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Evaluation of the bone repercussions of hyperlactacidaemia in patients with hereditary metabolic diseases

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Evaluation of the bone repercussions of hyperlactacidaemia in patients with hereditary metabolic diseases

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Abstract

Introduction: Children and adolescents with inborn errors of metabolism (IEM) are at risk for osteopenia. Preventive measures prior to reaching peak bone mass is critical.

We aimed to evaluate the changes in bone mineral density (BMD) and other parameters of bone assessment in children and adolescents with chronic hyperlactacidaemia.

Methods: Patients were selected among those who had lactate levels measured at the Paediatric Outpatient Clinics of the Reference Centre of Inherited Metabolic Diseases- CHUC in a ten-year period (2006-2016). Inclusion criteria were plasma lactate levels above 2,1 mmol/L in three or more different visits and availability of bone DXA. Patients' files were reviewed, including diagnosis, special diet and other therapies, ambulation, biochemical plasma and urine parameters of bone metabolism and BMD.

Results: Chronic hyperlactacidemia was disclosed in 43 patients, corresponding to 8,1% of the patients with lactacidaemia evaluated in the study period. Bone densitometry was available in 33 patients (18 boys) with chronic hyperlactacidaemia (76,7%). Eight patients (24,2%) had mitochondrial disease, seven (21,2%), glycogen storage disease type 1, five (15,2%), aminoacidopathy/organic aciduria and three (9,1%), other inherited metabolic disorders. Ten patients (30,3%) had suspected IEM. Mean ages at first lactate evaluation and at the end of the study period were 4,1±4,3 and 12±5,7 years-old, respectively. Median lactacidaemia was 3,2±2,4mmol/L. Fourteen individuals (42,4%) displayed acidosis. Most the patients presented normal values of plasma parameters, except for vitamin D, which was low in 62,5% of 24. Sixteen patients (48,0%) exhibited lumbar spine BMD *Z-Score* SD ≤-2. No significant association was found between BMD and lactacidaemia or pH levels.

Discussion:

This cross-sectional retrospective study included 33 patients with chronic hyperlactacidaemia and an IEM genetically proved or suspected. High lactate levels, lowering pH, causes

bone reabsorption and inhibits osteoblastic activity. DXA BMD exam is a well-recognized tool for bone investigation in Paediatrics.

No statistically significant correlations were found between BMD lactacidaemia or pH. Nevertheless, almost half of the patients presented lumbar spine BMD *Z-scores* ≤ -2 SD.

As in the general population, Vitamin D deficiency/insufficiency was common in the study group, although many patients were under supplements.

Analysis of the biochemical phospho-calcium metabolism plasma and urine parameters results was inconclusive.

Conclusion:

Although the present investigation pointed to a deleterious effect of hyperlactacidaemia on BMD, a statistical association could not be proved. This was a preliminary step to address the effects of chronic hyperlactacidaemia on bone heath in the field of paediatric IEM.

Key words

Hyperlactacidaemia, lactate, bone, paediatrics, inborn errors of metabolism

Abbreviations

AA-AO – Aminoacidopathy/organic aciduria

BMD – Bone mineral density

CHUC – Centro Hospitalar Universitário de Coimbra

DXA – Dual-Energy X-ray Absorptiometry

DXP – Urinary deoxypyridinoline

GSD1 – Glycogenosis type 1

HP – Hospital Pediátrico

IEM – Inborn Errors of Metabolism

MIT – Mitochondrial disease

OMD – Other metabolic disorder

SIMD – Suspicion of inherited metabolic disease

Background

Inborn errors of metabolism (IEM) are a protean group of monogenic disorders caused by a defect in a specific metabolic pathway (1). They are classically due to enzyme defects, with the block or modification of the pathway.

Lactic acid is the final by-product of anaerobic glycolysis, derived from pyruvate. Hyperlactataemia is a rather common situation, associated to hypoxia of any cause. Several IEM occur with hyperlactataemia, due to primary or secondary intra-mitochondrial energy production defects.

Bone health is dependent on a narrow balance between bone matrix protein formation with deposition of hydroxyapatite crystals and bone reabsorption.

As peak bone mass is established during the growing years, the prevention of adult osteoporosis is a paediatric issue. Especially, it should be a matter of concern in the follow-up of children and adolescents with chronic diseases whose pathophysiology and/or the therapeutic measures put them at an increased risk for osteopenia.

Several clinical and laboratory findings may indicate interfere with bone health, such as level of physical activity, calcium, vitamin D and parathormone (PTH) levels, and measuring those parameters are a reliable way of study (2–5). In recent years, dual-energy x-ray absorptiometry (DXA) has been stated as highly appropriate and a well-validated way for the study of bone health in paediatric patients through the evaluation of bone mineral density (BMD), being the lumbar spine the preferred measured site (6,7).

Acidosis, namely chronic acidosis, has an adverse effect on bone metabolism, promoting osteoclasia. Also, bone proteins may act as a pH buffer, absorbing the excess protons, with consequent calcium phosphate release (8,9) .

Children and adolescents with IEM, especially those with chronic acidosis, subjected to protein-restricted diets and/or with restricted physical activity are at risk for osteopenia.

The evaluation of bone density, with preventive and /or therapeutic measures taken prior to reaching peak bone mass, is critical in counteracting complications in adulthood (10).

This work is based on the hypothesis that the drop on pH levels due to the chronic excess of circulating lactate leads to changes in bone metabolism with clinical repercussions, namely osteopenia, with higher risk of bone pain and/or pathological fractures. (11)

The main objectives of this project are to evaluate the changes in BMD in children and adolescents followed at the CHUC Reference Centre of Inherited Metabolic Diseases Outpatient Clinics, in whom hyperlactataemia was detected in three or more different occasions, and to understand other relevant factors underlying the ultimate findings.

Materials and Methods

Patients followed at the Paediatric Outpatient Clinics of the Reference Centre of Inherited Metabolic Diseases- CHUC, who had their lactate levels measured in samples collected at ambulatory care were selected from the database of the Biochemistry Laboratory of the Paediatric Department - CHUC. The period of the study was between June 1st, 2006 and May 31st, 2016 (ten years).

Hyperlactacidaemia was defined as plasma lactate levels above 2,1 mmol/L for all ages. Levels above 5 mmol/L were considered severe hyperlactacidaemia (12). Elevated lactate levels observed in at least three separate occasions (visits) were classified as chronic hyperlactacidaemia.

Inclusion criteria were plasma lactate levels above 2,1 mmol/L detected in three or more different occasions and availability of bone densitometry study results.

Patients' files were retrospectively reviewed, comprising the following aspects: gender, current age (as of December 31st, 2016) and ages at first and last determination of blood lactate, main diagnosis, ambulation, type of diet and therapies, specifically anti-epileptics (sodium valproate), calcium and vitamin D supplements, lactate levels profile, blood gases, plasma levels of calcium, phosphorus, alkaline phosphatase, parathyroid hormone (PTH), calcitonin and 25(OH)D-vitamin, urinary calcium, phosphorus and deoxypyridinoline and bone densitometry parameters.

In patients with more than one bone densitometry exam, only the first was analysed. Data concerning ambulation, type of diet, therapies and analytic parameters were obtained contemporarily to the bone densitometry, during routine clinic visits.

Plasma lactate was measured by usual spectrophotometric methods. Other biochemical parameters were performed per standard methods (13).

Patients were categorized per their diagnostic group. Each was classified as ambulant or non-ambulant, per the capacity of independent walk. Dietary intake was classified as normal or specific diet (protein restricted; lipid restricted; carbohydrate supplemented; ketogenic). Acidosis and alkalosis were defined as blood pH levels under 7,35 and above 7,45, respectively. Vitamin D levels were classified as adequate (>30 ng/ml), insufficient (20-30) or deficient (<20) (14). BMD was evaluated by DXA. Normal BMD, matched by age and sex and expressed as a *Z-Score*, was defined as a *Z-Score* above -2.0 (15–18).

Statistical analysis

Data was recorded and coded on Microsoft Excel 2016® and further processed with *IBM SPSS Statistics software for Windows*® version 22 (Armonk, NY, USA: IBM Corp.). Normality tests were performed on all variables. The data description was based on the distribution of frequencies and measures of central tendency. Mean and standard deviation were used as measure of central tendency on continuous normally distributed variables. The median was used as measure of central tendency on non-normally distributed variables.

A chi-square test of independence was performed to examine the relation between pH and lactate levels, between vitamin D levels and its supplementation and between bone densitometry data and pH levels, median lactate levels, vitamin D categories and level of ambulation, in the total cohort and by main diagnosis. A Student t-test was employed to assess whether a difference existed between densitometry data of ambulant group and non-ambulant group.

A probability of type I (α) error of 0,05 was considered in all inferential analyses.

Results

During the period of the study (ten years), lactate plasma levels were determined on 1247 samples from 528 patients, collected at the Outpatient Metabolic Clinic, with a median of one sample per patient (1–23). Blood lactate levels varied from 0,7 to 16,9 mmol/L, with a median of 1,7±1,7 mmol/L.

Forty-three patients (8,1%) presented chronic hyperlactacidemia (lactate levels above 2,1 mmol/L in at least three different visits). Bone densitometry analysis was performed in 33 of the 43 patients with chronic hyperlactacidaemia (76,7%).

The files of the 33 patients with chronic hyperlactacidaemia and bone densitometry analysis available were retrospectively reviewed.

