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INBORN ERRORS OF FRUCTOSE METABOLISM

Georges Van den Berghe
Laboratory of Physiological Chemistry, International Institute of Cellular and Molecular Pathology and University of Louvain Medical School, B-1200 Brussels, Belgium

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INTRODUCTION

Fructose is one of nature's main sweetening agents. It is the free ketose in honey, fruit, and many vegetables and is added to several more foods and

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beverages in the form of the disaccharide sucrose (31). Sorbitol, also widely distributed in fruit and vegetables, is converted into fructose in the liver by sorbitol dehydrogenase. Mammalian fructose metabolism proceeds mainly by a specialized pathway, discovered by Hers (32), in which two inborn errors have been described in humans: essential fructosuria and hereditary fructose intolerance. The deficiency of the gluconeogenic enzyme, fructose 1,6-bisphosphatase, is also usually classified as an inborn error of fructose metabolism. The description of the clinical and laboratory findings, enzyme defect, genetics, pathophysiology, and treatment of these disorders is preceded by an outline of the normal metabolism of fructose. The toxicity of high doses of parenteral fructose in normal subjects, which results from the influence of the ketose on carbohydrate and purine metabolism, is also briefly reviewed. Knowledge of this toxicity is indeed essential for understanding the pathophysiology of hereditary fructose intolerance and of fructose 1,6-bisphosphatase deficiency.

METABOLISM OF FRUCTOSE

Given intravenously (46) or orally (70), fructose is taken up predominantly by the splanchnic bed, and therein mainly by the liver, but also by the kidneys (9) and (to a minor extent) by the small intestine. The predominance of fructose utilization in these organs is explained by their specialized pathway of fructose metabolism.

Enzymes of Fructose Metabolism

The specialized fructose pathway is composed of three enzymes—fructokinase, aldolase type B, and triokinase—that are only found in liver, kidney cortex, and small intestine mucosa. These enzymes convert fructose into intermediates of the glycolytic-gluconeogenic pathway (Figure 1). Fructose 1,6-bisphosphatase is found in many cell types, but its activity is highest in the gluconeogenic tissues, liver, and kidney cortex. The general properties of these enzymes are briefly reviewed. For more detailed information, see Refs. 26, 32, and 73.

Fructokinase (ketohexokinase, EC 2.7.1.3) catalyzes the phosphorylation of fructose to fructose 1-phosphate, using mainly adenosine triphosphate (ATP) but also guanosine triphosphate (GTP) as the phosphoryl donor. Fructokinase has a high affinity for fructose (K_m is ~ 0.5 mM) and a high V_{max} (~ 10 μmol/min per gram of liver at 37°C). Recently, a rat liver fructokinase cDNA was cloned (19). The 1342 bp sequence encodes a protein of 33 kDa, composed of 299 amino acids.

Aldolase (EC 4.1.2.13) B, also called liver aldolase, catalyzes the splitting

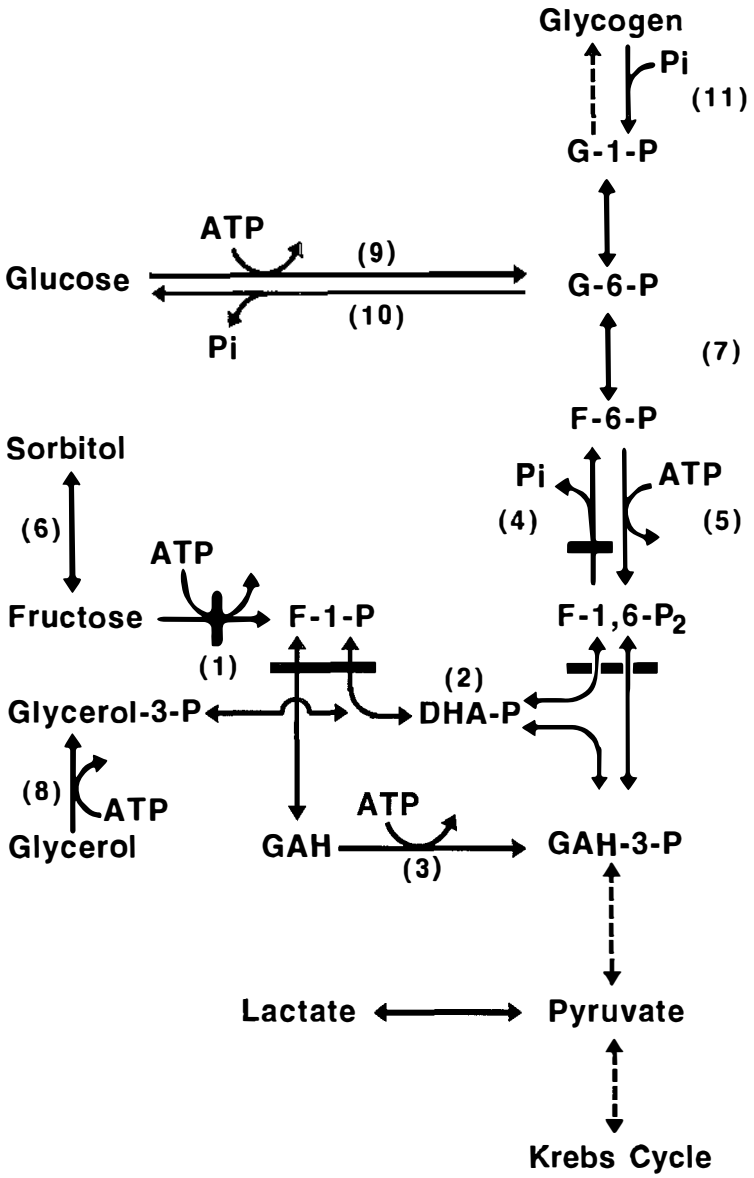

Figure 1 Pathway of fructose metabolism in liver. F, fructose; G, glucose; P, phosphate; Pi, inorganic phosphate; ATP, adenosine triphosphate; DHA, dihydroxyacetone; GAH, glyceraldehyde. (1) Fructokinase; (2) aldolase B; (3) triokinase; (4) fructose 1,6-bisphosphatase; (5) phosphofructokinase; (6) sorbitol dehydrogenase; (7) phosphoglucose isomerase; (8) glycerol kinase; (9) hexokinase and glucokinase; (10) glucose 6-phosphatase; (11) glycogen phosphorylase. The enzyme defects are depicted by bars across the arrows.

of fructose 1-phosphate into dihydroxyacetone phosphate and D-glyceraldehyde as well as the splitting of fructose 1,6-bisphosphate into dihydroxyacetone phosphate and D-glyceraldehyde 3-phosphate. Aldolase B also has a high V_{max} of ~ 10 μmol/min per gram of liver. An almost identical velocity is obtained with fructose 1-phosphate and with fructose 1,6-bisphosphate. In contrast, the muscle isozyme (aldolase A) and the brain isozyme (aldolase C) have a 50- and 10-fold lower V_{max}, respectively, with fructose 1-phosphate than with fructose 1,6-bisphosphate. The aldolases have a molecular weight of 160 kDa and are composed of 4 identical subunits. The complete 364–amino acid sequence of the human aldolase B subunit has been derived from its cDNA (58). The human aldolase B gene has been mapped to chromosome 9 and sequenced (69). It is 14,500 bp long and contains 9 exons.

