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Short Communication

The choice of biomarkers determines the selenium status in young German vegans and vegetarians

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Daily nutrition varies considerably among individuals. The number of vegetarians is increasing continuously due to ethical, environmental, religious or other reasons. There is growing concern over their nutritional status with respect to micronutrient deficiencies. Among the essential trace elements, Se is of prime importance as it is part of the active site in selenoproteins. European soil and plants are relatively poor sources of Se, while farm animals are generally supplemented with Se in order to improve their health and avoid deficiency syndromes. We therefore wondered whether German vegetarians display a measurable Se deficiency. To this end, we compared young vegetarians (*n* 54) and omnivores (*n* 53). We assessed their Se status by measuring extracellular glutathione peroxidase 3 (GPX3) activity, and concentrations of total serum Se and circulating Se-transport protein selenoprotein P (SEPP). GPX3 activities were not different between the groups, whereas both total Se and SEPP concentrations were reduced to 79·5 and 71·2 % in vegetarians compared with omnivores. When splitting the group of vegetarians into vegans (*n* 26) and vegetarians consuming egg and milk products (*n* 28), analyses of the Se-dependent biomarkers did not reveal significant differences. We conclude that low serum Se is mirrored by circulating SEPP concentrations, but not by GPX3 activities in marginally supplied individuals. The specific dietary Se sources, divergent metabolic routes of selenomethionine *v.* selenocysteine and the different saturation kinetics of GPX3 and SEPP probably underlie our contradictory findings. Whether German vegetarians and vegans need to be considered as a Se-deficient group depends on the biomarker chosen.

Trace elements: Selenoproteins: Glutathione peroxidase

Se is an essential trace element with important implications for human health[1–3]. Low Se intake may predispose to a variety of diseases including cancer[4,5]. In severe illness, Se status declines, presenting a prognostic marker for morbidity and mortality of sepsis patients[6–8]. However, regardless of this fundamental medical importance, a 'gold standard' to determine and interpret an individual's Se status is yet to be established[9].

Se quantification methods differ widely based on the use of different body fluids (whole blood, serum, plasma or urine) or body parts (hair and finger- or foot-nail clippings). Furthermore, when selenoproteins are quantified, their concentration or enzyme activity is measured in blood cells (glutathione peroxidase 1, GPX1) and in serum or plasma (GPX3 and selenoprotein P, SEPP)[5,9,10]. Serum or plasma samples are commonly chosen because they are easily obtained and used widely for other metabolic analyses. Also, interpretation of the results is complex. At least three different Se-containing fractions circulate in human blood, i.e. small Se-containing molecules including selenosugars, selenomethionine-containing proteins and selenoproteins containing the twenty-first proteinogenic amino acid selenocysteine[11]. The latter group is mainly or exclusively represented by GPX3 and SEPP in plasma or serum[11,12]. Thus far, conflicting results have been obtained, and they argue in favour of using a combination of parameters instead of a single analysis to assess the Se status in an individual[5,9].

Daily Se intake differs markedly between individuals depending on their geographical location, on the Se concentration and availability in soil and locally produced plant- and animal-based food stuffs, on whether farm animals are raised in captivity on well-balanced feed or on potentially poor soil in the environment, as well as on the degree to which food is imported from regions with higher or lower Se concentrations[13]. In addition, the major Se sources contain the trace element in different biochemical forms, i.e. selenomethionine and selenomethionine derivatives dominate in plants, and selenocysteine dominates in meat, fish or dairy products[14]. Consequently, vegetarians, vegans and omnivores consume both different amounts and forms of Se.

Abbreviations: GPX, glutathione peroxidase; SEPP, selenoprotein P.

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In general, Europeans must be considered as marginally supplied due to low Se concentrations in European soil[15]. Farm animals, on the other hand, are often supplemented with Se via food additives during breeding and maintenance. In order to improve the trace element status in the population of all the European countries, only Finland has taken systematic measures by supplementing fertilisers with sodium selenate since 1984, thereby increasing the Se concentrations in farm products and inhabitants[2]. In Germany, where no such systematic supplementation efforts are taken, vegetarians and especially vegans are at risk of consuming insufficient amounts of Se, and might develop a subclinical deficiency with the associated health risks.

