Osteoporosis and β-thalassemia major: Role of the IGF-I/IGFBP-III axis

A. Lasco*, N. Morabito*, A. Gaudio*, A. Crisafulli*, A. Meo**, G. Denuzzo***, and N. Frisina*

*Department of Internal Medicine, **Department of Pediatrics, ***Department of Radiological Sciences, University of Messina, Messina, Italy

ABSTRACT. Patients with β-thalassaemia major are susceptible to osteopenia due to several factors which interfere with bone remodeling. It is known that bone metabolism and skeletal consolidation result from a complex sequence of hormonal changes, where the concerted actions of GH, IGF-I and sex hormones and their receptors, are responsible for the timing and attainment of skeletal consolidation. IGF-I and the corresponding binding protein (IGFBP-III), markers of bone metabolism and lumbar and femoral neck BMD were measured in 28 adult patients, undergoing hormonal replacement and chelation therapy and a hypertransfusion program, with β-thalassaemia major (12 males with mean age 22.5±3.1 and 16 females with mean age 27.5±8.2), and in 28 healthy volunteers matched for age, anthropometric features and sex to the patients. BMD values, both at lumbar and femoral neck level were significantly lower ($p<0.001$ and $p<0.05$) by 18.7 and 4.2% respectively, in patients than in the controls. Markers of bone resorption [pyridinoline (Pyr) 78.1±15.7 *vs* 47.5±11.2 pmol/μmol urinary creatinine, $p<0.001$ and deoxypyridinoline (D-Pyr) 21.9±3.5 *vs* 14.5±5.4 pmol/ μmol urinary creatinine, $p<0.001$] were higher in patients than in controls, whereas the marker of bone formation was slightly lower [osteocalcin (BGP) 3.8±0.6 *vs* 4.6±1.7 pmol/ml, $p<0.05$]. Plasma levels of IGF-I (21.07±5.12 *vs* 35.25±8.33 nmol/ml, $p<0.001$) and IGF binding protein III (IGFBP-III) (1.9±0.4 *vs* 2.5±0.1 mg/ml, $p<0.001$) were lower in patients than in controls and positively correlated with BMD L2-L4 (r=0.57, $p<0.05$ and r=0.47, $p<0.05$ respectively), BMD neck (r=0.40, $p<0.05$ and r=0.34, $p<0.05$ respectively) and BGP (r=0.52, $p<0.05$ and r=0.34, $p<0.05$ respectively). Our β-thalassaemic patients, in spite of normalizing hemoglobin levels, adequate hormone replacement and chelation therapies, showed osteopenia and an unbalanced bone turnover with an increased resorptive phase and a decreased formation phase probably correlated to low levels of IGF-I and IGFBP-III observed in our study.

(J. Endocrinol. Invest. *25:* 338-344, 2002)

INTRODUCTION

Beta-thalassaemia major is an inherited blood disease which leads to severe bone deformities, originally described by Cooley *et al.* and Jensen *et al.* (1, 2). Regular blood transfusions and iron chelation therapy prevent these severe bone alterations, but osteopenia and in some patients severe osteoporosis, remain serious complications even in well-transfused and iron chelated patients (3). The major pathogenetic factors for the development of osteoporosis in thalassaemic subjects appear to be: 1) bone marrow hyperplasia due to ineffective erythropoiesis, which in spite of hypertransfusion, might not be completely suppressed (4); 2) genetic predisposition (5); 3) hemochromatosis (6); 4) low plasma vitamin D concentrations (7); 5) hypogonadotrophic hypogonadism (8). Other factors such as secondary hypoparathyroidism, hypothyroidism and diabetes may represent additional mechanisms (9).

However, the altered function of the hypothalamus-hypophysis-gonads axis is one of the more significant aspects of abnormal somato-sexual development of thalassemic patients. Gonadal insufficiency, observable in the majority of thalassemics of both sexes and imputable to functional damage to gonadotropins secreting cells,

Key-words: IGF-I, IGFBP-III, osteoporosis, thalassemia, bone turnover.

Correspondence: Prof. Antonino Lasco, Via Faustina e Tertullo 19, 98100 Messina, Italy.

