

Vitamin D accelerates epidermal keratinocyte migration

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Introduction: Keratinocyte migration is crucial during the re-epithelialization process of wound healing. The keratinocyte is a self-contained vitamin D endocrine system, containing the full machinery for the production of the hormonal form, calcitriol, from its parent compound 7-dehydrocholesterol, and a complete vitamin D response system. Previous studies reported a beneficial effect of hormonally active vitamin D derivatives on wound healing. We aimed to assess the notion that this effect is associated with accelerated keratinocyte migration.

Patients/ Methods: The non-tumorigenic immortal HaCaT keratinocytes were employed as an experimental model. Migration was assessed by the in-vitro scratch assay: confluent cultures in serum-free media were scratched and the area covered by migrating keratinocytes was photographed 15-24 hours later and quantified by Image-Pro Plus software. Signaling pathway activities were quantified by Western blotting of cell extracts derived from cultures subjected to multiple scratches.

Results: 24 hour pretreatment with calcitriol brought about a significant and consistent increase in the area covered by migrating keratinocytes (an increase of $79\% \pm 16\%$, 10 independent experiments $p < 0.01$). This effect was not secondary to increased proliferation rate, since migration was not affected by the addition of mitomycin C. Keratinocytes in the wound bed are exposed to serum following blood clotting. We found that the effect of calcitriol was more pronounced when human serum was added after the scratch. The potentiating agent in serum was also present in plasma of the same donor. The effect of calcitriol also persisted in the presence of growth factors known to be present in the wound bed such as: EGF, TGF α , TGF β , HB-EGF, KGF or in the presence of inflammatory cytokines. By employing specific inhibitors to the cytokines such as: IL-1, TNF α or IFN γ . EGF receptor (AG1478), Src (PP1), ERK cascade (U0126), p38 (SB203580), PKA (4-cyano-3-methylisoquinoline) and PKC (GF10290X), we found that EGFR and SRC are important for the migration process and that ERK, PKC and Src participate in the enhancing effect of calcitriol. Western blot analysis revealed that calcitriol increased ERK and Src activity in cultures 16 hours following scratch.

Conclusions: The results of this work present a hitherto unknown effect of the hormonal form of vitamin D, calcitriol, which may underlie the beneficial effect of hormonally active vitamin D derivatives in the course of epidermal wound healing.

Parathyroidectomy under Local Anesthesia

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Introduction: Parathyroidectomy began as bilateral neck explorative surgery in search of diseased glands. The extent of the surgery rendered general anesthesia necessary. Highly sensitive imaging techniques - neck ultrasonography and sestamibi scanning - made preoperative localization of the diseased gland, with high accuracy, possible. This permitted a reduction of the surgical field size and a shorter surgery time- making operation under local anesthesia possible. Furthermore, intraoperative PTH measurements in addition to a histopathological frozen section, ensures the curing of the disease by the end of surgery. In this work we review our experience in a new practice that has become a standard procedure at the Meir Medical Center: Parathyroidectomy under local anesthesia, enabling a shorter time for the patient in the operating room.

Patients/ Methods: Data from all 110 parathyroidectomies performed by the Otolaryngology department during five years, were collected. A study group of patients that were operated under local anesthesia was compared to a control group of patients that were operated by the traditional practice, under general anesthesia. We examined surgery duration and surgery success rate. We also studied and considered the reasons for cases that were not possible to conduct under local anesthesia.

Results: Patients and disease characteristics were similar between the two groups. The diseased glands were diagnosed as adenomas in 90%, hyperplasia in 5%, and carcinoma in 2% with the rest being double adenoma or atypical adenoma. The average time in the surgery room was much shorter for the study group than the control (82 minutes vs. 135 minutes, respectively) with a minimum time of 25 minutes in the study group compared to 60 minutes in the control group. The average and minimum hospitalization time were 0.9 days and 0.5 days for the study group and 2 days and 1 day for the control group, respectively. Surgical success rate, defined as postoperative normocalcemia, was 96.4% and no surgical complications were found in the study group.

