ORIGINAL ARTICLE

Bone mineral density in patients with classic galactosaemia

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Background: Diminished bone mineral density (BMD) is a well known complication in women with classic galactosaemia caused by premature ovarian failure. Diminished BMD in prepubertal patients of either sex has, however, only been reported once.
Aim: To assess BMD in children with classic galactosaemia.
Methods: Eleven treated patients (five males, six females, aged 2–18 years) had BMD determined by dual energy *x* ray absorptiometry. Two measurements were performed, an areal measurement of the total body and a volumetric measurement of the femoral neck. Results were expressed as Z scores. Dietary calcium intake, blood calcium, phosphate, vitamin D, parathormone, and markers of bone formation (bone alkaline phosphatase, osteocalcin) and bone resorption (NTX) were determined.
Results: All patients had a significantly diminished BMD. Mean Z score of the volumetric BMD was –1.76 (range –0.7 to –3.3), and of the areal BMD –0.99 (range –0.5 to –1.4). Dietary calcium intake and calcium, phosphate, parathormone, bone alkaline phosphatase, vitamin D metabolites, and osteocalcin (free and carboxylated) were normal in all patients. NTX levels in blood were significantly lower ($p < 0.001$) than in control subjects.
Conclusion: BMD in this group of children of both sexes with classic galactosaemia under dietary treatment was decreased. Lower NTX levels in galactosaemics point to an apparent decreased bone resorption.

Classic galactosaemia is an inborn error of metabolism caused by a near complete deficiency of galactose-1-phosphate uridyltransferase enzyme activity. The gene coding for this enzyme is located on chromosome 9. One of the most prevalent mutations is the Q188R mutation. The mode of inheritance is autosomal recessive. Treatment consists of a galactose free diet. Despite dietary treatment, delayed speech development, learning disabilities, impaired motor function, and premature ovarian failure are known late complications of patients with classic galactosaemia. Diminished bone mineralisation in female patients as a result of ovarian insufficiency is well known. However, abnormalities in bone density in treated prepubertal galactosaemics of either sex has only been reported once.[1] These findings suggest that treated galactosaemic children might be at risk of abnormal bone mineralisation.

The bone mineral content in children can be assessed by dual energy *x* ray absorptiometry (DXA) which is low in radiation dose, low in cost, accessible, and easy to use. The bone density measured in this manner is expressed in g/cm^2 (areal density). The use of this technique in children is rather limited because it cannot account for the changes in skeletal size that occur during childhood. Quantitative computed tomography (QCT) can measure volume and bone density without being influenced by skeletal size. The cost and the reduced accessibility of CT scanners limits its use for bone measurements. Recently "volumetric" DXA measurements have been performed at the mid femoral shaft and the femoral neck; these were found to be less dependent on skeletal size, making it a more valid method to assess BMD in children.[2 3]

Important factors which are known to influence BMD are physical activity, nutrition, hypogonadism, and mineral and vitamin status (D and K). The role of vitamin D in bone metabolism has been known for many years. The importance of vitamin K for bone mineralisation has become clear in the past decade.[4–6] Vitamin K acts as cofactor in the post-translational carboxylation of osteocalcin which has a regulatory role in the mineralisation and remodelling of bone. Carboxylation makes this protein capable of binding to calcium, and the ratio between the serum fraction of undercarboxylated osteocalcin (ucOC) and fully carboxylated osteocalcin (cOC) reflects the vitamin K status of bone tissue. Measurement of known parameters involved in bone formation and resorption, including osteocalcin, vitamin K, and type I collagen aminotelopeptide (NTX) has not been performed in galactosaemic patients.

A diminished BMD in childhood might predispose classic galactosaemia patients to osteoporosis and fractures in adult life.

