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Zinc supplementation reduced DNA breaks in Ethiopian women

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ABSTRACT

Assessment of zinc status remains a challenge largely because serum/plasma zinc may not accurately reflect an individual's zinc status. The comet assay, a sensitive method capable of detecting intracellular DNA strand breaks, may serve as a functional biomarker of zinc status. We hypothesized that effects of zinc supplementation on intracellular DNA damage could be assessed from samples collected in field studies in Ethiopia using the comet assay. Forty women, from villages where reported consumption of meat was less than once per month and phytate levels were high, received 20 mg zinc as zinc sulfate or placebo daily for 17 days in a randomized placebo-controlled trial. Plasma zinc concentrations were determined by inductively coupled plasma mass spectrometry. Cells from whole blood at the baseline and end point of the study were embedded in agarose, electrophoresed, and stained before being scored by an investigator blinded to the treatments. Although zinc supplementation did not significantly affect plasma zinc, mean (±SEM) comet tail moment measurement of supplemented women decreased from 39.7 ± 2.7 to 30.0 ± 1.8 ($P < .005$), indicating a decrease in DNA strand breaks in zinc-supplemented individuals. These findings demonstrated that the comet assay could be used as a functional assay to assess the effects of zinc supplementation on DNA integrity in samples collected in a field setting where food sources of bioavailable zinc are limited. Furthermore, the comet assay was sufficiently sensitive to detect changes in zinc status as a result of supplementation despite no significant changes in plasma zinc.

Abbreviations: AGP, α-1-acid glycoprotein; BMI, body mass index; DNA, deoxyribonucleic acid.

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1. Introduction

Zinc is a required cofactor for more than 200 enzymes involved in most major metabolic pathways and supports a wide range of biochemical and immunologic functions [1] including maintenance of deoxyribonucleic acid (DNA) integrity and antioxidant defense in cells [2]. Lack of adequate dietary zinc and effects of the subsequent zinc deficiency remain worldwide health problems disproportionately affecting populations in developing counties whose diets are heavily reliant on plant-based foods [3,4]. Inadequate dietary zinc continues to contribute to increased risk of infectious diseases in the developing world [5].

Although dietary intakes of zinc may correlate with plasma zinc concentrations, there are numerous dietary and nondietary factors that negatively affect zinc bioavailability, such as inhibitory ligands like phytic acid, as well as gastrointestinal diseases and malabsorptive syndromes [4,6–11]. In a meta-analysis of 3 observational studies involving 1184 participants, Lowe and colleagues [12] found a nonsignificant relationship between dietary Zn intake and plasma zinc concentration. Furthermore, based on a meta-analysis of 10 zinc supplementation randomized control trials, which included 1285 participants, Lowe and colleagues [12] concluded that doubling of zinc intake would increase plasma/serum zinc concentrations by only 6%. Hess and coworkers [13] and the International Zinc Nutrition Consultative Group suggested that plasma/serum zinc concentrations are useful to assess a population's risk of zinc deficiency or response to zinc supplementation but may not be reliable indicators of zinc status. Thus, no single biochemical assay has been validated that accurately reflects tissue or cellular zinc concentrations of individuals [4].

A particularly important function of zinc is protection of the cell against DNA damage through its role in proteins involved in DNA repair pathways. Zinc is required for optimal function of the repair proteins poly(adenosine diphosphate–ribose) polymerase 1, tumor protein 53, and apurinic endonuclease [14–16]. In a rat glioma C6 brain cell model, increased oxidative DNA damage and impaired nucleic acid repair mechanisms appeared to result, at least in part, from low zinc availability [17]. Indeed, zinc deficiency in both in vitro and in vivo models is associated with increased oxidative stress and increased DNA damage [2,18].

As a result of this relationship between cellular zinc levels and DNA damage, the comet assay, a method that measures DNA strand breaks in cells, may represent a sensitive functional tool to assess response to zinc supplementation. Because zinc is found predominantly as a cofactor or structural component in intracellular proteins, including transcription factors and proteins involved in DNA damage repair, changes in intake may affect intracellular processes such as DNA structure and repair more than plasma zinc concentration [17,19]. In a zinc depletion-repletion study conducted in healthy men, the comet assay reflected zinc status as a function of alterations in DNA integrity [20]. Importantly, increases in DNA damage preceded any change in fasting plasma zinc, suggesting that DNA damage is a more sensitive marker of zinc status.

The comet assay is based on a microelectrophoretic technique [21–23] and has been used in cancer research, as well as in toxicologic studies, to assess damage to nucleic acids [24,25]. The assay allows for the quantification of DNA damage within single agarose-embedded cells [26]. Under the appropriate assay conditions, electrophoresis of lysed cells may result in the generation of comet-like structures. Intact DNA is retained in the "head," whereas fragmented DNA forms the tail, resulting in the formation of so-called comets. The comets are stained and visualized using fluorescence microscopy [27,28]. Because of the essential role of zinc in DNA repair mechanisms, changes in zinc status can contribute to changes in the comet size and morphology.

Previous studies showed that rural women in subsistence farming households in the Sidama region consumed meat very rarely (less than once per month) [29] and that phytate/zinc molar ratios in women's diets averaged 17:1 [30]. Staple foods in southern Ethiopia are maize and enset (*Enset ventricosum*). Therefore, we hypothesized that women from the area would be at high risk for chronic zinc deficiency. Because of the limitations of assessing zinc status by measuring plasma zinc, we examined the use of the comet assay to assess the effects of a double-blind placebo-controlled supplementation with zinc sulfate in Ethiopian women drawn from this population. Additionally, we hypothesized that effects of zinc supplementation on intracellular DNA damage could be assessed from samples collected in field studies in Ethiopia using the comet assay. To test this hypothesis, changes in DNA strand breaks in peripheral blood cells at baseline and end point were compared between the placebo and zinc-supplemented groups.

2. Methods and materials

2.1. Study design, participants, and ethical approvals

This double-blind placebo-controlled zinc supplementation trial was conducted in the Finichawa community of the Sidama region in Ethiopia in January 2010. Women were recruited through community health workers by researchers from Hawassa University. A health care assistant excluded volunteers who self-reported pregnancy or malarial infection, or exhibited other clinical signs of impaired health. Details of the study were explained in the local language in an information session, and women between 18 and 50 years of age who chose to volunteer returned on another day to give consent and to be enrolled in the study. For women who could not read or write, the consent form was read in the local language, and oral consent was witnessed by a community volunteer able to sign her name as witness.

Approval for this study was provided by institutional review boards at both Oklahoma State University and Hawassa University. Approval was also granted through the Southern Nations, Nationalities and People's Regional Health Bureau, and the zonal health office of Hawassa. At the national level in Ethiopia, approval was granted by the National Health Research Ethics Review Committee and the Drug Administration and Control Authority.