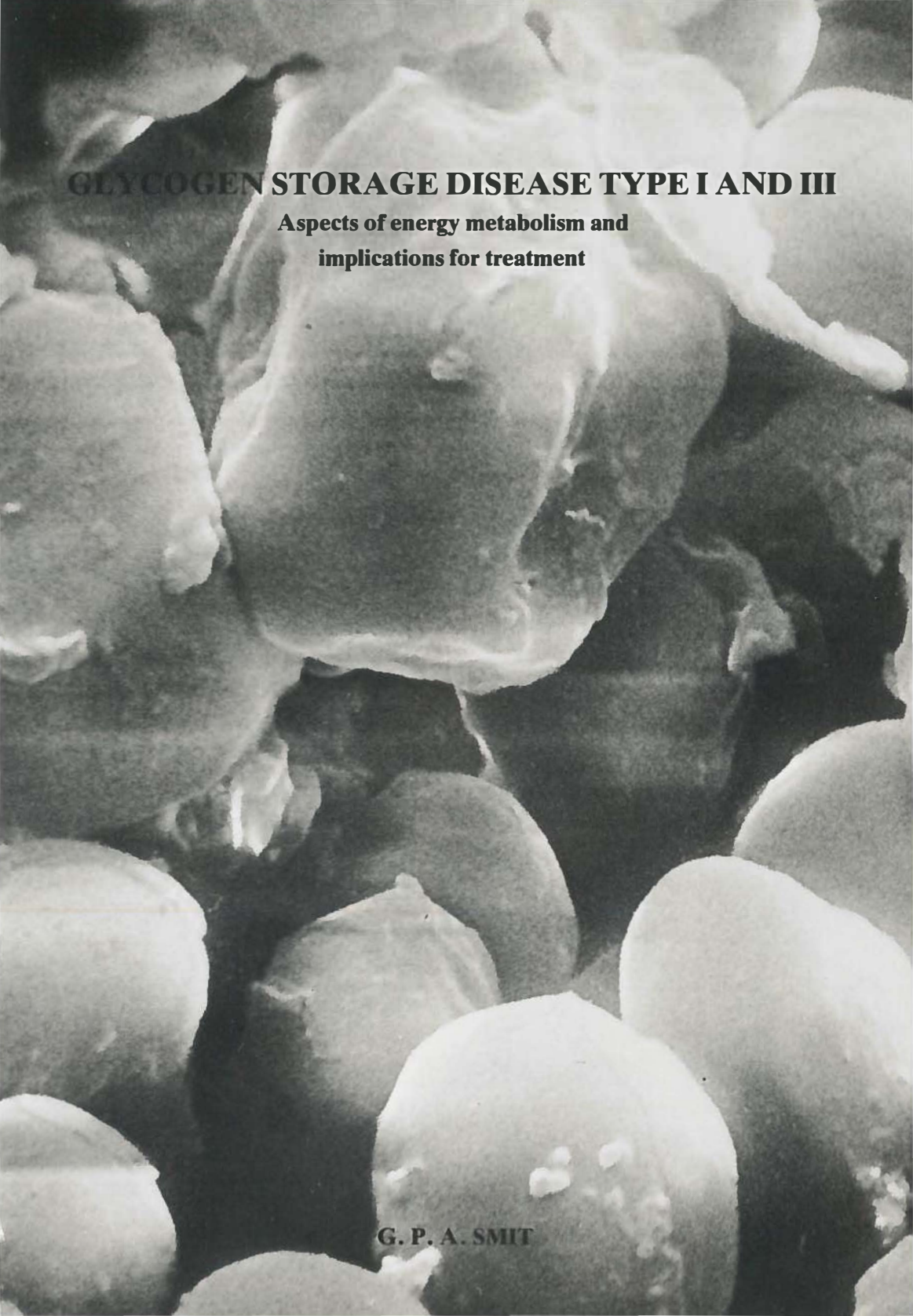
Glycogen storage disease type I and III : aspects of energy metabolism and implications for treatment

Smit, Gerrit Peter Arian



GLYCOGEN STORAGE DISEASE TYPE I AND III

Aspects of energy metabolism and implications for treatment

G. P. A. SMIT

GLYCOGEN STORAGE DISEASE TYPE I AND III

Aspects of energy metabolism and implications for treatment

Stellingen.

1.

Het verdient aanbeveling complexe koolhydraten toe te passen in de behandeling van die ziekten waarbij te hoge, dan wel te lage bloedsuikerwaarden moeten worden voorkomen.

dit proefschrift.

2.

De behandeling van patienten met glycogeenstapelingsziekte type I dient wat betreft de melkzuurconcentratie in bloed en urine niet op normalisering daarvan gericht te zijn.

dit proefschrift.

3.

De zogenaamde middenketen vetten verdienen een plaats in de behandeling van jonge patienten met glycogeenstapelingsziekte type III.

dit proefschrift.

4.

De eigen glucoseproductie van patienten met glycogeenstapelingsziekte type III is tot aan de volwassen leeftijd onvoldoende.

dit proefschrift.

5.

Bij patienten lijdende aan tyrosinaemie type I is orthotope levertransplantatie onvermijdelijk.

6.

De bij zuigelingen met een positieve Guthrie test uitgevoerde tetrahydrobiopterine belasting ter bevestiging c.q. uitsluiting van een tekort aan cofactor voor het enzym phenylalanine hydroxylase is niet waterdicht.

W. Endres et al, Lancet 1987, vol II : 222-223.

7.

Bij de behandeling van maternale PKU (phenylketonurie) dient normalisering van de phenylalanineconcentratie in het bloed te worden nagestreefd.

8.

Bij patienten met glycogeenstapelingsziekte type I wordt de prognose quoad vitam mede bepaald door de mate van nierfunctieverlies.

9.

Zonder een goed geoutilleerde afdeling voor functieonderzoek is universitaire pediatrische diagnostiek ondenkbaar.

10.

De variatie in het gedragspatroon van ernstig oligophrene patienten berust mogelijk op een afwijkend circadiaan ritme.

11.

Het effect van propaganda voor zogenaamde "lichte" sigaretten zou wel eens zwaar tegen kunnen vallen.

12.

Het Engelse gezegde "it 's raining cats and dogs" zou beter gewijzigd kunnen worden in "it 's raining vet's and doc's".

13.

Het doen opwaaien van stof is alleen al vanuit allergologisch standpunt bekeken onverstandig.

14.

"Bomvolle" voetbalstadions zijn ongewenst.

Stellingen behorende bij het proefschrift van

G.P.A.Smit, Groningen 1987.

RIJKSUNIVERSITEIT TE GRONINGEN

GLYCOGEN STORAGE DISEASE TYPE I AND III

Aspects of energy metabolism and implications for treatment

PROEFSCHRIFT

ter verkrijging van het doctoraat in de Geneeskunde

aan de Rijksuniversiteit te Groningen

op gezag van de Rector Magnificus Dr. E. Bleumink

in het openbaar te verdedigen op dinsdag 22 december 1987

des namiddags te 2.45 uur precies

door

GERRIT PETER ARIAN SMIT

geboren te Appingedam

1987

DRUKKERIJ VAN DENDEREN B.V.

GRONINGEN

Promotor : Prof. Dr. J. Fernandes

Referenten: Dr. R. Berger
Dr. D. J. Reijngoud

Promotiecommissie: Prof. Dr. C. J. de Groot
Prof. Dr. S. W. Moses
Prof. Dr. G. L. Scherphof

Aan mijn ouders

Voor Anne Marie
Rutger en
Maarten

Paranimfen: Drs. M. A. van Messel
Drs. J. Reinders

"Toutes les expériences s'enchaînent naturellement pour établir que le sucre, véritable produit d'une sécrétion intérieure, à laquelle j'ai donné le nom de glycogénie, prend naissance dans le foie aux dépens des éléments du sang et indépendamment de l'alimentation féculente et sucrée...."

Claude Bernard, 24 mars 1855, Vingt-cinquième leçon:

"Sur la glycogénie animale".

TEN GELEIDE

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Het verschijnen van dit proefschrift is mede mogelijk gemaakt door steun van: Maizena Diät GmbH Heilbronn, BRD.

CONTENTS.

CHAPTER I

GLYCOGEN METABOLISM.

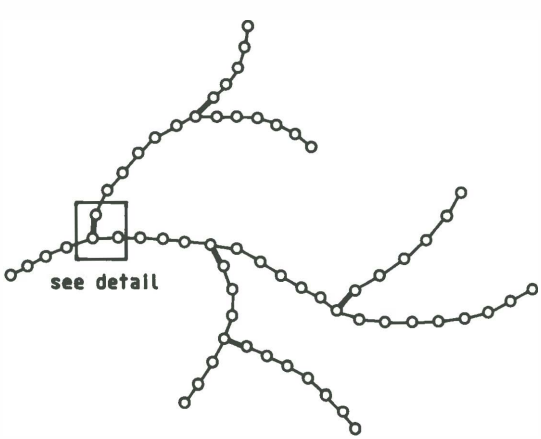
HISTORY.

In a ten years research period, Claude Bernard (1813-1878) discovered glycogen, isolated and characterized it both chemically and physiologically:
In 1848 he discovered a sugar-forming substance in the liver and called it glycogen (1). This research was performed on livers of dogs and rabbits in which he found glycogen after either starch-containing meals, or meals consisting of meat only. It was on this work that Bernard based his famous monography "Nouvelle fonction du foi", which contained many fundamental statements and hypotheses such as I.the liver is a "chemical laboratory", the center of metabolism, II. the liver forms a barrier between the blood from the intestines and the general circulation, III.the liver forms glucose and that this glucose synthesis is seen in all vertebrates, IV. after a meal rich in protein and carbohydrates the sugar concentration is increased, and V. that the liver has two main tasks: to excrete bile and produce glucose (2).
In 1857 Bernard isolated and characterized glycogen from liver tissue and described it as follows: "it is an amorphous white tasteless substance which does not dissolve in alcohol, it is free of nitrogen, and is degraded by diastase of either animal or vegetarian origin" (3).

STRUCTURE OF GLYCOGEN.

Glycogen is a polysaccharide with a molecular weight varying from a few million to well over several hundred million, depending on the type of tissue and the procedure of extraction. The molecules have a spherical form, and consist of D-glucose molecules which are attached to each other with α-1,4 or -1,6 linkages (figure 1 and 2).

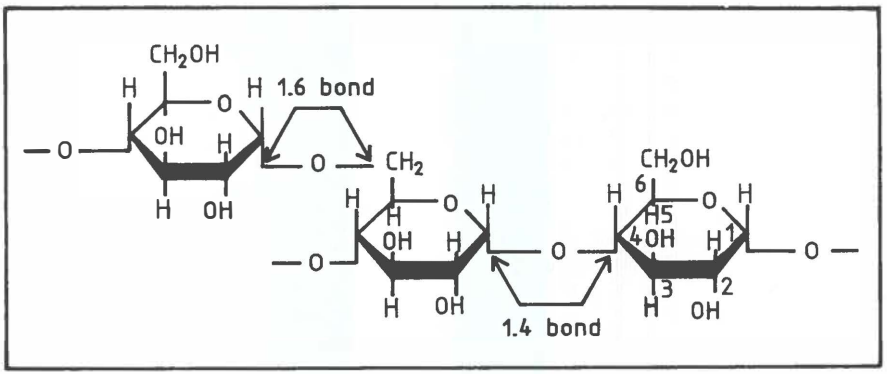
figure 1.



1.4(o—o)and 1.6(o—o)bonds of glucose molecules in glycogen

figure 2.

detail

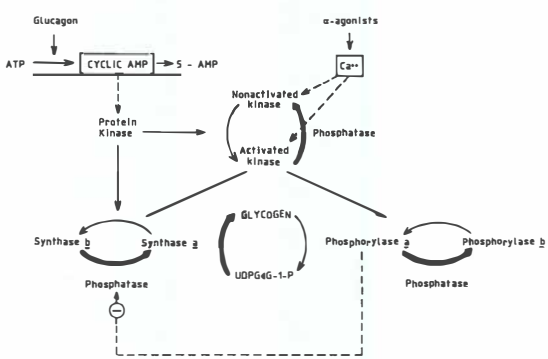


1.4 and 1.6 bonds of glucose molecules in glycogen

SYNTHESIS OF GLYCOGEN.

The primary stimulus that elicits glucose uptake or glucose output by the liver is the glucose concentration in the blood (4). Since most of the glucose uptake in the liver is for the purpose of glycogen synthesis, the activity of the enzymes involved in glycogen synthesis and breakdown is controlled by two main non-hormonal factors in the liver: the concentration of glucose and that of the end-product glycogen (4). The sensitivity of this system to glucose is under hormonal control (5). As soon as the glucose concentration in blood is increased, phosphorylase a is converted into phosphorylase b. Secondly, as a consequence of the disappearance of its inhibitor, phosphorylase a, synthase phosphatase is activated and synthase a is formed (5,6) (figure 3).

figure 3.



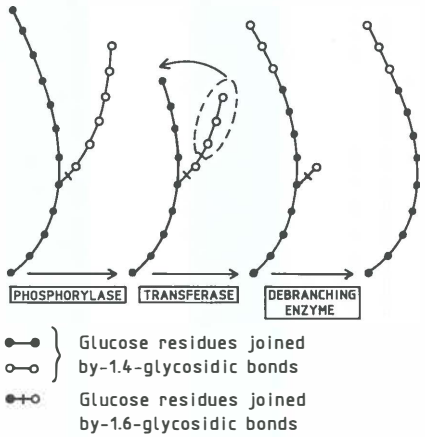
from: W Stalmans et al, 1974, ref 6.

Insulin is the most important hormonal factor, it decreases cAMP levels by inhibition of adenylate cyclase (7) and stimulation of cAMP-phosphodiesterase (8). Decreased cAMP concentrations are accompanied by low cAMP-dependent protein kinase activity and low phosphorylase activity. This causes a reciprocal increase in glycogen synthase activity (figure 3)(6,9). This enzyme promotes the biosynthesis of glycogen from uridine diphosphoglucose (UDPG), which is derived from glucose-1-phosphate (5,6). The newly formed α-1,4-glycosidic chains are subsequently branched by the transfer of at least six α-1,4-linked glucosyl units from the outer chains into a 1,6 position by the branching enzyme (α-1,4-glucan:α-1,4-glucan 6 α-glycosyltransferase EC 2.4.1.18) (22). This ultimately results in the multibranched glycogen molecule.

DEGRADATION OF GLYCOGEN.

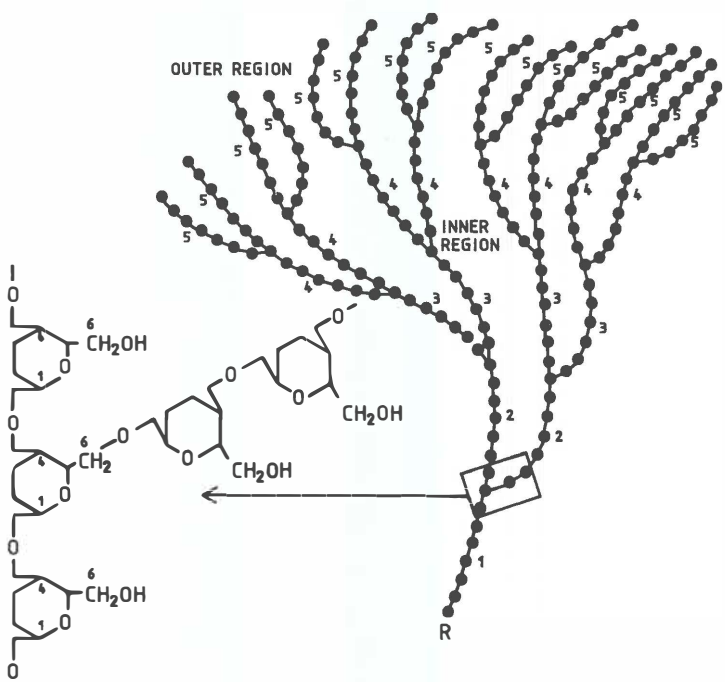
At low insulin concentrations phosphorylase b kinase activity is stimulated as glucagon, the insulin antagonist,increases the tissue concentrations of cyclic AMP (10). Phosphorylase b kinase catalyzes the conversion of phosphorylase b into phosphorylase a. Glycogen phosphorylase catalyzes the stepwise cleavage of glucosyl units from the nonreducing end of the α-1,4-glucosyl chain of glycogen, liberating glucose-1-phosphate until about four glucosyl-units remain on each branch.
After extensive phosphorolytic cleavage the glycogen molecule is characterized by four α-1,4-glycosidically linked glucose molecules attached by a 1,6 link to the inner region of the glycogen molecule (figure 5). This glycogen remnant is called phosphorylase limit dextrin (10). In order to allow further phosphorolytic cleavage three of the glucose residues must be removed to expose the 1,6-linked glucose at the branch point to further glycolytic cleavage. This is accomplished by oligo-α-1,4-glucan: α-1,4-glucan-4-glycosyltransferase (EC 2.4.1.25)/amylo-1,6-glucosidase (EC 3.2.1.33) (debranching enzyme), which catalyzes the transfer of these three glucose residues to another glycogen chain, with resynthesis of the α-1,4-bond (11). This activity also yields one free glucose molecule at the branch point (figure 4). The conversion of glucose-1-phosphate to glucose-6-phosphate is catalyzed by phosphoglucomutase. Glucose-6-phosphatase (EC 3.13.9), a microsomal enzyme, catalyzes the irreversible conversion of glucose-6-phosphate to glucose and phosphate (figure 6).

figure 4.



from: R R Howell, 1983, ref 22.

figure 5.

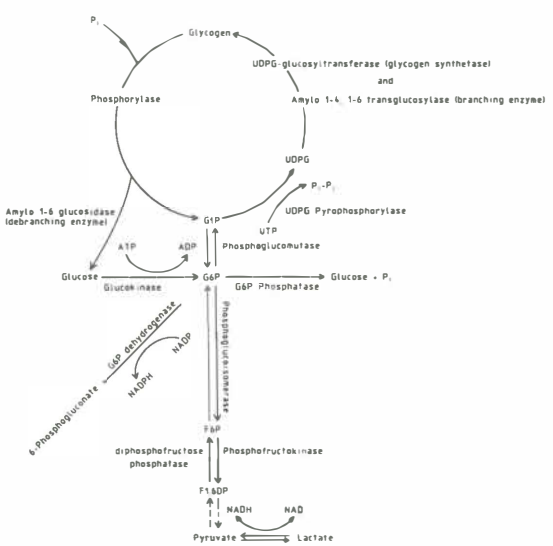


from: R R Howell, 1983, ref 22.

GLYCOGEN STORAGE DISEASE TYPE I.