E-mail: alasco@unime.it

Accepted November 8, 2001.

contributes, in fact, to determining an altered bone metabolism in these patients. One of our previous studies on thalassemic population (10), showed that thalassemics with gonadal failure have a more serious alteration of BMD, both at a vertebral and femoral level than eugonadal thalassemics. These observations support the importance of sex hormones on bone tissue and therefore, the importance of performing hormonal replacement therapy at an early stage.
It is not yet clear whether sex steroids act on bone tissue directly and/or through a mediation of local factors. One of the better characterized among these local factors in bone tissue is the IGF-I, that is considered a potent stimulator of bone formation and is present in the circulation and in extracellular spaces, and is especially bound to the IGF binding protein III (IGFBP-III), the prominent form of IGFBPs in man (11, 12).
Therefore, this study was designed in order to investigate the IGF-I/IGFBP-III axis and its relationship with bone metabolism and mineral density in a group of patients with thalassaemia major, undergoing hormonal replacement therapy and without other endocrine, liver or kidney disorders.

DESIGN AND METHODS

Patients

We recruited a total of 40 patients with β-thalassemia major attending the center for the prevention and cure of osteoporosis of the Department of Internal Medicine of the Messina University. Of these, patients with thyroid disease (3), diabetes mellitus (2), other endocrine disorders (2), liver disease (4), or on steroid treatment (1) were excluded from the study (12 patients). The remaining patients (28 subjects) were included in our cross-sectional. Twelve of these were male (mean age 22.5±3.1, BMI 22±2 kg/m^2) and 16 female (mean age 27.5±8.2, BMI 23±2 kg/m^2).
All the patients had been treated with hormone replacement therapy for 3.0±2.1 yr, with a good compliance, in the form of transdermal estrogen (17 β estradiol 50 μg/d for 21 days every month) and medroxyprogesterone acetate (10 mg for the last 11 days of the menstrual cycle) for female patients; in the form of metil testosterone (100 mg im every 28 days) for male patients.
Patients had been maintained on a regular transfusion program since early childhood, according to a monthly regimen with the aim of maintaining pretransfusional hemoglobin levels above 1.55 mmol/l. All thalassemic patients underwent an iron chelation program with desferioxamine (Desferal), administered subcutaneously by mini-pump, via an 8-h infusion/d. The dosage varied from 40 to 60 mg/kg/d and was recommended to be used 5 nights each week. Compliance to chelation therapy according to serum ferritin levels was monitored for 1 yr and considered acceptable in all patients. Thalassemic subjects supplemented their diet with colecalciferol (vitamin D_3) 400 IU/d.
In addition, we recruited 28 young volunteers from the nursing school of Messina. These were healthy subjects matched for age, height, weight and sex to the patients. Diagnosis of homozygous β-thalassaemia was made using hemoglobin electrophoresis to identify variant hemoglobins (13). The study was performed according to the principles of the Declaration of Helsinki, and informed consent was obtained from each person involved.

METHODS

Bone mass measurements

BMD was measured in all patients by a dual energy X-ray (DXA) densitometer (HOLOGIC QDR 4500 W) both at the lumbar spine (L2 to L4) in A-P projection and the proximal femur (neck and Ward's triangle). The instrument was calibrated on a daily basis according to the manufacturer's instructions. Reproducibility was calculated as a coefficient of variation (CV) obtained by weekly measurements of a standard phantom on the instrument and by repeated measurements obtained in 3 patients of different ages. The CV of our instrument was 0.5% with the standard phantom; *in vivo* we calculated a CV of 1.1% for the lumbar spine, 1.5% for the neck and 3.2% for Ward's triangle. BMD data were expressed as grams per centimeter squared and compared with BMD values of normal subjects of the same age.

