Evaluation of the use of the CBMN assay to determine inter-individual variation in spontaneous and folate deficiency-induced genome damage in humans

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Background – Folate deficiency causes genome and developmental defects in the fetus. However, the extent to which individuals vary in their sensitivity to the genome damaging effects of folate deficiency remains unknown. The cytokinesis-block micronucleus (CBMN) assay, one of the best validated and sensitive cytogenetic techniques for measuring chromosome breakage and chromosome malsegregation, appears to be sensitive enough to detect the genotoxic effects of moderate folate deficiency.

Objective – To test the capacity of the CBMN assay to detect inter-individual variation in base-line genome damage rates and sensitivity to the genome damaging effects of folate deficiency in the physiological range.

Design – Base-line and folate-deficiency induced micronuclei (MNi) in lymphocytes were measured in one individual on six different occasions and in six different individuals on single occasions. Other biomarkers within the CBMN assay such as nucleoplasmic bridges (NPB, a marker of chromosome rearrangement), nuclear buds (NBUD, a marker of gene amplification), necrosis and apoptosis were also measured. The effect of folate deficiency was investigated in long-term lymphocyte cultures in medium containing either 12nM or 120 nM folic acid.

Outcomes – MNi, NPB, NBUD and apoptosis were all significantly increased in 12nM folic acid cultures compared to 120nM folic acid cultures (all P<0.0001). Inter-individual variation was significantly greater than intra-individual variation for MNi (P = 0.0049) and apoptosis (P = 0.0208) only.

Conclusions – The CBMN assay is a robust and reproducible method for measuring genome damage caused by folate deficiency within the physiological range; however only the MNi and apoptosis measures are reliable enough for measuring inter-individual variation in spontaneous and folate-deficiency induced genome damage.

Dairy calcium and vitamin D stimulate postprandial thermogenesis: effect of sequential meals

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Background – Recent evidence suggests that an increase in dietary calcium may reduce adiposity. Breakfast meal composition has a significant influence on insulin sensitivity following lunch.

Objective – (i) To determine the acute effects of increasing calcium and vitamin D in breakfast meals on diet induced thermogenesis (DIT) and fat oxidation, (ii) to examine whether such changes had carry-over effects on a subsequent lunch meal.

Design – 11 subjects (mean ± SEM, age 54 ± 1.2 yr, weight 84.6 ± 5.39 kg, and BMI 31 ± 2.4 kg/m²) participated in a single blind, cross over study with a sequential-meal design. Volunteers were randomised to high dairy calcium (543 mg), high vitamin D (349 IU) breakfast (HCB) or a low dairy calcium (248mg), low vitamin D (12 IU) breakfast (LCB). Both breakfasts were followed by a very low calcium (48 mg), low vitamin D (25 IU) standard lunch (SL). Breakfast meals were isocaloric, with similar macronutrient profiles and identical volumes. Energy expenditure was assessed by indirect calorimetry and postprandial responses were calculated as the change from fasted values. Data was analyzed by a 2 x 2 repeated measures ANOVA with diet (HCD vs. LCD), meal (lunch vs. breakfast) and diet x meal interaction.

Outcomes – Non-esterified fatty acids (NEFA) were less suppressed following HCD compared to LCD: -18.2 ± 5.9% vs. –30.1 ± 4.7%, diet effect P<0.02). DIT was significantly higher on the HCD diet (6.8 ± 0.42 % vs. 4.4 ± 0.71%, diet effect P<0.01). Fat oxidation was less suppressed on the HCD diet (-1.6 ± 1.49 g/4h vs. – 4.3 ± 1.04 g/4h, diet effect P<0.05). Glucose and insulin responses were significantly higher at lunch compared to breakfast. This was accompanied by significantly higher carbohydrate oxidation at lunch (16.8 ± 2.7 g/4h vs. 11.5 ± 3.4 g/4h; meal effect P<0.03).

Conclusions – Higher calcium and vitamin D acutely stimulated postprandial thermogenesis and fat oxidation. Overall, the rank order of effects established at breakfast, were maintained over lunch.

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