The first description of glycogen storage disease type I is attributable to von Gierke in 1929 (12). In his paper titled "Hepato-Nephromegalia Glycogenica" von Gierke described the autopsy reports of two children who had a marked enlargement of the liver and kidneys due to the deposition of massive amounts of glycogen. Schoenheimer performed chemical tests on liver material of one of the original patients and found that the glycogen was exclusively composed of glucose residues and could be degraded by minced normal human liver (13). Because of this observation Schoenheimer and von Gierke concluded that a glycogen degrading ferment was missing in their patients. In 1952 deficiency of glucose-6-phosphatase (EC 3.1.3.9) was demonstrated by G.T.Cori and C.F.Cori in two patients with von Gierke's disease (14).
This was the first description of an inherited liver enzyme deficiency in humans.
Patients with glycogen storage disease type I are characterized clinically by massive hepatomegaly, stunted growth, severe hypoglycemia, xanthomata, and a bleeding diathesis (22). Metabolic derangements include hyperlactacidemia, hyperuricemia, hypertriglyceridemia, hypercholesterolemia, and elevated plasma concentrations of free fatty acids.
The primary enzymatic defect prevents the generation of free glucose from glucose-6-phosphate produced by glycogenolysis (figure 6).

figure 6.



from: R R Howell,
1983, ref 22.

After only a limited time of fasting a severe hypoglycemia ensues and a number of compensatory mechanisms are activated. One mechanism is that glucose-6-phosphate, which is released from glycogen is further metabolized, generating ultimately pyruvate and lactate as the end products of glycogenolysis. Hyperuricemia occurs due to both increased production and decreased excretion of uric acid. Increased production of uric acid occurs during hypoglycemia when secretion of glucagon is stimulated. Glucagon activates glycogen phosphorylase and promotes the formation of phosphorylated sugars. This phosphorylation utilizes ATP which is degraded to uric acid (36,37,38). Decreased excretion of uric acid results from decreased renal clearance of urate because of competition with lactate at the tubular level (37). The increased glycolytic flux results in an increased glycerol-3-phosphate concentration and increased production of acetyl-Coenzyme A. This leads in turn to increased synthesis of triglycerides by the liver. Obviously, the clinical symptoms and the laboratory findings can be attributed to decreased hepatic glucose release and the secondary metabolic derangements (22,23).

At present the classical form of glycogen storage disease due to deficiency of glucose-6-phosphatase is abbreviated to GSD IA. The variant, due to deficiency of glucose-6-phosphate translocase is abbreviated to GSD IB (16). Glucose-6-phosphatase is located on the inner luminal wall of the endoplasmatic reticulum. Therefore, a specific translocase is necessary to allow access to the glucose-6- phosphatase enzyme (20).

GSD IB is characterized by all the clinical features of GSD IA with additional neutropenia and disturbed neutrophil function. The latter is due to intracellular substrate limitation which affects both the hexose monophosphate shunt and anaerobic glycolysis (17,18,19,20,21).

GLYCOGEN STORAGE DISEASE TYPE III.

In 1928 Van Creveld gave the first clinical description of a patient with glycogen storage disease (24). The mother of this patient noticed shortly after birth an excessive increase in weight and a progressive enlargement of the abdomen. At the age of 8 months a very large smooth liver was found on exploratory laparotomy. No biopsy was performed at that time. At the age of 7 years the boy was re-investigated : he showed a peculiar adiposity giving the impression of "adiposo-genital dystrophy". The liver was excessively enlarged, the spleen could not be palpated. Routine urine investigations often revealed the presence of acetone, especially in fasting conditions. After injection of epinephrine a markedly increased ketosis developed instead of the normal glycemic response. At the same time the normal increase of the respiratory quotient after epinephrine injection was absent and a decrease was seen instead. From this finding " a particularly increased combustion of fat" was concluded. Van Creveld therefore proposed a defect in glycogen mobilization in order to explain the hepatomegaly, hypoglycemia and ketonuria (24).

Many years later a debranching enzyme deficiency could be demonstrated to underlie the patient's glycogen storage disease, which was subsequently denominated glycogen storage disease type III (25).

The observation of the peculiar structure of the accumulated polysaccharide by Illingworth and Cori in 1952 and their suggestion that its presence could be due to a deficiency of amylo-1,6-glucosidase were confirmed by the development of an assay for the activity of this enzyme in liver homogenates in 1956 (26,27).

Patients with glycogenosis type III cannot be reliably distinguished from patients with glycogenosis type I by physical examination. Both suffer from episodes of hypoglycemia, hepatomegaly and growth retardation. Usually the clinical findings in GSD III are less severe than those found in GSD I (22). The deficiency of debranching enzyme (amylo-1,6-glucosidase) limits the breakdown of glycogen resulting in an accumulation of a specific form of glycogen: limit dextrin in tissues.As soon as the outer branches of the glycogen molecule have been removed during fasting glycogenolysis stops. This leads to hypoglycemia unless gluconeogenesis and ketogenesis are switched on. In order to supply sufficient glucose the enhanced gluconeogenic activity may result in low concentrations of amino acids and citric acid cycle intermediates such as oxaloacetate (28). Especially young patients with GSD III are prone to hypoglycemia and hypoglycemic convulsions during fasting (28).

With increasing age these symptoms become less severe and adaptation to fasting develops gradually (28).

AIM OF THE STUDIES.

This thesis deals with glycogen storage disease. In particular studies on patients with deficiency of glucose-6-phosphatase (GSD IA) or deficiency of glucose-6-phosphate translocase (GSD IB), and deficiency of debranching enzyme (GSD III) are reported.

Glycogen Storage Disease Type I :

Since all clinical symptoms and laboratory findings can be attributed to decreased glucose output and secondary metabolic changes as a consequence of deficiency of glucose-6-phosphatase, the major goal of treatment in these patients is to maintain normoglycaemia. Initially this treatment consisted of frequent carbohydrate-enriched meals during day and night, later (1974) of a combination of frequent daytime meals and nocturnal gastric drip feeding (GDF) (30).
Especially the continuous nocturnal GDF improved physical growth and biochemical abnormalities: plasma concentrations of lactic acid, uric acid, free fatty acids and alanine, as well as triglycerides and cholesterol (31,32,33,34,35).
However, nocturnal GDF, especially with a high glucose formula, renders these patients more carbohydrate-dependent and serious symptoms of hypoglycemia may occur at blood glucose concentrations that had previously been well tolerated (33). As blood lactate concentrations are elevated in the phase before GDF is initiated, and tend to be lower when the patients are on frequent feedings combined with GDF, we wondered whether moderately elevated blood lactate concentrations would be an alternative substrate for energy for the brain, next to glucose, and thus protect the patient against the deterious effects of impending hypoglycemia.
For some patients and their parents the burden of positioning a naso-gastric tube every night may be too heavy. The initial report on the use of complex carbohydrates which, when given in the uncooked form slowly release glucose, prompted us to study their use as an alternative for GDF (39).
Therefore we wanted to study the following questions regarding the treatment of patients with GSD I :

1. Which complex carbohydrates might replace GDF during the night after the pubertal growth has been completed or earlier if indicated.

2. Which complex carbohydrates may be used during day-time in order to reduce the meal frequency.

3. Would moderately elevated lactate concentrations in blood be useful as an alternative energy substrate if the eventual harmful effects of chronic lactic acidosis could be prevented.

Glycogen Storage Disease Type III :

The clinical manifestations of debranching enzyme deficiency are limited to the consequences of decreased glycogenolysis and accumulation of glycogen in the liver, the muscles and the heart. Although the clinical picture is usually milder than that of GSD I, probably because glycogen can still be partially broken down and hepatic release of glucose from gluconeogenesis is unimpaired, young patients with GSD III appear to be very prone to hypoglycemia and hypoglycemic convulsions after only a short period of fasting (28). This fasting intolerance however, improves with increasing age. In the older patients relatively high ketone body concentrations in blood are observed during fasting, whereas in young patients relatively low ketone body concentrations are present. This might indicate a defect in the metabolism of fatty acids and /or glucose. Therefore regarding this age-related tendency for hypoglycemia in GSD III we wanted to study the following questions :

4. Would oxidation of fatty acids be insufficient , because of impaired:
   a. mobilization, b. activation in the cytosol,
   or c. transmitochondrial transport.

5. Is it possible to suppress the tendency for "hypoketotic hypoglycemia" in young patients by dietary manipulations.

6. Would the age-related tendency for hypoglycemia result from:
   a. decreased endogenous glucose production, or increased glucose requirement,
   b. lack of alternative substrate due to decreased turnover of fatty acids.

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CHAPTER II

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PEDIATRIC RESEARCH

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The Dietary Treatment of Children with Type I Glycogen Storage Disease with Slow Release Carbohydrate

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Summary

The effect of ingestion of uncooked cornstarch (2 g/kg body weight) in water, uncooked starch (1 g/kg) added to a meal, and glucose (2 g/kg) in water, was studied in eight patients with type I^{A} glycogen storage disease (GSD) and one patient with type I^{B} GSD. Blood glucose concentrations were determined at 30-min intervals during each tolerance test; blood lactate, blood insulin, and expiratory hydrogen were determined at 60-min intervals. The glucose levels remained in the normal range (≥1.8 mM) during approximately 6.5–9.0 h, 3.5–6.5 h, and 2.25–4.0 h during the three tolerance tests, respectively. The lactate levels differed markedly for the different tests per patient, and for the same type of test between the patients. Blood insulin concentrations after starch administration did not exceed values of 50 mU/liter above fasting levels and were markedly lower than those after glucose administration (maximum levels of 280 mU/liter). The expiratory hydrogen excretion did not increase or only slightly increased after cornstarch administration (<20 ppm).

Abbreviations

GSD, glycogen storage disease
GDF, gastric drip feeding

Patients with deficiency of glucose-6-phosphatase (GSD I^{A}) and glucose-6-phosphate translocase (GSD I^{B}) cannot produce glucose from glycogen stores or by gluconeogenesis because of their functional absence of glucose-6-phosphatase activity (14). Plasma glucose concentrations therefore completely depend on exogenous sources. The primary aim of treatment is prevention of hypoglycemia. Since the introduction of continuous gastric drip feeding (1), many pediatricians have treated their type I patients with overnight GDF and frequent daytime feeding, resulting in catch-up growth, decrease of hepatomegaly, and improvement of biochemical abnormalities (7, 8, 11, 12). Despite these good results, some major problems remain during long-term treatment. Nocturnal GDF, especially with a glucose amount over 8 mg/kg/min, renders these patients very carbohydrate-dependent and severe symptoms of hypoglycemia may occur at blood glucose concentrations which have previously been well tolerated (18). Such carbohydrate dependency may occur also at lower glucose quantities administered as kg/min, though less severely. For some patients and their parents, nocturnal GDF may be too complicated. The positioning of the gastric tube through the nose may ultimately cause irritation of the mucous membrane, nose bleeding, and nose and ear infections. The present studies were therefore undertaken to explore whether the use of slow release carbohydrate would decrease the carbohydrate dependency of the patient and whether its substitution for GDF would be feasible.

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PATIENTS AND METHODS

Eight patients with biopsy-documented glucose-6-phosphatase deficiency (GSD I^{A}) and one patient with glucose-6-phosphate translocase deficiency (GSD I^{B}) were studied. The nine patients, ages 5–13 years, included five male and four female children. All patients were on a low fructose, low galactose diet. Five of the nine patients were on a 12-h nocturnal GDF regimen (carbohydrate intake, 4.5–5.5 mg glucose/kg/min). All tests, carried out in the metabolic ward, started within 1 h after the night drip had been discontinued, and in the remaining four patients within 3 h after the last feeding. The following tolerance tests were performed.

1) Starch test. Uncooked cornstarch, 2 g/kg body weight, mixed in 100–150 ml water, was administered through the gastric tube, which was kept *in situ* in those patients who were on GDF. It was ingested as such by the other patients. The starch-water mixture had to be stirred repeatedly in order to prevent sedimentation of the starch.

2) Starch-meal test. Uncooked cornstarch, 1 g/kg body weight, was mixed in curd, the latter amounting to 0.5 g protein/kg body weight. Corn oil, 1 g/kg body weight, was added, no other nutrients or flavors being used. The energy content of the meal amounted to 15 kcal/kg body weight. The "meal" taken with a spoon was usually finished within 10 min.

3) Glucose test. Glucose, 2 g/kg body weight in a 20% solution, was administered orally. After the oral carbohydrate loading, blood glucose concentrations were determined at 30-min intervals; blood lactate, blood insulin levels, and expiratory hydrogen concentration were determined at 60-min intervals. Blood glucose concentrations were determined immediately by the glucose-oxidase method using a Beckman glucose analyzer. Blood lactate concentrations were determined by the Boehringer method using lactate dehydrogenase. Blood insulin levels were measured according to the double antibody procedure as described by Hales and Randle (13) with slight modifications. The breath samples were collected with a small pediatric size device according to Douwes *et al.* (6), and H_2 concentrations were determined gas chromatographically.

Tests were stopped at the first clinical signs of hypoglycemia or blood glucose concentrations below 1.8 mM. Informed consent was obtained from the parents.

RESULTS

Table 1 shows the periods of normal blood glucose levels after oral loading with uncooked cornstarch in water (a), uncooked cornstarch in curd (b) and glucose in water (c). Normoglycemia (blood glucose ≥1.8 mM) lasted 6.5–9.0, 3.5–6.5, and 2.25–4.25 h, respectively. Prolongation of normoglycemia after cornstarch therefore was 3.0–6.75 h; after cornstarch in curd, it was 0.5–4.25 h, as compared with glucose in water. The increments of blood glucose concentrations during the three tests are presented in Table 2. The glucose increments were lower during the cornstarch tests than during the glucose tests (except in L. S.). This difference applied also to the starch-meal test (except in T. D.).

Lactate concentrations after starch in water varied: in three patients (L. S., T. D., and M. R.), it decreased; in three patients (H. M., R. P., and M. v. D.), no change occurred; and in the other three patients (R. S., E. S., and D. D.), it increased, especially during the last part of the test (Fig. 1). Blood insulin concentrations in all patients were low during the cornstarch tests, and never exceeded values of 50 mU/liter above fasting levels, whereas peak insulin concentrations after oral glucose loading reached maximum levels of 280 mU/liter above fasting levels. Insulin levels during cornstarch in curd tests varied between the former and the latter values. Intestinal symptoms, due to starch indigestion, did not occur. Expiratory hydrogen concentrations were in the normal range (less than 20 ppm) or only slightly increased.

DISCUSSION

The use of cooked or partially hydrolized starch in the diets of GSD patients has rendered only moderate results until now (10), as it prolonged postprandial normoglycemia only slightly in comparison to that after glucose. In patients with diabetes mellitus, favorable results have been recently obtained with uncooked starch (3, 4). Postprandial blood glucose and insulin peak levels were much lower after its ingestion as compared to glucose. The effects of various starches differed according to their physical state (size of granules, presence of fiber, gelatinizing due to cooking) (5, 15–17). The best results were obtained with uncooked starches and of these cornstarch had the most pronounced effect.

Table 1. *Normoglycemic period in hours after dietary treatment*

Patient	Cornstarch in water	Cornstarch in curd	Glucose in water
L. S.	6.5		3.5
H. M.	8.0	3.5	4.25
R. S.	8.5	4.5	2.5
T. D.	7.0	4.0	3.5
E. S.	8.5	6.0	2.5
R. P.	8.0	6.5	4.0
D. D.	7.0	5.0	3.5
M. v. D.	9.0	6.5	2.25
M. R.	6.5	4.5	3.0

Table 2. *Δ Blood glucose after dietary treatment**

Patient	Cornstarch in water	Cornstarch in curd	Glucose in water
L. S.	4.8		4.5
H. M.	0.3	1.9	9.6
R. S.	3.2	1.9	8.6
T. D.	3.2	8.1	7.1
E. S.	1.7	0.6	6.8
R. P.	1.2	4.2	5.5
D. D.	3.7	3.8	5.8
M. v. D.	1.0	1.2	5.9
M. R.	1.1	4.7	3.2

* Δblood glucose, difference between initial and peak blood glucose concentration (mM).

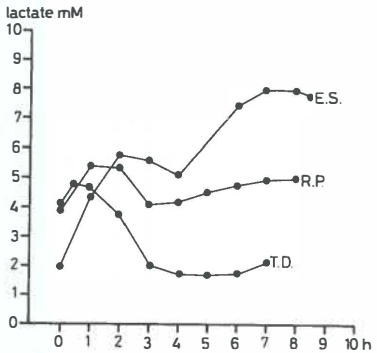

Fig. 1. Different responses of blood lactate concentrations after uncooked cornstarch (2 g/kg) in water in three patients.

In keeping with these observations, Sidbury tried uncooked cornstarch in water in GSD I patients who wanted to discontinue their GDF (personal communication). In the present study, we confirmed and enlarged the results of Chen *et al.* (2), by comparing the effects of uncooked cornstarch in water, uncooked cornstarch added to a meal, and glucose in water on glucose and lactate blood levels. After starch in water, all nine patients showed a marked prolongation of normoglycemia without the precipitous changes observed after the administration of glucose in water. The effects of uncooked cornstarch added to curd were intermediate between those after cornstarch in water and after glucose in water (Tables 1 and 2). Contrary to the similarity of the glucose curves after starch in water, the lactate curves showed marked variation among patients. For these differences, we have no explanation yet. A moderate hyperlactacidemia should be maintained as lactate appears to be a fuel for the brain as is glucose (9). The only means to achieve this would be to "titrate" the amount and type of starch added to the meals against blood lactate and glucose levels. The optimal dose of starch to be added to the meals varies between 1 and 2 g/kg body weight twice a day. The first dose should be administered either through the gastric tube just before its withdrawal after the night in patients with GDF, or added to curd or yoghurt at breakfast in patients without GDF. The composition of the latter mixture may serve for the second starch dose at luncheon, too. Simultaneous addition of mono- and disaccharides interferes with the steady state of blood glucose induced by starch due to the insulinogenic effect of the former carbohydrates (results not shown). These rapidly absorbed carbohydrates should therefore be limited or avoided. The two starch servings would not only make the patient less dependent on a strict timetable for his meals, but would also decrease the number of meals at daytime to the normal three to four.

There is no reason for apprehension on long-term effects of starch administration on somatic development of and biochemical abnormalities in the patient, but we prefer to postpone the substitution of starch for GDF till the puberal growth spurt of the child has been completed.

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CHAPTER III

SEMI-LENTE CARBOHYDRATES IN THE DIETARY TREATMENT OF PATIENTS WITH GLYCOGENOSIS CAUSED BY GLUCOSE-6-PHOSPHATASE DEFICIENCY.

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Brief title: Semi-lente carbohydrates in GSD type I.

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SUMMARY.

Carbohydrates with digestion characteristics between those of "lente" uncooked starches and rapidly digestible oligosaccharides were administered in a dose of 1.5 g.kg body weight to five patients with glycogenosis due to glucose-6-phosphatase deficiency.
Postprandial duration of normoglycemia, blood insulin and lactate concentrations were determined. Uncooked barley groats in water, or incorporated in a meal turned out to behave as "lente" carbohydrates. Uncooked couscous in water or incorporated in a meal, and partially cooked macaroni, given as a meal behaved as "semi-lente" carbohydrates as compared to uncooked cornstarch and glucose. The in vitro determination of the digestibility index, followed by the in vivo performance of a tolerance test, enables to choose and incorporate semi-lente carbohydrates in the daytime treatment of the patients.

KEYWORDS: complex carbohydrates, "lente" carbohydrates, "semi-lente" carbohydrates, glycogen storage disease type I, glucose-6-phosphatase deficiency.

ABBREVIATIONS:

GSD Ia	glycogen storage disease caused by glucose-6-phosphatase deficiency.
GDF	gastric drip feeding.
DI	digestibility index.

INTRODUCTION.