Biochemical analyses

On the day of admission, venous blood samples were obtained for determination of blood cell count and serum concentration of albumin, bilirubin, hemoglobin (Hb), ferritin and alanine aminotransferase (ALT). Following an overnight fast, venous blood samples were drawn through a polyethylene catheter inserted in a forearm vein between 8:00 h and 9:00 h. The serum was separated from the blood corpuscles by centrifugation and kept frozen at -20 C until analyzed for calcium (Ca), phosphorus (P), creatinine (Creat), IGF-I, IGFBP-III, intact PTH, 25-hydroxyvitamin D (25-OHD), osteocalcin (BGP), E2 and free-T. A 2-h fasting morning urine was collected at the same time for measurements of pyridinium crosslinks (pyridinoline - Pyr and deoxypyridinoline - D-Pyr) and creatinine.
Ca (normal range 2.2-2.6 mmol/l), P (normal range 1.2-1.7 mmol/l), Creat (normal range 63-133 μmol/l in serum and 0.13-0.22 mmol*kg^{-1} of body weight /24 h in urine), bilirubin (normal range 5.1 - 17 μmol), albumin (normal range 35-55 g/l), ALT (normal range 3-26 IU/l) and Hb (normal range 2.1-3.0 mmol/l) were determined by automated routine procedures. BGP (normal range 3.36-6.86 pmol/ml), PTH (normal range 1.17-6.77 pmol/l) and 25-OHD (normal range 25-125 nmol/l) were measured by an IRMA (Bouty SpA, Italiana Laboratori Bouty, Milan, Italy). Ferritin (normal range 3-12 mmol/l), E2 (normal range 73-367 pmol/l for the follicular phase of the menstrual cycle) and free-T (normal range 69-970 pmol/l) were valued using a solid phase immunoassay (Roche Diagnostics, Milan, Italy). Pyr (normal range 25-91 pmol/μmol urinary creat) and D-Pyr (normal range 3-21 pmol/μmol urinary creat) were measured by an high performance liquid chromatography (HPLC) (BIORAD Diagnostics,

Table 1 - *Hormonal parameters of patients and controls.*

	Patients		Controls	
	Males*	Females**	Males*	Females**
PTH (pmol/l)	4.3±0.7 (3.2-5.2)	4.2±0.6 (3.0-4.9)	4.4±1.3 (3.0-5.8)	4.3±0.9 (2.9-5.6)
25-OHD (nmol/l)	70.8±10.1 (58.7-82.9)	71.9±9.8 (60.1-82.6)	75.8±20.5 (52.3-98.5)	77.8±19.8 (56.2-98.9)
17βE_2 (pmol/l)	-	118.9±18.6 (98.3-139.6)	-	127.7±29.2 (96.4-159.6)
FreeT (pmol/l)	450.2±84.0 (355.5-539.8)	-	469.8±70.5 (397.5-544.2)	-

Data are expressed as mean±SD (range). *Dosage on the 12th day after the last injection of metil-testosterone. **Dosage on the 7th day of induced or spontaneous menstrual cycle. 25-OHD:25-hydroxyvitamin D.

Richmond, CA, U.S.A). The IGF-I and IGFBP-III levels were measured by RIA using reagents supplied by Bouty SpA, Milan, Italy. Mean intrassay coeffiecients of variations (CVs) were 7.4% and 5.7%, respectively, and interassay CVs were 8.3% and 6.1%, respectively.

Statistical analysis

Statistical analyses were performed using the Statview 512 statistical package. All values were expressed as mean±SD. Comparisons between the groups were performed using Student's *t* test for unpaired data when the data were normally distributed, or by non-parametric statistical analysis (Wilcoxon rank sum test) when they were not. A *p* value of <0.05 was considered significant. Pearson's correlation coefficient (r) was calculated to evaluate the correlation between 2 variables.

RESULTS

The hormonal and biochemical characteristics of subjects are shown in Tables 1 and 2. The circulating concentrations of albumin, Ca, P, PTH and 25-OHD did not differ significantly among the study groups. Levels of bilirubin and ferritin were significantly higher in thalassemic patients than in controls ($p<0.001$). These levels reflected respectively ineffective erythropoiesis and systemic hemosiderosis typical of thalassemics. Thanks to good compliance to the adequate HRT, levels of 17-β estradiol and free-T did not differ among thalassemics and control subjects.

