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BODY COMPOSITION AND FAT DISTRIBUTION USING DUAL-ENERGY X RAY ABSORTIOMETRY (DEXA) IN OBESE CHILDREN. COMPARISON WITH DEUTERIUM DILUTION (D2O), ANTHROPOMETRY AND BIA.

Recently dual-energy x ray absortiometry has been proposed for body composition analysis. Using a low dosage of x-ray it gives an accurate estimation of total body mineral content and a direct measurement of fat and lean mass both as total and regional amount. In order to evalute feasibility in childhood obesity we studied 17 obese children (7 males and 10 females), aged 7-13.7 years, BMI 21.4-29.6, using DEXA, D2O, anthropoplicometry and bioelectrical impedance (BIA). Fat distribution was not affected by sex; after the age of 10 years we observed an increase in trunk fat amount which was significantly different from the previous ages ($p<0.01$). Legs fat content was the best index of total fat mass($r=0.73$, $p<0.001$). Total fat content estimated by DEXA was significantly correlated with ponderal excess estimated according to Tanner ($r=0.6$, $p<0.02$) and to Rolland-Cachera ($r=0.7$, $p<0.001$), as well as with triceps skinfold ($r=0.73$, $p<0.001$). Fat free mass estimated by D2O and by BIA, using Battistini's formula specific for obese children, was highly related to lean mass detected by DEXA ($r=0.89$ $p<0.0002$ and $r=0.94$, $p<0.001$, respectively), while fat mass was not ($r=0.29$). Our data confirm feasibility of DEXA for body composition and fat distribution studies, even in children. Among standard anthropometric indexes of adiposity, triceps skinfold seems to assure the best prediction of total fat amount and BIA appears to be an accurate method for total body water estimation. Further investigations on fat hydration are needed for a more precise measurement of fat mass using indirect tecniques.

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EFFECT *IN VITRO* OF r-hGH AND CALCIOTROPIC HORMONES ON HUMAN OSTEOBLAST-LIKE CELLS. G. Saggese, G. Federico, L. Cinquanta. Chair of Preventive Pediatrics, Institute of Pediatrics, University of Pisa, 56125 Pisa, Italy.

Recent *in vitro* evidences indicate that factors as Parathyroid hormone (PTH) and its related peptide (PTHrp), 1,25-dihydroxyvitamin D_3 and 17β-estradiol (E_2) have regulatory effects on mouse- or rat-derived osteoblast-like cells. We studied the effects *in vitro* of various hormones, i.e. recombinant human growth hormone (r-hGH), PTHrp, 1,25-dihydroxyvitamin D_3 and E_2, on type I procollagen (IP) and IGFs binding proteins (IGFBPs) production by human osteosarcoma cells (SaOs).

Methods. Cells were cultured in 24-well plates in RPMI-1640 medium. Confluent cultures were added with medium alone (control cultures) or with the various hormones at 10^{-8} M final concentration in serum-free condition. After 3 days the supernates were recovered and assayed for IP by RIA and IGFBPs by the "ligand blotting" method, using ^{125}I-IGF-1 as probe.

Results and comment. In the supernates of hormonally-treated cultures IP levels almost doubled the values found in the control ones. The ligand blotting showed the presence of two major bands relative to IGFBP2 and BP4. Such binding proteins had higher levels in the supernates of the hormonally-treated than in the control cultures and, among the hormones used, PTHrp seems the most potent. Thus, our data indicate that PTHrp, r-hGH, 1,25-dihydroxyvitamin D_3 and E_2 have important regulatory effects on human osteoblast-like cells not only on bone matrix production but also on proteins that may play an autocrine/paracrine role in bone, as IGFBPs.