Patients with a deficiency of glucose-6-phosphatase (GSD Ia) depend completely on exogenous sources of glucose, which have to be administered either frequently or continuously by gastric drip feeding (GDF) (1,2).
This lays a heavy burden on the child and his parents. A socially more acceptable number of meals can be obtained by the inclusion of "slow release" starches in the diet. The ingestion of some of these starches in the uncooked form results in a normoglycemia of long duration (approximately 8 hours), accompanied by low insulin levels (3,4).
Starch consists of a mixture of amylose and amylopectin organized in semicrystalline granules (5,6,7). In general, the percentage of amylose in various starches is 20-30% .
An increase in amylose content renders the starch less digestible, probably because of increased viscosity (8,9,10).
The digestibility of starch depends not only on the percentage of amylose, but also on the size of the granules, the presence of fiber, and the amount of gelatinization due to thermoprocessing (cooking) (11,12,13,14,15). In keeping with this cornstarch behaves like a "rapid" carbohydrate if administered after cooking, but as a "lente" carbohydrate in the uncooked form. Lente carbohydrates such as cornstarch in the uncooked form slowly release glucose under the hydrolytic activity of pancreatic amylase (3) and thus ensure an exogenous glucose supply during many hours with hardly any insulin challenge. Lente carbohydrates, administered before the night may cover the relatively low glucose requirements of older children and adults during the night. During daytime, however, this provision falls short, especially in young children with relatively high glucose requirements. In this case the need for frequent meals might be reduced by the introduction of semi-lente carbohydrates, the digestion of which would proceed between those of lente uncooked starches and rapidly digestible oligosaccharides.
The present study was designed in order to investigate, whether such semi-lente carbohydrates would indeed have this supposed effect. In selecting suitable semi-lente carbohydrates for our experiments we made use of the digestibility index (DI). The DI is defined as the percentage of starch released as glucose after 60 minutes incubation with pig amylase at 37 °C (16). The DI of uncooked cornstarch is 21% , of uncooked rice starch 23% . Lente carbohydrates would therefore be characterized by a DI in the range of 20-30% . Precooked and ready-to-eat starches on the other hand have DI's between 95 and 100% . As a consequence, we looked for semi-lente carbohydrates among starches with DI'S between 40 and 60% . These are to be found among the starches that are modified by heating (steam or otherwise). In our pilot study we used quick cooking barley groats, couscous, and macaroni with DI's of 40, 47 and 50% respectively. We tested the suitability of these three presumably semi-lente carbohydrates on 5 children with GSD Ia by the administration thereof either in water or as a constituent of a meal. The

meal was more palatable and might prolong the digestion rate by retarding stomach emptying.

PATIENTS AND METHODS.

This study includes five patients with biopsy-documented glucose-6-phosphatase deficiency (GSD Ia), aged 7-15 years. All patients had the usual symptoms such as marked hepatomegaly, tendency to hypoglycemia, and hyperlactacidemia. Serum levels of uric acid, triglyceride and cholesterol varied from normal to markedly increased. The patients' height varied from markedly below normal to normal (height standard deviation scores -6.08 to +0.46). The nutritional state in terms of the weight as a percentage of the median weight-for-height was normal (93 to 132%).
During the tests all patients were free from diseases or abnormalities other than due to their primary disease. The dietary treatment consisted of frequent meals, sucrose-restricted and poly-unsaturated fats-enriched. The composition was approximately 60-65, 10-15, and 25 energy percent for carbohydrates, proteins and fats, respectively. The tests started within two hours after cessation of the GDF, and were carried out in the metabolic ward of the Department of Pediatrics. Informed consent was obtained from the parents.

The following tests were performed:

- uncooked quick-cooking barley groats, 1.5 g/kg body weight, mixed in 100-150 ml water (B1);

- uncooked quick cooking barley groats, 1.5 g/kg body weight as a constituent of a meal (B2);

- uncooked couscous, 1.5 g/kg body weight, mixed in 100-150 ml water (C1);

- uncooked couscous, 1.5 g/kg body weight, as a constituent of a meal (C2).

- partially cooked macaroni, 1.5 g/kg body weight. Macaroni particles were added to boiling water. Immediately thereafter the heating was stopped. After 10 minutes the partially cooked macaroni particles were removed to be administered as a constituent of a meal (M).

The meals in test B2, C2, and M consisted of curd or cheese with yoghurt added to make the meal more palatable. Both the protein and the fat content of each test meal amounted to 0.25 g/kg body weight. The additional small amount of lactose was neglected in the calculation of the carbohydrate intake.
All carbohydrates, whether administered in water or as a meal, were taken by mouth. The carbohydrate-water mixtures and the meals were usually ingested within 30 minutes. Blood

samples were taken from an indwelling venous catheter. The assays comprised: the determination of glucose at 30 min. intervals, and of lactate, insulin, and expiratory hydrogen at 60 min. intervals. The timing started at the beginning of the ingestion of the carbohydrates. Blood glucose concentrations were determined immediately by the glucose-oxidation method using a Beckman glucose analyzer. Blood lactate concentrations were determined by the Boehringer method using lactate dehydrogenase. Blood insulin concentrations were measured according to the double antibody procedure as described by Hales and Randle, with slight modifications (17). Δ-Glucose, Δ-insulin, and Δ-lactate were defined as the difference between the initial and the highest (lowest for lactate) postprandial concentration. Breath samples were collected with a small pediatric size device (18) and H_2 concentrations were determined by gaschromatography. Tests were stopped at the first signs of hypoglycemia or blood glucose concentrations below 2.0 mM. The results were expressed as mean ± SD, and the significance of the difference between the tests ($p < 0.05$) was calculated using "Student's t-test" for (un-)paired data.

RESULTS.

Table 1 shows the results of the five tolerance tests with modified starches as compared to glucose and uncooked cornstarch tolerance tests, both latter tests having been published earlier (3). The Table gives two cut-off points for normoglycemic blood glucose values:

- ≥ 2.0 mM for the lente carbohydrate tests because of the more slow blood glucose changes and gradual development of hypoglycemia.

- ≥ 3.0 mM for the semi-lente carbohydrate tests because of a more rapid blood glucose response and development of hypoglycemia.

B1 and B2 acted as lente carbohydrates with respect to Δ-glucose and Δ-insulin, but not with respect to Δ-lactate, the response of which corresponded more to the values of test C1 and C2. Noteworthy is the low Δ-insulin of B2. C1, C2 and M behaved as semi-lente carbohydrates because of the duration of normoglycemia and the Δ-glucose. Δ-Insulin and Δ-lactate values were between those after cornstarch and after glucose in water administration. Breath hydrogen concentrations were in the normal range (less than 20 ppm) during all tests.

DISCUSSION.

It appears that the in vitro DI values do not accurately predict the outcome of the in vivo tests. Only the results of the couscous and the macaroni tests were in the expected semi-lente range, whereas the results of the barley groats test were in the lente range for the duration of normoglycemia and for the Δ-values, with the exception of the Δ-lactate. In fact, as a constituent of a meal, barley groats proved to be an even more favorable lente carbohydrate than cornstarch: low Δ-insulin and decrease of Δ-lactate instead of increase. It is less palatable, however. In order to find other potential semi-lente carbohydrates, the in vitro test as described by Jenkins et al (19) may be more suitable. With this method, the carbohydrate is mixed with human digestive juice in a dialysis bag and the amount of glucose and oligosaccharides in the dialysate is determined at 1 and 5 hours. This gives information about the extend as well as on the rate of hydrolysis. It also imitates the in vivo situation because it is characterized by continuous exchange through the semi-permeable membrane of glucose and oligosaccharides liberated from starch by human enzymes in the dialysis bag.

In conclusion it can be said that barley groats as a lente carbohydrate might be a useful alternative to cornstarch, and might be suitable to ensure a prolonged period of normoglycemia during the night. Couscous and macaroni, figuring in the semi-lente range, may be alternatives for the usual rapid release carbohydrates as their use would lengthen the inter-meal interval and thus reduce the meal frequency at daytime.

Ultimately, lente and semi-lente carbohydrates may be of use in the diet of any patient with a disorder of glucose homeostasis.

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TABLE I.

Carbo-hydrate	n	normoglycemia[1] ≥2.0 mM	normoglycemia[1] ≥3.0 mM	Δ-glucose[2]	Δ-insulin[3]	Δ-lactate[2]
Glucose 2.0 g/kg	9	212±35	188±38	7.5±1.5	75.1±59.9	-4.58±3.13
Q.C.Barley Groats 1.5 g/kg	5	436±137+	334±86+	2.1±1.0+	12.4±5.1+	-3.67±1.19*
Q.C.Barley Groats meal 1.5 g/kg	5	467±130+	370±121+	2.2±0.9+	5.8±1.4*+	-2.00±3.53
Couscous 1.5 g/kg	5	304±101*	282±115	3.8±1.4*+	36.1±26.6	-3.81±3.26
Couscous meal 1.5 g/kg	5	334±89*	288±108+	3.0±1.2+	34.7±21.5	-3.75±3.24
Macaroni meal 1.5 g/kg	4	381±69*+	360±82+	2.8±1.0+	23.3±8.5+	-1.50±0.50+
Cornstarch 2.0 g/kg	9	470±53	-	2.3±1.0	15.6±12.6	+0.64±3.74

[1]: duration in minutes

[2]: difference (mM) between the initial and the highest postprandial concentration; for lactate these differences are usually negative.

[3]: difference (mU/l) between the initial and the highest postprandial concentration.

*: significant difference from results after cornstarch ($p < 0.05$).
+: significant difference from results after glucose ($p < 0.05$).

CHAPTER IV

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Lactate as a Cerebral Metabolic Fuel for Glucose-6-Phosphatase Deficient Children

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Summary

The main substrates for brain energy metabolism were measured in blood samples taken from the carotid artery and the internal jugular bulb of four children with glycogen storage disease caused by deficiency of glucose-6-phosphatase. Multiple paired arterial and venous blood samples were analyzed for glucose, lactate, pyruvate, D-β-hydroxybutyrate, acetoacetate, glycerol and O_2, and the arteriovenous differences of the concentrations were calculated. In the first three patients the substrates were measured in two successive conditions with lower and higher glucose-intake, respectively, inducing reciprocally higher and lower concentrations of blood lactate. In the fourth patient medium chain triglycerides were administered simultaneously with the glucose-containing gastric drip feeding.

Lactate appeared to be taken up significantly. It consumed, if completely oxidized, between 40–50% of the total O_2 uptake in most cases. Only once in one patient the uptake of lactate switched to its release, when the blood lactate level decreased to normal. D-β-hydroxybutyrate and acetoacetate arteriovenous (A-V) differences were small to negligible and these ketone bodies, therefore, did not contribute substantially to the brain's energy expenditure. Glycerol was not metabolized by the brain. Lactate thus appeared to be the second brain fuel next to glucose. It may protect the brain against fuel depletion in case of hypoglycemia.

Abbreviations

A-V, arteriovenous
AcAc, acetoacetate
G6Pase, glucose-6-phosphatase
GDF, gastric drip feeding
MCT, medium chain triglycerides
β-OHB, β-hydroxybutyrate
P, priming dose

Some children with hepatic glycogenosis caused by G6Pase deficiency show a striking tolerance for hypoglycemia. Their brain function remains unimpaired even when the blood glucose concentration is very low. Remarkably, those of our patients who show the most pronounced metabolic disturbances and the highest lactate levels in the blood, appear to be the least susceptible to clinical symptoms of hypoglycemia. On the other hand, patients with a less abnormal metabolic state and only moderately elevated lactate levels show cerebral symptoms as soon as their blood glucose drops to even moderately low levels. We, therefore, wondered whether in some patients, besides glucose, lactate might be utilized as an energy substrate by the brain and thus protect the child against the deleterious effects of glucose depletion. This would be opposite to the situation in normal children (18, 22, 24, 25, 26) and normal adults (4, 9, 23), in whom the brain releases lactate and consumes ketone bodies (β-OHB and AcAc) as soon as glucose, the preferential fuel, becomes insufficiently available. In G6Pase-deficient children, however, this is impossible because there is no physiologic hyperketosis during fasting. Their fasting hypoglycemia is accompanied by *hypo*ketosis (5) and *hyper*lactacidemia (6).

The ability to utilize lactate instead of ketone bodies would therefore be a useful mechanism. If, indeed, substantial cerebral utilization of lactate were found, this might influence the dietary treatment of the patients. The present treatment of frequent high carbohydrate meals during the day and a glucose-containing gastric drip feeding (GDF) at night (10) enhances the glucose dependency of the patients (20) as it suppresses the production of metabolic fuels other than glucose, especially lactate (11). This would deprive the patient of his only other possible brain fuel beside glucose. We considered this issue so important that we decided to test our assumption on four G6Pase deficient children. We measured the concentrations of possible brain fuels during two successive periods characterized by low and high glucose intake, which would induce a reciprocally higher and lower lactate availability, respectively. In one of these patients we administered MCT in order to stimulate ketone body production maximally (3) with the intention of providing the patient with another fuel beside glucose and lactate.

PATIENTS AND TEST PROCEDURES

Four children, aged 4, 5, 8, and 11 yr, with G6Pase deficiency diagnosed by enzyme assay of liver biopsy (type I-A) were investigated. They had the usual abnormalities such as marked hepatomegaly, tendency to hypoglycemia, hyperlactacidemia, hyperlipidemia, and hyperuricemia. They all were on GDF at night.

The investigations were carried out after approval from the medical ethical committee of the University Hospital of Leiden had been obtained, and with the informed consent of the parents. The children were carefully prepared for the test with the assistance of a psychologist, who participated in this study.

After local anesthesia two catheters were introduced by a pediatric cardiologist–one into the femoral artery, the other into the femoral vein–and positioned in the carotid artery and internal jugular bulb, respectively, as described earlier (23, 24). The position of the catheters was ascertained by x-ray with image intensification. Through the catheters, kept open with heparinized sodium chloride solution (10 U heparin/dl NaCl 0.9%, 3 ml/h), paired arterial and venous blood samples were taken and immediately prepared for analysis of oxygen content and determination of metabolic substrates. The tube for the nocturnal GDF was used to administer glucose solutions (three patients) and an emulsion of MCT (MIGLYOL 812, Dynamit Nobel, Troisdorf, W. Germany (one patient). Most fluids were administered by the P-continuous infusion technique in order to accelerate the transition from one condition into the other. Samples

from a peripheral vein were taken for immediate glucose and lactate assay at 30-min intervals when a fluid was being administered; as soon as the glucose assay indicated that a steady glucose concentration had been attained, samples for a complete analysis as described were taken.

The experimental design is presented in Figure 1A for the former three patients, and in Figure 1B for the latter patient. The patients were supervised by a pediatric cardiologist, a pediatrician, and the above mentioned psychologist. They were medicated with diazepam, 5 or 10 mg intramuscularly, at the beginning of the test. Arterial blood pressure and heart rate were monitored continuously. Most of the time the children were awake. They showed no fear. The younger children became rather restless towards the end of the test. Patient 1 vomited and became hypoglycemic 4 h after termination of the test. He recovered rapidly after intravenous administration of glucose and sodium bicarbonate. The other patients showed no abnormalities. A few weeks later the psychologist reexamined the patients and interviewed the parents for an evaluation of the test with regard to its psychologic impact on the children. She found no untoward sequelae.

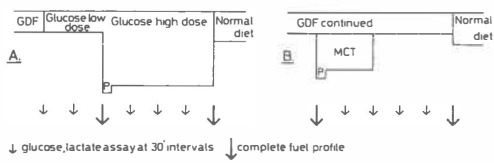

Fig. 1. Procedure of the cerebral substrate utilization test, (*A*) protocol for the first three patients and (*B*) protocol for the fourth patient. GDF, gastric drip feeding; MCT, medium chain triglycerides; and P, priming dose.

METHODS

Glucose, lactate, pyruvate, β-OHB, AcAc, and glycerol in blood (1.0 ml) were measured by spectrophotometry with $NAD(P)^+$, NAD(P)H coupled enzymic methods as described by Bergmeyer (1) with an Aminco-Chance Dual wavelength spectrophotometer. Blood gas analysis was performed on a Radiometer BMS 5Mk2 blood microsystem. Insulin was estimated with antiserum Cavis 51, [^{125}I]pork insulin as tracer, and human MRC insulin as standard. Separation of bound and free insulin was done with dextran-coated charcoal by the method of Herbert et al. (15). Glucagon was estimated by a modification of the method of Heding (13) with antiserum K5563 and ^{125}I-glucagon obtained from Novo Industries (København, Denmark).

RESULTS

The results of the tests are given in Tables 1–4. The A-V differences for glucose were positive irrespective of its concentration in arterial blood in all four patients. The A-V differences for lactate were positive except in patient 3 (period B) when the arterial concentration was low. The A-V differences for the other substrates were small to negligible except for βOHB and AcAc in patient 4 after MCT administration (period B). The sums of calculated O_2 equivalents assuming complete combustion of substrates by the brain, were in reasonable to good agreement with the O_2 actually taken up. The insulin concentration increased when the amount of glucose administered had been increased (period B in the first 3 patients). The glucagon concentration did not correlate with either glucose or insulin concentration.

The A-V differences of glucose and lactate of all patients have been plotted as functions of the respective arterial concentrations in Figure 2. The amount of glucose taken up (A-V difference) did not vary much and was not correlated with its arterial concentration or that of lactate (Fig. 2A and B). Lactate uptake,

Table 1. *Patient 1 (all concentrations in mmol/l, mean ± SD)**

	Arterial blood	A-V difference†	O_2 equiv. calculated‡	% of total O_2‡
Period A§				
Glucose	4.21 ± 0.06	+0.19 ± 0.07**	1.14	65
Lactate	8.83 ± 0.11	+0.21 ± 0.05***	0.63	35
Pyruvate	0.45 ± 0.02	+0.02 ± 0.02		
βOHB	0.68 ± 0.07	+0.03 ± 0.13		
Acac	0.26 ± 0.01	0.00 ± 0.003		
Glycerol	0.06 ± 0.002	+0.01 ± 0.001		
O_2	6.53	+1.93	1.77	
Insulin¶ venous	4			
Glucagon¶ venous	7			
Period B‖				
Glucose	10.81 ± 0.55	+0.15 ± 0.06**	0.90	55
Lactate	4.85 ± 0.07	+0.24 ± 0.05***	0.72	45
Pyruvate	0.36 ± 0.01	+0.04 ± 0.03		
βOHB	0.23 ± 0.08	−0.01 ± 0.11		
Acac	0.18 ± 0.02	−0.01 ± 0.03		
Glycerol	0.04 ± 0.001	0.00 ± 0.004		
O_2	6.60	+1.72	1.62	
Insulin¶ venous	45			
Glucagon¶ venous	<5			

* Part of the results of this patient has been published earlier (7).

† Triplicate paired arterial and venous blood samples were taken at the end of periods A and B for the assay of all substrates. Samples for O_2 determination were taken *in simplo*.

‡ O_2 equivalents and % of total O_2 were calculated if A-V differences of substrates were significant.

§ Period A, 3 mg glucose·kg^{-1}·min^{-1} during 2 h.

‖ Period B, 15 mg glucose·kg^{-1}·min^{-1} during 2 h.

¶ Insulin (mU/l) and glucagon (ng/l) were determined in peripheral venous blood. Significance (Student's *t* test): $^{**}P < 0.1$ and $^{***}P < 0.05$.