We decided to analyse the Se status in young Germans who follow vegetarian, vegan or omnivorous diets using different Se biomarkers. By comparing total Se concentrations and GPX3 activities and SEPP amounts, respectively, the question of functional deficiency *v.* nominal concentration differences comes to the fore. The present results indicate that vegetarians and vegans have lower Se status when judged according to their total serum Se concentrations or SEPP levels, while their GPX3 activities are not different from those of the omnivores. Therefore, vegans and vegetarians apparently lack some Se transport capacity without displaying a diminished antioxidative defence in serum. Hence, the choice of biomarkers decides whether young vegetarians and vegans in Europe must be considered as Se deficient or sufficiently supplied with Se.

Materials and methods

Study population and data collection

Serum samples from 107 apparently healthy individuals were collected during 2007 and 2008 in Berlin, Germany. Participants provided personal data on sex, age, health, diet, contraceptive medication use, BMI (kg/m^2), and smoking and drinking habits. The analyses were approved by the ethics committee of the Charité – Universitätsmedizin Berlin, Germany. The participants were informed about the study design, and they provided written consent before blood donation. Inclusion criteria were a general good health, an age of at least 18 years and no current medication use except for contraceptives. Dietary habits during the last 6 months before the analyses served as the distinctive criterion for allocating individuals to the nutritional categories. Vegans were defined as people living solely on food from plants, whereas lacto-ovo vegetarians were defined as people consuming dairy products and eggs in addition to plant products. Omnivores were defined as people consuming food of both plant and animal origins. Blood samples were obtained using peripheral venipuncture (BD Vacutainer® SST™). Samples were left to clot for 60 min on ice and centrifuged at 5000 ***g***, and the serum was separated and frozen in aliquots within 2 h of collection.

Quantification of selenoprotein P, glutathione peroxidase 3 and selenium

SEPP in the serum samples was determined by an immunoluminometric sandwich assay described recently[16]. Briefly, serum was diluted in an assay buffer, and samples of 50 μl (corresponding to 0·6 μl of serum) were applied to antibody-coated tubes, incubated with tracer antibodies and washed, and chemiluminescence was measured using a luminometer (LB 953; Berthold Technologies, Oak Ridge, TN, USA). All the samples were analysed in triplicate within the same assay run. The intra-assay CV was below 10 % for SEPP values >1 mg/l. GPX3 activities were determined in duplicate in a coupled enzymatic assay with *t*-butyl hydroperoxide as the substrate[17,18]. Briefly, 10 μl serum samples were incubated with NADPH (0·15 mmol/l), GSH (2·0 mmol/l), EDTA (0·6 mmol/l), potassium phosphate (20 mmol/l, pH 7·0) and four units of glutathione reductase (Sigma Aldrich, St Louis, MO, USA) at room temperature. Reactions were started with *t*-butyl hydroperoxide, and NADPH consumption was monitored at 340 nm with or without mercaptosuccinate to determine Se-dependent GPX activity. Total serum Se was quantified in triplicate using total reflection X-ray fluorescence analysis[19]. Briefly, 100 μl of serum were diluted with 895 μl of water and supplemented with 5 μl of a gallium standard (10 mg/l). Samples of 10 μl were placed on quartz glass sample carriers, dried and measured using a benchtop total reflection X-ray fluorescence spectrometer (S2 PICOFOX; Bruker AXS Microanalysis GmbH, Berlin, Germany) for 2000 s each. The method was validated with a Seronorm serum standard (Sero AS, Billingstad, Norway), and proved to be linear at 1:3, 1:10 or 1:20 dilutions of the standard serum; a standard sodium selenite solution was tested in addition, and signal linearity was verified by total reflection X-ray fluorescence analysis. The inter-assay CV was determined to be below 10 % in the concentration range of 50–150 μg Se/l serum.

Statistical analyses

Statistical calculations were done with SPSS, version 17.0 (SPSS Software; SPSS, Inc., Chicago, IL, USA). Data sets were verified for normal distribution using the Kolmogorov–Smirnov test. The unpaired two-tailed Student's *t* test was applied to two-group comparisons, and one-way ANOVA was used for variance analyses of more than two groups. Bonferroni's *post hoc* test was applied to test for significant differences compared with control groups.