Circulating concentrations of IGF-I (21.07±5.12 *vs* 35.25±8.33 nmol/ml, $p<0.001$) and IGFBP-III (1.9±0.4 *vs* 2.5±0.1 mg/ml, $p<0.001$) and their ratio (IGFI/IGFBP-III: 11.08±0.12 *vs* 14.10±0.15, $p<0.001$) were significantly lower in thalassaemic patients than in controls. Dual-Photon absorptiometry showed that thalassaemic patients had significantly lower BMD values (BMD L2-L4: 0.880±0.069 *vs* 1.083±0.090 g/cm^2, $p<0.001$ and BMD neck 0.894±0.060 *vs* 0.934±0.062 g/cm^2, $p<0.05$) than controls (Table 3). BMD L2-L4 values significantly correlated with hormonal treatment

Table 2 - *Biochemical parameters of patients and controls.*

	Patients	Controls
ALT (IU/l)	10.1±1.8 (7.8-13.4)	9.9±2.1 (7.5-12.5)
Bilirubin (μmol/l)	47.9±3.1* (41.8-52.3)	10.2±1.9 (8.0-12.3)
Albumin (g/l)	40.0±2.9 (35.0-44.7)	39.5±2.1 (37.1-42.2)
Ca (mmol/l)	2.51±0.08 (2.25-2.60)	2.49±0.09 (2.36-2.59)
P (mmol/l)	1.55±0.11 (1.42-1.69)	1.54±0.09 (1.41-1.68)
Creatinine (μmol/l)	84.5±4.7 (78.5-95.5)	83.7±5.4 (77.7-90.1)
Hb (mmol/l)	1.63±0.5* (1.07-2.14)	2.31±0.6 (1.68-2.99)
Ferritin (mmol/l)	57.34±50.70* (6.23-106.04)	5.21±3.01 (2.0-8.89)
IGF-I (nmol/ml)	21.07±5.12* (11-29)	35.25±8.33 (25-45)
IGFBP-III (mg/ml)	1.9±0.4* (1.1-2.5)	2.5±0.1 (2.3-2.8)
IGF-I/IGFBP-III (nmol/mg)	11.08±0.12* (10-11.2)	14.10±0.15 (10.86-16.07)

Data are expressed as mean±SD (range). *$p<0.001$; ALT: alanine aminotransferase; Ca: calcium; Hb: hemoglobin; IGFBP-III: IGF binding protein III; P: phosphorus.

Table 3 - *Parameters of bone turnover and BMD.*

	Patients	Controls
Pyr (pmol/mmol urinary creat)	78.1±15.7* (61.1-96.7)	47.5±11.2 (34.1-60.2)
D-Pyr (pmol/μmol urinary creat)	21.9±3.5* (15.2-28.4)	14.5±5.4 (8.2-20.7)
BGP (pmol/ml)	3.8±0.6** (2.6-4.7)	4.6±1.7 (2.5-6.8)
BMD L2-L4 (g/cm^2)	0.880±0.069* (0.620-0.960)	1.083±0.090 (0.987-1.184)
BMD femoral neck (g/cm^2)	0.894±0.060** (0.670-0.980)	0.934±0.062 (0.870-1.002)

Data are expressed as mean±SD (range). *p<0.001,**p<0.05; BGP: osteocalcin; D-Pyr: deoxypyridinoline; Pyr: pyridinoline.

duration (r=0.50, p<0.05), with IGF-I (r=0.57, p<0.05) and IGFBP-III levels (r=0.47, p<0.05). Similarly, BMD neck values significantly correlated with hormonal treatment duration (r=0.49, p<0.05), with IGF-I (r=0.40, p<0.05) and IGFBP-III levels (r=0.34, p<0.05) (Fig. 1).

Our thalassaemic patients had an increased bone resorptive phase, as shown by the fact that levels of Pyr, D-Pyr, were significantly higher than controls (Pyr 78.1±15.7 *vs* 47.5±11.2 pmol/μmol urinary creat, p<0.001 and D-Pyr 21.9±3.5 *vs* 14.5±5.4 pmol/ μmol urinary creat, p<0.001). On the contrary, levels of BGP were slightly, although significantly, lower in patients than controls (3.8±0.6 *vs* 4.6±1.7 pmol/ml, p<0.05) (Table 3). BGP showed a significant positive correlation with levels of IGF-I (r=0.52, p<0.05) and IGFBP-III and (r=0.34, p<0.05) (Fig. 1). No significant correlations were found between these growth factors and markers of bone resorption. No relationship was noticed with the other parameters considered in patients and controls.

