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Glycogen storage disease, Fanconi nephropathy, abnormal galactose metabolism and mitochondrial myopathy

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Abstract. We present a 4-year-old male suffering from profound muscular weakness, enzymatically undefined glycogen storage disease. Fanconi nephropathy and impaired galactose utilization. Distorted mitochondria, intramitochondrial fat droplets and partial deficiencies of pyruvate dehydrogenase complex, succinate: cytochrome c oxidoreductase, and cytochrome c oxidase have been found in muscle tissue. The causal relationship between mitochondrial myopathy, glycogen storage disease, Fanconi nephropathy and impaired utilization of galactose is discussed.

Key words: Glycogen storage disease – Fanconi nephropathy – Galactose – Mitochondrial myopathy

Introduction

The association of glycogen storage disease (GSD), Fanconi nephropathy (FN) and impaired metabolism of galactose has been previously described [2–5, 8, 11, 20, 22, 24, 27, 28]. Hitherto, an underlying defect which could lead to this combination has not been established. Some of the reported patients have an additional muscular involvement which seems to be an integral part of their primary disease. Neither the morphology nor the mitochondrial enzyme activities in muscle have been investigated in these patients. The present study concerns a boy with the above syndrome whose muscle findings contribute to the understanding of this metabolic disorder.

Case report

A 4-year-old boy was evaluated for failure to thrive and muscular weakness. His parents are first cousins of Moslem origin. There is one healthy sister, while three other pregnancies had terminated in stillbirths. He was born following an uneventful pregnancy; birth weight was 3 kg and length 50 cm. Despite a good appetite and the absence of vomiting or diarrhoea, his weight was only 9.5 kg and his length 77 cm at 4 years of age. Gross motor development was markedly delayed and he was unable to stand without support. His cognitive functions were adequate for age. On physical examination he appeared dystrophic. Tachypnoea, a grossly distended abdomen with hepatosplenomegaly and obvious manifestations of rickets were noted. There was severe hypotonia; deep tendon reflexes could not be elicited. Ophthalmological examination by slit lamp and fundoscopy was unremarkable.

Laboratory studies revealed a metabolic acidosis (pH = 7.13, HCO_3 = 7 meq/l, base excess −20), hypophosphataemia (0.5 mmol/l), reduced calculated reabsorption of phosphorus (63%), elevated alkaline phosphatase (500 U/l), and low serum carnitine (total = 13.4 μmol/l and free = 8.0 μmol/l; normal ≥ 40 and 33 nmol/l, respectively). Fasting hypoglycaemia (1.3 mmol/l), a mild elevation of liver transaminases (SGOT = 200 U/l, SGPT = 170 U/l), and lactic acidaemia (up to 6.3 mmol/l) were present. The concentration of acetoacetate was 444 μmol/l, and of β-hydroxybutyrate 2258 μmol/l, after 12 h fasting. Urinalysis revealed glucosuria, acetonuria, bicarbonaturia and generalized aminoaciduria. Urinary carnitine was (free/total) 35/69.5 μmol/mmol creatinine (normal = 9/16). Plasma urea, creatinine, sodium, potassium, chloride, calcium, uric acid, lactate dehydrogenase, creatine phosphokinase, albumin, globulin, prothrombin time, triglycerides, cholesterol, coeruloplasmin, amino acid chromatography and D-xylose test were all within the normal range.

X-ray studies showed severe rickets with osteoporosis; bone age was 3 years.

Administration of glucagon resulted in an appropriate elevation of urinary cyclic adenosine monophosphate, but plasma glucose and lactate remained unchanged. Insulin levels were consistent with hypoglycaemia. An oral galactose load (1.75 g/kg) resulted in a marked rise of plasma galactose, sustained for 2 h. Plasma glucose and lactate levels remained unaltered.

Tissue examination

A liver biopsy was obtained for histological studies, glycogen determination [18], and measurement of glucose-6-phosphatase activity [13].