Triokinase (EC 2.7.1.28) converts D-glyceraldehyde into D-glyceraldehyde 3-phosphate, using ATP as the preferential phosphoryl donor. The reaction allows the nonphosphorylated cleavage product of fructose 1-phosphate to enter the glycolytic-gluconeogenic pathway. The V_{max} of triokinase reaches ~ 1.5 μmol/min per gram of liver and is thus markedly lower than that of fructokinase and aldolase B.

Fructose 1,6-bisphosphatase (EC 3.1.3.11), also known as hexose bisphosphatase, catalyzes the irreversible splitting of fructose 1,6-bisphosphate into fructose 6-phosphate and Pi. In human liver, its V_{max} also reaches ~ 10 μmol/min per gram of tissue. Fructose 1,6-bisphosphatase possesses complex regulatory properties (reviewed in Ref. 67). In the absence of effectors, liver fructose 1,6-bisphosphatase displays hyperbolic kinetics for its substrate, with a K_m in the low micromolar range. It is inhibited competitively by physiological concentrations of its products (fructose 6-phosphate and Pi) and noncompetitively by physiological concentrations of adenosine monophosphate (AMP), which binds to an allosteric site. Inhibition by AMP is potently reinforced by low micromolar concentrations of fructose 2,6-bisphosphate that change the substrate saturation curve from hyperbolic to sigmoidal (reviewed in Ref. 79). Liver fructose 1,6-bisphosphatase is a tetramer with a molecular weight of ~ 140 kDa. The enzyme from pig kidney cortex has been crystalized, and its three-dimensional molecular structure in different conformational states, induced by binding of products and effectors, has been determined by X-ray crystallography (reviewed in Ref. 43). The gene of rat liver fructose 1,6-bisphosphatase has been cloned (21). It is 23 Kb long, contains 7 exons, and encodes a 362–amino acid sequence. The cDNA of the human liver enzyme has also been isolated and expressed in *Escherichia coli* (22). Although only a single fructose 1,6-bisphosphatase gene has been identified in the human genome, observations in fructose 1,6-bisphosphatase deficiency (see below) indicate the presence of at least one other gene.

Alternate Pathways of Fructose Metabolism

As reviewed in Ref. 26, muscle and adipose tissue can use fructose, but at a much lower rate than can liver, kidney, and small intestine. This utilization proceeds by way of hexokinase, which converts the hexose into fructose 6-phosphate. Several alternatives to the hepatic metabolism of fructose described in the previous section have been ruled out (also reviewed in Ref. 26). More recently, it was proposed that only 50% of the fructose 1-phosphate formed from fructose in liver is split by aldolase B and that the remainder is directly converted into fructose 1,6-bisphosphate by a fructose 1-phosphate kinase (27). This theory is based on studies in children who received uniformly labeled [^{13}C]fructose by gastric infusion. Approximately 50% of [^{13}C]glucose found in blood was uniformly labeled, although only C1-C2-C3- and C4-C5-C6-labeled glucose was expected given the cleavage of [^{13}C]fructose 1-phosphate by aldolase and recombination of its cleavage products with unlabeled triose phosphate molecules. Conversion of fructose into glucose via fructose 6-phosphate was ruled out because no uniformly labeled [^{13}C]glucose was formed from uniformly labeled [^{13}C]fructose in patients with fructose 1,6-bisphosphatase deficiency (41). However, no uniformly labeled [^{13}C]glucose was found following intravenous administration of [U–^{13}C]fructose in animals (28). This observation raised the suspicion, proven by studies of the fructose-to-glucose conversion in rabbit small intestine (8), that the results of the oral studies can be attributed to incomplete dilution of [^{13}C]triose phosphates by a smaller pool of unlabeled triose phosphates in the intestinal mucosa as compared with liver.

Recently, a novel fructose ester, fructose 3-phosphate, was identified in the lens and sciatic nerve of diabetic rats (66) and in erythrocytes of normal and diabetic humans, with concentrations tending to be higher in the latter (55). Incubation of human erythrocytes (56) and animal lenses (39) with fructose has shown that these tissues can phosphorylate, probably by means of a fructose 3-phosphate kinase, fructose into fructose 3-phosphate. Due to its potency as a protein-glycosylating and cross-linking agent, it has been hypothesized that fructose 3-phosphate may play a role in the etiology of diabetic complications. However, observations in subjects with essential fructosuria (see below) cast doubt on this hypothesis.

Stimulatory Effect of Fructose on Hepatic Glucose Uptake

The long-standing observation that fructose promotes hepatic glucose uptake and glycogen deposition (47) was recently explained by Van Schaftingen and colleagues (78, 80, 82, reviewed in Ref. 83). The effect of fructose was shown to be mediated by a 60-kDa protein regulator of glucokinase. Rat liver cDNAs

encoding this regulator have been cloned (17). The regulator decreases the affinity of glucokinase for glucose by forming a complex with the enzyme in the presence of physiological (10–40 μM) concentrations of fructose 6-phosphate. Fructose 1-phosphate, already at 10 μM concentration, opposes the formation of the complex and thereby antagonizes inhibition of glucokinase. Intragastric administration of as little as 20 mg/kg of fructose to rats increased their hepatic concentration of fructose 1-phosphate from < 5 nmol/g to ~ 30 nmol/g and doubled the rate of glucose phosphorylation in their liver. Fructose can thus be considered a signal that tells the liver when glucose, with which it is often associated, is absorbed from the gut (81).

TOXICITY OF PARENTERAL FRUCTOSE

The development of parenteral nutrition led to the recognition, in the late 1960s, of the toxicity of intravenous fructose to normal humans. Its main deleterious effects are lactic acidosis, caused by the rapidity of fructolysis as compared with glucolysis, and hyperuricemia, caused by catabolism of purine nucleotides. Both effects are the result of the characteristics of fructose metabolism in fructose-metabolizing tissues and have been most extensively investigated in liver (for reviews and primary references, see Refs. 26, 72 and 73).