Table 2. *Patient 2 (all concentrations in mmol/l mean ± SD)*

	Arterial blood	A-V difference*	O_2 equiv. calculated†	% of total O_2†
Period A‡				
Glucose	5.35 ± 0.12	+0.18 ± 0.06***	1.08	52
Lactate	9.21 ± 0.27	+0.33 ± 0.14***	0.99	48
Pyruvate	0.53 ± 0.03	−0.02 ± 0.04		
βOHB	0.35 ± 0.02	0.00 ± 0.01		
Acac	0.24 ± 0.02	+0.03 ± 0.03		
Glycerol	0.08 ± 0.01	−0.01 ± 0.01		
O_2	4.54 ± 0.03	+1.82 ± 0.03	2.07	
Insulin‖ venous	<1			
Glucagon ‖ venous	22			
Period B§				
Glucose	6.16 ± 0.12	+0.14 ± 0.08**	0.84	54
Lactate	3.46 ± 0.13	+0.24 ± 0.07***	0.72	46
Pyruvate	0.24 ± 0.01	−0.01 ± 0.04		
βOHB	0.08 ± 0.01	0.00 ± 0.01		
Acac	0.10 ± 0.02	0.00 ± 0.01		
Glycerol	0.07 ± 0.01	0.00 ± 0.01		
O_2	4.76 ± 0.10	+1.35 ± 0.15	1.56	
Insulin‖ venous	15			
Glucagon‖ venous	27			

* Quintuple paired arterial and venous blood samples were taken at the end of periods A and B for the assay of all substrates. Samples for O_2 determination were taken in triplicate.

† O_2 equivalents and % of total O_2 were calculated if A-V differences of substrates were significant.

‡ Period A, 3 mg glucose·kg^{-1}·min^{-1} during ½ h.

§ Period B, 15 mg glucose·kg^{-1}·min^{-1} during 2½ h.

‖ Insulin (mU/l) and glucagon (ng/l) were determined in peripheral venous blood. Significance (Student's *t* test): ** $P < 0.1$ and *** $P < 0.05$.

Table 3. *Patient 3 (determination of substrates, see Table 2).*

	Arterial blood	A-V difference	O_2 equiv. calculated	% of total O_2
Period A*				
Glucose	6.41 ± 0.17	+0.20 ± 0.15**	1.20	58
Lactate	4.24 ± 0.22	+0.25 ± 0.06***	0.75	36
Pyruvate	0.33 ± 0.02	+0.05 ± 0.01‡***	0.13	6
βOHB	0.07 ± 0.02	−0.02 ± 0.02		
Acac	0.05 ± 0.01	0.00 ± 0.01		
Glycerol	0.10 ± 0.01	+0.01 ± 0.01		
O_2	6.41 ± 0.07	+2.07 ± 0.22	2.08	
Insulin venous	11			
Glucagon venous	<5			
Period B†				
Glucose	7.66 ± 0.18	+0.29 ± 0.13**	1.74	100
Lactate	1.23 ± 0.16	−0.05 ± 0.08		
Pyruvate	0.11 ± 0.02	+0.02 ± 0.02		
βOHB	0.05 ± 0.03	0.00 ± 0.02		
Acac	0.05 ± 0.01	0.00 ± 0.01		
Glycerol	0.06 ± 0.01	−0.01 ± 0.01		
O_2	6.46 ± 0.10	+1.68 ± 0.14	1.74	
Insulin venous	29			
Glucagon venous	<5			

* Period A, 3 mg glucose·kg^{-1}·min^{-1} during 1 h.

† Period B, 15 mg glucose·kg^{-1}·min^{-1} during 2 h.

‡ ** $P < 0.1$ and *** $P < 0.05$.

however, varied greatly; it was correlated positively with the arterial lactate concentration (Fig. 2C) and negatively (not significantly) with the glucose concentration (Fig. 2D). Lactate release occurred only once in one patient, when the arterial lactate concentration decreased to a normal value, which is exceptional in these patients (Table 3).

DISCUSSION

Lactate is taken up by the brain of G6Pase-deficient children as is evident from the positive A-V differences found in all four patients. Its significant contribution to the total fuel supply of the brain can be inferred from its O_2 equivalent as percentage of

Table 4. *Patient 4 (determinations of substrates, see Table 2).*

	Arterial blood	A-V difference*	O_2 equiv. calculated	% of total O_2
Period A†				
Glucose	3.46 ± 0.14	+0.25 ± 0.17**	1.50	55
Lactate	9.99 ± 0.10	+0.35 ± 0.14**	1.05	38
Pyruvate	0.60 ± 0.01	+0.08 ± 0.03**	0.20	7
βOHB	0.12 ± 0.03	+0.01 ± 0.04		
Acac	0.08 ± 0.01	−0.01 ± 0.02		
Glycerol	0.11 ± 0.01	−0.01 ± 0.01		
O_2	5.19 ± 0.09	+2.95 ± 0.16	2.75	
Insulin venous	2			
Glucagon venous	107			
Period B‡				
Glucose	1.69 ± 0.06	+0.19 ± 0.06***	1.14	40
Lactate	12.86 ± 0.12	+0.46 ± 0.20**	1.38	49
Pyruvate	0.49 ± 0.01	0.00 ± 0.02		
βOHB	0.69 ± 0.02	+0.04 ± 0.02**	0.18	6
Acac	0.33 ± 0.01	+0.03 ± 0.01**	0.12	4
Glycerol	0.17 ± 0.01	+0.01 ± 0.01		
O_2	4.94 ± 0.04	+2.87 ± 0.04	2.82	
Insulin venous	3			
Glucagon venous	36			

* Quintuple paired arterial and venous blood samples were taken before starting the extra MCT drip and 1½ h after having stopped the MCT administration.

† Period A, gastric drip feeding (GDF) containing 6 mg glucose·kg^{-1}·min^{-1} throughout the night and the test.

‡ Period B, medium chain triglycerides (MCT) 1.5 g/kg, administered during 1 h, was added to the GDF (see Fig. 1B).

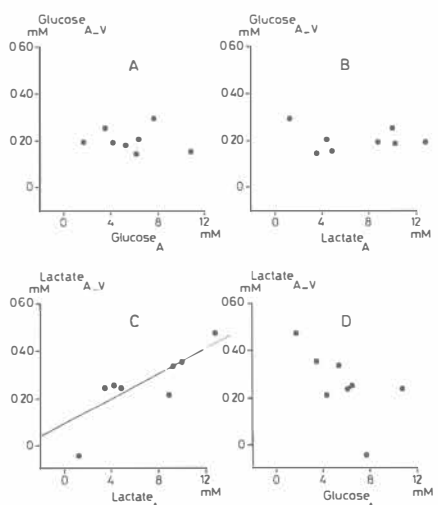

Fig. 2. A-V differences of glucose and lactate as functions of their arterial concentrations, (*A*) no correlation; (*B*) no correlation; (*C*) [lactate$_{A-V}$] = 0.031 [lactat $_A$] + 0.04, $r = 0.83$; $P < 0.01$; and (*D*) no correlation.

total O_2 consumption. Its contribution, together with the much smaller contribution of pyruvate, approached the share of glucose to the brain's total requirement. The prevailing lactate uptake in these patients is surprising because lactate release is the normal event both in children (18) and in adults (4) even during fasting (23). No exceptions from this rule have been described, except in newborn baboons (21), calves (8), and dogs (14), in which a temporary lactate uptake has been observed. There was only a slight, if any, contribution of βOHB and AcAc to brain energy metabolism in the G6Pase-deficient children. Only the combined stimulation of fatty acid oxidation by orally administered MCT and an unintentional hypoglycemia produced a moderate increase of blood ketone bodies and a low to moderate cerebral extraction of ketone bodies (Table 4). This finding is in line with the well-known fact that G6pase-deficient children, when hypoglycemic, are hypoketotic and *not* hyperketotic as is the case in other types of glycogen storage disease (5). As for glycerol, this substrate did not contribute significantly to brain energy metabolism (Tables 1–4), as had been found earlier (19). Free fatty acids have not been determined in our patients as they are not utilized by the brain (9, 19). Neither have amino acids been determined as their uptake by the brain is insignificant, arginine and histidine excepted (25). The error of not taking into account the contribution of amino acids in the cerebral energy expenditure of our patients cannot be large, because the oxygen needed for total combustion of the other substrates to carbon dioxide and water (calculated O_2 equivalents) was in good agreement with the O_2 equivalents actually taken up.

Variabilities in activity and mental alertness of the patients and particularly the possible influence of MCT ingestion on the rate of glucose absorption in patient 4 may have interfered with the attainment of steady state conditions during the experiments. Comparison of one period with another should, therefore, be made with more reservation than in anesthesized animals in which different amounts of lactate, administered intravenously entail steady conditions, which can be compared with more confidence (14). Taking this into account, it can still be concluded that lactate is the next important fuel after glucose for the cerebral energy requirement of patients with G6Pase-deficiency whereas ketone bodies make little or no contribution. The usefulness of lactate for these patients may be compared with the usefulness of ketone bodies as alternative fuels for the brain of the normal child. The development of a physiologic ketosis in a normal child is a gradual process that can compensate for a gradual glucose depletion during fasting. In contrast, the development of glucose depletion in G6Pase-deficient children is often precipitous; therefore, the continuous presence of lactate as an alternative fuel may be very important to save glucose and to compensate for glucose depletion. A more liberal glucose supply, exceeding the basic requirement, leads to an undesirable fall of lactate in the blood. Indeed, if this is done, the child is brought

into a more satisfactory metabolic state but becomes more susceptible to hypoglycemic convulsions. In the presence of an elevated lactate level, on the other hand, brain function would remain intact even when the glucose concentration becomes alarmingly low. Some caution may be in order, however, because the maintenance of an elevated lactate level may conceivably lead to other secondary metabolic abnormalities such as hyperlipidemia and hyperuricemia, the latter being elicited by excessive glycolysis (12) and impaired urinary excretion of urate (16).

We consider the reduction of susceptibility to hypoglycemic convulsions so important that the diet of the patients should be adjusted in such a way as to keep the blood lactate level moderately elevated, for instance from about 4–6 mM (Fig. 2C), in order to keep this alternative fuel available in case of sudden hypoglycemia. The simplest and safest way to maintain this lactate level would seem to restrict the glucose content of the night drip feeding to the basic production rate of glucose, as found in normal children (2), which is higher than the glucose production found in one G6Pase-deficient patient (17). This covers the brain's requirement for glucose, which is estimated to be 60–80% of the normale production rate (2).

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CHAPTER V

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Short Communication

The Lactate Concentration of the Urine, a Parameter for the Adequacy of Dietary Treatment of Patients with Glucose-6-phosphatase Deficiency

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Gastric drip feeding (GDF) during the night improves the clinical and metabolic abnormalities of patients with glycogen storage disease due to glucose-6-phosphatase (EC 3.1.3.9) deficiency (Greene *et al.*, 1980; Fernandes *et al.*, 1979; Stanley *et al.*, 1981). The normalization of blood glucose by GDF diminishes lactate overproduction by the liver and, therefore, suppresses the tendency for hyperlactacidaemia. Conversely, a (nearly) normal level of blood lactate is considered to be a parameter for adequate glucose supply by GDF (Stanley *et al.*, 1981). In our experience, however, the blood lactate concentration often fluctuates strongly. The lactate concentration of the urine averages these fluctuations and thus reflects lactate overproduction better than incidental blood lactate values do (Fernandes and Blom, 1976).

PATIENTS AND METHODS

Seventeen patients with glucose-6-phosphatase deficiency (type IA) and one with glucose-6-phosphate translocase deficiency (type IB) were treated with frequent meals at daytime and GDF during 10–12 hours each night, the eldest patient excepted. The meals were starch-enriched and sucrose- and lactose-restricted. The GDF consisted of a liquid lactose-restricted infant formula with maltose added.

Twelve hours' urine was collected from 8 a.m. till 8 p.m. and from 8 p.m. till 8 a.m. next day, synchronously with the frequent-meals regime in daytime and the GDF during the night. Each urine sample was immediately transferred to the collecting bottle and stored at −20 °C. The quantitative collection of urine, easy to carry out in hospital routine, is less suitable for conditions at home and when the child attends school. Then we asked the child to void before breakfast and before starting GDF. We assumed these two urine samples to reflect approximately the lactate concentration during the night and the day, respectively, after having observed the lactate concentrations of 12 hours' urine and the last sample of the same period to be in the same range under steady state conditions. The urinary lactate concentration was used without calculating lactate excretion per day or per kg bodyweight (Fernandes and Blom, 1976). Urinary lactate and creatinine were determined with routine chemical methods.

RESULTS AND DISCUSSION

A low exogenous glucose supply resulted in high lactate concentrations in the urine. This is shown in a longitudinal study of a patient (Figure 1). At high glucose infusion rates the lactate concentration decreased to a basal level between 0.3 and 1.0 mmol/l (normal upper limit at 0.3 mmol/l). This was first reached at an infusion rate of 4 mg glucose kg^{-1} min^{-1}, which is lower than the theoretical production rate of 6.7 mg kg^{-1} min^{-1}, calculated according to Bier *et al.* (1977). In most other patients the urinary lactate concentrations were in the same range, but markedly higher basal concentrations were found in five severely growth-retarded children (height less than 3rd percentile) (results not shown).

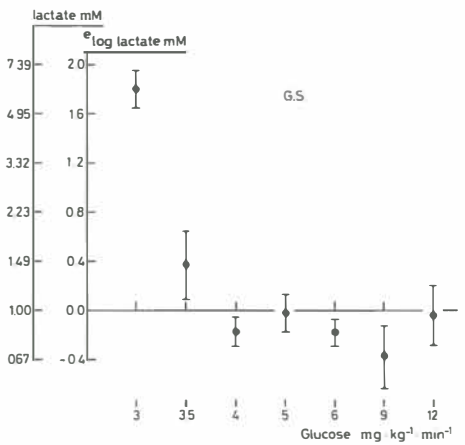

Figure 1 Urinary lactate concentration (mean ± SEM) for various dose levels of glucose in nocturnal gastric drip feeding administered to a patient with glucose-6-phosphatase deficiency. The glucose content of GDF was changed in a random order

The lactate/creatinine ratios were usually below 0.1 (normal range 0.010–0.058). Higher values were measured in five children with height percentiles below 25.

In conclusion we found the urinary lactate concentration to be a useful parameter to estimate the basal glucose requirement of glucose-6-phosphatase-deficient patients. Higher intakes have no additional benefit, as normalization of the lactate concentration in blood and urine can usually not be obtained, nor would this be desirable as it would reduce the availability of lactate as an alternate fuel for the brain in case of inadvertent

hypoglycaemia (Fernandes *et al.*, 1982). A hyperlactaciduria that persists despite adequate glucose supply is observed in some patients with severe growth retardation. The reason for this apparent correlation is unknown.

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CHAPTER VI

MEDIUM CHAIN TRIGLYCERIDES IN THE DIETARY MANAGEMENT OF PATIENTS WITH GLYCOGENOSIS CAUSED BY DEBRANCHING ENZYME DEFICIENCY.

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Running title:
Medium chain triglycerides administration and ketogenesis in GSD type III.

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ABSTRACT

When patients with glycogen storage disease due to de-branching enzyme deficiency are fasted, an age-related tolerance to fasting can be demonstrated: the tendency for hypoglycemia which is seen in young infants (<5 years of age) gradually disappears at older age (> 15 years).
In order to explore whether this age-dependent phenomenon was due to:

1. impaired mobilization of fatty acids, patients were given long-chain triglycerides (LCT), dose 1g.kg body weight orally,
2. impaired transport and/or activation of fatty acids, patients were given medium-chain triglycerides (MCT),dose 1g.kg body weight orally.

In the group of very young patients (<5 years) a striking improvement after MCT was demonstrated: all patients (n=5) were able to endure the maximal duration of the test (6h), instead of 90±30 min in the fasting test, or 150±30 min after LCT.
This improvement is due to an increase in ketogenesis in the first hour after MCT administration : 2.4±0.5 mM ketone bodies in blood after MCT, 1.3±0.8 mM after LCT and 1.1±0.7 mM during fasting.
The increased ketogenesis after MCT has a glucose-sparing effect and therefore prevents the development of hypoglycemia in the very young children as can be deduced from the change in blood glucose concentration in the first hour of the tests:+0.1±1.0 mM glucose after MCT, -1.6±0.8 mM after LCT,and -1.6±1.3 mM during fasting.
We conclude that in young children the age-related hypo-glycemia probably is due to deficient activation and/or transport of long-chain fatty acids in the mitochondria to maintain adequately high rates of ketogenesis. Their keto-genesis, which is insufficient, can be stimulated by MCT.

ABBREVIATIONS

GSD III	Glycogen Storage Disease caused by deficiency of debranching enzyme
MCT	Medium Chain Triglycerides
LCT	Long Chain Triglycerides
FFA	Free Fatty Acids
KB	Ketone Bodies.

INTRODUCTION

In glycogen storage disease caused by debranching enzyme deficiency (GSD III EC 3.2.1.33), the degradation of glycogen beyond the branching points is blocked. Accordingly, glycogen can only be used as a limited source of glucose in the postprandial state.
For this reason the patients have to be fed frequent carbohydrate-enriched meals during day and night. In particular very young patients with GSD III are prone to hypoglycemia and hypoglycemic convulsions during fasting. Fernandes and Pikaar (6) observed that the fasting hypoglycemia in these patients was accompanied by hypoketonemia. When the patients grow older the tendency for hypoglycemic symptoms during fasting decreases and hypoketonemia changes into hyperketonemia. This indicates that in very young patients the mobilization of an alternative fuel to compensate for the deficient availability of glucose is impaired.

In the present study we used oral tolerance tests with various triglycerides to explore these age-related changes in response to fasting. Starting from a fasted state the patients were given either a long-chain triglyceride emulsion (LCT) or a medium-chain triglyceride emulsion (MCT). LCT need to be converted to carnitine-esters before entering the inner mitochondrial space by means of the carnitine carrier system (12,13), whereas MCT pass the mitochondrial membrane independent from this carrier system.
Thus, in the LCT test the effect of fatty acid mobilization, cytosolic activation and carnitine-mediated transport on ketogenesis was studied. In the MCT test, that of fatty acids independent from cytosolic activation and carnitine-independent transport, as ultimately reflected by the formation of ketone bodies, was investigated.

PATIENTS AND METHODS.