The activity of α-1,6-amyloglucosidase was assayed in skin fibroblasts [14]. Cultured fibroblasts were also used for the investigation of galactose metabolism. The cells were incubated with 1-(^{14}C) glucose or 1-(^{14}C) galactose. $^{14}CO_2$ was collected and the radioactivity was determined. Labelled glycogen was isolated [16] and ^{14}C lactate was separated from the supernatant and quantitated [26].

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Abbreviations: COX = cytochrome c oxidase; FN = Fanconi nephropathy; GSD = glycogen storage disease; MM = mitochondrial myopathy; NADH = nicotinamide adenine dinucleotide reduced; PDHC = pyruvate dehydrogenase complex; SCOX = succinate: cytochrome c oxidoreductase

Muscle tissue was obtained from the quadriceps at the age of 4 years and was stored at −70°C for 10 months. Mitochondrial enzyme activities were measured in a total tissue homogenate. For the determination of pyruvate dehydrogenase complex (PDHC) activity the method of Van Laack et al. [32] was applied. Measurement of the other enzyme activities was performed as previously described [10].

Results

Liver glycogen content was found to be 100 mg/g tissue (normal ≤ 50 mg/g). Glucose-6-phosphatase activity was 17 μmol/min per gram (normal 2–10). The activity of α-1,6-amyloglucosidase in fibroblasts was within the normal range (0.6–0.9 μmol/min per gram wet weight). ^{14}C-lactate and $^{14}CO_2$ pro-

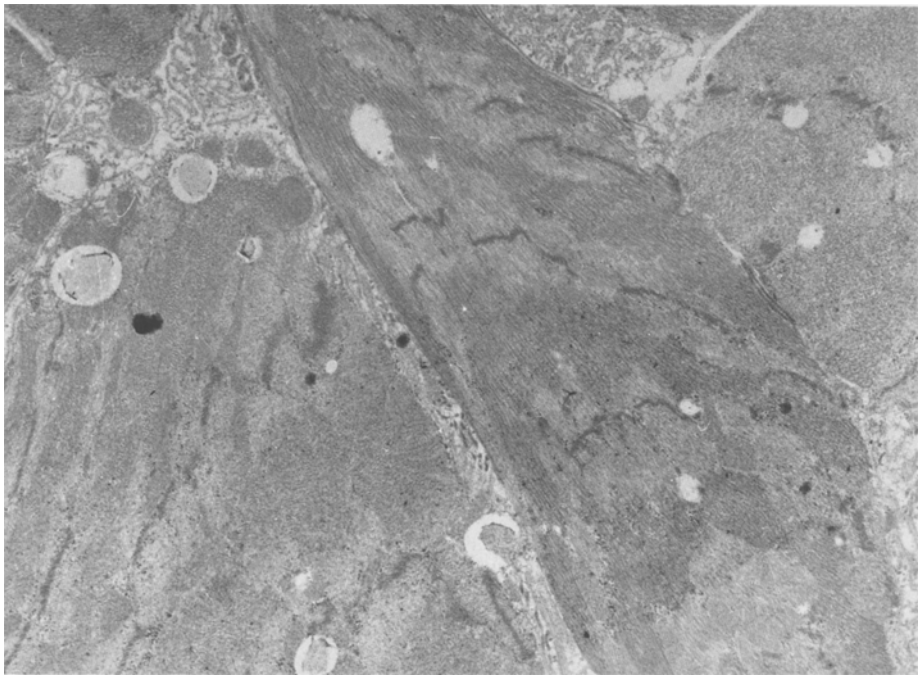

Fig. 1. Abundant fat droplets within muscle fibres. The muscle fibre shows irregularities of the Z lines with distortion of the myofibrillar arrangement. × 4600

