Chapter 1

General introduction and thesis outline


*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 small 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\(^3\)-\(^5\). 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 current approach in most cases results in a working diagnosis, it is prone to several limitations: relevant information on medical history may not 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 GSD types are recognised and classified based on enzyme or transporter deficiency and tissue involvement. The GSD 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.
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of hepatic GSDs. Additional symptoms and signs, biochemical disturbances and long-term complications vary widely between hepatic GSD types and can aid differential diagnosis.

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. Distinctive biochemical features include elevated blood concentrations of lactate, uric acid, and lipids. Additionally, patients with GSDIb show neutropenia/neutrophil dysfunction and recurrent infections. Long-term complications include liver neoplasms (mostly hepatocellular adenomas), renal disease (evolving to kidney failure), osteoporosis, anaemia, and inflammatory bowel disease (IBD).

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. Additionally, GSDIIIa patients show osteopenia and (cardio)myopathy worsening with age. Muscle involvement can include both proximal and distal muscle weakness and is likely overshadowed by fasting intolerance in childhood. Polyneuropathy caused by glycogen deposition in axons has also been described in adult GSDIIIa patients and may contribute to the muscle phenotype.

GSDIV is caused by glycogen branching enzyme deficiency and shows an extremely heterogeneous clinical presentation. The phenotypic continuum includes different degrees of hepatic involvement (from rapidly progressing liver cirrhosis to non-progressive liver disease), the neuromuscular system (e.g., foetal 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. Transaminase levels are increased in GSDIV patients with hepatic involvement.

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

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.

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.
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<table>
<thead>
<tr>
<th>Type</th>
<th>Name</th>
<th>Locus</th>
<th>OMIM#</th>
<th>Gene</th>
<th>Enzyme/Transporter</th>
<th>Glycogen structure</th>
<th>Guidelines*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0a</td>
<td>--</td>
<td>12p12.2</td>
<td>240600</td>
<td>GYS2</td>
<td>Glycogen synthase</td>
<td>Normal, decreased in quantity</td>
<td>No formal recommendations</td>
</tr>
</tbody>
</table>
| Ia   | Von Gierke       | 17q21.31       | 232200 | G6PC  | Glucose 6-phosphatase-α catalytic subunit | Normal             | Rake et al., 2002  
Kishnani et al., 2014  
Bali et al, 2016 |
| Ib   | Von Gierke       | 11q23.3        | 232200 | SLC37A4 | Glucose 6-phosphate transporter | Normal             | Visser et al., 2000  
Rake et al., 2002  
Kishnani et al., 2014  
Bali et al., 2016 |
| IIa/IIb | Cori/Forbes     | 1p21.2         | 232400 | AGL   | Glycogen debranching enzyme | Outer chains missing or very short | Kishnani et al., 2010  
Derks et al., 2021 |
| IV   | Andersen         | 3p12.31        | 232500 | GBE   | Glycogen branching enzyme | Very long unbranched chains | *Magoulas et al., 2019  
*Derks et al., 2021 |
| VI   | Hers             | 14q22.1        | 232700 | PYGL  | Liver glycogen phosphorylase | Normal             | Kishnani et al., 2019  
Labrador et al., 2019 |
| IXa  | --               | Xp22.13        | 306000 | PHKA2 | Phosphorylase kinase α-subunit | Normal             | Herbert et al., 2018  
Kishnani et al., 2019 |
| IXb  | --               | 16q12.1        | 261750 | PHKB  | Phosphorylase kinase β-subunit | Normal             | Herbert et al., 2018  
Kishnani et al., 2019 |
| IXc  | --               | 16p11.2        | 613027 | PHKG2 | Phosphorylase kinase γ-subunit | Normal             | Herbert et al., 2018  
Kishnani et al., 2019 |
| XI   | Fanconi-Bickel   | 3q26.2         | 227810 | SLC2A2 | GLUT2              | Normal             | *Pennisi et al, 2020 |

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 corn starch intake and/or gastric-drip feeding, is the cornerstone of the treatment\(^24\). 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. Serum triglycerides and cholesterol levels are therefore generally also included as biomarkers for all hepatic GSD types. Serum biotinidase activity and urine glucose tetrasaccharide can represent helpful diagnostic and dietary monitoring biomarkers, respectively. Additional biomarkers are monitored for specific hepatic GSD subtypes, such as lactate, uric acid, microalbuminuria in GSDI, neutrophil count and faecal calprotectin in GSDIb, ketones and creatine kinase in GSDIII.

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. Additional signs, symptoms and complications can be observed in specific subtypes, e.g., infections and diarrhoea in GSDIb, weakness and/or signs of cardiomyopathy in GSDIIIa, and neuromuscular symptoms in GSDIV. 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. Additional imaging studies may be required for specific hepatic GSDs subtypes, e.g., cardiac/muscle ultrasound in GSDIIIa. 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.

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 an 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.
REFERENCES


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PART I

Developing novel monitoring strategies