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INCREASED IDDM SUSCEPTIBILITY BY HLA-DR4 IN A PEDIGREE WITH PROLONGED QT SYNDROME. K. Hanaki, A. Tamura, H. Urashima, Y. Kohno, Y. Ogihara, Y. Tsuji, K. Tajima, H. Okuda, T. Ohzeki and K. Shiraki. Department of Pediatrics, Faculty of Medicine, Tottori University, Yonago 683, Japan

Prolonged QT(Romano-Ward) syndrome, characterized by prolonged QT interval in electrocardiogram and may result in fatal arrhythmia, is inherited as an autosomal dominant trait. It has recently been disclosed that the gene responsible for the syndrome (c-Harvey-ras 1) is located on chromosome 11p 15.5 and very close to a gene for insulin and IGF-2. [Case] A Japanese pedigree with the syndrome for subsequent three generations was referred to our hospital for the treatment of overt diabetes mellitus in a 13-year-old boy. He had hyperglycemia (739 mg/dl), ketosis and serious weight loss. insulin administration was then introduced. ICA and ICSA were negative. The boy, his father and his grandfather had definitely prolonged QT interval and clinical manifestations such as syncope. Hyperglycemia was seen only in the diabetic boy. HLA-DR4 was detected only in the boy with DM (DR4, DR9). His father had DR2 and DR9(mother not tested). [Conclusions] IDDM in the boy of the pedigree may be developed by the existence of HLA-DR4 and some definite changes around c-Harvey-ras 1 -insulin - IGF-2 genes that is responsible for the prolonged QT syndrome. The result is consistent with the hypothesis that the gene around insulin and IGF-2 affect the HLA-DR related IDDM susceptibility.

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BONE MINERAL METABOLISM IN PRECOCIOUS PUBERTY DURING TREATMENT. F. Antoniazzi, F. Bertoldo◊, G. Radetti°, E. Costantini, S. Sirpresi, G. Gambaro, R. Valentini, L. Tatò. Clinica Pediatrica, ◊Medicina Interna, University of Verona; °Pediatria, Bolzano General Hospital, Italy.

Gonadal steroids are known to play an important role in the control of GH-IGF-I axis and in bone mineral increase that occur during puberty. To better know the importance of estrogens on bone mineral metabolism during growth and puberty we studied ten girls (age range 6-7.2 yrs.) with true precocious puberty, before and after 1 year of LHRH analogs treatment. In these patients, before starting treatment with Leuprolide acetate (Enantone depot-Takeda), 0.2 mg/kg administered i.m. every 28 days, we performed: a clonidin stimulation test for GH; a 1,25dihydroxyvitaminD3 load (Rocaltrol-Roche) giving a dose of 2 μg/day for four consecutive days with the dosage before and after of serum Ca, P, IGF-I and Osteocalcin; a densitometric evaluation (dual photon absorptiometry) of BMC and BD (BMC/BW) in the distal and ultradistal region of the nondominant radius. After 1 year of therapy we repeated the same evaluations. During therapy serum estradiol levels decreased from pubertal (35.7 ± 3.5 pg/ml) (M± DS) to prepubertal levels (<20 pg/ml in each patient) as expected. GH peak after clonidin stimulation test after treatment was significantly lower than before (18.7 ± 2.2 vs 6.8 ± 3.5 ng/ml; $p=0.005$). IGF-I levels decreased not significantly at 12 months (from 278 ± 38 to 223 ± 53 ng/ml) and were not influenced by $1,25(OH)_2D_3$ load, while Osteocalcin levels were in the pubertal range before therapy ($35,8 \pm 6,2$ ng/ml), decreased not significantly after therapy, and showed a slight increase after $1,25(OH)_2D_3$ load both before and after LHRHa therapy. Before therapy, BMC and BD were increased for chronological age, but appropriate for bone age. After 12 months of LHRH analogs treatment, BMC and BD showed a significant decrease ($-4,9 \pm 1,0$ and $-5,1 \pm 0,9$ %; $p<0.05$) in the ultradistal region (measuring trabecular bone), while there was a not significant difference in the distal region (measuring cortical bone). These results indicate that contemporary decrease in estrogen and GH-IGF-I levels may reduce osteoblastic activity and produce a decrease in bone mass.