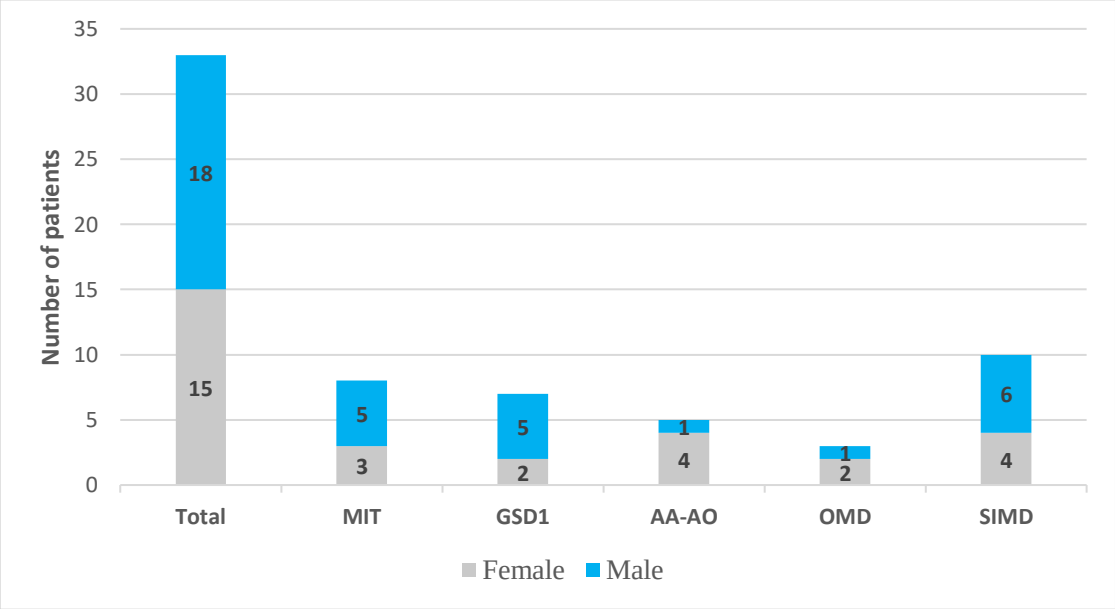

Figure 1 – Patients with chronic hyperlactacidaemia (N = 33): main diagnosis groups and gender distribution

MIT – Mitochondrial disease (N=8); GSD1 – glycogen storage disease type 1 (N=7); AA-AO – amino-acidopathy/organic aciduria (N=5); OMD – other inherited metabolic disorders (N=3); SIMD – suspicion of inherited metabolic disease (N=10);

The study group (33 patients) included 18 boys (54,5%) and 15 girls (45,5%) (Figure 1), with a mean current age of 12±5,7 years-old. Age at the first lactate determination varied from two weeks to 15-years-old, with a mean age of 4,1±4,3 years-old (Figure 2 and attachment 1). The age at last lactate determination ranged from two to 18-years-old, with a mean age of 8,3±5,1.

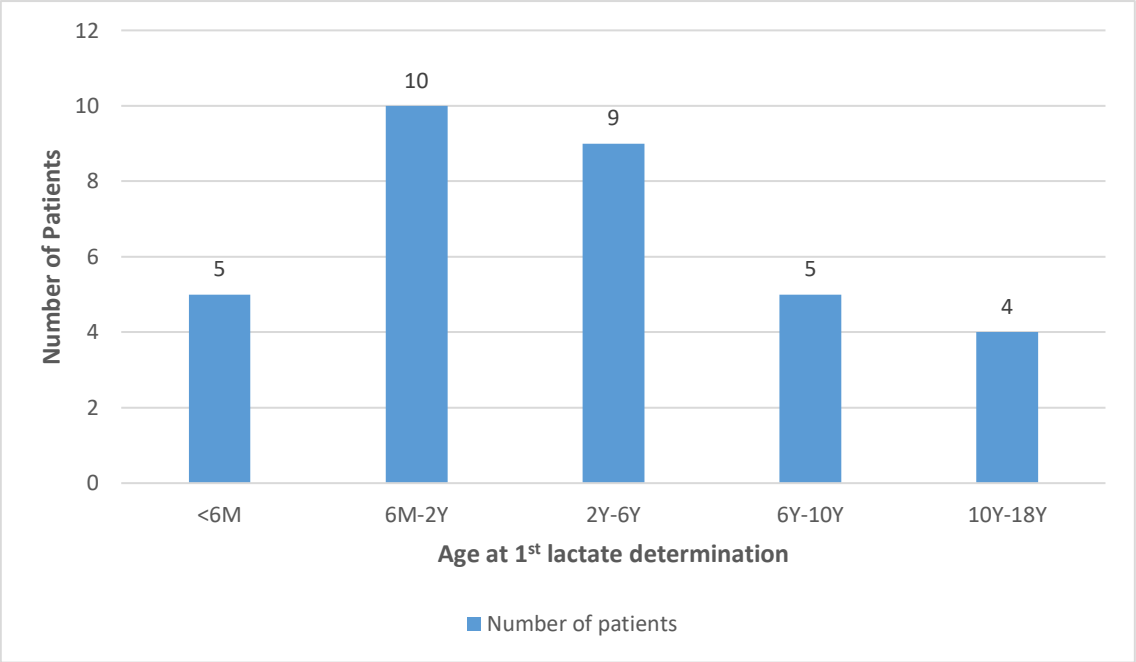

Figure 2 – Patients with chronic hyperlactacidaemia (N=33): age at first plasma lactate determination; M – Months; Y – Years

The cohort of patients with chronic hyperlactacidemia (33 patients) were divided in five groups, per their main diagnosis: eight (24,2%) with mitochondrial disease (MIT), seven (21,2%) with glycogen storage disease type 1 (GSD1), five (15,2%) with aminoacidopathy/organic aciduria (AA-OA), and three (9,1%) with other inherited metabolic disorders (OMD). Ten patients (30,3%) were being investigated due to suspected inherited metabolic disease (SIMD). (Figure 1)

The relevant clinical information is summarized in table 1 through 4. Five patients (four of the MIT and one of OMD groups) had no independent walk. Sixteen patients had

special diets: carbohydrate enriched (seven), protein restricted (five), ketogenic (three) and lipid restricted (one).

Table 1 – Patients with chronic hyperlactacidaemia: main clinical features – Mitochondrial disease (N=8)

Pt	*G*	*A*	*Diagnosis*	*Major clinical features*	*Ambulation*	*Diet*	*Supplements*	
1	F	16	Fumarase deficiency	Intellectual deficit	Yes	Normal		
2	M	9	Mitochondrial respiratory chain disorder	Motor delay, epilepsy	Yes	Normal	Ca	D
3	M	7	Mitochondrial respiratory chain disorder	Generalized hypotonia; tremor; psychomotor delay, apneas, growth delay	Yes	Normal		
4	M	6	Mitochondrial respiratory chain disorder	Generalized dystonia; psychomotor delay	No	Normal		
5	F	7	Mitochondrial respiratory chain disorder	Pancytopenia; steatorrhea; short stature; adrenal insufficiency; renal failure	Yes	Normal		
6	F	7	Pyruvate dehydrogenase deficiency	Hypotonia, psychomotor delay, strabismus	No	Ketogenic	Ca	D
7	M	6	Pyruvate dehydrogenase deficiency	Generalized dystonia; psychomotor delay	No	Ketogenic	Ca	D
8	M	6	Pyruvate dehydrogenase deficiency	Generalized hypotonia; psychomotor delay	No	Ketogenic		D

A – Age; G – Gender; Ca – calcium; D – vitamin D

Table 2 – Patients with chronic hyperlactacidaemia: main clinical features – Glycogen Storage Disease (GSD) (N=7)

Pt	*G*	*A*	*Diagnosis*	*Major clinical features*	*Ambulation*	*Diet*	*Supplements*	
9	M	18	Glycogen storage disease type 1a	Hepatomegaly; recurrent hypoglycemia; short stature	Yes	Carbohydrate enriched		
10	M	23	Glycogen storage disease type 1a	Hepatomegaly; recurrent hypoglycemia; short stature	Yes	Carbohydrate enriched	Ca	D
11	M	23	Glycogen storage disease type 1a	Hepatomegaly; recurrent hypoglycemia; short stature	Yes	Carbohydrate enriched	Ca	D
12	M	14	Glycogen storage disease type 1b	Hepatomegaly; recurrent hypoglycemia; short stature; recurrent infections	Yes	Carbohydrate enriched		
13	F	10	Glycogen storage disease type 1b	Hepatomegaly; recurrent hypoglycemia; short stature	Yes	Carbohydrate enriched	Ca	D
14	F	13	Glycogen storage disease type 1a	Hepatomegaly; recurrent hypoglycemia; short stature	Yes	Carbohydrate enriched	Ca	D
15	M	21	Glycogen storage disease type 1a	Hepatomegaly; recurrent hypoglycemia; short stature	Yes	Carbohydrate enriched	Ca	D

A – Age; G – Gender; Ca – calcium; D – vitamin D

Table 3 – Patients with chronic hyperlactacidaemia: main clinical features – Aminoacidopathies/Organic Acidurias (AA-OA) (N=5) and Other Metabolic Disorders (OMD) (N=3)

Pt	*G*	*A*	*Diagnosis*	*Major clinical features*	*Ambulation*	*Diet*	*Supplements*	
16	F	13	Glutaric aciduria type1	Macrocephaly	Yes	Protein restricted	Ca	D
17	F	15	3-Hydroxy-3 methylglutaric aciduria	Intellectual deficit; hypoketotic hypoglycemia; coma	Yes	Protein restricted	Ca	D
18	M	9	Methylmalonic aciduria	Metabolic acidosis, coma; renal failure	Yes	Protein restricted		D
19	F	5	Propionic aciduria	Psychomotor development delay; failure to thrive; coma	Yes	Protein restricted		D
20	F	9	Tyrosinemia type1	Learning disabilities	Yes	Protein restricted	Ca	D
21	F	4	Cobalamin deficiency	Nystagmus; retinopathy; coma	Yes	Normal		D
22	F	14	Long chain hydroxyacylCoA-dehydrogenase deficiency	Obesity; recurrent hypoglycemia, rhabdomyolysis crises	Yes	Lipid restricted	Ca	D
23	M	10	SLC35A2-CDG	Psychomotor development delay; epilepsy	No	Normal		

A – Age; G – Gender; Ca – calcium; D – vitamin D

Table 4 – Patients with chronic hyperlactacidaemia: main clinical features – Suspicion of inherited metabolic disease (SIMD) (N=10)

Pt	*G*	*A*	*Major clinical features*	*Ambulation*	*Diet*	*Supplements*	
24	M	20	Intellectual deficit; cardiomyopathy; growth retardation	Yes	Normal		
25	F	11	Epilepsy; intellectual deficit	Yes	Normal		
26	F	18	Intellectual deficit; epilepsy; retinopathy; ataxia	Yes	Normal		
27	M	3	Dysmorphisms; generalized hypotonia; encephalopathy	Yes	Normal		
28	M	11	Intellectual deficit; dysmorphisms	Yes	Normal		
29	M	11	Intellectual deficit; dysmorphisms; visual deficit	Yes	Normal		
30	F	15	Learning disabilities; strabismus	Yes	Normal		
31	M	3	Psychomotor development delay; hypotonia;	Yes	Normal		D
32	F	8	Intellectual deficit; failure to thrive; strabismus	Yes	Normal		D
33	M	13	Intellectual deficit; strabismus	Yes	Normal	Ca	D

A – Age; G – Gender; Ca – calcium; D – vitamin D

Nineteen patients were taking vitamin D supplements and 13 of these had oral calcium supplements. Five individuals (one MIT, one OMD and three SIMD) were treated with anti-epileptic drugs, of whom three with sodium valproate (two SIMD and the patient with GSD1).