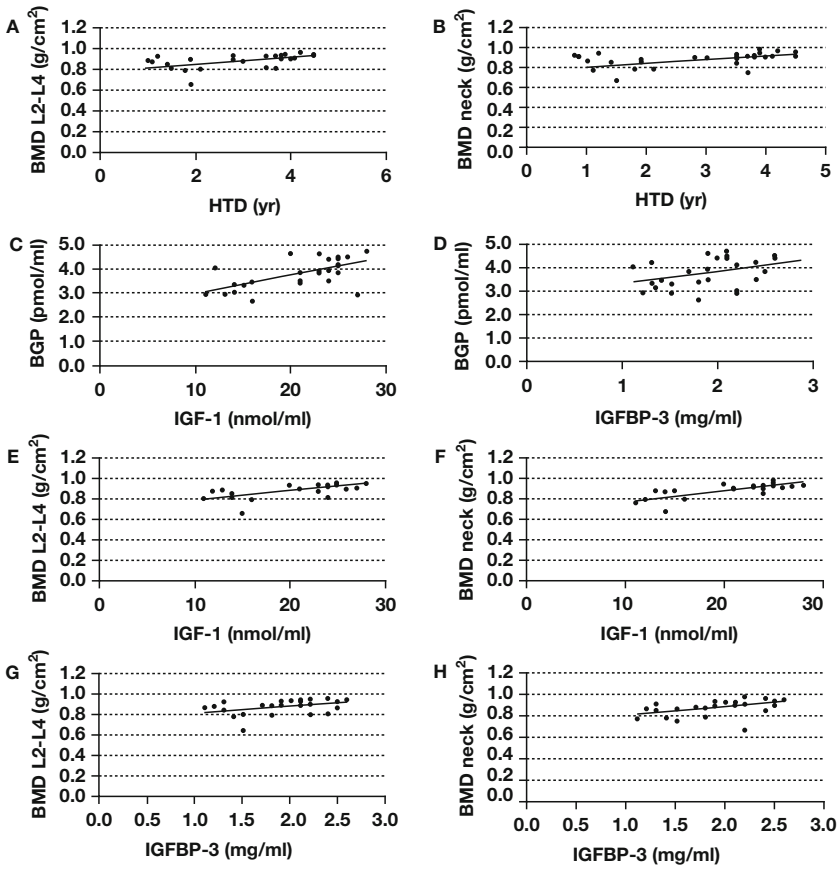

Fig. 1 - *Significant correlations in thalassemic patients. (A) Correlation between BMD L2-L4 (g/cm^2) and hormonal treatment duration (HTD) (yr) (r=0.50, p<0.05). (B) Correlation between BMD neck (g/cm^2) and HTD (yr) (r=0.49, p<0.05). (C) Correlation between osteocalcin BGP (pmol/ml) and IGF-I (nmol/ ml) (r=0.52, p<0.05). (D) Correlation between osteocalcin (BGP) (pmol/ml) and IGF binding protein-III (IGFBP-III) (mg/ml) (r=0.34, p<0.05). (E) Correlation between BMD L2-L4 (g/cm^2) and IGF-I (nmol/ml) (r=0.57, p<0.05). (F) Correlation between BMD neck (g/cm2) and IGF-I (nmol/ml) (r=0.40, p<0.05). (G) Correlation between BMD L2-L4 (g/cm^2) and IGFBP-III (mg/ml) (r=0.47, p<0.05). (H) Correlation between BMD neck (g/ cm^2) and IGFBP-III (mg/ml) (r=0.34, p<0.05).*

