Influence of the glutathione peroxidase 1 Pro200Leu polymorphism on the response of glutathione peroxidase activity to selenium supplementation: a randomized controlled trial1–3

Jody C Miller, Christine D Thomson, Sheila M Williams, Nathalie van Havre, Gerard T Wilkins, Ian M Morison, Jackie L Ludgate, and C Murray Skeaff

ABSTRACT

Background: A genetic variant at codon 200 (Pro200Leu) of the gene encoding for glutathione peroxidase 1 (GPx1), a selenium-dependent enzyme, is associated with lower enzyme activity; however, the evidence is limited to in vitro and observational studies.

Objective: The objective was to determine whether the GPx1 Pro200Leu genetic variants modify the response of whole-blood glutathione peroxidase (GPx) activity to selenium supplementation in patients with coronary artery disease in New Zealand.

Design: The results from 2 parallel-design, double-blind trials were combined. Participants were randomly assigned to receive a daily supplement of 100 µg Se as L-selenomethionine (n = 129) or placebo (n = 126) for 12 wk. Plasma selenium and whole-blood GPx activity were measured at baseline and at week 12. Participants were genotyped for the GPx1 Pro200Leu polymorphism.

Results: Selenium supplementation increased whole-blood GPx activity by 5 (95% CI: 4, 7) U/g hemoglobin (damage (2–4), endothelial dysfunction (5–7), inflammation (8–10), and accelerated development of atherosclerotic lesions (9, 11). Furthermore, an independent, inverse association between GPx1 activity and risk of recurrent coronary events over 5 y was reported in the Atherosclerosis Risk in Communities Study—a prospective study involving 636 patients with suspected coronary artery disease (12).

Dietary selenium intake is an important determinant of GPx1 activity (13). In populations with selenium intakes of 55 to 90 µg/d, GPx1 activity is submaximal, and supplementation with selenium leads to an increase in blood GPx1 activity (13–15). Large interindividual differences in the response of GPx1 activity to selenium supplementation have been observed (13, 16). These variations are due mainly to differences in baseline selenium status; however, interactions between genetic polymorphisms in the GPx1 gene and dietary selenium intake may account for some of the interindividual variations.

The gene encoding for GPx1 is located on chromosome 3p21.3 and contains a single nucleotide polymorphism within the coding region that results in a proline (Pro) to leucine (Leu) amino acid substitution (rs1050450); this variant is referred to as either Pro198Leu or Pro200Leu in the literature, because a variable number of alanine repeat codons in exon 1 of the GPx1 gene causes a shift in the amino acid position (17–19). Measurement of GPx1 enzyme activity in vitro showed that cells transfected with the Leu allele were less responsive to increasing selenium concentrations (20, 21). Cross-sectional studies have reported lower red and white blood cell glutathione peroxidase (GPx) activity in participants with the Leu allele, compared with Pro allele homozygotes (11, 19). However, the evidence is limited to in vitro and observational studies.

Conclusions: These results indicate that GPx1 Pro200Leu variants do not substantially modify the response of whole-blood GPx to selenium supplementation in individuals with relatively high plasma selenium concentrations. A nutrient-genotype interaction was observed when the baseline selenium concentration was low, but this requires independent confirmation. This trial was registered at www.actr.org.au as ACTRN12605000412639 and ACTRN12606000197538.

INTRODUCTION

Glutathione peroxidase 1 (GPx1)4 is an antioxidant enzyme that is expressed in most cells, including endothelial cells, and is an integral component of the mammalian endogenous antioxidant defenses (1). Evidence suggests that low GPx1 activity contributes to the development of atherosclerosis. In mice, GPx1 deficiency leads to increased susceptibility of vascular tissues to oxidative damage (2–4), endothelial dysfunction (5–7), inflammation (8–10), and accelerated development of atherosclerotic lesions (9, 11). Furthermore, an independent, inverse association between GPx1 activity and risk of recurrent coronary events over 5 y was reported in the Atherosclerosis Risk in Communities Study—a prospective study involving 636 patients with suspected coronary artery disease (12).

Dietary selenium intake is an important determinant of GPx1 activity (13). In populations with selenium intakes of 55 to 90 µg/d, GPx1 activity is submaximal, and supplementation with selenium leads to an increase in blood GPx1 activity (13–15). Large interindividual differences in the response of GPx1 activity to selenium supplementation have been observed (13, 16). These variations are due mainly to differences in baseline selenium status; however, interactions between genetic polymorphisms in the GPx1 gene and dietary selenium intake may account for some of the interindividual variations.