Plasma lactate levels

In the study group (33 patients), lactacidaemia was evaluated in 263 samples, with a median of seven samples evaluation per patient (3–23). Blood lactate levels presented median levels of 3,2±2,4 mmol/L (range 1,1-16,9) (Attachment 1). Normal concentrations were detected in 65 samples (24,7%). Severe hyperlactacidemia was detected in 61 determinations (23,2%) in 20 different patients (Figure 3).

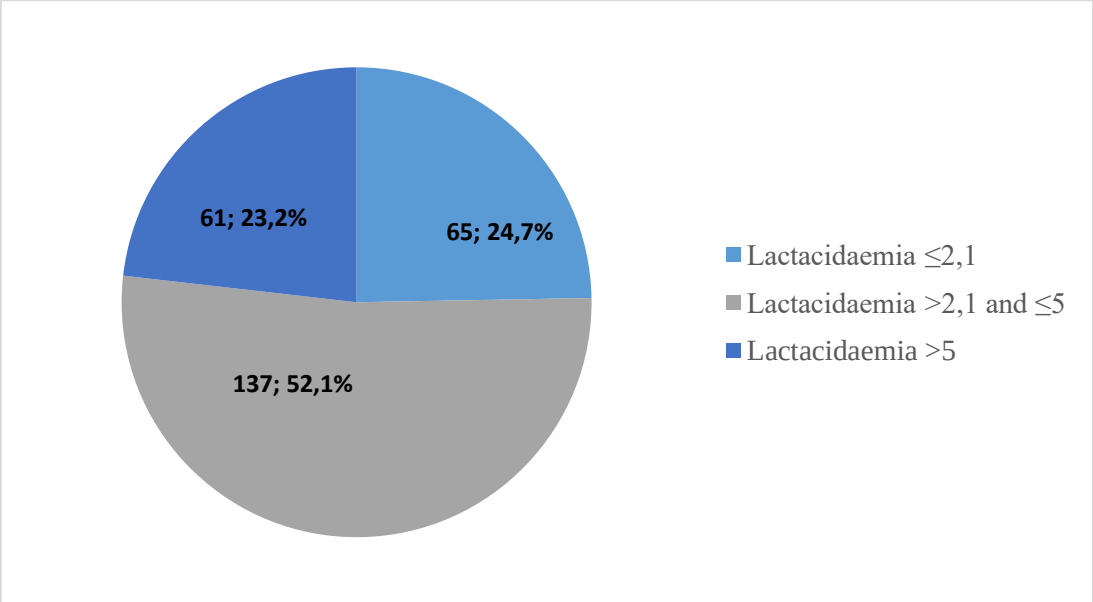

Figure 3 – Patients with chronic hyperlactacidaemia (N=33): lactate levels profiles, in the total cohort (N=263 lactate assessments).

In the MIT group (eight patients), severe hyperlactacidaemia was detected in 22 determinations (8,4%), in six different patients. Among the GSD1 group (seven patients), severe hyperlactacidemia was detected in 23 samples (8,8%), corresponding to six different patients. Among the AA-OA group (five patients), severe hyperlactacidaemia was detected in two occasions (0,9% of the samples) in one patient. Amid the patients categorized as OMD (three),

severe hyperlactacidemia was not detected. SIMD patients (ten) had severe hyperlactacidemia in 14 determinations (5,3%), in seven different patients. (Table 5)

Table 5 – Patients with chronic hyperlactacidaemia (N = 33): plasma lactate profiles in the main diagnosis groups

	Determinations				*Lactate levels*				
	N	Min.	Max.	Median	Min.	Max.	Mean	Median	SD
MIT	64	4	12	8	1,3	8,9	4,2	3,9	1,9
GSD1	70	3	18	11	1,1	16,9	4,6	4,2	3,0
AA-OA	53	2	23	8	1,1	8,6	2,7	2,4	3,0
OMD	15	4	6	5	1,4	4,9	2,7	2,4	0,9
SIMD	61	3	11	5	1,2	11,9	3,9	3,2	2,4

N – number of analyzed samples. MIT – Mitochondrial disease (N=8); GSD1 – glycogen storage disease type 1 (N=7); AA-OA – aminoacidopathy/organic aciduria (N=5); OMD – other inherited metabolic disorders (N=3); SIMD – suspicion of inherited metabolic disease (N=10).

Blood gases profile

In the study group, pH levels varied from 7,3 to 7,5, with a mean level of 7,4 ±0,1. Bicarbonate (HCO_3^-) concentration was 26,3±4,5, ranging from 11,3 to 33,4 mmol/L. Fourteen patients (42,4%) presented acidosis in, at least, one occasion (Figure 4). No significant association was found between pH and lactate levels (p=0,628). (Table 6)

Blood gases in the main diagnosis group are presented in table 7 and in detail in attachment 2. Acidosis was disclosed in three patients within the MIT group, one with GDS1, four with AA-OA, one with OMD and in five patients with SIMD (Figure 4).

Statistical significant correlation was not found between pH and lactate levels among the MIT group (p=0,375), the GSD1 group (p=0,429) and SIMD group (p=1,000). (Table 6)

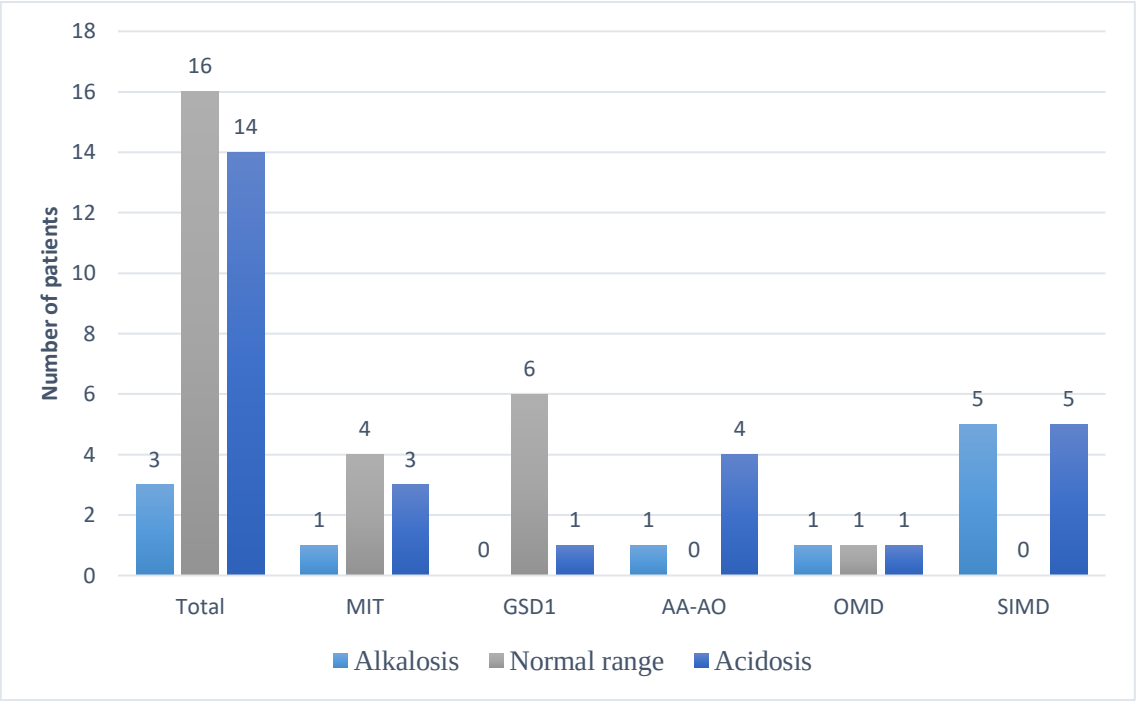

Figure 4 – Patients with chronic hyperlactacidaemia (N=33): pH profiles, in the total cohort and in the diverse diagnosis groups.

MIT – Mitochondrial disease (N=8); GSD1 – glycogen storage disease type 1 (N=7); AA-AO – aminoacidopathy/organic aciduria (N=5); OMD – other inherited metabolic disorders (N=3); SIMD – suspicion of inherited metabolic disease (N=10).

Other plasma parameters

The results of plasma creatinine, calcium, phosphorus, AP, PTH, calcitonin and 25(OH)D-vitamin evaluation are presented in table 8 and attachment 3 and 4. Most the patients presented normal values except for vitamin D.

Plasma calcium, evaluated in all patients was normal in 75,7% and elevated in six patients (18,2%). Five of the 6 patients with elevated plasma calcium also had vitamin D evaluation, varying from 6,1 to 23,1ng/ml. Three in the five hypercalcaemic patients also had hypercalciuria. Four of the nine patients with hypercalciuria presented high plasma calcium. Two had PTH measured, being high in one AA-OA case. Five patients with elevated calcium had vitamin D supplements associated with oral calcium in four. One of the two hypocalcaemic patients had calcium and vitamin D supplements.

Table 6 – Patients with chronic hyperlactacidaemia (N=33): Associations between Z-score of BMD and biochemical parameters

Main Diagnosis	***Biochemical parameters***	**p-value**
Total cohort	pH	1,000
	lactate	0,175
	calcium	0,398
	25(OH)D-vitamin	1,000
	PTH	0,217
	Ambulation	0,656
MIT	pH	1,000
	lactate	1,000
	calcium	*
	25(OH)D-vitamin	1,000
	PTH	1,000
	Ambulation	1,000
GSD1	pH	1,000
	lactate	1,000
	calcium	1,000
	25(OH)D-vitamin	1,000
	PTH	*
	Ambulation	*
AA-OA	pH	1,000
	lactate	*
	calcium	1,000
	25(OH)D-vitamin	1,000
	PTH	1,000
	Ambulation	*
SIMD	pH	1,000
	lactate	0,300
	calcium	*
	25(OH)D-vitamin	1,000
	PTH	*
	Ambulation	*

MIT – Mitochondrial disease (N=8); GSD1 – glycogen storage disease type 1 (N=7); AA-OA – aminoacidopathy/organic aciduria (N=5); SIMD – suspicion of inherited metabolic disease (N=10).
*Not calculated

Parathormone levels, studied in 25 (75,8%) patients, were elevated in two and low in one and calcitonin, in 19 (57, 8%) was elevated in ten. (Attachment 3 and 4)

Creatinine was elevated in two cases, one MIT and one with methylmalonic aciduria (AA-AO). Both patients had elevated levels of PTH.

Table 7 – Patients with chronic hyperlactacidaemia (N=33): blood gases profiles, in the total cohort and in the diverse diagnosis groups.

