+1G>T	Muscle weakness Exercise intolerance	High fat diet 1300-1500 Kcal /day,34% (110-137 g/day) carbohydrates, 36-37% (52-62 g/day) lipids, 29-30% (95-108 g/day) proteins	60 Poor compliance	Glucose: 3.7-6.2 Insulin: 2.9-7.9 Ketones: n/a TC: 2.6-5.1 TG: 1.2-1.7 CK: 792-2616 Other: AST: 65-114, ALT: 42-71, HDL: 1.1-1.5, LDL: 2.6-3.1, FFA, CK-MB, TnT, NT-proBNP: n/a	Glucose: 3.9-6.4 Insulin: 2.9-7.9 Ketones: n/a TC: 4-5.3 TG: 1.3-1.7 CK: 587-1400 Other: AST: 54-112, ALT: 31-67, HDL:1-1.4, LDL: 2.5-3.6,FFA, CK-MB, TnT, NT-proBNP: n/a	*BEFORE* Liver US: hepatomegaly, fatty liver, cirrhosis Cardiac US: normal ECG: n/a *AFTER* Liver US: hepatomegaly, fatty liver, cirrhosis Cardiac US: normal ECG: n/a	*BEFORE* Weight: 66.7 (0.7 SD) Height: 166 (0.4 SD) BMI: 24.2 (0.6 SD) *AFTER* Weight: 73 (1.2 SD) Height: 166 (0.4 SD) BMI: 26.5 (1 SD) Muscle strength: n/a Refused to further increase lipid intake

28	1 F IIIa	AGL, c.2590C>T (p.Arg864Ter*)	Cardiomyopathy	High fat diet Never taken UCCS 710 Kcal/day, 11% carbohydrates, 70% lipids, 19% proteins	12 Lost to follow-up	Glucose: 2.3-5.4 Insulin: 0.3-6.6 Ketones: n/a TC: 4.2 TG: 3.9 CK: 430 Other: AST: 225, ALT: 240, HDL, LDL, FFA, CK-MB, TnT, NT-proBNP: n/a	Glucose: 3.6-4.8 Insulin: 23.4 Ketones: n/a TC: 3.9-5.5 TG: 4.1-10.3 CK: 181-295 Other: AST: 205, ALT: 265, HDL, LDL, FFA, CK-MB, TnT, NT-proBNP: n/a	*BEFORE* Liver US: hepatomegaly, fatty liver Cardiac US: obstructive hypertrophy; IVS thickness: 4.5, LVW thickness: 4.2 ECG: n/a *AFTER* Liver US: hepatomegaly, fatty liver Cardiac US: reduced hypertrophy (no more obstructive); IVS thickness: 4.2, LVW thickness: 4.4 ECG: n/a	*BEFORE* Weight: 7 (0.7 SD) Height: 60 (1 SD) BMI: 19.4 (2 SD) *AFTER* Weight: 9 (-0.7 SD) Height: 71 (-1.1 SD) BMI: 17.9 (0.1 SD) Muscle strength: n/a

Supplementary File S3. Table unpublished cases.

ALT: alanine aminotransferase, AST: aspartate aminotransferase, BHB: beta -hydroxybutyrate, CK: creatine kinase, CK-MB: creatine kinase isoenzyme MB, ECG: electrocardiogram, FFA: free fatty acids, HDL:High-density lipoprotein, IVS: interventricular septum, LCT: long-chain triglycerides, LD: liver longitudinal diameter, LDL: Low-density lipoprotein, LVW: left ventricular wall, Mb: myoglobin, MCT: medium-chain triglycerides, MRI: magnetic resonance imaging, NT-proBNP: N-terminal prohormone of brain natriuretic peptide, UCCS: uncooked cornstarch, TC: total cholesterolTG: triglycerides, TnT: Troponin T, US: ultrasound, ω-3FA: omega-3 fatty acids.

Supplementary file S4

	Δ Glucose (%)	Δ Insulin (%)	Δ Ketones (%)	Δ Total Cholesterol (%)	Δ Triglycerides (%)	Δ AST (%)	Δ ALT (%)	Δ CK (%)
P1	-	-	+	-	-	-	-	-
P2	-	-	+	-	-	-	-	-
P3	-	-	-	-	-	-	-	-31
P4	+6	-	-	+24	-1	-21	-6	-88
P5	+7	-	-	-9	+30	-71	-37	-51
P8	-	-	+2800	-	+39	-	-	-77
P9	-	-	+6000	-	-	-	-	-27
P10	-	-	-	-	-	-	-	-
P11	-	-	-	-	-	-	-	-
P12	-16	-55	+1000	0	0	0	0	-^
P13	-	-	-	0#	0	-	-	-
P14	-	-	-	-+	-	-	-	-
P15*	+28	-	+463	+33	+26	-25	-22	-33
P16*	+24	-	+2600	-12	-18	-53	-34	-41
P17	+7	-	+250	-5	-20	+13	10	-38
P18	-16	-35	+450	+9	+41	+0	+8	-65
P19	-4	-	-	+29	-11	+179	+61	-20
P20*	-2	0	-	-3	+4	-	-	-65
P21*	-6	-32	-	+7	-42	-60	-68	-66
P22	+12	-	-	-17	0	-44	-63	-73
P23	+9	-	-	+9	+22	+15	+39	-31
P24	0	-	-	+9	+93	-19	-19	-22
P25	+21	-	-	+22	+42	+304	+83	+32"
P26	+2	-	-	+915	-10	+27	+42	0"
P27	+4	0	-	+31	0	-7	-13	-36
P28	+23	+580	-	+12	+85	-9	+10	-45
Stable (%)	***59***	***33***	***0***	***50***	***37***	***29***	***31***	***5***
Increased (%)	***29***	***17***	***100***	***39***	***42***	***29***	***31***	***5***
Decreased (%)	***12***	***50***	***0***	***11***	***21***	***41***	***38***	***90***

Supplemental file S4, Individual percentual changes in laboratory parameters of metabolic control for all GSDIII patients.

* high fat diet + MCT supplementation, # increased after MCT supplementation," within the reference range, + increased, no raw data available, ^ decreased, no raw data available

Supplementary file S5

Recommendations for clinical follow-up of dietary lipid manipulations in patients with glycogen storage diseases type III.

Based on: 'Dietary lipids in glycogen storage disease type III: a systematic literature study, case studies and future recommendations.'

Introduction

Prospectively designed dietary intervention studies are strongly needed to strengthen our knowledge on dietary management in hepatic GSD. With this recommendations document we aim to provide guidance to clinicians and researchers in the field of metabolic disease who intend to study a dietary lipid manipulation, either in clinical management or in the setting of a clinical trial.

Target audience

The present recommendation document is addressed to all health care professionals (physicians and dieticians) who take care of hepatic GSD patients.

Disclaimer

Recommendations are derived from retrospective data collection. Recommendations only refer to dietary lipid manipulations in GSD type IIIa. However, general principles provided here could help arranging a dietary lipid manipulation in all hepatic GSD.
To date, recommendations on dietary management from international management guidelines are still the key in management in hepatic GSD patients.

Index

A- General study recommendations

I. Patient selection

1) Reasons to start dietary lipid manipulation

Development of cardiomyopathy and/or muscle weakness despite optimal dietary regimen[1].

2) Rationale to start dietary lipid manipulation

Reverse/improve cardiomyopathy and/or myopathy

3) Check contra-indications

-Liver and/or kidney dysfunction

-Osteoporosis

-Current pregnancy, or breastfeeding

-Diabetes mellitus (excluding isolated insulin-resistance)

II. Dietary intervention

- Interventions should be standardized. The amount of fat should be uniform (e.g. high-fat diet, ketogenic diet) as well as the type of fat administered (e.g. high-fat only, high-fat + MCT) and duration of the supplementation.

- Three-day food diaries are recommended to study dietary compliance and analyze exact distributions of macronutrients.

- Amount and duration herein suggested are based on the results of the present study (median value among patients showing beneficial effect)

[1]*Guidelines recommend a minimal protein intake of 3 grams per kilogram bodyweight in pediatric GSDIIIa patients.*

III. Outcome measures

Outcome measures should be uniform and blood samples should be taken under similar conditions (i.e. specific number of hours after meal and/or specific time during the day).

Standard outcome markers should be assessed to make future studies comparable. Taking into account the results of the present study, specific markers are suggested on the next pages.

Improvement should be defined if:

- [CK] decreased by 10% (or more) or normalized
- IVSd Z-scores decreased or normalized

IV. Recommendations on safety

In compliance with Good Clinical Practice (GCP) all adverse events should carefully be assessed and documented.

Possible adverse events:

-Hypoglycemia

-Gastrointestinal symptoms

B- Lipid manipulation in GSDIIIa patients

Dietary intervention: high fat diet

Amount: lipids 60% of daily E-% (children 6 g/kg/day, adults 1.7 g/kg/day)

Minimal duration of intervention: 24 months [range: 3 – 60]

Outcome measures:

Clinical markers: height SDS, weight SDS, BMI, clinical picture (e.g. fatigue, exercise intolerance, dyspnea, muscle strength), comorbidities, QoL questionnaire, International physical activity questionnaire.

Biochemical markers:

- Blood glucose homeostasis: home site continuous glucose monitoring. Number of hypoglycemia (n), mean [range] glucose concentration, percentage of the day [glucose] < 4.0 mmol/L, percentage of the day [glucose] > 8.0 mmol/L. Mean morning ketone concentrations (mmol/L) as assessed with handheld device.

-Blood markers*:* beta-hydroxybutyrate, acetoacetate, triglycerides, total cholesterol, HDL, LDL, FFA, insulin, AST, ALT, CK, CK-MB, NT-proBNP, TnT, calcium, phosphorus, alkaline phosphatase, parathyroid hormone, calcitonin, osteocalcin, vitamin D, prealbumin, creatinine, estimated glomerular filtration rate, vitamins*, minerals*

- urine: proteinuria, microalbuminuria

- metabolic investigations: plasma acylcarnitines, plasma biotinidase, urine organic acids, urine glucose tetrasaccharide

Imaging markers: liver ultrasound (liver size in cm, liver longitudinal diameter), cardiac ultrasound (IVS thickness, SF, outflow obstruction, diastolic function parameters, left ventricular mass), muscle ultrasound, bone mineral density (DXA), liver/heart/muscle MRI*

Muscle markers: six-minute walking test, muscle ultrasound (muscle density for all muscle groups), dynamometry (strength Z-scores according to references) *

Dietary markers: diet composition (total Kcal/day, E-% and exact amount (g/kg/day) for each macronutrient. Dietary compliance; three-day food diary.

Frequency of follow-up:

Check clinical, blood, and dietary markers monthly for the first 3 months. According to individual outcomes frequency of follow-up can be expanded to every 6 months. Specific metabolic investigations, muscle markers and imaging measures should be at least assessed at the beginning and at the end of the intervention.

* consider

Supplementary File S5. Recommendations for clinical follow-up of dietary lipid manipulations in patients with glycogen storage diseases type III.

Chapter 5.

A generic emergency protocol for patients with inborn errors of metabolism causing fasting intolerance: a retrospective, single-center study and the generation of www.emergencyprotocol.net

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ABSTRACT

Patients with inborn errors of metabolism causing fasting intolerance can experience acute metabolic decompensations. Long-term data on outcomes using emergency letters are lacking. This is a retrospective, observational, single-center study of the use of emergency letters based on a generic emergency protocol in patients with hepatic glycogen storage diseases (GSD) or fatty acid oxidation disorders (FAOD). Data on hospital admissions, initial laboratory results, and serious adverse events were collected. Subsequently, the website www.emergencyprotocol.net was generated in the context of the CONNECT MetabERN eHealth project following multiple meetings, protocol revisions, and translations. Representing 470 emergency protocol years, 127 hospital admissions were documented in 54/128 (42%) patients who made use of emergency letters generated based on the generic emergency protocol. Hypoglycemia (here defined as glucose concentration < 3.9 mmol/L) was reported in only 15% of hospital admissions and was uncommon in patients with ketotic GSD and patients with FAOD aged >5 years. Convulsions, coma, or death was not documented. By providing basic information, emergency letters for individual patients with hepatic GSD or the main FAOD can be generated at www.emergencyprotocol.net, in nine different languages. Generic emergency protocols are safe and easy for home management by the caregivers and the first hour in-hospital management to prevent metabolic emergencies in patients with hepatic GSD and medium-chain Acyl CoA dehydrogenase deficiency. The website www.emergencyprotocol.net is designed to support families and healthcare providers to generate personalized emergency letters for patients with hepatic GSD and the main FAOD.

INTRODUCTION

Fasting intolerance is a critical feature of several rare inborn errors of metabolism (IEMs), including hepatic glycogen storage diseases (GSD) and fatty acid oxidation disorders (FAOD). If inadequately treated or not prevented, fasting intolerance can lead to acute lifethreatening complications, such as (severe) hypoglycemia; metabolic acidosis; and eventually convulsions, coma, or death. For these reasons, it is crucially important that following the diagnosis of such IEMs, metabolic decompensations and emergencies are effectively prevented, risk situations are recognized in a timely fashion, and that prompt safe treatment is established rapidly to stop and reverse catabolism[1]. Catabolism is a key trigger of clinical and metabolic decompensation and is often induced by (the combination of) fever, prolonged fasting (e.g, decreased oral/enteral intake due to illnesses, or surgery-related fasting protocols), increased enteral losses resulting from vomiting and/or diarrhea, or alcohol excess. Therefore, the key initial measure in IEM emergency protocols is to stop catabolism and promoteanabolism[2-4].

Information about IEM-specific emergency protocols is available at multiple online resources, such as the New England Consortium of Metabolic Programs, British Inherited Metabolic Diseases group (https:// www.newenglandconsortium.org/acute-illness), and INVEST (in Dutch: Internisten voor volwassenen met een erfelijke stofwisselingsziekte) (https://investof.nl/noodprotocollen/), and scientific publications, such as for urea cycle defects[5], maple syrup urine disease[6], organic acidemias[7], FAOD[8], or incorporated in guidelines for glutaric aciduria type I[9] and subtypes of hepatic GSD[10-14]. These guidelines and emergency protocols are largely based on expert opinions. Follow-up studies are not available on practices of emergency treatments.

The question “How should sickness and emergency situations be managed for patients with liver GSD?” has been recently ranked as a top priority for research in the international priority setting partnership for liver GSD[15]. Given the geographical distance between centers of expertise and the home address of patients with IEMs, local or regional healthcare providers are often the ones starting the initial emergency treatment. However, it is recognized that most (pediatrics) residents/physicians consider they have insufficient knowledge to start emergency treatment for patients with IEMs in the absence of expert advice or written protocols[16].Finally, keeping emergency letters up to date for large cohorts of patients with IEMs can be labor intensive.

The aim of this report is 2-fold. First, we describe a retrospective, observational, single-center study about the application of emergency letters based on a simple generic emergency protocol for patients with IEMs causing fasting intolerance. Second, we report the development of the website www.emergencyprotocol.net, where personalized emergency letters can be automatically created for and by (families and) patients with FAOD and hepatic GSD.

METHODS

Ethics

For the retrospective, observational, single-center medical chart review in patients with hepatic GSD or FAOD, the Medical Ethical Committee of the University Medical Center Groningen (UMCG)

stated that the Medical Research Involving Human Subjects Act was not applicable. Official study approval by the Medical Ethical Committee was not required (METc 2019/119) because the study involved retrospective, anonymous data collection of standardized care.