Influence of Fructose on the Glycolytic Pathway

Intravenous infusion of fructose in healthy volunteers and patients may elevate blood lactate by two- to fivefold, whereas intravenous glucose does not increase blood lactate more than twofold on average. The hyperlactacidemia induced by fructose results from the latter's more rapid metabolism via the glycolytic pathway, which can be explained by several factors: (*a*) the ~ 10-fold higher V_{max} of fructokinase compared with the maximal glucose-phosphorylating capacity of glucokinase; (*b*) the fact that fructolysis bypasses phosphofructokinase, the principal regulatory enzyme of glycolysis; and (*c*) the stimulation by fructose 1-phosphate of pyruvate kinase, another regulatory enzyme of the glycolytic pathway. In addition, fructose may, under certain conditions (35), increase the concentration of fructose 2,6-bisphosphate, the main physiological stimulator of liver glycolysis (79). The fructose-induced increase in lactic acid may provoke metabolic acidosis, which may prove life-threatening, particularly in liver failure (84).

In recent years, several studies have shown that administration of 5–20 mM fructose to perfused rat livers or to isolated hepatocytes exerts a protective effect against anoxia (2, 3, 25) and a variety of mitochondrial toxins (18, 51), as determined by a decrease in the release of cytosolic liver enzymes and by other parameters of cell damage. This protective effect of fructose is most

likely due to its high rate of use, resulting in the anaerobic generation of ATP by the glycolytic pathway, which compensates to a certain extent the anoxia- and toxin-induced impairment of mitochondrial ATP synthesis. However, this protective effect is accompanied by a high rate of production of lactate, which rules out its clinical application, with the possible exception of liver preservation prior to transplantation.

Influence of Fructose on Purine Catabolism

Infusion of fructose in humans at rates above 1.0–1.5 g/kg per hour usually results in hyperuricemia and hyperuricosuria. These disorders reflect catabolism of the purine nucleotides, which can be elucidated as follows. As discussed above, the V_{max} of both fructokinase and aldolase reaches ~ 10 μmol/min per gram of liver. This rate is several-fold more than the V_{max} of triokinase (1.5 μmol/min per gram) and than that of the fluxes through the glycolytic and gluconeogenic pathways (2 μmol of C-6 units/min per gram). Owing to this difference, fructose 1-phosphate can easily accumulate. However, the formation of fructose 1-phosphate is not only determined by the kinetic characteristics of fructokinase, but is also dependent on the hepatic transport of fructose. This process has a K_m of ~ 100 mM (as opposed to ~ 0.5 mM for fructokinase) and a V_{max} of ~ 30 μmol/min per gram of liver (59). Oral ingestion of fructose, which results in fructose levels of maximally 2.5 mM in the portal vein (34, 70), will therefore not induce a marked accumulation of fructose 1-phosphate. Conversely, intravenous fructose, which results in higher fructosemias (4.8 mM at 1.0 g/kg per hour and 7 mM at 1.5 g/kg per hour) (61), causes a pronounced buildup of hepatic fructose 1-phosphate that can reach millimolar concentrations (10).

Accumulation of millimolar concentrations of fructose 1-phosphate occurs at the expense of ATP and GTP, which are used as phosphate donors, and of inorganic phosphate, which is used to regenerate ATP inside the mitochondria (Figure 2). Both GTP and inorganic phosphate are inhibitors of liver AMP deaminase, the rate-limiting enzyme of the catabolism of the adenine nucleotides in this tissue (76, 77). By depleting ATP and inorganic phosphate, fructose infusion causes a degradation of the hepatic adenine nucleotide pool, which is manifested by hyperuricemia and hyperuricosuria (44). The depletion of liver inorganic phosphate is reflected by a decrease of plasma inorganic phosphate, and that of ATP is reflected by an increase of plasma Mg^{++}, which is due to the potent Mg^{++} chelating effect of ATP. Increased production of uric acid induced by fructose is therefore not a harmless phenomenon but indicates degradation of ATP, the main energy currency of the cell. This decrease in ATP leads to a series of disturbances, including inhibition of the synthesis of RNA and protein, disaggregation of ribosomes, and ultrastructural lesions (reviewed in Refs. 26, 72, and 73). Fructose is thus a potentially dangerous

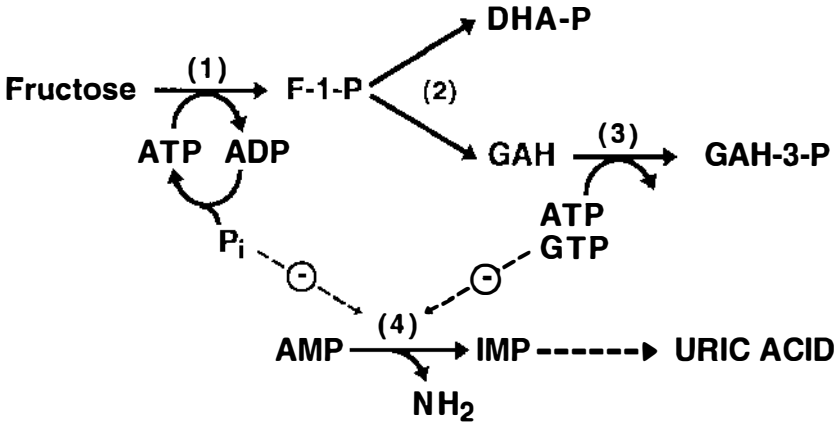

Figure 2 Mechanism of fructose-induced hyperuricemia. F, fructose; P, phosphate; Pi, inorganic phosphate; ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; GTP, guanosine triphosphate; IMP, inosine monophosphate; DHA, dihydroxyacetone; GAH, glyceraldehyde. (1) Fructokinase; (2) aldolase B; (3) triokinase; (4) AMP deaminase. Inhibition is depicted by (-). Further explanations are given in the text.

compound in parenteral nutrition. The same holds true for mixtures of glucose and fructose, known as invert sugar, and for sorbitol, which is converted into fructose in the liver by sorbitol dehydrogenase. As a result, several authors have strongly discouraged intravenous administration of these compounds (12, 26, 74, 84).

ESSENTIAL FRUCTOSURIA

Clinical and Laboratory Findings

First described in 1876, independently by Czapek (16) and Zimmer (86), essential fructosuria is characterized by the appearance of fructose in the urine following the intake of fructose- and/or sorbitol-containing foods. The disorder is usually discovered on routine urine analysis for reducing sugars. It has no other clinical consequences and is therefore completely harmless. Although essential fructosuria may remain undetected in some individuals, the disorder is considered very rare, since less than 80 cases have been reported (62) in nearly a century.

Following a high dose of oral or intravenous fructose (1 g/kg body weight or more), blood fructose rises rapidly beyond the level of 10–25 mg/ml recorded in control subjects (64). However, blood glucose, lactate, and uric acid, which increase significantly in normal individuals, are barely altered, reflecting

the absence of formation of fructose 1-phosphate. In the ensuing 2–6 hours, 10–20% of administered fructose is excreted in the urine, compared with 1–2% in control subjects.