Results

Characteristics of study participants

Participant characteristics in the different groups were similar, and the female:male ratio was close to 1:1 in the different groups. All of them were recruited from the authors' circle of friends and peers. The level of education of the participants was above average (most of them hold a university degree), but it was similar in each of the groups. Of the participants, 94 of 107 were of normal weight (BMI between 18·0 and 24·9 kg/m^2), and the average age was 25·3 (SD 5·4) years for omnivores and 31·1 (SD 11·2) years for vegans and vegetarians.

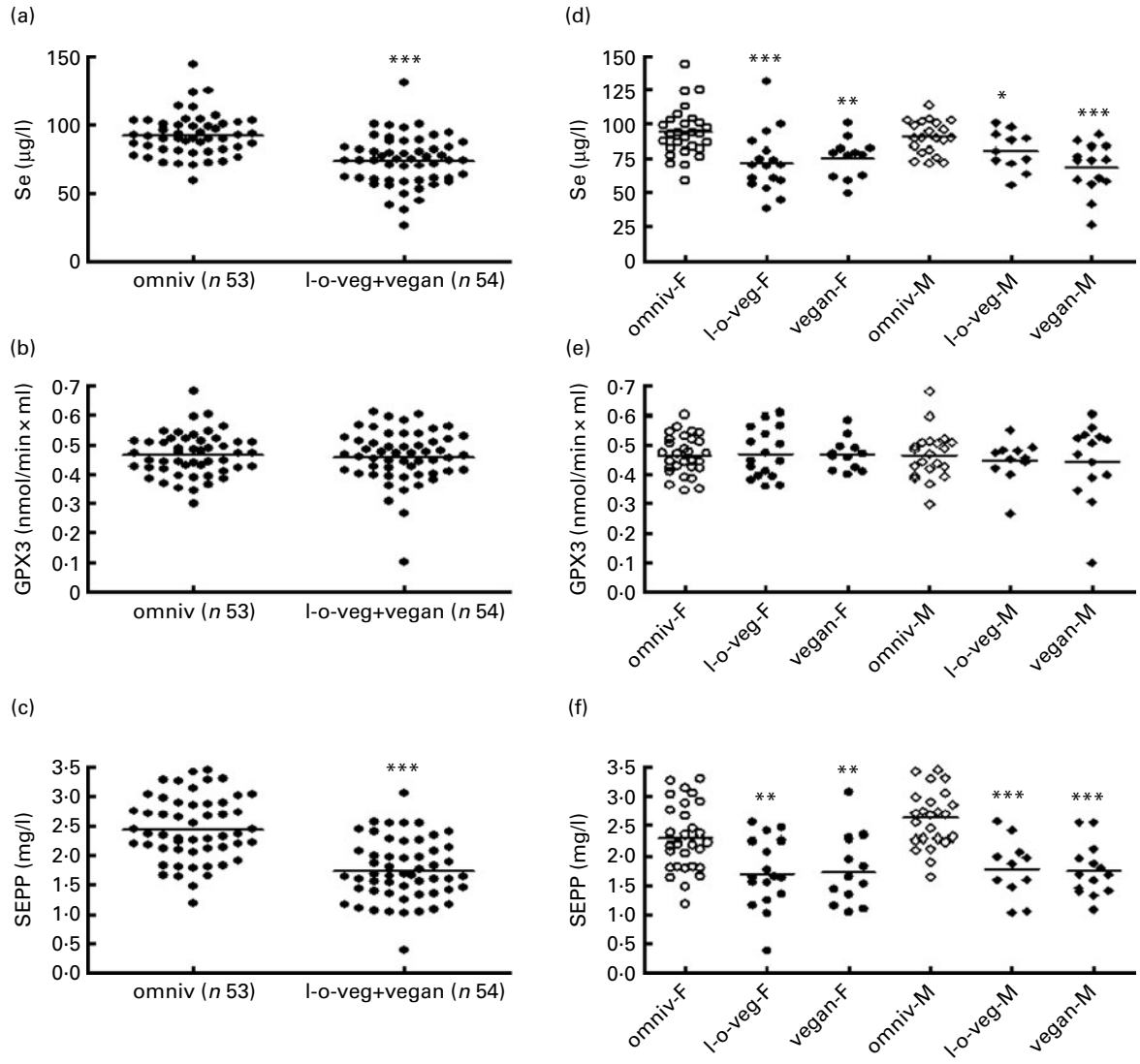

Fig. 1. Biomarkers of Se status in serum of omnivores and vegetarians (a)–(c), and their subanalysis with respect to sex and degree of vegetarianism (d)–(f). Serum selenium concentrations ((a) and (d)) and glutathione peroxidase 3 (GPX3) activities ((b) and (e)) were determined along with selenoprotein P (SEPP) levels ((c) and (f)) from serum of healthy male (M) and female (F) omnivores (omniv), lacto-ovo vegetarians (l-o-veg) and vegans (vegan). Data are compared by Student's *t* test ((a)–(c)) or by ANOVA ((d)–(f)). Mean values were significantly different from those of the omnivores: *$P<0{\cdot}05$, **$P<0{\cdot}01$, ***$P<0{\cdot}001$.

Status of selenium-dependent biomarkers

Participants were first categorised as omnivores or vegetarians to address the primary study aim, i.e. the question of whether vegetarians are Se deficient (Fig. 1(a)–c). Secondary analyses were performed on the data with respect to sex differences and whether vegetarians were considered separately as lacto-ovo vegetarians or vegans (Fig. 1(d)–(f)).

Serum Se concentrations differed significantly between omnivores and vegetarians (Fig. 1(a)). This difference persisted when the group of vegetarians was subdivided into lacto-ovo vegetarians and vegans, or when males and females were considered separately (Fig. 1(d)).

In contrast, activities of circulating GPX3 were not different between the groups of omnivores and vegetarians (Fig. 1(b)). Average activities showed no significant differences even when comparing lacto-ovo vegetarians and vegans or when separating males and females (Fig. 1(e)). This result is in contrast to the data on the second circulating selenoprotein, i.e. SEPP. Vegetarians displayed SEPP concentrations of only 71·2 % in comparison with the omnivores (Fig. 1(c)). There were no sex-specific differences when male and female omnivores, lacto-ovo vegetarians or vegans were compared, and SEPP concentrations in vegans were not different from those of the lacto-ovo vegetarians in a subgroup analysis (Fig. 1(f)).