Calcium/Phosphorus, Bone

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THERAPY WITH AMINOHYDROXYPROPYLIDENE BISPHOSPHONATE IN YOUNG PATIENTS WITH SEVERE OSTEOPOROSIS. S.Marinoni, B.Bembi, G.Tonini, G. Nassimbeni, E. Agosti (Isituto per l'Infanzia, Trieste, Italy).

Osteoporosis in the youth most often accompanies severe illness, such as inborn errors of metabolism, or therapy affecting bone metabolism (e.g. steroid). Calcium supplementation, sodium fluoride, vitamin D3 and derivatives, calcitonin, while effective in relenting the progression of the disease, are not satisfactory as to recover from conspicuous loss of bone mass. By contrast, increasing experience is being collected in the use of bisphosphonates, a class of molecules able to inhibit osteoclastic activity of bone resorption. We have treated with 3amino 1hydroxy propylidene 1,1bisphosphonate (APD) 5 patients aged 6.5 to 20 years, with severe osteoporosis: two had primitive bone disease (Gaucher's disease, familial hypophosphatasia), one was affected by cystic fibrosis, two received steroid (for rheumatoid arthritis and after bone marrow transplantation for acute leukemia). Two of them had experienced spontaneous fractures, the others had mostly limb and/or back pain. The calcium content of bone had been assessed by means of computed densitometry of lumbar vertebrae: the figure was reduced to 4-125 mg/ml of bone (normal range for age approx. 150-200). The APD was administered intravenously, 5-7.5 mg/day for 5 days, then 10 mg once every three weeks. Moderate hypocalcemia observed after the first infusions was prevented by oral calcium supplementation, 500-1500 mg/day for a few days following each infusion. After a follow-up ranging 5-12 months, the vertebral CT scan showed an increase of calcium content by 26 - 1325 %, the figure attaining normality in two cases: the best result was obtained in the boy with the lowest pretreatment value. Symptoms and regional radiographs also showed comparable improvement. Side effects were self-limited fever following infusions in one patient, and transient calf pain in another. We conclude that APD can be effectively and safely used in children and adolescents to cure severe osteoporosis, both primitive and iathrogenic.

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BONE MINERAL DENSITY IN ANOREXIA NERVOSA PATIENTS. RELATION TO WEIGH LOSS, BODY MASS INDEX AND PERIOD OF AMENORRHEA.

Bone mineral density, gr/cm2, (BMD) of the lumbar spine, L2-L4, was measured by dual energy X-ray absorptiometry (Lunar DPX) in 9 anorexia nervosa female patients aged 14 to 17 years, and compared with that of 54 normal age-and sex-matched controls.

All patients had had regular menses one or more years before the start of the amenorrheic period. At the time of BMD evaluation amenorrhea ranged from 2 to 12 months, weight loss from 13% to 41%, mean value 33.9% in relation to ideal height/weight ratio, and body mass index from 13.41 to 17.70, mean value 15.21.

BMD was less than -1 SD of normal age-matched controls in five patients and higher in four. No correlation was found between BMD values and weight loss, or with body mass index. However, a significant and statistically negative correlation was found between BMD values and the period of amenorrhea ($r= -0.80$, $p< 0.01$, $n=9$).

Follow-up (6-14 mo.) of three patients with BMD values less than - 1SD, showed no increase in BMD values despite a significant weight gain (body mass index changed from 13.68 +/- 0.3 to 17.88 +/- 2.27 , $p<0.01$, $n = 3$). All three patients remained amenorrheic during this period.

In conclusion, BMD values are decreased in some patients with anorexia nervosa. BMD values correlated negatively with the duration of amenorrhea. The significant weight increase in these patients was not accompanied by a parallel BMD increase since amenorrhea persisted during this period. Our results underline the importance of estrogens in the maintenance of BMD values in female adolescent patients.