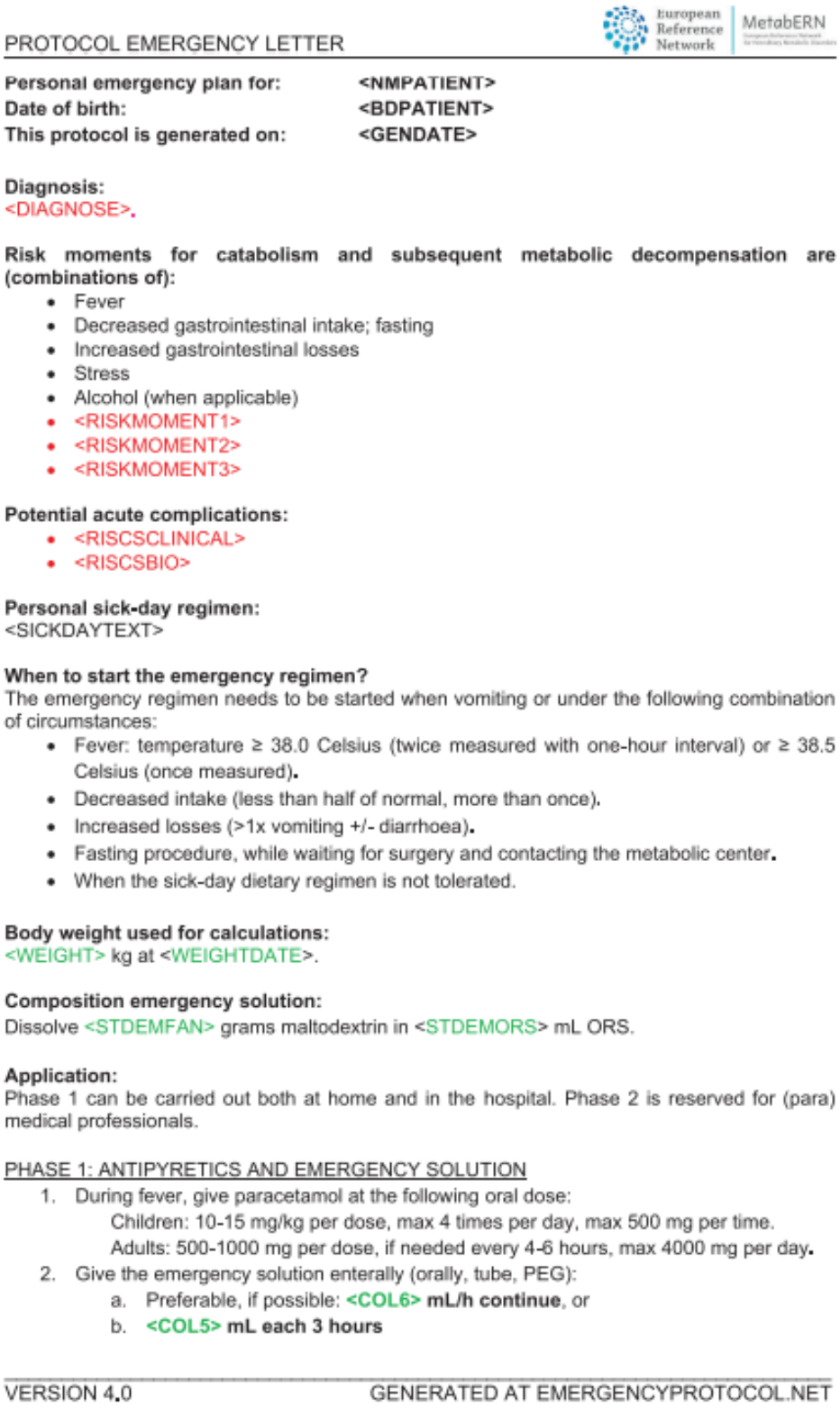
The UMCG generic emergency letters

Since February 2014, individualized, IEM-specific emergency letters have been replaced by emergency letters based on the generic "Emergency protocol for children at risk for acute metabolic decompensation" at UMCG (Figure 1). Patients (and their families) have been instructed at the outpatient clinic and in hospital about the prevention of catabolism, how to use the emergency letter, and how to directly seek for healthcare professional support during acute hospital admissions. In brief, the protocol includes two phases. Phase I can be initiated by caregivers or patients at home under the following circumstances: (1) more than one-time vomiting, or (2) a combination of (a) fever >38.5C, (b) decreased enteral intake, and (c) increased enteral losses. Phase I prescribes (a) a weight-dependent dose of paracetamol (acetaminophen) to reduce fever, and (b) the administration of the "emergency solution" to provide enough carbohydrates.

Before 2014, several patients with IEMs at UMCG had reported emergency treatments in local hospitals, which were complicated by hypoglycemia after administration of oral rehydration salt solutions (relatively low in calories and thus unable to stop or reverse catabolism). Therefore, in our generic protocol, we have ensured that total fluid maintenance requirements per 24 hours include glucose polymer enrichment, as described by Van Hove et al[4], with slight simplifications. In this so-called emergency solution, total carbohydrate prescriptions are based on experimental data on carbohydrate requirements using stable isotopes[17]. The emergency solution provides, in 500 mL of oral rehydration salt solution (ORS), 75 g of maltodextrin (15 g per 100 mL of solution) for patients weighing up to 12 kg and 110 g (20 g per 100 mL of solution) for patients weighing 12 kg or more, respectively. A stand-alone product is currently lacking. Therefore, in the Netherlands, the maltodextrin is currently provided by the metabolic dietitian through a facility company, while the ORS can be purchased in local drugstores. The protocol is updated when the body weight changes more than 10%. If phase I is not tolerated or ineffective, the protocol moves to phase II. For phase II, local physicians (pediatricians, internal medicine specialists) are asked to provide patients with direct access to the emergency or general department to ensure prompt enteral or parenteral carbohydrate administration. The protocol advises physicians to contact the metabolic consultant on call when the initial laboratory results are available, usually within 1 hour after hospital admission. At this point, the generic approach and emergency letter treatment change into personalized management plan, based on the specific IEM and patient.

Subjects

Clinical and laboratory data from emergency department visits and hospital admissions were retrieved from the electronic health record (EHR) system of the UMCG for the period 1 February 2014 to 24 April 2019. Inclusion criteria were a confirmed diagnosis of hepatic GSD or FAOD, and the presence of an emergency letter based on the generic emergency protocol. Patients for whom UMCG was not the primary responsible center in the entire healthcare chain were excluded. Patients were classified as children (age < 16 years) or adults (age ≥ 16 years). Data were abstracted on the number of admissions due to a metabolic emergency, the percentage of patients with hypoglycemia at



PROTOCOL EMERGENCY LETTER

European Reference Network | MetabERN

Personal emergency plan for: **<NMPATIENT>**
Date of birth: **<BDPATIENT>**
This protocol is generated on: **<GENDATE>**

Diagnosis:
<DIAGNOSE>.

Risk moments for catabolism and subsequent metabolic decompensation are (combinations of):

- Fever
- Decreased gastrointestinal intake; fasting
- Increased gastrointestinal losses
- Stress
- Alcohol (when applicable)
- <RISKMOMENT1>
- <RISKMOMENT2>
- <RISKMOMENT3>

Potential acute complications:

- <RISCSCLINICAL>
- <RISCSBIO>

Personal sick-day regimen:
<SICKDAYTEXT>

When to start the emergency regimen?
The emergency regimen needs to be started when vomiting or under the following combination of circumstances:

- Fever: temperature ≥ 38.0 Celsius (twice measured with one-hour interval) or ≥ 38.5 Celsius (once measured).
- Decreased intake (less than half of normal, more than once).
- Increased losses (>1x vomiting +/- diarrhoea).
- Fasting procedure, while waiting for surgery and contacting the metabolic center.
- When the sick-day dietary regimen is not tolerated.

Body weight used for calculations:
<WEIGHT> kg at <WEIGHTDATE>.

Composition emergency solution:
Dissolve <STDEMFAN> grams maltodextrin in <STDEMORS> mL ORS.

Application:
Phase 1 can be carried out both at home and in the hospital. Phase 2 is reserved for (para) medical professionals.

PHASE 1: ANTIPYRETICS AND EMERGENCY SOLUTION

1. During fever, give paracetamol at the following oral dose:
   Children: 10-15 mg/kg per dose, max 4 times per day, max 500 mg per time.
   Adults: 500-1000 mg per dose, if needed every 4-6 hours, max 4000 mg per day.
2. Give the emergency solution enterally (orally, tube, PEG):
   a. Preferable, if possible: **<COL6> mL/h continue**, or
   b. **<COL5> mL each 3 hours**

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Figure 1. "Emergency protocol for children at risk for acute metabolic decompensation" template. Variables depending on patients' body weight are shown in blue; variables depending on the specific IEM are shown in green

PROTOCOL EMERGENCY LETTER

3. Evaluate each 3 hours (also during the night).
4. Consult our team within 24-48 hours: during working hours the dietician and outside office hours the metabolic paediatrician.
5. Start PHASE 2 when (continuously) vomiting, or clinical decline. These are indications for hospital admission**.** Under these circumstances, the following health care providers can be directly contacted: <DEPARTMENT>**.**

PHASE 2: HOSPITAL – (GLUCOSE INFUSION)

1. Assess every 3 hours whether the standard emergency solution is well tolerated, or intravenous glucose infusion needs to be started.
2. Place an intravenous drip and request the following laboratory tests:
   Blood: <LABBLOOD>,
   Urine: <LABURINE>
3. Infusion policy depending on the clinical condition and/or bedside glucose concentration:
   a. Glucose < 3 mmol/L (< 54 mg/dL) or symptoms like hypotonia, encephalopathy, coma, hyperventilation, convulsions or any doubt:
      i. Give glucose 200 mg/kg as a bolus intravenously in 5-10 minutes as either **2 mL/kg glucose 10% (= <COL12> mL),** or **1 mL/kg glucose 20% (= <WEIGHTAM> mL).**
      ii. Subsequently immediately start a maintenance glucose infusion (including sodium and potassium) of at least the estimated requirements or **<COL7> mg/kg/min** as **<COL8> mL/h glucose 10%** (<COLMLW8> ml/kg/day), increased with 10% extra for each degree Celsius from 37.0°C (at 38.0°C **<COL9> mL/h (<COLMLW9> ml/kg/day)**, at 39.0°C **<COL10> mL/h (<COLMLW10> ml/kg/day)**, at 40.0°C **<COL11> mL/h (<COLMLW11> ml/kg/day)**).
   b. Glucose > 3 mmol/L:
      i. Immediately start a maintenance glucose infusion (including sodium and potassium) of at least the estimated requirements or **<COL7> mg/kg/min** as **<COL8> mL/h** glucose 10% **(<COLMLW8> ml/kg/day)**, increased with 10% extra for each degree Celsius from 37.0°C (at 38.0°C **<COL9> mL/h (<COLMLW9> ml/kg/day)**, at 39.0°C **<COL10> mL/h (<COLMLW10> ml/kg/day)**, at 40.0°C **<COL11> mL/h (<COLMLW11> ml/kg/day)).**
4. Disease specific considerations:
   - Administration of glucagon is contraindicated.
   - Symptomatic metabolic acidosis (pH<7.25) may require active buffer treatment with sodium bicarbonate 4.2% (0.5 mmol/mL) intravenously. Dose in mmol = 0,33 x body weight in kg x base deficit.
   - <CONSIDERATION1>
   - <CONSIDERATION2>
   - <CONSIDERATION3>
5. Consult one of the following health care providers concerning the subsequent metabolic treatment. <CONTACTINFO>

Yours sincerely,

<SIGNATURE>

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Figure 1 (continued)

admissions, and the occurrence of serious adverse events (defined as intensive care unit [ICU] admission, coma, or death). Neurological symptoms (convulsions, lethargy) and blood concentrations of creatine kinase (CK) and ammonia were also recorded.

Data analysis

Study data were collected and managed using REDCap electronic data capture tools hosted at the UMCG[18]. Hypoglycemia was conservatively defined as blood glucose concentrations <3.9 mmol/L, based on glycemic thresholds for activation of counterregulatory systems[19].

Generation of www.emergencyprotocol.net

This project aimed at being as inclusive as possible. Invitations were sent to healthcare professionals and patient organization representatives after the society for the study of inborn errors of metabolism Sponsored Satellite Symposium “Emergency regimes: current status and options for improvement”, the European Metabolic Group meeting Workshop “Dietary management in GSD type I", and the IGSD2017 Networking session “Emergency protocols for hepatic GSD”. Additional contribution came from (national/international) patient organizations meetings.

In the CONNECT MetabERN eHealth project, activity 3 was focused on the automatical generation of emergency letters for patients with GSD and FAOD. After initial meeting in Hannover on 2 December 2019, the UMCG emergency protocol has been revised by multiple healthcare providers during discussions in online meetings on 27 February 2020 and 1 April 2020, input by emails, and via a SurveyMonkey questionnaire (sent on 20 March 2020; 36 responses).

After agreement on the English template to generate emergency letters, since 15 April 2020, the website www.emergencyprotocol.net has been designed and published on 23 June 2020 during a webinar for families and healthcare providers. Meanwhile, translations have been created for the patient information leaflets (providing instructions on how to use the emergency letter) and the emergency letter templates into the following languages: Dutch, English, French, German, Greek, Italian, Polish, Portuguese, Spanish, Swedish, and Turkish. The international validity of the protocol was guaranteed by the contribution of native tongue language editors/healthcare professionals/patient organizations who are part of the CONNECT MetabERN collaboration group. After agreement among native tongue language editors for each of the abovementioned languages, the translated versions were released on the website.

RESULTS

Subjects

In total, 128 patients (66 males, 62 females) with hepatic GSD or an FAOD were included. Of these, 95/128 (74%) were children, 33/128 (26%) were adults. Median age at implementation of the generic emergency protocol was 12 years (range: 0-50 years): <12 months (*n* = 10 patients); 1 to 5 years (*n* = 35); 6 to 10 years (n = 35); 11 to 15 years (*n* = 14); and >16 years (*n* = 34 patients), respectively. The cohort contributed a total of 470 emergency protocol years. The type and distribution of the specific IEM were as follows: medium-chain Acyl CoA dehydrogenase deficiency (MCADD) (*n* =

63, 49%), hepatic GSD (*n* = 59, 46%), multiple-chain Acyl CoA dehydrogenase deficiency (MADD) (*n* = 3, 2%), long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHADD) (*n* = 2, 2%), and very long-chain Acyl CoA dehydrogenase deficiency (VLCADD) (*n* = 1, 1%). One patient was excluded from data analysis, because of severe medical and psychosocial comorbidities that complicated the interpretation of hospital admissions.

Outcomes of the application of generic emergency letters

Table 1 presents an overview of the 127 hospital admissions documented in 54 of 128 patients (42%). Patients' ages at admission were as follows: < 12 months (n = 2 admissions); 1 to 5 years (n = 71); 6 to 10 years (n = 20); 11 to 15 years (n = 7); >16 years (n = 19), respectively. Exact information on age was not available for eight admissions. Hospital admission was considered unnecessary in 11 presentations at the emergency department, representing seven individual patients.

Data on initial plasma glucose concentrations at admission were available for 64% of the admissions (81/127). Hypoglycemia was reported in 15% (19/127) of such admissions (Figure 2). 84% (16/19) of hypoglycemic events occurred in patients with GSDIa and Ib, and 11% (2/19) of hypoglycemic events occurred in patients with FAOD (Figure 2A). When stratifying for age, hypoglycemia was detected in all age groups in patients with GSDI, but it was uncommon in patients with FAOD aged >5 years (Figure 2B). No convulsions, coma, or death due to a metabolic decompensation were reported. One GSDIb patient died in the data collection period because of a severe dilated cardiomyopathy unrelated to metabolic decompensations. An ICU admission was documented for two patients with GSDIa, to support safe monitoring in one adult patient, and for central venous line placement in a 1-year-old patient. The duration of ICU treatment was 1 day in both patients and no long-term complications due to these admissions were reported.

Lethargy was reported in patients with GSDIa (n = 2), GSDIb (n = 1), GSDXI (n = 1), MCADD (n = 4), LCHADD (n = 1), and VLCADD (n = 1). In three out of four patients with MCADD in whom lethargy was documented, glucose concentrations were above 3.9 mmol/L. No hyperammonemia was documented. Acute rhabdomyolysis was reported in two patients with LCHADD (n = 1) and VLCADD (n = 1), with maximum CK concentrations of 63 238 and 3200 U/L, respectively.

www.emergencyprotocol.net

The website www.emergencyprotocol.net is now freely accessible to patients and healthcare providers. The page “Leaflet” provides translated instruction leaflets on how to use the emergency protocol. The page “Emergency letter” allows the generation of personalized emergency letters. These personalized emergency letters are based on a protocol version resulting from revisions of the original UMCG generic emergency protocol, after multiple discussions and reaching final agreement on topics such as drugs and solutions calculations, laboratory tests, and aims (Figure 1).

For the generation of the emergency letters, the following basic information should be provided: patient's name, disease type, date of birth, weight, language, and primary metabolic center. To date, emergency letters can be generated for patients with the following IEMs: GSD 0, GSD Ia, GSD Ib, GSD IIIa, GSD IIIb, GSD IV, GSD VI, GSD IX, GSD XI, MCADD, VLCADD, MADD, and LCHAD/MTP deficiency. The option GSD* is added to offer a solution to modify the template for IEMs that are not listed explicitly.

