VITAMIN D INCREASES THE LEVEL OF THE ADHESION MOLECULE E-CADHERIN AND INHIBITS IL-4-INDUCED CCL11 PRODUCTION BY HUMAN BRONCHIAL EPITHELIAL CELLS

Carmela Rotem[1], Ruth Koren[1,2], Amiram Ravid[1]

1 FACULTY OF MEDICINE, FELSENSTEIN MEDICAL RESEARCH CENTER, TEL AVIV, Israel

2 FACULTY OF MEDICINE, TEL AVIV UNIVERSITY, TEL AVIV, Israel

The physical and functional barrier functions of the airway epithelium are defective in asthma leading to deleterious inflammatory and immune responses. This is at least partially due to the action of diverse noxious agents on airway epithelial cells (ECs). The activation of ECs leads to excessive expression of chemokines and cytokines and consequent recruitment of effectors cells . Epithelial physical barrier function is maintained by intercellular contact formation mediated by adherens junctions and tight junctions. Epidemiological studies suggest that vitamin D reduces the frequency and ameliorates exacerbation episodes in asthmatic patients. We aimed to assess the notion that these putative effects are associated with modulation of the barrier function and inflammatory response of ECs by the hormonal metabolite of vitamin D, calcitriol.

Our experimental model was the human bronchial airway epithelial cell line BEAS-2B. Protein levels were assessed by Western blotting and mRNA level by real time PCR.

Treatment of BEAS-2B cells with calcitriol (100 nM) resulted in a marked increase in the level of the major protein in adherens junction, E-cadherin in the presence and absence of TNF, which on its own reduced E-cadherin level. The hormone increased E-cadherin mRNA levels in a time dependent manner, apparent 6 hours after treatment. Exposure of BEAS-2B cells to IL-4 dramatically increased gene expression of the chemokine CCL11 . This increase was markedly inhibited by pretreatment with calcitriol In a dose dependent manner significant already at 10 nM .

The results of this study suggest that the airway epithelium is a target for the action of calcitriol. Up-regulation of E-cadherin can contribute to the integrity of the epithelium and down-regulation of CCL11 may attenuate the inflammatory response of the epithelial cells. Both of these actions can underlie the observed association between vitamin D status and amelioration of the deleterious effects of asthma.

INTRA-DUODENAL 25-HYDROXYVITAMIN D_3-1 -HYDROXYLASE EXPRESSION, ACTIVITY AND 1,25$(OH)_2D_3$ METABOLISM.

Aneta Gawlik[1], Nimrod Rozen[2], Aviva Dahan[1], Dafna Ben- Yosef[1], Ron Shaoul[1], Yehuda Hovers[1], **Dov Tiosano[1]**

1 Pediateric Endocrinology, Rambam medical center
2 3. Bone and mineral research Division, Aemeck Hospital

Context: Duodenal calcium absorption is mainly regulated by 1,25$(OH)_2D_3$ through the Vitamin D receptor. Vitamin D deficiency (VDD), the most common cause of rickets, is associated with low 25(OH)D, low serum calcium and high PTH levels, but normal or high1,25$(OH)_2D_3$. If 1,25$(OH)_2D_3$ levels are normal in VDD, why then does VDD cause reduced calcium absorption leading to rickets?

Hypothesis: Calcium absorption requires intra-duodenal generation of 1,25$(OH)_2D_3$ by 1α-OHase, and its activity is 25(OH)D dependent.

Methods: Duodenal 25OH D-1α-hydroxylase activity was assessed by the generation of 1,25$(OH)_2D_3$ to added substrate 25(OH)D in duodenal samples from 15 individuals aged 4-73 years. CYP27B1, CYP24A1 and CYP3A4 expression was assessed in 22 duodenal samples from subject's ages 4-61 years.

Results: Basal generation of 1,25$(OH)_2D_3$ was 8.2 2.4pg/ml/mg protein/60 min. In the young group (4-14 years, n=7) Vmax was 246$\pm$20 pg/ml/mg protein at 300 ng/ml of 25(OH)D while in adult subjects (25-73 years,n=8) Vmax was much lower 27.5$\pm$4.5 pg/ml/mg protein, $p<0.03$. $K_{0.5}$ was similar in both groups 242$\pm$23 ng/ml and 270$\pm$30 ng/ml 25(OH)D (NS). CYP27B1 expression was elevated at ages 10-14(n=9)12.9±43.4 compared to patients younger than 8 years 1.2±4.3 (n=5), $p<0.003$ and older than 30 years 0.9±6.8,(n=8), $p<0.003$. Duodenal CYP3A4 expression was similar in all subjects and CYP24A1 expression was barely detected.