In the red cells of three subjects with essential fructosuria, fasting concentrations of fructose 3-phosphate were 3- to 15-fold higher than in controls and were further increased by an oral fructose load (57). The fact that the three subjects' glycated hemoglobin levels were normal and that diabetic complications have not been reported in fructosuria casts doubt on the suggested role of fructose 3-phosphate in the pathogenesis of diabetic complications.

Enzyme Defect and Genetics

Essential fructosuria results from a deficiency of fructokinase (60). This defect suppresses fructose metabolism via fructose 1-phosphate and its specialized, preferential pathway. Consequently, the ketose is partially metabolized by conversion into fructose 6-phosphate and is partially excreted as such in the urine. The study of a number of families with essential fructosuria has revealed that the disorder is inherited as an autosomal recessive trait (42).

HEREDITARY FRUCTOSE INTOLERANCE

Hereditary fructose intolerance was first reported by Chambers & Pratt (11) in a 24-year-old woman who complained of vomiting after ingesting fruit or sugar. This disorder was recognized as an inborn error of metabolism by Froesch et al (24), who discovered that the administration of fructose provoked profound hypoglycemia in the affected subjects.

Clinical Picture

Subjects with hereditary fructose intolerance do not exhibit any symptoms of the enzyme defect, provided they do not ingest foods containing fructose. Typically, affected babies can breast-feed without any ill effects. Symptoms first appear upon introduction of cow's milk formulas sweetened with sucrose or at weaning, when fruits and vegetables are given (6, 26). In infants and small children, signs of the disorder include gastrointestinal discomfort and hypoglycemia following meals containing fructose. Nausea, vomiting, pallor, sweating, trembling, lethargy, and ultimately jerks and convulsions may also be observed. If the condition is not recognized, and fructose is not excluded from the diet, failure to thrive, liver disease characterized by hepatomegaly, jaundice, bleeding tendency, proximal renal tubular dysfunction, and eventually edema and ascites occur. The younger the child and the higher the fructose intake, the more severe the symptoms, which progress to reflect liver and kidney failure and end in death if their cause is not recognized and treated.

In certain cases, hereditary fructose intolerance is recognized (although not medically diagnosed) and adequately treated when fructose-containing nutri-

ents are suppressed. Some mothers quickly learn that their baby does not tolerate certain foods and suppress these from the diet, which enables the infant to develop normally. Older children acquire a distinct aversion to foods containing fructose. This aversion protects them but is at times considered anomalous behavior. Occasionally, hereditary fructose intolerance is detected during preschool or school age, following the finding of hepatomegaly or growth delay (48). Other cases are only diagnosed after life-threatening perfusions with fructose (40) or sorbitol. Because approximately 50% of adults with hereditary fructose intolerance are completely free of caries, diagnoses have also been made by dentists. These observations indicate that subjects with hereditary fructose intolerance remain undiagnosed in the general population.

Laboratory Findings

When hereditary fructose intolerance is suspected, an intravenous fructose tolerance test should be performed after 6–8 weeks of fructose withdrawal. Oral loading tests are not recommended because they provoke more ill effects and are less reliable. Fructose should be administered as a 20% solution at a dose of 200 mg/kg body weight (26, 63). Blood glucose and serum phosphate should be measured, preferably at two 1–min intervals before the administration of fructose and 10, 20, 30, and 45 min thereafter. In normal children, a slight (5–20 mg/dl) increase in blood glucose is recorded, with little change in serum phosphate. In affected children, glucose diminishes progressively (by 30–50 mg/dl over 30–45 min), whereas phosphate decreases more rapidly (by 1–2 mg/dl over 10–20 min). It is thus useful to include serum phosphate among the parameters measured during a fructose tolerance test. Other modifications that can be measured during the test include an increase in serum magnesium, which is not observed in normal children, and an increase in serum urate, which is more pronounced in hereditary fructose intolerance patients than in normal children.

Other laboratory findings in patients with hereditary fructose intolerance in whom fructose intake has not been suppressed include signs of liver disease (elevations of serum transaminases and bilirubin, depletion of blood clotting factors) and of proximal tubular dysfunction (proteinuria, melituria, generalized hyperaminoaciduria, metabolic acidosis).

Enzyme Defect

In patients with hereditary fructose intolerance, the capacity of aldolase B to split fructose 1-phosphate is reduced to a few percent of normal on average (33, 63). This enzyme's activity toward fructose 1,6-bisphosphate is also reduced, but to a lesser extent. Consequently, the ratio of the V_{max} to fructose 1,6-bisphosphate vs the V_{max} to fructose 1-phosphate, which is approximately 1 in control liver, is increased to 2–∞ in patients. Kinetic studies have revealed

that mutant aldolase B has an abnormally high K_m for both fructose 1-phosphate and fructose 1,6-bisphosphate (38). The defect of aldolase B has been most extensively investigated in the liver of patients but can also be demonstrated in the kidney cortex (63) and jejunal mucosa (65). The activities of aldolase A and C are normal.

Genetics

Hereditary fructose intolerance is genetically heterogeneous, which is evidenced by several observations: (*a*) Clinically, certain patients are very sensitive to fructose, whereas others can tolerate moderate intakes (up to 250 mg/kg per day, compared with an average intake of 1-2 g/kg per day in Western societies). (*b*) The activity of adolase B toward fructose 1-phosphate may vary from undetectable to 15 (63) and even 30% of normal (40). (*c*) Immunologically reactive aldolase B is detectable in all affected tissues, but the amount of cross-reacting material found may vary from less than 3 to a full 100% of controls (30). In recent years, studies of the molecular basis of hereditary fructose intolerance have confirmed its genetic heterogeneity. Studies of the aldolase B gene in 50 patients from 5 European countries have shown that the most frequent mutation, accounting for 67% of alleles, is a G → C substitution, which results in an Ala149 → Pro change (15). This mutation also creates a new recognition site for the restriction enzyme AhaII, which renders it easily detectable. The second most frequent mutation, accounting for 16% of alleles, is a C → A substitution, which results in an Ala174 → Asp change. In the other families, deletions range in size from a single base pair to 1.65 Kb (14). In patients from the US and Canada, A149P and A174D mutations occurred in the same order of prevalence but at a slightly lower frequency than in patients from Europe (68). DNA analysis has also allowed for postmortem diagnosis of hereditary fructose intolerance in a patient who died after fructose and sorbitol infusions during minor surgery (1).