IEM	Total of patients, n	Total admissions, n	Unique patients with admission, n (%)[a]	Number of patients with ≥ 1 admission x number of admissions	Median age[b], years [range]
GSDIa	23	25	8 (35%)	1 x10 1 x 5 1 x 3 2 x 2 3 x 1	18 [1– 39]
GSDIb	7	10	4 (57%)	2 x 4 2 x 1	13 [4 – 19]
GSDIIIa	8	7	3 (38%)	1 x 3 2 x 2	8 [6 – 11]
GSDIIIb	3	0	-	-	-
GSDVI	1	0	-	-	-
GSDIX	15	16	7 (47%)	1 x 4 3 x 3 3 x 1	3 [0 – 6]
GSDXI	2	1	1 (50%)	1 x 1	6 [NA]
MCADD	63	50	26 (41%)	4 x 4 3 x 3 6 x 2 13 x 1	3 [0 – 13]
MADD	3	14	2 (67%)	2 x 7	4 [0 – 21]
LCHADD	2	3	2 (100%)	1 x 2 1 x 1	4 [0 – 5]
VLCADD	1	1	1 (100%)	1 x 1	4 [NA]
Total population	128	127	54 (42%)	54 x 127	8 [0 – 39]

TABLE 1. Overview of hospital admissions during metabolic decompensation in 128 patients with an IEM associated with fasting intolerance.

[a]% is the number of unique patients with admission divided by total number of patients with a specific IEM.
[b]Age at hospital admission

The emergency letter can be generated in three different file types (i.e, pdf, Word, or HTML) and currently in the following languages: Dutch, English, French, German, Greek, Italian, Polish, Portuguese, Spanish, Swedish and Turkish.

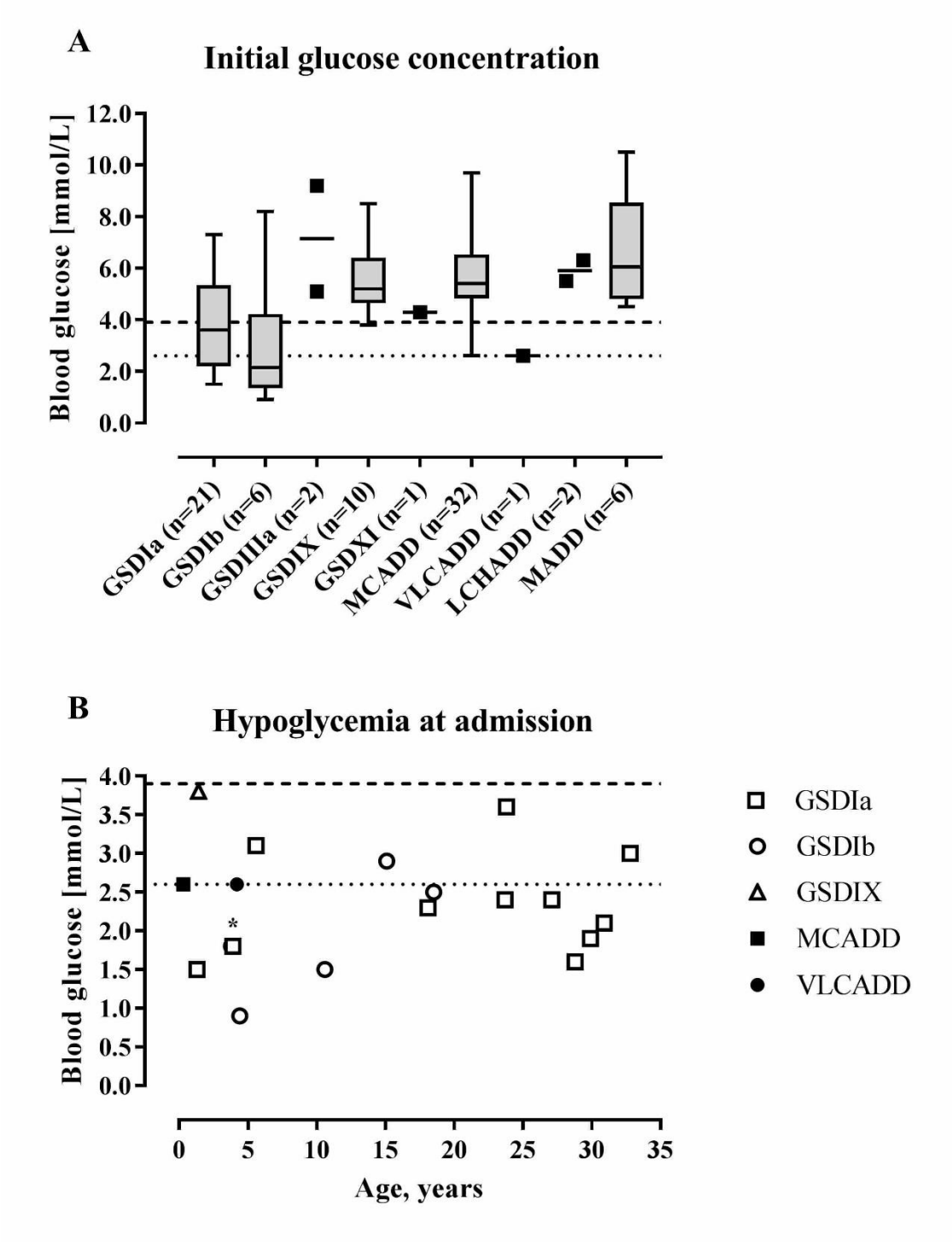

FIGURE 2 Blood glucose concentrations at hospital admission. A, Initial glucose concentrations at hospital admission per IEM (*n* = 81). The boxes represent the 25th to 75th percentiles, the whiskers represent the range. **B**, Characteristics of hypoglycemic glucose concentrations at hospital admission (*n* = 19). Dashed lines represent the cutoff values for hypoglycemia at 2.6 mmol/L[22] and 3.9 mmol/L[19], respectively.

**data point represents two patients with a glucose concentration of 1.8 mmol/L at the age of 4 years with GSD types Ia and Ib*

DISCUSSION

Preventing acute metabolic decompensation by timely, prompt, and safe treatment and communication is crucially important in optimizing outcomes in IEM patients with fasting intolerance. We herein report single-center experience with the use of a generic emergency protocol in a subgroup of IEM patients. Collected data suggest that emergency letters based on a generic emergency protocol can safely prevent metabolic emergencies in patients with hepatic GSD and MCADD (the most common IEM and the one for which more data were available). We also describe the development and functionality of a public website aimed at creating personalized emergency letters for patients with hepatic GSD and the main FAOD.

In the study cohort, few patients were hypoglycemic at hospital admission. Hypoglycemia was uncommon in patients with ketotic GSD and patients with FAOD aged >5 years. This is notable because an important subset of patients with IEMs has severe fasting intolerance with regular events of hypoglycemia in their daily life[20]. Because a key objective of the study was to assess the safety of the protocols, we used a conservative definition of hypoglycemia <3.9 mmol/L (< 70 mg/dL)[21] compared to 2.6 mmol/L as used in some other studies[22]. Before autonomic system and neuroglycopenia-related symptoms and signs are perceived, this threshold is commonly used in family instructions for recognizing and initiating hypoglycemia treatment. The approach taken in the emergency protocol emphasizes prevention and reversal of a catabolic state, through early intervention, timely, and relatively high carbohydrate intake (estimated based on actual body weight[17]), and prompt communication with IEM experts as needed. Meanwhile, the approach acknowledges the high level of self-management by many IEM families. To our opinion, this combined approach likely has prevented hypoglycemias in many patients with IEMs in this study.

Convulsions, coma, or death were not reported at acute hospitalizations in the 128 patients during the 5-year study period. Nonetheless, preventive hospital admissions were frequent among all studied IEMs. Although newborn screening for FAOD has led to a significant reduction in deaths and serious adverse events, utilization of acute care services remains high in these patients compared to age-matched controls. In line with the present study, a retrospective cohort study in patients with IEMs identified through newborn screening between 2006 and 2007 reported that 44% (27 out of 61) of patients with an FAOD had IEM-related acute care utilization during their first year of life[23]. In a recent study from Canada, children with MCADD experienced on average 0.6 hospital admissions per year, between 6 and 12 months of age[24]. Long-term data on hospital admissions in patients diagnosed with hepatic GSD are lacking. However, an international questionnaire showed that hospital admission due to complications of dietary management occurred in 32% (79 out of 249) of patients with GSD[20]. In the latter study, 61% of the respondents eported using an emergency letter.

Preventing catabolism and recognizing the early stages of metabolic decompensation in patients with IEM is challenging because of the IEM-specific pathophysiology of fasting. For instance, in patients with GSDI, lactate can function as an alternative energy substrate to glucose for the brain[25]. Consequently, overt neurologic symptoms and signs of hypoglycemia (neuroglycopenia) may be delayed in patients experiencing hypoglycemia. By contrast, in patients with FAOD hypoglycemia is a relatively late finding of metabolic decompensation and often preceded by lethargy and vomiting[26]. Indeed, in the present study, we found that lethargy was reported in three out of four patients with MCADD in whom glucose concentrations were above the stated cutoff values for hypoglycemia. In

addition, the symptoms and signs associated with fasting intolerance likely depend on the patients' ages[12]. Although our protocol is generic, these findings underscore the importance of individualizing instructions for caregivers and patients, as the combination of education and practical and explicit clinical pathways are crucial to prevent emergency situations[27,28]. In addition, healthcare providers should be aware of the potential risks related to suboptimal emergency treatment, including electrolyte imbalance and iatrogenic hypoglycemia (if the emergency solution or glucose infusion are given late or stopped too early).

The study has some potential limitations. First, the retrospective design and the lack of interoperability and interconnectivity between different EHR systems may have introduced selection bias and information bias. For example, hospital admissions and initial laboratory studies may not always have been communicated to our center or documented in the EHR system. However, it is unlikely that metabolic decompensations causing death, coma, convulsions, and/or ICU admissions would have been missed, as the patient cohort is closely followed and shared care with the local hospitals is well organized. Second, for both organizational and ethical reasons, the study did not include a control group. Therefore, we were not able to compare the events and outcomes with, for example, a patient cohort with IEM-specific emergency etters. Third, the study design did not allow us to assess if and to what extent starting phase I of the emergency protocol at home prevented hospital admissions (however, a higher number of [potentially unrecorded] prevented admissions would argue in favor of the protocol presented here). Conversely, delay in starting the protocol due to various reasons (e.g, lack of materials at home, sociodemographic factors, patient-related factors) might have resulted in an increased number of hospitalizations. Both early and late starting of phase I may be caused by individual patient-related factors and should be addressed during prospective monitoring. Fourth, the study cohort included relatively few patients with FAOD other than MCADD, limiting the generalizability of the findings to all FAOD. Additionally, the study did not include patients with IEMs of the intoxication type (e.g, the organic acidemia, urea cycle defects), for which outcomes after using generic emergency protocols remain to be assessed. Because preventing and reversing catabolism is crucially important also in intoxication type IEMs, we hypothesize that the generic emergency protocol that include the use of the emergency solution can be useful also in such IEMs, with the addition of further measures specific for those types of IEMs. It should also be noted that our generic emergency protocols, which emphasize the use of carbohydrate rich enteral or parenteral intake are contraindicated in patients on a ketogenic or carbohydrate restricted diet. Although the current version of the protocol is the result of agreement among 54 participants from 32 centers and 15 countries, the consensus could not be formally validated (e.g, by Delphi methodology).

Real-world evidence (clinical evidence derived from the analysis of real-world data) plays an increasing role in supporting decision-making for rare disorders. Randomized clinical trials are often not feasible, for many reasons. In rare diseases, patients are relatively few, many are children, and clinical endpoints may not have regulatory precedence. The US Food and Drug Administration (FDA) defines real-world data as data that are routinely collected from several sources, such as HER and disease registries. For real-world data using EHR, data reliability and relevance are key requirements. Retrospective studies can be efficient tools to begin collecting and analyzing real-world data. Despite several potential limitations (e.g, missing elements, lack of comparability after improvements in

standard-of-care management, referral bias), retrospective studies can be performed relatively quickly and may provide the background for longer and laborious prospective studies[29].

We have previously digitalized the emergency protocol as part of the GSD Communication Platform, a telemedicine platform for patients with hepatic GSD[30]. The website www.emergencyprotocol.net supports a shared care model, which uses the medical and communication competences of all stakeholders: the metabolic center of expertise, the local healthcare providers, the caregivers, and the patients, who all share joint responsibility. In this respect, the emergency protocol does not to replace expert metabolic advice; the connection with the responsible metabolic center remains an important step in patients' management. These emergency letters and the website can help to focus decision taking. However, emergency letters, clinical care pathways, and evidence-based guidelines can never replace clinical expertise when making treatment decisions for individual patients. The doctor-patient relationship needs to guarantee that personal values, preferences, and individual circumstances (including psychosocial and cultural aspects) are taken into account. For these reasons, next steps may include a value-based healthcare process toward personalized medicine, by implementing patient's perspectives, to strike the most effective balance between timely management and avoiding overtreatment. Since www.emergencyprotocol.net is constantly updated as part of a continuous process, future discussion, revision, and validation within the IEM (professional and patient) community are also expected to lead to further improvements of the emergency letters.

CONCLUSION

A generic emergency protocol can be safe for home management by caregivers and for initial (first hour) in-hospital management of metabolic emergencies in patients with hepatic GSD and MCADD. Even though IEM-specific emergency letters are widely used, a simple generic emergency protocol, which can be generated online at any time, can be easier to use for families and local physicians before contacting the metabolic specialist. Disseminating such emergency protocol methods and assessing outcomes are crucial next steps aimed at improving further care and prevention, developing an international consensus among healthcare providers, and fostering prospective research studies in patients with IEMs.

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Chapter 6.

Crohn disease-like enterocolitis remission after empagliflozin treatment in a child with glycogen storage disease type Ib: a case report

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ABSTRACT

Background: Besides major clinical/biochemical features, neutropenia and inflammatory bowel disease (IBD) constitute common complications of Glycogen storage disease type Ib (GSD Ib). However, their management is still challenging. Although previous reports have shown benefit of empagliflozin administration on neutropenia, no follow-up data on bowel (macro/microscopic) morphology are available. We herein present for the first time longitudinal assessment of bowel morphology in a GSD Ib child suffering from Crohn disease-like enterocolitis treated with empagliflozin.

Case presentation: A 14-year-old boy with GSD Ib and severe IBD was (off-label) treated with empagliflozin (20 mg/day) after informed oral and written consent was obtained from the patient's parents. No adverse events were noted. Clinical symptoms and stool frequency improved within the first week of treatment. Pediatric Crohn disease activity index (PCDAI) normalised within the first month of treatment. Abdomen magnetic resonance imaging (MRI) performed 3 months after treatment initiation showed dramatic decrease in disease activity and length. Similar findings were reported on histology at 5.5 months. At 7.5 months hemoglobin levels normalised and fecal calprotectin almost normalised. Improved neutrophil count, metabolic control and quality of life were also noted. G-CSF dose was decreased by 33% and the patient was partly weaned from tube feeding.

Conclusions: This is the first report presenting extensive gastrointestinal morphology follow-up in a GSD Ib patient receiving empagliflozin. The present case suggests that empagliflozin can be safe and effective in inducing IBD remission in GSD Ib patients and can even postpone surgery. Future studies are required to confirm its effect over time and assess its benefit in various disease stages. The development of an international collaborating networks for systematic data collection is worthy

BACKGROUND

Glycogen storage disease type Ib (GSD Ib, MIM#232220) is an inherited disorder of carbohydrate metabolism due to microsomal glucose-6-phosphate transporter (G6PT) deficiency (SLC37A4 gene). The ubiquitously expressed G6PT transports glucose 6-phosphate (G6P) from cytosol to endoplasmic reticulum (ER) where it is oxidized to glucose to ensure glucose homeostasis. G6PT defect results into both glycogenolysis and gluconeogenesis defect[1]. Major clinical features of GSD Ib include fasting hypoglycaemia, hyperlactatemia, hyperuricemia, hyperlipidaemia, hepatomegaly, growth retardation, renal disease[2]. Additionally, GSD Ib patients show neutropenia/neutrophil dysfunction[3] and increased risk of inflammatory bowel disease (IBD) (i.e., Crohn disease-like enterocolitis)[4], and autoimmune disorders[5,6].