		Minimum	*Maximum*	*Mean*	*Median*	*SD*
MIT	pH	7,3	7,5	7,4	7,4	0,1
	HCO_3^-	22,5	33,4	27,5	28,2	3,5
GSD1	pH	7,3	7,4	7,4	7,4	0,0
	HCO_3^-	11,3	25,4	21,9	24,0	4,6
AA-OA	pH	7,3	7,5	7,3	7,3	0,1
	HCO_3^-	16,4	30,0	25,7	27,5	4,8
OMD	pH	7,3	7,5	7,4	7,4	0,1
	HCO_3^-	22,6	33,4	26,8	24,3	4,7
SIMD	pH	7,3	7,4	7,4	7,4	0,0
	HCO_3^-	25,4	32,2	28,4	27,7	2,0

MIT – Mitochondrial disease (N=8); GSD1 – glycogen storage disease type 1 (N=7); AA-OA – aminoacidopathy/organic aciduria (N=5); OMD – other inherited metabolic disorders (N=3); SIMD – suspicion of inherited metabolic disease (N=10); CO_2 (mmHg); HCO_3^-, BEb, BEecf (mmol/L)

Table 8 – Patients with chronic hyperlactacidaemia (N=33): other plasma and urine parameters profiles, in the total cohort.

		r.v.	*N**	*Min.*	*Max.*	*Mean*	*Median*	*SD*
Plasma	Creatinine	27-62	33	23,0	92,0	40,4	35,0	16,8
	Calcium	2,2-2,5	33	2,1	2,8	2,4	2,4	0,2
	Phosphorus	1,2-1,8	33	1,1	2,2	1,6	1,6	0,2
	AP	150-380	25	73,0	379,0	178,5	164,0	67,2
	PTH	9-72	23	11,0	404,0	53,6	33,0	80,8
	25(OH)D-vitamin	>30	24	14,0	46,0	26,4	24,5	8,8
	Calcitonin	<5	18	2,0	15,5	6,2	5,2	4,4
Urine	Calcium/Creatinine	≤ 0,7	24	0,0	2,2	0,7	0,5	0,5
	PRR	>85	18	88,5	98,2	92,4	91,2	3,3
	Deoxypyridinoline	3,0-7,4	18	11,1	36,6	22,9	23,0	6,6

N* – number of patients who had parameter tested; AP – Alkaline Phosphatase; PTH – Parathyroid Hormone; PRR – Phosphorus Reabsorption Rate

Creatinine, Calcium, Phosphorus (mmol/L); AP (UI/L); PTH, calcitonin (pg/mL); 25(OH)D-vitamin (ng/ml); Deoxypyridinoline (nmol/mmol creat); PRR (%)

Twenty-four patients (72,7%) had vitamin D evaluation. Six (25%) registered a deficient level, and nine (37,5%) presented an insufficient level (Figure 5). Vitamin D supplementation was present in 57,6% of patients. No significant correlation was found between vitamin

D levels and supplementation (p=0,589). No significant association was disclosed between PTH and vitamin D levels (p=1).

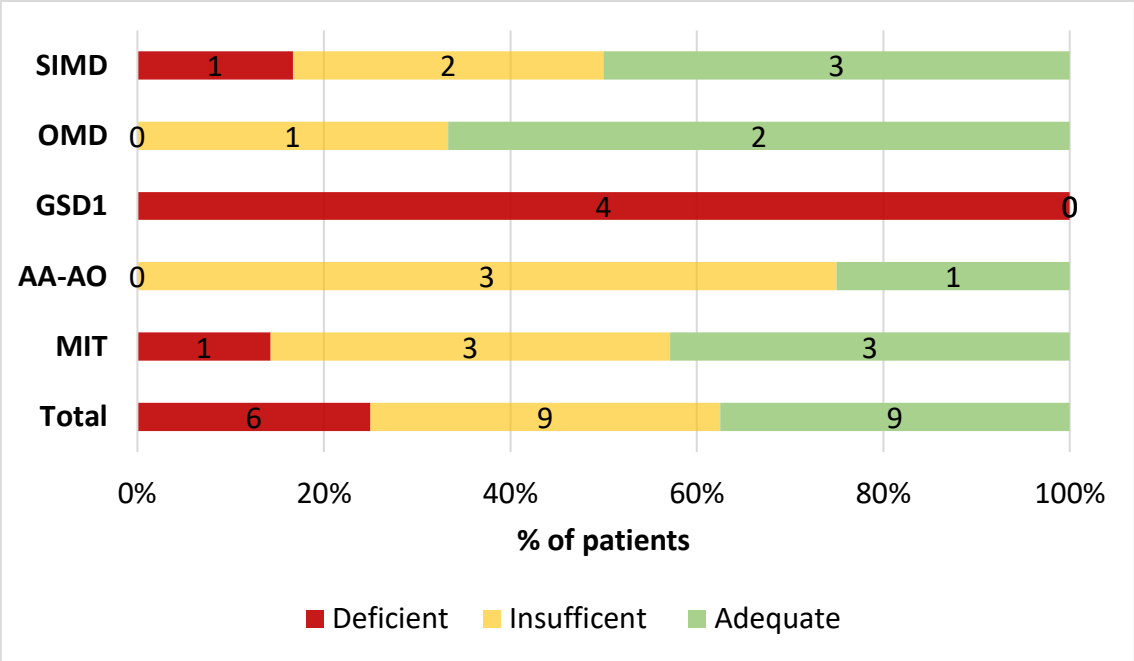

Figure 5 – Patients with chronic hyperlactacidaemia (N=25): vitamin D levels adequacy, in the total cohort and in the diverse diagnosis groups.
MIT – Mitochondrial disease (N=7); GSD1 – glycogen storage disease type 1 (N=5); AA-AO – aminoacidopathy/organic aciduria (N=4); OMD – other inherited metabolic disorders (N=3); SIMD – suspicion of inherited metabolic disease (N=6).

Plasma parameters of the patients per main diagnosis group are detailed in attachment 7 and 8. Deficient levels of vitamin D was found in one patient with MIT (out of 7 patients), in all four patients with GSD1 who had their vitamin levels tested and in one patient with SIMD. No deficient levels of vitamin D were discovered in the AA-OA or OMD groups (Figure 5).

Urine parameters

Urinary parameters were evaluated in random urine samples in most cases. PRR was normal in all (18) patients who had it evaluated.

Calciuria was evaluated in 24 patients (72,7%), with a median of 0,53 (0,02-2,2 mmol/ umol creat). Values were elevated (> 0,7 mmol/umol creat) in nine cases (37,5%) (attachment 3 and 4).

In 18 patients (54,5%), urinary deoxypyridinoline was measured (in nmol/mmol creat). In two cases, no reference range for age and gender were available. High levels for were depicted in the majority of the other patients (13; 81,3%) (attachment 3, 4 and 5).

The results of urine analysis and their distribution by diagnostic group are presented in attachment 6 and 7.

Bone densitometry

Evaluation of the BMD of lumbar spine and of femoral neck was performed in 33 and 20 patients, respectively. (Figure 6 and Attachment 8) The mean age at BMD assessment was 9,6±4,4 years-old (range 3–18). Sixteen patients (48,0%) presented lumbar spine BMD *Z-Score* SD ≤-2 (Figure 7) In five patients (25%) the femoral neck BMD *Z-Score* was SD ≤-2. (Attachment 8)

Only lumbar spine BMD results were further analysed.

Lumbar spine BMD *Z-score* ≤ -2 SD was found in five patients with MIT, five patients with GSD1, three patients with AA-OA, three patients with SIMD. All patients with OMD had a *Z-score* > -2 SD. (Figure 8)

No significant association between BMD and any of the covariates selected for analysis was found: pH values, lactacidaemia and plasma calcium levels (p=1,000, p=0,175, p=0,398, respectively); plasma 25(OH)D-vitamin (p=1,000); PTH levels (p=0,217); ambulation (p=0,656). A statistically significant difference was not found between mean BMD Z-score SD of ambulant group (-2±-2) and non-ambulant group (1,7±1), t(33)=-0,186, p=0,854. (Table 6)

No significant association between the variables compared to BMD were found within the groups. (Table 6)

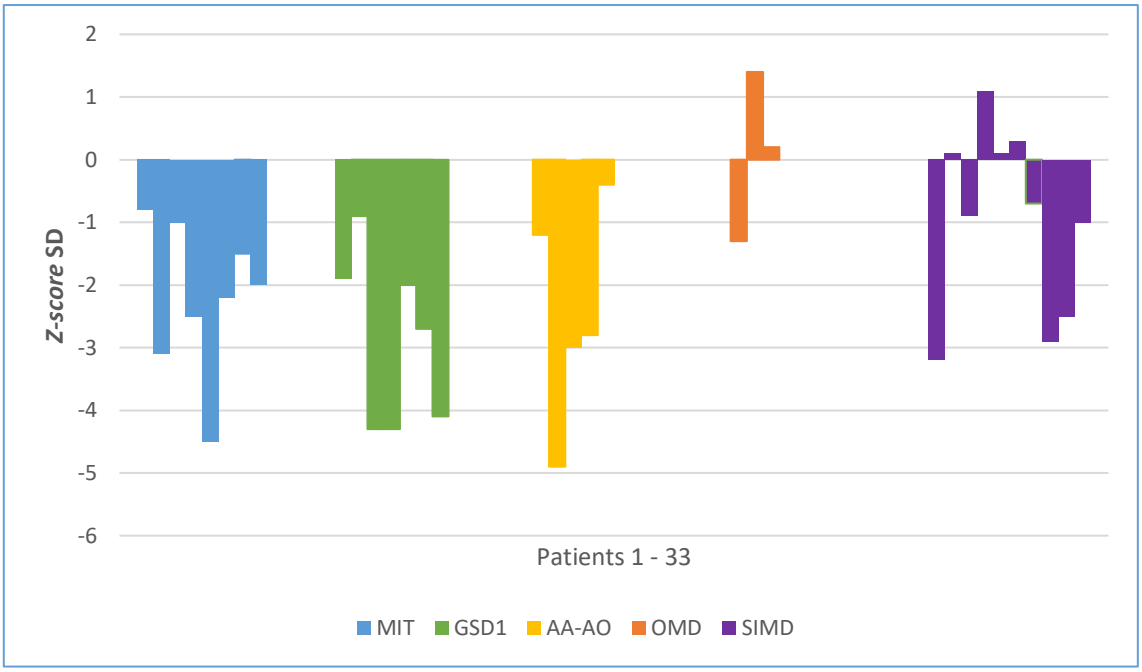

Figure 6 – Patients with chronic hyperlactacidaemia (N=33): lumbar spine bone densitometry results, in the diverse diagnosis groups
MIT – Mitochondrial disease (Pt 1-8); GSD1 – glycogen storage disease type 1 (Pt 9-15); AA-OA – aminoacidopathy/organic aciduria (Pt 16-20); OMD – other inherited metabolic disorders (Pt 20-23); SIMD – suspicion of inherited metabolic disease (Pt 23-33)