The aims of this study were: (1) to measure BMD in children with classic galactosaemia on dietary treatment, using areal and volumetric DXA measurements; and (2) to assess blood parameters involved in bone formation and resorption such as calcium, phosphate, parathormone, bone alkaline phosphatase, vitamin D metabolites (25-hydroxy and 1,25-dihydroxy vitamin D), osteocalcin, and NTX levels. The ratio ucOC:cOC was assessed to evaluate the vitamin K status of bone tissue.

METHODS

Patients

Eleven children with classic galactosaemia on dietary treatment were included in the study. Table 1 lists patient characteristics. All but one patient had been diagnosed in the

Abbreviations: BAP, bone alkaline phosphatase; BMD, bone mineral density; cOC, carboxylated osteocalcin; DXA, dual energy *x* ray absorptiometry; NTX, type I collagen aminotelopeptide; QCT, quantitated computed tomography; ucOC, uncarboxylated osteocalcin

Table 1 Characteristics of study group

Patient no.	Age (y)	Gender	Weight (kg)	Height (cm)	Enzyme activity (μmol/uur/g Hb)	Mutations
1	18.0	M	64	175	0.2	Q188R/Q252H
2	14.6	M	53	182	n.d.	n.p.
3	12.8	M	42	156	1.4	n.p.
4	10.8	F	28	132	n.d.	Q188R/Q188R
5	9.3	F	27	133	1.3	Q188R/Q188R
6	7.3	F	19	114	n.d.	Q188R/Q188R
7	7.1	F	20	129	4.5	L195P/K229N
8	7.1	F	20	129	2.7	L195P/K229N
9	4.3	F	15	103	1.2	L195P/?
10	3.4	M	14	95	n.d.	Q188R/L195P
11	2.5	M	13	86	n.d.	Q188R/L195P

n.d., not detectable; n.p., not performed.

neonatal period and had good dietary control. An 18 year old male had been diagnosed at age 16 years and showed severe psychomotor retardation.

Dietary calcium intake was assessed by our dietician by means of a standard questionnaire.

Biochemical measurements

Parameters in blood known to be involved in bone formation and resorption were assessed, and DXA performed on the same day.

Calcium, phosphate, parathormone, bone alkaline phosphatase, and vitamin D metabolite levels (25-hydroxy and 1,25-dihydroxy vitamin D) were determined according to standard methods and compared to the reference ranges.

Total immunoreactive osteocalcin was measured with the h-Ost EASIA kit from BioSource (Nivelles, Belgium); carboxylated and undercarboxylated osteocalcin fractions were determined using the conformation specific EIA kits from Takara Shuzo (Tokyo, Japan). Serum NTX levels were assessed with the ELISA from Ostex International (Seattle, Washington), and intact parathormone with the ELISA from Sangui Biotech (Santa Ana, California).

Bone specific alkaline phosphatase was determined with the Alkphase-B kit from Metra Biosystems (Mountain View, California).

Concentrations in the patients were compared with those of 19 healthy children, age and sex matched, available at our laboratory.

Bone mineral density

The bone mineral density was assessed by DXA[7] (Model DPX-L, Lunar Radiation Corp., Madison, Wisconsin, USA) using an areal measurement of the total body and a volumetric measurement of the femoral neck. Total body BMD results were expressed in Z scores and compared to the reference population. Z score for areal bone density measurements could not be calculated for children under 5 years of age (software version 4.7). Using this measurement only six patients could be analysed.

Volumetric BMD was measured as reported previously.[3] Briefly, the shape of the femoral neck was regarded as a cylinder. The diameter and the height were used for volumetric BMD, which was expressed as g/cm^3. Mean and standard deviation of femoral neck BMD measured in 40 young adults in our department corresponded exactly (mean 0.41, SD 0.06 g/cm^3) to values reported for both children and young adults.[3] We consequently expressed volumetric results in Z scores, making use of the above mentioned mean and SD. Weight and height Z scores of the study group were calculated, making use of normal paediatric Dutch reference data.[8]

Statistical analysis

The Statistical Package for the Social Sciences (SPSS 7.5 for Windows package 1996, SPSS Inc., Chicago, Illinois, USA) was used for the analysis. Comparison of Z score results with the 0 mean reference value was made using the one sample test. The non-parametric Wilcoxon signed ranks test was used to compare results of galactosaemia patients with those of our control group. The non-parametric Kendall correlation coefficient was used for calculating the correlation between areal and volumetric BMD results and both height and age. Multiple regression analysis was used to evaluate the relation between both age and diagnosis on NTX results in galactosaemia and control subjects as a group.

