Long-term low dose calcitriol treatment reduces blood pressure and decreases diet-induced atherosclerosis in Tsukuba hypertensive mice (THM)

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Introduction: Epidemiologic studies have suggested vitamin D might have favorable cardiovascular effects. Indeed, there is evidence that vitamin Dlevels are inversely correlated with blood pressure, and with cardiovascular mortality. Suppression of the renin-angiotensin system (RAS), through downregulation of renin, its rate-limiting step, has been proposed as one of several possible mechanisms. We reported that calcitriol administered for 3 weeks to young Tsukuba Hypertensive Mice (THM) prevented hypertension in this model of hypertension and atherosclerosis secondary to the transgenic expression of the human renin (hRen) and angiotensinogen (hAGT) genes. We questioned whether long-term calcitriol teatment would have a sustained effect on blood pressure ,and whether it would affect the atherosclerosis that develops in these mice.

Patients/ Methods: Starting at 7-9 weeks, THM animals were fed an atherogenic Western diet. 17 animals received calcitriol as an intraperitoneal injection at a dose of 0.25 ng/g body weight every other day for 12 weeks (1/2 the dose used in our previous study, and shown not to cause hypercalcemia in the short-term). 19 control mice received the vehicle only. BP was measured noninvasively. The extent of atherosclerosis at the aortic sinus was assessed by quantification of Oil-Red-O-stained lesions. Expression of the RAS in the aorta was studied by real-time PCR.

Results: BP was unchanged in control animals but was significantly lower in calcitriol-treated mice: systolic 142.7+1.8 vs 111.9+3.2 mm Hg, diastolic 88.6+1.4 vs 69.6+2.5, $P<0.0001$ for both. Calcitriol treatment significantly increased cholesterol concentrations 186.4+8.7 vs. 144.8+8.3 mg/dl, $P=0.0028$, and serum calcium 10.3+0.2 vs 8.9+0.3 mg/dl, $P<0.001$. In addition, calcitriol-treated animals weighed more. Nonetheless, the extent of atherosclerosis was reduced by 42.9% with calcitriol, $P=0.015$. This was accompanied by a significant 77% suppression of the hRen gene at the aorta, $P=0.005$. hAGT, ACE and the angiotensin II type 1 receptor were not affected.

Conclusions: Long-term calcitriol treatment provided sustained BP reduction as well as a significant attenuation of atherosclerosis in THM animals. The concomitant suppression of hRen is in agreement with the notion that the beneficial effect is mediated by downregulation of the RAS. However, even at this lower dose which had no untoward effect in the short-term, prolonged treatment resulted in hypercalcemia. Moreover, the unfavorable metabolic profile seen with treatment is a major concern. Animal studies with analogs devoid of these effects will have to confirm the above findings before clinical trials can be contemplated.

The mechanism of the regulation of the epidermal vitamin D endocrine system by inflammatory cytokines

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Introduction: The epidermal keratinocyte contains the machinery for the production of the hormonal form of vitamin D, calcitriol, from 7-dehydrocholesterol, and a vitamin D response system.Our previous findings indicate that calcitriol takes part in the stress and inflammatory responses in the skin and we argue that to fulfill these roles the epidermal vitamin D endocrine system should be unregulated when exposed to inflammatory stresses. The vitamin D endocrine system includes the enzymes responsible for the two hydroxylations of vitamin D at position 25 and 1 alpha, the vitamin D receptor (VDR). The in situ overall activity of this system can be assessed by exposing the keratinocyte to the parent compound vitamin D and monitoring the up-regulation of the sensitive calcitriol target gene CYP24A1. We aimed to examine the effect of inflammatory agents on the epidermal vitamin D endocrine system and to identify the signaling pathways involved in its regulation.

Patients/ Methods: HaCaT keratinocytes, cultured in the absence of exogenous growth factors or active mediators, were our experimental model. Cultures were exposed to TNF or interferon gamma (IFN) for 24 hours. mRNA levels were quantified by real time PCR. The overall activity of the vitamin D endocrine system was assayed by exposing the cultures to vitamin D3 for 5 hours and assaying CYP24A1 mRNA levels. 25-hydroxy 1a hydroxylase (CYP27B1) and VDR mRNA levels were quantified following exposure to the cytokines. This was done in the presence of inhibitors of the following signaling pathways: ERK(U0126), c-Jun N-terminal kinase (SP600125), p38 MAPK(SB203580), NF kappaB(BMS) and PKA (4C3M).