Fourteen patients with deficiency of amylo-1,6-glucosidase (EC 3.2.1.33) as documented by enzyme assays in either leukocytes or liver biopsies were admitted in the Pediatric Department for at least 14 days. The patients were of the age of 10 months to 26 years, and comprised 8 males and 6 females. All patients received frequent carbohydrate-enriched diets with a high content of linoleic acid. Eight patients (N.P.,J.V., D.S., R.K., O.O., C.H., E.R.) also received a late evening meal (protein- and carbohydrate-enriched), and one patient (L.L.) received nocturnal gastric drip feeding.
The tests were carried out in the metabolic ward and were started after a fast of different duration, depending on the patient's propensity for hypoglycemia. The tests lasted ultimately six hours. They were stopped earlier if symptoms of hypoglycemia developed or if the blood glucose concentration dropped below 1.2 mM. Informed consent was obtained from the parents and/or the patients.
Three tests were performed, a fasting test, an oral tolerance test with LCT, and an oral tolerance test with MCT. The dose of the triglycerides amounted to 1 g.kg body weight. The triglycerides were ingested as an emulsion in water (1:1 v/v).
For LCT Calogen® was used, for MCT Liquigen® both from Scientific Hospital Supplies Ltd. , (Liverpool, GB). The composition of the acylchains in the LCT emulsion was: C16:0 10% ; C18:0 5% ; C18:1 60% and C18:2 25%, and in the MCT emulsion: C6:0 1% ; C8:0 81% ; C10:0 16% ; C12:0 2%.
The age-matched control groups consisted of 16 healthy volunteers and children with the Lennox epilepsy syndrome. As part of the treatment in the last group a ketogenic diet is used.
The blood glucose concentrations were measured at 30 min intervals by the glucose oxidation method with a Beckman glucose analyzer. Concentrations of 3-hydroxybutyrate and acetoacetate in blood were determined enzymatically at 60 min intervals (1). Plasma immunoreactive insulin was measured by the double anti-body method of radioimmunoassay (11), plasma immunoreactive pancreatic glucagon by radioimmunoassay with the talc method (16). The timing of taking the blood samples started from the beginning of the ingestion of the fat emulsions.
Statistical analysis was done using the Student's test for (un-) paired samples. The data are given as mean±standard deviation. Differences were considered to be significant at $p < 0.05$.

RESULTS.

Clinical observation during fasting and oral tolerance tests.

The fourteen patients in this study were divided retrospectively into three age groups, depending on their tolerance to fasting (Table I). We grouped the very young patients till 5 years (# 1- 5) together since they developed clinical symptoms of hypoglycemia early in the fasting test. In this group the patients could fast for 90±30 min. In an age-matched control group the fasting test lasted for the maximal duration (6h) in all cases.
A second group comprised patients between 5 and 15 years (#6-9), all of which were able to tolerate fasting for 6h, except that some of them developed clinical symptoms of an impending hypoglycemia at the end of the fasting test.
The last group comprised patients older than 15 year (#10-14). These patients could fast for 6h without clinical symptoms of hypoglycemia.
Oral administration of LCT resulted in a small but significant improvement of the tolerance to fasting in the very young children: the mean duration of the test increased from 90±30 to 150±30 min. Administration of MCT resulted in a further improvement. All patients could fast the maximal duration of 6h of the test.

A. The patient group under 5 years of age.

In Table II the mean plasma concentrations of glucose and ketone bodies (KB) during fasting, and after ingestion of an emulsion of LCT or MCT are given for the patients under 5 years of age. The mean glucose concentration of the patients during fasting was significantly lower than that of the control group; whereas the KB concentration did not differ significantly from that of the control group. However, it should be taken into account that the patients could only fast for 90±30 min, while the controls could fast for the whole test period (6h).
Ingestion of LCT did not result in a significant change in the mean concentrations of glucose and KB. Oral administration of MCT resulted in a small increase of glucose and KB concentrations although this was not significant.

In Table III the mean changes are given in the concentration of glucose and KB in plasma during the first hour of the test. Oral administration of MCT almost abolished the changes in the average glucose concentration and caused the KB concentration to increase significantly when compared to both fasting and the LCT test.

B. The patient group between 5 and 15 years of age.

During fasting the patients exhibited a significantly lower mean glucose concentration in plasma accompanied by a higher KB concentration when compared to an age-matched control group (Table II). It is also clear that in this patient group the KB concentration is increased over that measured in the patient group aged under 5 years, although not significantly. Oral administration of LCT or MCT did not change the mean glucose concentrations. In the latter test the mean KB concentration increased slightly, but not significantly.

C. The patient group above 15 years of age.

In this group the mean glucose concentration was similar to that of an age-matched control group and not influenced by either LCT or MCT administration (Table II). In contrast, the KB concentration was significantly higher as compared to the control group, but not influenced by the challenge of either triglyceride.

In all groups FFA concentrations were measured in plasma in all tests, the mean maximum concentration being 982±45 uM. No significant difference of the maximum FFA concentrations, either between the three tests or between the three age groups could be detected. At the end of the test period the glucagon/insulin ratio was determined. In none of the groups this ratio was lower than 6.0. This indicates a catabolic state (14).

DISCUSSION.

Young patients with GSD III suffer from hypoglycemia upon fasting (6). Glycogen is only a limited source of glucose in these patients due to the lack of debranching enzyme. With age the propensity to fasting hypoglycemia disappears gradually.
Two main factors might contribute to this increasing resistance :
In the first place KB concentration in blood rises with age. In a previous study (6), KB concentrations were found to be greatly elevated over age-matched controls in older patients. In the present study this observation has been confirmed, especially in the older patients (over 5 years). It appears that this hyperketosis represents an excessive response to the decrease in glucose concentration in plasma (8,9,17,19). Since the patient's glycogenolysis is hampered, increased gluconeogenesis from amino acids might be the first means to compensate for shortage of glucose (4,5,7,18). Secondly,

lipolysis is stimulated and β-oxidation of fatty acids is enhanced as an alternative for glucose-oxidation. Presumably, the lack of glycolytic substrate in the cell results in a low malonyl-CoA level which is known to stimulate fatty acid transport into the mitochondria (12). A subsequent high production of acetyl-CoA from fatty acids combined with a small oxaloacetate pool due to the presumed high gluconeogenesis then results in an increased ketogenesis (13). If a similar relationship exists in GSD III patients, high KB blood levels would not only indicate increased KB production, but would also reflect increased KB utilization (8,15), if the production rate and utilization rate are in balance. This means increased availability of KB as a metabolic fuel alternative to glucose, at least in the intermediate and oldest patient groups.

A second factor influencing the demand for glucose is the basal metabolic rate (3). With increasing age this rate decreases and this is a contributing factor to the gradual disappearance of hypoglycemia. Therefore the demand for glucose and KB diminishes. This is in agreement with measurements of the glucose turnover in humans, which decreases, too, with increasing age (2). A defective glucose release would then be less of a problem at older age, and the demand for KB as an alternative "fuel" becomes less.

In the group of the very young patients (less than 5 years) these compensatory mechanisms appear to be inadequate. No excessive rise in KB concentration in plasma was observed in this group upon a decrease in glucose concentration. The low steady state concentration of KB in blood might indicate an imbalance between the low production rate and the high requirement.

LCT were given to ensure a high supply in FFA. However, no significant differences were observed as compared to fasting. Administration of MCT on the other hand was followed by a significant improvement in the patient's resistance to hypoglycemia. Although the average blood glucose concentration did not change, the KB concentration did increase considerably. The major difference in metabolism of the two types of fatty acids is the difference in the site of activation. Whereas long-chain fatty acids are activated in the cytosol before transport into the mitochondria, medium-chain fatty acids are activated inside the mitochondria (10,13).

It thus appears that in the very young patients the activation of long-chain fatty acids in the mitochondria, and/or the rate of transport over the mitochondrial membrane is limited to allow a high rate of fatty acid oxidation and ketogenesis in the mitochondria. In view of a high demand for metabolic fuels the rate of production becomes limiting, which results in a lower steady-state concentration of KB.

In conclusion, in very young patients with GSD III the low KB concentration in blood during fasting is due to a discrepancy between the (low) production rate and the high demand for energy. KB production, if deficient, can be stimulated by MCT administration. This might have practical consequences

for the diet, as MCT inclusion in a late evening meal may improve the resistance to fasting during the night. In older patients, whose KB levels during fasting are already high without additional MCT, the use of complex carbohydrates like uncooked cornstarch in the diet, may improve their resistance to fasting.

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Table I Patients grouped after their age-related tolerance to fasting.

	Patients No	Name	Age in Years/ Months*	Sex	Fasting tolerance
	1.	NP	10/12*	♀	--
	2.	LL	2	♀	--
A	3.	JV	2	♂	--
	4.	DS	3	♂	--
	5.	OO	5	♂	--
	6.	RK	5	♂	-
	7.	JK	10	♀	+
B	8.	CH	11	♀	-
	9.	ER	15	♀	+
	10.	AJ	16	♂	+
	11.	BJ	16	♂	+
C	12.	JA	23	♀	+
	13.	BN	23	♂	+
	14.	SB	26	♂	+

Symbols:
-- clinical symptoms of hypoglycemia in the beginning of the fasting test.
- clinical symptoms of hypoglycemia at the end of the fasting test.
+ no clinical signs of hypoglycemia during the fasting test.

Table II

The mean concentrations of all plasma glucose and KB concentrations in GSD III patients and age-matched controls during fasting and after administration of LCT and MCT.

age group (yr)	category	glucose concentration (mM)			ketone body concentration (mM)		
		fasting	+ LCT	+ MCT	fasting	+ LCT	+ MCT
<5	patients (n=5)	2.1±1.1 *	2.2±1.0	2.7±1.2	2.8±1.8	2.8±1.7	4.3±2.3
	controls (n=6)	3.4±0.5	N.D.	3.5±0.9	1.5±0.7	N.D.	2.5±1.4
5-15	patients (n=4)	1.8±0.5 *	2.3±0.4	2.0±0.5 *	5.3±2.3 *	4.1±1.7	6.6±2.0 *
	controls (n=6)	4.3±0.4	N.D.	4.0±0.4	0.7±0.4	N.D.	1.6±1.0
>15	patients (n=5)	4.5±0.6	4.6±0.6	4.7±0.6	5.1±1.7 *	4.0±1.8	4.8±2.3 *
	controls (n=4)	4.6±0.2	N.D.	4.7±0.3	0.2±0.1	N.D.	0.3±0.1

*: significant difference between patient group and age-matched control group.
N.D. is not determined.

Table III

The change in plasma glucose and KB concentration in the very young patients (<5 yr) in the first hour of fasting, and in the first hour after oral administration of LCT and MCT.

test	Δ-glucose concentration (mM)	Δ-ketone body concentration (mM)
fasting	-1.6±1.3 *	+1.1±0.8 *
+LCT	-1.6±0.8 #	+1.3±0.8 #
+MCT	+0.1±1.0	+2.3±0.5

*: significant difference between fasting and MCT.
#: significant difference between LCT and MCT.

CHAPTER VII

GLUCOSE AND PALMITATE TURNOVER IN GLYCOGEN STORAGE DISEASE CAUSED BY DEBRANCHING ENZYME DEFICIENCY.

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ABSTRACT.

Glucose and palmitate turnover rates were determined simultaneously in nine patients with glycogen storage disease caused by debranching enzyme deficiency in order to explore whether the age-related tendency for hypoglycemia resulted from:

a. decreased endogenous glucose production, or increased rate of glucose turnover.
b. lack of alternative energy substrate due to decreased turnover of fatty acids.

The tests were performed in the postabsorptive state, after a fast of different duration depending on the patient's propensity for hypoglycemia. During the tests the blood glucose concentration was kept at a constant level (between 2.3-3.0 mM) by intravenous glucose administration at a rate of 0.5-2.0 $mg.kg^{-1}.min^{-1}$, except in the oldest two patients, who maintained normal blood glucose concentrations during fasting. During steady state conditions glucose and palmitate turnover rates were estimated with [6,6-$^{2}H_{2}$]-glucose and [1-^{13}C]-palmitate, respectively. The endogenous glucose production rate was calculated by subtracting the individual glucose infusion rate from the measured glucose turnover rate. The basal metabolic rate was determined by indirect calorimetry. The measured glucose turnover rates : 6.7±0.6 - 1.7±0.2 $mg.kg^{-1}.min^{-1}$, were in the normal range when compared to fasting predicted values: 6.1-1.7 $mg.kg^{-1}.min^{-1}$. The endogenous glucose production rates, amounting to: 4.9±0.6 - 1.7±0.1 $mg.kg^{-1}.min^{-1}$ gradually met the glucose consumption rate for adults.
The measured palmitate turnover rates varied between 2.00±0.08 and 0.51±0.03 $mg.kg^{-1}.min^{-1}$ versus 1.6±0.5 - 1.2±0.5$mg.kg^{-1}.min^{-1}$ for predicted values. Basal metabolic rates were normal and varied between 9.7±0.2 - 4.5±0.2 $kJ.kg^{-1}.min^{-1}$ against 9.5-4.7 $kJ.kg^{-1}.min^{-1}$ for predicted values.
It was concluded that the deficit in endogenous glucose production is compensated for by increased ketogenesis at young age, although in the young patients this compensatory mechanism is still insufficient.

ABBREVIATIONS

GSD III	Glycogen storage disease caused by debranching enzyme deficiency
FFA	Free Fatty Acids
KB	Ketone Bodies
BMR	Basal Metabolic Rate
MPE	Mole Percent Excess
Ra	Rate of appearance
E-Ra	Endogenous rate of appearance
BW	Body Weight
BrW	Brain Weight
LW	Liver Weight

Glycogen storage disease type III (GSD III) is characterized by a deficiency of the debranching enzyme, amylo-1,6-glucosidase, which limits glycogen breakdown beyond the branch points of the molecule. Especially young GSD III patients suffer from hypoglycemia and hypoglycemic convulsions during fasting. This tendency for fasting hypoglycemia improves with age and the majority of adult GSD III patients develops tolerance against fasting although the blood glucose concentration remains at the lower level of the normal range (1,2,3,12). Upon fasting, the hepatic glucose production from glycogen in these patients is limited, and hypoglycemia ensues unless sufficient glucose is produced by gluconeogenesis or if alternative energy substrates resulting from lipolysis (KB) become available (1,2,3,6,7).

It is not known whether this age-related propensity for hypoglycemia results from increased demand for glucose with respect to a decreased endogenous glucose production, or a lack of alternative energy substrate due to decreased turnover of fatty acids. Therefore, we studied simultaneously the glucose and palmitate turnover rates in the postabsorptive state by a stable isotope dilution method, together with the BMR.

MATERIALS AND METHODS.

Patients.

Nine patients, four females and five males, aged 1 to 28 years with GSD III were included in the study, and admitted in the Pediatric Department. The diagnosis of GSD III was based on deficient liver or leukocyte amylo-1,6-glucosidase activity.

All patients received frequent carbohydrate enriched meals with a high content of linoleic acid. Six patients (# 1, 3, 4, 5, 6 and 7) also received a late evening meal, one patient (# 2) received nocturnal gastric drip feeding. All tests were carried out in the metabolic ward of the Pediatric Department. The room where the tests were performed was kept quiet, the temperature was 22°C, and the lighting was dimmed. The patients were given sufficient time to become familiar with the equipment and the procedures. Patient # 8 and 9 were studied in Israel (Soroka Medical Center, University of the Negev, Beer-Sheba). The study was approved by the University

Medical Ethical Committee. Informed consent was obtained from the parents and/or the patients.

Chemicals.
1-Butaneboronic acid (99%) was purchased from Janssen Chemica (Beerse, Belgium). Pyridine and acetic anhydride were obtained from Applied Science (Alltech Europe, Eke, Belgium). All other chemicals used were of analytical grade and supplied by Merck (Darmstadt, FRG). Stable isotopically labeled [6,6-$^2H_2$]-glucose (99 atom% deuterium),and [1-^{13}C]-palmitate (99 atom% ^{13}C)were obtained from Merck, Sharp and Dohme Isotopes (Sanbio B.V. Uden, The Netherlands).

Study design.
After a fast of different duration depending on the patient's propensity for hypoglycemia, an intravenous infusion of glucose at a constant rate to prevent hypoglycemia was administered, except in the oldest two patients (# 8 and 9), who maintained normal blood glucose levels during fasting. The individual infusion rate of glucose was determined in a previously performed glucose titration test during which the amount of glucose, infused per kg body weight per minute, needed to maintain steady state blood glucose concentrations was estimated. Blood glucose concentrations were maintained at about 2.5 mM.
[6,6-$^2H_2$]-Glucose was dissolved in 0.5N saline and prepared for human use as described (4). [1-^{13}C]-Palmitic acid was bound to human albumine for intravenous administration as described (8).
Prior to the measurements blood samples were taken for the determination of the natural enrichment of glucose and palmitate.
At approximately 10.00 A.M. the tests started with a primed-dose constant-rate infusion of [6,6-$^2H_2$]-glucose (dose: 5mg/kg body weight) for 90 min, followed by a constant-rate infusion of [6,6-$^2H_2$]-glucose (dose: 0.045 mg/kg bodyweight.min^{-1}). The start of the constant-rate infusion of [6,6-$^2H_2$]-glucose was taken to be time: 0 min (see Fig I). At time -30 min the [1-^{13}C]-palmitate constant-rate infusion at a dose of 0.05 mg/kg body weight.min^{-1}) was started and continued for 120 min.
Blood samples were taken for the determination of the concentration of glucose, palmitate, KB, and [6,6-$^2H_2$]-glucose and [1-^{13}C]-palmitate enrichments. The samples amounted to a total of 3 ml blood per sample and were taken at 15-min intervals during 90 minutes. The BMR was measured at time: 0, 30, and 60 min.

Analytical methods.
The BMR was measured by indirect calorimetry with a diaferometer of Noyons (Kipp en zonen, Delft Holland) (19). Blood glucose concentrations were determined with a Beckman glucose analyzer. Blood 3-hydroxy-butyrate and acetoacetate concentrations were determined enzymatically (10).

Sample extraction and derivatization.

Glucose. The blood samples were immediately centrifuged at 2500 rpm for 5 min, and the plasma samples were stored at -70 °C until analysis. The plasma sample (50 ul) was deproteinized by mixing with 500 ul methanol and storing at 0°C for 30 min. The samples were then centrifuged for 10 min at 2500 rpm, the supernatants were decanted into a clean test tube and the fatty acids were removed by extraction with 5 ml of hexane. After centrifugation the hexane layer was removed. The remaining methanol solution was evaporated to dryness under nitrogen at 60°C. To the dried residue 150 ul butaneboronic acid (25 mg/2 ml pyridine) was added and heated to 95°C for 30 min. The test tubes were then allowed to cool to room temperature and 50 ul acetic anhydride was added, and after 30 min the derivative was washed with 0.1 N HCL after dissolving in 1 ml hexane. The upper hexane layer was removed to a 1 ml vial and evaporated to dryness and reconstituted with 50 ul of hexane. A volume of 1-4 ul was injected into the gas chromatograph/mass spectrometer combination (GC/MS) for analysis.

Palmitic acid. A plasma sample (500 ul) was added to a test tube containing 25 ul heptadecanoic acid (100 mg/100 ml hexane) and 50 ul 2,6-ditert-butyl-p.cresol (1g/100 ml), and subsequently vortexed for 1 min. Thereafter the mixture was allowed to stand for 5 min. To the mixture 750 ul of buffer was added (0.53 M potassium phosphate and 0.27 M sodium phosphate). The FFA were then extracted with 6.25 ml solvent (chloroform: heptane: methanol, 49:49:2 v/v) by vortexing for 2 min. The mixture was allowed to stand for 15 min, and then centrifuged for 15 min at 2500 rpm. The lower organic phase was removed and pipetted into a clean test tube and evaporated under nitrogen at 60°C. Methylation was carried out using diazomethane (23). The fatty acid methylester derivatives were evaporated to dryness and reconstituted with 50 ul of hexane. A volume of 1-4 ul was injected into the GC/MS for analysis. Profiling of the fatty acid methylesters was performed by gas chromatography as described. (24).