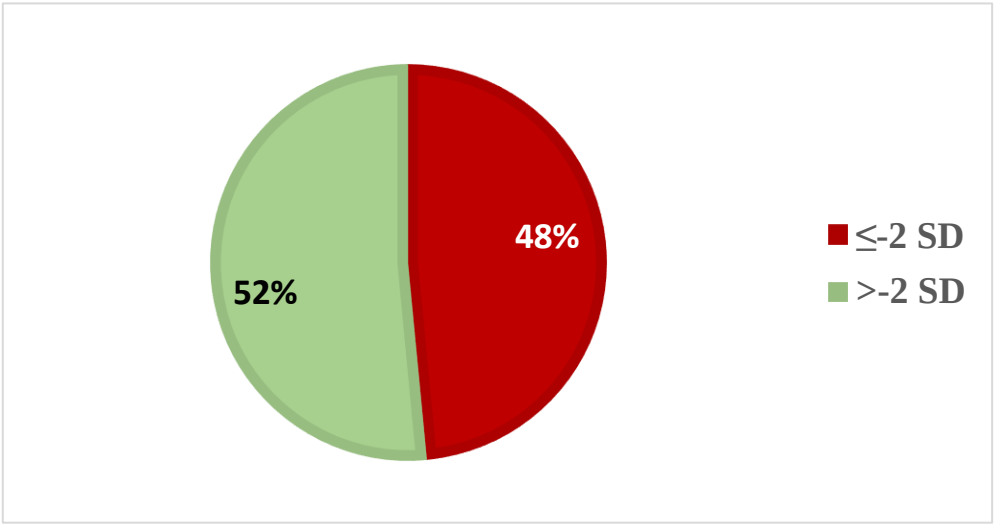

Figure 7 – Patients with chronic hyperlactacidaemia (N=33): lumbar spine bone densitometry Z-score SD.

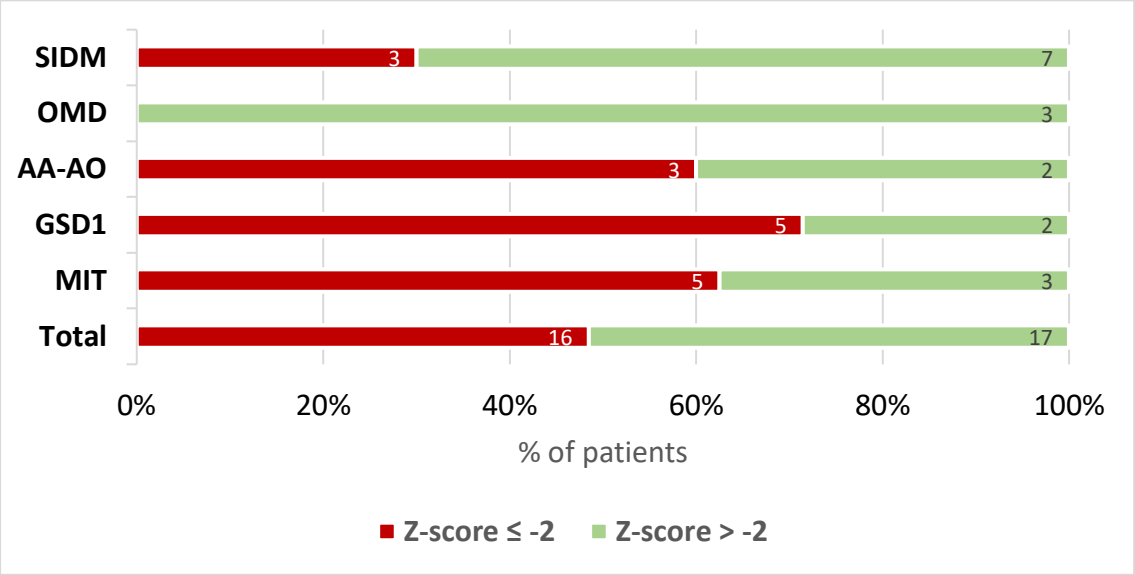

Figure 8 – Patients with chronic hyperlactacidaemia (N=33): lumbar spine bone densitometry Z-score SD, in the diverse diagnosis groups.
MIT – Mitochondrial disease (N=8); GSD1 – glycogen storage disease type 1 (N=7); AA-AO – aminoacidopathy/organic aciduria (N=5); OMD – other inherited metabolic disorders (N=3); SIMD – suspicion of inherited metabolic disease (N=10).

Discussion

The main objective of this cross-sectional retrospective study, that included 33 patients with chronic hyperlacticaemia and an IEM genetically proved or suspected, was to establish a correlation between lactate accumulation and bone disease.

Hyperlactacidaemia is a common finding both in intermediary metabolism inherited disorders and in patients suspected of IEM. Although high plasma lactate levels are frequently found in the context of acute disease states associated with tissue hypoxia, chronic hyperlactacidaemia is much rarer and points to a persistent disturbance in aerobic glycolysis, raising the possibility of an IEM. In this group of rare disorders, elevated lactate levels can be primary, due to direct excess pyruvate production (as in GSD1 patients) or accumulation (as in MIT patients) or secondary to an impairment of other intramitochondrial metabolic pathways (as in AA-AO patients). In many patients with IEM, the diagnosis process is a long one and a provisory diagnosis of SIMD is usual, as seen in 30% of the patients in this study. Interestingly, their mean and median lactate levels are similar to the other patients with a genetically confirmed IEM (table 5).

Lactate levels analysis is complex, due to methodological issues that go from sample collection and transport conditions to the plasma separation and results interpretation. This is critical in paediatric clinics due to the difficulty of young (and occasionally older) children's cooperation and the variation of reference range levels, which decreases with age. In fact, falsely high levels may emerge due to due to haemolysis induced by difficulty in blood collection or late plasma isolation. Likewise, it is advisable that tubes contain sodium fluoride, a glycolysis inhibitor, as anticoagulant. This problem was addressed in our hospital and a lactate protocol was established and reference values for the diverse age groups published. (19) Being this study retrospective, we can only admit that the protocol was followed, namely with avoidance of struggle and the use of tourniquets during venepuncture.

The cut-off value of 2,1mmol/L, regardless of age, seems adequate even the younger patients in our study, since the normal value for healthy control children under one year of age in our Hospital was 1,61 ± 0,35 (19)

On the other hand, the choice of lactate levels above 2,1mmol/L in at least three different visits precludes spurious lactate elevations. Woolf N et al. have used lactate levels above 2 mmol/L in three or more occasions as a minor criterion of mitochondrial respiratory disorders. (20,21)

It is recognized that numerous children with chronic illnesses are at high risk for osteopenia (2,12,20,21). Osteopenia in children and osteoporosis in adulthood are multifactorial syndromes. One of the factors is acidosis, even if extracellular pH is maintained in the normal range (8,9). In fact, bone acts a giant proton buffer and low pH causes mineral loss from bone by direct bone mineral dissolution, enhancement of osteoclast-mediated bone reabsorption, and inhibition of osteoblastic activity (8,19). This seems to be more important in high proton levels of metabolic origin (primary lowering of bicarbonate) than of respiratory causes (8).

We have hypothesized that patients with chronic hyperlactacidaemia, causing the drop on pH levels, were prone to osteopenia. In several IEM, such as AA-AO, other non-volatile organic acids may contribute to lower pH. The same goes for ketogenic diet treated patients (three patients) or patients under anti-epileptic drugs (five patients), namely sodium valproate (three patients). (22) The limited number of patients under those conditions in our cohort did not allow a thorough analysis of those factors' influence.

Although we have used only one blood gases evaluation per patient for the analysis, many (42,4%) were in an acidotic state, as expected. Also, almost half (48%) of the patient's cohort presented lumbar spine *Z-scores* ≤ -2 in the first bone DXA evaluation.

We have chosen the availability of bone DXA as a criterion for inclusion in the present investigation, since several studies report it to be a useful method for bone health evaluation, namely in children and adolescents. (2–5,23,6,7,24).

We have analysed exclusively the lumbar spine BMD, since the femoral study was available in a reduced number of patients, due to methodological issues, including diverse laboratory protocols and lack of reference values for the younger children.

We could not prove a statistically significant correlation between lactate accumulation and low BMD and thus bone disease in the selected patient cohort. In fact, no significant association was found, either between the pH and lactate levels (p=0,628), between BMD and pH levels (p=1) or, most importantly, between BMD and lactacidaemia (p=0,175).

Evaluation of bone health can be a most complicated process due to many known (and still unknown) contributing factors, such has diet, physical exercise, vitamin and hormonal status and genetic background. It is even more difficult in children and adolescents, were other influences like those associated to growth should be considered (25).

In our cohort, this task was even more complex due to the impact of chronic multisystem disease on bone health. This impact is well known, although not fully understood, in same disorders, like GSD1. (21) Interestingly, a PubMed search matching organic aciduria or mitochondrial respiratory chain disorders with osteopenia or bone density retrieved no results.

Physical activity and weight bearing is one of the main factors in establishing peak bone mass (3,21). It is important to notice that our retrospective review is limited by the inability to include more detailed information of activity level in our analysis, being the patients simply classified in two groups if ambulant (84,8%) or non-ambulant (15,2%). Mean BMD Z-score SD was not significantly different between ambulant group (-2±-2) and non-ambulant group (1,7±1; p = 0,8).

Many patients (16 patients) in our cohort were under special diets, namely those with protein restriction (five patients). In the context of IEM, the effects of protein restricted diets on bone have been addressed in phenylketonuria, which, contrary to many IEM of aminoacid catabolism causes no acidosis (20). As in our outpatient clinics we have multidisciplinary teams, which include nutritionist, at the time of the data collection, many of the patients had calcium (39,4%) and vitamin D (57,6%) supplements, among others, to adjust intake to estimated average requirements.

We have investigated other bone metabolism parameters besides BMD, namely plasma calcium, vitamin D, PTH and calcitonin levels. Contrary expectance, no significant association between BMD and any of these parameters was found. As occurs in the general population, vitamin D deficiency/insufficiency was frequent in the study group, although many patients were already supplemented.

Due to the clinical heterogeneity of the sample and the fact that some parameters have not been assessed in all patients, analysis of the phospho-calcium metabolism became a difficult and inconclusive task.

Hypercalcaemic patients had low vitamin D levels, contrary to what could be expected. On the other hand, only 37,5% of the patients had sufficient levels of vitamin D, even with supplementation. No significant correlation was found between vitamin D levels and supplementation (p=0,589). The relation between BMD and serum 25OHD concentration was non-significant (p=1,000), being the literature conflicting on whether or not this association exists (12,21).

