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Reduced bone mineral density in glycogen storage disease type III: evidence for a possible connection between metabolic imbalance and bone homeostasis

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ABSTRACT

Introduction: Glycogen storage disease type III (GSDIII) is an inborn error of carbohydrate metabolism caused by deficient activity of glycogen debranching enzyme (GDE). It is characterized by liver, cardiac muscle and skeletal muscle involvement. The presence of systemic complications such as growth retardation, ovarian polycystosis, diabetes mellitus and osteopenia/osteoporosis has been reported. The pathogenesis of osteopenia/osteoporosis is still unclear.
Objectives: The aim of the current study was to evaluate the bone mineral density (BMD) in GSDIII patients and the role of metabolic and endocrine factors and physical activity on bone status.
Methods: Nine GSDIII patients were enrolled (age 2–20 years) and compared to eighteen age and sex matched controls. BMD was evaluated by Dual-emission-X-ray absorptiometry (DXA) and Quantitative ultrasound (QUS). Clinical and biochemical parameters of endocrine system function and bone metabolism were analyzed. Serum levels of the metabolic control markers were evaluated. Physical activity was evaluated by administering the International Physical Activity Questionnaire (IPAQ).
Results: GSDIII patients showed reduced BMD detected at both DXA and QUS, decreased serum levels of IGF-1, free IGF-1, insulin, calcitonin, osteocalcin (OC) and increased serum levels of C-terminal cross-linking telopeptide of type I collagen (CTX). IGF-1 serum levels inversely correlated with AST and ALT serum levels. DXA Z-score inversely correlated with cholesterol and triglycerides serum levels and directly correlated with IGF-1/IGFBP3 molar ratio. No difference in physical activity was observed between GSDIII patients and controls.
Discussion: Our data confirm the presence of reduced BMD in GSDIII. On the basis of the results, we hypothesized that metabolic imbalance could be the key factor leading to osteopenia, acting through different mechanisms: chronic hyperlipidemia, reduced IGF-1, Insulin and OC serum levels. Thus, the mechanism of osteopenia/osteoporosis in GSDIII is probably multifactorial and we speculate on the factors involved in its pathogenesis.

1. Introduction

Glycogen storage disease type III (GSDIII) is an autosomal recessive disease caused by deficient activity of glycogen debranching enzyme (GDE) [1]. It primarily affects the liver, heart and skeletal muscle and it is characterized by hepatomegaly, elevated serum concentrations of transaminases, hypoglycemia, hyperlipidemia, myopathy and cardiomyopathy [2]. Two major subtypes, based on difference in tissue expression, are recognized: GSDIIIa (85% of all GSDIII), resulting from GDE deficiency in both liver and muscle and GSDIIIb (15% of all GSDIII), resulting from GDE deficiency in liver [3,4]. Several systemic complications such as growth retardation [1], ovarian polycystosis [5], diabetes mellitus [6–8] and osteopenia/osteoporosis have been noted [9]. The

Abbreviations: GDE, Glycogen debranching enzyme; BMD, bone mineral density; DXA, dual-emission-X-ray absorptiometry; QUS, quantitative ultrasound; OC, osteocalcin; CTX, C-terminal cross-linking telopeptide of type I collagen; IPAQ, International Physical Activity Questionnaire.

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association of osteopenia and hepatic glycogenosis have been reported [10–13], but only a little is known about its pathophysiology in GSDIII. It has been proposed that osteopenia in GSDIII is multifactorial, playing a role the altered muscle physiology, abnormal metabolic environment and altered nutrition [9]. Different hormones and proteins cooperate in determining bone mineral density (BMD). Bone status is directly controlled by parathyroid hormone (PTH), calcitonin, osteocalcin (OC) and Vitamin D. However several other hormonal factors are known to interact to regulate bone metabolism: the GH-IGF-1 system [14], Insulin [15], thyroid hormones [16,17], sexual hormones [18,19] and adrenal hormones [20]. The effect of hyperlipidemia on bone resorption is also known [21,22]. Finally, the strict connection between muscle activity and BMD has been extensively reported: physical activity can improve bone mass acquisition and it is considered another regulating factor [23,24].

The aim of the present study was to investigate the presence of osteopenia/osteoporosis in patients with GSDIII and to study the role of the above-mentioned factors in its pathogenesis.

2. Subjects

Nine patients (6 males and 3 females, median age 10.43 ± 5.21 years) with biochemically or genetically confirmed GSDIII were enrolled. They were compared to eighteen age and sex matched controls. All patients were on dietary treatment. Dietary regimens varied among different patients according to their families' requests and attitudes. All patients received cornstarch supplementation both during the day and the night. The dose of cornstarch ranged between 0.50 and 0.80 g/kg/meal. Four patients also received protein supplementation (0.11–0.60 g/kg/day).

3. Methods

The study protocol was in accordance with the Italian regulations on privacy protection and with the Helsinki Doctrine for Human Experimentation. Informed consent to participate in the study was obtained from participants or their parents in the case of children under 16.

