

752 Calcium And Vitamin D Supplementation By Outpatient Allergy Patients

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RATIONALE: To determine if age 18 to 55 women with allergic disorders and/or asthma are taking calcium and vitamin D supplementation and assess associations, if present, between supplementation and history of asthma, systemic corticosteroid use, or tobacco use.

METHODS: 140 women aged 18 to 55 attending a private allergy practice completed voluntary questionnaires assessing self reported history of asthma, corticosteroid use, use of calcium and vitamin D supplements, and history of tobacco use.

RESULTS: Calcium and vitamin D supplement use was increased in women with history of asthma, and with past exposure to systemic steroids but was not associated with history of tobacco use.

31.9% of patients took daily calcium and vitamin D supplements, 56.7% took no nutritional supplements, 14.2% took multivitamins. Calcium and vitamin D supplementation was associated with 29.2 and 70.8% tobacco use and no tobacco use, 54.2% and 45.8% positive and negative asthma histories ($p < 0.01$), and 54.2 and 45.8% positive and negative systemic steroid exposure ($p < 0.001$). Respective evaluation of those not taking calcium and vitamin D were 37.4 and 62.6%, 26.4 and 71.4%, and 23.1 and 74.7%.

CONCLUSION: This is the first evaluation calcium and vitamin D supplementation by premenopausally aged women with allergic disease. Women with a history of asthma and/or systemic steroid use are cognizant of their risk for low bone mass. These findings are particularly pertinent to our field given the recent findings that postulate vitamin D status impacting rates of allergy and asthma.

753 Resistin-like Molecule (RELM- β) Is Up-regulated In Asthmatic Airways: Potential Role In Airway Remodelling

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RATIONALE: RELM- β is a member of cysteine-rich cytokine family involved in insulin resistance and inflammation. RELM- β is expressed *in vitro* in human airway epithelial cells, fibroblast cells and smooth muscle cells. It was shown RELM- β enhanced airways inflammation and remodelling in a mouse model. However, its function in human asthma is uncertain. We hypothesized that RELM- β is elevated in asthmatic airways and plays a role in the pathogenesis of airways remodelling.

METHODS: *In situ* hybridization, immunohistochemistry (IHC) and ELISA were employed to evaluate the expression RELM- β in bronchial biopsies and bronchoalveolar lavage fluid (BAL) obtained from asthmatics and controls ($n = 5$ for each group), respectively. Effects of RELM- β on human lung fibroblast cell (MRC5) proliferation and differentiation, and on human endothelial cells *in vitro* was evaluated by MTT assay, Western blot and angiogenesis assay, respectively.

RESULTS: Expression of RELM- β , both at the mRNA and protein levels was increased in the asthmatic bronchial mucosa as well as BAL compared with controls. After 48 hours of stimulation, RELM- β significantly increased MRC-5 fibroblast cell proliferation and expression of α -smooth muscle actin ($p < 0.05$). RELM- β also enhanced formation of microvessel structures by endothelial cells co-cultured with fibroblasts.

CONCLUSIONS: Our data suggest that increased expression of RELM- β in asthmatic airways, especially in epithelial cells, may contribute to airways remodelling by inducing proliferation of airway fibroblast cells and differentiation of these cells into myofibroblasts, as well as angiogenesis.

Funding: Medical Research Council

754 Sugar Consumption Increases Susceptibility to Allergic Airway Inflammation and Activates the Innate Immune System in the Lung

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RATIONALE: The rise in asthma has been attributed to a number of risk factors including poor eating habits such as high sugar intake, especially in children. Interactions between molecular and cellular components of the pulmonary innate immune system such as the carbohydrate recognition molecule surfactant protein-D (SP-D) and dendritic cells may regulate susceptibility to airway inflammatory diseases. We hypothesize that a high sucrose diet impairs the immunoprotective action of SP-D and increases susceptibility to airway inflammation.

METHODS: C57BL/6 and SP-D deficient mice were studied. A short-term (overnight) high sugar consumption-induced murine model of intermittent hyperglycemia was combined with a well-established model of allergic sensitization and challenge using *Aspergillus fumigatus* (*Af*) extract. Cellular inflammation, lung function, cytokine and SP-D levels in the airways of mice on sugar diet were compared to animals receiving normal diet during allergic sensitization and challenge.

RESULTS: Compared with wild-type mice, SP-D deficient animals displayed increased baseline glucose levels and developed elevated serum IgE in response to sensitization with *Af*. In *Af*-sensitized wild type mice, high sucrose diet induced intermittent spikes of hyperglycaemia, significantly enhanced levels of TNF α , increased number of dendritic cells and eosinophilic inflammation 36 hours after *Af* challenge. Further, sensitized mice on sucrose developed a significant eosinophilia and elevated lung resistance even without allergen challenge. Unlike mice on normal diet, sucrose-fed mice did not up-regulate SP-D protein in the BAL 36 hrs after allergen challenge.

CONCLUSION: A sugar rich diet may prime the innate immune system of the airways to allergic inflammation.

Funding: NIH

755 *Foxp3* Expression is Induced *In Vitro* and *In Vivo* By Probiotic Bacteria

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RATIONALE: Evidence supporting the benefits of probiotics in improving atopic dermatitis and other atopic conditions is emerging, yet mechanisms have not been elucidated. T Regulatory cells (TRegs), expressing *Foxp3*, may be central in decreasing allergic inflammation and could be a mechanism supporting this hypothesis.

METHODS: *In Vitro:* Peripheral blood mononuclear cells from healthy humans were incubated in: media, media containing lipopolysaccharide (LPS), or probiotic (*B. Lactis*) conditioned media with and without TGF-beta for 18 hours. *Foxp3* expression was measured by relative RT-PCR compared to a control gene, HPRT.

In Vivo: Conventional CD4⁺/*Foxp3*- T-cells from transgenic mice co-expressing EGFP/*Foxp3* were injected intraperitoneally into C57BL/6 RAG1 knockout mice. These mice were fed 3 different probiotics or water daily for 1 month. Flow cytometry was used to identify EGFP⁺ cells, indicating induced TRegs. The Mann-Whitney test was used for nonparametric analysis.

RESULTS: *In Vitro:* Without TGF-beta, *Foxp3* expression was induced more by LPS-Media (1.99 fold, SD 0.21) and *B. Lactis* (1.62 fold, SD 0.2) versus media alone (1.17 fold, SD 0.14).

In Vivo: Probiotic fed mice had 2- to 4-fold higher proportions of TRegs [*L. Rhamnosus* 1.4%, *B. Longum* 1.2% ($p = 0.004$), *B. Infantis* 0.6%] in mesenteric lymph nodes compared to those fed water (0.3%). There was no significant difference in TRegs in spleen or intestinal lamina propria. Taken together, probiotic fed mice induced TRegs greater than water fed mice (1.19% vs. 0.3%, $p = 0.01$).

CONCLUSION: This data suggests that selective probiotics may induce the expression of *Foxp3* and development of TRegs in atopic individuals. Further investigation is underway.

Funding: Thrasher Research Fund & Nestle Corporation