Brazil nuts: an effective way to improve selenium status¹–³

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ABSTRACT

Background: Brazil nuts provide a rich natural source of selenium, yet no studies have investigated the bioavailability of selenium in humans.

Objective: We investigated the efficacy of Brazil nuts in increasing selenium status in comparison with selenomethionine.

Design: A randomized controlled trial was conducted with 59 New Zealand adults. Participants consumed 2 Brazil nuts thought to provide ≈100 μg Se, 100 μg Se as selenomethionine, or placebo daily for 12 wk. Actual intake from nuts averaged 53 μg Se/d (possible range: 20–84 μg Se). Plasma selenium and plasma and whole blood glutathione peroxidase (GPx) activities were measured at baseline and at 2, 4, 8, and 12 wk, and effects of treatments were compared.

Results: Plasma selenium increased by 64.2%, 61.0%, and 7.6%; plasma GPx by 8.3%, 3.4%, and −1.2%; and whole blood GPx by 13.2%, 5.3%, and 1.9% in the Brazil nut, selenomethionine, and placebo groups, respectively. Change over time at 12 wk in plasma selenium (P < 0.0001 for both groups) and plasma GPx activity in the Brazil nut (P < 0.001) and selenomethionine (P = 0.014) groups differed significantly from the placebo group but not from each other. The change in whole blood GPx activity was greater in the Brazil nut group than in the placebo (P = 0.002) and selenomethionine (P = 0.032) groups.

Conclusion: Consumption of 2 Brazil nuts daily is as effective for increasing selenium status and enhancing GPx activity as 100 μg Se as selenomethionine. Inclusion of this high-selenium food in the diet could avoid the need for fortification or supplements to improve the selenium status of New Zealanders. Am J Clin Nutr 2008;87:379–84.

KEY WORDS Selenium status, Brazil nuts, bioavailability, New Zealand

INTRODUCTION

Selenium functions as a component of several selenoproteins in antioxidant and redox reactions, thyroid hormone metabolism, immune function, and reproduction (1). Marginal selenium status resulting in suboptimal amounts of one or more selenoproteins may be associated with increased risk from a number of conditions, including cancer, cardiovascular disease, altered immune function, male infertility, inflammatory disorders, autoimmune thyroid disease, and viral infection (2). Moreover, there is growing evidence that higher than recommended intakes may confer additional health benefits such as reduction in chronic disease and enhancement of immune function (2, 3). Thus, the argument is strong for augmenting selenium intakes, and interest in foods containing high amounts of selenium or supplements is increasing (4).

Food sources are preferable to alternative supplementation practices for improving the nutritional status of a population, because they are sustainable, less expensive, and have lower risk of toxicity (5). The bioavailability of selenium from a variety of foods and their efficacy for increasing selenium status has been investigated, including that in high-selenium wheat bread (6), fish (7), and meat (8). Because of the relatively low selenium content of some of these foods, however, large quantities need to be consumed to improve selenium status. Brazil nuts (Bertholletia excelsa, family Lecythidaceae) are the richest known food source of selenium, with mean concentrations reported in the literature between 8 and 83 μg Se/g (4, 9–12). Concentrations in unshelled nuts are reported to be greater than in shelled nuts (4, 9, 10). Rat studies suggest that the bioavailability of selenium in Brazil nuts is equal to that in sodium selenite for the restoration of both tissue selenium and selenoprotein activity and that these nuts may be successful in tumor prevention (13). However, to our knowledge, no studies have assessed the efficacy of Brazil nuts in increasing selenium status in humans.

New Zealand soils are low in selenium, resulting in generally low concentrations of this trace element throughout the food system (14). The selenium status of the New Zealand population is marginal, and, despite improvements in dietary selenium intakes and status in recent years, blood selenium concentrations remain lower than those reported in many other Western countries (15, 16). Selenium supplementation of New Zealanders still results in an increase in glutathione peroxidase (GPx) activity (17, 18), suggesting that selenium intakes are inadequate for optimal functioning of some selenoproteins (16). Thus, investigating the efficacy of specific foods for improving selenium status is of particular relevance to New Zealanders. The aim of this study was to assess the efficacy of Brazil nuts in comparison with a supplement of 100 μg Se as selenomethionine in increasing selenium status, as measured by the response of plasma selenium and GPx activity in New Zealand residents with low selenium status.

