J. Inher. Metab. Dis. 16 (1993) 441–450

Osteoporosis in Lysinuric Protein Intolerance

K. PARTO[1,2], R. PENTTINEN[2], I. PARONEN[4], L. PELLINIEMI[3] and O. SIMELL[1]
[1]*Department of Pediatrics, University Central Hospital, University of Turku;* [2]*Department of Medical Biochemistry, University of Turku;* [3]*Laboratory of Electron Microscopy, University of Turku, 20520 Turku, Finland;* [4]*Department of Pathology, University of Tampere, 33520 Tampere, Finland*

Summary: Lysinuric protein intolerance (LPI) is an autosomal recessive disease characterized by defective transport of cationic amino acids. Patients have an increased incidence of fractures and their skeletal radiographs show osteoporosis. The aim of the study was to characterize the osteopenia in LPI.

Twenty-nine Finnish LPI patients (age range 3.7–44.4 years) were screened for parameters of bone metabolism. Morphometric analysis of bone was carried out in specimens of 9 patients. Collagen synthesis was studied with cultured skin fibroblasts (4 patients) and collagen fibril sizes (3 patients) were measured using electron microscopy.

Most histological bone specimens (8/9) showed osteoporosis. Osteomalacia was excluded. Routine clinical laboratory tests were unrevealing. The concentrations of free hydroxyproline and type III procollagen N-propeptide in serum and the urinary excretion of hydroxyproline were increased in almost all patients during their growth and in about half of adult patients. Collagen synthesis in LPI fibroblast cultures was significantly decreased compared with that in age-matched controls at 5 ($p < 0.01$), 14 ($p < 0.01$) and still at 30 years ($p < 0.01$), whereas no difference was observed at the age of 44 years (p = N.S.).

Osteoporosis in LPI might reflect defective matrix protein synthesis caused by protein deprivation and deficiency of cationic amino acids. Increased collagen turnover can also contribute to the osteoporosis.

Lysinuric protein intolerance (LPI; McKusick 222700) is an autosomal recessive disease in which the transport of cationic amino acids lysine, arginine and ornithine is defective (Simell et al 1975; Rajantie et al 1981; Simell 1989). To date, about 80 LPI patients have been diagnosed worldwide, 38 of them in Finland, where the prevalence of this disease is 1 in 60 000. The urinary excretion of the cationic amino acids in LPI is markedly increased, whereas their plasma concentrations are normal or low. The patients cannot tolerate usual amounts of dietary protein and often present with hyperammonaemic episodes. Other signs of LPI are retarded growth, muscular hypotonia and enlarged liver and spleen.

Osteopenia is an outstanding feature in the clinical picture of LPI and it can even

MS received 14.5.92 Accepted 21.12.92

be the main sign of the disease (Carpenter et al 1985). In our previous study (Svedström et al 1993) 29 Finnish LPI patients had 57 fractures during a mean follow-up time of 18.1 years. Two-thirds of the fractures occurred in patients before the age of 15 years and most fractures in childhood were caused by minor trauma. Thirteen of the 29 patients studied showed signs of osteoporosis in skeletal radiographs.

The aim of the present study was to characterize the type and possible pathogenetic mechanism of osteopenia in LPI. Our hypothesis is that dietary protein restriction and the suggested defect in cationic amino acid transporter protein in LPI cause a functional deficiency of essential cationic amino acids, decreased matrix protein synthesis and impaired capacity for maintenance of normal bone remodelling.

METHODS

Patients: The study group consisted of 29 Finnish LPI patients (12 men and 17 women), age range 3.7–44.4 years (mean 21.3 years). The diagnosis of LPI was based on massively increased urinary excretion of lysine, arginine and ornithine, their decreased concentrations in plasma, increased concentration of ferritin in serum, increased activity of lactate dehydrogenase in serum, postprandial hyperammonaemia and clinical findings of LPI (Perheentupa and Simell 1974). In addition to LPI, one patient had congenital rubella syndrome, one had hypothyroidism, one had rheumatoid arthritis, one had a history of stroke and five patients were hypertensive needing medication. All patients used L-citrulline 0.1–0.5 g/kg for improvement of urea synthesis and protein tolerance (Rajantie et al 1980; Mizutani et al 1984). Calcium intake was at least 1 g/day. Supplements were used, if necessary.