Results: Exposure to TNF (10 ng/ml) and IFN (15 ng/ml) for 24 hours resulted in a marked increase in the overall activity of the keratinocyte vitamin D endocrine system as demonstrated by the induction of CYP24A1 by vitamin D. Upregulation of CYP27B1 by both cytokines and upregulation of the VDR by TNF underlie this effect. The signaling pathways involved are shown in the table.

Conclusions: These findings demonstrate that the all over activity of the epidermal vitamin D endocrine system increases following exposure to stimuli commonly present in the stressed and inflamed epidermis. Taken together with the capacity of the hormone to exert protective and anti-inflammatory actions on keratinocytes, we maintain that the epidermal vitamin D endocrine system may serve as a hormonal stress system in the skin.

Vitamin D increased E-cadherin synthesis and inhibited E-cadherin cleavage by TNF in epidermal keratinocytes

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Introduction: Cell-cell adhesion plays an important role in tissue and organ pattern formation, maintenance of specific tissue architecture and in the regulation of cell migration and proliferation. E-cadherin, which is the prototypical member of the classic cadherin family, is a major component of adherens junctions, at which it provides cell-cell adhesion through Ca+2-dependent, homophilic binding between molecules on adjacent epithelial cells. E-cadherin is trafficked to and from the cell surface by various pathways. E-cadherin can be cleaved by different metalloproteases induced under various pathological conditions including skin inflammation. It was previously shown that treatment with the hormonal form of vitamin D increased the formation of adherens junctions in cultured keratinocytes. This work was undertaken in order to explore the effect of calcitriol on E-cadherin turnover and cleavage by pro-inflammatory cytokines.

Patients/ Methods: The non-tumorigenic immortal HaCaT keratinocytes were employed as an experimental model, and cultures in the absence of serum, exogenous growth factors and active mediators were treated with calcitriol (100 nM) for 24 and 48 hours. mRNA levels were quantified by real time PCR and protein levels by western blot analysis. E-cadherin fraction present in adherens junctions was quantified by Triton-X100 solubilization. E-cadherin fraction present on cell surface was distinguished from the intracellular fraction by the trypsin protection assay.

Results: Treatment with calcitriol increased E-cadherin mRNA levels in HaCaT cells. In accordance, calcitriol increased the level of an intracellular protein with a higher molecular weight recognized by E-cadherin antibody. This protein almost disappeared following a 2h treatment with cycloheximide. These features support the identification of this protein with the short-lived pro-E-cadherin that is processed in the Golgi apparatus to the mature protein. In addition, immunobloting with E-cadherin antibody revealed a calcitriol inducible protein, of a slightly lower molecular weight, which may be an E-cadherin variant as it co-localized with the wild type protein in the different cellular fractions. Simulation of the inflammatory state by exposure to TNF brought about cleavage of E-cadherin, which was markedly inhibited by pretreatment with calcitriol.

Conclusions: Vitamin D increased E-cadherin synthesis and inhibited its cleavage by a pro-inflammatory cytokine. These effects could contribute to its known anti inflammatory action and suggest novel therapeutic modalities in ailments involving defects in epidermal cell adhesion.

Vitamin D up-regulates the expression of MMP-1 in human keratinocytes

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Introduction: The collagenase, matrix metalloproteinase 1 (MMP-1) plays a major role in wound healing by mediating keratinocyte migration and remodeling scar tissue. Low expression of MMP-1 may result in delaying the first stage of wound healing on the one hand and is associated with fibroproliferative disorders such as keloid and hypertrophic scars on the other hand. Calcitriol, the hormonally active form of vitamin D, enhances wound healing by an unknown mechanism. We aimed to examine the effect of calcitriol on MMP-1 expression in human keratinocytes and to identify the signaling pathways involved in this process.

Patients/ Methods: The immortalized, non-tumorigenic HaCaT keratinocytes, cultured in the absence of exogenous growth factors or active ingredients served as an experimental model. These cells represent the mitotic basal keratinocyte layer. MMP-1 mRNA levels were quantified by real-time PCR and protein levels in the culture medium by western blotting. Rates of mRNA decay were determined following 5h treatment with the transcription inhibitor actinomycin D (1 mic.M).