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SUBJECTS AND METHODS
Sixty healthy subjects aged 18–60 y, with plasma selenium concentrations < 1.27 μmol/L, were recruited from a group of 92 volunteers in the Dunedin area in June 2004. At baseline subjects completed a questionnaire to obtain information on dietary habits, supplement use, and demographic details. Height and weight were measured at baseline. All participants gave their informed consent, and the Ethics Committee of the University of Otago, Dunedin, New Zealand, approved the protocol.

Participants were randomly assigned to 1 of 3 groups and consumed 2 Brazil nuts (supplied by Leith Distributors, Dunedin, New Zealand), a tablet providing 100 μg Se as l-selenomethionine, or placebo (Alaron Products Ltd, Nelson, New Zealand) daily for 12 wk. One participant was lost to follow-up, leaving 59 subjects (30 men and 29 women), with 20, 19, and 20 in the Brazil nut, selenomethionine, and placebo groups, respectively. The study was double blinded for those in the tablet groups, but this was not possible for the Brazil nut group. Compliance was monitored with the use of a self-administered checklist and from the number of tablets or nuts returned at the conclusion of the study. Reported high compliance suggests that participants adhered to their prescribed treatments. Subjects were asked to avoid selenium-rich foods such as fish, liver, kidney, or additional Brazil nuts during the study period, but otherwise they consumed their normal diets.

Fasting, morning blood samples were drawn by venepuncture in EDTA evacuated tubes at baseline and at 2, 4, 8, and 12 wk. Blood was centrifuged (3000 rpm, 15 min) for preparation of plasma, and the aliquots of plasma and whole blood were stored at −80 °C until analysis.

Analytic methods
Plasma selenium concentrations were measured by graphite furnace atomic absorption spectroscopy with Zeeman background correction (Model 3100; Perkin-Elmer, Norwalk, CT), with the use of a modified version of the method of Jacobson and Lockitch (19). Analysis of an external quality control, Utak Reference Plasma (batch no. 66816, lot 4102; Nycomed Pharma Diagnostics, Oslo, Norway), with a certified selenium concentration of 1.60 μmol/L, gave a mean (±SD) of 1.58 ± 0.06 μmol/L (CV: 5.6%; n = 69). Analysis of aliquots of pooled plasma gave a mean of 1.04 ± 0.05 μmol Se/L (CV: 5.1%; n = 18).

GPx activity was measured in whole blood and plasma with the use of a modification (20) of the coupled enzyme procedure (21) on a Cobas Fara autoanalyzer (Hoffman-La Roche, Basel, Switzerland). Whole blood GPx was assayed as a measure of erythrocyte GPx activity, which was shown previously by us to constitute 95% of whole blood activity with the use of this assay method (22). Analysis of pooled plasma and whole blood was assayed with each batch of samples gave mean activities of 2.7 ± 0.3 U/g protein (CV: 4.9%; n = 15) and 56.6 ± 6.2 U/g hemoglobin (CV: 6.9%; n = 15), respectively.