Despite the progress in the (medical and dietary) treatment of GSD Ib over the past years, such immunological complications still heavily impact on patients' prognosis and quality of life. While evidence regarding the pathogenesis of neutropenia/neutrophil dysfunction and autoimmune disorders has accumulated[7–9], the pathomechanism of IBD in GSD Ib is still unclear; the disturbed immune response may play a role in its pathogenesis[4]. Granulocyte-colony stimulating factor (G-CSF) for neutropenia and conventional drugs for IBD and autoimmune disorders still constitute the current treatment options for most GSD Ib patients. For IBD, conventional treatments are sometimes ineffective and/ or associated with side effects and patients might eventually need surgery[1]. Notably, improved prevention/treatment of IBD in GSD Ib ranked as a top priority for research in the international priority setting partnership for liver glycogen storage diseases[10].

Recent evidence has shown a major role for plasma 1,5- anhydroglucitol (1,5AG) in causing neutropenia/neutrophil dysfunction in GSD Ib. 1,5AG enters neutrophils where it is phosphorylated to 1,5-anhydroglucitol-6-phosphate (1, 5AG6P). 1,5AG6P is transported by G6PT into the ER, where it is physiologically dephosphorylated. G6PT defect results into cytosolic toxic 1,5AG6P accumulation thus affecting neutrophils survival and function[11].

Empagliflozin is a sodium glucose co-transporter 2 (SGLT2) inhibitor approved for the treatment of type 2 diabetes which reduces renal 1,5AG resorption by increasing urinary glucose excretion. Notably, empagliflozin administration decreased 1,5AG (plasma) and 1,5AG6P (neutrophils) concentrations in GSD Ib mice[11]. Two recent reports have shown same effect in GSD Ib patients with improved neutrophil count/ function. Possible benefit on gastrointestinal symptoms have also been reported[12,13]. However, no follow-up data on bowel (macro/microscopic) morphology are available.

We herein present for the first time longitudinal assessment of bowel morphology in a GSD Ib child suffering from Crohn disease-like enterocolitis treated with empagliflozin.

CASE PRESENTATION

Methods

Study design

Empagliflozin is a SGLT2-inhibitor registered and marketed for type 2 diabetes in adults. Its most common adverse effects include low blood pressure and urogenital infections[14]. In the case herein described informed oral and written consent for the off-label treatment with empagliflozin was obtained from the patient and patient's parents after discussing potential benefits and adverse effects of such treatment. Baseline data were collected 1 (day − 1) or 2 (day − 2) days before starting the treatment during in-hospital admission under medical supervision (day 0). Vital parameters were checked every 2 hours within the first 12 hours after treatment initiation and subsequently every 8 hours. The patient was discharged on day + 5. Regular assessments of his GSD Ib and related conditions were performed at the outpatient clinic every 1 week within the first 2 months of treatment; subsequent evaluations were performed based on the patient's conditions and medical advice. Blood samples were collected at the maximum distance after last C-GSF administration (day − 2 to day 30: 48 h; day 37 to day 51: 72 h; day 64 to day 71: 96 h; day 78 to day 115: 72 h). Physical examination included: weight, height and body mass index, signs/symptoms of infections, abdominal pain, mouth ulcers and perianal lesions. Adverse events were also recorded. For all results, the specific day of collection (i.e., day before/after starting the treatment) is reported in the main text, tables or figures.

Gastrointestinal assessment

An expert endoscopist performed the colonoscopy. During colonoscopy, 4 biopsies were taken from each colonic segment and from the terminal ileum, if entered. The histologic features were assessed by an experienced IBD pathologist, who was blinded to the patient's endoscopic features and clinical history. Magnetic Resonance Imaging (MRI) was performed by an experienced IBD radiologist (who was blinded to the patient's morphological features and clinical history) using a high-field (3.0-Tesla) scanner (Trio, Siemens) using a body coil with four channels; the following sequences were acquired: T2-weighted HASTE triggered on the axial plane (TR/TE 2000/91 ms; thickness 6 mm; flip angle 150; matrix: 256 × 157; acquisition time: 64 s), T2-weighted HASTE triggered on the coronal plane (TR/TE 2000/92 ms; thickness 4 mm; flip angle 121; matrix 320 × 256; acquisition time 80 s) with and without fat saturation, T1 weighted in-phase on the axial plane (TR/TE 1500/2.3 ms; thickness 6 mm; flip angle 20; matrix 256 × 154; acquisition time 50 s), T1- weighted out-of-phase on the axial plane (TR/TE 1500/1.37 ms; thickness 3.5 mm; flip angle 20; matrix 256 × 160; acquisition time 58 s) before and after intravenous injection of paramagnetic contrast (gadopentetate dimeglumine, Magnevist, Bayer HealthCare Pharmaceuticals). Disease activity was assessed using the pediatric Crohn disease activity index (PCDAI)[15]. Stool consistency was assessed with the Bristol stool chart.

Biochemical tests

Fecal calprotectin was assessed through ELISA assay. Plasma 1,5AG and granulocytes 1,5AG6P were assessed as previously described[12]. Blood (glucose, lactate, cholesterol, triglycerides (TG), uric acid, AST, ALT, albumin, complete blood count, absolute neutrophil count (ANC), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) within the first hour, creatinine, blood urea nitrogen) and urine tests (creatinine, (24 h-proteins, 24 h-glucose, urinalysis) were performed by using assays

with commercially available kits.

Glucose monitoring

Besides capillary glucose measurements, flash glucose monitoring (FGM) was performed through an intermittent scanning FGM device (Freestyle Libre2) during the following time frames: 1) baseline to day + 10; 2) + 3 months; 3) + 5.5 months; 4) + 7.5 months; 5) + 8 months. Low-glucose threshold was set at 3.3 mmol/L. In case of glucose concentrations below threshold, capillary glucose was also checked. Hypoglycemia was defined as capillary glucose < 3.3 mmol/L. Due to possible interference of daily life activities, physical activity and the risk of temporary sensor disconnection, both 24-h and night-time (1 a.m. to 5 a.m.) FGM data were analyzed for each time frame by using descriptive statistics. Only days with > 15 time points available were considered for the analysis. Time below range (TBR), time in range (TIR) and time above range (TAR) were defined according to current consensus glucose monitoring recommendations[16].

Quality of life (QoL)

Health-related QoL was assessed at baseline and on day + 232 through the Italian version of the Short Form Health Survey (SF-36) questionnaire which has been previously used for GSDI patients. The SF-36 questionnaire consists of 36 items combined into eight scaled scores. The raw score is transformed into a 0–100 scale to generate a summary measure, with higher scores indicating better QoL[17].

Case presentation

A 14-year-old boy was diagnosed with GSD Ib at age 7 months due to fasting hypoketotic hypoglycemia with high lactate, enlarged liver and neutropenia. The molecular diagnosis of the SLC37A4 gene showed homozygosity for the mutation c.742C > T (p.Gln248X). A diet based on frequent meals and nocturnal gastric drip-feeding was started and the patient was included in a follow-up program at Section of Pediatrics, University of Naples "Federico II". Several unsuccessful attempts with uncooked cornstarch were made in order to extend his fasting time (all associated with diarrhea, abdominal pain, vomiting). After such attempts, the patient developed avoidant/restrictive food intake disorder. Starting from age 6 years, 24-h gastric drip feeding (GDF) was required. Due to neutropenia, he was also started with i.m. G-CSF.

During the follow-up several complications appeared. At 9 years of age juvenile idiopathic arthritis was diagnosed following the development of arthralgia and arthritis in the right knee and hip. From that age he also experienced recurrent aphthous stomatitis. Since Naproxen (15 mg/Kg/day) administration showed no benefit, i.m. methotrexate (15 mg/m2 every 1–3 weeks) was started. At age 13 limitation of range of motion and arthritis in the left knee required intra-articular injection of triamcinolone acetonide. At age 14 hyperuricemia was detected, requiring allopurinol administration (200 mg twice per day). Kidney function was regularly assessed and found normal. Plasma cholesterol (range 1.4–2.5 mmol/L) and TG (range 0.4–1.3 mmol/L) concentrations were constantly decreased.

Since 10 years of age, he suffered from Crohn-like IBD (PCDAI:75 at the diagnosis). Chronic anemia was also detected requiring several (partly beneficial) intravenous iron infusions (Hb 7.6–10.5 g/dL).

Despite methotrexate (15 mg/m2 every 1–3 weeks) administration, he experienced 2 disease relapses during the following 3 years. At age 13 switching to adalimumab (40 mg every 2 weeks) was decided after additional bowel and joint relapse. At age 14, further relapse occurred: moderate/severe abdominal pain, 2–5 liquid stools (with mucus) per day, perineal pain due to anal fissure, pain in the left foot with limitation of range of motion due to left metatarsal joints arthritis. No oral lesions were noted. Therefore, the patient was admitted, and extensive reassessment was performed (Table 1). Stricture at the ileocecal valve was detected at ileocolonoscopy (Fig. 1A). Histology showed active disease with crypt abscesses (Fig. 1B). Abdomen Magnetic resonance imaging (MRI) showed active disease with increased wall thickness and contrast enhancement in the distal ileum (total length:15–20 mm) with ileal stricture (Fig. 1C). 7-day ciprofloxacin (2 mg/Kg/ day) and metronidazole (15 mg/Kg/day) treatment showed no benefit. Ibuprofen patch was partly effective on arthralgia. Since anti-adalimumab antibodies together with undetectable plasma adalimumab were also detected (Table 1), this treatment was withdrawn (hospital day 5) and ileocecal resection was proposed. 4.8 μg G-CSF/Kg every other day (i.e., 2.4 μg G-CSF/Kg/day) was continued.

Off-label treatment with empagliflozin was also discussed with the patient's family. After oral and written informed consent for this individual treatment, ileocecal resection was postponed, and the patient was started with empagliflozin (day 0, hospital day 16). The starting dose was 5 mg/day (0.1 mg/Kg/day); the dose was further increased to 5 mg twice a day (0.2 mg/Kg/day) on day +3 and 10 mg twice a day (0.4 mg/Kg/day) on day + 7. Ciprofloxacin was stopped on day − 1; metronidazole was withdrawn on day + 3. The dietary regimen was continued as usual (24-h GDF). No significant changes in vital signs and no serious adverse events were observed. On day + 22 urinary nitrites (with no leukocytes) were detected, with no associated symptoms. Urine culture was ordered and oral cefixime (8 mg/Kg/day) was started (being urinary tract infections common side effects of empagliflozin and considering the risk of metabolic decompensation in case of infection). On day + 27 urine culture tested negative and oral cefixime was withdrawn. Subsequent urinalysis tested normal. 24-h urine glucose was absent on day − 2 and tested constantly increased after treatment initiation (range 13,212–30,775 mg/24 h).

Perineal pain and anal fissure improved after 3 days of treatment and disappeared on day + 6. On day + 3 pain in the left foot improved and on day + 5 metatarsal swelling was reduced; starting from day + 20 no signs or symptoms of arthritis were noted. On day + 232 his weight (Z-score: − 0.49), height (Z-score: − 1.03) and BMI (Z-score:0.01) were comparable to baseline. The stool frequency went down to 1–2 x day after 1 week of treatment and 1 x every 2 days after 1 month of treatment. The stool consistency switched from type 6 to type 5 after 2 weeks of treatment and type 4 after 1 month of treatment. The PCDAI decreased from 50 (day − 1) to 20 (day + 7) to 5 (day + 15). Fecal calprotectin increased up to + 40% during the first month of treatment with subsequent decrease. 7.5 months after starting with empagliflozin its value was − 48% compared to baseline and almost normalised (Fig. 2A). Similarly, CRP values increased during the first 2 week of treatment and eventually normalised (occasional spikes occurred). ESR values decreased by 34% after 2 weeks of treatment and normalised after 3 months of treatment. Hemoglobin concentrations constantly increased from the first week of treatment and eventually normalised 5.5 months after starting with empagliflozin (Fig. 2B).

	Result	Reference Range
Weight (Kg)	50	--
Weight (Z-score)	-0.46	-2-+2
Height (cm)	159	--
Height (Z-score)	-1.10	-2-+2
BMI	20	--
BMI (Z-score)	0.10	-2-+2
PCDAI	50	<10
Stool consistency type	6	3-4
Glucose (mmol/L)	4.5	3.3-6.1
Lactate (mmol/L)	1.8	<2.2
Total cholesterol (mmol/L)	1.4	3.4-5.3
Triglycerides (mmol/L)	0.4	0.5-1.6
Uric acid (mmol/L)	0.26	0.13-0.39
AST (U/L)	12	0-34
ALT (U/L)	7	0-55
Albumin (g/L)	37	34-48
Creatinine (mg/dL)	0.66	0.60-1.10
Blood Urea Nitrogen (mg/dL)	13	18-45
eGFR (ml/min/1.73 m2)	132.6	100.9-133.3
White blood cells (WBC)/µL	3010	5000-15000
Neutrophils/µL	1490	1300-8500
Lymphocytes/µL	1370	1300-8500
Hemoglobin (g/dL)	8.8	11.5-14.0
Hematocrit (%)	33	33-35
Platelets/µL	274000	140000-440000
Fibrinogen (mg/dL)	233	160-350
1,5AG (µM)	155	--
1,5AG6P (µM)	1.35	--
CRP (mg/dL)	2.8	<0.5
ESR (mm/h)	35	<20
Adalimumab (µg/ml)	< 0.5	5-10
Anti-adalimumab IgG (ng/ml)	62.3	< 2.5
24-hour urine protein (mg/24h)	< 200	< 200
24-hour urine glucose (mg/24h)	not detected	not detected
Fecal calprotectin (µg/g)	253	< 100

TABLE 1. Baseline clinical and biochemical data.

PCDAI: Pediatric Crohn's Disease Activity Index; eGFR: estimated glomerular filtration rate; 1,5AG:1,5-anhydroglucitol; 1,5AG6P: 1,5-anhydroglucitol-6- phosphate; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate within the first hour

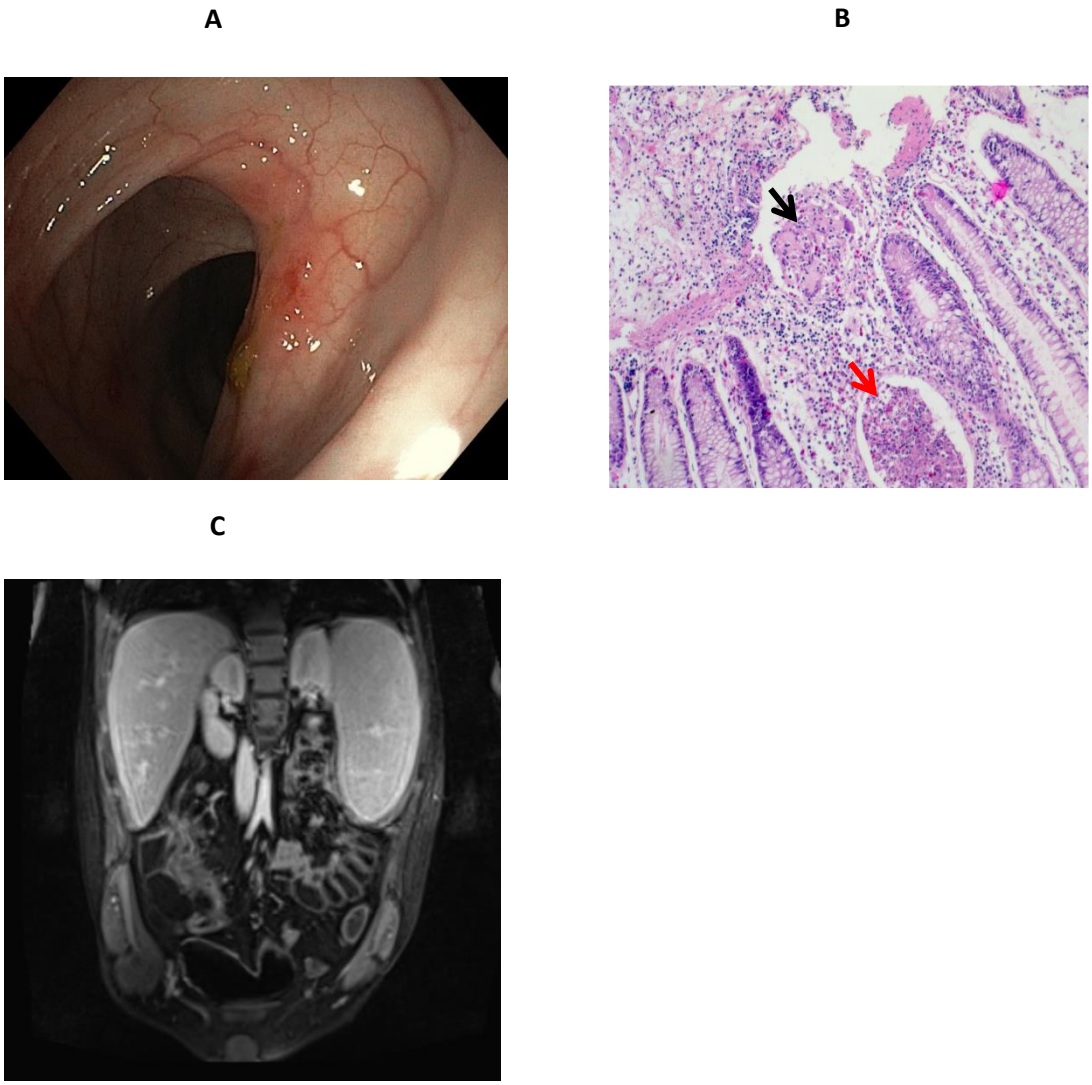

FIGURE 1. Bowel morphology at baseline. (**A)** Ileocolonoscopy: ulcerated and ileocecal valve stricture with impossibility to pass through with the scope (Paris classification A1b, L1, B2, G0; SES-CD: 3). (**B)** Histology (colonic mucosa): architectural irregularity and a mild patchy increase of lamina propria cells with neutrophilic and eosinophilic infiltration, crypt abscesses (red arrow) and an epithelioid cell granuloma (black arrow) indicating active disease. **(C)** Abdomen MRI: active disease with increased wall thickness (max: 10 mm), diffusion restriction and contrast enhancement in the distal ileum (total length:15–20 cm) and ileal stricture; mesenteric hypertrophy (creeping fat) and lymphadenopathy and conglomerated bowel loops (right lower quadrant) are also shown. *SES-CD: simplified endoscopic score for Crohn's disease*