3.1. Clinical evaluation

Clinical evaluations included height, height Standard Deviation Score (SDS), growth velocity, weight, Body Mass Index (BMI), BMI SDS, pubertal stage. Total daily protein and calcium intakes were also assed using a questionnaire developed for a pediatric population.

3.2. Bone mineral density

BMD was studied using Dual-emission X-ray absorptiometry (DXA) and Quantitative ultrasound (QUS). DXA (Hologic QDR 1000, Hologic Inc., Waltham, USA) was measured at the L1-L4 vetrebrae, considering that the hip is not a reliable site for measurement in growing children. Z-scores were calculated by comparing BMD with age and sex matched reference values according to the manufacturer's internal reference database. In order to achieve an appropriate interpretation of densitometry data, DXA results must be analyzed in terms of relevant patient factors, including age, gender, height, weight, pubertal development and bone age, particularly in children with short stature or maturation delay. However to date there is no consensus regarding which of these adjustments methods is the best [25]. In the present study height, height SDS, weight, BMI, BMI SDS, growth velocity and pubertal stage were evaluated before considering bone age assessment. Four QUS probes were applied to the lateral surfaces of the fingers (II-V, non-dominant hand) in the proximity of the condyles using a DBM Sonic 1200 apparatus (IGEA, Carpi, Italy). The amplitude-dependent speed of sound (AD-SoS. m/s) was measured and expressed as a Z-score, on the basis of the standards provided by the manufacturer, derived from a large group of Italian subjects aged 2–21 years.

3.3. Hormonal studies

Blood samples were obtained at 8 a.m. The different endocrine axes were evaluated. The somatotropic axis was evaluated by analyzing basal plasma GH, IGF-1, IGFBP3 and free IGF1 (IGF-1/IGFBP3 molar ratio) levels. IGF-1 and IGFBP3 were measured by Enzyme-Linked Immunosorbent Assay (ELISA) commercially available kits (Mediagnost-Germany) in human plasma stored at −80 °C. IGF-1 was measured in non-extracted plasma, diluted 1:21. The interference dues to IGFBPs was eliminated by IGF-2 excess. The immunoassay was calibrated against the International Standard WHO NIBSC 02/254. The analytical sensitivity of the assay was 0.09 ng/mL, whereas the inter- and intra-assay coefficients of variation (CVs) were 6.8 and 6.7% respectively. IGFBP-3 was measured in 1:505 diluted plasma. The analytical sensitivity was 0.1 ng/mL. The inter- and intra- assay variation coefficients were found lower than 6.3 and 4.5% respectively. GH was measured by using immunoassay with commercially available kits. The thyroid profile was evaluated by analyzing TSH, fT3, fT4, T3, T4, thyroglobulin (Tg), thyroid antithyroglobulin antibody (ATA), anti-thyroid peroxidase antibodies (anti-TPO) serum levels. The adrenal function was evaluated by analyzing ACTH, cortisol, androstenedion, 17hydroxyprogesteron (17OHP), dehydroepiandrosteron sulphate (DEHA-S), renin, aldosteron serum levels and 24-h urinary free cortisol (UFC). The gonadotropic axis was evaluated by analyzing FSH, LH, 17β-estradiol, testosterone serum levels. Pancreatic function was analyzed by evaluating basal Insulin serum levels. Thyrotropic, adrenocorticotropic and gonadotropic hormones and Insulin serum levels were measured by using immunoassay with commercially available kits.

3.4. Biochemical markers of bone metabolism

Bone metabolism was studied by evaluating serum calcium, phosphorus, alkaline phosphatase, PTH, calcitonin, 25-hydroxyvitamin D, C-terminal cross-linking telopeptide of type I collagen (CTX) and OC and urinary calcium/creatinine ratio (UCa/UCr). Fasting peripheral blood samples were obtained at 8 a.m. and were collected on plastic tubes containing a cloth activator. The serum was obtained by centrifugation at 900 g and immediately stored at −20 °C until used. CTX were measured using a serum crosslaps ELISA kit (Immunodiagnosticsystems, Frankfurt, Germany) and OC was measured using a Microvue Osteocalcin ELISA kit (Quidel Corporation, San Diego, CA, USA). ELISA tests were automated on a DSX system (Dynex technologies-Technogenetics-Milan-Italy) following the manufacturer's instructions. The other markers of bone metabolism were measured by using immunoassay with commercially available kits.

3.5. Biochemical studies

Blood samples were obtained at 8 a.m. Fasting time ranged between 4 and 9 h. Serum glucose, cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides, AST, ALT, creatine kinase (CK) (only in GSDIIIa patients) were considered markers of metabolic control as well as morning urine ketones.

3.6. Physical activity

Physical activity was evaluated by administering the International Physical Activity Questionnaire (IPAQ) [26]. Data were scheduled considering for each patient the time spent experiencing different kind of activity (vigorous-intensity, moderate-intensity, walking) and the time spent sitting every day during the previous week.

3.7. Statistical analysis

All data mentioned in the text or shown in the figures are expressed as mean ± SD. Statistical analysis was performed using Statistical