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Trace element status and fatty acids metabolism during healthy ageing: An example of a population from the Tunisian eastern coast

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ABSTRACT

Micronutrients as well as essential fatty acids are indispensable for the correct functioning of the organism. The risk of disturbance in the associated nutrition and metabolism is expected to increase during ageing. In addition, it seems that trace elements are involved in the fatty acids metabolism. The aim of the present study was then to assess age-related changes in trace elements status and in plasma essential fatty acids composition with an emphasis on the desaturase activity estimation. Two hundred healthy Tunisian subjects (30–85 years old) were recruited and separated into two subgroups: elderly (65–85 years old) and middle-aged (30–60 years old). The findings revealed that plasma zinc and calcium concentrations significantly decreased according to age. The prevalence of zinc deficiency was therefore shown to increase in old age (over 60% of elderly subjects were deficient or at risk of deficiency). No age-related changes were obtained for copper or magnesium status. The Δ6 desaturase, involved in the EFAs conversion, was shown to decrease according to age and to be associated with the plasma zinc level. Since elderly subjects were at risk of nutritional imbalance, it would be interesting to set optimal dietary proportion. This will help to prevent age-associated alterations and diseases for a better and healthy ageing.

1. Introduction

Lifestyle factors such as smoking, physical activity and nutrition play important roles in the onset and progress of chronic diseases including cardiovascular and neurodegenerative diseases, cancer and diabetes. It may be especially important in the elderly who are at an increased risk of developing such diseases (Waijers et al., 2006). In addition, the acceleration of functional decline caused by external factors is generally believed to be reversible at any age (De Groot et al., 2004). Because society is ageing, it is important to investigate specific biological aspects of healthy elderly.

Trace elements (zinc, copper, selenium, etc.) and minerals (magnesium, calcium, potassium, etc.) are essential for the correct functioning of an organism. Zinc is a structural constituent of many proteins, hormones and hormone receptors and has a fundamental role in the function of biological membranes, many enzymatic activities, cell division and differentiation, programmed cell death and gene transcription, and preservation of bone density (Shankar and Prasad, 1998 ; Savarino et al., 2001 ; Saltman and Strause, 1993).

Copper has an important role in human growth, development and immune function, and wound healing (Chan et al., 1998 ; Galan et al., 1997 ; Berger and Chiolero, 1995). The metal calcium is an important component of bone and acts in many other physiological functions and may alter trace element status (Nieves, 2003 ; Nielsen, 2009). Magnesium is involved in bone metabolism and osteoporosis (Paunier, 1992 ; Koh et al., 1993). It has also been shown to play an important role in the prevention of cardiovascular disease (Barbagallo et al., 2010). Some of the beneficial effects of these minerals may be related to their role in the activity of antioxidant enzymes. In fact, normal micronutrients status is required for stability and optimal activity of superoxide dismutase and glutathione peroxidase (Sfar et al., 2009). The risks of disturbances in trace element nutrition and metabolism are high during ageing (Ferry and Roussel, 2011). In most studies, micronutrient status is assessed by measuring the plasma or serum level. Various investigations concerning the plasma micronutrient levels of healthy individuals have been carried out (Rukgauer et al., 1997; Mariani et al., 2006; Sfar et al., 2009). However, data regarding the mineral status of healthy, elderly populations and the influence of the associated parameters (dietary habits, age, physiological and psychological state, life style, etc.) are very scarce.

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Besides, essential fatty acids (EFAs) are necessary for human life metabolism, but cannot be synthesized de novo by man. As a result, humans rely on the dietary intake of these FAs (Bezard et al., 1994). Plasma fatty acid compositions can fairly reflect the dietary habits as has been reported in many studies (Lopes et al., 1991 ; Sfar et al., 2010). The n−3 and n−6 EFAs can be desaturated and elongated to long-chain polyunsaturated fatty acids. However, Δ5 and Δ6 desaturases activity is expected to be related to several parameters (nutritional status, age, gender, etc.) (Bezard et al., 1994). In particular, the evidence for an interaction between trace elements and essential fatty acid (EFA) metabolism has been reported (Huang et al., 1982). It has been shown, for example, that zinc could be needed as a cofactor in the Δ6 enzyme activity in rats (Horrobin, 1981). Other factors important in regulating the Δ6 desaturase and the conversion of gamma-linolenic acid (GLA) to prostaglandin E1 (PGE1) are pyridoxine, ascorbic acid, melatonin and vitamin B3 (Nakamura and Nara, 2004). GLA administration to humans has been found to lower blood pressure and cholesterol and to cause clinical improvement in patients with Sjogren's syndrome, scleroderma and alcoholism (Das, 2008). Such diseases are associated with some features of accelerated ageing. Nevertheless, data on the described interactions in healthy, elderly subjects is still poorly reported. Thus, a study of the possible effects of trace element status and deficiency on EFA metabolism is relevant to widen our understanding of harmonious ageing. The main objectives of the current study were, therefore, to establish age-related deficiencies of trace element status and to test the possible interactions of plasma micronutrients levels and fatty acids metabolism in a Tunisian healthy population.