Pathophysiology

The pathophysiology of hereditary fructose intolerance can be explained by the fact that the aldolase B defect causes not only an impairment of the conversion of fructose into glucose but also a marked accumulation of fructose 1-phosphate in the fructose-metabolizing tissues, even upon minimal ingestion or infusion of fructose. As demonstrated by ^{31}P nuclear magnetic resonance spectroscopy of the liver, this accumulation is accompanied by a depletion of ATP and inorganic phosphate (52). The hepatic accumulation of fructose 1-phosphate provokes a characteristic, progressive hypoglycemia by a compounded mechanism involving (*a*) a block of glycogenolysis; (*b*) inhibition of gluconeogenesis; and (*c*) possible stimulation of the uptake of glucose. The block of glycogenolysis is evidenced both by the absence of dilution of infused

radioactive glucose by endogenous glucose (20) and by the inability of glucagon to raise blood glucose during fructose-induced hypoglycemia (13). Detailed investigations of the effects of fructose on the glycogenolytic mechanism (71, 75) have revealed that fructose administration decreases the capacity of the liver to form cyclic AMP and results in an inhibition of the activity of phosphorylase a. The decreased capacity to form cyclic AMP can be attributed to the loss of ATP, the substrate of adenylate cyclase, and is characterized by a marked reduction of the glucagon-induced urinary excretion of cyclic AMP. However, the decreased formation of cyclic AMP is not sufficient to explain the absence of response to glucagon, because fructose-induced hypoglycemia also cannot be corrected by dibutyryl cyclic AMP. The block of glycogenolysis occurs as a result of an inhibition of liver phosphorylase a, caused by the accumulation of fructose 1-phosphate combined with the depletion of inorganic phosphate, a substrate of the enzyme.

The decreased activity of aldolase B toward fructose 1,6-bisphosphate in hereditary fructose intolerance has no clinical effects on gluconeogenesis in the sense that patients do not exhibit fasting hypoglycemia, in contrast to patients with defects of gluconeogenesis, e.g. fructose 1,6-bisphosphatase deficiency. Nevertheless, during fructose-induced hypoglycemia, inhibition of gluconeogenesis is evidenced by the fact that glycemia cannot be corrected by dihydroxyacetone, which enters the glycolytic-gluconeogenic pathway by way of triokinase. Inhibition of gluconeogenesis is explained by the inhibitory effect of fructose 1-phosphate on glucose 6-phosphate isomerase (85) and on the condensation of the triose phosphates to fructose 1,6-bisphosphate by aldolase (C Bally, F Leuthardt, unpublished data cited in Ref. 26). Stimulation of the hepatic uptake of glucose might also play a role in fructose-induced hypoglycemia, owing to the stimulatory effect of fructose 1-phosphate on the protein regulator of glucokinase, discussed above. The other toxic effects of fructose recorded in hereditary fructose intolerance, namely gastrointestinal discomfort and hepatic and renal dysfunction (49), are most likely due to the loss of ATP, GTP, and Pi, caused by the accumulation of fructose 1-phosphate in these tissues.

Treatment and Prognosis

Treatment of hereditary fructose intolerance consists of immediate elimination of all sources of fructose from the diet, i.e. all foods in which fructose and/or sucrose and sorbitol are present naturally or have been added. Lists of fructose content of various foodstuffs are available (31). The presence of fructose in medications and in infant formulas, although declining, should also be checked. Despite adequate treatment, small children with hereditary fructose intolerance usually exhibit hepatomegaly for months and even years (53). The reason for this disorder is unclear and has been linked both to an overly strict and an

insufficiently stringent limitation of fructose intake. Inadequate restriction of fructose has been reported to cause isolated growth retardation, as evidenced by catch-up growth on a stricter diet (48). Therefore, fructose intake should not be determined by subjective tolerance, at least during childhood. Upon adequate suppression of fructose, the further course of the affected subjects is uneventful. Patients (and their parents) should be made aware that infusions containing fructose, sorbitol, or invert sugar are life-threatening to them, and they should report fructose intolerance on any hospital admission.

FRUCTOSE 1,6-BISPHOSPHATASE DEFICIENCY

Fructose 1,6-bisphosphatase deficiency was first identified in 1970 by Baker & Winegrad (7) in a 5-year-old girl with a history of episodes of metabolic acidosis and hypoglycemia since the age of 6 months. Since then, over 100 cases have been diagnosed.

Clinical Picture

Approximately half of subjects with fructose 1,6-bisphosphatase deficiency present in the immediate neonatal period with life-threatening episodes of hypoglycemia accompanied by profound acidosis (4, 26). These episodes are characterized by hyperventilation, irritability, somnolence or coma, dyspnea and apneic spells, tachycardia, and muscular hypotonia. The other half of affected children experience attacks of hypoglycemia and acidosis during the first months or years of life. These attacks are usually triggered by prolonged fasting or febrile infections and are accompanied by refusal to eat and vomiting. Hyperventilation, trembling, lethargy, and ultimately coma and convulsions may be followed within a few hours by apnea and cardiac arrest. Moderate hepatomegaly and muscle weakness often occur as well.

In contrast to subjects with hereditary fructose intolerance, patients with fructose 1,6-bisphosphatase deficiency tolerate normal amounts of fructose and sucrose in their diet and do not develop an aversion to sweet foods. As described in the next section, loading tests nevertheless reveal a reduced tolerance for fructose and related compounds.

Laboratory Findings

During acute episodes, blood glucose may fall below 40 mg/dl, whereas lactic acid may accumulate up to 20 mM, and pH may drop below 7.1. Alanine, ketones, and uric acid are elevated in blood and urine, and glycerol and glycerol 3-phosphate may be detected in the latter. Between attacks, prolonged fasting results in a progressive decrease of blood glucose, accompanied by elevation of lactate, alanine, and ketones.

In patients with fructose 1,6-bisphosphatase deficiency, hypoglycemia can be provoked by oral or intravenous administration of fructose or sorbitol, but

higher doses are generally required than in subjects with hereditary fructose intolerance. Fructose at a dose of 1 g/kg orally or 500 mg/kg intravenously causes a marked decrease in blood glucose and serum phosphate accompanied by an increase in serum magnesium and urate (7, 26, 54). Oral glycerol at a dose of 1 g/kg also induces hypoglycemia and hypophosphatemia owing to accumulation of glycerol 3-phosphate (Figure 1), but both disorders tend to be less marked than after the same dose of fructose (5, 7, 29, 54). In further contrast to hereditary fructose intolerance, disturbances of liver function tests are rare in fructose 1,6-bisphosphatase deficiency.

Enzyme Defect

The deficiency of fructose 1,6-bisphosphatase can be demonstrated in liver (5, 7, 36, 54), jejunum (29), and kidney (29, 45). Whether the defect can be diagnosed in leukocytes remains a matter of debate because of the enzyme's low activity in these cells. In patients with deficiency of the liver enzyme, activity of muscle fructose 1,6-bisphosphatase is normal (45, 54), which indicates that this enzyme is encoded by a different gene.