Results: Treatment with calcitriol (1-100 nM for 6-24 hours) increased MMP-1 mRNA and protein levels in a dose and time dependent manner. The increase in mRNA was significant at a concentration of 1nM and detectable already following 6h treatment. This effect was partially due to increase in mRNA stability. The increase in MMP-1 expression by calcitriol was significantly higher than that due to treatment with the pro-inflammatory cytokine TNF, a classical inducer of MMP-1. The effect of co-treatment with the two agents was additive. The involvement of intracellular signaling pathways in the up-regulation of MMP-1 by calcitriol was examined by using pharmacological inhibitors. The signaling pathways examined are known participants in the regulation of MMP-1 expression and also known to be affected by calcitiriol in keratinocytes. The up-regulation of MMP-1 mRNA by calcitriol was found to be fully inhibited by a Src kinase inhibitor (PP1), and partially inhibited by the ERK inhibitor (U0126) and EGFR inhibitor (AG1478). In contrast, a pan-PKC inhibitor (GF109203x), PLC inhibitor (U73122) and PI3K inhibitor (Wortmannin) had no inhibitory effect on MMP-1 mRNA levels in calcitriol-treated cultures.

Conclusions: Taken together, these results indicate that calcitriol is a potent regulator of MMP-1 expression in human keratinocytes. Src kinase, EGFR, and ERK are involved in the up-regulation of MMP-1 by calcitriol. This finding supports the notion that treatment with hormonally active vitamin D derivatives may enhance the initial steps of wound healing while reducing the risk of hypertrophic scars.

Angiotensin 1-7 prevents the metabolic syndrome, hepatosteatosis and adipose tissue inflammation (Adipositis) in the fructose-fed rat

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Introduction: The metabolic syndrome (MetSyn) usually evolves in the context of visceral adiposity which fosters insulin resistance, hypertension, dyslipidemia, fatty liver and white adipose tissue (WAT) inflammation induced by adipocyte enlargement and macrophage infiltration. We have previously shown that the receptor for angiotensin 1-7 (Ang 1 -7), Mas, is expressed in the adipose tissue. Mas knockout mice were shown to feature MetSyn, we therefore reasoned that treatment with Ang 1-7 may prevent the development of MetSyn in animals fed on high-fructose/low magnesium diet over 24 wks.

Patients/ Methods: The experimental setting was as follows: one group of animals (n= 6) received a continuous infusion of Ang 1-7 (576 μg/kg/day, s.c., via an Aldzet pump for 6 months) and the other served as a control group (n=9, no treatment).

Results: By the end of the treatment period, Ang 1-7 -treated animals had lower final body weight (457+/-8.9 vs 483+/-7.8g, $p=0.03$), lower fat mass (detected by MRI , t-test $p<0.05$) and lower serum triglycerides (97.1±16.3 vs 227.5±21.7 mg/dl, t-test $p<0.001$). Additionally, Ang 1-7 treatment markedly lowered serum aldosterone levels (11.1+/- 2.2 vs 19.1+/-2.1 ng/dl, $p<0.01$). Ang 1-7 treatment did not induced changes in basal serum glucose or insulin, while it did attenuate increase in serum glucose ($P<0.05$) that normally occurs in response to acute intraperitoneal glucose challenge (2 grams/kg). Histological examination of the liver revealed that fructose-fed rats developed hepatosteatosis which was nearly absent in the fructose fed, Ang1-7-treated rats. Mean adipocyte size in epididymal fat sections was significantly larger in untreated than in Ang1-7 treated, fructose fed rats (4133+/-729 vs 8370+/-3934 μm2, respectively, $p=0.008$). Additionally, macrophage infiltration was present in white adipose tissue (WAT) from untreated, but not from Ang1-7 treated rats. This was associated with reduced epididymal fat tissue pP65 protein expression ($p<0.05$), suggesting lower activation of the NFkB pathway in Ang1-7-treated rats. Finally, based on lucigenin-enhanced chemiluminescence, WAT from Ang1-7 treated rats showed reduced NADPH stimulated superoxide production.

Conclusions: We show that Ang 1-7 had an ameliorating effect on insulin resistance, hypertriglyceridemia, fatty liver, obesity and adipositis in the high fructose fed rats. These beneficial effects could be related, at least partially, to the anti-oxidative and anti-inflammatory influence in adipose tissue and to the prevention of hepatosteatosis by Ang1-7.

Differential effect of antidepressants on body mass regulation and food intake in rats exposed to chronic mild stress as compared to unstressed - evidence for a role of leptin

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Introduction: Depression is associated with alteration in food intake and in body mass regulation. Treatment with different antidepressants (AD) such as selective serotonin reuptake inhibitors (SSRIs), noroadrenaline reuptake inhibitors (NRIs) and NE and DA reuptake inhibitor (NDRIs) was reported to modify appetite and body weight.