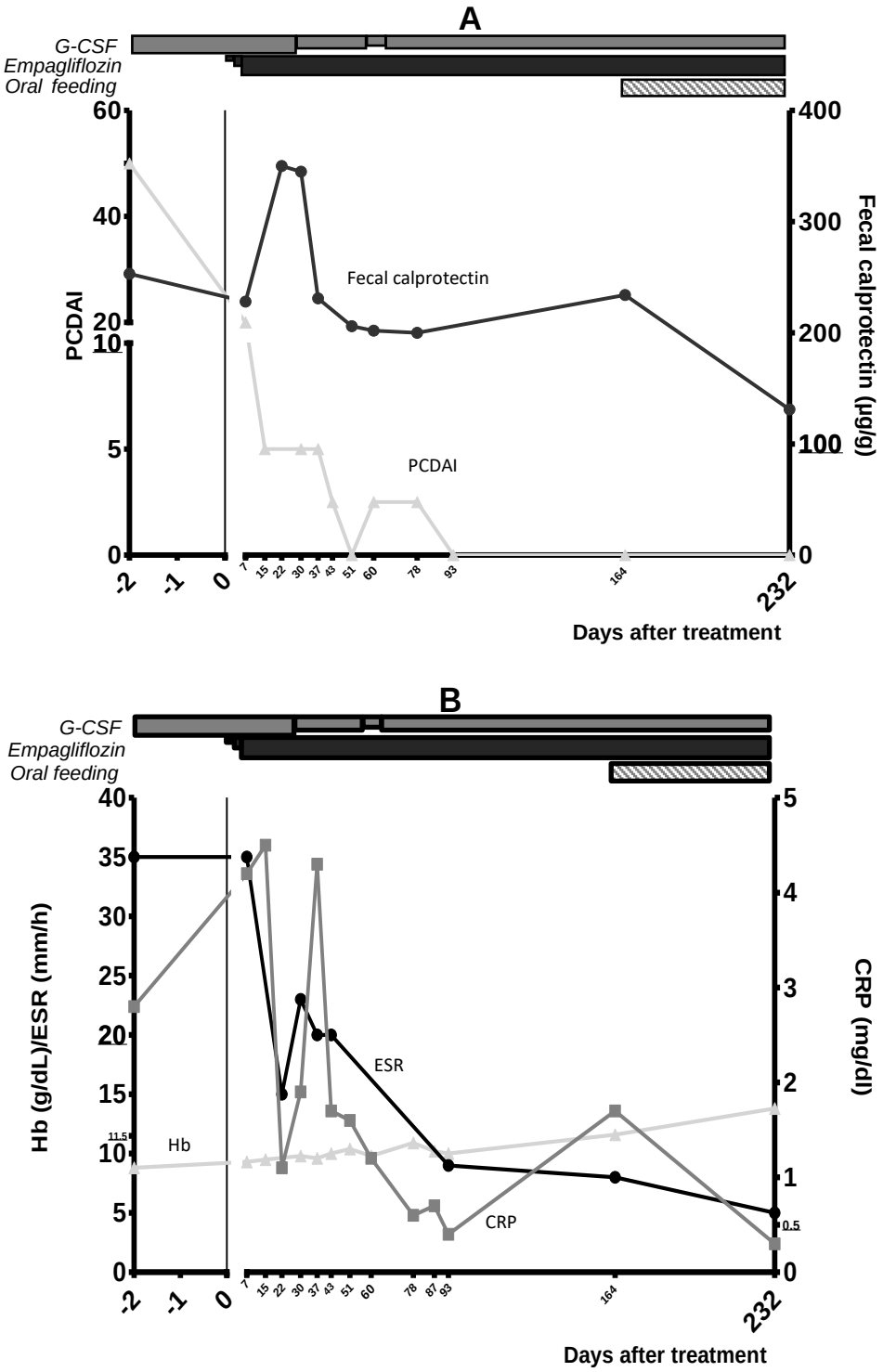

FIGURE 2. Paediatric Crohn's Disease Activity Index (PCDAI) and biochemical assessment before and after empagliflozin. (**A)** PCDAI (light grey triangles) and fecal calprotectin (dark grey circles) values before and after empagliflozin (upper references values for PCDAI (10) and fecal calprotectin (100) are underlined; (**B)** Hemoglobin (light grey triangles), ESR (Black circle) and CRP (dark grey squares) values before and after empagliflozin (upper reference values for ESR (20) and CRP (0.5) and lower reference value for Hb (11.5) are underlined. *CRP: C-reactive protein; ESR: erythrocyte sedimentation rate within the first hour.*

Abdomen MRI performed at + 85 days showed − 45% wall thickness and − 63% disease length in the distal ileum (total length 5.5 cm) together with ileal stricture (Fig. 3A). At + 161 days ileocolonoscopy showed unchanged stricture at the ileocecal valve (Fig. 3B); histology showed no signs of active disease (Fig. 3C). At the time no significant change in spleen longitudinal diameter was noted (Z-score: baseline: + 8.75; + 161 days: + 7.52).

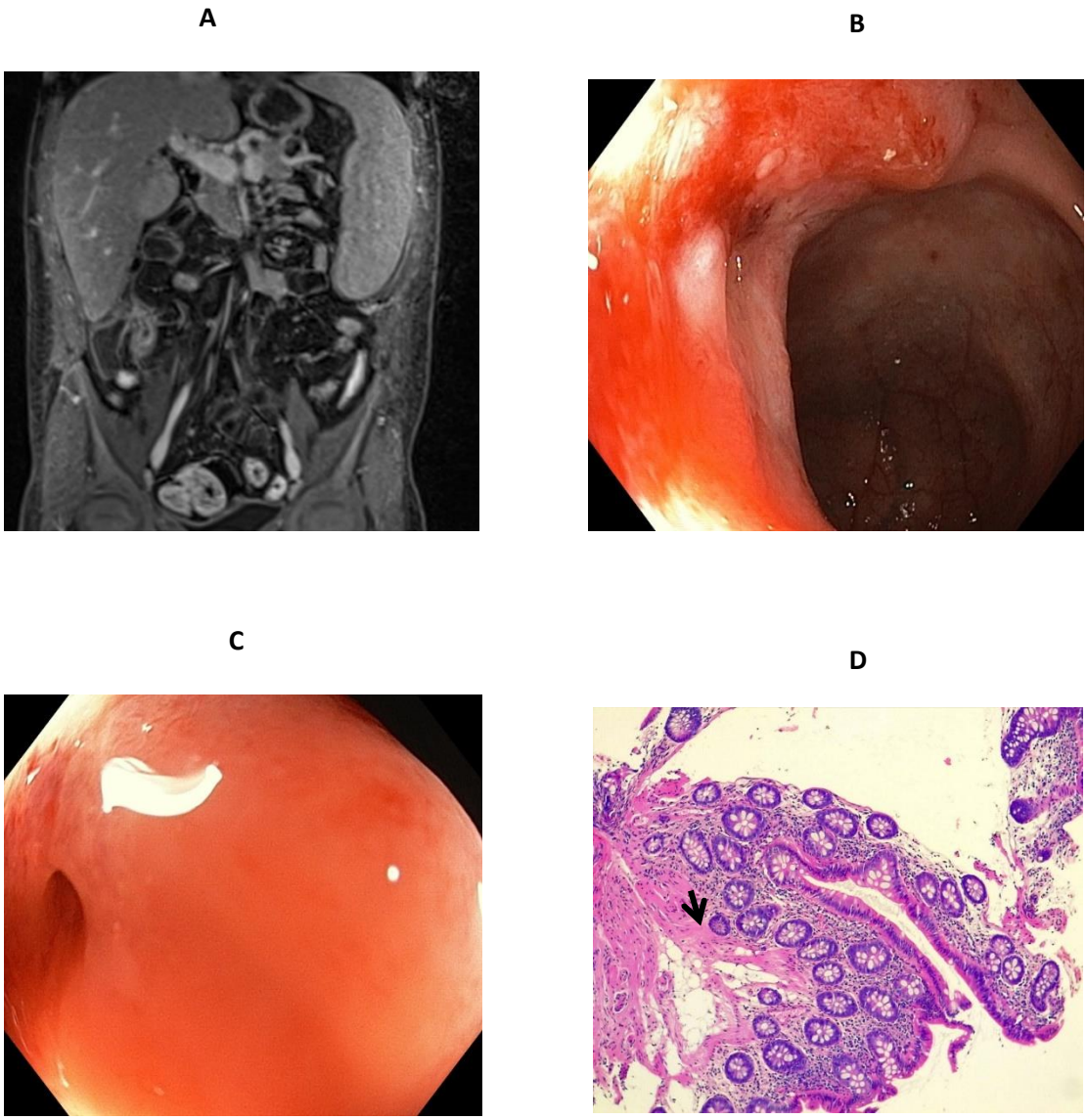

FIGURE 3. Bowel morphology after empagliflozin treatment. (**A**) MRI (day + 85): decreased wall thickness (max 6.5 mm), decreased diffusion restriction, decreased contrast enhancement in the distal ileum (total length: 5.5 cm) together with ileal stricture; stable mesenteric hypertrophy (creeping fat) and lymphadenopathy with no evidence of conglomerated bowel loops (right lower quadrant) are also shown. **(B-C)** Ileocolonoscopy (day + 161): ileocecal valve ulcer and stricture with the impossibility to pass through with the scope (Paris classification A1b, L1, B2, G0; SES-CD: 3). **(D)** Histology (day + 161, colonic mucosa): minimal architectural distortion, increase of lamina propria, associated with muscularis mucosae hypertrophy

(black arrow) and adequate gland representation indicating chronic mild colitis with histologic remission. SES-CD: simplified endoscopic score for Crohn's disease

On day + 163 oral refeeding was proposed. Following discussion with the family, the patient was switched from 24-h (5 mg carbohydrates/Kg/min) to 19-h (4 p.m.- 11 a.m.) (5 mg carbohydrates/Kg/min) GDF. In the "GDF-free" hours one morning snack (11 a.m.) and lunch (01.30 p.m.) were included in the hospital setting, providing an overall carbohydrate intake of 2.2 g/Kg (i.e., 7.6 mg carbohydrates/Kg/min). With such a scheme, the patient's fasting time changed from 0 to 2.5 h during the "GDF-free" hours. Capillary glucose concentrations (checked every 30 min on 3 consecutive days during the 5 hours without gastric drip feeding) were > 3.9 mmol/L (range 4.9–7.2 mmol/L). Neither gastrointestinal symptoms/signs nor changes in stool frequency/features were reported during the subsequent 2-month follow-up; on day + 232 fecal calprotectin almost normalised (Fig. 2A).

ANC showed wide variations before empagliflozin (340–4720/μL) with reduced fluctuations (580–2990/μL) after empagliflozin was started. 1,5-AG and 1,5-AG6P concentrations are presented in Additional file 1. The G-CSF dose was gradually decreased and finally set to 4.8 μg G-CSF/Kg every 3 days (i.e. 1.6 μg G-CSF/Kg/day). Lactate concentrations stayed within the reference range (1.3–2.3 mmol/L). A slight increase in cholesterol and TG concentrations (which reached the reference range) was observed (Additional file 2A). Uric acid concentrations stayed normal and allopurinol was gradually discontinued (Additional file 2B). Liver and kidney function were regularly checked and tested normal.

Capillary glucose values were below < 3.3 mmol/L (range 2.6–3.3 mmol/L) on 20/32 low-glucose events measured by FGM within the first week of treatment and promptly increased upon glucose administration via the feeding tube. No signs or symptoms of hypoglycemia were reported. Occasional (2–4 times per month) asymptomatic mild hypoglycemias (range 2.7–3.3 mmol/L) occurred during the subsequent 6-month capillary glucose self-monitoring. Data on FGM monitoring are shown in Additional file 3. A substantial decrease in TBR as well as an increase in TIR were observed (also after oral refeeding was started). The patient's QoL score improved from 37.64 (baseline) to 74.44 (day + 232).

DISCUSSION AND CONCLUSIONS

The management of IBD is still challenging in GSD Ib as its pathogenesis remains unresolved[1,3]. Conventional treatments (i.e., corticosteroids, immunomodulators, biological agents) are sometimes ineffective and/or associated with side effects (e.g. leucopenia, anemia, diarrhea) and patients might eventually need surgery[18]. Being the cornerstone of treatment for neutropenia, G-CSF has also led to IBD remission in some GSD Ib patients[19]. However, its efficacy is variable and long-term G-CSF administration may cause side effects (e.g. enlarged spleen) and increased risk for malignancies (e.g. acute myeloid leukemia, myelodysplastic syndrome)[20, 21]. Therefore, more effective treatments are required to improve patients prognosis and quality of life.

Although previous reports have shown possible benefit of empagliflozin on gastrointestinal symptoms[12,13], follow-up data on bowel (macro/microscopic) morphology are not available. In the

case herein reported, we presented comprehensive gastrointestinal assessment in a child with GSD Ib, showing clear benefit of empagliflozin administration on Chron disease-like enterocolitis. No symptomatic hypoglycemias and no adverse events were associated with empagliflozin administration.

Clinical improvement was noted within the first week of treatment and eventually led to normal stool frequency/consistency and PCDAI normalisation. Clinical remission occurred within the first month of treatment. Biochemical improvement occurred within the first 2 weeks of treatment and remission was documented after 2 months of treatment. Strikingly, the benefit on clinical picture and hemoglobin concentrations appeared as soon as the end of the first week. Morphology studies also showed partial IBD remission within 3–6 months of treatment. Notably, a dramatic improvement in the disease length and activity was documented on the abdomen MRI after 3 months of treatment as well as histologic remission after 5.5 months of treatment. Empagliflozin allowed to postpone ileocecal resection (and possibly decrease the length of the bowel segment to be resected) in the present case. However, no major endoscopic changes were noted 5.5 months after starting treatment. Those data show that empagliflozin may be effective in healing the inflammatory lesions/strictures but might not be able to reverse fibrotic strictures once established. Such conclusion suggests early empagliflozin administration in GSD Ib patients with IBD before the onset of (irreversible) intestinal fibrosis. 0.4 mg/Kg/day empagliflozin were administered in the present case. Previous study showed 0.3–0.7 mg/Kg/day to lay within the therapeutic window for neutropenia[12]. It is still unclear if higher doses can be more effective or whether a specific dose range might exert special benefit on other disease complications (e.g., IBD, arthritis). Future studies should address this issue.