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1From the Departments of Human Nutrition (JCM, CMS, and CDT), Preventive and Social Medicine (SMW), Medical and Surgical Sciences (GTW), and Pathology (IMM and JLL), University of Otago, Dunedin, New Zealand, and the School of Medicine and Mater Health Services, Brisbane, Australia (NvH).

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3Address correspondence to JC Miller, Department of Human Nutrition, University of Otago, PO Box 56, Dunedin, New Zealand 9054. E-mail: jody.miller@otago.ac.nz.

4Abbreviations used: GPx, glutathione peroxidase; GPx1, glutathione peroxidase 1; SeCath Study, Selenium and Catheterization Study; SeHeart Study, Selenium and Heart Study.

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homozygotes (18, 21–24), although others have shown no differences (25, 26). Confounding by differing selenium intakes may explain some of the discrepancies in the findings from these observational studies. Randomized controlled trials reduce confounding bias (27) and are needed to test the hypothesis that the GPx1 Pro200Leu variants influence enzyme activity. Currently, the evidence in humans for this nutrient-gene interaction is limited to observational studies; therefore, we performed 2 randomized, placebo-controlled trials to determine whether the GPx1 Pro200Leu polymorphism influences the response of whole-blood GPx activity to selenium supplementation in New Zealanders with coronary artery disease.

**SUBJECTS AND METHODS**

This study involves the combined results from 2 unrelated, but similar, randomized, placebo-controlled, double-blind selenium-supplementation trials involving coronary artery disease patients from the lower South Island of New Zealand. The first study, the Selenium and Heart Study (SeHeart Study), was designed to assess the effect of selenium supplementation on the occurrence of clinical coronary events in participants recruited from the Otago and Southland cardiac surgery waiting list. Patients had undergone diagnostic coronary angiography within 2 y of enrolling in the study. Recruitment for the SeHeart Study began in October 2004 and finished in December 2006. The second study, the Selenium and Catheterization Study (SeCath Study), involved patients who had undergone percutaneous coronary angioplasty. This trial was designed to assess the effect of selenium supplementation on markers of inflammation associated with increased restenosis risk. Recruitment began in June 2006 and finished in August 2007. Sample sizes for each study were calculated on the basis of the primary endpoint described. Here we report the findings of a prespecified principal secondary endpoint, which was to investigate the influence of the GPx1 Pro200Leu polymorphism on the response of whole-blood GPx activity to selenium supplementation.

Potential participants for each study were identified from the Dunedin Hospital cardiac surgery waiting list or cardiology angiography register. Patients with ≥2-vessel disease and ≥50% coronary stenosis on each vessel were included. Patients with valvular or congenital heart disease, who were receiving dialysis, or who resided outside of the Southland and Otago provinces were excluded. Letters and information sheets were sent to potential participants. Patients were telephoned 1 wk later and were invited to participate in the study if they were not already taking medications prescribed. Medications prescribed at the time of angiography were also recorded. *Current smoker* was defined as cigarette smoking at the start of the study or within 6 mo before starting the study.

**Treatments**

The study tablets were provided by New Zealand Nutritional Ltd, exclusively for these studies, and were identical in size, shape, and color. The selenium tablets contained l-selenomethionine as the active ingredient and lactose monohydrate, silicon dioxide, and magnesium stearate as the excipients. The placebo tablets contained cellulose, silicon dioxide, and magnesium stearate. Ten tablets were randomly selected from each group and were analyzed for selenium content by Hill Laboratories Ltd by using inductively coupled plasma mass spectrometry with a detection limit 0.01 μg/tablet. Placebo tablets contained <0.01 μg Se, and the selenium tablets contained a median of 108 (IQR: 100, 110) μg Se. Participants were asked to consume one tablet per day for 12 wk. Participants in the SeCath Study began taking tablets 2–3 wk after percutaneous coronary intervention. One week after starting the study, the participants were telephoned to ensure that they had no difficulties taking their tablets.

**Blood sample collection**

Eligible and interested patients attended the Human Nutrition clinic at Otago University or their closest community laboratory. For both trials, participants provided a fasting blood sample at baseline and after 12 wk of selenium supplementation and were genotyped for the GPx1 Pro200Leu variants. Blood was drawn into 4-mL EDTA Vacutainers (Becton Dickinson) for separation of whole blood and plasma. Serum was collected in 4-mL additive-free Vacutainers. RNA was extracted from whole blood for participants of the SeHeart Study. For both studies, samples were centrifuged at 3000 rpm (1500 × g) for 15 min, and aliquots of whole blood, plasma, and serum were stored at −80°C in polyivials until analyzed.