Conclusions: Human calcium absorption requires 25OHD as a substrate for local generation of 1,25$(OH)_2D_3$ by duodenal 1α-OHase for intracrine activity. This intracrine effect promote calcium absorption and explains why calcium is reduced in the presence of normal circulating 1,25$(OH)_2D$ levels in VDD.

CAROTENOID DERIVATIVES INHIBIT THE DELETERIOUS NFKB ACTIVITY IN BONE AND CANCER CELLS BY AFFECTING STRATEGIC THIOL GROUPS

karin Linnewiel Hermoni[1], Hansgeorg Ernst[2], Catherine Caris-Veyrat[3], Joseph Levy[1], Yoav Sharoni[1]

1 Clinical biochemistry, Ben Gurion University, Beer Sheva, Israel
2 BASF, Company, Ludwigshafen, Germany
3 Human Nutrition, INRA, Avignon, France

Activation of the NFkB transcription system contributes to cancer progression, and has a harmful effect on bone health. Under un-stimulated conditions, the NFkB transcription factor is retained in the cytoplasm by the inhibitory protein IkB. Phosphorylation of IkB by IkB kinase (IKK) activates the system. Importantly, both IKK and the NFkB subunits, contain cysteine residues that are critical for their activity. The interaction of various electrophyles with these cysteines results in inhibition of NFkB. Intact carotenoids such as lycopene and beta-carotene lack such electrophylic groups and we have recently demonstrated that carotenoid derivatives, but not the intact carotenoids, activate the Nrf2 transcription system.

The aim of the current study was to examine whether carotenoid derivatives prevent cancer and improve bone health by inhibiting the NFkB transcription system, and to determine the molecular mechanism of this inhibition.

To this end, we analyzed the structure-activity relationship of a series of dialdehyde carotenoid derivatives in NFkB inhibition. These compounds inhibited NFkB-driven reporter gene expression as well as several stages of the NFkB pathway in both cancer and bone cells. Moreover, similar to our previous findings regarding the Nrf2 system, the activity of the carotenoid derivatives depended on the reactivity of the electrophylic group in reactions such as Michael addition to SH groups in proteins. Importantly, carotenoid derivatives directly affected the NFkB machinery at two stages: the key regulatory enzyme IKK, as well as the p65 subunit of the transcription factor. Direct interaction with IKKbeta was found in acellular, in vitro kinase assay with a recombinant enzyme. Inhibition of p65 transcriptional activity was found in a reporter gene assay conducted in the presence of excess p65. Interestingly, the same structure activity relationship was evident in both p65 and IKK inhibition. Furthermore, pretreatment with SH donors such as N-acetyl cystein or dithiothreitol prevented part of the inhibition, altogether, supporting an SH group dependent mechanism.

In conclusion, we suggest that electrophylic carotenoid derivatives contribute to cancer prevention as well as bone health maintenance by inhibition of the NFkB transcription system, a process that may include modification of SH groups in both IKK and p65.

ASSOCIATION BETWEEN BONE MINERAL DENSITY AND INCIDENCE OF BREAST CANCER IN SOUTHERN ISRAEL

Merav Fraenkel[1], Yair Liel[1], Michael Koretz[2], Ethel Siris[3], Larry Norton[4], Tali Shafat[5], Shraga Shani[6], David B. Geffen[7]

1 Endocrine Unit, Soroka University medical Center and the Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel
2 Breast Health Center, Soroka Univ. Medical Center, Faculty of Health Science Ben Gurion University of the Negev, Beer Sheva
3 Division of Endocrinology, Columbia University Medical Center, New York, USA
4 Breast Center, Memorial Sloan Kettering Cancer Center, New York, USA
5 Clinical Research Center, Soroka University Medical Center, Facultiy of Health Sience Ben Gurion University of the Negev, Beer Sheva, Israel
6 Department of Clinical Biochemistry, Faculty of Health Sciences, Ben Gurion University of the Negev, Beer Sheva., Beer Sheva, Israel
7 Department of Oncology, Soroka University Medical Center, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel, Beer Sheva, Israel

Introduction: Osteoporosis and breast cancer affect many postmenopausal women. Estrogen deficiency leads to accelerated bone resorption and decreased BMD. Some studies have suggested an inverse relationship between BMD and breast cancer incidence. The objective of this study was to assess whether BMD is associated with risk of subsequent development of breast cancer in the female population of southern Israel treated at Soroka University Medical Center (SMC).