PTH plays a vital part in calcium homeostasis and elevated PTH may lead to mobilization of calcium from bone, resulting in low BMD (7). PTH levels were elevated in two patients with moderate renal failure, who also had elevated plasma creatinine. In our

group of 25 patients with PTH results, no statistically significant relation between BMD and PTH levels was found (p=0,217).

A functional relationship between vitamin D and PTH is well established: this hormone is an important stimulator of vitamin D synthesis, via positive feedback, and vitamin D has a negative feedback on PTH secretion. (26) However, we found no significant association between PTH and vitamin D levels in the 20 patients in whom both were performed (p=1).

The analysis of the relation between the former plasma tests by each main diagnosis group was not applied in many due to the reduced size of the samples.

In the Outpatient Paediatric Clinics urine analysis is frequently done in randomly collected samples. Calciuria, measured in 72,7% of the patients, was elevated in more than one third (37,5%) of the individuals submitted to the analysis.

Hypervitaminosis D is a well-known cause of hypercalcaemia and hypercalciuria. In our cohort, none of those abnormalities could be attributed to it.

Deoxypyridinoline (DXP) in urine is one of the most reported markers of bone resorption. Its levels have been evaluated in in more than half of the patients in our study, with high levels in the majority (81%) pointing to an elevated osteoclastic activity. In two cases, we had no reference values for age. Furthermore, this results should be interpreted with caution, since children, due to the normal growing process of bone remodelling, have a high excretion of DXP (up to five times higher, compared to adults), and present a high variation throughout the day, which precludes its usefulness to bone health evaluation in clinical daily practice. (27–30) Due to those facts and not having controlled the type of urine sample analysed, we did not attempt to correlate this parameter to bone density and its meaning to bone health.

In this investigation, the absence of statistically significance correlations, contrary to some evidences could be due to several reasons, including: the small cohort and clinical heterogeneity of the sample; patients' good metabolic control, as hypothesized by others (21);

not all data being available due the retrospective investigation; the cross-sectional type of the study, some of the analytic parameters represent a single point in time, even though data was collected before or on the day of the DXA test, to prevent any influence of possible treatments on the BMD.

Although biochemical markers analysis and DXA are relevant, complementary methods of bone remodelling, the former seems to be an early indicator, emerging before changes in bone density can be determined by DXA. This time lag might have contributed to the non-significant results achieved in the present cross-sectional investigation.

The main limitation of our study was being a retrospective, cross-sectional analysis. The reduced size of the patients' cohort, associated to the rarity of each IEM, even if grouped, also contributed to make the statistical analysis poorly reliable. Likewise, the lack of a gender and age matched control group limited the final conclusions.

Conclusion

There have been significant advances in understanding of paediatric bone disease and its mechanisms and, thus, how to better monitor it. One major progress was the widespread availability of DXA and its validation for the paediatric population. Yet, still more research is needed for a greater consensus on the pathophysiology of low BMD and methods of study and treatment in the paediatric population. In fact, extensive studies concerning osteopenia in paediatric population are scarce. (23,25)

This investigation was a preliminary step to address the consequences of chronic hyperlactacidaemia on bone heath in the field of paediatric IEM. Its main limitation was being a retrospective, cross-sectional survey. The reduced size of the patients' cohort, associated to the rarity of each IEM, even if grouped, also contributed to make the statistical analysis poorly reliable. Likewise, the lack of a gender and age matched control group limited the final conclusions.

Further studies with larger and more homogeneous groups of patients are needed, since many factors must be considered for an adequate analysis. The scarce number of patients with each IEM in present study limited it. Although our investigation pointed to a deleterious effect of hyperlactacidaemia on BMD, a statistically significant association could not be proven. Since IEM are rare disorders, a multicentre study on chronic hyperlactacidaemia effects on bone heath is warranted. Contrary to the present one, it should be prospective, with focus on the major factors to be analysed, and longitudinal, since it comprises paediatric populations.

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References

1. Mak CM, Lee HCH, Chan AYW, Lam CW. Inborn errors of metabolism and expanded newborn screening: Review and update. Crit Rev Clin Lab Sci. 2013;50(6):142–62.
2. Ma NS, Gordon CM. Pediatric osteoporosis: Where are we now? J Pediatr. 2012;161(6):983–90.
3. Uziel Y, Zifman E, Hashkes PJ. Osteoporosis in children: pediatric and pediatric rheumatology perspective: a review. Pediatr Rheumatol Online J. 2009;7:16.
4. Ward LM, Konji VN, Ma J. The management of osteoporosis in children. Osteoporos Int. 2016;27(7):2147–79.
5. De Groot MJ, Hoeksma M, Van Rijn M, Slart RHJA, Van Spronsen FJ. Relationships between lumbar bone mineral density and biochemical parameters in phenylketonuria patients. Mol Genet Metab. 2012;105(4):566–70.
6. Binkovitz LA, Henwood MJ. Pediatric DXA: Technique and interpretation. Pediatr Radiol. 2007;37(1):21–31.
7. Sopher AB, Fennoy I, Oberfield SE. An update on childhood bone health: mineral accrual, assessment and treatment. Curr Opin Endocrinol Diabetes Obes. 2015;22(1):35–40.
8. Bushinsky DA. Acid-base imbalance and the skeleton. Eur J Nutr. 2001;40(5):238–44.
9. Brandao-Burch A, Utting JC, Orriss IR, Arnett TR. Acidosis inhibits bone formation by osteoblasts In Vitro by preventing mineralization. Calcif Tissue Int. 2005;77(3):167–74.
10. Buist NRM, Steiner RD, Grompe M, Harding CO, Harding CO, Enns GM, et al. Inborn errors of metabolism : biochemical genetics. In 2016.
11. Krapf R, Seldin DW, Alpern RJ. Clinical Syndromes of Metabolic Acidosis. Fifth Edit. Seldin and Giebisch's The Kidney. Elsevier Inc.; 2013. 2049-2112 p.

12. Seheult J, Fitzpatrick G, Boran G. Lactic acidosis: an update. Clin Chem Lab Med. 2016;
13. White R, Yaeger D, Stavrianeas S. Determination of Blood Lactate Concentration: Reliability and Validity of Lactate Oxidase-Based Method. Dep Exerc Sci Willamette Univ Salem, OR, USA. 2009;2(2):83–93.
14. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: An endocrine society clinical practice guideline. J Clin Endocrinol Metab. 2011;96(7):1911–30.
15. ISCD. Combined Official Positions. 2013;
16. Estrada A, Ramnitz MS, Gafni RI. Bone densitometry in children and adolescents. Curr Opin Obstet Gynecol. 2014;26(5):339–46.
17. Coakley K, Douglas T, Goodman M, Ramakrishnan U, Dobrowolski S, Singh R. Modeling correlates of low bone mineral density in patients with phenylalanine hydroxylase deficiency. J Inherit Metab Dis. 2016;39:363–72.
18. Geiger KE, Koeller DM, Harding CO, Huntington KL, Gillingham MB. Normal vitamin D levels and bone mineral density among children with inborn errors of metabolism consuming medical food-based diets. Nutr Res. 2016;36(1):101–8.
19. Diogo L, Drago H, Fernandes A, Carvalho G, Canha J, Mota H et al. Avaliação do Estado Redox Plasmático na Criança. Acta Ped Port. 1999;30(2):129–33.
20. Mendes AB, Martins FF, Cruz WMS, Da Silva LE, Abadesso CBM, Boaventura GT. Bone development in children and adolescents with PKU. J Inherit Metab Dis. 2012;35(3):425–30.
21. Badalà F, Nouri-mahdavi K, Raoof DA. Bone mineral density in glycogen storage disease type Ia and Ib. Genet Med. 2008;144(5):724–32.
22. Ułamek-Kozioł M, Pluta R, Bogucka-Kocka A, Czuczwar S. To treat or not to treat

drug-refractory epilepsy by the ketogenic diet? That is the question. Ann Agric Environ Med. 2016;23(4):533–6.

23. Eapen E, Grey V, Don-Wauchope A, Atkinson A. Bone health in childhood: usefulness of biochemcial markers. eJIFCC. 2008;19(2):1–14.

24. Kafadar I, Kilic B, Yilmaz F, Kilic M. Bone mineral density in pediatric patients with meningomyelocele. Child's Nerv Syst. 2016;32(1):111–9.

25. Szulc P, Seeman E, Delmas P. Biochemical measurements of bone turnover in children and adolescents . Osteoporos Int. 2000;11(4):281–94.

26. Khundmiri SJ, Murray RD, Lederer E. PTH and Vitamin D. Compr Physiol. 2016;6(2):561–601.

27. Shaw N, Dutton J, Fraser W, Smith C. Urinary pyridinoline and deoxypyridinoline excretion in children . Clin Endocrinol (Oxf) 1995 Jun;42(6)607-12.

28. Robins S, Woitge H, Hesley R, Ju J, Seyedin S, Seibel M. Direct , enzyme-linked immunoassay for urinary deoxypyridinoline as a specific marker for measuring bone resorption . J Bone Min Res. 1994;9(10):1643–9.

29. Crofton P, Kelnar C. Bone and collagen markers in paediatric practice . Int J Clin Pr. 1998;52(8):557–65.

30. Husain SM, Mughal Z, Williams G, Ward K, Smith CS, Dutton J, et al. Urinary excretion of pyridinium crosslinks in healthy 4 – 10 year olds. Arch Dis Child. 1999;80:370–3.