**Analytic methods**

Plasma selenium concentrations were measured by graphite furnace-atomic absorption spectroscopy with a Zeeman background correction (AA–800; Perkin-Elmer Corp) in the Trace Element Laboratory, Department of Human Nutrition, by using a modified version of the Jacobson and Lockitch (1988) method (28). Samples were diluted with a 0.1% Triton X-100 and 6% glycerine solution, which allowed the absorbance to be read with greater accuracy. Accuracy was assessed by using an external certified reference material (Seronorm Reference Plasma, lot JL4409, Seronorm Trace Element Serum; Laboratories of SERO AS) with a mean certified selenium value of 1.04 μmol/L (95% CI: 0.92, 1.14 μmol/L). Our analysis gave a mean (±SD) of 1.04 ± 0.05 μmol/L with a CV of 5% (n = 78). In addition, multiple aliquots of control pooled plasma samples were analyzed during each batch to determine the interassay CV, which was 6% (n = 78).

GPx activity was measured in whole blood by using the commercially available RANSEL kits (catalog no. RS505; Randox Laboratories Ltd) on a Cobas Mira Plus auto analyzer (Roche Diagnostic Systems Inc). This method is based on the coupled
GPx1 genotyping

The methods for nucleic acid extraction and genotyping of the GPx1 Pro200Leu variant (rs 1050450) differed between the 2 study groups. For the SeHeart Study, RNA extraction was performed with peripheral leukocytes from 7.5 mL of an EDTA-treated blood sample by using Dextran sedimentation and Tri-Reagen. For the SeCath Study and the first 61 participants of the SeCath Study, was 605 (range: 511–691) U/L, and our analysis gave a mean (±SD) 601 ± 55 U/L with a CV of 9% (n = 58). The certified reference value for lot 291RS, used for the SeHeart Study, was 732 (range: 622–842) U/L, and our analysis gave a mean (±SD) 663 ± 25 U/L with a CV of 4% (n = 24). In addition, multiple aliquots of control pooled whole-blood samples were analyzed during each batch to determine the inter-assay CV, which was 9% (n = 120). For all analyses, baseline and follow-up samples from each participant were analyzed in the same batch and in duplicate.

RESULTS

Participant characteristics and baseline selenium status

Of the 289 randomized participants, 255 participants (88%) completed the 12-wk trial and were genotyped for the GPx1 polymorphism and selenium supplementation. 925

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Statistical analysis

The analysis was performed by using Stata (version 11; StataCorp). All tests were 2-sided, and the statistical significance was assessed at P < 0.05. Because of the small number of participants who were homozygous for the Leu allele (n = 14), we combined the Leu/Leu and Pro/Leu genotypes (Leu group), for comparison with participants homozygous for the Pro genotype (Pro/Pro).

Descriptive statistics for baseline characteristics are presented by study group and by treatment group. Continuous baseline variables that were normally distributed are presented as means ± SDs or medians (25th, 75th percentiles) for skewed data. Categorical data are presented as the number of participants and percentages. Deviations of observed frequencies from expected Hardy-Weinberg frequencies were tested by using the chi-square test (31). Because we had combined 2 different study groups, differences in baseline variables between study groups were tested by using ANOVA for continuous variables and the chi-square test for categorical variables. In addition, differences in baseline values of selenium status (plasma selenium concentration and whole-blood GPx activity) by genotype were tested by using ANOVA.

Multiple linear regression models, adjusted for baseline values and including an interaction term for treatment-by-study group, were used to estimate the difference in week 12 plasma selenium concentration and whole-blood GPx activity between the placebo and treated groups. To estimate the treatment effect for each genotype, we used a regression model that included an interaction term for genotype (Pro/Pro coded as 0 and Leu group coded as 1) and treatment group.

In a further analysis to examine the influence of baseline selenium status on the treatment response of GPx to selenium supplementation in different genotypes, a model that included an interaction effect between baseline selenium concentration, treatment group, and genotype was considered. The model included the 2-factor interactions and main effects for each variable. It also included a term for the study group and a quadratic or squared term for baseline selenium concentration, because the effect of baseline selenium concentration on treatment response was expected to be nonlinear. Because the interaction effect of interest was statistically significant, the results are presented graphically as the differences between the treatment and control groups for the different genotypes at various baseline selenium concentrations; the differences were estimated from the regression model. A sensitivity analysis was performed by imputing missing values; because this did not change the results, we present only the data without the imputed values.

RESULTS

Participant characteristics and baseline selenium status

Of the 289 randomized participants, 255 participants (88%) completed the 12-wk trial and were genotyped for the GPx1
Pro200Leu polymorphism (Figure 1); 126 and 129 participants in the placebo and selenium groups, respectively, were included in the primary analysis.