Preparation of the GC/MS standards.

Standards containing 0,1,2,3,4, and 5 mole percent excess (MPE) [6,6-$^{2}H_{2}$]-glucose and [1-^{13}C]-palmitate were prepared by weighing aliquots of the same stable isotopes that were administered to the patients, and mixing them with weighed amounts of natural glucose and palmitate. Each day samples were analyzed by GC/MS, a calibration graph containing 0 to 5% was constructed.

Gas chromatography / mass spectrometry.

The isotope enrichment (mole percent excess, MPE) of [6,6-$^{2}H_2$]- glucose and [1-^{13}C]-palmitate was determined by GC/MS, using selective ion monitoring (SIM) at 70 eV. GC/MS of the palmitate derivatives were carried out using a HP 5995B system (Hewlett Packard, Palo Alto, USA). The glucose derivatives were analyzed using either the HP 5995B system or a Finnigan MAT 212 system (Finnigan MAT GmbH,Bremen, FRG). Gas chromatography of both derivatives was carried out using a 25m x 0.32 mm ID fused silica column (CP Sil 5 CB), with a film thickness of 0.12 um (Chrompack B.V.,Middelburg, The Netherlands). The HP 5995B system was connected to a HP 9825B desktop computer with two HP flexible disk drives controlled by the HP flexible disk software for data collection and computation. Helium served as the carrier gas, the injector temperature was 280°C, and the ion source pressure was 5 x 10^{-6} Torr. The column was directly coupled to the ion source at a temperature of 180°C.
Before analysis with the HP 5995 the mass spectrometric conditions were first optimized for m/z 414 by means of the "autotune" program. Thereafter the mass spectrometer was tuned for a resolution of 0.6 u peak width at half height.
The Finnigan MAT 212 system consisted of a Varian 3700 gas chromatograph, helium served as the carrier gas, and the injector temperature was 280°C. The column was directly coupled to the ion source of the Finnigan MAT 212 mass spectrometer at a temperature of 250°C, at a pressure of 10^{-5} Torr. Analyses were performed at a mass spectrometric resolution of 1000. Data collection and computation was performed by the Spectro System MAT 200 (Finnigan MAT GmbH, Bremen, FRG).

Selective ion monitoring.

a. For glucose. The dibutyl borate acetate derivatives were monitored at m/z 297 $(M-57)^{+}$ for non-labelled glucose and at m/z 299 for labelled glucose (4). The oven temperature was 250°C. Calibration graphs were prepared by plotting the measured percent ion current ratio's (peak height) m/z 299/297 (y) versus known mole percent excess in the [6,6-$^{2}H_2$]-glucose derivative standards (x). The slope (m) and y-intercept (b) were calculated according to the linear relationship for a straight line as expressed by the equation: $y = mx + b$.
b. For palmitate. The methylester derivatives were monitored at m/z 270 (M^{+}) for non-labeled palmitate and at m/z 271 for labelled palmitate (20,21). The oven temperature was 240°C. Calibration graphs were prepared by plotting the measured ion current ratio's (peak area) m/z 271/270 (y) versus the known mole percent excess in the [1-^{13}C]-palmitate derivative standards (x).
All samples were injected at least three times and the coefficient of variation was always lower than 5% .

CALCULATIONS.

All rates are expressed as $mg.kgBW^{-1}.min^{-1}$ except when otherwise stated.

The BMR was measured by indirect calorimetry and was calculated from the oxygen uptake as described (19):

$$BMR = K \times S \qquad (I)$$

in which: BMR is the basal metabolic rate in $kJ.kg^{-1}.h^{-1}$
K is the oxygen uptake in liters $O_2.kg^{-1}.h^{-1}$
S is the number of kJ corresponding to the respiratory quotient in $kJ.lO_2^{-1}$.

The results were compared to published values (18).

Rate of appearance for glucose was calculated according to Steele (5) from the mean mole % of enrichment.

$$Ra = \frac{MPE\ inf}{MPE\ pl} \times V\ inf \qquad (II)$$

in which: Ra is the rate of appearance into the circulation
MPE inf is the mole percent excess of [6,6-$^2H_2$]-glucose in the infusion solution
MPE pl is the mole percent excess of [6,6-$^2H_2$]-glucose in the plasma
V inf is the rate of infusion in $mg.kg^{-1}.min^{-1}$

The endogenous rate of appearance (E-Ra) was calculated by subtracting the individual glucose infusion rate (R inf) from the rate of appearance of glucose (Ra) :

$$E\text{-}Ra = Ra - R\ inf \qquad (III)$$

The turnover rate is defined as the rate of disappearance of tracer from the blood compartment, which in steady state is equal to the rate of appearance.

The consumption rate is defined as the fraction of the turnover rate which is used for energy production.

The measured rates of appearance of glucose were compared to predicted rates of appearance of glucose at different degrees of ketosis as calculated using a modified equation deduced from the Tables III and IV in ref (13) :

$$Ra = -9.69 \times \frac{BrW}{BW} \times [KB] + 125.7 \times \frac{BrW}{BW} \qquad (IV)$$

in which: BrW is brain weight in kg as calculated from the head circumference as described (15)
[KB] is the mean concentration of ketone bodies in blood in mM.

The rate of appearance for palmitate was calculated according to (II).

The measured rates of appearance of palmitate were compared to predicted rates of appearance of palmitate, and were calculated according to (9) :

$$Ra = 2.56 \times [palm] + 0.58 \qquad (V)$$

in which: [palm] is the mean palmitate concentration in plasma in mM.

The rate of appearance of KB was calculated from the concentration of KB in blood, using the equation (29) :

$$Ra = 0.39\ [KB] + 0.39 \qquad (VI)$$

All numbers were expressed as mean ± SD. Statistical analysis was performed using the Student's test for (un-)paired samples. Differences were considered significant at $p < 0.05$.

RESULTS.

In Table I physical patient parameters are given, together with the BMR values. The data in this Table show that the measured BMR value decreased with age, from 9.7±0.2 kJ.kg-BW^{-1}.min^{-1} at the age of 1 year to 4.6±0.2 at the age of 28.5 years. These measured values compared well with those published (Table I). The oldest patients had BMR values near to that measured in normal adults.

In Fig I a typical measurement is shown (patient # 7). As can be deduced from this figure steady-state is reached at 30 min after the start of the constant-rate infusion of [6,6-$^{2}H_2$]-glucose. The concentrations in plasma of glucose, KB, and palmitate were steady during the experiment. During the same period isotopic steady-state was reached for [6,6-$^{2}H_2$]-glucose and [1-^{13}C]-palmitate enrichments. For each patient the reported concentrations in plasma are the average of the measurements over this period of time. The rates of appearance of glucose and palmitate into the circulation were calculated from the average of the mole percent excess of the two metabolites measured over this period.

The average plasma glucose concentrations of the patients varied between 2.3±0.2 and 4.1±0.2 mM (Table II). In the patients who needed a glucose maintenance infusion the plasma glucose concentration varied between 2.3±0.2 and 3.0±0.1 mM. Adequate lipid oxidation rates were observed at these plasma glucose concentrations as indicated by the average respiratory quotient value of 0.78±0.08 after correction for protein oxidation.

In Table II the individual rates of infusion of glucose needed to maintain the plasma glucose concentrations, the total rate of appearance calculated from the mean mole percent enrichment and the endogenous rate of appearance of glucose are given. The rate of the glucose infusion tended to decrease with increasing age from 1.8 in the youngest patient to 0.5 mg.kgBW^{-1}.min^{-1} in the patients 15 y of age. The total rate of appearance decreased from 6.7±0.6 to 1.7±0.2 mg.kg-BW^{-1}.min^{-1} , whereas the endogenous rate decreased from 4.9±0.6 to 1.7±0.2 mg.kgBW^{-1}.min^{-1}. When compared to predicted values at different degrees of ketosis the total rate of appearance of glucose in GSD III patients was in the same range: 6.7±0.6 to 1.7±0.2 mg.kg^{-1}.min^{-1} for GSD III patients against 6.1-1.7 mg.kg^{-1}.min^{-1} for predicted values (13).

KB concentrations in plasma were elevated in all patients during the measurements as could be expected in view of the glucose concentration in blood and the low respiratory quotients (Table III). Some of the patients even displayed hyperketosis when compared to control values (2) (patients # 4 and 5 in Table III). Accordingly, the KB concentrations in plasma varied over a wide range: between 1.1±0.2 and 6.5±1.0 mM.

From the mole percent enrichment of palmitate in plasma the rate of appearance of palmitate was calculated. In Table III these rates are given for each patient together with the palmitate concentration in plasma and the corresponding published value for the rate of appearance of palmitate. As is clear from this Table, the observed rates of appearance are not related to the age of the patients and varied between 0.51±0.03 and 2.00±0.08 $mg.kgBW^{-1}.min^{-1}$. Neither was there a relation with the palmitate concentration or KB concentration in plasma, as is particularly clear for the values in patients #4 and #5. Comparison of the observed with the published rates of appearance shows that the average palmitate rate of appearance (1.1±0.5 $mg.kgBW^{-1}.min^{-1}$) was not significantly different ($p > 0.1$) from the average of the predicted values (1.3±0.3 $mg.kgBW^{-1}.min^{-1}$) (Table III,13). The correlation between the observed and the predicted rate of appearance in the individual patients is rather poor. In neither case a significant difference could be demonstrated between the observed and the predicted value for the rate of appearance for palmitate due to the error in the estimated rate of appearance (Table III).

DISCUSSION.

Patients with GSD III exhibit fasting hypoglycemia. This diminishes gradually in childhood. Adult patients can tolerate fasting without any clinical symptoms. In this study the rate of appearance of two major metabolic fuels have been measured, i.e. glucose and palmitate as a representative tracer for FFA (11). Since all measurements were performed under steady-state conditions the rate of appearance of tracer as derived from the mole percent excess in plasma equals the rate of disappearance from the blood compartment and may be regarded as the rate of turnover.

For all patients the observed total rate of turnover of glucose was compared to published values. When compared to postprandial published values as described by Bier et al (4) the measured values in these patients were systematically lower. This discrepancy could be accounted for when the measured values were compared to published values at various degrees of ketosis as derived from Haymond et al (13) (Table II). Therefore, the rates of turnover of glucose in GSD III patients are regarded to be normal. The endogenous rates of glucose appearance which are equal to the endogenous rates of glucose production however, were lower than the measured and predicted rates of glucose turnover except in the oldest patients (#8 and #9). Since no significant difference between the measured and predicted rates of appearance for palmitate were found (Table III), the rates of turnover of palmitate in GSD III patients are regarded to be normal. As [1-^{13}C]-palmitate is a representative tracer for FFA, the rates of turnover of FFA in GSD III patients are regarded to be normal.

The mechanism responsible for the age-related increase in tolerance to fasting is not clearly understood. In this mechanism two organs are of major importance, the brain and the liver. A shortage of oxidative substrates in brain tissue elicits the clinical symptoms accompanying hypoglycemia. For brain tissue two oxidative substrates are of importance, glucose and KB. The liver is the only organ able to release glucose into the circulation during fasting, either by glycogenolysis or by gluconeogenesis (7,16,17,25). In GSD III patients the former process of glucose production is virtually blocked due to the deficiency of debranching enzyme. As may be deduced from this study the latter process of glucose production is limited until adulthood.

The liver is also the only source of KB, which arise during β-oxidation of FFA (14,25). Thus a metabolic axis exists between the liver as a producing organ and the brain as a consuming organ of metabolic fuel. In GSD III patients high KB concentrations in plasma have been observed in this as well as in previous studies (1,2). It has therefore been speculated that KB serve as an alternative substrate for brain tissue to compensate for the insufficient glucose production by the liver.

Thus, in order to understand the mechanism of the age-related increase in tolerance to fasting the glucose production-consumption must be discussed in relation to that of FFA and KB.

The rate of ketogenesis in man under various conditions (e.g. fasting up to six weeks, diabetic ketosis) correlates well with the concentration in plasma (29). Starting from the measured KB concentration this correlation was used to estimate the production rate of KB (Table IV). To evaluate these estimated production rates two comparisons were made. In the first comparison the fraction of FFA necessary for this ketogenesis was calculated. In this calculation 1 mole of FFA produces 4 moles of KB. In Table IV the calculated fraction of FFA necessary for ketogenesis in the individual patients is given as a percentage of the observed FFA turnover. As is clear from the Table the fraction of FFA turnover that served for ketogenesis averaged 33±14% . Similar values of 29±3% and 31±2% have been published (28,29). In the second evaluation of the estimated KB turnover the following comparison was made. Since oxidation is the major metabolic fate for KB, the fraction of FFA entering ketogenesis should at least be equal to the fraction of FFA oxidized. The amounts of the FFA oxidized were calculated from the O_2 consumption and the CO_2 production as measured by indirect calorimetry. In Table V these amounts are given as a fraction of FFA turnover. The average fraction of FFA turnover oxidized was 35±10% , which is equal to the fraction of FFA turnover entering ketogenesis. Thus, the calculated values of the KB turnover represent a reliable estimate.

During fasting KB replace glucose as oxidative substrates in brain tissue (27,28). In the patients studied the data on the glucose and KB consumption allow a quantitative evaluation of the participation of the KB in brain metabolism. At rest glucose consumption by brain tissue has been determined to amount approximately 80% of the total glucose production (4,26). In the absence of KB, in the postprandial state, glucose utilization is maximal and the participation of brain tissue can be estimated at 80% of the total postprandial glucose consumption. If the actual glucose consumption by brain tissue during various degrees of ketosis in GSD III patients is also estimated at 80% of the observed total glucose turnover, the difference between the postprandial and fasting state can be transformed into KB consumption when isocaloric replacement is assumed. In this calculation the oxidation of glucose is equivalent to 15.7 $kJ.g^{-1}$, the oxidation of KB to 19.1 $kJ.g^{-1}$. In Table V the estimated KB consumption of brain tissue is given during the measurements as a fraction of the total KB production. As is clear from this Table for all patients, the two oldest patients included, the degree of utilization averaged at 29±9% . Similar values have been published previously (27). For neonates and infants it was estimated that about 25% of the total KB production was used by brain tissue (29). This degree of KB utilization by brain tissue probably represents a maximum. In

view of the severe shortage of metabolic fuels in the young infants no significant increase in estimated utilization of KB was observed. Thus in the GSD III patients tested KB replace glucose as an alternative metabolic fuel for brain tissue only to a limited extent. The degree of replacement was estimated to be similar to that observed under normal physiological conditions after a short period of fasting (27,29). Therefore, no specific adaptations could be discerned in the consumption of metabolic fuels to cope with the glucose deficit.

Accordingly, we speculate that normal physiological mechanisms account for the age-related increase in fasting tolerance in GSD III patients. Most likely the change in body composition brings about this age-related effect. Although liver to body weight and brain to body weight ratios both decrease with age, the decrease of the two ratios is quantitatively different (4,13,15,22). This results in an increase in liver to brain weight ratio with increasing age. The effect of this is shown in Fig. 2 in which the normal total glucose consumption as estimated on a brain weight basis in $mg.min^{-1}$ postprandially is shown together with the observed total glucose production. The total glucose consumption increases less with age. This diminished increase of total glucose consumption is then gradually met by the increased total glucose production due to an increase in liver weight. Although the glucose production expressed in $mg.kgBW^{-1}.min^{-1}$ decreases with age, the total production of glucose in $mg.min^{-1}$ increases from birth to adulthood.

In conclusion, the age-related increase in fasting tolerance in patients with GSD III results from the age-related increase of the liver to brain weight ratio. In adult patients the defective glucose production of the liver can meet the normal glucose consumption of the brain. No adaptive mechanisms are operative. KB can serve as an alternative fuel in addition to glucose only to an extent as observed under physiological conditions.

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TABLE I.

Patients	sex	age (y)	BW (kg)	BrW (kg)	BMR measured ($kJ.kg^{-1}.h^{-1}$)	BMR predicted(* ($kJ.kg^{-1}.h^{-1}$)
1.	♀	1.0	8.6	0.572	9.7±0.2	9.5
2.	♀	6.4	23.5	1.040	7.9±0.1	7.3
3.	♂	6.5	19.0	1.100	9.7±0.1	8.3
4.	♂	6.6	21.7	1.166	9.7±0.2	7.9
5.	♂	8.8	25.5	1.179	6.5±0.2	7.3
6.	♀	14.3	47.7	1.232	4.9±0.2	5.1
7.	♀	15.7	50.4	1.265	4.9±0.2	5.0
8.	♂	26.3	66.2	1.370	4.5±0.2	4.7
9.	♂	28.5	64.0	1.471	4.6±0.2	4.8

Patient population: physical parameters concerning the patients studied, together with the measured and predicted BMR values (* (18).

BrW : Brainweight calculated according to (15).

BMR : Basal metabolic rate.

TABLE II.

Patients	blood glucose concentration (mM)	rate of glucose infusion ($mg.kg^{-1}.min^{-1}$)	rate of appearance of glucose ($mg.kg^{-1}.min^{-1}$)		
			measured		predicted(*
			total	endogenous	
1.	2.5±0.5	1.8	6.7±0.6	4.9±0.6	6.1
2.	2.8±0.2	1.4	4.3±0.3	2.9±0.3	4.4
3.	3.0±0.1	2.0	5.8±0.3	3.8±0.3	5.2
4.	2.6±0.1	0.9	5.2±0.2	4.3±0.2	3.8
5.	2.9±0.2	1.3	3.6±0.3	2.3±0.3	2.9
6.	2.4±0.1	0.5	2.3±0.3	1.8±0.3	3.0
7.	2.3±0.2	0.5	2.2±0.1	1.7±0.1	2.5
8.	3.5±0.3	0.0	1.7±0.2	1.7±0.2	1.7
9.	4.1±0.2	0.0	2.2±0.3	2.2±0.3	2.4

Mean±SD for blood glucose concentrations, rates of glucose infusion, and observed and predicted (* rates of glucose appearance. The predicted rates of glucose appearance were corrected for the individual degree of ketosis as described (13) according to (IV).

TABLE III.

Patients	ketone body concentration	palmitate concentration	rate of appearance of palmitate measured	(* predicted
	(mM)	(mM)	($mg.kg^{-1}.min^{-1}$)	
1.	3.5±0.5	0.39±0.03	(**	1.6±0.5
2.	2.8±0.5	0.41±0.09	0.97±0.27	1.6±0.5
3.	3.7±0.4	0.41±0.05	1.77±0.21	1.6±0.5
4.	5.7±0.5	0.16±0.01	2.00±0.08	1.0±0.5
5.	6.5±1.0	0.29±0.09	0.98±0.34	1.3±0.5
6.	1.1±0.2	0.26±0.02	1.02±0.20	1.2±0.5
7.	2.6±0.5	0.34±0.03	0.69±0.07	1.5±0.5
8.	4.4±0.3	0.33±0.03	1.06±0.05	1.4±0.5
9.	2.1±0.5	0.22±0.03	0.51±0.03	1.1±0.5

(** no isotopic equilibration was obtained.

Ketone body and palmitate concentrations in blood in GSD III patients (mean±SD), together with the observed and the predicted (* rates of appearance of palmitate (mean±SD). The predicted values were calculated as described by Bougneres et al (29) according to (V).

TABLE IV.