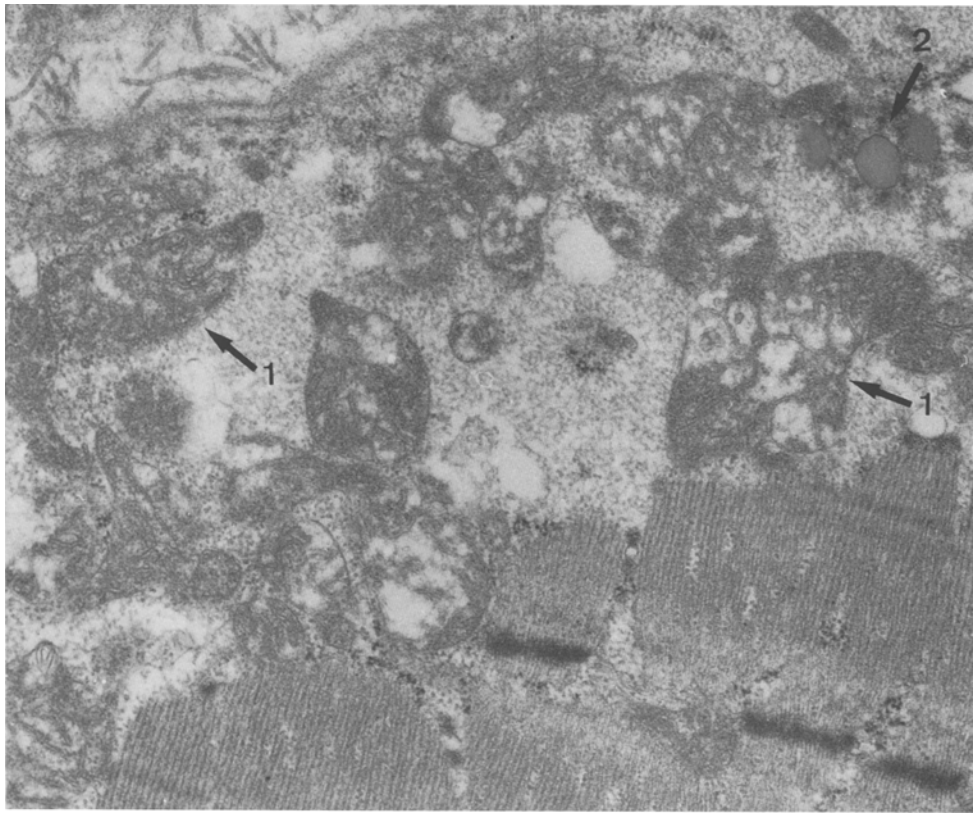

Fig. 2. Accumulation of mitochondria in subsarcollemal region. These mitochondria show irregular pattern and their cristae are dilated, elongated and distorted *(arrow 1)*. Few fat droplets are seen within the mitochondria and adjacent to the Z lines *(arrow 2)*. × 10000

Table 1. Activity of mitochondrial enzymes in muscle tissue

	Patient[a]	Control range[a]
Cytochrome c oxidase	31	73 –284
NADH: Q_1 oxidoreductase	5.8	4.7–19
Succinate: cyt c oxidoreductase	1.9	4.2–16
Pyruvate dehydrogenase complex	0.92	2.8–6.2
Citrate synthase	69	48 –146

[a] Values are in mU/mg homogenate protein

duction from labelled galactose and glucose and their incorporation into labeled glycogen were similar in both patient and control fibroblasts.

The pathological findings in the muscle tissue are shown in Figs. 1 and 2. No glycogen granules were seen within the muscle fibres.

The activities of pyruvate dehydrogenase (PDH), cytochrome c oxidase (COX), and succinate:cytochrome c oxidoreductase (SCOX) were diminished to 32%–45% of the lowest normal values, while the other two mitochondrial enzyme activities were normal, as shown in Table 1.

Discussion

Our patient presented with the typical findings of FN including hypophosphataemic rickets, metabolic acidosis, glucosuria and aminoaciduria. Cystinosis, glactosaemia, hereditary fructose intolerance, tyrosinaemia, Wilson disease and severe malabsorption were all excluded.

In addition to the classical signs of FN, the patient had marked hepatomegaly, fasting hypoglycaemia, mild elevation of liver transaminases, no response to glucagon and increased liver glycogen content. GSD types I and III were excluded by enzyme determinations.