Patients/ Methods: Male SD rats were exposed to 8 weeks of unpredictable chronic milled stress (Ucms) or to normal conditions (unstressed). Animals (8/group) were treated daily with vehicle, reboxetine (NRI), paroxetine (SSRI), or bupropion (NDRI) (5, 5 and 20 mg/kg respectively). Body weight and food intake were followed weekly. After sacrificing, trunk blood was collected for leptin determination (ELISA), and brains were dissected for neurotrophic protein determination using western blot analysis.

Results: Body weight gain was significantly suppressed in all the UCMS groups as compared to unstressed rats. In the stressed group, the SSRI paroxetine showed impaired body weight gain on the 7th and the 9th week. Whereas, in the unstressed rats food intake and body weight gain was significantly suppressed by bupropion. Plasma leptin levels did not differ between the vehicle groups of stressed and unstressed rats, however, bupropion caused a significant decease in plasma leptin levels in both UCMS and unstressed groups. Paroxetine also caused a significant decrease in leptin levels in the stressed rats.

Conclusions: Stress caused a marked decease in body weight gain and in food intake. The AD bupropion seems to inhibit appetite and body weight gain mainly in unstressed conditions.This effect is accompanied by a decrease in plasma leptin levels. Bupropion, therefore might have a role in the therapy of obesity and hyperphagia in humans.

Effect of weight loss maintenance on arterial compliance, metabolic and inflammatory parameters

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Introduction: Data related the long-term vascular impacts of purpose weight loss are limited. Even less information is available on whether the positive vascular effects of weight reduction are maintained after weight regain following treatment termination. The aim of the present study was to evaluate the effect of long-term (three years) weight loss maintenance on arterial compliance, metabolic and inflammatory parameters in obese patients who participated in a 6-month weight loss program featuring nutritional and exercise intervention.

Patients/ Methods: Open prospective study, 67 obese subjects who participated in a 6-month weight loss program featuring nutritional and exercise intervention followed additional 30 months. The 47 patients that fully completed the three year follow-up were divided into two groups according BMI: group 1 included 22 patients which decreased or did not change BMI after weight loss program discontinuation, group 2 included 25 patients which increased BMI from visit 3 to 4. Arterial compliance as well as metabolic parameters were evaluated at baseline, 3-, 6- and 36-months of follow up.

Results: BMI changed from 35.4 ± 6.9 kg/m2 to 33.0 ± 6.5 kg/m2 after 3 months, to 32.6 ± 6.6 kg/m2 after 6 months and to 33.4 ± 7.0 kg/m2 after 36 months. While 53 % of participants regained weight after 6-month initial weight loss program discontinuation, the mean weight at 3 years remained lower than mean weight at entry into the study (p=0.01). Although SAEI did not differ significantly between the groups at baseline, at the end of the study SAEI was greater in patients who decreased or did not change BMI than in subjects who gained weight (p=0.025). LAEI was greater in group 2 compared to group 1 at baseline, after 3 and 6 months of follow-up, however, at the end of the study no significant difference in LAEI was detected. Repeated measures analysis indicated that LAEI changed significantly over time (p=0.022).

Conclusions: Obese subjects who completed behavioral weight loss program and decreased or maintained weight during 30 additional months future improved arterial stiffness in comparison to subjects who regained weight.

Prevalence of polycystic ovary syndrome in non-classical 21 hydroxylase deficiency females by age at initiation of glucocorticoids therapy and genotype

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Introduction: Non-classical 21-hydroxylase deficiency (NC21OHD) is a mild form of congenital adrenal hyperplasia associated with different degrees of postnatal virilization developing from infancy to adulthood. The genotype might be either homozygous or compound heterozygous for mild mutations, or compound heterozygous for one mild and one severe mutation of the gene encoding 21-hydroxylase (CYP21). Compound heterozygosity has been associated with an earlier and more severe presentation: females with NC21OHD might develop polycystic ovarian syndrome (PCOS) secondary to chronic adrenal androgen hypersecretion. Aims: To determine the prevalence of secondary PCOS and identify clinical parameters associated with the development of PCOS in females with NC21OHD.

Patients/ Methods: Medical records of females with NC-21OHD were retrospectively reviewed for presenting signs, age at diagnosis, timing of puberty, age at initiation of therapy, CYP21 genotype and pelvic ultrasound reports. PCOS was diagnosed according to clinical signs of hyperandrogenism and ultrasound findings. The ultrasound criteria included the presence of ≥12 follicles in each ovary, or increased ovarian volume.