Selenium content of Brazil nuts and supplements
Initially the selenium content of Brazil nuts was estimated with the use of the value for selenium concentration of 127 μg Se/g in the 2003 New Zealand Food Composition Tables (23) and the average weight of a nut (4.0 g). Two Brazil nuts were estimated to provide ≈100 μg of selenium. Subsequent laboratory analysis of a random sample of 20 Brazil nuts used in this study was undertaken by Roger Hill Laboratories, Hamilton, New Zealand, with the use of inductively coupled plasma mass spectrometry. Brazil nuts were prepared for analysis by digestion with 12.5% aqueous tetramethylammonium hydroxide pentahydrate at 90 °C (24). Because of difficulties in analyzing individual nuts (first 10 nuts ranging from 0.816 to 1390 μg Se/g), the selenium content of 2 distinct composite samples was determined in triplicate. These composite samples were prepared by removing slices from a random selection of Brazil nuts with the use of a vegetable peeler. The range of selenium concentrations in the composite samples was 2.35–10.2 μg Se/g with a mean of 6.4 μg Se/g. The mean weight of a single Brazil nut was 4.1 ± 0.4 g. Thus, on average, 2 Brazil nuts provided 53 μg Se, with a possible range of 20–84 μg Se. However, the large differences in selenium concentrations within and between the 2 composite samples of Brazil nuts indicated that there was a wide range of selenium content within this batch of nuts. Therefore, the range in selenium content of 2 nuts, and consequently the daily selenium intakes, was potentially large. The mean selenium content of selenomethionine supplements was 97.5 ± 11.1 μg/tablet (n = 10) and that of placebo tablets was 0.038 ± 0.036 μg/tablet (n = 10).

Statistical analysis
Descriptive statistics were calculated for all baseline characteristics and were described with the mean (±SD) for each treatment group. Baseline measures were compared with the use of one-factor analysis of variance in STATA 8.2 (Stata Corp, College Station, TX). Random coefficients models were used to compare the effects of the 3 treatments with time on changes in plasma selenium concentration and changes in whole blood and plasma GPx activities. In a random coefficients model, it is assumed that there is a linear relation between the outcome variable and time. If there was a nonlinear relation between the outcome variable and time, we investigated the use of a quadratic curve. The random coefficients model allows the slopes and intercepts to vary randomly between the study subjects; hence, a separate regression line is fitted for each subject. Time, treatment, and the treatment-by-time interaction were included as fixed effects in the model, and participant and participant-by-time interaction were included as random effects in the model. If a quadratic model was fitted to the data, time squared and the interaction treatment-by-time squared were also included in the model as fixed effects. Age, sex, and body mass index (BMI; in kg/m²) were adjusted for in all analyses by including them as fixed effects. The primary measure of interest was the change in mean response from baseline to week 12 for the outcome variables. Pairwise comparisons were made between groups if the treatment-by-time interaction or treatment-by-time squared interaction was statistically significant. These statistical analyses were conducted with the use of SAS 9.1.3 (SAS Institute Inc, Cary, NC). The mixed models were fitted with the use of the SAS procedure SAS PROC MIXED. Statistical significance was assessed at the 5% level, and an intention-to-treat analysis was used.

RESULTS
Mean ages and BMI of participants in the 3 groups were similar, as were baseline concentrations of plasma selenium and
TABLE 1
Baseline plasma selenium concentrations and plasma and whole blood glutathione peroxidase (GPx) activities in 59 New Zealand residents screened for low plasma selenium.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Age (y)</th>
<th>BMI (kg/m²)</th>
<th>Plasma selenium (μmol/L)</th>
<th>Plasma GPx (U/g protein)</th>
<th>Whole blood GPx (U/g hemoglobin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brazil nut (n = 20)</td>
<td>49.5 ± 8.6</td>
<td>28.2 ± 5.3</td>
<td>1.09 ± 0.16</td>
<td>2.46 ± 0.26</td>
<td>19.5 ± 4.4</td>
</tr>
<tr>
<td>Selenomethionine (n = 19)</td>
<td>46.6 ± 11.0</td>
<td>26.0 ± 3.6</td>
<td>1.12 ± 0.17</td>
<td>2.91 ± 0.42</td>
<td>18.1 ± 3.5</td>
</tr>
<tr>
<td>Placebo (n = 20)</td>
<td>42.5 ± 9.9</td>
<td>25.9 ± 4.2</td>
<td>1.08 ± 0.17</td>
<td>2.84 ± 0.43</td>
<td>18.8 ± 5.1</td>
</tr>
</tbody>
</table>

1 All values are ± SD. 
2 Significant difference from the selenomethionine group, P = 0.011 (ANOVA).

whole blood GPx activities (Table 1). Mean baseline plasma GPx activity of the Brazil nut group was significantly lower than that of the selenomethionine group (P = 0.011, analysis of variance). Mean plasma selenium concentrations, plasma GPx activities, and whole blood GPx activities during the 12 wk of intervention are summarized in Table 2.