Discussion

There are a number of studies comparing Se concentrations between vegetarians and omnivores from different countries worldwide. These studies have not yielded consistent results, but serum or plasma Se concentrations in vegans and lacto-ovo vegetarians tend to be lower, especially in regions with marginal Se supply[20,21]. The data presented here indicate

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that the Se status in vegetarians is not a simple issue. This notion is partly related to the fact that a clear definition of Se status is missing, and differences in circulating selenoproteins do not correlate with blood Se concentrations or Se intake[10]. In the present study, we found significantly lower serum Se and circulating SEPP concentrations in vegetarians, both in male and in female lacto-ovo vegetarians and vegans, in comparison with omnivores. Nevertheless, serum GPX3 activities were similar in all the groups.

A likely explanation for these seemingly conflicting data needs to take into account the oddities of selenoprotein biosynthesis. At a given Se supply, not all selenoproteins are synthesised to the same extent[22]. It has been shown earlier that with Se supplementation in individuals with marginal Se intake, GPX3 expression rapidly reaches a plateau, while SEPP concentration continues to rise[23]. SEPP is mainly produced in the liver, and accounts for the majority of circulating Se in human blood[24]. SEPP expression in our group of vegans and lacto-ovo vegetarians was at a submaximal level compared with that in the omnivores, which is in line with their reduced serum Se concentrations. Serum Se concentrations differ only slightly between vegetarians and omnivores, despite the fact that SEPP levels vary and that every SEPP molecule accounts for several Se atoms. This finding is likely to be related to the relatively high proportion of selenomethionine within a lacto-ovo vegetarian or a vegan diet leading to random incorporation of Se into proteins. Still, Se supply from the liver to the kidney appears undisturbed, as normal expression of GPX3 in both lacto-ovo vegetarians and vegans is observed. Whether the differences in SEPP or total serum Se concentrations are important for health issues and disease risk or for the course of pathologies remains to be demonstrated. Our data highlight that the choice of biomarkers is crucial for the categorisation of the Se status in an individual, and support the view that the combination of biomarkers might represent a better option to classify an individual's Se status.

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