Genetics

Fructose 1,6-bisphosphatase deficiency is inherited as an autosomal recessive trait. More girls than boys (1.5:1) have been diagnosed, and parental consanguinity is found in several families. Intermediate activities of fructose 1,6-bisphosphatase in the liver of the parents, who do not display symptoms, have been reported. The recent isolation of a cDNA encoding human liver fructose 1,6-bisphosphatase (22) will allow for studies of the molecular basis of the defect.

Pathophysiology

Fructose 1,6-bisphosphatase deficiency results in an inhibition of the formation of glucose from all physiological gluconeogenic substrates, including those that enter the gluconeogenic pathway immediately before the enzyme, namely fructose and glycerol (Figure 1). Maintenance of normoglycemia thus depends on glucose intake and on degradation of hepatic glycogen. Consequently, when the glycogen reserve is limited, as in newborns, or exhausted, as after prolonged fasting, hypoglycemia occurs, accompanied by elevation of the main gluconeogenic precursors (lactate, alanine, and glycerol).

The mechanism of hypoglycemia induced by loading tests with fructose, sorbitol, or glycerol is similar to the mechanism that operates in hereditary fructose intolerance. Owing to the fructose 1,6-bisphosphatase defect, fructose and sorbitol cause a buildup of fructose 1-phosphate, whereas glycerol induces an accumulation of glycerol 3-phosphate in the liver (Figure 1). Both phosphate esters inhibit phosphorylase (37, 75). This inhibition, together with the de-

crease in inorganic phosphate, results in a block of glycogenolysis on which maintenance of normoglycemia in the affected subjects depends. Fructose tolerance is higher in fructose 1,6-bisphosphatase deficiency than in hereditary fructose intolerance because with a given amount of ketose, less fructose 1-phosphate accumulates in fructose 1,6-bisphosphatase deficiency; although its conversion into glucose is blocked, it can still be metabolized into lactate (Figure 1).

Treatment and Prognosis

Acute episodes of hypoglycemia and acidosis should be corrected by intravenous infusion of glucose and sodium bicarbonate. Maintenance therapy (e.g. frequent feeding, gastric drip) should be aimed at avoiding fasting and providing adequate dietary management during febrile infections. Although elimination of fructose and sucrose from the diet is not required, restriction of these compounds is advocated until the sensitivity of the individual patient has been assessed. Because of its inhibitory effect on gluconeogenesis, alcohol should also be restricted. Once fructose 1,6-bisphosphatase deficiency has been diagnosed and adequate management established, its course is generally benign. Growth and development are normal, and tolerance for fasting improves with age (7, 23, 26, 50).

Literature Cited

1. Ali M, Rosien U, Cox TM. 1993. DNA diagnosis of fatal fructose intolerance from archival tissue. *Quart. J. Med.* 86:25–30
2. Anundi I, de Groot H. 1989. Hypoxic liver cell death: critical PO_2 and dependence of viability on glycolysis. *Am. J. Physiol.* 257:G58–64
3. Anundi I, King J, Owen DA, Schneider H, Lemasters JJ, Thurman RG. 1987. Fructose prevents hypoxic cell death in liver. *Am. J. Physiol.* 253: G390–96
4. Baerlocher K. 1990. Disorders of gluconeogenesis. In *Inborn Metabolic Diseases. Diagnosis and Treatment*, ed. J Fernandes, J-M Saudubray, K Tada, pp. 113–23. Berlin/Heidelberg: Springer-Verlag. 730 pp.
5. Baerlocher K, Gitzelmann R, Nüssli R, Dumermuth G. 1971. Infantile lactic acidosis due to hereditary fructose 1,6-diphosphatase deficiency. *Helv. Paediatr. Acta* 26:489–506
6. Baerlocher K, Gitzelmann R, Steinmann B, Gitzelmann-Cumarasamy N. 1978. Hereditary fructose intolerance in early childhood: a major diagnostic challenge. Survey of 20 symptomatic cases. *Helv. Paediatr. Acta* 33:465–87
7. Baker L, Winegrad AI. 1970. Fasting hypoglycaemia and metabolic acidosis associated with deficiency of hepatic fructose-1,6-diphosphatase activity. *Lancet* 2:13–16
8. Bismut H, Hers HG, Van Schaftingen E. 1993. Conversion of fructose to glucose in the rabbit small intestine. A reappraisal of the direct pathway. *Eur. J. Biochem.* 213:721–26
9. Björkman O, Felig P. 1982. Role of the kidney in the metabolism of fructose in 60-hour fasted humans. *Diabetes* 31: 516–20