Plasma selenium concentration
At 12 wk the estimated mean (95% CI) percentage changes in plasma selenium concentrations were 64.2% (54.5%, 73.8%) in the Brazil nut group and 61.0% (51.3%, 70.7%) in the selenomethionine group compared with 7.6% (−1.7%, 16.8%) in the placebo group, an increase of 58.0, 59.3, and 61.0% (51.3%, 70.7%) in the Brazil nut group and 6.7% (0.7%, 6.2%) in the selenomethionine group compared with −1.2% (−3.8%, 1.3%) in the placebo group. This increase was statistically significant for both the Brazil nut and the selenomethionine groups (P < 0.0001). A quadratic curve was used to describe the relation between plasma GPx activity and time for each of the treatment groups after adjusting for age, sex, and BMI (Figure 1). The change over time in plasma selenium concentration depended on the treatment group (P < 0.0001 for treatment-by-time and treatment-by-time squared interactions). Further comparison between the treatment groups showed that overall changes in plasma selenium concentrations in the Brazil nut and the selenomethionine groups differed significantly from the placebo group (P < 0.0001), but they did not differ from each other (P = 0.840).

Plasma glutathione peroxidase activity
At 12 wk the estimated mean percentage changes in plasma GPx activities were 8.3% (5.3%, 11.4%) in the Brazil nut group and 3.4% (0.7%, 6.2%) in the selenomethionine group compared with −1.2% (−3.8%, 1.3%) in the placebo group. This increase was statistically significant for both the Brazil nut and the selenomethionine groups (P < 0.0001 and P = 0.013, respectively) but not for the placebo group (P = 0.351). A quadratic curve was used to describe the relation between plasma GPx activity and time for each of the treatment groups (Figure 2). The change over time in plasma GPx activity depended on the treatment group (P < 0.0001 for treatment-by-time and treatment-by-time squared interactions). Further comparison between the treatment

TABLE 2
Plasma selenium concentrations and plasma and whole blood glutathione peroxidase (GPx) activities at baseline and during 12 wk of supplementation with Brazil nuts, selenomethionine, or placebo.

<table>
<thead>
<tr>
<th>Week of supplementation</th>
<th>Baseline</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma selenium (μmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brazil nut (n = 20)</td>
<td>1.15 ± 0.16</td>
<td>1.51 ± 0.24</td>
<td>1.78 ± 0.18</td>
<td>1.91 ± 0.32</td>
<td>1.91 ± 0.22</td>
</tr>
<tr>
<td>Selenomethionine (n = 19)</td>
<td>1.16 ± 0.18</td>
<td>1.64 ± 0.17</td>
<td>1.81 ± 0.26</td>
<td>1.96 ± 0.28</td>
<td>2.08 ± 0.29</td>
</tr>
<tr>
<td>Placebo (n = 20)</td>
<td>1.13 ± 0.18</td>
<td>1.16 ± 0.18</td>
<td>1.15 ± 0.20</td>
<td>1.22 ± 0.18</td>
<td>1.20 ± 0.18</td>
</tr>
<tr>
<td>Plasma GPx (U/g protein)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brazil nut (n = 20)</td>
<td>2.55 ± 0.26</td>
<td>2.81 ± 0.33</td>
<td>2.95 ± 0.35</td>
<td>2.89 ± 0.38</td>
<td>2.83 ± 0.36</td>
</tr>
<tr>
<td>Selenomethionine (n = 19)</td>
<td>2.91 ± 0.42</td>
<td>3.21 ± 0.47</td>
<td>3.19 ± 0.46</td>
<td>3.14 ± 0.49</td>
<td>3.12 ± 0.47</td>
</tr>
<tr>
<td>Placebo (n = 20)</td>
<td>2.80 ± 0.43</td>
<td>2.75 ± 0.46</td>
<td>2.73 ± 0.42</td>
<td>2.79 ± 0.44</td>
<td>2.74 ± 0.44</td>
</tr>
<tr>
<td>Whole blood GPx (U/g hemoglobin)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brazil nut (n = 20)</td>
<td>20.6 ± 4.4</td>
<td>20.7 ± 4.2</td>
<td>21.2 ± 4.3</td>
<td>21.8 ± 4.3</td>
<td>22.8 ± 5.0</td>
</tr>
<tr>
<td>Selenomethionine (n = 19)</td>
<td>17.4 ± 3.5</td>
<td>18.1 ± 4.2</td>
<td>18.1 ± 4.3</td>
<td>18.1 ± 4.1</td>
<td>18.4 ± 4.3</td>
</tr>
<tr>
<td>Placebo (n = 20)</td>
<td>18.6 ± 5.1</td>
<td>18.7 ± 5.4</td>
<td>18.8 ± 5.6</td>
<td>19.2 ± 5.7</td>
<td>18.9 ± 5.3</td>
</tr>
</tbody>
</table>