Results: Ultrasound results were available for 52 females [mean age at diagnosis (SD): 10.5 (7.6) years]. Thirteen (25%) had clinical signs and ultrasound findings compatible with PCOS. PCOS was more prevalent (67%) in girls who were diagnosed and started on therapy after the age of 12 years compared with those diagnosed and started on therapy earlier (33%, $p=0.02$ and $p=0.04$, respectively). PCOS was less prevalent in girls with presenting signs of precocious pubarche or precocious puberty, compared with girls presenting with post-pubertal virilization ($p<0.001$). There was no significant correlation or association between PCOS and other clinical parameters, including genotype.

Conclusions: The prevalence of PCOS is higher in females with NC21OHD compared to the normal population. Early diagnosis and initiation of therapy in subjects with NC21OHD might prevent the development of postpubertal PCOS. The lack of correlation with genotype might be due to our small sample size.

Recurrent episodes of iatrogenic Cushing's syndrome due to inhaled steroids in ritonavir-treated HIV-infected child

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Background: Ritonavir is protease inhibitor used in the treatment of HIV infection. It is an extremely potent inhibitor of cytochrome P450 3A4, and may increase the bioavailability of drugs metabolized by this pathway such as glucocorticoids. Although inhaled steroids have allegedly local effects, they may cause systemic complications when administer with ritonavir. We describe the hormonal and auxological effects of inhaled steroids over seven years in a ritonavir-treated HIV-infected child.

Case report: The patient is a 12.5 y.o. girl of Ethiopian origin who was diagnosed with AIDS at 2.5 years of age. Since 1999 she has been treated with Zidovudine and Lamivoudine with Lopenavir, boosted with ritonavir. Additionally, she was also treated with inhaled bodesonide and fluticasone as preventive therapy for asthma. In her last admission for asthma exacerbation, remarkable Cushingoid features were noticed and her hormonal profile was compatible with iatrogenic Cushing's syndrome, with suppression of the hypothalamic-pituitary-adrenal axis. Her morning cortisol level was <20 nmol/L and raised to a peak of 70 nmol/L and 99 nmol/l in response to low and high dose Synacten, respectively. Her corresponding ACTH level was 4.7 pmol/L (N 2.2-11.0) and urinary free cortisol was remarkably suppressed at a levels of 13 nmol/L (N 42-254). Two months after cessation of inhaled steroids, her morning cortisol and ACTH levels were raised to 307 nmol/L and 18 pmol/L, respectively. Notably, the patient initially demonstrated severe insulin resistance (glucose 93 mg%; insulin 152.9 mU/L) that was resolved following cessation of inhaled steroids (glucose 64 mg%; insulin 12.4 mU/L). Height and weight measurements recorded over the last 7 years have shown periods of remarkable growth decelerations (Fig 1) and reciprocally opposite weight gain (Fig 2), in association with inhaled steroids treatment (at 6, 8, & 12 years of age).

Conclusion: In ritonavir-treated HIV-infected children, inhaled steroids should be avoided as they may cause iatrogenic Cushing's syndrome with suppressing effect on growth.

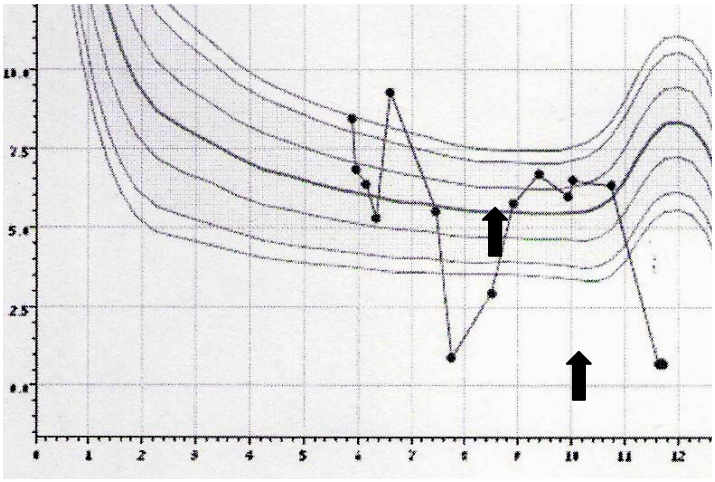

Fig 1: Growth velocity (cm/y)