Conclusions: Parathyroidectomy has become more efficient in cases of preoperative imaging localization of suspected adenoma. This is achieved through local anesthesia with sedation. Our results point out that our method of minimally invasive parathyroidectomy under local anesthesia has the advantages of a shorter surgery time and avoidance of general anesthesia- and all that without compromising therapeutic success or patient comfort.

Choice of treatment for vitamin D deficiency in residents of an institution for developmentally disabled, sunshine or supplement?

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Introduction: Vitamin D deficiency is common in the population of mentally disabled. Unfortunately, it mostly remains untreated. The objectives of this study were: assessment of vitamin D and PTH status in residents of a rural institution for developmentally disabled, evaluation of two therapeutic intervention strategies aimed to normalize vitamin D status.

Patients/ Methods: Stage I: Assessment of vitamin D and PTH status in residents of an institution for developmentally disabled. Stage II: An open randomized, 3 months (July-September) interventional trial, comparing between supplementation with 50,000 IU vitamin D3 once a month, supervised sun exposure, 15 minutes per day at midday, 25% of body area and a control group.

Results: Stage I – 153 residents – 86 men and 67 women –aged 37.6±12.1, were included. Serum 25(OH)D was 10.7 ± 6 ng/ml, 25(OH)D < 20ng/ml was observed in 92.2%. Plasma PTH concentration was 34.22 ± 17.95 pg/ml, (normal 11 – 62), 7(4.6%) of the participants displayed secondary hyperparathyroidism Multiple regression analysis (stepwise regression) revealed a significant negative correlation between Teril use (p<0.001, n=32), Prothiazin (p=0.02, n=18), behavioral disturbances (p=0.031, n=27) and serum 25(OH)D concentration. These 3 variables explain 17.7% of the decrease in 25(OH)D concentration. No significant correlation was established with any other parameter. Stage II – At the end of the 3 months intervention period 25(OH)D significantly increased (p<0.0001) by 16.02 +/- 6.3 ng/ml to 26.83 ± 5.6 ng/ml, by 15.53 +/- 7.4 ng/ml to 25.52 +/- 5.7 ng/ml, and by 8.23 +/-6 ng/ml to 18.16 +/- 8.5ng/ml, in groups I, II, and III respectively. Both types of therapeutic intervention contributed to a significant rise in serum 25(OH)D concentration compared to a control group (p<0.0001), with no significant difference between the treatment groups (p<0.397). The implementation of the sun exposure treatment was more complex (high number of caregivers) and costly than oral vitamin D supplementation (5850 vs. 886 NIS). From the assessment of oral supplement treatment - an intake of 104 IU of the vitamin corrected serum 25(OH)D concentration by 1 ng/ml. After controlling for seasonal (summer) vitamin D production, 214 IU of the vitamin were needed in order to induce 25(OH)D serum concentration increase by 1 ng/ml.

Conclusions: Vitamin D deficiency appears to be extremely common in residents of an institution for mentally disabled, partly due to medication use. Both types of treatment improved vitamin D status, but were unable to achieve optimal levels of > 30 ng/ml. The treatment with sun exposure was more complex and difficult to implement, in comparison with the oral supplement given once a month. Higher supplement doses may be needed for achievement of vitamin D adequacy.

Hospitalization in the Department of Internal Medicine, a Window of Opportunity for vitamin D replenishment

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Introduction: Vitamin D deficiency is common in the elderly in Israel. Awareness of this condition and adherence to vitamin D supplementation is low. It has been recently demonstrated that administration of a single dose of 100000 IU of vitamin D can effectively correct vitamin D status without toxicity. The aims of this study were: to assess vitamin D status in patients hospitalized in the Departments of Internal Medicine in Rambam Medical Center and to detect factors connected to vitamin D deficiency, to compare the efficacy of vitamin D supplementation initiated during hospitalization to the currently practiced treatment in the community.

Patients/ Methods: During hospitalization data was collected about patients' health status, sun exposure, medications, nutritional habits, functional status, falls and routine laboratory evaluation. 25(OH)D and PTH were assessed at inclusion and after 2 months. Patients were randomly assigned to oral vitamin D supplementation of 100000 IU/monthly, 20 cc of aqueous solution of vitamin D₃, CTS, for 2 months, the second dose was administered during an outpatient clinic visit or at patient's home under study physician's supervision, (group I) or treatment in the community, following an information letter to the family physician about patient's vitamin D status (group II).