Methods: The electronic medical charts of women who underwent DEXA BMD studies at SMC between February 2003 and March 2011 were screened for breast cancer diagnosed **after** DEXA was performed, using ICD-9 codes. Demographic, BMD and laboratory findings were compared between those subsequently diagnosed with breast cancer and those who remained cancer-free. Women were divided into groups based on the tertiles of BMD at 3 sites: lumbar spine (LS,L1-4), total hip (TH) and neck of femur (FN). Breast cancer incidence was calculated for each group.

Results: Of 15268 women who underwent BMD testing, 807 had a previous diagnosis of breast cancer and 86 were diagnosed with breast cancer **after** BMD testing. The mean age of those diagnosed with breast cancer was higher than those without (68.8±9.1 vs. 65.1±11 years; $p<0.001$) and they had a higher BMI (30.9±5.5 vs. 29.1±5.7 $p=0.004$). Most women in both groups were older than 50 (94.2% and 92.7% $p=0.597$). The mean Z-score BMD of breast cancer patients was significantly higher than controls in all 3 sites (LS: 0.36±1.58 vs. -0.12±1.42, $p=0.002$; TH: 0.371.08± vs. 0.03±1.02, $p=0.002$; FN: 0.04±0.99 vs.-0.18±0.94; $p=0.026$). Women with higher Z scores had a significantly higher chance of developing breast cancer with an odds ratio of 2.1, 2.2 and 1.85 for the FN, TH and spine (per tertile, p value 0.006, 0.005 and 0.03 respectively). No correlation was found between the BMD Z-score and the stage, histology or grade or survival from breast cancer.

Conclusions: This study confirms previously published data on a direct association between BMD and the risk for breast cancer.

IS AGING MODULATED BY ANGIOTENSIN1-7? SYSTEMIC ADMINISTRATION OF ANGIOTENSIN1-7 INDUCES METABOLIC, ENDOCRINE AND MUSCULOSKELETAL "REJUVENATION" IN OLD FEMALE MICE: INTERACTION WITH LONG-TERM ENDURANCE EXERCISE

Yonit Marcus1, Gabi Shefer2, Rona Limor1, Zipora Yablonka-Reuveni2, Naava Nevo3, Keren Sasson3, Stav Brown4, Eli Carmeli4, Naftali Stern1

1 Institute of Endocrinology, Metabolism and Hypertension, Sourasky Medical Center, Tel Aviv, Israel

2 Department of Biological Structure, University of Washington School of Medicine, Seattle, Washington, USA

3 Departments of Biological Regulation & Biological Chemistry, Weizmann Institute of Science

4 Department of Physical Therapy, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv

As aging progresses, loss of skeletal muscle, bone mass and the appearance of decelerated metabolism predominate, resulting in declining health and gradual incapacitation. We previously reported that angiotensin1-7 (Ang1-7) favorably affects the metabolic profile in fructose-fed rats, owing partly to downregulation of adipose tissue NOX-4. Exercise seems a reasonable additional intervention as it exerts positive effects on aging. Here we tested the separate effects of 3 months endurance training via treadmill exercise (6 days/week, 20 minutes/day) alone, continuous Ang1-7 infusion alone (0.576mcg/k/d) or their combined effect in aged female mice (~17 months). First, exercise exerted beneficial effects on all studied parameters, including enhanced total bone density & content (determined by Dual energy X-ray absorptiometry), increased locomotor distance and speed and restoration of serum glucose and triglycerides to the range seen in younger mice. Second, combination of exercise and Ang1-7 resulted in significant elevation in estradiol levels and further increase in bone mineral density and content compared to exercise alone. Third, Ang1-7 alone supplemented to sedentary mice, induced a significant reduction in the distance and speed of locomotion, increased behavioral signs of anxiety, reduced glucose sensitivity and induced the intramuscular fat deposition. In elderly female mice, the key additive effect of Ang1-7 appears to reside in the induction of increased estradiol secretion, which enhances bone quality. Central / peripheral effects of Ang1-7 and /or estradiol likely also potentiate locomotor activity. In contrast, Ang1-7 treatment to sedentary mice is potentially harmful through deleterious effects on skeletal muscle resulting in enhanced intramuscular fat accumulation, reduced locomotor distance and speed. By alternate regulation of the renin-angiotension system, formerly unknown metabolic and endocrine effects may be set to motion to affect not only cardiovascular disease and basic components of the metabolic syndrome such as reduced locomotor activity, but also key elements of the aging process.