DISCUSSION

Our study shows that, in spite of adequate sex hormone replacement therapy, thalassaemic patients exhibit lower BMD values than controls, both at a vertebral and femoral level, as has already been reported by Jensen *et al.* (2) and Giardina *et al.* (3), and that these values are significantly correlated with replacement treatment duration. These findings implicate that sex steroids are important, but not sufficiently to prevent bone loss in these patients. Other factors could play an important role. Because our patients were maintained on a hypertransfusion program and received hormone replacement and chelation therapies, we expected a normal bone turnover; whereas our thalassaemic patients had an unbalanced bone turnover with an increased resorption phase, as is shown by high levels of pyridinium crosslinks and a decreased neoformation phase, as shown by the slightly low levels of osteocalcin. This data is also in accordance with histomorphometric studies performed by De Vernejoul *et al.* (14), that showed decreased bone formation in thalassemic patients. The depression of bone formation, even if slight, is surprising because an increase in resorption is generally followed by a corresponding increase in bone formation (15, 16). These findings suggest that the decrement in bone density in thalassemics patients may be a consequence of uncoupling bone turnover. With regard to the potential etiology for this unbalanced bone turnover, that has been reported also in a recent work of Voskaridou *et al.* (17), we found lower serum levels of IGF-I and IGFBP-III in thalassemic patients than in age-matched controls (-40.2% and -24.0% respectively) and that levels of these growth factors show significant correlations with BGP, a marker of bone formation and BMD values. Low levels of IGF-I in thalassemic adults and its correlation with BMD have been similarly reported by Dresner Pollak *et al.* (18), but our data, for the first time, relate unbalanced bone turnover to a decrease in the components of the IGF-I/IGFBP-III system. Previously, Sartorio *et al.* (19) demonstrated that 12 months' rec-GH therapy in GH deficient children with thalassaemia major significantly increased levels of IGF-I and markedly of BGP. The mechanisms responsible for reduction of IGF-I/IGFBP-III axis in thalassemics are still being debated. Danesi *et al.* (20) found in a considerable proportion of thalassemic patients an impairment of GH secretion, compatible with a hypothalamic and/or pituitary damage. But, it is unclear whether the IGF-I decrease occurs before or after GH secretion dysfunction (21-26). In this regard, recently, Chrysis *et al.* (27) suggest that impaired GH secretion rather than GH insensitivity is the cause of growth retardation in thalassemic patients. Another important aspect is that nutritional state has profound actions at all levels of the GH/IGF-I axis and GH has potent effects on nutritional state (28). But our thalassemic patients had a normal diet, as is shown by the normal values of albumin and BMI.

IGF-I, a polypeptide synthesized mostly by the liver but also by skeletal cells, is a molecule that stimulates bone tissue formation, but it is also a product of the osteoblasts, on which it acts as an autocrine and paracrine modulator of bone formation, stimulating function and proliferation of bone forming cells. It is interesting to note how sex hormones are all involved in IGF-I production by bone cells, also influencing the cellular responsiveness to IGF-I (29-36). Moreover, all bone cells are capable of producing binding proteins for IGF-I, that modulate its final effect. In particular IGFBP-III seems to have the role of enhancing IGF-I life span and of protecting from its hypoglycemic effects (37). Bone metabolism and skeletal consolidation result from a complex sequence of hormonal changes in interaction with nutritional factors, where the concerted actions of GH, IGF-I and sex hormones and their receptors, besides other factors, are responsible for the timing and attainment of skeletal consolidation (38).

We hypothesize that IGF-I/IGFBP-III decrement is the "missing link" between sex hormone action and bone metabolism in these patients. In other words, hormone replacement therapy, that in other cases is associated with a beneficial and protective effect on the bone, in these patients could not completely act on bone because of the alteration of the IGF-I/IGFBP-III axis. In conclusion, the essential mechanism of cortical and trabecular bone loss in thalassemic patients seems to be an uncoupling of bone resorption and bone formation, with a decrease in osteoblast recruitment and activity. This uncoupling could be caused by the above described alteration of the IGF-I/IGFBP-III axis, that determines a decreased peripheral action of sex hormones.

In the light of this new evidence, IGF-I therapy may represent a valid therapeutic option in thalassemic patients. In fact, we have today a variety of factors with inhibitory effects on bone resorption in the treatment of osteopenic pathologies such as bisphosphonates (39), but there is no substance capable of stimulating bone neoformation. Different situations, such as thalassemic osteoporosis, could benefit from this

therapy. Moreover, systematic administration of IGF-I and IGFBP-III could prevent undesirable systemic effects of the IGF-I molecule.

