

# The Cytokinesis-Block Micronucleus (CBMN) Cytome Assay as a Bioefficacy Biomarker for Zinc Status

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## INTRODUCTION

Zinc is required as a co-factor for Cu/Zn superoxide dismutase, endonuclease IV, function of p53, Fapy glycosylase and in Zn-finger DNA repair proteins such as PARP and OGG1. Deficiency in Zn may result in increased DNA oxidation, DNA breaks and elevated chromosome damage rate. Damage to the genome is an important risk factor for developmental and degenerative diseases. It is therefore essential to determine the optimal concentration of Zn in vitro and in vivo that corresponds with optimisation of genome stability. We investigated the relationship between plasma Zn-status, mitogenic response and DNA damage in human peripheral blood lymphocytes in vivo using the Cytokinesis-Block Micronucleus (CBMN) Cytome Assay.

## METHODS

The CBMN Cytome Assay is a comprehensive test system used to measure chromosomal stability (micronuclei, biomarker of chromosome breakage or loss; nucleoplasmic bridges, biomarker of chromosome rearrangement; nuclear buds, a biomarker of gene amplification), DNA repair capacity, nuclear division rate, mitogenic response and prevalence of necrotic and apoptotic cells in human and mammalian cells.

We used the CBMN Cytome assay to determine the relationship between plasma-Zn and genome stability, cell death and mitogen response in peripheral blood lymphocytes in obese healthy adult males. We also performed a controlled intervention on 190 healthy individuals (mean age 47.8 years, 46% males), to examine whether supplementation with vitamins A, C and E together with Zn (ACEZn) for 6 months improves genome stability.

## RESULTS AND DISCUSSION

Using the CBMN Cytome technique we showed that even within the narrow range of plasma-Zn concentration observed in our cohort of obese males (25<sup>th</sup> percentile 0.835 ug ml<sup>-1</sup>, 75<sup>th</sup> percentile 1.04 ug ml<sup>-1</sup>, min 0.66 ug ml<sup>-1</sup>; max 1.52 ug ml<sup>-1</sup>) it was possible to detect a significant negative correlation with nuclear division rate in lymphocytes which is a biomarker of mitogenic and immune response ( $r = 0.34$ ,  $P < 0.05$ ). In addition, a significant negative correlation with necrosis rate was also observed ( $r = -0.41$ ,  $P < 0.05$ ). Supplementation with ACEZn significantly reduced the micronucleus frequency index by 13% ( $P = 0.038$ ).

The results of these investigations indicate that the CBMN Cytome assay has good potential as a bioefficacy biomarker to investigate the effects of Zn on genome health maintenance, cell death and immune function of peripheral blood lymphocytes. Other studies have shown that the micronucleus index in buccal cells is lowered after supplementation with supplements containing Zn. These observations of genome protective effects of Zn need to be confirmed in placebo-controlled interventions using both organic and inorganic forms of Zn in biofortified and/or fortified food products as well as supplements and the optimal intake and tissue concentrations for genome health maintenance need to be clearly defined.

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