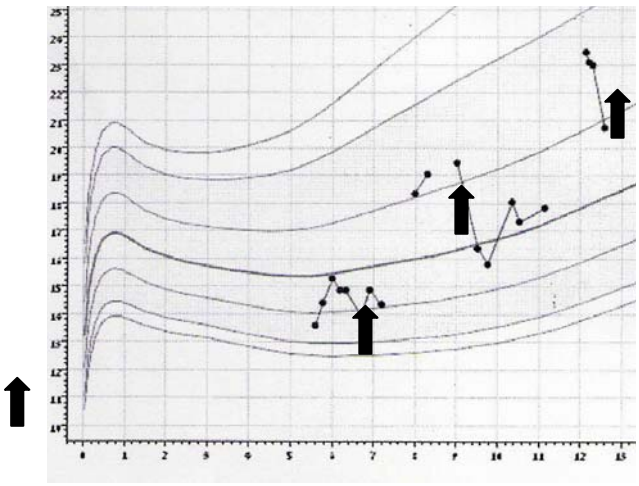

Fig 2: Body mass index (BMI)

Effect of treatment with insulin sensitizer on arterial properties, metabolic parameters and liver function in patients with nonalcoholic fatty liver disease: A randomized, placebo-controlled trial

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Introduction: Insulin resistance has an important role in the development of nonalcoholic fatty liver disease (NAFLD) and is involved in both pathological processes: hepatic steatosis and atherosclerosis. Therefore, treatment of NAFLD with insulin sensitizers is likely to have a favorable effect towards hepatic steatosis and cardiovascular outcomes. Measurement of arterial stiffness provides useful information regarding vascular health and may serve as a marker of atherosclerosis as well as a surrogate for cardiovascular morbidity and mortality risk. The present study investigated the effect of metformin on arterial properties, metabolic parameters and liver function in patients with NAFLD.

Patients/ Methods: In randomized, placebo controlled study, 63 patients with NAFLD were assigned to one of two groups: Group 1 received daily metformin, Group 2 received placebo. Pulse wave velocity (PWV) and augmentation index (AI) and were performed using SphygmoCor (version 7.1, AtCor Medical, Sydney, Australia). Metabolic measures were determined at baseline and at the end of 4-month treatment period.

Results: Among metformin treated patients: PWV and AI decreased significantly during the study. Significant declines in fasting glucose, triglyceride, alkaline phosphatase and a significant increase in HDL-cholesterol were observed. CRP, ALT and GGT decreased and serum adiponectin increased marginally. In placebo group: neither PWV nor AI improved significantly during the treatment period. ALT, AST and adiponectin did not change in placebo group.

Conclusions: Metformin treatment was associated with significant decrease in PWV and AI in NAFLD patients. This beneficial vascular effect was accompanied by an improvement in glucose and lipid metabolism as well as liver function.

Implications of vitamin D status in patients with primary hyperparathyroidism

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Introduction: Primary hyperparathyroidism (PHP) and vitamin D deficiency are connected on several levels. Epidemiological data suggests that vitamin D deficiency is more prevalent in PHP patients than in matched control subjects. Moreover, there is data indicating, that PHP manifestations are more severe in patients with vitamin D deficiency. This is probably true in respect to the severity of the bone involvement and parathyroid adenoma weight. Scant data exists regarding vitamin D status normalization in this patient population, which shows that PTH might even be lowered by vitamin D supplementation. Little is known about the safety aspects of vitamin D replenishment in PHP patients, and there has been some concern about worsening hypercalcemia and hypercalciuria.

Patients/ Methods: We retrospectively examined the electronic database of patients treated in our department since 2005. Medical records of patients with a diagnosis of "hyperparathyroidism" were reviewed. Normocalcemic patients, those with impaired kidney function and patients with only one measurement of 25-OH- D or without increment of vitamin D during the years of follow up were excluded from the analysis. The remaining cohort was analyzed for calcium metabolism parameters at two different time points: lowest and highest vitamin D measurement. The parameters selected were blood calcium, phosphorus, albumin, PTH concentration, and 24-hour urinary calcium and creatinine.

Results: Thirty-three patients met the sample criteria. The mean age was 63 and eighty-nine percent were women. Vitamin D status changed significantly during the follow up years, due to supplementation. Mean vitamin D level at the lowest point was 16.4 + 6 ng/ml. The mean 25-OH-D concentration was 33.8+8.9 ng/ml at the highest measurement. Neither of the parameters examined was influenced by vitamin D status. Serum calcium, phosphorus, and PTH were not significantly changed by vitamin D replenishment: 10.7+ 0.4 mg/dl, 3+0.4 mg/dl and 130+39.5 pg/ml at lowest vitamin D value as compared with 10.7+0.36, 2.9 +0.4 and 133+55, at the highest, respectively. Twenty-four hour urinary calcium excretion, calculated as 24-hour calcium/24-hour creatinine, had not significantly changed between the two time points (0.2+0.1 vs. 0.26 + 0.13 mg Ca/mg Cr, respectively).

