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Hypoplasia osteolysis, epiphyseal
muscle atrophy skeleton

Selective Muscle Fiber Hypoplasia and Epiphyseal Osteolysis

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Summary

This paper reports the investigation of a case associating grotesque skeletal deformities, selective hypoplasia of muscle fiber types I and IIA, and a decrease in skeletal mineral plus increased skeletal resorption. The investigation also included administration of synthetic salmon calcitonin, a hormone that reduces bone resorption. The patient, a 10-year-old female, presented with foreshortened, clawlike hands and feet, joint stiffness with contractures, marked muscle atrophy, and severe joint pain. Radiologic survey revealed muscle atrophy, osteoporosis and epiphyseal osteolysis. ⁴⁷Ca turnover was accelerated. Muscle biopsy revealed a population of hypoplastic muscle fibers, 10-22 μ m in diameter, scattered singly or in small groups among a majority of larger morphologically normal fibers. Myofibrillar ATPase stains showed that all types I and IIA fibers present were hypoplastic, and all large fibers present were type IIB. Electron microscopic study of the skeletal muscle showed normal myofibrils but vacuolated mitochondria with disorganized cristae in the hypoplastic fibers. Synthetic salmon calcitonin given at 2 MRC units/kg 3 days a week produced a marked improvement in bone pain and reduced urinary excretion of hydroxyproline from 110.3 and 155.9 mg/24 hr during the two control periods to 136.2 and 84.2 mg/24 hr. Calcium metabolic balance changed from an average control value of +124.4 to +248 mg/day during calcitonin therapy. Iliac crest biopsy before calcitonin revealed increased uncalcified bone matrix and increased osteoclastic activity. After 3 months of calcitonin there was a decrease in the amount of uncalcified matrix and an apparent decrease in osteoclastic activity. Skin biopsy was normal.

Speculation

One may speculate as to the relationship between the muscle and bone abnormalities in the present case. Joint contractures

are not uncommon in children with muscle disease, and it is likely that the joint deformities are at least in part related to the muscle hypoplasia and weakness. The osteomalacia and epiphyseal osteolysis, on the other hand, have not been reported in association with other types of congenital muscle disease, and appear much too severe to be secondary phenomena. The selective muscle fiber hypoplasia and severe osteomalacia are apparently two associated aspects of one clinical syndrome. Whether the mitochondrial abnormalities noted in the muscle reflect the underlying metabolic defect of this syndrome remains to be investigated.

We report a girl of Oriental-American Negro descent who was found to have the clinical features of foreshortened, clawlike hands and feet, joint stiffness, and contractures with severe joint pain and muscle atrophy. This characteristic clinical picture is explained by a selective muscle fiber hypoplasia affecting types I and IIA fibers, associated with a marked osteolytic process. To our knowledge, this is the first time that carpal and tarsal osteolysis has been associated with a selective muscle fiber hypoplasia.

MATERIALS AND METHODS

CASE REPORT

A 10-year-old girl was born by normal vaginal delivery after an uncomplicated full term pregnancy. The mother is Oriental and the father is an American Negro. The family history revealed a mother, two sisters, and a brother in good health. The father had muscle weakness during childhood and adolescence, and he was refused admission to the army because of it. A recent physical examination revealed normal muscle strength and mass. He refused a muscle biopsy. There was no history of musculo-

skeletal disease in any other member of the family. Neonatal period was uneventful. Psychomotor development was normal except that the child was only able to walk with bilateral long braces because of "weakness" in both legs. At age 2 years, she suffered a fracture of the left leg. The first available hospital records at age 5 years documented the presence of diffuse weakness in arms and legs, ulnar deviation of both wrists, and essentially no motion in the knees; a radiologic study revealed absence of the tarsal and carpal bones. From 5–10 years there has been a progressive increase in weakness of upper and lower extremities with limitation of movements at elbow, wrist, knee, and ankle joints. At 7 years of age, she suffered a fracture of the left femur, and has, since then, been confined to a wheel chair. At 10 years of age, the patient was referred to us for evaluation.