1 All values are ± SD. Random coefficients models were used to compare the effects of the 3 treatments with time on changes in plasma selenium concentration and in whole blood and plasma GPx activities. Adjusted for age, sex, and BMI. Time-by-treatment effects were significant (P < 0.0001 for plasma selenium and plasma GPx; P = 0.005 for whole blood GPx).

2 Subgroup analysis (pairwise comparisons between groups): 2 changes in the Brazil nut and selenomethionine groups were significantly greater than in the placebo group, P < 0.0001; 4 Changes in the Brazil nut and selenomethionine groups were significantly greater than in the placebo group (P < 0.001 and P = 0.014, respectively); 6 The change in the Brazil nut group was significantly greater than in the placebo (P = 0.002) and selenomethionine (P = 0.032) groups.
Time-by treatment interaction was significant, thionine (selenium supplementation in populations of low selenium status been used to investigate bioavailability of organic and inorganic period of 12 wk. Both plasma selenium and GPx activity have of a 100- selenium concentration and GPx activities as is the consumption supplementation of 2 Brazil nuts daily is as effective in raising plasma selenomethionine and placebo groups were significant, P < 0.0001 for each comparison. There was no significant difference between the Brazil nut and selenomethionine groups, P = 0.840. Error bars represent SEs.

Whole blood glutathione peroxidase activity

At 12 wk the estimated mean percentage changes in whole blood GPx activities were 13.2% (8.2%, 18.2%) in the Brazil nut group and 5.3% (−0.4%, 11.1%) in the selenomethionine group, compared with 1.9% (−2.9%, 6.8%) in the placebo group. This increase was statistically significant for the Brazil nut group (P < 0.0001), but the increase in the selenomethionine group did not quite reach significance (P = 0.070). The change in whole blood GPx activity in the placebo group was not significant (P = 0.431). A linear random coefficients model was used to describe the relation between whole blood GPx activity and time for each of the treatment groups (Figure 3). The change over time in GPx activity depended on the treatment group (P = 0.005 for treatment-by-time interaction). Further comparison between the treatment groups showed that overall changes in whole blood GPx activity in the Brazil nut group differed significantly from the placebo group (P = 0.002), but there was no significant difference between the selenomethionine and placebo groups (P = 0.395). The change was greater in the Brazil nut group than in the selenomethionine group (P = 0.032).

DISCUSSION

Our study is the first to investigate the response of both blood selenium concentration and activity of the selenoprotein GPx to Brazil nut supplementation in humans. We have shown that consumption of 2 Brazil nuts daily is as effective in raising plasma selenium concentration and GPx activities as is the consumption of a 100-μg selenium selenomethionine supplement during a period of 12 wk. Both plasma selenium and GPx activity have been used to investigate bioavailability of organic and inorganic selenium supplementation in populations of low selenium status (6, 17, 22, 25). Plasma selenium reflects short-term changes in tissue selenium concentrations, whereas plasma and whole blood GPx activities are functional indexes of selenium status. Plasma GPx again reflects relatively short-term changes, whereas whole blood activity reflects longer-term changes because of the long lifetime of erythrocytes of 120 days. This might account for the continuing increase in whole blood GPx activity in the Brazil nut group, which may not have reached maximal activity after only 84 days of observation. However, investigation of longer-term effects of Brazil supplementation is warranted.