Conclusions: Vitamin D replenishment in patients with primary hyperparathyroidism does not cause worsening of hypercalcemia and hypercalciuria, and thus, is safe. As opposed to previous reports, we did not find significant improvement in the degree of PTH elevation.

Neonatal panhypopituitarism: A unique presentation

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Introduction: Infantile cholestasis should be evaluated completely to exclude genetic, metabolic, infectious, obstructive and endocrine causes. Congenital hypopituitarism is an uncommon cause of neonatal cholestasis, and can present with hypoglycaemia. Treatment with glucocorticoid and thyroid hormones, play a significant role in the resolution of cholestasis and hepatosplenomegaly. we report on an infant with panhypopituitarism, presenting with cholestatic jaundice, hypoglycaemia and high serum ferritin level suggesting neonatal hemochromatosis.

Patients/ Methods: Comprehensive clinical and laboratory investigations were performed to establish the etiology of the presenting complaints. This included genetic, metabolic, infectious , as well as thorough hormonal profile (LH, FSH, PRL, TSH, FT4, ACTH and growth hormone stimulation tests).

Results: Hormonal evaluation revealed cortisol and growth hormone deficiency with central hypothyroidism. Other causes of cholestasis were ruled out. In addition there was a high serum ferritin level of 2315ng/ml suggesting neonatal hemochromatosis that was excluded by the absence of hemosidrin deposition in buccal mucosal biopsy. Treatment with cortisol and eltroxin resulted in dramatic improvement of the liver function tests, resolution of cholestatic jaundice and significant reduction of serum ferritin level.

Conclusions: To our knowledge this is the first description of an infant with congenital panhypopituitarism, presenting with cholestasis, hypoglycaemia and high serum ferritin level. Panhypopituitarism should be considered in any infant who presents with cholestasis, hypoglycaemia, and other manifestations of pituitary malfunction. High serum ferritin level most probably suggests acute phase reactant.

Single intrauterine L-Thyroxin treatment of fetal goiter secondary to fetal dyshormonogenesis and maternal hypothyroidism

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Introduction: Fetal goitrous hypothyroidism is rare. Only few cases of fetal goitrous hypothyroidism are described in the English literature.

Patients/ Methods: The diagnosis of fetal goiter was made in the 32th week of geststion by sonography. Size of the goiter was 3.3X2X1 cm with calculated volume of 5.7 ml (normal 0.4). Apparent hypereflexion of the neck with severe polyhidramnion were noted. Fetal blood sampling showed TSH 744 mIU/L(normal 8-10) , free T4 7.1 pmol/L(normal 8-10). The common therapeutic regime of repeated fetal blood sampling for determination of thyroid hormones and intra-amniotic administrations of 250-300 µg levothyroxine (LT4) weekly was not used. A single intramniotic LT4 dose of 500 µg was injected . Maternal hypothyroidism was treated by oral LT4 treatment.

Results: During the 3 following weeks there was no change in fetal condition but after the 3rd week the fetal goiter size, hypereflexion of neck and polyhidramnion were all reduced as seen by repeated fetal sonography . Normal fetal growth and an uncomplicated course of pregnancy between the 32th and 39th week of gestation were observed. After elective Cesarean birth small goiter was observed without any respiratory distress. Neonatal thyroid function tests showed TSH 924 mIU/L(normal 1-20) , free T4 6.8 pmol/L(normal 7-15). and oral L T4 treatment was initiated.

Conclusions: Fetal goiter secondary to fetal dyshormonogenesis and maternal hypothyroidism was treated by a single higher LT4 and careful sonography followup. Monitoring of repeated intramniotic LT4 therapy by determination of TSH in fetal serum or TSH in amniotic fluid may not be necessary,as the change in fetal goiter size was observed only 3 weeks after therapy.

