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Micronutrients intake associated with DNA damage assessed by in a human biomonitoring study

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Nutrition science has evolved into a multidisciplinary field that applies molecular biology and integrates individual health with the epidemiologic investigation of population health (Go *et al.,* 2003). Nutritional genomics studies the functional interaction of food and its components, macro and micronutrients, with the genome at the molecular, cellular, and systemic level (Ordovas & Corella, 2004). Diet can influence cancer development in several ways, namely direct action of carcinogens in food that can damage DNA, diet components (macro or micronutrients) that can block or induce enzymes involved in activation or deactivation of carcinogenic substances (Willett & Giovannucci, 2006). Moreover, inadequate intake of some molecules involved in DNA synthesis, repair or methylation can influence mutation rate or changes in gene expression. Several studies support the idea that diet can influence the risk of cancer; however information concerning the precise dietary factor that determines human cancer is an ongoing debate (Ames, 2001; Key *et al.,* 2004; Anand *et al*., 2008; Couto *et al.*, 2011). A lot of epidemiological studies, involving food frequency questionnaires, have been developed providing important information concerning diet and cancer, however, diet is a complex composite of various nutrients (macro and micronutrients) and non-nutritive food constituents that makes the search for specific factors almost limitless.

Micronutrients are a set of approximately 40 substances, including vitamins, essential minerals and other compounds required in small amounts for normal metabolism, that are essential for human health (Ames, 1998; Lal & Ames, 2011). Micronutrients are capable of acting via a number of mechanisms to block DNA damage, mutation, and carcinogenesis by oxygen radicals, PAHs, and other chemical carcinogens (Perera, 2000; Collins & Ferguson,

2004). Micronutrient deficiency or excess can have modifying effects on genomic integrity that may involve nutrient-nutrient or nutrient-gene interactions and may depend on an individual's genetic constitution (Fenech *et al.,* 2005; Thomas *et al.*, 2011). Therefore, determining the intake levels of micronutrients required to maintain genome stability is an essential step in the definition of optimal diets for the prevention of cancer and other diseases caused by genome damage (Fenech *et al*., 2005).

Comet assay has become one of the standard methods for assessing DNA damage, with a wide range of applications, namely in genotoxicity testing, human biomonitoring and molecular epidemiology, as well as fundamental research in DNA damage and repair (Collins, 2004, 2009); studying the mechanisms of action of genotoxic chemicals; investigating oxidative damage as a factor in disease; monitoring oxidative stress in animals or human subjects resulting from exercise, or diet, or exposure to environmental agents; studying the effects of dietary antioxidants; and monitoring environmental pollution by studying sentinel organisms (Dusinska & Collins, 2008; Azqueta et al., 2009).

This research was conducted as a part of a human biomonitoring study to assess genotoxic effects in workers occupationally exposed to cytostatics drugs, and verify how micronutrients can influence a genotoxic response. Therefore, there were constituted two groups, 46 subjects occupationally exposed to antineoplastic drugs and 46 subjects non-exposed. For analysis of DNA damage and oxidative damage a modification of the comet assay originally described by Singh *et al.* (1988) was used to measure the basal level of DNA oxidation in lymphocytes (Collins, 2009). Percentage of DNA in the tail and oxidative damage parameters were measured using Zeiss AxioScope.A1 fluorescence microscope and Comet Assay IV capture system (Perceptive Instruments ® software) and 50 nucleoids were scored per gel. Dietary intake was assessed using a self-administered Food Frequency Questionnaire (FFQ) (Lopes, 2000; Lopes *et al.,* 2007). The FFQ included type and quantity of food intake, namely some food items, which allowed for the quantification of different macronutrients and micronutrients. The FQQ is a 3-page booklet including a list of 92 common food and beverage items and questions relating to food preparation and dietary habits. Participants were required to indicate how often each food and beverage was usually consumed per month, week, or day. Average daily consumption was based on the participants' reports on how often a specified serving size of each food or beverage item was consumed. This information, along with the nutrient composition of the food item/unit weight taken from 92 selected items, allowed participants' daily micronutrient and macronutrient intake to be calculated using the FREQUAN dietary analysis program (Baghurst and Record, 1984).