REFERENCES

1. Cooley T.B., Witwer E.R., Lee P. Anemia in children with splenomegaly and peculiar changes in bones. Am. J. Dis. Child. 1927, *34:* 347-363.
2. Jensen C.E., Tuck S.M., Agnew J.E., *et al.* High prevalence of low bone mass in thalassaemia major. Br. J. Haematol. 1998, *103:* 911-915.
3. Giardina P.J., Schneider R., Lesser M., *et al.* Abnormal bone metabolism in thalassaemia. In: Andò S., Brancati C., (Eds.), Endocrine Disorders in Thalassaemia. Springer Verlag, Berlin-Heidelberg, 1995, p. 39-46.
4. Cazzola M., De Stefano P., Porchio L., *et al.* Relationship between transfusion regimen and suppression of erythropiesis in beta-thalassemia major. Br. J. Haematol. 1995, *89:* 473-478.
5. Wonke B., Jensen C., Hanslip J.J., *et al.* Genetic and acquired predisposing factors and treatment of osteoporosis in thalassaemia major. J. Pediatr. Endocrinol. Metab. 1998, *11:* 795-801.
6. El Hazmi M.A., Warsy A.S., Al Fawaz I. Iron-endocrine pattern in patients with β-thalassaemia. J. Trop. Pediatr. 1994, *40:* 219-224.
7. Pratico G., Di Gregorio F., Caltabiano L., Palano G.M., Caruso-Nicoletti M. Calcium phosphate metabolism in thalassemia. Pediatr. Med. Chir. 1998, *20:* 265-268.
8. Anapliotou M.L., Kastanias I.T., Psara P., Evangelou E.A., Liparaki M., Dimitriou P. The contribution of hypogonadism to the development of osteoporosis in thalassaemia major: new therapeutic approaches. Clin. Endocrinol. 1995, *42:* 279-287.
9. Vullo C., De Sanctis V., Katz M., *et al.* Endocrine abnormalities in thalassaemia. Ann. NY. Acad. Sci. 1990, *612:* 293-310.
10. Lasco A., Morabito N., Gaudio A., Buemi M., Wasniewska M., Frisina N. Effects of hormonal replacement therapy on bone metabolism in young adults with β-thalassemia major. Osteoporos. Int. 2001, *12:* 570-575.
11. Rechler M.M., Nissley S.P. Insulin-like growth factors. In: Sporn M.B., Roberts A.B. (Eds.) Peptide growth factors and their receptors. Springer-Verlag, Berlin-Heidelberg, 1990, p. 263-346.
12. Clemmons D.R. IGF Binding proteins: regulation of cellular actions. Growth Regul. 1992, *2:* 80-87.
13. Working Party of the General Haematology Task Force of the British Committee for Standards in Haematology. The laboratory diagnosis of haemoglobinopathies. Br. J. Haematol. 1998, *101:* 783-792.
14. De Vernejoul M.C., Girot R., Gueris J., *et al.* Calcium phosphate metabolism and bone disease in patients with homozygous thalassemia. J. Clin. Endocrinol. Metab. 1982, *54:* 276-281.
15. Mohan S., Baylink D.J. Bone growth factors. Clin. Orthop. 1991, *263:* 30-48.
16. Hayden J.M., Mohan S., Baylink D.J. The insulin-like growth factor system and the coupling of formation to resorption. Bone 1995, *17:* 93S-98S.
17. Voskaridou E., Kyrtsonis M.C., Terpos E., *et al.* Bone resorption is increased in young adults with thalassaemia major. Br. J. Haematol. 2001, *112:* 36-41.
18. Dresner Pollack R., Rachmilewitz E., Blumenfeld A., Idelson M., Goldfarb A.W. Bone mineral metabolism in adults with beta-thalassaemia major and intermedia. Br. J. Haematol. 2000, *111:* 902-907.
19. Sartorio A., Conte G., Conti A., *et al.* Effects of 12 months rec-GH therapy on bone and collagen turnover and bone mineral density in GH deficient children with thalassaemia major. J. Endocrinol. Invest. 2000, *23:* 356-361.
20. Danesi L., Scacchi M., De Martin M., *et al.* Evaluation of hypothalamic-pituitary function in patients with thalassemia major. J. Endocrinol. Invest. 1992, *15:* 177-184.
21. Pintor C., Cella S.G., Manso P., *et al.* Impaired growth hormone (GH) response to GH-releasing hormone in thalassemia major. J. Clin. Endocrinol. Metab. 1986, *62:* 263-267.
22. Leger J., Girot R., Crosnier H., Postel-Vinay M.C., Rappaport R. Normal growth hormone response to GH-releasing hormone in children with thalassemia major before puberty: a possible age related effect. J. Clin. Endocrinol. Metab. 1989, *69:* 453-456.
23. Shehadeh N., Hazani A., Rudolf M.C., Peleg I., Benderly A., Hochberg Z. Neurosecretory dysfunction of growth hormone secretion in thalassemia major. Acta Paediatr. Scand. 1990, *79:* 790-795.
24. Katzos G., Harsoulis F., Papadopoulou M., Athanasiou M., Sava K. Circadian growth hormone secretion in short multitrasfused prepubertal children with thalassemia major. Eur. J. Pediatr. 1995, *154:* 445-449.
25. Chatterjee R., Katz M., Cox T., Bantock H. Evaluation of growth hormone in thalassemic boys with failed puberty: spontaneous versus provocative test. Eur. J. Pediatr. 1993, *152:* 721-726.
26. Postel-Vinay M.C., Girot R., Leger J., *et al.* No evidence for a defect in growth hormone binding to liver membranes in thalassemia major. J. Clin. Endocrinol. Metab. 1989, *68:* 94-98.
27. Chrysis D.C., Alexandrides T.K., Koromantzou E., *et al.* Novel application of IGF-I and IGFBP-III generation tests in the diagnosis of growth hormone axis disturbances in children with beta-thalassaemia. Clin. Endocrinol. (Oxf.) 2001, *54:* 253-259.
28. Ross R.J. GH, IGF-I and binding proteins in altered nutritional states. Int. J. Obes. Relat. Metab. Disord. 2000, *24:* S92-S95.
29. Jones J.I., Clemmons D.R. Insulin-like growth factors and their binding proteins: biological actions. Endocr. Rev. 1995, *16:* 3-34.