2. Materials and methods

2.1. Subjects

The considered population included 200 healthy Tunisian volunteers (104 women and 96 men) aged between 30 and 85 years old. The recruitment was performed from the district of Mahdia (located at the eastern central coast of Tunisia). Two subject groups were considered: adults aged 30–60 years (50 women and 50 men) and elderly persons aged over 65 years (54 women and 46 men). Health status was evaluated by a specific questionnaire filled in by a general practitioner after medical examination. The inclusion criteria were: BMI between 22 and 30 kg/cm^2, normal clinical laboratory tests, no chronic and degenerative diseases or functional impairment. Exclusion criteria were: smoking (>10 cigarettes/day), alcohol consumption (<30 g/day), mineral and vitamin additives, drug intake which can affect nutrient consumption or absorption. Anthropometric measurements were performed according to standardized procedures. The body weight (kg) was measured with an electronic scale (accurate to ±0.1 kg). The height (cm) was estimated to the nearest 0.5 cm with a stadiometer. Clinical laboratory test data included albumin (reference values [RV]: 35–45 g/l), glucose (RV: 4–6 mmol/l), creatinine (RV: 53–120 μmol/l), uric acid (RV: 0.1–0.35 mmol/l), total cholesterol (RV: 4–6.5 mmol/l) and triglycerides (RV: 1–2 mmol/l).

The study protocols were approved by the Local Ethical Committee of the hospital of Mahdia.

2.2. Blood samples collection

For every volunteer, two blood samples were collected by venipuncture after an overnight fasting respectively into EDTA anticoagulation (for fatty acid analysis) and heparinized trace element specific tubes (BD Vacutainer System). All the blood samples were immediately carried to the laboratory of the teaching hospital of Mahdia in a crushed ice block. Erythrocytes and plasma were separated by centrifugation at 3600 *g* for 10 min, aliquoted, coded, frozen and stored at −80 °C until tests.

2.3. Micronutrient analysis

Plasma zinc, copper, magnesium and calcium were determined by flame atomic absorption spectrometry using analysis methods described by Arnaud et al. (1985, 1986). The plasma was diluted (1:5) in 0.1 M hydrochloric acid solution for each micronutrient. Calibration solutions were prepared with different concentrations: 5 to 30 μmol/l for zinc and copper, 5 to 50 mg/l for magnesium and 40 to 140 mg/l for calcium. All the obtained solutions were then nebulized in the spectrometer flame. The solution absorptions were directly measured by the atomic absorption spectrophotometer (Analytikjena, ZEEnit700). In the standard procedure described in Arnaud et al. (1985, 1986), air and acetylene flow rates were adjusted in order to obtain a slightly oxidizing flame (blue colored). The hollow cathode lamp wavelength and spectral width were regulated at the operating values for every analyzed element. Nebulizer flow rate was optimized with 6 to 30 μmol/l solutions to obtain maximal absorbance. Linearity of the method varied respectively from 0.23 to 150 μmol/l for zinc and copper and 1.2 to 150 mg/l for magnesium and calcium concentrations (Arnaud et al., 1986). The intra- and inter-assay coefficients of variation (CV) were below 8% for all analyzed elements. Obtained zinc and copper concentrations were both expressed as μmol/l, whereas magnesium and calcium concentrations were calculated in mg/l. SeronormR Trace Element (Nycomed, Oslo, Norway) plasma was used as interval quality control.

2.4. Analysis of the plasma fatty acids composition

The fatty acids composition was measured in each plasma sample using the Gas Chromatography method (Burdge et al., 2000). Aliquots (150 μl) of plasma were first spiked with 200 μl of internal standard (I.S.) C17:0 and 350 μl of physiological serum. 8 ml of working solution (hexane/isopropanol 3:2 by volume) was then added, vortex-mixed for 30 min and centrifuged at 3000 *g* for 10 min. 7 ml of the supernatant was extracted and evaporated under nitrogen gas. 4 ml of the working solution was added to the previous liquid solution, vortex mixed for 15 min and centrifuged at 3000 *g* for 10 min. 4 ml of extracted supernatant was added to the first evaporated fraction. Evaporation to dryness of the obtained solution was performed again under nitrogen gas. 1 ml of sodium hydroxide in methanol 0.5 (sponification) was added to the dried extract, vortex-mixed and put in an oven at 100 °C for 10 min. After cooling down (to room temperature), fatty acids were transesterified with 700 μl of 14% boron trifluoride in methanol at 100 °C for 10 min. After extraction with 2 ml of saturated sodium chloride (5.6 M), 5 ml of 2 M n-heptane were added, vortexed for 15 min and centrifuged at 3000 *g* for 10 min. After collecting 4 ml of the residuary phase, samples were evaporated to dryness under nitrogen gas. The samples were then immediately introduced in the Gas Chromatograph for analysis. The concentrations of individual fatty acids were determined by identification and measurement of the peak area. Each fatty acid level was expressed as a proportion of the total plasma lipids (%).

Desaturase activities were estimated by the following FA product-to-ratios: Δ5-desaturase (C20:4n−6/C20:3n−6) and Δ6-desaturase (C18:3n−6/C18:2n−6) (Okada et al., 2007 ; Petersson et al., 2010).

2.5. Statistical analysis

The statistical analysis was performed using the SPSS package (12.0 for Windows). The findings were presented as means ± SD. Z-scores were used to describe standardized variations in anthropometrical data (weight, height and BMI). Differences in mean values were evaluated using the one-way analysis of variance (ANOVA). Pearson's model was used to test associations between the considered variables. Statistical significance was considered at $p<0.05$.