Attachments

Attachment 1 – Patients with chronic hyperlactacidaemia (N=33): lactate determinations

Pt #	*Present Age[A]*	*Age at first determination*	*Lactate concentration*			
			N	**Mean**	**Median**	**SD**
1	16	8 Y	4	4,65	5,00	2,89
2	9	1 Y	12	5,54	5,55	1,54
3	7	3 Y	5	2,72	2,80	0,48
4	6	1 Y	9	4,06	3,90	1,48
5	7	1 Y	12	4,70	4,55	1,03
6	7	1 Y	11	3,35	1,80	2,69
7	6	2 Y	4	2,58	2,70	0,63
8	6	1 Y	7	4,36	3,70	1,93
9	18	9 Y	6	3,67	3,65	1,28
10	23	13 Y	8	7,89	8,00	2,15
11	23	13 Y	12	5,58	5,40	2,62
12	14	4 Y	11	2,79	2,20	1,82
13	10	7 M	18	2,63	2,30	1,54
14	13	5 Y	3	12,73	11,40	3,69
15	21	11 Y	12	4,58	4,60	1,29
16	13	3 Y	3	3,00	2,50	1,14
17	15	9 Y	7	2,36	2,40	0,84
18	9	2 W	23	2,62	2,30	1,60
19	5	4 W	12	3,08	2,75	0,84
20	9	3 Y	8	2,35	2,00	0,93
21	4	1 M	4	2,90	2,40	1,35
22	14	4 Y	6	2,72	2,60	0,93
23	10	1 Y	5	2,48	2,70	0,67
24	20	15 Y	4	6,70	6,35	1,17
25	11	1 Y	5	3,80	3,60	0,78
26	18	8 Y	5	4,50	4,80	3,28
27	3	1 M	11	4,56	2,90	3,23
28	11	3 Y	3	4,70	4,20	1,80
29	11	1 Y	5	2,82	2,20	1,70
30	15	6 Y	5	3,80	2,80	2,99
31	3	4 Y	10	4,03	3,30	2,67
32	8	1 Y	4	3,23	3,25	0,50
33	13	5 Y	9	2,34	1,80	1,09

A –in years; Y – years; M – months; W – weeks; N – number of lactate determinations

Attachment 2 – Patients with chronic hyperlactacidemia (N=33): blood gases profile, in the diverse diagnosis groups

		Minimum	***Maximum***	***Mean***	***Median***	***SD***
MIT	pH	7,3	7,5	7,4	7,4	0,1
	CO_2	38,0	59,0	50,4	51,5	7,0
	HCO_3^-	22,5	33,4	27,5	28,2	3,5
	BEb	-2,6	9,6	3,2	3,6	3,6
	BEecf	-3,0	8,6	2,2	2,7	3,2
GSD1	pH	7,3	7,4	7,4	7,4	0,0
	CO_2	34,9	42,0	38,0	37,0	2,5
	HCO_3^-	11,3	25,4	21,9	24,0	4,6
	BEb	-5,6	0,0	-1,3	-1,0	1,8
	BEecf	4,9	1,0	-1,0	-1,0	1,7
AA-AO	pH	7,3	7,5	7,3	7,3	0,0
	CO_2	35,0	61,0	47,4	52,0	9,9
	HCO_3^-	16,4	30,0	25,7	27,5	4,8
	BEb	-10,3	3,6	0,0	2,1	5,2
	BEecf	-9,5	3,2	-0,9	1,3	5,0
OMD	pH	7,3	7,5	7,4	7,4	0,1
	CO_2	43,0	48,0	46,3	47,8	2,3
	HCO_3^-	22,6	33,4	26,8	24,3	4,7
	BEb	-4,1	9,6	1,5	-1,1	5,9
	BEecf	-4,4	8,6	1,0	-1,2	5,5
SIMD	pH	7,3	7,4	7,4	7,4	0,0
	CO_2	42,0	60,0	51,0	52,0	4,9
	HCO_3^-	25,4	32,2	28,4	27,7	2,0
	BEb	0,4	4,8	2,6	2,1	1,5
	BEecf	0,3	3,7	1,8	1,3	1,2

CO_2 – Carbon dioxide; $HCO3^-$ - Bicarbonate; BEecf/BEb – base excess and in the extracellular fluid compartment/ base excess;

MIT – Mitochondrial disease (N=8); GSD1 – glycogen storage disease type 1 (N=7); AA-OA – aminoacidopathy/organic aciduria (N=5); OMD – other inherited metabolic disorders (N=3); SIMD – suspicion of inherited metabolic disease (N=10); CO_2 (mmHg); HCO_3^-, BEb, BEecf (mmol/L)

Units: $HCO3^-$, BEecf/BEb (mmol/L); CO_2 (mmHg);

Attachment 3 – Patients with chronic hyperlactacidemia (N=33): Biochemical parameters

	MD	***Age[A]***	***Gender***	***L***	***pH***	***CO_2***	***HCO^{3-}***	***BEecf***	***BEb***	***Cr[B]***	***Ca^{2+}***	***P***	***AP[B]***	***PTH***	***Calcitonin***	***Vit. D***	***PRR***	***Ca/cr ur***	***DXP[B]***
	r.v.			≤2,1	7,35-7,45	35-45	22-26			27-62	2,2-2,5	1,2-1,8	150-380	9-72	<5	>30	>85	≤0,7	17,6 ±5
1	MIT	15	F	4,65	7,38	51	30,2	5,1	3,8	57	2,25	1,37		41	2	24	98,16	0,19	
2	MIT	9	M	5,54	7,34	55	29,7	3,9	2,6	27	2,39	1,72	131	22	2	21	90,94	0,81	31
3	MIT	7	M	2,72	7,29	59	28,4	1,8	0,4	31	2,46	1,72	133	21	4	24	92,62	0,38	20
4	MIT	6	M	4,06	7,41	44	27,9	3,3	2,7	42	2,44	1,36	155	32	10,7	14			11,1
5	MIT	6	F	4,70	7,46	47	33,4	9,6	8,6	92	2,06	1,66	137	404*					
6	MIT	6	F	3,35	7,40	38	22,5	-2,6	-2,3	23	2,37	1,61	98	4,8		41		0,20	
7	MIT	6	M	2,58	7,38	40	23,7	-1,4	-1,3	30	2,28	1,58	153	38	5,2	34	88,49	0,50	36,6
8	MIT	6	M	4,36	7,28	52	24,4	-2,3	-3	29	2,48	1,57	126	11	7,2	31			
9	GDS1	9	M	3,67	7,44	34,9	24	-0,4	1	26,5	2,56	1,24	213				96,85	1,58	
10	GDS1	16	M	7,89	7,36	35	19,8	-5,6	-4,9	40	2,6	1,34				16,6	98,16	0,85	
11	GDS1	17	M	5,58	7,41	37	23,5	-1,1	-1	34	2,63	1,38	214			14,1		1,16	
12	GDS1	7	M	2,79	7,37	42	24,3	-1	-1	30	2,3	1,5	164	33,1	15,5	19,4			27,6
13	GDS1	4	F	2,63	7,39	42	25,4	0,4	0,3	31	2,5	1,58	207						
14	GDS1	5	F	12,73	7,30	23	11,3	-15,1	-13,5	28	2,84	1,51	314	11,4		14,7			16,6
15	GDS1	15	M	4,58	7,40	40	24,8	0	0	31,1	2,71	1,51	272			6,1		0,60	

MD – Main Diagnosis; r.v. – reference range values; L – Lactate (median values); CO_2 – Carbon dioxide; $HCO3^-$ - Bicarbonate; BEecf/BEb – Base excess; Cr – Creatinine; Ca – calcium; P – Phosphorus; AP – Alkaline Phosphatase; PTH – Parathyroid Hormone; vit. D – vitamin D; PRR – Phosphorus Reabsorption Rate; DXP – Deoxypyridinoline

MIT – Mitochondrial disease (N=8); GSD1 – glycogen storage disease type 1 (N=7); AA-OA – aminoacidopathy/organic aciduria (N=5); OMD – other inherited metabolic disorders (N=3); SIMD – suspicion of inherited metabolic disease (N=10)

Units: Lactate, Bicarbonate, BEecf/BEb, Creatinine, Calcium, Phosphorus (mmol/L); CO_2 (mmHg); AP (UI/L); PTH, calcitonin (pg/mL); vit. D (ng/ml); PRR (%); Deoxypyridinoline (nmol/mmol creat)

A- Present age in years; B - reference range values for patients' group median age (9 years-old) (*) value before peritoneal dialysis

Attachment 4 – Patients with chronic hyperlactacidemia (N=33): Biochemical parameters (continuation)

	MD	Age[A]	Gender	L	pH	CO_2	HCO^{3-}	BEecf	BEb	Cr[B]	Ca^{2+}	P	AP[B]	PTH	Calcitonin	Vit. D	PRR	Ca/cr ur	DXP[B]
	r.v.			≤2,1	7,35-7,45	35-45	22-26			27-62	2,2-2,5	1,2-1,8	150-380	9-72	<5	>30	>85	≤0,7	17,6 ±5
16	AA-AO	12	F	3,00	7,33	52	27,5	1,5	0,5	42	2,37	1,55	213					0,27	
17	AA-AO	14	F	2,36	7,47	37	26,9	3,2	3,2	39	2,47	1,23	209	33		27			
18	AA-AO	8	M	2,62	7,28	35	16,4	-10,3	-9,5	78	2,59	1,77	189	173		23,1	90,41	0,22	
19	AA-AO	5	F	3,08	7,30	61	30	3,6	2,1	31	2,43	2,17	73	32	2	33	88,94	0,33	27
20	AA-AO	8	F	2,35	7,33	53	27,9	2	1	43	2,41	1,64	134	28	5,5	30		0,44	23
21	OMD	4	F	2,90	7,36	43	24,3	-1,1	-1,2	31	2,46	1,77	127	38	2,6	33,7	93,01	0,46	30
22	OMD	12	F	2,72	7,46	47,8	33,4	9,6	8,6	92*	2,06	1,66	60	42		25			
23	OMD	10	M	2,48	7,28	48	22,6	-4,1	-4,4	28	2,42	1,67	155	31,6		46		2,20	
24	SIMD	16	M	6,70	7,31	60	30,2	3,9	2,3	42	2,28	1,57	379		6,7		91,35	0,59	28
25	SIMD	11	F	3,80	7,33	52	27,4	1,5	1	43	2,34	1,59	234	51	14,4	21	88,65	1,03	16
26	SIMD	18	F	4,50	7,39	42	25,4	0,4	0,3	35	2,5	1,58	207						
27	SIMD	3	M	4,56	7,34	55	29,7	3,9	2,6	27	2,39	1,72	131	22	2	21	90,94	0,81	21
28	SIMD	11	M	4,70	7,37	52	30,1	4,8	3,7	39	2,48	1,31	186	37	2	15	98,05	0,27	23
29	SIMD	11	M	2,82	7,38	46	27,2	2,1	1,5	46	2,35	1,36		41	4,1	37	91,15	0,57	26
30	SIMD	15	F	3,80	7,36	57	32,2	6,8	5,1	49	2,38	1,32	126	26	8,1	34	91,86	0,46	11,7
31	SIMD	3	M	4,03	7,37	46	26,6	1,3	0,9	31	2,49	1,66	76	17	3,7	35	90,20	1,42	24
32	SIMD	8	F	3,23	7,33	52	27,4	1,5	1	43	2,34	1,59	234	51	14,1	14,82	88,65	1,03	16
33	SIMD	13	M	2,34	7,33	53	27,9	2	1	43	2,41	1,64	134	28	5,5	46,6	94,36	0,44	23

MD – Main Diagnosis; r.v. – reference range values; L – Lactate (median values); CO_2 – Carbon dioxide; $HCO3^-$ - Bicarbonate; BEecf/BEb – Base excess; Cr – Creatinine; Ca – calcium; P – Phosphorus; AP – Alkaline Phosphatase; PTH – Parathyroid Hormone; vit. D – vitamin D; PRR – Phosphorus Reabsorption Rate; DXP – Deoxypyridinoline