On physical examination, the patient was an alert, cooperative, prepubescent girl with short stature (height 120 cm; -3.5 SD from the mean for age). She was found to be unable to ambulate because of grotesque deformities of upper and lower extremities, characterized by foreshortened clawlike hands and feet, and stiffness of elbows, wrists, knees, and ankle joints (Figs. 1 and 2). Marked kyphoscoliosis, generalized muscle atrophy, and tenderness of both knees and ankle joints were present. Examination of the teeth revealed absence of the lamina dura. There was hypoplasia of the external ear canal. After 2 weeks of hospitalization, therapy was started with synthetic salmon calcitonin (Armour lot no. K735062), which was given subcutaneously at 2 MRC units/kg 3 days a week. Oral calcium in the form of Neocalglucon was given at the dose of 5 ml three times a day (equivalent to a supplement of 45 mg calcium daily). After 2 weeks of therapy, joint pain was markedly reduced. There was increased joint mobility. After 6 weeks of hospitaliza-

Fig. 2. Small foreshortened, clawlike hands.

tion the patient was discharged on synthetic salmon calcitonin and oral calcium at the dose described above.

RADIOLOGIC FINDINGS

The radiologic studies of the skeleton included spine, skull, hips, pelvis, long bones, and hand and feet. In addition, there was a chest examination as well as an intravenous urogram, which was normal. The studies of the joints showed severe destruction in both the ankles and feet and elbows and wrists (Figs. 3 and 4). The carpal bones were totally destroyed and there was erosion of the proximal metacarpals. The phalanges were expanded and appeared to have a coarsened trabecular pattern. The destructive arthritis was seen in the metacarpal, phalangeal, and interphalangeal joints. Resorption of the small bones was also noted with severe osteoporosis and destruction of the tarsal bones. Interestingly, the soft tissues about the hands and feet were comparatively swollen in comparison to the soft tissues of the upper and lower extremities, which were markedly diminished. There were also destructive changes in the long bones with irregularities of the epiphyseal centers, osteoporosis, and the shaft of the long bones markedly thinned with the metaphyseal areas widened. The hips and pelvis were misshapen with bilateral coxa valga and destruction of the femoral heads. The clavicles were also widened but there were no destructive changes at the acromial junction. Contracture deformities were severe about the knees and elbows. The spine showed evidence of a kyphoscoliosis in the thoracolumbar area. A lateral view of the skull showed a brachiocephalic configuration. No subluxation of C_1 and C_2 was present.

Bone density was measured by radiologic studies of the left hand and wrist following the method of Colbert (17) before calcitonin therapy and 3 months after. There was a marked

Fig. 1. Patient at age 10 years. Note the foreshortened clawlike hands and feet; marked stiffness of lower and upper extremities and inability to stand.

Fig. 3. The carpals are almost totally destroyed with erosion of the proximal metacarpals as well as the distal portion of the radius and ulna. The distal radius is flared. There is soft tissue swelling about the hand with markedly diminished soft tissues about the forearm and upper arm. Contracture deformity is present at the elbow. The bones show a severe osteoporosis with a coarsened cystic trabecular pattern with joint changes throughout.

increase of bone density after 3 months of calcitonin therapy. Before calcitonin bone density measured 159 (normal for sex and age is 195). The standard score corresponded to -1.19 at the 12th percentile. After 3 months of calcitonin bone density measured 180 (normal for sex and age is 200). The standard score corresponds to -0.65 at the 26th percentile.

LABORATORY FINDINGS

Determinations of serum sodium, potassium, chloride, bicarbonate, glutamic oxaloacetic transaminase, total protein, albumin, bilirubin, glucose, and blood urea nitrogen concentrations were determined by the SMA-12 Technicon AutoAnalyzer method and were found to be normal. Plasma creatine phosphokinase and aldolase were normal.