RESULTS

Compared to the mean reference value of 0, mean areal BMD (total body) Z score was significantly decreased ($p < 0.05$), showing a value of –0.99, range –0.5 to –1.4. The volumetric BMD (femoral neck) Z score was significantly decreased

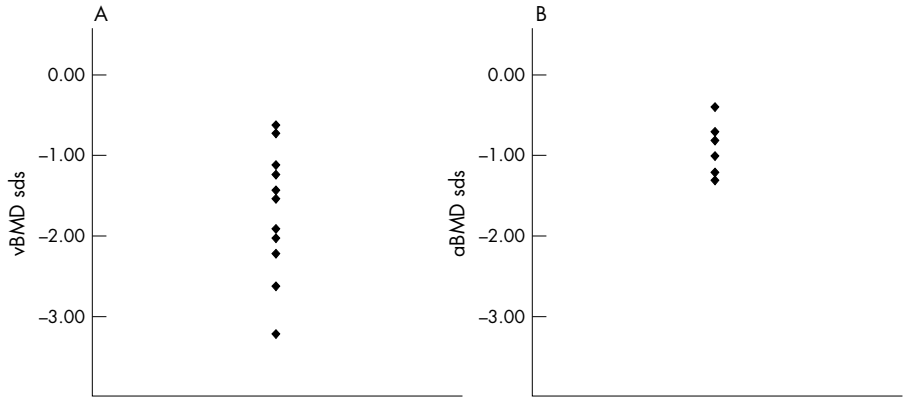

Figure 1 Results of BMD measurements in study group, expressed in Z scores. (A) BMD of the femoral neck. (B) BMD of the total body.

Table 2 Results for blood osteocalcin, BAP, and NTX in the study group and in healthy controls

Patient no.	Age (y)	ucOC (ng/ml)	cOC (ng/ml)	BAP (U/l)	NTX (nmol/l)
Study group					
1	18	30.7	6.0	130.7	24.3
2	14	30.2	11.3	146.9	62.7
3	12	29.1	18.9	161.5	60.5
4	10	28.1	18.4	132.4	47.0
5	9	29.1	15.6	94.6	54.9
6	7	28.0	14.1	127.2	46.0
7	7	21.5	13.6	99.2	42.0
8	7	19.8	13.1	92.8	42.4
9	4	29.9	14.4	132.4	53.3
10	3	26.8	11.2	107.2	36.5
11	2	24.9	16.4	95.0	61.8
Control group					
1a	9	49.3	25.3	149.3	65.8
2a	7	57.6	17.2	230.0	57.4
3a	10	26.2	13.6	132.3	54.0
4a	11	28.5	27.7	101.6	52.0
5a	7	24.7	16.9	63.4	52.5
6a	7	27.1	26.1	96.5	57.7
7a	13	84.0	23.1	166.7	148.7
8a	12	75.4	26.3	149.2	109.6
9a	6	39.1	29.9	128.0	126.3
10a	8	17.5	16.3	94.9	87.1
11a	9	21.3	10.7	130.8	100.1
12a	3	13.3	8.6	92.5	84.7
13a	6	50.2	12.8	76.6	105.3
14a	6	34.3	19.0	104.1	74.5
15a	13	12.8	5.8	163.0	71.4
16a	11	51.6	29.7	169.5	101.3
17a	12	61.8	25.3	140.0	113.3
18a	16	13.5	6.4	23.2	24.1
19a	3	63.3	10.9	104.6	99.2

ucOC, uncarboxylated osteocalcin; cOC, carboxylated osteocalcin; BAP, bone alkaline phosphatase; NTX, type I collagen aminotelopeptide.