Results: 150 patients aged 76.2 +/- 12.8 (range 65-89), 85 women and 65 men were included in the study. 25(OH)D serum level was 16.2 +/- 17.8. A significant correlation between vitamin D deficiency and age ($p=0.03$) and female gender ($p=0.018$) was observed. No correlation was established between any other parameter. 107 (71.3%) patients completed the study. At the end of the study period 25(OH)D serum level >32 ng/ml was observed in 25(50.9%) vs. 4 (7.7%), 25(OH)D serum level 20 – 32 ng/ml in 26(49.1%) vs. 23(44.2%), 25(OH)D serum level <10 in none versus 5 (9.6%) in group I and II respectively. The likelihood of achieving 25(OH)D serum level > 32 ng/ml in patients with initial serum level <10 ng/ml was 40% vs. 0%, with 10-20 ng/ml 30.4% vs. 0%, with 20-32 ng/ml 79% vs. 18.2% in group I and II respectively.

Conclusions: Vitamin D deficiency is common in patients, especially in women and in older patients, hospitalized in the Departments of Internal Medicine. Treatment with a monthly dose of 100000 IU of vitamin D for 2 months, initiated during hospitalization, is superior to the treatment administered in the community in treating vitamin D deficiency, although it has not ensured vitamin D adequacy in all patients.

A Novel Loss-of-function mutation, Gln459Arg, of the Calcium-Sensing Receptor Gene Associated with Apparent Autosomal Recessive Inheritance of Familial Hypocalciuric Hypercalcemia

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Introduction: Mutations that inactivate one allele of the gene encoding the calcium sensing receptor (CASR) cause autosomal dominant familial hypocalciuric hypercalcemia (FHH) and neonatal severe hyperparathyroidism. We describe the identification and biochemical characterization of a novel CASR gene mutation that caused apparent autosomal recessive FHH in extended consanguineous kindred.

Patients/ Methods: Direct sequence analysis of the CASR gene, clinical and biochemical analyses of 14 family members, and in vitro immuno-biochemical studies of the mutant CASR.

Results: A novel inactivating mutation (Q459R) was identified in exon 4 of both alleles of the CASR in the proband, who presented with asymptomatic hypercalcemia and hypocalciuria at age 2 years. The proband was consanguineous, and both of his parents were heterozygous for the Q459R mutation consistent with autosomal recessive inheritance of FHH. Among thirteen family members that were studied, eight subjects were heterozygous for the Q459R mutation and five had normal genotype. All heterozygous subjects were asymptomatic and normocalcemic apart from one subject, who was mildly hypercalcemic. The Q459R mutant CASR was transfected into HEK293 cells and receptor expression and function were assessed. The mutant receptor was normally expressed at the cell membrane in HEK293 cells, but retained only 30 to 50% of the calcium-dependent activity of the wild-type CASR. The mild reduction in calcium sensitivity as was confirmed by the in-vitro analyses is consistent with the mild phenotypes in this kindred.

Conclusions: We identified a novel loss-of-function Q459R mutation in the CASR gene that exhibits mildly reduced sensitivity to calcium, and which is associated with apparent autosomal recessive transmission of FHH. This study demonstrates the importance of genetic testing in FHH to distinguish between de novo and inherited mutations of the CASR gene and to assist in management decisions.

microRNAs are involved in catch up growth in the epiphyseal growth plate.

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Introduction: MicroRNAs (miRNAs) are endogenous small RNAs, 18-22 nucleotides long, that can regulate target mRNAs by binding to their 3'-UTRs. MiRNAs have been reported to be involved in a variety of functions, including skeletal development and longitudinal growth. Catch-up (CU) growth is a period of accelerated growth that occurs after correction of a temporary growth-retarding disorder. Most of the studies on CU growth were focused on changes in weight and only few showed the effect on the growth plate (EGP). The exact mechanism by which nutrition affects growth is still not elucidated.