Besides the effect on IBD, decreased 1,5AG and 1,5AG6P concentrations as well as higher/more stable neutrophil count were also documented after starting with empagliflozin. Based on those data, the G-CSF dose was decreased by 33% in the present case. Undoubtedly, reducing G-CSF dose can decrease the risk of side effects and malignancies associated with its long-term administration. However, not all GSD Ib patients treated with empagliflozin are able to discontinue G-CSF[12]. The reason for such discrepancy is still unclear. Possible role of additional factors contributing to empagliflozin response (e.g., genotype, renal function, glycosylation status) should be further addressed for optimal patient selection. The results herein reported also support possible role of disrupted immune response in the pathogenesis of IBD in GSD Ib. Indeed, a role for 1,5AG and 1,5AG6P in modulating other peripheral blood mononuclear cells has been postulated[11].

Despite 33% reduction in G-CSF dose, no change in spleen size was observed in the current patient. In 3 out of the 5 previous GSD Ib patients that have been previously described to be treated with empagliflozin and who presented with splenomegaly, 2 showed decreased spleen size only 9 months after starting empagliflozin (G-CSF was discontinued or decreased by 81%, respectively). Despite G-CSF discontinuation, 1 patient showed stable spleen size 3 months after starting empagliflozin[12]. Longer follow-up studies are warranted to clarify when to expect benefit on splenomegaly.

Additionally, improved metabolic control was noted in the present case. Increase of (low) cholesterol and TG levels and reversal of hyperuricemia (leading to allopurinol discontinuation) were detected after empagliflozin administration. Likely, normalisation of plasma cholesterol and TG were secondary to intestinal healing in the present case. FGM data showed stable glucose levels and eventually no hypoglycemia was detected. Strikingly, the patient also experienced (limited) amount

of time in the "above-range" (Additional file 3). Such findings are in line with previous report[12] suggesting that IBD might concur to metabolic control in GSD Ib patients by limiting intestinal glucose absorption. Interestingly, recent research has shown the impact of life-long diet on gut microbiota in GSD Ib[22]. FGM may constitute an additional, minimally invasive monitoring tool for GSD Ib patients.

Not only led empagliflozin to improved clinical conditions and biochemical/morphological markers but also allowed drug dose reduction/discontinuation in the present case. As a matter of fact, the patient was switched from 4-drug (i.e., adalimumab, allopurinol, G-CSF, ibuprofen patch) to 2-drug (G-CSF, empagliflozin) regimen with a simplified drug schedule. Notably, the number of painful G-CSF injections was decreased. Benefits on healthcare costs (empagliflozin is less expensive than G-CSF and biologic therapies for IBD) as well as reduced healthcare use by GSD Ib patients can also be expected. Indeed, the gross monthly financial burden for medication decreased by 59% in the present case (3586 € vs 1467€).

Improved QoL was also observed after empagliflozin administration. Patients with IBD show increased prevalence of psychological disturbances like depression and anxiety[23]. In the present case, the patient agreed on restarting oral feeding after 8 years, allowing (in part) weaning from tube feeding. This result suggests that, by improving the IBD-related symptoms, empagliflozin can also exert a positive effect on psychosocial health and well-being in patients with GSD Ib.

Although renal function was constantly normal in the present case, future studies should assess the effect of empagliflozin on renal function in GSD Ib patients[24,25].

Overall, the present case shows that empagliflozin administration is safe and effective in inducing IBD remission in GSD Ib patients and can postpone surgery. It also improves neutrophil count and metabolic control. Since this is the first case documenting comprehensive longitudinal IBD morphology follow-up in a patient with GSD Ib treated with empagliflozin, future studies are needed to confirm its safety and efficacy over time and assess its benefit in various disease stages. As empagliflozin has the potential to change the natural history and management of GSD Ib patients, the development of an international collaborating networks for systematic data collection on its safety and efficacy is worthy.

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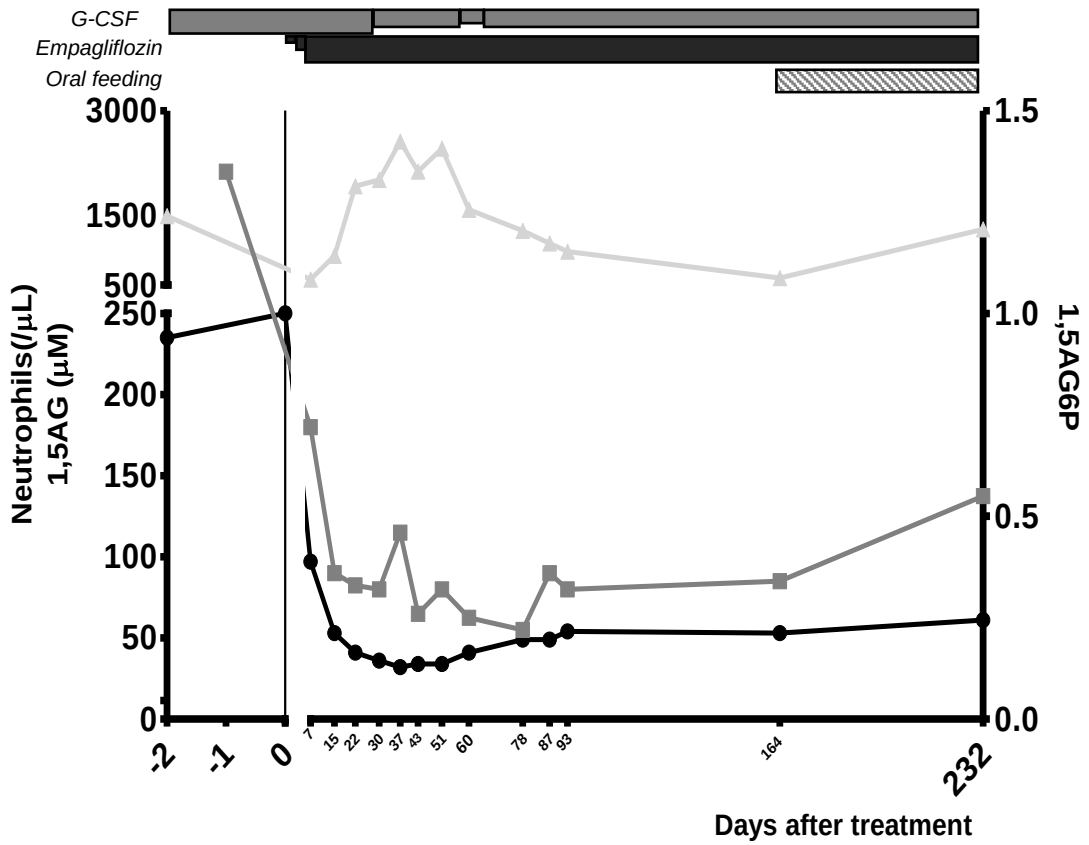
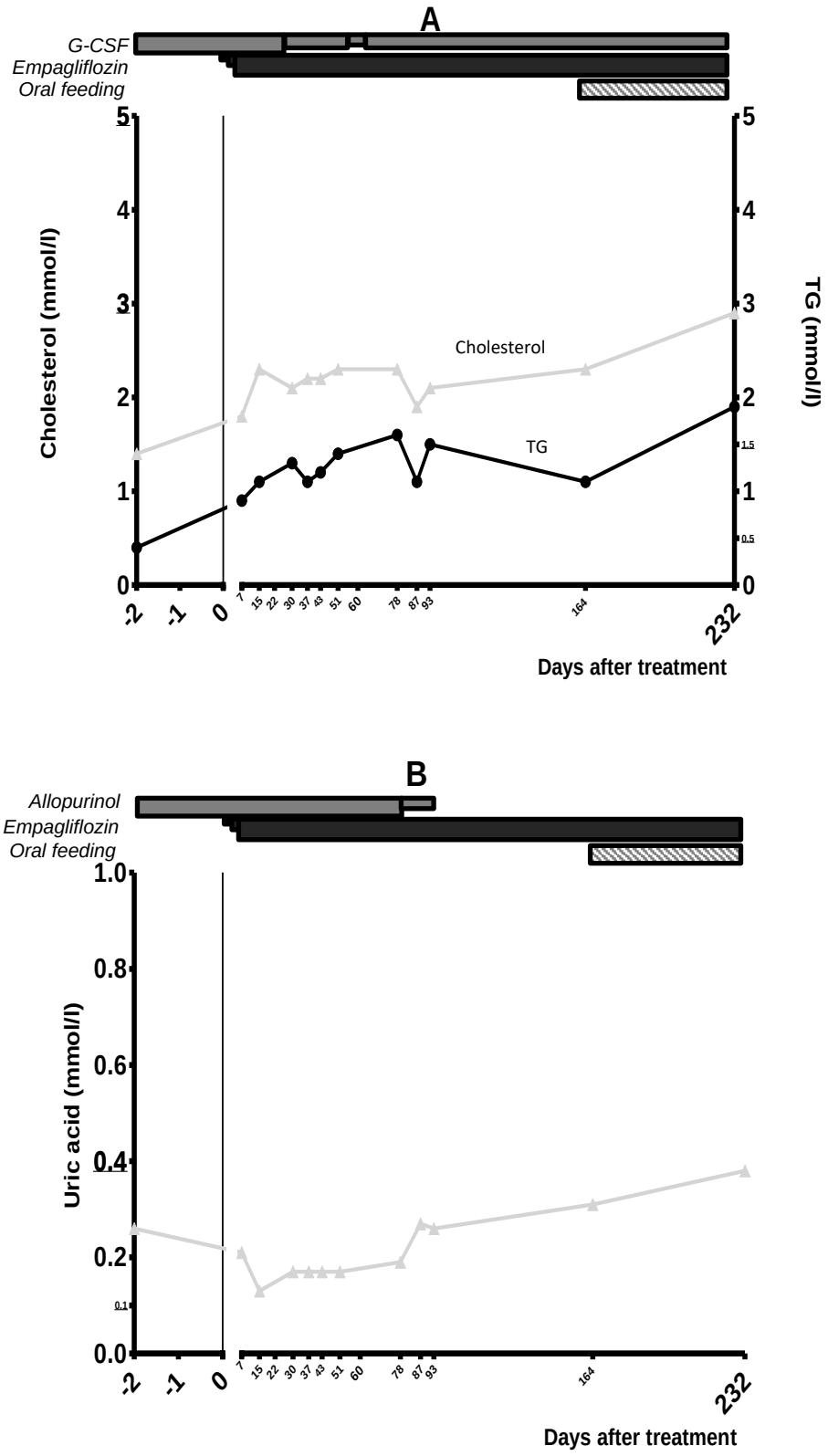
SUPPLEMENTARY MATERIAL

Additional File 1



Additional file 1. Neutrophil count (light grey triangles), 1,5AG (black circles) and 1,5AG6P (dark grey squares) before and after empagliflozin. Plasma 1,5-AG concentration dropped from ±250 μM before treatment to ±50 μM after 2 weeks on empagliflozin. Concentration of 1,5-AG stayed relatively constant until day + 164, before a change in the diet introducing a daily oral intake of carbohydrates. On day + 232, approximately 2 months after this change, plasma 1,5-AG was only very slightly increased to 60 μM. After treatment, 1,5-AG6P present in leukocytes and measured in whole blood samples was reduced by 4- to 5-fold when compared to values before starting empagliflozin. *1,5AG: 1,5-anhydroglucitol; 1,5AG6P: 1,5-anhydroglucitol-6-phosphate.*

Additional File 2



Additional file 2. (A) Plasma cholesterol (grey triangles) and TG (black circles) before and after empagliflozin (reference values for cholesterol (3–5) and TG (0.5–1.5) are underlined); **(B)** Plasma uric acid concentrations before and after empagliflozin (reference values are underlined). *TG: triglycerides.*

Additional file 3

										TIME BELOW RANGE (TBR)		TIME IN RANGE (TIR)		TIME ABOVE RANGE (TAR)	
Time frame	**Study day**	**Days analysed**	**Time points analysed**	**median**	**min**	**max**	**variance**	**SD**	**Coefficient of Variation**	**<3.0 mmol/L**	**≥ 3 < 3.9 mmol/L**	**≥ 3.9 ≤ 7.8 mmol/L**	**≥ 3.9 ≤ 10 mmol/L**	**> 7.8 mmol/L**	**>10 mmol/L**
				mmol/L	mmol/L	mmol/L	mmol/L	mmol/L	%	%	%	%	%		
24-h	-1 to +10	12	1151	4.44	2.61	7.33	14.52	0.90	1.11	3,2	25,5	71,2	71,2	0,0	0,0
	+85 to +96	12	794	5.17	2.67	8.67	25.74	1.20	1.30	2,4	16,4	80,5	81,2	0,8	0,0
	+164 to +176	13	1178	5.55	2.94	8.50	18.07	1.00	1.00	0,1	5,8	93,3	94,1	0,8	0,0
	+214 to +226	13	1175	5.44	2.89	9.78	18.54	1.02	1.00	0,6	5,9	92,2	93,5	1,4	0,0
	+245 to +257	13	1174	5.94	2.94	10.33	23.07	1.13	1.10	0,1	3,5	91,7	96,3	4,7	0,2
Night	-1 to +10	12	195	4.78	2.94	6.94	16.87	0.97	1.11	1,0	17,9	81,0	81,0	0,0	0,0
	+85 to +96	12	195	5.61	2.94	7.89	29.78	1.29	1.32	1,5	13,3	83,6	85,1	1,5	0,0
	+165 to '+176	12	193	5.44	3.17	7.44	14.69	0.90	0.93	0,0	6,7	93,3	93,3	0,0	0,0
	+215 to +226	12	192	5.44	3.17	7.28	12.84	0.84	0.87	0,0	6,3	93,8	93,8	0,0	0,0
	+246 to +257	12	194	6.06	3.00	8.33	17.87	1.00	0.92	0,0	3,6	93,8	96,4	2,6	0,0

Additional file 3. Flash glucose monitoring data.

Chapter 7

General discussion and future perspectives

Hepatic GSDs are ultra-rare, inherited disorders of carbohydrate metabolism, which usually present in early childhood[1]. Their multisystem involvement, requiring multidisciplinary professionals, raises huge organizational, logistic, and financial obstacles for affected families and healthcare providers. The potentially life-threatening nature of hepatic GSDs symptoms and high variability in hepatic GSDs patients' phenotypes, treatment interventions and outcomes emphasize the need and urgency for improved monitoring options. The current diversity in management guidelines for hepatic GSDs causes unwanted variations in diagnosis and treatment, and necessitates standardisation of clinical care, aiming at the most favourable patient's outcomes and healthy ageing.

Despite the increase in life expectancy over the past years, there is an unmet clinical need for improved management of hepatic GSDs. Standardisation of the patients' management (including emergency situations), development of strategies to prevent and/or treat intestinal problems in GSDIb and muscle problems in GSDIII have been identified as top research priorities by patients, carers, and healthcare professionals[2]. Potential solutions for these priorities have been investigated in the present thesis.

Innovative management strategies and novel monitoring tools for hepatic GSDs are closely related challenges. The need for improved monitoring strategies can be considered as a direct consequence of the need for novel management strategies. Paragraph 7.1 focuses on the strategies to improve and (possibly) standardise management of patients with hepatic GSDs. In paragraph 7.2 an opportunity for minimally invasive monitoring and a possible novel biomarker for GSDI are discussed.

7.1 Developing novel management strategies

Current therapies for hepatic GSDs appear untargeted and generally? do not target the primary metabolic defect but rather the related symptoms and signs. Frequent feedings and uncooked cornstarch (UCCS) have been the cornerstone of the treatment since the 1970s[3-5]. Some patients may require complementary continuous nocturnal gastric drip feeding (CNGDF) to avoid hypoglycaemia[6]. Additional and distinct dietary recommendations are provided for specific hepatic GSDs subtypes, such as avoidance of sucrose and lactose in GSDI[3], and high-protein intake for GSDIII[4]. Such strict dietary regimens may heavily challenge patient's quality of life and compliance. The development of dietary treatments has resulted in a dramatic reduction in hypoglycaemic events and changed the clinical focus of hepatic GSDs from mortality to morbidity. At the same time, several long-term complications have emerged which are not (entirely) prevented by currently available treatments. Among these, renal disease and liver adenomas in GSDIa[3], neutropenia and inflammatory bowel disease (IBD) in GSDIb[7], and (cardio)myopathy in GSDIIIa[4] still heavily impact on patients' prognosis and quality of life. In this respect, several novel and innovative treatments, such as gene (NCT03517085) and mRNA therapy[8] are currently under investigation. Whether such treatments can provide an effective and permanent cure for patients with hepatic GSDs or realistically broaden the available therapeutic options is yet unclear.