Laboratory investigations: All patients underwent extensive biochemical screening reflecting bone metabolism. Serum calcium, phosphate, magnesium, alkaline phosphatase, creatinine, total protein, albumin, lactate dehydrogenase, oestradiol, testosterone, cortisol, thyroid-stimulating hormone, parathyroid hormone, vitamin D metabolites, osteocalcin, calcitonin and free hydroxyproline and urinary excretion of calcium, phosphate and hydroxyproline were measured using standard automated methods. Carboxyterminal propeptide of type I procollagen (PICP) and aminoterminal propeptide of type III procollagen (PIIINP) in the serum were measured by radioimmunoassay (Trivedi et al 1989; Melkko et al 1990). The results were compared case by case to age-related reference values.

Bone histomorphometry: Bone biopsy specimens were taken from 9 patients (4 men and 5 women, aged 14–44 years). Adult patients were given tetracycline 25 mg/kg/per day for two 3-day periods with a 14-day interval. The biopsy specimens were obtained in patients under local anaesthesia from the posterior iliac crest. The volumetric density of trabecular bone and osteoid seams, surface area per tissue volume, extent of osteoid along bone surfaces, extent of bone surfaces covered with osteoblasts, extent of bone surfaces covered with osteoclasts, mean trabecular plate thickness and osteoid seam width were measured. Bone specimens obtained at autopsy were used as controls in this part of the study (Hoikka and Arnala 1981).

Collagen synthesis by cultured skin fibroblasts: Collagen metabolism was studied in 4 LPI patients aged 5, 14, 30 and 44 years and compared to controls matched for age and sex. Fibroblasts were cultured from forearm skin biopsy specimens. Cells (passages 3–10) were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 50 μg/ml ascorbic acid, 100 U/ml penicillin and 100 μg/ml streptomycin. Cultures were regularly screened for mycoplasm infection (Chen 1977).

Cell lines from patients and controls were used for the determination of total protein and collagen synthesis. Fibroblasts (250 000) were plated on 8.9 – cm^2 plastic Petri dishes. Eight to ten parallel cultures were analysed in each experiment. Confluent cultures were labelled with Dulbecco's minimal essential medium containing [^{3}H]proline 20 or 50 μCi/dish without serum but containing sodium ascorbate 50 mg/L. Media were collected after 24 h and dialysed against water until free from non-incorporated radioactivity. The cells were washed with PBS, supplied with protease inhibitors (40 mmol/L phenylmethylsulphonyl fluoride and 40 mmol/L *p*-chloromercurobenzoate), collected with a cell harvester and dialysed against water as above. Dialysed samples were hydrolysed for 3 h in 6 mol/L HCl at 130°C and used for the determinations of hydroxyproline (Juva and Prockp 1966). Parallel dishes were used for counting of the cells.

Radioactivity was measured using a liquid scintillation spectrometer. The mean values of total protein synthesis (total ^{3}H-radioactivity) and collagen accumulation (hydroxyproline radioactivity) were measured and the ratios were compared using Student's paired *t*-test. The activity of prolyl 4-hydroxylase was measured (Pihlajaniemi et al 1991) using the same cell lines.

Electron microscopy of collagen fibrils: Collagen fibrils in skin specimens of three LPI patients (ages 4, 30 and 44 years) were studied by morphometry and compared to controls matched for age and sex.

Skin biopsy specimens were fixed in 5% glutaraldehyde in 0.16 mol/L s-collidin-HCl buffer (pH 7.4), postfixed in 1% OsO_4 and 1.5% potassium ferrocyanide, dehydrated through a graded series of ethanol and embedded in epoxy resin (Glysidether 100, Merck Co. Darmstadt, Germany). Samples were examined in a JEM-100C (JEOL, Tokyo, Japan) transmission electron microscope. The diameters of 200 collagen fibril cross-sections in reticular dermis were measured from electron micrographs (magnification × 54 000) in each analysis.