Standard tests for screening inborn errors of metabolism (horizontal paper chromatography for amino acids, Clinitest for reducing substances, Phenistix for keto acids, nitroprusside test for sulfur-containing amino acids, and cetyltrimethylammonium bromide test for mucopolysaccharides), with the use of a modification of the methods described by Renuart (48) and by Efron

and associates (21), as well as dermatoglyphics and chromosome studies, were normal. Electromyographic studies were within normal limits. An insulin stimulation test, performed as previously described (14), produced a peak immunoreactive human growth hormone value of 7 ng/ml (normal response). There was no evidence of hypothyroidism as documented by the normal concentration of plasma thyroxine. The basal 24-hr urinary 17-ketogenic steroid excretion was normal. An ACTH and metyrapone test done as previously described (14), elicited normal adrenal responses.

CALCIUM METABOLISM

Serum concentrations of calcium, measured by automated atomic absorption spectrophotometry to a precision and accuracy of $\pm 1\%$ and phosphorus by the method of Fiske and SubbaRow (24) (9.6 and 5.2 g/100 ml, respectively), were normal. ^{47}Ca turnover studies were done as previously described (12). The samples were analyzed for radioactivity and stable calcium. A standard, containing a known fraction of the administered dose in the same volume as the sample, was also measured. The activity of the sample, expressed as the percentage of

the administered dose per g calcium, was calculated. The values for normal children were obtained from the estimation of the 24-hr specific activity by McPherson (43) and Bronner *et al.* (5).

The patient's 24-hr specific activity (percentage of dose $^{45}\text{Ca}/\text{g Ca}$) was 5.80 (normal control children: 11.10 ± 3.1). The reciprocal of the 24-hr ^{45}Ca specific activity was used as a measure of the exchangeable calcium pool. The patient's exchangeable calcium pool was 17.20 g (normal control children: 9.70 ± 2.4); when related to ideal body weight; the pool size was 574 mg/kg body weight (normal control children: 328 ± 89).

Calcium and phosphorus balance studies were carried out as previously described (12). Calcium metabolic balance changed from an average of the 2-week control value of +124.5 mg/day to an average of 2 weeks of calcitonin therapy of +248 mg/day (Fig. 5). There were no significant changes in phosphorus balance studies (Fig. 5). Urinary hydroxyproline, measured by the method of Baker and associates (1), declined during calcitonin therapy from 110.3 and 155.9 mg/24 hr during the 2 weeks before calcitonin to 136.2 and 84.2 mg/24 hr during calcitonin therapy (Fig. 5). Alkaline phosphatase, found to be 160 and 166 IU/liter (normal 6–110) before calcitonin therapy, declined to 136 and 135 IU/liter during calcitonin administration.

PATHOLOGIC FINDINGS

A biopsy of the gastrocnemius muscle was obtained. Part of the specimen was immediately frozen in isopentane cooled to -160° in liquid nitrogen, and part was fixed in neutral formalin.

Sections of formalin-fixed tissue were stained with hematoxylin and eosin, Gomori trichrome, and phosphotungstic acid-hematoxylin stains. Serial frozen sections of rapidly frozen unfixed tissue were cut at $10\text{-}\mu$ in a cryostat and stained with hematoxylin and eosin, the modified Gomori trichrome method (22), techniques to demonstrate succinic dehydrogenase, and NADH dehydrogenase (45, 29), and the myofibrillar ATPase reaction (46). Brooke's modification (6) of the ATPase technique was used, in that all sections were preincubated at pH 9.3 in a medium containing 0.4 M Calcium. Additional preincubation of some sections at pH 4.5 and 4.3 was done to demonstrate the type II fiber subtypes characterized by Brooke and Kaiser (7). Frozen sections were also stained with Oil Red O to show neutral fat droplets, and with the periodic acid-Schiff technique for glycogen.

For electron microscopy, strips of muscle about 1 mm in thickness were fixed in 3% glutaraldehyde buffered with 0.1 M cacodylate (pH 7.3) (50), postfixed in osmium tetroxide, and embedded in an Araldite-resin mixture (40). Thick sections were made with glass knives on a Sorvall MT-2B ultramicrotome, stained with methylene blue (49), and used for screening by light microscopy. Thin sections of selected regions were then prepared with diamond knives, and examined in a Siemens Elmiskop Ia at 80 kv.