In the "real world" amongst health care providers, controversies still exist on specific management topics, such as treatment of hyperlipidaemia, risks related to long-term G-CSF administration, and many recommendations are based on so-called best practice and expert opinions. Current guidelines mainly focus on the long-term management while little attention is paid to acute treatment[3-7].

Consequently, wide differences in patients' management still exist among clinical centres impacting on patients' outcomes and access to healthcare services.

7.1.1. Towards standardization of the management of patients with hepatic GSDs

One of the research questions for this thesis was: *Can patients with hepatic GSDs benefit from dietary lipid manipulation?* In recent years, various case studies have described reversal of cardiac hypertrophy and myopathy in GSDIII patients in response to ketogenic diets[9-10]. Addressing this question will therefore contribute to development of novel treatment options to prevent and/or treat muscle problems in GSDIII patients.

In chapter 4 results on dietary lipid manipulations in patients with GSDIII are presented and discussed. A high fat diet represented the most common dietary lipid manipulation. Cardiomyopathy and myopathy were the main indications for switching to a high fat diet in GSDIII patients.

Firstly, a high fat diet appears safe in patients with GSDIII; also, no increase in patients' BMI was observed. Secondly, significantly lower CK concentrations were observed after dietary lipid manipulation. Thirdly, reduced interventricular septum thickness was observed in paediatric but not adult GSDIII patients following dietary lipid manipulation. Lastly, most patients reported subjective improvements of exercise tolerance and/or muscle strength after dietary lipid manipulation. Overall, these findings suggest that a high fat diet can exert beneficial effects on the muscle phenotype in GSDIII patients. Available data support the inclusion of a high fat diet among the possible treatment options for GSDIII in future guidelines.

Interestingly, the benefit of the high fat diet on cardiomyopathy in GSDIII appears to be age dependent. Likely, an early switch to a high fat diet can reverse, or at least reduce cardiac glycogen accumulation. Following our literature search in December 2018, two additional reports were published that describe improvements in cardiac and skeletal muscle function after starting a ketogenic diet in adult and adolescent GSDIII patients, respectively[11-12].

Although the effect of a high fat diet on muscle outcomes seems promising in GSDIII patients, the underlying mechanism remains currently largely unresolved. In all reported dietary interventions, carbohydrates were replaced by lipids. Thus, the observed benefit may be driven by reduced carbohydrate intake[13]. Alternatively, the properties of fat as an alternative energy substrate for muscle may contribute. In addition, a general improvement in dietary compliance could also play a role, regardless of the type of intervention. Therefore, it would be interesting to compare the efficacy of a high fat diet to that of improved metabolic control combined with appropriate protein intake on muscle signs/symptoms in GSDIII patients.

Notably, the long-term effect of a high-fat diet in patients with GSDIII should be monitored as these may increase the risk to develop steatohepatitis[14] and osteoporosis[15]. On the other hand, liver cirrhosis[16] and osteopenia[17] can naturally occur in patients with GSDIII, independent of a switch to a high fat diet. Follow-up studies are therefore warranted.

Chapter 5 presents a potential approach to optimise and uniform the initial, preventive management of emergency situations for patients with hepatic GSDs.

Although local or regional healthcare providers that lack specific metabolic training are often the first actors in metabolic decompensations, current disease-specific recommendations are not always easily accessible to non-metabolic specialists. Taking into account that such recommendations are largely based on expert opinions, standardisation of the initial emergency management for patients with hepatic GSDs is challenging.

After multiple revisions of the original "generic emergency protocol" which has been used at University Medical Centre Groningen (UMCG) since 2014, an international collaborating group consisting of healthcare providers and patient representatives from 32 centres and 15 countries worldwide released a shared emergency protocol for hepatic GSDs patients (and also fatty acid oxidation disorders (FAOD) patients) in 10 languages. This new protocol has several advantages. First, by providing shared and endorsed recommendations, it represents a major step towards standardised global management of emergency situations for hepatic GSDs patients[24]. Second, it adds to current guidelines where practical instructions on prehospital phase management and communication are scarce[3-7]. In fact, prevention of metabolic emergency is one of its main aims. Third, it provides simple instructions which can be followed by both patients during the prehospital phase and local healthcare providers upon hospitalisation. Fourth, the personalised emergency letter can be easily generated and updated at any time via www.emergencyprotocol.net, only requiring the patient's weight and diagnosis. This can be done either by the patient or the healthcare providers and in multiple languages.

Five-year single-centre experience suggests that the generic emergency protocol can safely prevent metabolic emergencies in patients with hepatic GSDs. The electronic emergency letters can be particularly helpful in case patients are far away from the metabolic centre of expertise. This is relevant for both prevention in the home setting and for in-hospital management. Taking into account its benefits on prevention of metabolic decompensations and guidance for local healthcare providers, we propose to include this protocol in future management recommendations.

Although the generic protocol provides simple instructions and can guide families and healthcare professionals, it does not to replace expert metabolic advice. The generic emergency protocol can guide decision making during the first hours of a metabolic emergency before reaching out to the metabolic specialist. Importantly, the proposed management strategies should not be executed extensively. Both the emergency solution and the glucose infusion rates are tailored to meet carbohydrate requirements under these circumstances, but the treatments do not provide sufficient calories in the long-term. Therefore, prolongation of the emergency treatments poses the risk of (protein) catabolism. This can be prevented by good communication between health care providers and families, for instance repeatedly during the intercurrent illness episode, in a shared care model including the metabolic centre of expertise, the local healthcare providers, the caregivers and the patients. In this respect, personal values, preferences, and individual circumstances (including psychosocial and cultural aspects) should at all times be considered to ensure optimal care.

Although a generic emergency protocol appears effective in hepatic GSDs and FAOD, it is as yet not in place for inherited metabolic disorders of the intoxication type (e.g., organic acidemias, urea cycle

defects) or disorders associated with perturbed carbohydrate metabolism (e.g., idiopathic ketotic hypoglycaemia, congenital hyperinsulinism, and patients using ketogenic diets). Future studies may evaluate the cost-effectiveness of this emergency protocol.

7.1.2. Repurposing empagliflozin to treat inflammatory bowel disease (IBD) in GSDIb

Conventional treatments for IBD (i.e., corticosteroids, immunomodulators, biological agents, G-CSF) can be ineffective and/or associated with side effects (e.g., leucopenia, anemia, diarrhea) in GSDIb patients. As patients sometimes even require abdominal surgery, improved prevention and treatment of intestinal problems is a compelling need in GSDIb[2]. After elucidation of the pathogenic role of 1,5-anhydroglucitol accumulation in neutropenia/neutrophil dysfunction in GSDIb[25], two recent papers have shown that repurposing the antidiabetic drug empagliflozin exerts beneficial effect on neutropenia/neutrophil dysfunction in 5 patients with GSDIb[26-27].

Although non-invasive markers of IBD, i.e., Crohn disease activity index (CDAI), stool consistency and faecal calprotectin, were shown to be beneficially affected by empagliflozin, the chronic relapsing and remitting course of IBD warranted additional investigation to confirm efficacy of this treatment on IBD in GSDIb patients.

In chapter 6 the first evidence on the benefit of empagliflozin treatment on bowel (macro/microscopic) morphology in a GSDIb patient is presented. A significant decrease in disease length and activity as well as histological remission were documented 3 months and 5.5 months after empagliflozin treatment was started. Nonetheless clinical and biochemical improvements were observed, i.e. improved perineal pain and anal fissure as well as reduced stool frequency and increased haemoglobin levels within one week and the first month of treatment, respectively. Notably, as empagliflozin treatment in this patient was started during a IBD flare-up, these findings suggest its potential efficacy also during the acute phase. Still, the persistence of ileal stricture after the treatment suggests that empagliflozin may be effective in healing the inflammatory lesions/strictures but might not be able to reverse established fibrotic strictures. It can also be speculated that empagliflozin, by counteracting neutrophil dysfunction, and G-CSF, which mobilises neutrophils from the bone marrow, may exert a synergistic effect on neutropenia and bowel inflammation in GSDIb.

Bowel morphology can provide significant insight on the effect of empagliflozin in GSDIb patients. Results from the present case and literature data support an early empagliflozin administration in GSDIb patients with IBD before the onset of (irreversible) intestinal fibrosis. Interestingly, empagliflozin use has been proposed to prevent bleomycin-induced lung fibrosis[28] and will likely reduce the healthcare costs for GSDIb (-59% medication-related costs in the present case).

Although empagliflozin appears to be a promising drug for IBD in GSDIb, several questions remain. Its effect on lymphocytes and/or macrophages likely explains its beneficial actions on bowel inflammation. In this respect, the proposed role for 1,5-anhydroglucitol (1,5AG) and 1,5-anhydroglucitol-6-phosphate (1,5AG6P) in modulating other peripheral blood mononuclear cells may be relevant for its effects on IBD in GSDIb patients[29]. Interestingly, improved colonic inflammation through TNFα- and IL1β-independent mechanisms has been recently shown in IL-10$^{-/-}$ mice (experimental models of colitis) after 14 days treatment with empagliflozin[30]. Follow-up

research elucidating the effects of empagliflozin on bowel inflammation (both in GSDIb and non-GSDIb patients) is needed.

7.2 Developing novel monitoring strategies

Several novel treatments options for hepatic GSDs are currently being explored (Table 1). Some studies aim for refinement of traditional treatments (e.g., optimisation of macronutrient composition) and novel/repurposed drugs, in addition to (potentially curative) treatments which can possibly restore enough working enzyme.

Longitudinal monitoring of patients with hepatic GSDs receiving such novel treatments may include a combination of (1) assessment of traditional biochemical biomarkers, (2) assessment of enzyme activities *ex vivo*, (3) execution of (invasive, clinical) fasting challenges *in vivo*, (4) continuous glucose monitoring (CGM), and (5) application of stable isotope methods to longitudinally quantify endogenous glucose production (EGP) rates *in vivo*[31].

Disease	Drug	Study Sponsor	Approach	Age (years)	Study phase	Study identifier
GSDIa	AAV8-G6PC	Ultragenyx Pharmaceutical Inc (Novato, CA, USA)	Gene replacement	> 18	1/2	NCT03517085
GSDIa	--*	Ultragenyx Pharmaceutical Inc (Novato, CA, USA)	Gene replacement	> 18	*	NCT03970278
GSDI	Triheptanoin	Ultragenyx Pharmaceutical Inc (Novato, CA, USA)	Anaplerotic therapy	0.08-65	1/2	NCT03665636
GSDIb	Empagliflozin	Hong Kong Children's Hospital (Hong Kong)	Drug repurposing	0.5-18	Observational	NCT04986735
GSDIb	Empagliflozin	Cliniques universitaires Saint-Luc- Catholic University Leuven (Leuven, Belgium)	Drug repurposing	1-18	2	NCT04138251
GSDIb	Empagliflozin	Department of Internal Medicine, Hypertension and Vascular Diseases, The Medical University of Warsaw (Warsaw, Poland)	Drug repurposing	>0.08	2	NCT04930627
GSDIII	LNP-mRNA	Ultragenyx Pharmaceutical Inc (Novato, CA, USA)	RNA replacement	> 18	1/2	NCT04990388

Table 1. Ongoing clinical trials assessing novel medical treatment options for hepatic GSDs. Data retrieved from Clinicaltrials.gov, last access on October 3rd, 2021. *LNP: lipid nanoparticle. *This is an observational study of patients enrolled in the trial NCT03517085. No drug is administered during this study.*

Although various biochemical markers of metabolic control are included in current guidelines[3-7] and clinical trial protocols, they do not always appear sufficiently reliable to dissect the phenotypic heterogeneity and to assess the (expected) efficacy of novel treatment on the affected tissues[32]. Enzyme testing may involve invasive procedures (e.g., liver biopsy for G6Pase)[3], may not always retrieve conclusive results (e.g., phosphorylase kinase)[33] and the results may be dependent on the sample type (e.g., whole cell or endoplasmic reticulum fractions for G6Pase). Additionally, for most

hepatic GSDs no clear correlation between residual enzyme activity and clinical phenotypes is established [3-7,33]. A controlled fasting challenge can dynamically assess the metabolic changes that occur upon fasting and postprandially, but poses a significant organizational burden, e.g., hospital admission, a designated team and a fully equipped laboratory are required, and potential safety issues, as the test is continued until patients develop signs/symptoms of hypoglycaemia[34].

CGM represents a potential tool for minimally invasive monitoring of patients with hepatic GSDs. It constitutes a highly attractive approach to longitudinally monitor glucose trends both in the hospital and in the home-setting. Previous research has confirmed the efficacy of CGM to monitor the efficacy of novel/optimized dietary and/or medical treatments in patients with hepatic GSDs[35-37]. Reference values for the CGM-related outcome measures in GSDIa patients, however, are as yet lacking.

Chapters 2 and 3 address the generation of reference values for CGM-derived parameters in GSDIa patients, therefore implementing the CGM use into clinical research, and the identification of a novel biomarker for GSDIa.

7.2.1 Towards minimally invasive monitoring of patients with GSDIa

The research question: *Can CGM reference values be defined for adult GSDIa patients?* a was addressed in chapter 2. This study allowed to generate reference values for CGM-derived outcomes in adult GSDIa patients. Unlike diabetes mellitus (DM), in which CGM is a recognised tool for patients' monitoring and to support decision making on dietary and/or medical adjustments[42-43], the lack of CGM reference values for hepatic GSDs currently limits the use of this technology for follow up and monitoring during regular healthcare or clinical trials.

Prospective CGM data on 10 GSDIa patients and 10 age-, gender- and BMI-matched healthy volunteers were collected. Reference values for major CGM-derived outcome parameters, (i.e., descriptive parameters, glycaemic variability, time below range, time in range, time above range, were generated.

This study also revealed that GSDIa patients display higher time below range (TBR) and time above range (TAR), lower time in range (TIR) and larger glycaemic variability (GV) compared to healthy controls. Furthermore, 9/10 GSDIa patients did not demonstrate level 2 hypoglycaemia (i.e., glucose values <3.0 mmol/L) overnight.

This work demonstrates that individual GSDIa patients' CGM outcomes can be compared to reference values obtained from a matched GSDIa reference population, as well as from matched healthy volunteers. Separately analysis of CGM data collected during day and night-time may allow for optimal interpretation of the CGM results. Obviously, other factors that may influence glucose homeostasis, such as dietary management and exercise, should be considered when interpretating the glucose patterns. The correlation between CGM results and dietary intake or physical activity was not considered in the current study. Follow-up studies investigating the major determinants of CGM-derived parameters are warranted.

Collecting CGM data from GSDIa patients poses major challenges. First, no general agreement on CGM outcome parameters in GSDIa exists. In this study we referred to parameters commonly used

for DM[42]. Second, GSDIa patients display large variability with regard to their clinical phenotype, dietary scheme and physical activity[32]. It is not clear whether the sample size in the present study adequately covers such variability. Third, several types of CGM devices have been previously used in patients with hepatic GSDs presenting with different functionalities, advantages, and limitations (Table 2). Notably, none of them has been formally licensed for use in patients with hepatic GSDs. Additionally, data management applications currently vary depending on the type of CGM device installed and interconnectivity between CGM softwares and digital health records is lacking. Fourth, it is currently unknown how many measurements are minimally required to generate reliable CGM profiles in GSDIa patients. In DM patients 14-day data collection is recommended to adequately predict the GV over a 3-month period[46]. Within the current study, this was unfortunately not feasible. Based on the above-mentioned considerations, this work provides insights that are of great importance towards the (routine) use of CGM in clinical research and care for GSDIa patients.