Patients/ Methods: To study the mechanisms governing CU growth in the EGP, we subjected prepubertal rats to 10 days of 40% food restriction (FR), followed by a renewal of the regular food supply. Humeri were extracted, fixed and used for immunohistochemistry and morphological analysis. The EGP was excised, total RNA was extracted and loaded on miRNA microarrays. Results were confirmed by realtime PCR and changes in the predicted targets of these miRNAs were followed using Western immunoblotting.

Results: A significant weight difference between the control and FR group was observed. When the food restriction was removed, the CU group showed an instantaneous increase in weight, later accompanied by an increase in EGP and humeri length. The expression of several hundred genes was affected by nutritional manipulation, as shown by Affymetrix microarray 1. However, while immunohistochemistry studies showed dramatic changes in the level of growth related proteins such as GHR and IGFIR 2, no parallel differences in the respective gene expression level was detected. One possible explanation for this discrepancy may be regulation by miRNA. Indeed we found by miRNA microarray that numerous miRNAs are expressed in the EGP. Furthermore, the nutritional manipulation led to significant changes in the expression of several miRNAs in the EGP, including miR21, miR22, miR126 and miR140.

Conclusions: Dysregulation of mir-21 and 126 was previously described in cancer, mir-22 in cellular differentiation and mir-140 was shown to be cartilage specific, thus these results may have important implications for the growth of the EGP. These results are the first, to our knowledge, to show the involvement of miRNA in growth regulation in the mature EGP and the first to show the effect of nutrition on miRNA expression in vivo. Involvement of microRNA in the regulation of growth may open a new era of research and may enable the development of new treatment regime for children with growth abnormalities.

Changes in estrogen receptor alpha and in Nrf2 transcription factor are involved in the differential modulation of estrogenic activity by phytonutrients in bone vs. breast cancer cells

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Introduction: The advantages of fruit and vegetable rich diet for human health are well known. Phytonutrients from such diet were found by us to inhibit sex steroid signaling, the major risk factor in breast and endometrial cancer. In contrast, in bone cells, where estrogen is beneficial, phytonutrients supported estradiol induced activity and combination of phytonutrients with estradiol elevated transcription of markers for bone differentiation. In addition, carotenoid derivatives, polyphenols and isothiocyanate stimulate the electrophile/antioxidant response element (EpRE/ARE) transcription system and the Nrf2 transcription factor in both cell types. Furthermore, we showed that Nrf2 is involved in the inhibition of estrogen activity in breast cancer cells. Our aim in the current study was to determine how phytonutrients, which inhibit the harmful signals of estrogen in cancer cells, do not inhibit its positive action in bone cells.

Patients/ Methods: We used MC3T3-E1 mouse calvaria osteoblast like cells which respond to estrogen and MG-63 human osteosarcoma cells stably transfected with estrogen receptor alpha (ER α). Breast cancer T47D cells were used for comparison. Phytonutrients effect on estrogen induced activity was measured by ERE reporter gene assay. Estrogen receptor alpha level was detected by Western blotting in cells after separation of nuclei and cytosol.

Results: In both the mouse and human bone cells phytonutrients such as lycopene derivative, sulforaphane and carnosic acid increased alkaline phosphatase activity, the main marker for osteoblastic differentiation, relative to estradiol alone. In contrast, in breast cancer cells, these phytonutrients decreased the level of estrogen-induced proteins such as progesterone receptor. The phytonutrients increased estrogen receptor alpha levels in bone cells nuclei but reduced its levels in nuclei of breast cancer cells. Nrf2 was found to be involved in the inhibition of estrogenic activity in breast cancer cells. In contrast, in bone cells, over-expression of Nrf2 enhanced estrogen induced transcription whereas reduction of Nrf2 level, by siRNA, leads to a decrease in phytonutrient supported activity of estrogen.

Conclusions: Dietary phytonutrients, which inhibit estrogenic activity in cancer cells, do not inhibit and even stimulate estrogen signaling in bone cells. The results suggest that the Nrf2 transcription factor is involved in this differential activity of plant-derived phytonutrients.