Patient	estimated rate of KB production ($mg.kgBW^{-1}.min^{-1}$)	fraction of FFA flux that served for ketogenesis	fraction of FFA oxidized
1.	1.79	(*	(**
2.	1.50	0.31	0.32
3.	1.89	0.22	(**
4.	2.63	0.27	0.36
5.	2.91	0.61	0.24
6.	0.85	0.17	0.25
7.	1.41	0.42	0.45
8.	2.16	0.42	0.47
9.	1.22	0.24	(**

(* (** no data available.

The rates of ketone body production were calculated from the ketone body concentration in blood as described by Bougneres et al (29). The fractions of FFA flux that served for ketogenesis were calculated from the palmitate turnover rates and the estimated ketone body production rates as described by Bougneres et al (29). The fraction of FFA that was oxidized was calculated from the respiratory quotients and the FFA turnover rates.

TABLE V.

estimated KB consumption
by the brain as a fraction
of the total KB production

Patients	
1.	0.30
2.	0.31
3.	0.25
4.	0.23
5.	0.36
6.	0.46
7.	0.29
8.	0.18
9.	0.22

The estimated ketone body consumption by the brain as a fraction of total ketone body production was calculated from the differences between estimated postprandial and actual brain glucose consumption, as expressed in ketone bodies and the total ketone body production.

FIG 1. Blood glucose, ketone bodies, palmitate concentration, and mole percent excess of [6,6-$^{2}H_2$]-glucose and [1-^{13}C]-palmitate in patient #7 with glycogen storage disease type III during tracer equilibration and steady-state.
* Primed dose infusion of tracer. ** Constant rate infusion of tracer. MPE : Mole percent excess of tracer in plasma.

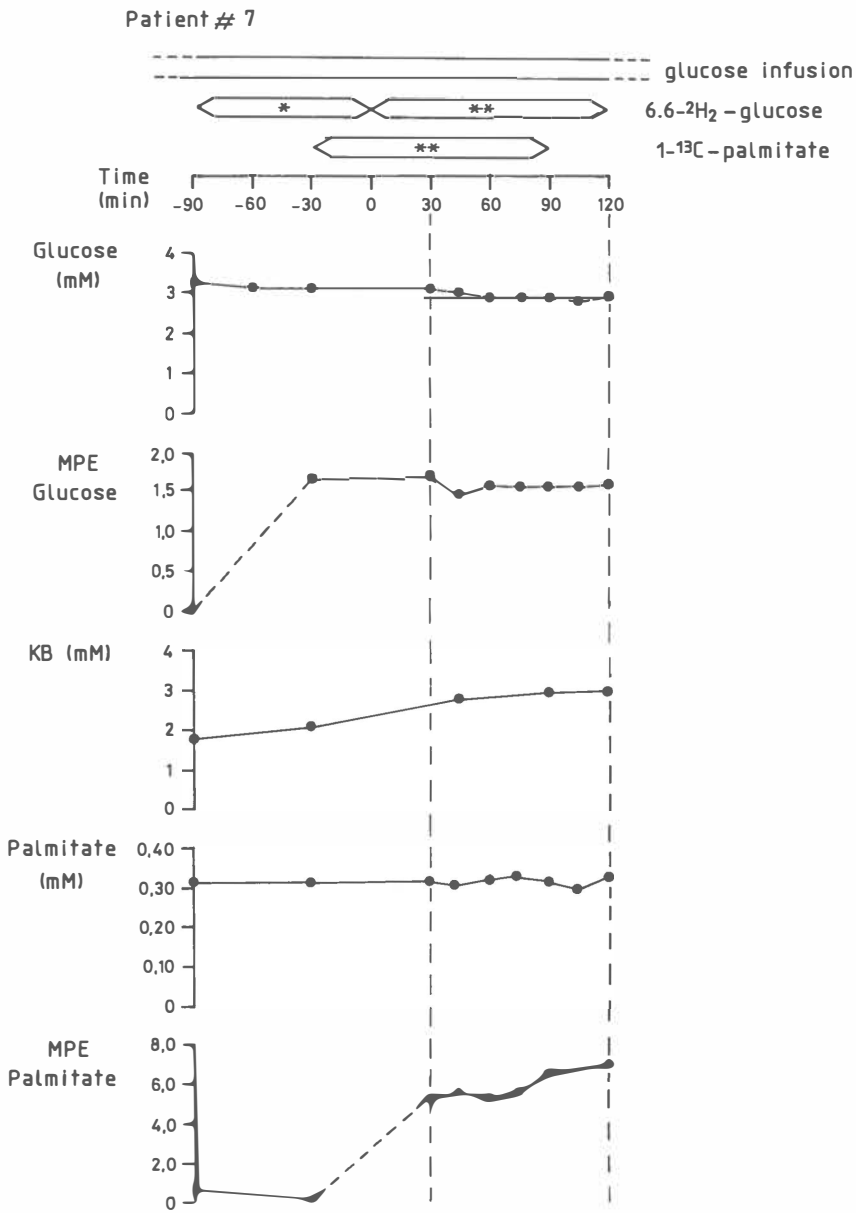
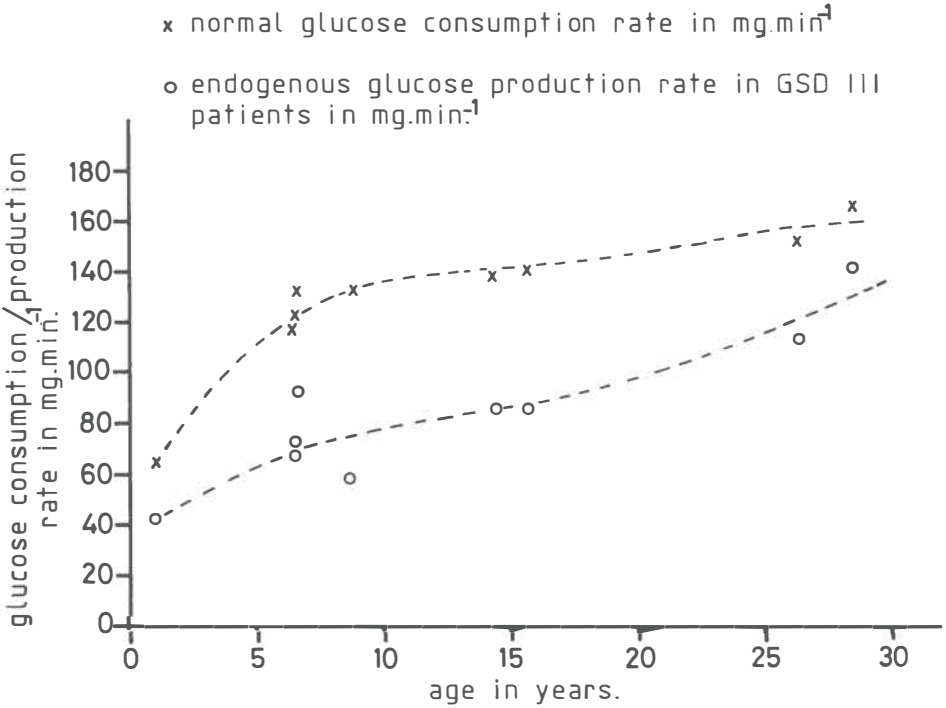


FIG 2. Estimated total body glucose consumption rates compared to observed total glucose production rates in nine patients with glycogen storage disease type III (see text).



APPENDIX

GLYCOGEN STORAGE DISEASE: RECOMMENDATIONS FOR TREATMENT.

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ABSTRACT.

A workshop was held on "Aspects of treatment of patients with glycogen storage disease" within the framework of the Concerted Action "Inborn Errors of Metabolism" of the European Community. Consensus was reached on the main issues of treatment of patients with deficiency of glucose-6-phosphatase, glucose-6-phosphate translocase, debranching enzyme, liver phosphorylase and phosphorylase-b-kinase. The resulting recommendations are reported.

KEYWORDS.

glycogen storage disease

glucose-6-phosphatase

glucose-6-phosphate translocase

debranching enzyme

phosphorylase

phosphorylase-b-kinase.

INTRODUCTION.

At the occasion of the annual meeting of the European Society for Pediatric Research a workshop was held on "Aspects of treatment of patients with glycogen storage disease" in Groningen, The Netherlands, on September 10th 1986, within the framework of the Concerted Action "Inborn Errors of Metabolism" of the European Community. Specialists from several European Countries and Israel presented their work and views on the treatment of patients with glycogen storage disease due to deficiency of glucose-6-phosphatase, glucose-6-phosphate translocase and the debranching enzyme system. Consensus on the main issues of treatment was reached and the resulting recommendations reported. A note about the management of liver phosphorylase and phosphorylase-b-kinase deficiency has been added.

Glucose-6-phosphatase deficiency (GSD-IA).

The aim in treating patients with GSD-IA is to correct hypoglycemia and hyperlactacidemia, achieve normal growth and prevent nephropathy. It is recognized that the metabolic abnormalities such as hyperlactacidemia, hyperlipidemia and hyperuricemia cannot be completely normalized (1).

Infants.

Glucose or appropriate alternatives (see below) should be administered at 2-3 hours intervals around the clock giving the equivalent of 8-9 $mg.kg^{-1}.min^{-1}$, a rate which meets the glucose requirement at that age (2). Some specialists recommend that lactose and sucrose should be restricted as galactose and fructose exacerbate the hyperlactacidemia (3), but not all agree on this. Two strategies can be used for the treatment, either the oral administration of glucose polymers that are slowly digested, or gastric drip feeds. They can be used in combination. Some specialists prefer to use gastric drip feeds from the time of the diagnosis, whilst others prefer to postpone this until the child is 1-2 years of age. If gastric drip feeds are not being used, regular feeds are given at 2-3 hours intervals around the clock. Precooked starches such as rice and corn flour may be added and gradually increased to 6% in order to prolong gastric emptying time. The meal frequency can often be decreased to 3-hourly intervals during the day and 4-hourly intervals at night at the age of 6-12 months. However, if there is recurrent hypoglycemia, gastric drip feeding at night should be used, but it should be realized that not all parents are able to cope with the technical and emotional stresses of infusion pumps and tube feeding. Furthermore, nocturnal gastric drip feeding may render the patient more sensitive to hypoglycemia as a result of the failure of the glucose supply because of an accident with the pump or the tube (4).

Toddlers and older children.
The amount of glucose and its polymers given should be gradually decreased to 5-7 $mg.kg^{-1}.min^{-1}$ during the day and to 5 $mg.kg^{-1}.min^{-1}$ at night as the glucose requirements of the child are known to decrease with age (2). It is important to adjust the rate of glucose administration accurately as excessive glucose administration makes the patient vulnerable to hypoglycemia. On the other hand insufficient glucose administration may lead to hyperlactacidaemia and growth retardation (1). A moderately increased lactate concentration (up to 6.0 mM) is considered to be desirable, since lactate is a useful alternative substrate, especially for the brain, at low glucose concentrations (5). The glucose requirement of the patient can be determined as follows:

a) The glucose-lactate profile of the blood during 24 hours. The glucose concentration should not be less than 2.2 mM (40 $mg.dl^{-1}$), and the lactate concentration should be kept between 4.0 and 6.0 mM (36-54 $mg.dl^{-1}$).
b) The lactate concentration in 12-hour urine or the lactate-creatinine ratio of two urine specimens per day, spaced approximately 12 hours apart and taken on 5-10 successive days, probably gives a better assessment of lactate production at home than a 24-hour blood profile in the hospital. The urinary lactate concentration should be kept between 0.2 and 1.0 mM, the urinary lactate-creatinine ratio (mM:mM) between 0.06 and 0.20 (6).

Gastric drip feeding is usually given for about 12 hours each night to promote or maintain normal growth and to prevent nocturnal hypoglycemia. The energy intake from gastric drip feeding should be approximately 35% of the total 24 hour intake. A complete milk formula lactose-restricted and without sucrose, containing the required amount of dextrin-maltose, is preferable for young children, but for older children a solution of a glucose polymer in water is usually adequate (7).
Uncooked cornstarch should be introduced during childhood, but below the age of 2 years the children are nor able to digest it adequately (8). Slow-releasing carbohydrates like uncooked cornstarch, rice starch and barley groats (lente carbohydrates), partially cooked couscous and macaroni (semi-lente carbohydrates) can be very effective at prolonging the period of normoglycemia between the meals (9). If there is a good glycemic response they should be included in the diet during the daytime, but not at night because the rate at which glucose is supplied does not meet the requirement of young children throughout the night. Uncooked starch may be administered during or after breakfast or lunch. The dose necessary is up to 2 $g.kg^{-1}$ per meal for young children, 1.5 $g.kg^{-1}$ for older children (larger doses are difficult to consume). The starch can be mixed with water, milk, yoghurt

or curd. In some children good metabolic control and growth can be achieved by administering the uncooked cornstarch 4 times a day (3 times after the meals and once after midnight).
Adolescent and adults require 3-4 $mg.kg^{-1}.min^{-1}$ during basal conditions at night (2). This can be adequately covered by a starch meal at bedtime (≈1.5 $g.kg^{-1}$), which may replace gastric drip feeding as soon as pubertal growth spurt tapers off. No other special dietary measures are indicated. The protein content of the diet is kept at 10-15% of energy, the fat content at 25%, the carbohydrate content being usually not less than 60%. The inclusion of polyunsaturated fat in excess of the linoleic acid requirement does not influence the hyperlipidemia (10).
Prevention of complications. Gout and urate kidney stones can be prevented by appropriate medication with allopurinol which should be started whenever there is persistent hyperuricemia (>7 $mg.dl^{-1}$ or 400 uM). Additional measures are helpful including the administration of sodium bicarbonate to reduce urinary acidity, and a high water intake. Adenomata of the liver, occurring particularly in patients who have not been treated adequately, are difficult to treat. Regression of the size of the adenomata has been reported after intensive dietary treatment (11). Malignant transformation has also been reported (12).

Glucose-6-phosphate translocase deficiency (GSD-IB).

The dietary treatment of GSD-IB patients is similar to that of GSD-IA patients as the metabolic abnormalities are similar. The additional immune disturbance is characterized by neutropenia and susceptibility to bacterial infections. The neutrophil abnormality is caused by reduced flux in both the hexose-monophosphate shunt and anaerobic glycolysis (13,14). Treatment with lithium has been tried to stimulate the production of neutrophils, but this treatment is still experimental and, if instituted, should be reserved for severely neutropenic patients. Particular care should be taken with dosage and control of blood lithium levels as patients may be more prone to side effects.
Treatment of bacterial infections with antibiotics according to the sensitivity of the pathogens is essential, preferably with antibiotics which penetrate into the interior of phagocytes (co-trimoxazole, clindamycin, rifampicin). Prophylaxis with antibiotics, most commonly co-trimoxazole, is often necessary. Initial reports on the portocaval shunt suggested that it improved growth and corrected metabolic abnormalities. A lasting favorable effect of portocaval shunt on leucocyte function has been reported in a single case of GSD-IB (15). It was, however, not observed or only temporarily in other cases (16). Therefore, there is at present no indication present for performing a portocaval shunt in any patient.

Debranching enzyme deficiency (GSD-III).

The dietary treatment of GSD-III patients is generally similar to that of GSD-I patients, but as the tendency to hypoglycemia is less marked, the treatment is usually less demanding. Although glycogenolysis is reduced, gluconeogenesis is normal or even increased. Carbohydrates should be given frequently around the clock. The indications for gastric drip feeding and starch are similar to those of GSD-I patients. No restrictions of lactose and sucrose are necessary as galactose and fructose can be converted normally into glucose. Protein should be administered in liberal amounts, because some amino acids serve as substrates for gluconeogenesis (17). It has been emphasized that protein is an important adjunct for treating the myopathic form of GSD-III (18). Fat intake is usually reduced for two reasons. Long chain fat may be less efficiently oxidized at a young age, since there is often hypoketonemia at that age (19), and the high carbohydrate and protein intake entails a reciprocal reduction of fat intake. MCT in moderate amounts can be included in the diet for their ketogenic effect, which may provide supplementary fuel for the brain (20). The composition of the diet for young patients with GSD-III should be approximately: carbohydrate 50-55%, protein 25%, fat (MCT included) 20-25% of total energy intake.

Prevention of complications.

The infantile form of myopathy can be prevented or improved by gastric drip feedings (containing protein) at night and a high protein intake (18). The effect of this treatment on the late adult form of myopathy is less well established (21). Prevention and treatment of cardiomyopathy is not possible at present.

Phosphorylase and phosphorylase kinase deficiency (GSD-VI and GSD-VIII).

Patients with a deficiency of either liver phosphorylase or phosphorylase-b-kinase are very similar to GSD-I and GSD-III patients with respect to clinical features (hepatomegaly, muscle hypotonia at young age), but very different as regards their mild metabolic abnormalities. Hypoglycemia is mild and does not require any treatment except for the prevention of prolonged fasting during infections with vomiting or anorexia. The mild hypercholesterolemia can be corrected by a diet, enriched with polyunsaturated fat (10).

Future developments for treatment.

Ready-to-eat starches.
Pilot studies with kitchen-prepared meals, in which starch, uncooked or briefly heated, is a main nutrient, have shown that this type of food can be used in meals. There is an urgent need for ready-to-eat, palatable starch meals in the semi-lente range which prolong normoglycemia. Such products should contain few additional carbohydrates and it is hoped that the industry will develop such products.

Portable pumps.
Portable pumps for gastric drip feeding usually do not have a sufficiently refined alarm system to enable them to be used safely in very glucose-dependent GSD-I patients. Infusion pumps for intravenous use, on the other hand, are adequately equipped with an alarm system, but difficult to transport. It is hoped that cheap portable pumps are developed that will combine the advantage of both.

Lactate "stick".
A stick for the estimation of the lactate level in the blood and urine, similar to the glucose stick which indicates the appropriate glucose concentration in blood and urine for the patient with diabetes mellitus, would be useful. This would provide the GSD-I patient with information about the metabolic control.

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GENERAL DISCUSSION AND CONCLUSIONS.

Glycogen storage disease type I and III (GSD I and GSD III) have many clinical similarities and are sometimes difficult to distinguish from each other. In young patients, they both manifest episodes of hypoglycemia, hepatomegaly and growth failure. The clinical findings in GSD I are usually more severe than those found in GSD III. Biochemically, there are similarities and differences between the two conditions. The latter are the consequence of the different localization of the deficient enzyme in each disease. In GSD I, there is a deficiency of the enzyme glucose-6-phosphatase, which catalyzes the conversion of glucose-6-phosphate to glucose. This enzyme is normally present in liver, kidney, and intestinal mucosa and is deficient in these tissues in GSD I. Glucose-6-phosphate, which cannot be converted into glucose is consequently metabolized along the glycolytic pathway leading to increased synthesis of pyruvate and hence lactate, alanine, acetyl-CoA and triglycerides.

In GSD III, the deficiency of amylo-1,6-glucosidase (debranching enzyme) limits glycogen breakdown, resulting in an increased accumulation of limit dextrin in various tissues. Once the outer branches of the glycogen molecule have been removed during fasting, glucose production from glycogen is blocked. This leads to hypoglycemia unless gluconeogenesis is switched on, or production of alternative energy substrate for instance ketogenesis is stepped up.

The aim of treatment of glycogen storage disease type I and III is to maintain the concentration of metabolic fuels in blood, primarily glucose, within certain limits in order to maintain normoglycemia and to suppress secondary metabolic derangements.