Informed consent for the studies was obtained from all patients or their guardians. The study was approved by the Ethics Committee of the University of Turku and the University Central Hospital.

RESULTS

Laboratory investigations: The results of routine clinical laboratory tests reflecting calcium metabolism were unrevealing. Most patients showed normal concentrations of calcium (29 patients of 29 studied), phosphate (28/29) and magnesium (28/28) in serum, as well as normal daily urinary excretion of calcium (26/27) and phosphate (27/27). Serum oestradiol (10/10), testosterone (6/6), thyroid-stimulating hormone (29/29), cortisol (21/21), vitamin D metabolites (1,25-$(OH)_2$-D (27/27); 25-OH-D

(27/27); 24,25-$(OH)_2$-D (18/18)), parathyroid hormone (27/27), calcitonin (27/27) and osteocalcin (27/27) concentrations were also within the normal range.

The daily urinary excretion of hydroxyproline was most markedly increased during the active growth period (6/10); small children showed only moderately increased values. About half of the adults with total bone maturation (6/15) also showed increased excretion (mean of all adults 212 ± 103 μmol/m^2, reference range for adults 60–180 μmol/m^2). The serum hydroxyproline concentrations were increased in almost all (7/8) patients in the active growth spurt. Also, 10 of 14 adults showed increased concentrations (mean of adults 20 ± 9 μmol/L, reference range for adults 5–12 μmol/L). The serum concentration of carboxyterminal propeptide of type I procollagen (PICP) was normal in all patients (20/20). The aminoterminal propeptide of type III procollagen (PIIINP) was normal in all child patients and again increased in the active growth period (4/7), and in adults (10/11; mean of adults 6.50 ± 2.18 μg/L, reference range for adults 1.70–4.20 μg/L).

Serum alanine aminotransferase activity as well as albumin, protein and creatinine concentrations were normal in all patients (29/29). The activity of lactate dehydrogenase (mean 2760 ± 1210 U/L, reference range for adults 200–400 U/L) and serum concentration of ferritin (mean 3260 ± 6507 μg/L, reference range for adults 13–230 μg/L) were massively increased in all patients (29/29).

Histomorphometry of bone: Eight of the nine patients studied showed histological evidence of osteoporosis; five of them had severe and three had mild disturbances. Only one patient showed normal histomorphometric findings. The mean trabecular bone volume and osteoid volume were markedly reduced. Tetracycline labels in the biopsy samples were barely identifiable and double lines were not detected. The number of osteoblasts and osteoclasts was low. The extent of osteoid along the bone surfaces was normal or decreased in all specimens (Table 1).

Collagen metabolism: Collagen synthesis (the ratio of hydroxyproline radioactivity to total protein radioactivity) in LPI fibroblast cultures was significantly decreased compared with that in age-matched controls at the ages of 5 ($p < 0.01$), 14 ($p < 0.01$) and 30 years ($p < 0.01$), whereas no difference was observed at the age of 44 years (p = N.S.) (Table 2). There was no difference in the total radioactivity between LPI and control cultures.

The activity of prolyl 4-hydroxylase in LPI fibroblasts was moderately decreased compared with controls (Table 2).

The morphometric analysis of skin collagen fibrils showed no differences between patients and controls (Table 3).

DISCUSSION

The patients with LPI have an increased incidence of fractures and their skeletal radiographs show morphological abnormalities and signs of osteoporosis. However, no correlation was found between fracture incidence and radiologically abnormal bone structure (Svedström et al 1993). The histomorphometry of bone in 8 patients of the 9 studied showed signs of osteoporosis. The normal or reduced extent of

Table 1 Histomorphometry of bone in nine LPI patients. The existing radiological signs of osteoporosis[a] and the number of fractures are also shown