10. Burch HB, Lowry OH, Meinhardt L, Max P Jr, Chyu K-J. 1970. Effect of fructose, dihydroxyacetone, glycerol, and glucose on metabolites and related compounds in liver and kidney. *J. Biol. Chem.* 245:2092–102
11. Chambers RA, Pratt RTC. 1956. Idiosyncrasy to fructose. *Lancet* 2:340
12. Collins J. 1993. Time for fructose solutions to go. *Lancet* 341:600
13. Cornblath M, Rosenthal IM, Reisner SH, Wybregt SH, Crane RK. 1963. Hereditary fructose intolerance. *New Engl. J. Med.* 269:1271–78
14. Cross NCP, Cox TM. 1990. Hereditary fructose intolerance. *Int. J. Biochem.* 22:685–89
15. Cross NCP, DeFranchis R, Sebastio G, Dazzo C, Tolan DR, et al. 1990. Molecular analysis of aldolase B genes in hereditary fructose intolerance. *Lancet* 335:306–9
16. Czapek F. 1876. Eine seltene Form von Diabetes mellitus. *Prager Med. Wochenschr.* 1:245–48
17. Detheux M, Vandekerckhove J, Van Schaftingen E. 1993. Cloning and sequencing of rat liver cDNAs encoding the regulatory protein of glucokinase. *FEBS Lett.* 321:111–15
18. Di Monte D, Sandy MS, Blank L, Smith MT. 1988. Fructose prevents 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)–induced ATP depletion and toxicity in isolated hepatocytes. *Biochem. Biophys. Res. Commun.* 153:734–40
19. Donaldson IA, Doyle TC, Matas N. 1993. Expression of rat liver ketohexokinase in yeast results in fructose intolerance. *Biochem. J.* 291:179–86
20. Dubois R, Loeb H, Ooms HA, Gillet P, Bartman J, Champenois A. 1961. Etude d'un cas d'hypoglycémie fonctionnelle par intolérance au fructose. *Helv. Paediatr. Acta* 16:90–96
21. El-Maghrabi MR, Lange AJ, Kümmel L, Pilkis SJ. 1991. The rat fructose-1,6-bisphosphatase gene. Structure and regulation of expression. *J. Biol. Chem.* 266:2115–20
22. El-Maghrabi MR, Gidh-Jain M, Austin LR, Pilkis SJ. 1993. Isolation of a human liver fructose-1,6-bisphosphatase cDNA and expression of the protein in *Escherichia coli*. Role of ASP-118 and ASP-121 in catalysis. *J. Biol. Chem.* 268:9466–72
23. Elpeleg ON, Hurvitz H, Branski D, Barash V. 1989. Fructose-1,6-diphosphatase deficiency: a 20-year follow-up. *Am. J. Dis. Child.* 143:140–42
24. Froesch ER, Prader A, Labhart A, Stuber HW, Wolf HP. 1957. Die hereditäre Fructoseintoleranz, eine bisher nicht bekannte kongenitale Stoffwechselstörung. *Schweiz. Med. Wschr.* 87: 1168–71
25. Gasbarrini A, Borle AB, Farghali H, Francavilla A, Van Thiel D. 1992. Fructose protects rat hepatocytes from anoxic injury. Effect on intracellular ATP, Ca^{2+}, Mg^{2+}, Na^{+}, and pH. *J. Biol. Chem.* 267:7545–52
26. Gitzelmann R, Steinmann B, Van den Berghe G. 1989. Disorders of fructose metabolism. In *The Metabolic Basis of Inherited Disease*, ed. CR Scriver, AL Beaudet, WS Sly, D Valle, 1:399–424. New York: McGraw-Hill. 3006 pp.
27. Gopher A, Vaisman N, Mandel H, Lapidot A. 1990. Determination of fructose metabolic pathways in normal and fructose-intolerant children: a ^{13}C NMR study using [U–^{13}C]fructose. *Proc. Natl. Acad. Sci. USA* 87:5449–53
28. Gopher A, Lapidot A. 1990. The effect of mode of fructose administration on its metabolic pathways in a Rhesus monkey and rabbits, studied by [U–^{13}C]fructose and ^{13}C NMR. *Ninth Annual Scientific Meeting and Exhibition, Society of Magnetic Resonance in Medicine, New York, 1990,* 2:941 (Abstr.)
29. Greene HL, Stifel FB, Herman RH. 1972. "Ketotic hypoglycemia" due to hepatic fructose-1,6-diphosphatase deficiency. *Am. J. Dis. Child.* 124:415–18
30. Grégori C, Schapira F, Kahn A, Delpech M, Dreyfus JC. 1982. Molecular studies of liver aldolase B in hereditary fructose intolerance using blotting and immunological techniques. *Ann. Hum. Genet.* 46:281–92
31. Hardinge MG, Swarner JB, Crooks H. 1965. Carbohydrates in foods. *J. Am. Diet. Assoc.* 46:197–204
32. Hers HG. 1957. Le métabolisme du fructose dans le foie. Brussels: Arscia 200 pp.
33. Hers HG, Joassin G. 1961. Anomalie de l'aldolase hépatique dans l'intolérance au fructose. *Enzymol. Biol. Clin.* 1:4–14
34. Holdsworth CD, Dawson AM. 1965. Absorption of fructose in man. *Proc. Soc. Exp. Biol. Med.* 118:142–45
35. Hue L, Bartrons R. 1984. Role of fructose 2,6-bisphosphate in the control by glucagon of gluconeogenesis from various precursors in isolated rat hepatocytes. *Biochem. J.* 218:165–70
36. Hülsmann WC, Fernandes J. 1971. A child with lactacidemia and fructose diphosphatase deficiency in the liver. *Pediatr. Res.* 5:633–37

37. Kaufmann U, Froesch ER. 1973. Inhibition of phosphorylase-a by fructose-1-phosphate, a-glycerophosphate and fructose-1,6-diphosphate: explanation for fructose-induced hypoglycaemia in hereditary fructose intolerance and fructose-1,6-diphosphatase deficiency. *Eur. J. Clin. Invest.* 3:407–13
38. Koster JF, Slee RG, Fernandes J. 1975. On the biochemical basis of hereditary fructose intolerance. *Biochem. Biophys. Res. Comm.* 64: 289–94
39. Lal S, Szwergold BS, Kappler F, Brown T. 1993. Detection of fructose-3-phosphokinase activity in intact mammalian lenses by ^{31}P NMR spectroscopy. *J. Biol. Chem.* 268:7763–67
40. Lameire N, Mussche M, Baele G, Kint J, Ringoir S. 1978. Hereditary fructose intolerance: a difficult diagnosis in the adult. *Am. J. Med.* 65:416–23
41. Lapidot A, Gopher A, Gutman A. 1990. Does fructose metabolize to fructose-6-P by hexokinase in human liver? In vivo studies of fructose metabolism in patients with fructose-1,6-diPase deficiency. *Ninth Annual Scientific Meeting and Exhibition, Society of Magnetic Resonance in Medicine, New York.* 2:938 (Abstr.)
42. Lasker M. 1941. Essential fructosuria. *Hum. Biol.* 13:51–63
43. Liang J-Y, Zhang Y, Huang S, Ke H, Lipscomb WN. 1992. Catalysis and allosteric regulation in fructose-1,6-bisphosphatase. *Proceedings of the RA Welch Foundation Conference on Chemical Research. XXXVI. Regulation of proteins by ligands. Houston, Texas, 1992,* 36:57–99
44. Mäenpää PH, Raivio KO, Kekomäki MP. 1968. Liver adenine nucleotides: fructose-induced depletion and its effect on protein synthesis. *Science* 161:1253–54
45. Melancon SB, Khachadurian AK, Nadler HL, Brown BI. 1973. Metabolic and biochemical studies in fructose 1,6-diphosphatase deficiency. *J. Pediatr.* 82: 650–57
46. Mendeloff AI, Weichselbaum TE. 1953. Role of the human liver in the assimilation of intravenously administered fructose. *Metabolism* 2:450–58
47. Minkowski O. 1893. Untersuchungen über den Diabetes mellitus nach Exstirpation des Pankreas. *Arch. Exper. Pathol. Pharmakol.* 31:85–189
48. Mock DM, Perman JA, Thaler MM, Morris, RC Jr. 1983. Chronic fructose intoxication after infancy in children with hereditary fructose intolerance. A cause of growth retardation. *New Engl. J. Med.* 309:764–70
49. Morris RC Jr, Nigon K, Reed EB. 1978. Evidence that the severity of depletion of inorganic phosphate determines the severity of the disturbance of adenine nucleotide metabolism in the liver and renal cortex of the fructose-loaded rat. *J. Clin. Invest.* 61:209–20
50. Moses SW, Bashan N, Flasterstein BF, Rachmel A, Gutman A. 1991. Fructose-1,6-diphosphatase deficiency in Israel. *Israel J. Med. Sci.* 27:1–4
51. Nieminen AL, Dawson TL, Gores GJ, Kawanishi T, Herman B, Lemasters JJ. 1990. Protection by acidotic pH and fructose against lethal injury to rat hepatocytes from mitochondrial inhibitors, ionophores and oxidant chemicals. *Biochem. Biophys. Res. Commun.* 167: 600–6
52. Oberhaensli RD, Rajagopalan B, Taylor DJ, Radda GK, Collins JE, et al. 1987. Study of hereditary fructose intolerance by use of ^{31}P magnetic resonance spectroscopy. *Lancet* 2:931–34
53. Odièvre M, Gentil C, Gautier M, Alagille D. 1978. Hereditary fructose intolerance in childhood. Diagnosis, management and course in 55 patients. *Am. J. Dis. Child.* 132:605–8
54. Pagliara AS, Karl IE, Keating JP, Brown BI, Kipnis DM. 1972. Hepatic fructose-1,6-diphosphatase deficiency. A cause of lactic acidosis and hypoglycemia in infancy. *J. Clin. Invest.* 51:2115–23
55. Petersen A, Szwergold BS, Kappler F, Weingarten M, Brown TR. 1990. Identification of sorbitol 3-phosphate and fructose 3-phosphate in normal and diabetic human erythrocytes. *J. Biol. Chem.* 265:17424–27
56. Petersen A, Kappler F, Szwergold BS, Brown TR. 1992. Fructose metabolism in the human erythrocyte. Phosphorylation to fructose 3-phosphate. *Biochem. J.* 284:363–66
57. Petersen A, Steinmann B, Gitzelmann R. 1992. Essential fructosuria. Increased levels of fructose 3-phosphate in erythrocytes. *Enzyme* 46:319–23
58. Rottmann WH, Tolan DR, Penhoet EE. 1984. Complete amino acid sequence for human aldolase B derived from cDNA and genomic clones. *Proc. Natl. Acad. Sci. USA* 81: 2738–42
59. Sestoft L, Fleron P. 1974. Determination of the kinetic constants of fructose transport and phosphorylation in the perfused rat liver. *Biochim. Biophys. Acta* 345: 27–38
60. Shapira F, Shapira G, Dreyfus JC. 1961–62. La lésion enzymatique de la fructosurie bénigne. *Enzymol. Biol. Clin.* 1: 170–75