Clinical consequences of prolonged subclinical hypothyroidism

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Introduction: Subclinical hypothyroidism is typically considered a benign condition, whose main importance lies in progression to overt thyroid disease. Data supporting associations of subclinical thyroid disease with symptoms or adverse clinical outcomes are few. Whether subclinical thyroid disease should be treated is controversial, there is little robust evidence that treatment is beneficial and it is not routinely recommended as long as TSH is <10 mU/l. Recently is has been suggested that overweight and obesity may be associated with sub-clinical hypothyroidism in pediatric patients

Patients/ Methods: We describe a patient with prolonged iatrogenic subclinical hypothyroidism associated with growth deceleration and overweight, reversed after correction of thyroid function.

Results: The patient was diagnosed with brain stem glioma at age 2 years. He was treated with Temozolomide and CNS irradiation and was subsequently put on a PTU induced hypothyroidism protocol. As his tumor was stable, treatment was continued for 7 years. Thyroid function was maintained for most of the time in the mild sub-clinical range. Over the past 5 years, TSH ranged from 6 to10 mIU/L and FT4 range from 10 to13 pmol/L. During the treatment period, height percentile decreased from the 75th to the 10th percentile, weight increased from the 50th to the 75th percentile, and BMI increased from 5th to the 95th percentile. IGF-I was consistently low. PTU treatment was discontinued at age 9 years. Shortly after, precocious puberty was noted and treatment with a GnRH agonist was started. Subsequently, growth acceleration and a decrease in weight and BMI were noted. At last measurement (one year after discontinuation of PTU and 9 months after starting GnRH agonist) height was at the 25th percentile, weight at the 60th percentile and BMI at the 75th percentile. IGF-I levels are now normal.

Conclusions: This case offers a unique opportunity to observe the clinical implications of sustained sub-clinical hypothyroidism. Contrary to current thinking, subclinical hypothyroidism per se may be associated with growth retardation and weight gain.

Vitamin D status, calcium intake and bone density in young HIV infected Israeli women

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Background: Decreased bone mineral density (BMD) was reported in HIV infected patients. Mechanisms leading to this decrease are poorly understood.

Aim: To assess sun exposure, clothing habits, vitamin D status and BMD in young HIV infected Israeli women of Ethiopian (ET) and Caucasian (CA) origin.

Patients and Methods: 75 HIV infected women aged 34.5±8.5 years with regular menses who were followed up at the Institute of Allergy, Clinical Immunology & AIDS. Data about the HIV status and treatment was collected from the patients' charts; the patients filled a questionnaire about sun exposure, daily calcium intake and dress habits. Laboratory evaluation: routine chemistry, 25(OH)D by ^{125}I-radioimmunoassay, PTH (Intact) by STAT; bone turnover by plasma total procollagen type1amino-terminal peptide (P1NP) and collagen beta cross-laps (CTX). BMD measurements of the lumbar spine (LS), femoral neck (FN) and total hip (TH) by using dual energy X-ray absorptiometry (Lunar DPX scanner). The BMD results were expressed in comparison to aged matched (Z-scores).

Results: 43 (57.3%) patients were Ethiopian (ET) and 32 (42.6%) Caucasian (CA). There were no significant differences in demographics, actual and past HIV status, antiretroviral treatment and bone turnover markers between the groups.
25(OH)D serum levels<10 ng/ml (severe vitamin D deficiency),were observed in 28(66.7%) of ET vs 2 (6.5%) of CA, p =0.001. Plasma PTH was72.14±57.37 ng/l (normal 12-65), in ET vs 31.23±14.21 in CA, p<0.001. 17 (40.4%) of the ET had sun exposure <1 hour/day, vs 6 (19.4%) of CA patients, p= 0.07; daily calcium intake was 514 vs 164 mg, p=0.001. Avoidance of sun exposure was observed in 21 (67.7%) ET, vs 16 (39%) CA, p= 0.019.
Z-scores in ET and CA were: at LS -1.8±1.1 vs -0.79±0.88, respectively, p=0.001; at FN -1.12±1.1 vs -0.59±0.87, p=0.02, at TH -0.94±1.1 vs -0.25±1.1, p=0.007. BMD Z scores<-1 at LS were observed in26 (89.7%) vs 20 (48.8%), p<0.01, at FN- 20(69%) vs 17 (41.5%), p<0.03, at TH 17 (58.6%) vs 9 (22%), p<0.001 of severely vitamin D deficient pts vs pts with 25(OH)D >10 ng/ml respectively. Logistic regression: risk for LS Z-scores <-1 SD was 5.74-fold higher in pts with vitamin D levels <10 ng/ml.

Conclusion: Osteopenia is frequent in young HIV infected women. Vitamin D deficiency, low calcium intake, limited sun exposure and clothing habits might affect