Age (years)	*Sex*	*Volumetric density of trabecular bone* (mm^3/cm^3)[b]	*Osteoid/bone surface* (mm^2/cm^3)[b]	*Volumetric density of osteoid seams* (mm^3/cm^3)[b]	*Histomorphometric diagnosis*	*Radiological signs of osteoporosis*[a]	*Number of fractures*
14	F	153 (199 ± 53)	138 (167 ± 79)	4 (19 ± 9)	Severe osteoporosis	−	1
19	M	110 (222 ± 49)	181 (210 ± 67)	4 (17 ± 6)	Severe osteoporosis	+	0
23	F	135 (199 ± 53)	16 (167 ± 79)	16 (18 ± 9)	Mild osteoporosis	+	2
25	M	193 (220 ± 49)	278 (210 ± 67)	16 (17 ± 6)	Mild osteoporosis	+	1
33	F	230 (226 ± 72)	222 (188 ± 43)	2 (19 ± 15)	Normal bone	−	0
34	M	160 (175 ± 43)	50 (174 ± 97)	5 (11 ± 6)	Severe osteoporosis	−	4
35	F	84 (226 ± 72)	0 (188 ± 143)	0 (19 ± 15)	Severe osteoporosis	+	0
37	F	166 (226 ± 72)	137 (188 ± 143)	15 (19 ± 15)	Severe osteoporosis	−	3
44	M	207 (162 ± 43)	423 (268 ± 101)	1.3 (22 ± 11)	Mild osteoporosis	+	0

[a]Svedström et al 1993
[b]Normal range (means ± SD) in parentheses

Table 2 Collagen metabolism in four LPI patients and foure age- and sex-matched controls. The existing radiological signs of osteoporosis and the number of fractures are also shown

Patient	*Age* (years)	*n*[a]	*Collagen accumulation*[b] (mean ± SD, $\times 10^{-3}$)	*Activity of prolyl 4-hydroxylase* (dpm/mg protein)	*U-HOP*[c] (μmol/m^2 per day)	*S-HOP*[d] (μmol/L)	*S-PIIINP*[e] (μg/L)	*S-PICP*[f] (μg/L)	*Radiological signs of osteoporosis*[g]	*Number of fractures*
LPI 1	5	8	44.2 ± 10.2	10920	220	17	NT[h]	NT	+	2
Control 1	5	8	66.8 ± 23.8	30050						
LPI 2	14	8	58.9 ± 18.6	27720	710	42	9.6	274	+	7
Control 2	14	8	93.5 ± 35.7	29600						
LPI 3	30	9	51.4 ± 8.4	24050	240	10	5.0	86	+	0
Control 3	30	9	82.0 ± 18.6	35600						
LPI 4	44	10	38.4 ± 10.5	22810	343	14	3.5	60	+	3
Control 4	44	10	41.8 ± 5.8	23730						

[a] n = number of culture dishes used for testing
[b] mean ratio of hydroxy-[^{3}H]proline radioactivity to total protein radioactivity
[c] U-HOP = urinary excretion of hydroxyproline; normal range for children aged < 15 years 300–700 and for adults 60–180 μmol/m^2 per day
[d] S-HOP = serum concentration of hydroxyproline; normal range for adults 5–12 μmol/L
[e] S-PIIINP = concentration of aminoterminal propeptide of type III procollagen in serum; normal range for age 14 years 12.9 ± 4.1 and for adults 1.7–4.2 μg/L
[f] S-PICP = concentration of carboxyterminal propeptide of type I procollagen in serum; normal range for adults 50–200 μg/L
[g] Svedström et al 1993
[h] NT = not tested

Table 3 Mean diameters of collagen fibrils in reticular dermis of patients with LPI and controls

	Age (years)	*Diameter* (Mean ± SD; nm)
LPI 1	4	99.2 ± 5.8
Control 1	4	105.5 ± 5.8
LPI 2	30	102.7 ± 5.8
Control 2	30	99.5 ± 4.6
LPI 3	44	102.8 ± 4.3
Control 3	45	97.5 ± 4.4

osteoid along the bone surfaces excludes osteomalacia as a cause of osteopenia. The weak labelling after tetracycline exposure indicates severely impaired new bone formation. The histological findings resemble those of malnourished rats (Shieres et al 1980) and rhesus monkeys (Jha et al 1968), which also show reduced quantity of compact bone, osteoid, osteoblasts and osteoclasts as well as failure to incorporate tetracycline label in a normal way. Again, no correlation was found between the severeness of histomorphometric findings and the number of fractures or osteoporotic changes detected in radiographs.