On galactose containing diet, the patient did not excrete galactose. However, an oral galactose load resulted in an abnormally prolonged elevation of plasma galactose, no increase in plasma glucose or lactate, and massive urinary excretion of galactose.

The coexistence of GSD, FN and impaired galactose metabolism has previously been reported [2–5, 8, 11, 20, 22, 24, 27, 28] and is also known as Fanconi-Bickel syndrome [25] and GSD type XI [17]. The underlying defect of this metabolic association is still unknown.

Brivet et al. [5] observed that persistent galactosaemia following a galactose load was seldom found in patients with GSD, unless associated with FN, thus suggesting a linkage between the FN and the abnormal galactose metabolism. Galactose by itself, however, does not seem to have a toxic effect on the renal tubuli, since an oral galactose load did not worsen a pre-existing renal tubular defect, and a lactose-free diet did not normalize the tubular function in similar patients [1, 27].

The coexistence of FN and defects in galactose metabolism is well documented in galactose-1-phosphate uridyl transferase deficiency and has also been observed in systemic uridyl diphosphate galactose-4-epimerase deficiency [15]. Both enzymatic defects were excluded in our patient. FN has also been reported in the rare cases of galactose intolerance with all galactose metabolizing enzymes having a normal activity [1, 6, 23]. The possibility of a galactose transport defect, common to the renal tubuli and the hepatocyte membrane, has been suggested [23]. Challenging this hypothesis, we have studied galactose metabolism in fibroblasts and found it to be intact. Therefore, either there is a tissue specificity for galactose metabolism [5], or the abnormal in vivo galactose load is the result of a functional impairment of its metabolism, not being expressed in fibroblasts. Nevertheless, such a common transport defect would not explain the coexistence of GSD.

Considering the clinical and biochemical findings, our patient shares some features with GSD type XI patients. This type of GSD has been attributed to a functional defect of phosphoglucomutase [17], which is an essential enzyme in the common degradative pathways of both glycogen and galactose. Thus, its inhibition would result in the unresponsiveness of both lactate and glucose to glucagon and galactose loads observed in our patient.

Nevertheless, in GSD type XI patients, in spite of the accompanying FN, with the resultant metabolic acidosis, hypophosphataemia, and low serum carnitine, the muscles are usually unaffected [18]. These factors may all contribute, but not fully explain, the prominent and most disabling muscular weakness in our patient. This muscular involvement does not seem to be directly linked to the GSD, since no glycogen granules have been found within the muscle fibres.

The morphological findings of distorted mitochondria and intramitochondrial fat droplets were suggestive of mitochondrial myopathy (MM). The finding of partial deficiencies of PDHC, COX and SCOX is intriguing, since this combination of mitochondrial defects has not been previously described. Yet, the normal activity of reduced nicotinamide adenine dinucleotide (NADH): Q_1 oxidoreductase, possibly the most labile component of the respiratory chain and the normal activity of citrate synthase point toward a real, although not necessarily primary, MM.

The association of MM with FN is well documented and has been reported in PDHC, COX, SCOX and multiple cytochrome deficiencies [7, 9, 21, 30, 31]. Furthermore, decreased NADH oxidation is known to impair galactose utilization, since the uridyl diphosphate galactose-4-epimerase activity is inhibited by increased NADH [19]. In addition, seeing that increased galactose-1-phosphate is known to inhibit phosphoglucomutase activity [29], this, in turn, might result in GSD.

Thus, a mitochondrial defect could theoretically explain the association of FN, abnormal galactose metabolism and GSD. This hypothesis, however, fails to explain the absence of significant muscular weakness in many of the patients with GSD, FN and abnormal galactose metabolism. Moreover, MM has not been previously associated with hepatic GSD or with impaired galactose utilization.

Searching for an explanation for the combination of the metabolic disorders in this highly consangineous family, the possibility of two distinct mutations cannot be excluded.

In order to elucidate the molecular mechanism of this complicated metabolic disorder it will be necessary to investigate the mitochondrial enzyme activities in similar patients and galactose and glycogen metabolism in MM patients.

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