The introduction of nocturnal gastric drip feeding (GDF) in 1974 by Burr, although initially intended to maintain normal blood glucose concentrations, resulted in considerable improvement of both clinical and biochemical parameters in virtually every patient. Perhaps the most striking improvement in both glucose-6-phosphatase and debranching enzyme deficiency has been the improvement in linear growth. Although the exact mechanism which causes this dramatic improvement is yet poorly understood, the elimination of on-off effects of intermediary metabolism during the night may be an important beneficial factor, because nocturnal GDF keeps the blood glucose concentration in the normal range without undue fluctuations. This is supported by Yudkoff et al, who were able to demonstrate increased whole body protein synthesis during nasogastric feedings using [^{15}N]-glycine.

Probably this study solves the old controversy as regards the choice of the composition of the nasogastric feeding in favor of a complete formula feeding instead of a solution containing only glucose.

In patients with glycogenosis type III the endogenous glucose production, as measured by stable isotope dilution, is insufficient until adulthood when it meets the total body demand for glucose. At earlier age this shortage in glucose production should be compensated for by increased fatty acid oxidation and ketone body formation. However, in the very

young patients this compensatory mechanism is still limited, although the turnover of fatty acids, as measured by stable isotope dilution, was within normal limits. Probably the cytosolic activation and/or the transport of fatty acids into the mitochondria is limited to meet the high energetic requirement, as ketogenesis in these young patients may be increased by the administration of medium chain triglycerides.
Indeed, the application of stabile isotopes in the study of intermediary metabolism has enhanced the possibilities to gain more insight into the origin and consequence of metabolic derangements with only minimal risk and discomfort of the patient. Extended knowledge of these derangements is of major importance as it might change the current concepts of treatment. A strong support of this allegation is the significance of the lactate concentration in blood and urine in GSD I as discussed in this thesis (chapter IV and V).

As regards the aims of this thesis stated in chapter I concerning GSD I and GSD III the following conclusions may be drawn:

1. Some starches can be used as lente carbohydrates. Cornstarch and barley groats in the uncooked form released glucose slowly to achieve normoglycemia for approximately eight hours. This enables their use as a substitute for nocturnal gastric drip feeding, especially in older children (chapter II,III and Appendix).

2. Other starches can be used as semi-lente carbohydrates. The glucose release from couscous, in water or incorporated in a meal, and partially cooked macaroni given as a meal, proceeds between the lente uncooked starches and rapidly digestible carbohydrates " (chapter II,III and Appendix).

3. Lactate appeared to be taken up significantly by brain tissue of patients with GSD I during low-glucose intake. This would be an argument against excessive glucose administration which suppresses lactate production, and pro a regime that keeps the lactate concentration in the blood moderately elevated (chapter IV). For this monitoring of the lactate concentration in the urine turned out to be a more reliable parameter than the lactate concentration in the blood as the latter may fluctuate considerably (chapter V). We advocate lactate-/creatinine ratio's in urine between 0.06-0.20, and blood lactate levels of 4.0-6.0 mM, in order to reduce the risk of sudden substrate deficiency and to ensure normal growth (chapter V and Appendix).

4. Hypoketosis, and as a result, hypoglycemia in young patients with GSD III (0-5 years of age) is due to insufficient activation in the cytosol and/or transmitochondrial transport of long-chain fatty acids to allow adequately high rates of fatty acid oxidation and ketogenesis in the mitochondria (chapter VI).

5. The tendency for "hypoketotic hypoglycemia" in young patients with GSD III (0-5 years of age) can be amended by administration of medium chain triglycerides (chapter VI). These triglycerides stimulate ketogenesis.

6. Patients with GSD III have normal rates of basal metabolism, glucose turnover, and fatty acid turnover (chapter VII). The endogenous glucose production rate however is insufficient, but gradually normalizes at adulthood when it meets the total body demand for glucose (chapter VII). No specific adaptations could be detected in the consumption of metabolic fuels to cope with the deficit in glucose production. The determinating factor for this normalization is most likely the age-related decrease in the glucose consumption rate. This can be deduced from the age- related decrease of the brain to liver ratio, the brain being the main glucose consumer. The deficit in endogenous glucose production before adulthood is compensated for by ketogenesis. This compensatory mechanism is still limited in the very young patients, and this explains their tendency for impending hypoglycemia.

SUMMARY.

In this thesis clinical and biochemical studies concerning parameters of energy metabolism and their implications for treatment in glycogen storage disease type I (glucose-6-phosphatase deficiency EC 3.1.3.9) and type III (debranching enzyme deficiency EC 2.4.1.25 / 3.2.1.33) are described.

In chapter I the aims of the thesis are outlined, preceded by a historical review on clinical and biochemical aspects of the two types of glycogen storage disease. Furthermore synthesis, structure and degradation of the glycogen molecule are described, because the following chapters present biochemical parameters and their clinical impact both primarily based on this knowledge.

In chapter II the application of uncooked cornstarch in the dietary treatment of glycogen storage disease is presented. The normoglycemic period after ingestion of this complex carbohydrate ranged from 6.5-9.0 hours. This enables us to use this starch as a substitution for nocturnal gastric drip feeding in older patients.

In chapter III the use of two categories of complex carbohydrates is described. The first, defined as "lente carbohydrates" were characterized by a long postprandial normoglycemic period like that of uncooked cornstarch.
The second, defined as "semi-lente carbohydrates" were characterized by a shorter postprandial normoglycemic period between that of uncooked cornstarch and that of rapidly digestible carbohydrates like glucose. The method of administration of all lente or semi-lente carbohydrates, either suspended in water or mixed in a curd-yoghurt-cheese meal did not appreciably affect the duration of the postprandial normoglycemic period. The application of these carbohydrates allows the reduction of the meal frequency during the day in the dietary treatment of glycogen storage disease.
From the knowledge about carbohydrates with protracted digestibility applied to GSD I patients, patients with other diseases in which the prevention of hypo- and hyperglycemia is the major goal of treatment may benefit as well.

In chapter IV the importance of lactate as a substrate for brain energy metabolism next to glucose in four patients with glycogen storage disease type I is demonstrated. The practical consequence of this finding is not to lower the blood lactate concentration to the normal range, but to adjust the diet in such a way as to keep the lactate concentration in blood moderately elevated (4.0-6.0 mM).

In chapter V the use of the lactate concentration in urine as a parameter for the adequacy of the dietary treatment in patients with glycogen storage disease type I is advocated. The blood lactate concentration turned out to be less reliable as it fluctuates strongly. Urinary lactate concentrations in GSD I patients are advocated between 0.2 and 1.0 mM, urinary lactate/creatinine ratios (mM/mM) between 0.06 and 0.20.

In chapter VI stimulation of ketogenesis by medium chain triglycerides is demonstrated in young patients with glycogen storage disease type III. These patients are susceptible to hypoglycemia and exhibit relatively low ketone body concentrations because of limited ketogenesis from oxidation of long chain fatty acids.

In chapter VII the age-related tendency for hypoglycemia in patients with glycogen storage disease type III was studied by stable isotope dilution, using [6,6-$^{2}H_{2}$]-glucose and [1-^{13}C]- palmitate as tracers. The rate of: 1. glucose turnover and endogenous glucose production, and 2. fatty acid turnover, and 3. basal metabolism was determined simultaneously.
The endogenous glucose production rate was insufficient, except in the oldest patients. The glucose turnover rate, the fatty acid turnover rate and the basal metabolic rate were all within normal limits, indicating a normal demand for energetic substrates. The insufficient endogenous glucose production rate is age-dependent and gradually normalizes at adulthood when it meets the total body demand for glucose. No specific adaptations could be detected in the consumption of metabolic fuels to cope with the deficit in glucose production. The determinating factor for this normalization is most likely the age-related increase in the liver to brain weight ratio. The liver being the main producer, the brain the main consumer of glucose and ketone bodies in the fasting and resting state. The deficit in endogenous glucose production before adulthood is compensated for by ketogenesis. This compensatory mechanism is still limited in the very young patients, and explains their tendency for hypoglycemia upon fasting.

In the Appendix recommendations for treatment of glycogen storage disease are reported as were agreed upon during a workshop held within the framework of the Concerted Action "Inborn errors of metabolism" of the European Community at the 1986 meeting of the European Society for Pediatric Research in Groningen.

SAMENVATTING.

In dit proefschrift worden de resultaten beschreven van klinisch en biochemisch onderzoek naar parameters betreffende de energie stofwisseling bij patienten met glycogeenstapelingsziekte type I (glucose-6-fosfatase deficientie EC 3.13.9) en type III (debranching enzyme deficientie EC 2.4.1.25/ 3.2.1.33), en de daarvan afgeleide therapeutische consequenties.
Door de vele klinische overeenkomsten tussen glycogeenstapelingsziekte type I en III (GSD I en GSD III) zijn deze aandoeningen op het eerste gezicht vaak moeilijk van elkaar te onderscheiden. Op jonge leeftijd staat bij beide ziekten de neiging tot hypoglycaemieën, de hepatomegalie, en de groeiachterstand op de voorgrond. Veelal zijn de klinische symptomen bij GSD I echter ernstiger dan bij GSD III. Ook bij biochemisch onderzoek bestaan er naast overeenkomsten verschillen tussen de twee aandoeningen. Een en ander is het gevolg van de specifieke lokalisatie van het deficiente enzym in de koolhydraatstofwisseling.
Bij GSD I is door deficientie van het enzym glucose-6-fosfatase de omzetting van glucose-6-fosfaat in glucose gestoord. Hierdoor is tijdens vasten niet alleen de vorming van glucose uit glycogeen, maar ook door middel van gluconeogenese gestoord, met als gevolg een snelle daling van de glucoseconcentratie. Het enzym glucose-6-fosfatase ontbreekt bij deze patienten in de lever, de nieren, en de mucosa van de dunne darm, hetgeen tot stapeling van glycogeen leidt in voornamelijk lever en nieren. Het tijdens vasten in overmaat gevormde glucose-6-fosfaat geeft aanleiding tot verhoogde productie van pyruvaat, en als gevolg daarvan lactaat, acetyl-CoA, en triglyceriden.
Bij GSD III is door deficientie van het debranching enzyme de afbraak van het glycogeenmolekuul slechts mogelijk tot de buitenste vertakkingen. Dit leidt tot stapeling van het zogenaamde limit dextrin in verschillende weefsels ondermeer in lever en spieren. Wanneer tijdens vasten de buitenste takken van het glycogeenmolekuul zijn omgezet in glucose, is verdere afbraak van het glycogeenmolekuul onmogelijk geworden. Dit leidt tot lage glucoseconcentratie, tenzij glucose wordt gevormd door gluconeogenese en/of door de vorming van ketonlichamen alternatief substraat voor de energiehuishouding wordt aangeboden.
De behandeling van patienten met glycogeenstapelingsziekte I en III is gericht op de handhaving van normale concentraties van metabole brandstoffen in bloed, met name de concentratie van glucose. Hierdoor is het mogelijk de door lage glucoseconcentraties ontstane ongewenste secundaire metabole veranderingen te beperken. De introductie van de continue sondevoeding gedurende de nacht door Burr et al in 1974, hoewel oorspronkelijk uitsluitend bedoeld om de glucoseconcentratie binnen normale grenzen te handhaven, resulteerde bij de meerderheid van de behandelde patienten na enige tijd in een sterke verbetering van zowel klinische als biochemische parameters. De meest opvallende verbetering was zowel bij GSD I als GSD III patienten het optreden van

inhaalgroei in lichaamslengte. Hoewel de verklaring voor deze dramatische verbetering nog niet duidelijk voorhanden is, lijkt het voorkomen van de plotselinge overgang van te veel naar te weinig glucose, na en voor de volgende maaltijd een belangrijke factor. Dit wordt ondersteund door het onderzoek van Yudkoff et al, waarbij door gebruik te maken van [^{15}N]-glycine een toename van de eiwitsynthese tijdens continue sondevoeding bij deze patienten werd vastgesteld. Bovendien lijkt als gevolg van deze waarneming het twistpunt rond de samenstelling van de sondevoeding, glucose alleen of een complete voeding met inbegrip van eiwitten, in het voordeel van de laatste samenstelling beslist.
De vervanging van de nachtelijke sondevoeding op latere leeftijd, bij voorkeur na de groeispurt in de puberteit, is mogelijk geworden door gebruikmaking van complexe koolhydraten. Deze koolhydraten worden 's avonds in ongekookte vorm toegediend. Door de vertraagde afgifte van glucose blijft de glucoseconcentratie binnen normale grenzen gehandhaafd gedurende de nacht. Daarbij wordt de verbetering van klinische en biochemische parameters zoals bereikt door de behandeling met continue sondevoeding nagestreefd. De hoge frequentie van maaltijden overdag kan worden verminderd door toepassing van gedeeltelijk gehydrolyseerde (gekookte) koolhydraten. Het onderzoek naar voor dit doel bruikbare complexe koolhydraten is zeer recent begonnen. Het ziet er echter naar uit dat het toepassingsgebied zich niet zal beperken tot de glycogeenstapelingsziekten alleen, maar zal uitbreiden tot alle ziektebeelden waarbij de behandeling bestaat uit het voorkomen van te hoge, dan wel te lage glucoseconcentratie in bloed.
Met behulp van stabiel isotoop gemerkt glucose werd bij patienten met GSD III de endogene glucose productie vastgesteld.
Deze productie blijkt op jonge leeftijd onvoldoende te zijn. Jongere patienten compenseren dit tekort aan glucosevorming door een toename van de synthese van ketonlichamen uit vetafbraak, waardoor het optreden van hypoglycaemieën meestal wordt voorkomen. Op zeer jonge leeftijd is dit compensatiemechanisme echter nog onvoldoende ontwikkeld, maar kan worden gestimuleerd door het geven van vetten met kortere koolstofketens, zogenaamde middenketenvetten.
De toepassing van stabiel gemerkte isotopen voor in vivo onderzoek opent nieuwe mogelijkheden om inzicht te verkrijgen in de pathogenese van metabole afwijkingen en de gevolgen daarvan voor het intermediaire metabolisme. Vergroting van de kennis van de intermediaire stofwisseling kan aanleiding zijn tot aanpassing van de behandelingsnormen. Dit blijkt uit het aanpassen van de behandeling van GSD I na het aantonen van het belang van lactaat als alternatieve brandstof voor de hersenen tijdens lage glucoseconcentratie.

In Hoofdstuk I van het proefschrift worden de vraagstellingen van dit onderzoek geformuleerd, voorafgegaan door een historisch overzicht betreffende de klinische en biochemische aspecten van de twee typen glycogeenstapelingsziekte. Tevens worden synthese, structuur, en afbraak van het glycogeenmolekuul toegelicht. Dit is noodzakelijk, omdat in de volgende hoofdstukken biochemische parameters en hun betekenis voor klinische toepassing worden gepresenteerd, welke gebaseerd zijn op deze kennis.

In Hoofdstuk II wordt de toepassing beschreven van ongekookt maiszetmeel in de behandeling van glycogeenstapelingsziekte type I. De periode van normoglycaemie na toediening van dit complexe koolhydraat varieert van 6.5-9.0 uren. Dit zetmeel blijkt daardoor een goede vervanging te zijn voor de behandeling door middel van continue nachtelijke sondevoeding.

In Hoofdstuk III worden twee categorieën complexe koolhydraten beschreven. Een eerste categorie, gedefinieerd als "lente" koolhydraten, wordt gekenmerkt door een periode van postprandiale normoglycaemie overeenkomend met die na toediening van ongekookt maiszetmeel. Een tweede categorie, gedefinieerd als "semi-lente" koolhydraten, wordt gekenmerkt door een kortere periode van normoglycaemie welke ligt tussen die verkregen na ongekookt maiszetmeel en snel verteerbare koolhydraten (bijvoorbeeld glucose). De toedieningsvorm van de lente of de semi-lente koolhydraten, hetzij gegeven als suspensie in water hetzij deel uitmakend van een maaltijd, heeft geen significante invloed op de uiteindelijk bereikte periode van postprandiale normoglycaemie. De toepassing van deze koolhydraten in de behandeling van patienten met glycogeenstapelingsziekten maakt vermindering van de frequentie van de maaltijden overdag mogelijk.

In Hoofdstuk IV wordt het belang van lactaat als alternatieve energiebron voor de hersenen naast glucose aangetoond bij vier patienten met glycogeenstapelingsziekte type I. De klinische consequentie van deze waarneming is, dat de behandeling van dit type glycogeenstapelingsziekte erop gericht moet zijn de lactaatconcentratie in bloed niet te normaliseren, maar te streven naar matig verhoogde waarden (4.0-6.0 mM).

In Hoofdstuk V wordt het belang van de lactaatconcentratie in de urine als maat voor het al dan niet goed ingesteld zijn op de therapie van patienten met een glycogeenstapelingsziekte type I beschreven. De lactaatconcentratie in bloed blijkt daarvoor een veel minder betrouwbare parameter te zijn, omdat zij onderhevig is aan grote fluctuaties. Als maat voor goed ingesteld zijn op de therapie wordt een lactaatconcentratie in de urine tussen 0.2 en 1.0 mM, of een lactaat/creatinine ratio (mM/ mM) tussen 0.06 en 0.20 aanbevolen.

In Hoofdstuk VI wordt het stimulerende effect op de ketogenese door middenketenvetten bij zeer jonge patienten met glycogeenstapelingsziekte type III (0-5 jaar) beschreven. Deze patienten neigen tot lage glucoseconcentratie in bloed en beschikken nog niet over de relatief hoge concentratie ketonlichamen zoals die op oudere leeftijd bij dit ziektebeeld kan worden gevonden. Bij deze patienten is de oxidatie van langketenvetzuren te gering door onvoldoende activering in het cytosol, en/of onvoldoende transmitochondriaal transport. Deze factoren spelen bij de oxidatie van middenketenvet geen of slechts een ondergeschikte rol.

In Hoofdstuk VII wordt het onderzoek beschreven naar de leeftijd-afhankelijke neiging tot hypoglycaemie bij patienten met glycogeenstapelingsziekte type III. Hierbij werd stabiel isotoop gemerkt glucose en palmitaat tegelijkertijd toegediend. Uit de eveneens gemeten waarden voor het basaal metabolisme blijkt dat patienten met een glycogeenstapelingsziekte type III een normale energetische behoefte hebben. De voor glucose turnover en palmitaat turnover, (als maat voor de vetzuur turnover), gevonden waarden zijn eveneens normaal. De waarden voor de endogene glucose productie blijken echter onvoldoende te zijn, maar normaliseren op de volwassen leeftijd. De bepalende factor voor de normalisering op volwassen leeftijd is waarschijnlijk de relatieve toename van het gewicht van de lever als producent ten opzichte van de andere organen, met name de hersenen, als gebruikers van glucose. Als gevolg daarvan wordt de glucose consumptie geleidelijk gedekt door de glucose productie. Het deficit aan endogene glucose productie wordt op jongere leeftijd gecompenseerd door productie van ketonlichamen, behalve bij de zeer jonge patienten (zie Hoofdstuk V en Hoofdstuk VI).

In de Appendix worden aanbevelingen voor de behandeling van glycogeenstapelingsziekten gerapporteerd, vastgesteld op een workshop gehouden tijdens het congres van de European Society for Pediatric Research in 1986 in Groningen.