The greater increase in whole blood GPx activity after consumption of Brazil nuts than after consumption of selenomethionine suggests that selenium from Brazil nuts may be more bioavailable for functional selenoprotein activity. Although whole blood GPx activity might not be the most appropriate measure to assess the bioavailability during a period of <120 days, superior bioavailability of Brazil nuts is supported by the trend for a greater increase in plasma GPx activity. The observation is further supported because participants in the Brazil nut group were probably consuming considerably less than the 100 μg selenium consumed by the selenomethionine group. Because of the difficulty in determining the selenium content of the Brazil nuts, however, it is possible that the Brazil nut group consumed >100 μg Se/d from the nuts. Other researchers have also reported a wide range in the selenium concentration in Brazil nuts, with concentrations in individual nuts varying from 0.2–253 ppm (26) and 0.03 to 512 ppm (10). It was also reported that selenium concentrations in unshelled nuts are greater than in shelled nuts (4, 9, 10), but in 2 of these reports the shelled and unshelled nuts were sourced from different areas of South America (9, 10), whereas the source was not reported in the third study (4). The differing selenium concentrations in Brazil nuts, therefore, more likely reflect the availability of selenium in the soils in the areas in which they are harvested (10). Characterization of the selenium species in Brazil nuts indicates that selenomethionine is the principal species (4, 24, 27), but attempts to identify other selenium species present have so far been unsuccessful (4). It is possible that selenium in uncharacterized selenium species in Brazil nuts is more bioavailable than that in selenomethionine.
Our finding is of particular relevance to New Zealand and countries where both dietary intakes of selenium and selenium status of its residents are low (16). Daily consumption of just 1 Brazil nut would raise current New Zealand dietary selenium intakes, which was estimated in the National Nutrition Survey to be 56 μg for men and 39 μg for women (28), to recommended intakes of selenium (29, 30). Given the relatively low cost, high bioavailability, and increasing popularity of Brazil nuts, inclusion of this high-selenium food in the diet would appear to be a suitable option to improve the selenium status of New Zealanders. However, consumption on a daily basis should be limited to no more than a few nuts to avoid accumulation of selenium in the tissues. Furthermore, Brazil nuts provide unusually high and variable concentrations of barium and radium (31), which accumulate because of the extensive root system of the tree. Although adequate chronic oral studies in rats and mice have not been reported in a worker in the United Kingdom who consumed 25 g Brazil nuts/d (33). Consideration also needs to be given to the uncertainty of the future supply of Brazil nuts, which at present is mainly from wild trees (34). Production has declined in recent years as a result of deforestation, and, although several Brazil nut plantations have been established in Brazil, their production is still low (34, 35).

The increasing interest in possible health benefits of higher selenium intakes is of concern where both dietary intakes of selenium and growing evidence that such intakes protect against cancer and other chronic conditions provide a compelling argument for increasing selenium intake. Brazil nuts are a convenient source of selenium and were shown to prevent mammary cancer in rats (13). A simple public health recommendation to include as few as 1 Brazil nut/d in the diet would avoid the need for fortification of foods or for expensive supplements to improve the selenium status of New Zealanders. However, although there is still uncertainty about the intake of barium and radium that might be harmful, this recommendation should be made with caution.

We thank the participants for both their interest in the project and their enthusiastic cooperation. We thank Ms Claire Heslop for technical assistance in carrying out the study, Mr Mark Wohlers and Ms Vicki Livingstone for statistical support, and Mrs Margaret Waldron for assistance with venepuncture.

The author’s responsibilities were as follows—CDT: designed the study, obtained funding support, and was responsible for interpretation of the data and writing the manuscript; AC: contributed to the study design and interpretation of the data; SKM: was responsible for recruitment of subjects, data collection, and laboratory analyses; JMC: assisted in data analyses and in drafting the manuscript. None of the authors had a personal or financial conflict of interest.

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