MIT – Mitochondrial disease (N=8); GSD1 – glycogen storage disease type 1 (N=7); AA-OA – aminoacidopathy/organic aciduria (N=5); OMD – other inherited metabolic disorders (N=3); SIMD – suspicion of inherited metabolic disease (N=10) Units: Lactate, Bicarbonate, BEecf/BEb, Creatinine, Calcium, Phosphorus (mmol/L); CO_2 (mmHg); AP (UI/L); PTH, calcitonin (pg/mL); vit. D (ng/ml); PRR (%); Deoxypyridinoline (nmol/mmol creat) A- Present age in years; B - reference range values for patients' group median age (9 years-old) (*) normal for age

Attachment 5 – Patients with chronic hyperlactacidaemia (N=18): urine deoxypyridinoline

Pt	*MD*	*Age at exam*	*Gender*	*DXP(*)*	*DXP (**)*
2	MIT	9	M	31	>N
3	MIT	7	M	20	>N
4	MIT	6	M	11,1	N
7	MIT	6	M	36,6	>N
12	GDS1	7	M	27,6	>N
14	GDS1	5	F	16,6	N
19	AA-AO	5	F	27	>N
20	AA-AO	8	F	23	>N
21	OMD	4	F	30	>N
24	SIMD	16	M	28	>N
25	SIMD	11	F	16	>N
27	SIMD	3	M	21	?
28	SIMD	11	M	23	>N
29	SIMD	11	M	26	>N
30	SIMD	15	F	11,7	>N
31	SIMD	3	M	24	?
32	SIMD	8	F	16	N
33	SIMD	13	M	23	>N

MD – Main Diagnosis; DXP – Deoxypyridinoline (*) in nmol/mmol creat, (**) age reference values

Attachment 6 – Patients with chronic hyperlactacidaemia (N=33): other plasma and urine parameters profiles, in the diverse diagnosis groups.

			r.v.	*N**	*Min.*	*Max.*	*Mean*	*Median*	*SD*
MIT	Plasma	Creatinine	27-62	8	23,0	92,0	41,4	30,5	21,6
		Calcium	2,2-2,5	8	2,1	2,5	2,3	2,4	0,1
		Phosphorus	1,2-1,8	8	1,3	1,7	1,5	1,6	0,2
		AP	150-380	5	126,0	155,0	139,6	133,0	12,0
		PTH	9-72	7	11,0	404,0	81,3	32,0	132,1
		25(OH)D-vitamin	>30	7	4,0	10,7	7,5	7,7	2,4
		Calcitonin	<5	6	2,0	10,7	5,2	4,6	3,1
	Urine	Calcium/Creatinine	≤ 0,7	5	0,2	0,8	0,4	0,4	0,2
		PRR	>85	4	88,5	98,2	92,6	91,8	3,6
		Deoxypyridinoline	3,0-7,4	4	11,1	36,6	24,7	25,5	9,9
GSD1	Plasma	Creatinine	27-62	7	26,5	40,0	31,5	31,0	4,1
		Calcium	2,2-2,5	7	2,3	2,8	2,6	2,6	0,2
		Phosphorus	1,2-1,8	7	1,2	1,6	1,4	1,5	0,1
		AP	150-380	5	164,0	314,0	222,4	213,0	49,4
		PTH	9-72	2	11,4	33,1	22,3	22,3	10,9
		25(OH)D-vitamin	>30	4	14,1	19,4	16,2	15,7	2,1
		Calcitonin	<5	1	15,5	15,5	15,5	15,5	0,0
	Urine	Calcium/Creatinine	≤ 0,7	4	0,6	1,6	1,0	1,0	0,4
		PRR	>85	2	96,9	98,2	97,5	97,5	0,7
		Deoxypyridinoline	3,0-7,4	2	16,6	27,6	22,1	22,1	5,5
AA-OA	Plasma	Creatinine	27-62	5	31,0	78,0	46,6	42,0	16,3
		Calcium	2,2-2,5	5	2,4	2,6	2,5	2,4	0,1
		Phosphorus	1,2-1,8	5	1,2	2,2	1,7	1,6	0,3
		AP	150-380	5	73,0	213,0	163,6	189,0	53,4
		PTH	9-72	4	28,0	173,0	66,5	32,5	61,5
		25(OH)D-vitamin	>30	4	23,1	33,0	28,3	28,5	3,7
		Calcitonin	<5	2	2,0	5,5	3,8	3,8	1,8
	Urine	Calcium/Creatinine	≤ 0,7	3	0,22	0,4	0,3	0,3	0,2
		PRR	>85	2	88,9	90,4	89,7	89,7	0,7
		Deoxypyridinoline	3,0-7,4	3	23,0	27,0	25,0	25,0	2,0

N* – number of patients who had parameter tested; AP – Alkaline Phosphatase; PTH – Parathyroid Hormone; PRR – Phosphorus Reabsorption Rate

MIT – Mitochondrial disease (N=8); GSD1 – glycogen storage disease type 1 (N=7); AA-OA – aminoacidopathy/organic aciduria (N=5); OMD – other inherited metabolic disorders (N=3); SIMD – suspicion of inherited metabolic disease (N=10). Creatinine, Calcium, Phosphorus (mmol/L); AF (UI/L); PTH, calcitonin (pg/mL); 25(OH)D-vitamin (ng/ml); Deoxypyridinoline (nmol/mmol creat); PRR (%)

Attachment 7 – Patients with chronic hyperlactacidaemia (N=33): other plasma and urine parameters profiles, in the diverse diagnosis groups (continuation)

			r.v.	*N**	*Min.*	*Max.*	*Mean*	*Median*	*SD*
OMD	Plasma	Creatinine	27-62	3	28,0	92,0	50,3	31,0	29,5
		Calcium	2,2-2,5	3	2,1	2,5	2,3	2,4	0,2
		Phosphorus	1,2-1,8	3	1,7	1,8	1,7	1,7	0,0
		AP	150-380	1	127,0	127,0	127,0	127,0	0,0
		PTH	9-72	2	38,0	42,0	40,0	40,0	2,0
		25(OH)D-vitamin	>30	3	25,0	46,0	34,9	33,7	8,6
		Calcitonin	<5	1	2,6	2,6	2,6	2,6	0,0
	Urine	Calcium/Creatinine	≤ 0,7	2	0,5	2,2	1,3	1,3	0,9
		PRR	>85	1	93,0	93,0	93,0	93,0	0,0
		Deoxypyridinoline	3,0-7,4	1	30,0	30,0	30,0	30,0	0,0
SIMD	Plasma	Creatinine	27-62	10	27,0	49,0	38,8	42,5	29,5
		Calcium	2,2-2,5	10	2,3	2,5	2,4	2,4	0,1
		Phosphorus	1,2-1,8	10	1,3	1,7	1,5	1,6	0,1
		AP	150-380	9	76,0	379,0	189,7	186,0	84,1
		PTH	9-72	8	17,0	51,0	34,1	32,5	12,1
		25(OH)D-vitamin	>30	6	15,0	37,0	27,3	27,5	8,5
		Calcitonin	<5	9	2,0	14,4	6,7	5,5	4,4
	Urine	Calcium/Creatinine	≤ 0,7	9	0,3	1,4	0,7	0,6	0,4
		PRR	>85	9	88,7	98,0	91,7	91,1	2,8
		Deoxypyridinoline	3,0-7,4	9	11,7	28,0	30,0	23,0	5,0

N* – number of patients who had parameter tested; AP – Alkaline Phosphatase; PTH – Parathyroid Hormone; PRR – Phosphorus Reabsorption Rate

MIT – Mitochondrial disease (N=8); GSD1 – glycogen storage disease type 1 (N=7); AA-OA – aminoacidopathy/organic aciduria (N=5); OMD – other inherited metabolic disorders (N=3); SIMD – suspicion of inherited metabolic disease (N=10). Creatinine, Calcium, Phosphorus (mmol/L); AF (UI/L); PTH, calcitonin (pg/mL); 25(OH)D-vitamin (ng/ml); Deoxypyridinoline (nmol/mmol creat); PRR (%)

Attachment 8 – Patients with chronic hyperlactacidaemia (N=33): bone densitometry results, in the diverse diagnosis groups.

			Lumbar spine			*Femoral neck*		
Main Diagnosis	*Pt #*	*Age at exam*	BMD (g/cm2)	*Z-score* SD	*Z-score %*	*BMD (g/cm2)*	*Z-score SD*	*Z-score %*
MIT	1	15	0,868	-0,80	-9	1,007	0,6	7
	2	9	0,381	-3,10	-36	0,379	-4,4	-49
	3	7	0,483	-1,00	-11	0,604	-1	-10
	4	6	0,375	-2,50	-27			
	5	6	0,307	-4,50	-41			
	6	6	0,400	-2,20	-22	0,385	-2,9	-33
	7	6	0,427	-1,50	-17	0,498	-2,1	-22
GSD1	8	6	0,411	-2,00	-22			
	9	9	0,439	-1,90	-21			
	10	16	1,074	-0,90	-11			
	11	17	0,559	-4,30		0,557		
	12	13	0,338	-4,30	-51			
	13	9	0,417	-2,00				
	14	5	0,468	-2,70	-29			
	15	15	0,529	-4,10				
AA-OA	16	12	0,642	-1,20	-15	0,815	0	0
	17	14	0,517	-4,90	-44	0,56	-3,2	-39
	18	8	0,371	-3,00	-33	0,599	-1,3	-13
	18	5	0,355	-2,80	-27	0,438	-1,9	-21
	20	8	0,553	-0,40	-5	0,617	-0,5	-6
OMD	21	4	0,419	-1,30	-13	0,468		
	22	12	0,977	1,40	22	0,943	1	12
	23	10	0,639	0,20	3	0,635	-1,4	-16
	24	16	0,566	-3,20	-41	0,641	-2,9	-38
	25	11	0,674	0,10	1	0,695	-1,1	-13
	26	18	0,918	-0,90	-9	0,831	-1,4	-15
SIMD	27	3	0,506	1,10	11			
	28	11	0.674	0,10	1	0,695	-1,1	-13
	29	11	0,694	0,30	4	0,801	0	0
	30	15	0,887	-0,70	-7	0,86	-0,8	-9
	31	3	0,323	-2,90	-30			
	32	8	0,412	-2,50	-27	0,568	-0,9	-10
	33	13	0,652	-1,00	-14	0,796	-0,7	-9

MIT – Mitochondrial disease (N=8); GSD1 – glycogen storage disease type 1 (N=7); AA-OA – aminoacidopathy/organic aciduria (N=5); OMD – other inherited metabolic disorders (N=3); SIMD – suspicion of inherited metabolic disease (N=10). Pt # – Patient number