30. Schmid C., Steiner T., Froesch E.R. Insulin-like growth factor I supports differentiation of cultered osteoblast like cells. FEBS Lett. 1984, *173:* 48-52.
31. Hock J.M., Centrella M., Canalis E. Insulin-like growth factor I has independent effects on bone matrix formation and cell replication. Endocrinology 1988, *122:* 254-260.
32. Ernst M., Froesch E.R. Growth hormone-dependent stimulation of osteoblast-like cells in serum free cultures via local synthesis of insulin-like growth factor I. Biochem. Biophys. Res. Commun. 1988, *151:* 142-147.
33. Canalis E., McCarthy T., Centrella M. Isolation of growth factors from adult bovine bone. Calcif. Tissue Int. 1988, *43:* 346-351.
34. Kream B.E., Petersen D.N., Raisz L.G. Cortisol enhances the anabolic effects of insulin-like growth factor I on collagen synthesis and procollagen messenger ribonucleic acid levels in cultered 21-day fetal rat calvariae. Endocrinology 1990, *126:* 1576-1583.
35. Margolis R.N., Canalis E., Partridge N.C. Anabolic hormones in bone: basic research and therapeutic potential. J. Clin. Endocrinol. Metab. 1996, *81:* 872-877.
36. Fiorelli G., Formigli L., Zecchi Orlandini S., *et al*. Characterization and function of the receptor for IGF-I in human preosteoclastic cells. Bone 1996, *18:* 269-276.
37. Martin J.L., Baxter R.C. Insulin-like growth factor binding protein-3: biochemistry and physiology. Growth Regul. 1992, *2:* 88-99.
38. Soliman A.T., EL Banna N., Abdel Fattah M., El Zalabani M.M., Ansari B.M. Bone mineral density in prepubertal children with beta-thalassemia: correlation with growth and hormonal data. Metabolism 1998, *47:* 541-548.
39. Wonke B. Bone disease in beta-thalassaemia major. Br. J. Haematol. 1998, *103:* 897-901