($p < 0.001$) with a mean of −1.76, range −0.7 to −3.3. Figure 1 shows the results of the bone mineral density (areal and volumetric) of the study group, expressed in Z scores.

Mean Z scores for length and weight were slightly but significantly decreased to respectively −0.88 (range +1.00 to −2.60, $p < 0.05$) and −1.1 (range 0.04 to −2.00, $p < 0.05$). A significant positive correlation was found between areal BMD results (g/cm^2) and both age ($r = 0.83$) and height ($r = 0.88$). No correlation was found between volumetric BMD and either height or age.

The dietary calcium intake for all patients appeared sufficient for all subjects, mean 839 mg per day, range 500 to 1070 mg. There was no correlation between BMD results and dietary calcium intake.

Serum concentrations of calcium, phosphate, parathormone, and vitamin D metabolites were all within the normal laboratory reference values. Except for NTX, no significant differences between galactosaemia and control subjects were found for osteocalcin (ucOC and cOC), and bone alkaline phosphatase (table 2). NTX levels in blood were significantly lower ($p < 0.001$) than in control subjects. By multiple regression analysis using NTX as dependent variable and diagnosis and age as independent variables, the NTX values were found to be solely dependent on the diagnosis and independent of age.

DISCUSSION

The decreased BMD found in our patients with galactosaemia is in agreement with the earlier report which made use of quantitative computed tomography for the measurement of BMD. In our study DXA measurements have been used and both areal and volumetric BMD shown to be severely abnormal. Volumetric DXA measurement at both the mid femoral shaft and the femoral neck have been found to be practically independent of age, height, and gender. This makes standardisation for these variables unnecessary, resulting in easier interpretation of BMD data in children. Our mean volumetric BMD of −1.7 is severely abnormal when compared to reported results using the same methodology in children with PKU, chronic renal insufficiency, and chronic asthma,[2] and correspond to the Z scores found by quantitative computed tomography in galactosaemia.[1]

The lack of correlation between volumetric BMD and either age or length confirms that these variables are independent, in agreement with previous reports.[2 3] This was also shown in our young adult group (unpublished results).

Other than the fact that these children follow a diet, no known risk factors apply for this group as the six females included in this study were prepubertal. All children had a normal physical activity except for the late diagnosed patient who is neurologically severely affected. Blood parameters of importance for bone formation were in the normal range. Dietary calcium may have been inadequate earlier in life. To our knowledge osteocalcin has not been investigated before in galactosaemic patients.

Surprisingly, NTX was significantly lower in the galactosaemic group. Kaufman and colleagues[1] suggested an intrinsic defect in collagen, the major component of bone matrix. Collagen contains uridine diphosphate galactose, a galactose residue which might be decreased because of the deficient activity of galactose-1-phosphate uridyl transferase in galactosaemics. This could lead to abnormal collagen formation and interference in bone formation, mineralisation, and/or resorption. The lower levels of NTX in these patients might be the result of different digestion of the abnormal collagen, leading to fragments with a distinct immunological reactivity. Possibly, the NTX fragments in these patients are not recognised with the ELISA test used. Sugars are very immunogenic groups, and a different sugar composition in these patients' collagen could change the immunogenic reactivity.

Another possibility is that the lower bone resorption is an adaptive mechanism to protect bone integrity. This seems unlikely, however, as bone formation markers were normal, pointing to a normal bone turnover.

The findings in this study strongly suggest that children with classic galactosaemia are at risk of decreased BMD. Further studies are necessary to elucidate the pathophysiological mechanism leading to the decreased BMD and to design therapeutic strategies. Studies are also needed to determine whether adults with galactosaemia have an increased number of fractures.

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