As aetiological factors for osteoporosis we could exclude the common causes of increased bone resorption, i.e. hypocalcaemia, hyperparathyroidism, vitamin D deficiency, hyperthyroidism and hypercortisolism. Further, serum concentrations of oestradiol, testosterone and calcitonin, all important regulators of bone formation, were within the normal range.

LPI patients develop spontaneous aversion to protein at an early age. During the whole growth period they have to balance adequate caloric and protein intake and hyperammonaemia. Although L-citrulline increases their protein tolerance, the daily protein intake is often lower than the recommended 0.8–1.2 g/kg. Protein deficiency is relatively most severe during the active growth period, which is also reflected in skeletal findings and fracture incidence. Besides protein deprivation, deficiency of a single essential amino acid also affects protein synthesis. Rats suffering from dietary lysine deficiency show marked retardation of bone growth (Likins et al 1957). Although the plasma concentrations of cationic amino acids in LPI are normal or only slightly decreased, the transport defect reduces their availability for protein synthesis. Since bone is constantly a highly active tissue, alterations in protein synthesis are prone to have skeletal manifestations (Robey 1989; Boskey 1990).

The proportion of dairy products and meat in the diet of patients with LPI is very small and most patients need supplementation to get the recommended minimum daily intake of 800 mg calcium (Consensus Development Conference 1991). Despite the supplementation, insufficient intake of calcium cannot be excluded as a contributing factor to the genesis of osteoporosis in LPI.

Immobilization leads to osteoporosis, whereas exercise has been shown to increase bone mass in osteoporotic women (Rigotti et al 1984). All our patients are physically active except one, who suffered a stroke and hemiplegia at the age of 33 years.

Several studies suggest an altered collagen metabolism in osteoarthritis and osteoporosis (Gevers and Dequeker 1987; Jimenez 1991), and decreased synthesis of collagen has been reported to be associated with aspartylglucosaminuria, another inherited metabolic disease with skeletal manifestations (Näntö-Salonen and Penttinen 1982). Mutations in procollagen type I gene cause osteogenesis imperfecta and have been detected in some other inherited disorders resulting in structural or functional abnormalities of connective tissue (Holbrook and Byers 1982; Prockop and Kivirikko 1984; Kuivaniemi et al 1991). In LPI the decreased hydroxyproline biosynthesis as a measure of collagen and the decreased activity of prolyl 4-hydroxylase might reflect a generally impaired matrix protein synthesis.

The concentration of free serum hydroxyproline (Minisola et al 1985) and its urinary excretion (Epstein 1988) correlate with the rate of collagen degradation. As hydroxyproline is a neutral amino acid, its renal tubular reabsorption in LPI is unaffected. Our patients were also hospitalized for 24 hours on a gelatin-free diet before urine collection to reduce the day-to-day variation of hydroxyproline excretion (Gasser et al 1979). The increased serum hydroxyproline concentrations and increased daily urinary excretion in LPI patients suggest increased collagen degradation.

Collagen propeptides have been suggested to regulate collagen synthesis by feedback inhibition (see Fouser et al 1991 for references). The serum concentrations of type I procollagen carboxyterminal propeptides were normal, suggesting no major alteration in type I collagen synthesis. Theoretically, however, their uptake from the circulation by liver endothelial cells (Bentsen et al 1990) could be affected. Type III collagen is detected in bone in only marginal amounts, and the increased serum concentrations of aminoterminal propeptide of procollagen type III in adults might indicate increased turnover of soft-tissue collagen due, for example, to inflammatory processes (Risteli and Risteli 1990). All LPI patients show hepatomegaly; severe pulmonary and hepatic complications are common, and the massively increased activity of lactate dehydrogenase might reflect general cell damage. The PIIINP concentration might thus be increased because of its defective uptake by the receptor-mediated endocytosis of liver cells (Smedsröd 1988).