Reference	**Device**	**Country**	**Population (**n. of patients)	**Age (**range in years)	**GSD subtype**
Hershkovitz ea. J Inherit Metab Dis. 2001.	MiniMed (Medtronic)	Israel	4	2-15	Ia
Maran ea. Diabetes Metab Res Rev. 2004.	Glucoday® (Menarini)	Italy	4	14-47	Ia
			1	22	Ib
			1	10	III
White ea.J Inherit Metab Dis. 2011.	iPro™ (Medtronic)	UK	1	6	0
			6	0-13	Ia
			2	0-3	Ib
			7	4-20	III
			4	5-16	IX
			2	2-24	GLUT2
Kasapkara ea. Eur J Clin Nutr. 2014.	MiniMed (Medtronic)	Turkey	15	2-18	Ia
			1		Ib
Herbert ea. J Inherit Metab Dis. 2018.	Dexcom G4 Platinum (Dexcom)	USA	7	2-56	Ia
			2	9-17	Ib
			6	6-44	III
			5	7-17	IX
Kaiser ea. Mol Gen Metab. 2019.	iPRO2® (Medtronic) Guardian® (Medtronic) FreeStyle Libre® (Abbott)	Switzerland	12 2	11-49	Ia Ib
Peeks ea. J Inherit Metab Dis. 2021.	Dexcom G6 (Dexcom) Dexcom G4 (Dexcom) Dexcom G6 (Dexcom)	Netherlands	1	9	Ia
			12	2-22	Ia, III, IX
			3	2-11	Ib

Table 2. Previous studies using CGM in hepatic GSDs patients.

7.2.2 Cortisol metabolism: a novel biomarker for GSDI

Although endocrine abnormalities have been extensively reported in GSDI[47-51], adrenal cortex function had not been assessed systematically. Chapter 3 presents our study of baseline and ACTH-stimulated adrenal cortex hormone responses in 17 GSDI patients (10 GSDIa, 7 GSDIb) and 34 age- and gender-matched healthy volunteers.

This study reveals that imbalanced serum cortisol levels are a feature of GSDI. Specifically, GSDIa patients exhibit increased baseline and ACTH-stimulated cortisol levels while GSDIb patients show reduced baseline cortisol levels. We hypothesise that cortisol imbalance in GSD I may result from deregulation of adrenal cortex or hepatic 11βhydroxysteroid dehydrogenase type 1 (11βHSD1) activity. 11βHSD1 is an ER-bound enzyme that catalyses the conversion of inactive cortisone in active cortisol. Its activity is determined by G6P levels within the ER[52]. Thus, 11βHSD1 activity potentially links endocrine regulation and metabolic derangement in GSDI. Notably, cortisol imbalance may also increase the risk for metabolic syndrome development in GSDIa patients[53].

A direct correlation between baseline cortisol serum levels and both cholesterol and TG serum levels is also found in GSDI patients. Since cellular glucocorticoid synthesis involves the shuttling of (lipid) precursors between mitochondria and the ER[54], high cortisol levels might represent a mechanism to divert lipid excess within the mitochondria in GSDIa. Interestingly, increased G6P levels in ER[55] and mitochondrial dysfunction[56] have been proposed as the cause and the effect of hypercholesterolemia in GSDIa, respectively.

Notably, GSDIb patients exhibited lower basal cortisol levels while they generally display remarkably less severe hyperlipidaemia as compared to GSDIa patients[50,53]. A reduction of 11βHSD1 activity in GSDIb immune cells may represent one of the factors contributing to impaired immune cell function and chronic inflammation[57] as 11βHSD1 deficiency is associated with delayed resolution of inflammation[58] while glucocorticoids, which are amongst the major modulators of regulatory T cells[59], can protect from the development of autoimmunity[60].

Overall, we demonstrate for the first time that GSDI patients display imbalanced cortisol homeostasis. This result is particularly relevant as it extends the current disease phenotype. Also, cortisol might serve as an additional biomarker for patients' monitoring. Although we hypothesise a role for tissue 11βHSD1 activity in mediating imbalanced cortisol homeostasis in GSDI, the underlying mechanisms remain largely unresolved. Besides being regulated by 11βHSD1 activity, serum cortisol levels are also subject to regulation by the hypothalamo-pituitary-adrenal axis as well as by circulating glucose concentrations. The unaltered adrenal cortex hormone levels suggest absence of hypothalamo-pituitary-adrenal axis dysfunction in GSDI patients. Baseline glucose levels were comparable between patients and healthy volunteers and the ACTH stimulation test was designed not to exceed patients' fasting tolerances. Although glucose concentrations were not routinely monitored during the ACTH stimulation test, available data from 4 patients indicate relatively stable glycaemia during the test suggesting that changes in glucose concentrations likely did not affect the measured cortisol levels in the present study.

7.3 Future perspectives

Despite the progress made over the past decades, there still exists a compelling need to improve clinical outcomes and to uniformly deliver state-of-the-art healthcare to patients with hepatic GSDs[2]. In addition, novel promising treatments for hepatic GSDs are becoming available, requiring more reliable, safer, and easier monitoring tools. Surrogate endpoints need to be defined in order to identify early treatment responses. Moreover, methodologies to integrate standardised and structured data for so-called real-world databases are urgently warranted. The research presented in this thesis aimed to provide possible solutions to these needs. Future research challenges can hence? be summarised in three categories: novel treatment strategies, novel monitoring tools and (re)organization of healthcare.

Developing **novel treatment strategies** is a compelling need for patients with hepatic GSDs[2]. Although switching to a high fat diet appears to be promising for patients with GSDIII many uncertainties remain on the long-term effect of dietary modifications as well as on the optimal dietary regimen. Recordings of dietary intake are often complicated by poor documentation and compliance. Also, discrepancies between described and consumed diets often exist. These factors make it challenging to identify correlations between dietary patterns and long-term complications. In this respect telemedicine platforms allowing for home-site monitoring of dietary habits could provide valuable tools[61]. In addition, evidence should be collected to define the recommended daily amount of lipids and to determine whether specific lipids (e.g., medium chain triglycerides) may provide additional benefits. Furthermore, assessment of the potential synergistic effects of combined therapeutic approaches (e.g., high-fat diet together with exercise training[62] or acute nutritional ketosis[63]) is worthwhile. Finally, the efficacy of dietary lipid modifications in other hepatic GSDs subtypes remains to be assessed.

Our work furthermore expands the evidence on the benefits of empagliflozin treatment for GSDIb patients. Shortly after release of our paper, a short report was published which confirmed improvement of IBD symptoms in response to empagliflozin treatment[64]. Yet, current experience and evidence is limited, while three clinical trials are currently ongoing (Table 1). In addition, an international collaborative retrospective study on empagliflozin treatment in GSDIb patients is currently integrating all published and unpublished cases. One major issue is that as empagliflozin is currently an off-label drug for GSDIb each healthcare professional takes the responsibility of independently treat his/her patients after obtaining a written informed consent. This can result in supply/reimbursement issues depending on the local healthcare policies. Expansion of current evidence on the efficacy of empagliflozin is expected to ameliorate these challenges by including this drug as a licensed treatment option for GSDIb. Besides these regulatory issues, aspects that need further investigation include the optimal daily dosing, and the potential of baseline 1,5AG concentrations to predict treatment responses in GSDIb patients. Additionally, the (dis)advantages of other SGLT2-inhibitors (i.e., dapagliflozin, canagliflozin), and the (side) effects of long-term empagliflozin treatment remain to be established.

The novel promising therapies that are currently being investigated aim to target GSDIa disease pathophysiology rather than its symptoms and/or signs. Among these, gene-based therapies are of particular interest. Gene therapy (GT) with adeno associated (AAV) vectors was shown to restore G6Pase activity and ameliorated disease sequelae in murine and canine models for GSDIa[65-66].

Although challenged by the large size of the human gene, hepatic correction and rescued muscle function was also observed after dual-vector *AGL* administration in GSDIII mice[67-68]. Reduced glycogen content and improvement liver and muscle function was also observed in the murine model for GSDIV after AAV9-GBE infusion[69]. GT appears to be less effective in GSDIb as the loss of vector genomes during cell division only allows for transient reversal of neutropenia[70]. Alternative approaches are therefore also explored. For example, mRNA therapy delivered to the target tissue via lipid nanoparticles improved fasting glucose concentrations and prevented the occurrence of liver neoplasms in the murine model of GSDIa[8]. Following these promising preclinical results, several clinical trials were initiated to assess the safety and efficacy of novel therapies in hepatic GSDs patients (Table 1).

Since current tools do not always appear sufficiently reliable and/or safe/simple to assess treatment efficacy, development of **novel monitoring tools** is essential. In this thesis two tools that could potentially expand current monitoring of hepatic GSD patients have been presented.
Previously collected evidence supporting the (routine) use of CGM in clinical research and care for GSDIa patients was extended by the study described in chapter 2. Although several studies on hepatic GSDs made use of CGM (Table 2) a number of unsolved questions prevent widespread and optimal CGM use in hepatic GSDs patients. First, consensus should be reached on the definition of "low-glucose" and "high-glucose" thresholds. Second, the relationship between CGM-derived outcome parameters and traditional biomedical outcomes as well as the prognostic role of these parameters should be investigated. Third, a thorough integration of CGM data and information on the diet and physical activity should be attained. Fourth, harmonising the CGM data management systems and developing infrastructures to integrate CGM data into EHR is a compelling need. Fifth, reference values for CGM parameters should also be generated for the hepatic GSDs subtypes other than type Ia .

Extension of the GSDI phenotype with perturbed cortisol homeostasis (chapter 3) may have intriguing implications. Our findings are particularly remarkable considering that hormonal perturbations in GSDI patients potentially complicate monitoring of clinical trials on novel therapies which involve corticosteroid treatment to prevent potential treatment side-effects (Table 1). As next research step it is critical to investigate (1) the relationship between chronic hypercortisolism and growth problems in GSDIa, and (2) cortisol homeostasis in hypoglycaemic GSDI patients.

As previously mentioned, the application of stable isotope methods to longitudinally quantify EGP rates in humans[71] could provide a means for minimally invasive monitoring of patients with hepatic GSDs. Changes in EGP may reflect specific enzyme defects in hepatic GSDs. Therefore, at least in theory, stable isotope-based EGP assessment could enable monitoring efficacy of novel treatments. Yet, the need for intravenous (or via nasogastric tube) tracer administration and repeated venous blood sampling[72-74] currently limits the application of this method and its use in the home-setting. Future studies exploring alternative administration routes and sampling modalities are therefore warranted. The ongoing ENGLUPRO GSDIa study (NCT04311307) is aiming to assess glucose homeostasis in GSDIa patients using an orally administered stable isotope and dried blood spot sampling.

The use of emerging high-throughput analytical technologies such metabolomics, proteomics and lipidomics greatly enhances the potential for identification of novel disease biomarkers[75]. Integration of clinical, nutritional, biochemical, CGM, imaging, and multi-omics data will subsequently contribute to establishment of personalised disease profiles for hepatic GSDs patients. The availability of novel biomarkers may also open new perspectives for population newborn screening for hepatic GSDs. In this respect, identifying sensitive and specific biomarkers for specific disorders to be assessed on dried blood spots is crucial.

Delivering standardized high-quality healthcare to patients worldwide is amongst the top research priorities for hepatic GSDs[2]. To achieve this aim, **re(organization) of healthcare** over the next years is essential. The development of a generic emergency protocol (chapter 6) constitutes a major step towards this aim. Formal agreement between all contributors is required prior to protocol inclusion in consensus guidelines. Expansion of knowledge and experience will drive protocol revisions. Since ethical and organizational aspects prohibited direct comparison of "generic" and "personalised" emergency protocols, additional data on worldwide protocol utilization are warranted to confirm its efficacy. Prevention and reversal of catabolic states are critically important in hepatic GSDs as well as other disorders in which carbohydrate metabolism is perturbed, such as idiopathic ketotic hypoglycaemia, congenital hyperinsulinism, and patients using ketogenic diets and patients with inherited metabolic disorders of the intoxication type, e.g., organic acidemias, urea cycle defects[76-77]. It is therefore worthwhile to investigate whether the approach herein presented may also benefit the management of such disorders.

Major challenges to ensure optimal healthcare and perform high-quality research for patients with hepatic GSDs remain. Research and clinical expertise on hepatic GSDs are generally fragmented and confined to personal interest of a limited number of experts, and interaction between stakeholders, i.e., healthcare professionals, fundamental/translational scientists, patients, industry, regulatory agencies, is not sufficiently guaranteed. In the everyday reality, directing research agendas towards the most urgent needs and getting access to state-of-the-art healthcare and therapeutic options, is extremely challenging for patients with hepatic GSDs. Traditionally health care systems are focused on local delivery of care, while hepatic GSDs patients usually do not live close to expert health care providers. Large clinical and dietary heterogeneity in GSD patients furthermore challenges the interpretation of traditional outcome parameters[32]. Moreover, due to the rapid fluctuations in glucose homeostasis, there is high degree of self-management while monitoring and management guidelines for GSD patients still differ between parts of the world, causing unwanted variations in diagnosis, treatment, and outcomes[3-7]. Finally, information on guidelines and care pathways does not always reach every patient. There is a strong compelling need to develop inclusive collaborative networks to efficiently provide knowledge to individual GSD patients at any time or place. Ideally, such networks would integrate and expand existing patient registries and telemedicine/knowledge dissemination platforms.

Integrating healthcare and research, homeside monitoring and establishement of standard outcome measures are key for effective characterisation of rare diseases. It is increasingly recognised that patients and families need to be involved when prioritizing (patient-reported) outcome measures[78]. Notably, the generic emergency protocol presented in chapter 5 included patients' input, underlining

that active participation of patients at early stages of biomarkers/management strategies/drug development processes is paramount.
By organising healthcare according to the needs and preferences of the individual patient, value-based healthcare (VBHC) intends to achieve better outcomes for patients and hence, improve quality of patient care, at reduced cost. In the VBHC approach patients' relevant medical outcomes define their view, whereas Integrated Practice Units (IPU) define the organizational view. An IPU can be defined as "organized around the patient and providing the full cycle of care for a medical condition, including patient education, engagement, and follow-up and encompass inpatient, outpatient and rehabilitative care as well as supporting services"[79]. In this respect, development of collaborative networks organised according to a VBHC approach, including health care providers, scientists, patients and their families has the potential to radically impose positive changes on the future of research and healthcare.

7.4 Concluding remarks

Until the 1970s, hepatic GSDs were mostly fatal diseases. Subsequently frequent feeding, continuous nocturnal gastric drip-feeding and UCCS contributed to maintain normoglycaemia, hence increasing patients' life expectancies[3-7]. The availability of these treatment options and establishment of biomarkers and guidelines have improved patients' prognosis and outcome, changing the clinical focus of hepatic GSDs from mortality to morbidity over the past decades. Nowadays additional efforts are needed to improve quality of life and healthy ageing for hepatic GSDs patients. This thesis combined studies on novel modalities for monitoring and management. Our studies have opened doors for future research directing precision medicine for individual GSD patients. Multistakeholder meetings in which of variety of professionals (including scientists, healthcare professionals, data analysts and software developers), patients, industry and regulators interact should be facilitated to prioritise strategies for research and care[80]. Patient value will be maximized when patients and their families actively participate during all phases of the process.

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Propositions

Novel monitoring and management strategies for hepatic glycogen storage diseases

by Alessandro Rossi

1. Continuous glucose monitoring should be considered standard of care for hepatic GSDs patients (Chapter 2)

2. Cortisol levels can constitute a novel monitoring biomarker for GSDI (Chapter 3)

3. A high-fat, low-carbohydrate diet can be beneficial in children with GSDIII and cardiomyopathy (Chapter 4)

4. A generic emergency protocol is instrumental to prevent metabolic decompensation and to facilitate communication in patients experiencing fasting intolerance due to an inherited metabolic disease (Chapter 5)

5. Empagliflozin improves inflammatory bowel disease in patients with GSDIb (Chapter 6)

6. Both “glucose control” and “metabolic control” should be assessed in hepatic GSDs patients (this thesis)

(00)7. Vodka Martini. Shaken, not stirred (Chapter 2).

8. Integration of research and healthcare is essential to improve quality of life and outcomes for hepatic GSDs patients (this thesis)

9. Each patient carries his/her own story. The availability of a range of monitoring and management tools allows healthcare professionals to offer patients optimal care based on individual preferences and needs (this thesis)

10. Collaboration between scientists, healthcare professionals, patients, policy makers and companies is crucial to define and achieve relevant outcomes (this thesis)

11. Chi conosce tutte le risposte non si è fatto tutte le domande (Confucius)

12. Quid quisque posset nisi temptando non didicit (Lucius Annaeus Seneca)

13. In het Nederlands zeg je “met de deur in huis vallen”, in het Italiaans zeg je liever "indori la pillola"

14. Nederlanders hechten aan organisatie en agenda’s, in Italië praktiseert men eindeloze flexibiliteit en creativiteit.