Nondecalfied sections of iliac crest bone were prepared by Dr. Micheline Federman, New England Deaconess Hospital, Boston, Massachusetts, using tissues fixed in 70% ethanol, dehydrated through absolute alcohol and methyl benzoate, and

Fig. 4. There is marked loss of soft tissues in the lower extremities. The end of the long bones is flared. The density of the bone is markedly diminished throughout. Destruction of the small bones of the feet is present.

Fig. 5. Calcium and phosphorus balance before and during 2 weeks of salmon calcitonin administration. Fecal excretion (stippled area) is plotted upward from the baseline and urinary excretion (cross-hatched area) is plotted above fecal excretion. A clear space below the dietary intake line indicates positive balance. The 24-hr hydroxyproline excretion rate is represented by a dark area. The vertical broken line indicates the initiation of calcitonin.

embedded in methyl methacrylate. The plastic embedment was hardened by incubation at 40° for 1–4 days. Sections were cut at 4 μ m thickness with a Jung microtome (model K). Staining was by the Goldner modified trichrome method. A skin biopsy was also obtained and it was reported as normal.

RESULTS

Frozen and paraffin sections of the skeletal muscle biopsy revealed a population of hypoplastic muscle fibers, 10–22 μ m in diameter, scattered singly or in small groups among a majority of larger normal appearing fibers (Fig. 6). The large fibers measured 26–44 μ m in smallest diameter, and constituted about half the muscle fibers present. Myofibrillar ATPase and NADH dehydrogenase stains showed that all the large fibers were type II, staining light in the dehydrogenase preparations and dark in the ATPase preparations which had not been preincubated at a low pH. The small fibers, however, were a mixed population of both type I and type II fibers. Sections stained with the ATPase reaction preincubated at pH 4.5 revealed that the large fibers showed the characteristics of IIB fibers, retaining some of their staining after the preincubation, whereas the smaller type II fibers were IIA, having lost their staining. The small type I fibers characteristically stain dark in this preparation (Fig. 7). A count of 500 fibers revealed that 41% were type I, 12% were type IIA, and 47% were type IIB.

Sections stained for succinic dehydrogenase showed very sparse staining in all fiber types, and confirmed the observation that all type I fibers were hypoplastic.

Preparations stained with Oil Red O and periodic acid-Schiff reactions showed no abnormalities of fat or glycogen distribution in the muscle. Review of many frozen and paraffin sections with the light microscope showed no evidence of any degeneration or regeneration of muscle fibers, and no inflammation or fibrosis was found in the sections. The intramuscular blood vessels appeared unremarkable.

Electron microscopy of skeletal muscle showed well preserved general architecture, with only very small foci of myofibril degeneration. The plasma membranes of the fibers appeared intact, and there was no scalloping of basement membranes such as is seen in atrophic fibers. Many mitochondria were vacuolated, especially in the smaller fibers, and contained disorganized cristae and dense granules (Fig. 8). Varying degrees of vacuolization were seen, suggesting a progressive mitochondrial lesion. In some fibers mitochondria appeared normal, indicating that the changes, when present, were not artifactual.

A nondecalfied iliac crest bone biopsy showed predominantly osteomalacia, *i.e.*, a relative increase in the amount of uncalcified bone matrix (osteoid) associated with an increase in osteoblastic activity. Some osteoblasts and osteoclasts showed hydropic enlargement which was interpreted as artifactual. There was no evidence of osteoporosis, *i.e.*, there was no discernible reduction in the size of medullary bone spicules ("osteoporosis" on x-ray was probably a reflection of osteomalacic demineralization of bone rather than a true osteoporotic reduction in bone spicule size). After 3 months of calcitonin therapy, a repeated iliac crest bone biopsy, indicated a reduction in the severity of osteomalacia (Fig. 9).

DISCUSSION

This child presents with severe osteolysis associated with a selective muscle fiber hypoplasia. The skeletal abnormalities were characterized by a marked osteolytic process as indicated by the radiologic findings, the increase in serum alkaline phosphatase concentration and in 24-hr urinary hydroxyproline excretion rate and an elevated exchangeable calcium pool.