61. Smith LH Jr, Ettinger RH, Seligson D. 1953. A comparison of the metabolism of fructose and glucose in hepatic disease and diabetes mellitus. *J. Clin. Invest.* 32:273–82
62. Steinitz H, Mizrahy O. 1969. Essential fructosuria and hereditary fructose intolerance. *New Engl. J. Med.* 280:222
63. Steinmann B, Gitzelmann R. 1981. The diagnosis of hereditary fructose intolerance. *Helv. Paediatr. Acta* 36:297–316
64. Steinmann B, Baerlocher K, Gitzelmann R. 1975. Hereditäre Störungen des Fruktosestoffwechsels: Belastungsproben mit Fruktose, Sorbitol und Dihydroxyaceton. *Nutr. Metab.* 18 (Suppl. 1):115–32
65. Skreb H, Posselt HG, Wolter K, Bender SW. 1981. Aldolase activities of the small intestinal mucosa in malabsorption states and hereditary fructose intolerance. *Eur. J. Pediatr.* 137:5–10
66. Szwergold BS, Kappler F, Brown TR. 1990. Identification of fructose 3-phosphate in the lens of diabetic rats. *Science* 247:451–54
67. Tejwani GA. 1983. Regulation of fructose-bisphosphatase activity. *Adv. Enzymol.* 54:121–94
68. Tolan DR, Brooks CC. 1992. Molecular analysis of common aldolase B alleles for hereditary fructose intolerance in North Americans. *Biochem. Med. Metabol. Biol.* 48:19–25
69. Tolan DR, Penhoet EE. 1986. Characterization of the human aldolase B gene. *Mol. Biol. Med.* 3:245–64
70. Topping DL, Mayes PA. 1971. The concentrations of fructose, glucose and lactate in the splanchnic blood vessels of rats absorbing fructose. *Nutr. Metab.* 13:331–38
71. Van den Berghe G. 1975. Biochemical aspects of hereditary fructose intolerance. In *Normal and Pathological Development of Energy Metabolism*, ed. FA Hommes, CJ Van den Berg, pp. 211–26. London: Academic Press. 246 pp.
72. Van den Berghe G. 1978. Metabolic effects of fructose in the liver. *Curr. Top. Cell. Regul.* 13:97–135
73. Van den Berghe G. 1986. Fructose: metabolism and short-term effects on carbohydrate and purine metabolic pathways. *Progr. Biochem. Pharmacol.* 21: 1–32
74. Van den Berghe G, Hers HG. 1978. Dangers of intravenous fructose and sorbitol. *Acta Paediatr. Belg.* 31:115–23
75. Van den Berghe G, Hue L, Hers HG. 1973. Effect of the administration of fructose on the glycogenolytic action of glucagon. An investigation of the pathogeny of hereditary fructose intolerance. *Biochem. J.* 134:637–45
76. Van den Berghe G, Bronfman M, Vanneste R, Hers HG. 1977. The mechanism of adenosine triphosphate depletion in the liver after a load of fructose. A kinetic study of liver adenylate deaminase. *Biochem. J.* 162:601–9
77. Van den Berghe G, Bontemps F, Hers HG. 1980. Purine catabolism in isolated rat hepatocytes. Influence of coformycin. *Biochem. J.* 188:913–20
78. Vandercammen A, Van Schaftingen E. 1990. The mechanism by which rat liver glucokinase is inhibited by the regulatory protein. *Eur. J. Biochem.* 191: 483–89
79. Van Schaftingen E. 1987. Fructose 2,6-bisphosphate. *Adv. Enzymol.* 59:315–95
80. Van Schaftingen E. 1989. A protein from rat liver confers to glucokinase the property of being antagonistically regulated by fructose 6-phosphate and fructose 1-phosphate. *Eur. J. Biochem.* 179: 179–84
81. Van Schaftingen E, Davies DR. 1991. Fructose administration stimulates glucose phosphorylation in the liver of anesthetized rats. *FASEB J.* 5:326–30
82. Van Schaftingen E, Vandercammen A. 1989. Stimulation of glucose phosphorylation by fructose in isolated rat hepatocytes. *Eur. J. Biochem.* 179: 173–77
83. Van Schaftingen E, Vandercammen A, Detheux M, Davies DR. 1992. The regulatory protein of liver glucokinase. *Adv. Enz. Regul.* 32:133–48
84. Woods HF, Alberti KGMM. 1972. Dangers of intravenous fructose. *Lancet* 2: 1354–57
85. Zalitis J, Oliver IT. 1967. Inhibition of glucose phosphate isomerase by metabolic intermediates of fructose. *Biochem. J.* 102:753–59
86. Zimmer K. 1876. Levulose im Harn eines Diabetikers. *Dtsch. Med. Wochenschr.* 2:329–31