SIRT1 ACTIVATOR 2183 INCREASES OSTEOBLASTOGENESIS AND DECREASES OSTEOCLASTOGENESIS *IN VITRO.*

Irina Gurt[1], Eva Abramowitz[1], Gilad Hamdani[1], James Ellis[2], Jennifer Cermak[2], Rivka Dresner-Pollak[1]

1 Endocrinology and Metabolism, Hadassah-Hebrew University Medical Center, Jerusalem, Israel

2 Sirtris, a GSK company, Boston, USA

Background: We have recently reported that Sirt1 haplo-insufficiency results in decreased bone mass in female mice (Endocrinology 2011 Dec;152(12):4514-24, PMID: 21952235). We sought to investigate whether Sirt1 activation affects osteoblastogenesis and osteoclastogenesis in vitro.

Methods: The murine embryonic stem cell line C3H10T1/2, primary bone marrow mesenchymal stem cells (BM-MSCs) and bone marrow macrophages (BMMs) were used for this study. Osteoblastogenesis and osteoclastogenesis were induced by 50µg/ml ascorbic acid/10mM β-glycerophosphate and 10ng/mlRANKL and M-CSF, respectively.

Results: SRT2183 increased the osteoblastic markers: alkaline phosphatase activity (50%), osteocalcin mRNA expression and Von-Kossa positively-stained mineralized nodules. A decrease in p53 acetylation supported Sirt1 activation. SRT2183 inhibited BMMs- derived osteoclastogenesis in a dose dependent manner as determined by TRAP staining.

Conclusions: SRT2183 decreases osteoclastogenesis and increases osteoblastogenesis in vitro and may be considered as therapy for diseases such as osteoporosis.

ATYPICAL ACUTE ORAL CALCIUM LOAD HAS NO ADVERSE EFFECT ON VASCULAR COMPLIANCE AND ENDOTHELIUM REACTIVITY ASSESSED NON-INVASIVELY IN HEALTHY SUBJECTS

Marianna Yaron[1], Vanessa Roach[1], Elena Izkhakov[1], Maya Ish-Shalom[1], Jessica Sack[1], Yael Sofer, Ibrahim Azzam, Assaf Ray[1], Naftali Stern[1], Karen Tordjman[1]

1 Institute of Endocrinology, Metabolism, and Hypertension, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel

Studies have recently implicated calcium supplementation as a potential cause for an increased incidence of cardiovascular events among older women. It was proposed that transient elevations of serum calcium could have an adverse effect on vascular function. It was further suggested that, coming from food, calcium might be devoid of such an effect, as it is typically accompanied by far milder perturbations of serum calcium. The aim of this study was to non-invasively assess vascular function in response to an acute calcium load in young, healthy subjects.

Methods: Eleven healthy, vitamin D-sufficient, volunteers (8 females and 3 males) aged 33 ± 6.1 years participated in this random-order, cross over study. Biochemical and vascular parameters before and 3h after a typical acute oral calcium (600 mg) load, administered either as calcium citrate or as non-fat dairy products, were compared. Arterial stiffness was studied non-invasively by measuring Pulse wave velocity (PWV), Augmentation index (Alx) and Large (C1) and Small (C2) arterial compliance. Endothelial function was assessed by reactive hyperemia-induced flow mediated dilation (FMD). Paired t-test, or paired ANOVA were used to analyze the results.

Results: The acute calcium supplement raised serum calcium from 9.14 ± 0.3 to 9.64 ± 0.4 mg/dl, $P<0.005$, while the same amount from food resulted in an insignificant increase. The degree of urinary calcium excretion (expressed as Ca/Cr) was similar after both challenges. Moreover, PTH was equally and significantly suppressed: from 29.9±11.2 to 13.6±7.4 pg/ml for the supplement ($P<0.001$), and from 31.8±11.5 to 12.6±6.6 for the food study ($P<0.001$). Despite clear biochemical evidence for effective calcium loading on both occasions, none of the vascular parameters were affected by either challenge.

Conclusions: An acute oral calcium load appears to have no appreciable untoward effect on the vascular properties of young healthy subjects, regardless of the way it is provided.