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A short 18 items food frequency questionnaire biochemically validated to estimate zinc status in humans

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ABSTRACT

Inadequate dietary zinc intake is wide-spread in the world's population. Despite the clinical significance of zinc deficiency there is no established method or biomarker to reliably evaluate the zinc status.

The aim of our study was to develop a biochemically validated questionnaire as a clinically useful tool that can predict the risk of an individual being zinc deficient.

From 71 subjects aged 18–55 years blood and urine samples were collected. Zinc concentrations in serum and urine were determined by atomic absorption spectrometry. A food frequency questionnaire (FFQ) including 38 items was filled out representing the consumption during the last 6 months obtaining nutrient diet scores. Latter were calculated by multiplication of the particular frequency of consumption, the nutrient intake of the respective portion size and the extent of the consumed quantity. Results from the FFQ were compared with nutrient intake information gathered in 24-h dietary recalls. A hemogram was performed and cytokine concentrations were obtained using Enzyme-linked Immunosorbent Assay.

Reducing the items of the primary FFQ from 38 to 18 did not result in a significant variance between both calculated scores. Zinc diet scores showed highly significant correlation with serum zinc ($r = 0.37$; $p < 0.01$) and urine zinc concentrations ($r = 0.34$; $p < 0.01$). Serum zinc concentrations and zinc diet scores showed a significant positive correlation with animal protein intake ($r = 0.37$; $p < 0.01$/$r = 0.54$; $p < 0.0001$). Higher zinc diet scores were found in omnivores compared to vegetarians (213.5 vs. 111.9; $p < 0.0001$).

The 18 items FFQ seems to be a sufficient tool to provide a good estimation of the zinc status. Moreover, shortening of the questionnaire to 18 items without a loss of predictive efficiency enables a facilitated and resource-saving routine use. A validation of the questionnaire in other cohorts could enable the progression towards clinical utilization of this promising tool.

1. Introduction

Zinc is a component of various enzymes and structural proteins and of major importance for the function and regulation of the immune system [1–3]. The total body content of zinc is 2–4 g, distributed mainly in the musculoskeletal system [4]. In the plasma, the zinc concentration is around 12–16 μM and almost all zinc is bound to proteins, mainly albumin [5]. There is no storage system, thus a daily intake of around 0.14 mg/kg for males/0.12 mg/kg for females is recommended [6,7].

Subjects with severe zinc deficiency display skin lesions, paronychia, alopecia, neuropsychiatric disorders, ophthalmic abnormalities, weight loss, growth retardation, male hypogonadism [8], impaired reproductive performance [9] and a high susceptibility towards infectious diseases through functional abnormalities of the immune system [8]. The immunodeficiency itself can manifest in lower concentrations of TH-1-associated cytokines [10]. However, even moderate zinc deficiency causes several negative effects on human health [11].

Epidemiological data revealed an estimated prevalence of inadequate dietary zinc intake in more than 20% of adolescents worldwide [12]. One-third to one-half of the world's population is at risk of low dietary intake of absorbable zinc and the development of zinc deficiency [9]. Despite the clinical significance of zinc deficiency there is no established method or biomarker to reliably evaluate the zinc status [13,14]. The clinical practice most widely used is the measurement of serum/plasma [15] zinc concentrations [16]. Amongst several other indicators analyzed for reflecting human zinc status, urinary and hair

Abbreviations: FFQ, food frequency questionnaire; IFN-γ, interferon-γ; IL-2, interleukin-2; LPS, lipopolysaccharide; PHA, phytohemagglutinin

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zinc concentrations have been shown to reflect responses to zinc supplementation in a systematic review [15]. Nevertheless data from studies applying those is still insufficient for proving the validity [17,15]. Serum zinc only represents 0.1% of whole body zinc pool [18]. While it is well-established and solid for severe zinc deficiency [16], its reliability is questionable in mild to moderate deficiency through its oligosymptomatic clinical presentation and the lack of specific deviations in laboratory findings [17,14]. Besides, serum/plasma zinc concentrations are influenced by the time of day, inflammation, hormones, certain drugs and recent meal consumption. Another limitation is its considerable interindividual variability with changes in dietary zinc intake. It has been also demonstrated that serum/plasma zinc concentrations show different responses towards changes in dietary zinc intake compared to zinc supplementation [15].

Foods containing the highest zinc concentrations are meat, offal, fish, seafood and cheese [1], though the bioavailable zinc contents themselves vary in a wide range [19]. The major dietary factor that influences bioavailability of zinc from dietary sources is phytate, followed by calcium and fiber [4]. The mechanism through which phytates lead to a decreased resorption of zinc is chelation [4]. The mechanism and extent of the influence of calcium and fiber on intestinal zinc absorption remains uncertain and still under discussion [20,21].

The aim of the present study was to develop a biochemically validated questionnaire as a diagnostic tool that can predict the risk of an individual being zinc deficient. Information drawn from this questionnaire was compared with zinc concentrations in serum and urine. This diagnostic tool could be valuable in identifying subjects with moderate zinc deficiency. Another possible implication of such a questionnaire is a better adjusted therapy concept and surveillance of zinc substitution.