The nutritional items selected from the FFQ for analysis were calories, retinol, vitamin B12, folate, vitamins D and E, iron, and selenium. All the items presented concentrations above the dietary references intakes of Food and Nutrition Board, Institute of Medicine, National Academies. In order to investigate the association between genotoxicity biomarkers and nutritional items, multiple linear regressions were conducted by group (exposed/controls). It was found in the exposed group negative associations between %DNA in tail and calories (B=-0.011, p=0.013) and folate (B=-0.078, p<0.001), and positive associations between vitamin E (B=1.912, p=0.002) and iron (B=2.345, p<0.001). In control group, it was found for DNA oxidative damage a positive association with retinol (B=0.004, p=0.020) and negative associations with calories (B=-0.006, p=0.002) and vitamin B12 (B=-0.589, p=0.006).

Retinol was positively correlated with oxidative DNA damage in controls. The study by van Helden *et al.* (2009) demonstrated that vitamin A enhances OH radical formation in the Fenton reaction, showing that vitamin A can act as pro-oxidant or antioxidant, depending on the type of radicals involved, and may lead to DNA oxidative damage (Alakhras *et al.,* 2011). Azqueta & Collins (2012) clearly distinguished between effects of vitamin A, pro-vitamin A carotenoids, and non-vitamin A carotenoids; being the latter group almost invariably reported to protect against DNA damage, whether endogenous or induced by exogenous agents, the pro-vitamin A carotenoids show a wider spectrum of effects, sometimes protecting and sometimes enhancing DNA damage.

Vitamin E was found to be positively correlated with % DNA in tail. Watters *et al.* (2007) also found a positive association of vitamin E and oxidative DNA damage in a healthy, non-smoking population of young adults. A possible explanation for this result stems from some evidence that in the presence of copper or in smokers with a fat rich diet, vitamin E can act as a strong pro-oxidant, nevertheless it remains an unexpected result.

Results found a positive correlation between iron and % DNA in tail, meaning that higher intake of iron associates with higher DNA damage. Oxidative lesions, and more specifically 8-OHdG, is one of the most prevalent lesions induced by iron containing substances (Prá *et al.,* 2012), however the FPG biomarker was not statistically associated with iron. There is sound evidence that iron deficiency increases genome instability, among other mechanisms, by impairing enzymes involved in antioxidant and nuclei acid metabolism (Prá *et al.,* 2012). Results presented herein found that the amount of calories ingested was negatively correlated with both biomarkers assessed by comet assay. This was somewhat unexpected, as calories restriction reduces metabolic rate and oxidative stress, meaning that lower calories ingestion

decreases DNA damage and DNA oxidative damage (Hart *et al.,* 1999; Heilbronn & Ravussin, 2003).

A significant negative correlation was found between folate and % DNA in tail. Courtemanche *et al.* (2004) also found that folate deficiency leads to increased DNA damage in primary lymphocytes, and that deficiency in the physiological level of folate caused more DNA damage than low-dose radiation in primary T lymphocytes.

A significant negative correlation between vitamin B12 and DNA oxidative damage (FPG) was found, suggesting that vitamin B12 acts like a protective factor (Ames, 2001; Ames & Wakimoto, 2002; Ames, 2006). Minnet *et al.* (2011) also found a negative correlation between DNA damage and vitamin B12 levels, meaning that higher levels of vitamin B12 decrease DNA damage, in good agreement with the results herein.

Comet assay allows for the study of the effects of nutrients with known anti- or pro-oxidant capacities on different cell types and in different concentrations. These studies have revealed an apparent paradox, or at least a hormetic effect, whereby many of these antioxidant compounds seem to protect against DNA damage at low doses while actually causing DNA damage at higher doses (Wasson *et al.*, 2008). There are several possible reasons why significant associations are difficult to find. First, samples usually comprise mostly healthy persons; second, it is possible that a synergistic effect exists involving all antioxidants which is not seen for each individual nutrient (Watters *et al.*, 2007). Third, it is plausible that associations between some of the antioxidants examined and oxidative DNA damage may be better captured using other measures of oxidative DNA damage. Fourth, it is possible that the range of antioxidant concentrations and/or oxidative DNA damage in this study was not wide enough to detect associations or that the associations simply do not exist (Watters *et al.,* 2007). Previous studies have suggested a significant moderating effect of long-term antioxidant supplementation on endogenous and exogenous oxidative DNA damage in lymphocytes, supporting the hypothesis that dietary antioxidants may protect against cancer.

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