Different types of skeletal disease with osteolysis have been described. Carpal and tarsal osteolysis is a group of syndromes with painful swelling of multiple joints associated with osteolysis and skeletal deformities mostly affecting the carpal and tarsal bones. The osteolytic process has, in some cases, been associated with nephropathy (41, 42, 52, 54), angiomas (27, 28) or described alone as autosomal dominant (2, 3, 26, 38, 39, 44, 53) or autosomal recessive (3) or no known genetic trait (9, 15, 20). Winchester *et al.* (57) have described the association of carpal and tarsal osteolysis with corneal opacities and considered the disorder a mucopolysaccharide storage disease. Hollister *et al.* (31) have studied three cases of carpal and tarsal osteolysis associated with corneal opacities and concluded that there was not enough evidence for a mucopolysaccharidosis or lysosomal storage disease.

Our case had striking similarities of clinical, radiologic, and ultrastructural features to those described by Winchester *et al.* and Hollister *et al.* but is differentiated from their cases by the absence of corneal opacities and the lack of characteristic histopathological skin lesions. Of these differences, the absence of corneal opacities is the most compelling since all of the five previously reported cases of the Winchester syndrome did indeed have corneal opacities. Yet, it is by no means certain that all patients with this syndrome will invariably have the corneal opacities. The reported normal skin histology in our case may be a reflection of sampling since Hollister *et al.* (31) have found that areas of clinically normal skin are normal by light microscopy.

The selective muscle fiber hypoplasia observed in the present patient is suggestive of a new syndrome of carpal and tarsal osteolysis associated with a selective muscle atrophy. Observa-

Fig. 6. Cross section of skeletal muscle biopsy showing scattered hypoplastic fibers. Frozen section, modified Gomori trichrome stain (650 $\times$).

Fig. 7. Cross section of muscle stained for ATPase, preincubated at pH 9.3 and 4.5. Note that the hypoplastic type I fibers stain dark after acid preincubation. The normal size type II fibers are IIB, retaining some staining after incubation at pH 4.5, whereas the hypoplastic type IIA fibers have lost their staining after acid preincubation (650 $\times$).

Fig. 8. Electron micrograph of skeletal muscle showing mitochondria with sparse and disoriented cristae and with dense deposits (indicated by arrows) (33,000 $\times$).

tions on muscle have not been reported in any of the other cases of carpal-tarsal osteolysis, including the patients described by Winchester *et al.* and Hollister *et al.* Therefore, there is no data in reference to distribution of muscle fiber types for comparison.

The histochemical characterization of fiber types in human muscle, and the changes in these patterns observed in disease states, have been studied most extensively by W. K. Engel, whose review of the subject (23) lists a number of conditions which selectively involve one fiber type. Thus, type I fibers are preferentially involved in central core disease, in the target fiber degeneration observed in denervation, and in certain "mitochondrial" myopathies associated with loosely coupled oxidative phosphorylation or abnormal fatty acid metabolism. In some congenital cases of rod (nemaline) myopathy, and centronuclear myopathy, only type I fibers are affected. Type I atrophy is characteristic of myotonic dystrophy and of experimental tenotomy, whereas type II atrophy occurs in cachexia and in experimental acute total denervation. By contrast, disease states such as polymyositis and muscular dystrophy involve both fiber types equally. Because muscle fiber types are dependent on innervation and can be changed through changes in innervation (35, 36) it is likely that some of these differences in fiber type susceptibility, for example, in congenital myopathies, will ultimately be related to abnormalities of innervation.

It is more germane to the present case to examine reported cases in which hypoplasia of one or another fiber type, or marked disproportion between fiber types, was the major abnormality. Congenital type I fiber hypoplasia (congenital fiber type disproportion) has been reported in 12 cases by Brooke (4), and in one family by Kinoshita *et al.* (37). These cases showed congenital, nonprogressive weakness and hypoplasia of muscle, and some had myopathic facies, scoliosis, and high arched palates and feet. Osteomalacia and/or epiphyseal osteolysis were not reported in any case. However, in some of the cases reported by Brooke (4), congenital dislocation of the hip and joint contractures occurred. In some instances they were severe enough at birth to warrant a diagnosis of arthrogryposis. Our case is apparently unique, in that selective muscle fiber hypoplasia has not previously been associated with such severe osteolysis, or severe, progressive, and permanent deformity of joints.