2. Materials, methods and subjects

2.1. Subjects and study design

The protocol for this trial was reviewed by the institutional ethics committee (EK089/08). All the subjects provided written informed consent before enrollment. This study was conducted from January 2017 until June 2017. The participants were recruited through an email-list server from the RWTH Aachen University and notices in the Aachen University Medical Faculty. The inclusion criteria for participants were 1) 18–55 years of age 2) no current chronic diseases concerning malabsorption, gastrointestinal diseases, kidney-/liver diseases or diabetes 3) no diagnosed zinc deficiency or high risk for deficiency (for example presence of persistent diarrhea, HIV infection) [22] 4) consumption of omnivorous, vegetarian or vegan diet, but no fruitarian or raw food diet. Pregnant and lactating women were also excluded. 5) Zinc supplementing subjects were not excluded, even if supplemental daily doses were exceeded.

71 study participants (38 female, 33 male) were handed out dietary intake questionnaires. They received instructions on completing the protocol from a nutritional scientist prior to presentation. After overnight fasting (minimum 8 h) blood and urine samples were collected and the completed questionnaires were gathered. Furthermore the participants were asked not to perform physical activity in the morning before sample collection [23].

2.2. Sample collection and analysis

Blood was collected in 9 ml serum monovettes suitable for zinc measurement, 2.7 ml EDTA-containing tubes and 2.7 ml lithium-heparin monovettes (Sarstedt, Nürnbrecht, Germany). Serum samples were centrifuged for 10 min at 1841 g at room temperature. In EDTA-anticoagulated blood a hemogram was obtained through Sysmex XS-800i fluorescence flow cytometry analyzer (Sysmex Europe GmbH, Norderstedt, Germany).

Urine was collected in urine beakers (Sarstedt), decanted, transferred to tubes and centrifuged 1 min at 20,000*g* at room temperature. The supernatant was removed for analysis.

Blood sampled in lithium-heparin tubes was used to perform a whole blood assay for evaluation of cytokine production. For untreated control samples 100 µl whole blood was added to 900 µl RPMI-1640 medium (Sigma-Aldrich, St. Louis, Missouri, USA) (containing 10.000 U/ml penicillin, 10.000 µg/µl streptomycin, 200 mM L-glutamine, Sigma) in sterile 5 ml polypropylene tubes (Greiner, Vippula, Finland). For Phytohemagglutinin L (PHA) (Biochrom) stimulation samples contained 850 µl medium, 100 µl blood and 50 µl PHA working solution containing 200 µg/ml dissolved PHA in medium, resulting in a final concentration of 10 µg/ml PHA. For stimulation with Lipopolysaccharides from *Escherichia coli* 0111:B4 (LPS) (Sigma) samples contained 850 µl medium, 100 µl blood and 50 µl LPS working solution containing 5 µg/ml LPS resulting in a final concentration of 250 ng/ml LPS. Stimulated and control samples were incubated for 24 and/or 48 h, respectively at a temperature of 37 °C, 5% CO_2 in a humidified atmosphere (Sanyo, Moriguchi, Japan). After incubation the samples were mixed by a vortex and centrifuged at 300*g* for 10 min at room temperature. The supernatants were removed for cytokine analysis [24].

Interleukin-2 (IL-2) and Interferon-γ (IFN-γ) concentrations were measured by enzyme-linked immunosorbent assay (ELISA) (BD Biosciences, San Diego, CA, USA).

Additionally the zinc contents of human serum samples were measured subsequently in different laboratories at two different times, respectively. 16 samples were determined in a commercial laboratory for clinical chemistry and parallel aliquots were analyzed in an academic laboratory specialized on trace element research.

Zinc concentrations in serum and urine samples were determined via flame atomic absorption spectrometry by an AAnalyst 800 (Perkin-Elmer, Waltham, United States). Prior to zinc concentration measurement the serum was diluted 1:5 and 1:10 in deionized water. The average of both measurements was applied in a standard curve for calculation of zinc concentration. The zinc concentration measurement of urine supernatants was handled in the same way with undiluted samples.

2.3. Questionnaires for the assessment of dietary zinc, phytate and other nutrient intake

A food frequency questionnaire (FFQ) was applied for calculating the average zinc and phytate diet scores. The time period the FFQ referred to was the past 6 months. Frequency, servings and quantity of different foods were inquired for analyzing the dietary habits. Additionally, details about the intake of supplementary zinc were evaluated. Every row contained 1) the food type 2) the frequency identified through the selection of a checkbox ranging from “daily” to “less than 1 time monthly or never” in five steps and thus quantified with numbers ranging from one to five (1: „less than 1 time monthly or never”; 2: „2–3 times monthly”; 3: „1 time weekly“; 4: „2–4 times weekly“; 5: „1 time daily“) 3) a free text field for precise description of the serving (for example: one bar, one loaf, one bowl etc.) 4) the amount identified through the selection of the checkbox defined as low, medium or large and thus quantified with numbers ranging from one to three (1: small, 2: medium, 3: large). A standardized overview for the calculation of quantities of consumed foods from specified dimensions − such as one tablespoon − was applied [25].

The equation for calculating the diet scores was derived from the zinc diet score developed and validated by Kanoni et al. [26] (zinc or phytate diet score = frequency index * quantity index * zinc or phytate content [mg]). A nutritional value and calorie table by Elmadfa et al. (2015) was used as a main source for zinc contents [27] (Supplementary Table B.1-2) and a review of Schlemmer et al. (2009) served as a main source for phytate contents [28] (Supplementary Table B.1-2).

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