Patients with LPI show extensive variation in the severity of organ-specific manifestations of the disease, suggesting the possible existence of more than one mutation. Among the other inborn errors of metabolism in Finns, aspartylglycosaminuria is caused by practically one single mutation (AGU_{Fin}), whereas gyrate atrophy, another Finnish metabolic disease, has been shown to be caused by several mutations (Brody et al 1992).

After excluding the well-known causes of secondary osteoporosis we suggest that osteoporosis in LPI reflects defective extracellular matrix protein synthesis due to dietary protein deprivation and functional deficiency of cationic amino acids. The accumulation of bone collagen is decreased during the growth period and collagen turnover might also be increased.

ACKNOWLEDGEMENTS

The authors thank Drs Leila and Juha Risteli, Collagen Research Unit, University of Oulu, for determination of serum procollagen propeptides, Dr Raili Myllylä,

Department of Medical Biochemistry, University of Oulu, for determination of the activity of prolyl 4-hydroxylase and Mr Urpo Reunanen for technical help with electron microscopy. The study was supported with a grant from the Foundation for Pediatric Research and from the Sigrid Jusélius Foundation, Finland.

REFERENCES

Bentsen KD, Henriksen JH, Bendtsen F, Horslev-Petersen K, Lorenzen I (1990) Splanchnic and renal extraction of circulating type III procollagen aminoterminal propeptide in patients with normal liver function and in patients with alcoholic liver disease. *Hepatology* **11**: 957–963.

Boskey AL (1990) Bone mineral and matrix. *Orthop Clin N Am* **21**: 19–29.

Brody LC, Mitchell GA, Obie C et al (1992) Ornithine delta-aminotransferase mutations in gyrate atrophy. Allelic heterogeneity and functional consequences. *J Biol Chem* **15**: 3302–3307.

Carpenter TO, Levy HL, Holtrop ME, Shih VE, Anast CS (1985) Lysinuric protein intolerance presenting as childhood osteoporosis. *N Engl J Med* **31**: 290–294.

Chen T (1977) In situ detection of mycoplasma contamination in cell cultures by fluorescent Hoechst 33258 stain. *Exp Cell Res* **104**: 255–262.

Consensus development conference: prophylaxis and treatment of osteoporosis. Conference report (1991) *Am J Med* **90**: 107–110.

Epstein S (1988) Serum and urinary markers of bone remodeling: assessment of bone turnover. *Endocrine Rev* **9**: 437–449.

Fouser L, Sage EH, Clark J, Bornstein P (1991) Feedback regulation of collagen gene expression: A Trojan horse approach. *Proc Natl Acad Sci USA* **88**: 10158–10162.

Gasser A, Celada A, Courvoisier B et al (1979) The clinical measurement of urinary total hydroxyproline excretion. *Clin Chim Acta* **95**: 487–491.

Gevers G, Dequeker J (1987) Collagen and non-collagenous protein content (osteocalcin, sialoprotein, proteoglycan) in the iliac crest bone and serum osteocalcin in women with and without hand osteoarthritis. *Collagen Rel Res* **7**: 435–442.

Hoikka V, Arnala I (1981) Histomorphometric normal values of the iliac crest cancellous bone in a Finnish autopsy series. *Ann Clin Res* **13**: 383–386.

Holbrook KA, Byers PM (1982) Structural abnormalities in the dermal collagen and elastic matrix from the skin of patients with inherited connective tissue disorders. *J Invest Dermatol* **79**: 7–16.

Jha GH, Deo MG, Ramalingaswami V (1968) Bone growth in protein deficiency, a study in rhesus monkeys. *Am J Pathol* **53**: 1111–1121.

Jimenez SA (1991) Molecular biological approaches to the study of heritable osteoarthritis. *J Rheumatol* **18**: 7–9.