The vacuolated appearance of the muscle mitochondria on electron microscopic examination is of additional interest in view of the reported findings of vacuolated mitochondria in fibro-

blasts cultured from a patient with Winchester syndrome (31). However, our patient's muscle mitochondria appear somewhat different from those in the Winchester fibroblasts, because of the presence of dense bodies (Fig. 8). They are also quite different from the inclusion- and glycogen-containing mitochondria described in many "mitochondrial myopathies" (51). It has been noted that changes in mitochondrial morphology in muscle may occur in many types of neuromuscular disease, and that in general these changes are nonspecific, merely serving to point out a site of subcellular injury or abnormal function (18, 51). Therefore, in our patient, as in patients with the Winchester syndrome, the abnormal appearance of the mitochondria probably accompanies abnormal function of these organelles. Whether this abnormality is causally related to the accompanying defects, and whether it is similar in the two instances, cannot be established from the evidence now at hand.

The lack of an effective treatment has remained the major practical problem presented by the osteolytic syndromes. Salicylates have not proved to be of any value except as needed for analgesia. Steroids have given symptomatic relief of the inflammatory joint symptoms without significantly altering the progressive osteolysis. Physical therapy and splints worn for stabilization have afforded some relief and maintain functional positions but have not influenced the progression of the osteolytic process.

Of important therapeutic implication was the improvement of the osteolytic process after 3 months of therapy with synthetic salmon calcitonin. Calcitonin is a polypeptide hormone secreted in mammals by the parafollicular cells of the thyroid gland (30). The major action of calcitonin is to inhibit bone resorption (47), which leads to a definite increase in total bone mass in experimental animals (25, 56). Information concerning the effectiveness of calcitonin in metabolic bone disease of man with high resorption rates is accumulating. Calcitonin appears to be of value in the management of adult osteoporosis (8, 10, 16, 33, 55), osteoporosis of osteogenesis imperfecta (11, 13), and especially in Paget's disease (19). The marked improvement in our patient after three months of therapy suggests that calcitonin may be of value for treatment of childhood osteolytic disease. In the present case, 2 MRC units/kg body weight of synthetic salmon calcitonin given three times a week, produced a marked clinical improvement, as determined by the disappearance of joint pains and increased joint mobility during therapy. The

Fig. 9. *Upper*: cancellous bone from iliac crest before administration of calcitonin. A layer of noncalcified bone matrix is abnormally prominent and appears as a dark band on the surfaces of the bone spicule. (Undecalcified section stained by the Goldner-modified Masson trichrome procedure which stains uncalcified matrix red and calcified matrix green; photographed in green light (280 $\times$).) *Lower*: cancellous bone from iliac crest after administration of calcitonin. The amount of noncalcified bone matrix at the surface of bone spicules is reduced. (Undecalcified section stained with Goldner-modified Masson stain; photographed in green light) (590 $\times$).)

serum alkaline phosphatase concentrations and the urinary hydroxyproline excretion returned towards normal values during calcitonin therapy.

A bone biopsy done on the third month of therapy with calcitonin, compared to a bone biopsy before initiation of therapy, revealed a diminution of osteomalacia with an apparent diminution of the osteoclastic activity. These findings correlate well with *in vitro* studies indicating that calcitonin inhibits osteoclastic activity (32, 34).

CONCLUSION

Muscle atrophy and osteolysis were investigated in a girl who presented with grotesque skeletal abnormalities. A selective

hypoplasia of muscle fiber types I and IIA was found. Electron microscopic study of the muscle revealed normal myofibrils, but vacuolated mitochondria with disorganized cristae, in the hypoplastic fibers. The osteolytic process was found to improve with administration of synthetic salmon calcitonin.

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