Juva K, Prockop DJ (1966) Modified procedure for the assay of H^3- or C^{14}-labeled hydroxyproline. *Anal Biochem* **15**: 77–83.

Kuivaniemi H, Tromp G, Prockop DJ (1991) Mutations in collagen genes: Causes of rare and some common diseases in humans. *FASEB J* **5**: 2052–2060.

Likins RC, Bavetta LA, Posner AS (1957) Calcification in lysine deficiency. *Arch Biochem Biophys* **70**: 401–412.

Melkko J, Niemi S, Risteli L, Risteli J (1990) Radioimmunoassay of the carboxyterminal propeptide of human type I procollagen. *Clin Chem* **36**: 1328–1332.

Minnisola S, Antonelli R, Mazzuoli G (1985) Clinical significance of free plasma hydroxyproline measurement in metabolic bone disease. *J Clin Chem Clin Biochem* **23**: 515–519.

Mizutani N, Kato T, Maehara M, Watanabe K, Ban M (1984) Oral administration of arginine and citrulline in the treatment of lysinuric protein intolerance. *Tohuko J Exp Med* **142**: 15–24.

Näntö-Salonen K, Penttinen R (1982) Metabolism of collagen in aspartylglycosaminuria: Decreased synthesis by cultured fibroblasts. *J Inher Metab Dis* **5**: 197–203.
Perheentupa J, Simell O (1974) Lysinuric protein intolerance. *Birth Defects* **10**: 201–207.
Pihlajaniemi T, Myllylä R, Kivirikko K (1991) Prolyl-4-hydroxylase and its role in collagen synthesis. *J Hepatol* **13** (Suppl.): 2–7.
Prockop DJ, Kivirikko KI (1984) Heritable diseases of collagen. *N Engl J Med* **9**: 376–386.
Rajantie J, Simell O, Rapola J, Perheentupa J (1980) Lysinuric protein intolerance: A two-year trial of dietary supplementation therapy with citrulline and lysine. *J Pediatr* **97**: 927–932.
Rajantie J, Simell O, Perheentupa J (1981) Lysinuric protein intolerance: basolateral transport defect in renal tubuli. *J Clin Invest* **67**: 1078–1082.
Rigotti N, Nussbaum S, Herzog D, Neer R (1984) Osteoporosis in women with anorexia nervosa. *N Engl J Med* **311**: 1601–1606.
Risteli L, Risteli J (1990) Noninvasive methods for detection of organ fibrosis. In Rojkind M, ed. *Focus on Connective Tissue in Health and Disease*. Boca Raton: CRC Press, 61–98.
Robey PG (1989) The biochemistry of bone. *Endocrin Metab N Am* **18**: 859–891.
Shieres R, Avioli LV, Bergfeld MA, Fallon MD, Slatopolsky E, Teitelbaum SL (1980) Effects of semistarvation on skeletal homeostasis. *Endocrinol* **107**: 1530–1535.
Simell O (1989) Lysinuric protein intolerance and other cationic aminoacidurias. In Scriver C, Beaudet AL, Sly W and Valle D, eds. *The Metabolic Basis of Inherited Disease*, 6th edn. McGraw-Hill, New York, 2497–2513.
Simell O, Perheentupa J, Rapola J, Visakorpi J, Eskelin L (1975) Lysinuric protein intolerance. *Am J Med* **59**: 229–240.
Smedsröd B (1988) Aminoterminal propeptide of type III procollagen is cleared from the circulation by receptor-mediated endocytosis in liver endothelial cells. *Collagen Rel Res* **8**: 375–388.
Svedström E, Parto K, Marttinen M, Virtama P, Simell O (1993) Skeletal manifestations of lysinuric protein intolerance. A follow-up study of 29 patients. *Skeletal Radiol* **22**: 11–16.
Trivedi P, Hindmarsh P, Risteli J, Risteli L, Mowat AP, Brook CGD (1989) Growth velocity, growth hormone therapy, and serum concentrations of the amino-terminal propeptide of type III procollagen. *J Pediatr* **114**: 225–230.