Baseline characteristics of the participants by recruitment and treatment groups are presented in Table 1. Some differences in the characteristics of participants by study group were observed, including a higher proportion of patients with 3-vessel disease in the SeHeart Study ($P < 0.001$) and a higher proportion of current smokers ($P = 0.001$) and lower mean whole-blood GPx activity ($P < 0.0001$) in the SeCath Study. However, the treatment and placebo groups were balanced in terms of age, sex, cardiovascular disease risk factors, disease severity, prescribed medications, and selenium status.

The frequencies of the GPx1 Leu and Pro alleles were 0.26 and 0.74, respectively. Forty-six percent of the participants carried at least one Leu allele, and 5% were homozygous for the Leu allele. The frequencies of the GPx1 Pro200Leu genotypes did not differ from Hardy-Weinberg equilibrium [$\chi^2(1\ df) = 0.13$ $P = 0.966$] and did not differ between the 2 treatment groups [$\chi^2(1\ df) = 2.21$ $P = 0.331$] (Table 1). Baseline selenium status did not differ between the genotype groups; mean (±SD) plasma selenium concentrations were 1.32 ± 0.40 and 1.38 ± 0.30 μmol/L ($P = 0.163$), and mean (±SD) whole-blood GPx activities were 38 ± 11 and 39 ± 11 U/g hemoglobin ($P = 0.557$) for Pro/Pro and Leu participants, respectively.

Effect of intervention and GPx1 Pro200Leu polymorphism on selenium status

After 12 wk of supplementation, plasma selenium concentrations ($P < 0.001$) and whole-blood GPx activity ($P < 0.001$) were significantly higher in the treatment group than in the placebo group, after adjustment for baseline GPx activity (Table 2). The treatment effect of selenium supplementation on GPx activity did not differ by study group ($P = 0.758$ for the treatment-by-study group interaction).

When the analysis was stratified by genotype and adjusted for baseline values, selenium supplementation significantly increased plasma selenium concentrations, compared with placebo, in both the Pro/Pro ($P < 0.001$) and Leu participants ($P < 0.001$) (Table 2); however, there was no difference in the increase between genotype groups ($P = 0.291$ for treatment-by-genotype interaction).

**FIGURE 1.** Flow diagram of participants in the SeHeart and SeCath studies. SeHeart, Selenium and Heart; SeCath, Selenium and Catheterization.
which 12 wk of selenium supplementation increased GPx activity difference in the increase between genotype groups (adjustment for baseline GPx activity, in both higher in the selenium group than in the placebo group, after Similarly, at week 12, whole-blood GPx activity was significantly higher in the selenium group than in the placebo group, after adjustment for baseline GPx activity, in both Pro/Pro participants (P < 0.001) and Leu participants (P < 0.001); again, there was no difference in the increase between genotype groups (P = 0.165 for the treatment-by-genotype interaction).

Baseline plasma selenium concentration affected the extent to which 12 wk of selenium supplementation increased GPx activity (P < 0.001 for the treatment-by-baseline selenium concentration and P = 0.001 for the treatment-by-baseline selenium concentration squared interactions) (Table 3). When the baseline selenium concentration was 1.05 μmol/L, the mean increase (95% CI) in GPx activity was 7.7 (5.8, 9.7) U/g hemoglobin (P < 0.001), whereas when baseline plasma selenium was 1.55 μmol/L the increase in GPx activity was 2.8 (0.8, 4.7) U/g hemoglobin (P = 0.005).

Genotype was found to affect the GPx response to selenium supplementation, when baseline selenium status was included in the regression model (P = 0.006 for the treatment-by-genotype × baseline plasma selenium concentration interaction). In both genotype groups, the greatest treatment effect on GPx activity occurred in participants with the lowest baseline selenium concentration; however, the increase in GPx activity was greater in Pro/Pro participants than in Leu participants when baseline plasma selenium concentrations were lower (Figure 2). At the lowest plasma selenium concentration, the increase in GPx activity was 5 (95% CI: 1, 9) U/g hemoglobin higher in supplemented Pro/Pro participants than in supplemented Leu participants, after adjustment for baseline GPx activity and change in placebo (P = 0.015). In Pro/Pro participants, GPx activity was no longer responsive to supplementation at higher baseline selenium concentrations (≥1.55 μmol/L), whereas the increase in GPx activity in Leu participants was statistically significant at all baseline selenium concentrations (Figure 2).

**DISCUSSION**

In this randomized, double-blind, placebo-controlled trial, a daily supplement of 100 μg Se for 12 wk increased whole-blood GPx activity; however, the mean increase in whole-blood GPx activity with selenium supplementation did not differ by GPx1 Pro200Leu genotype. Given that the lower bound of the

### TABLE 1
Baseline characteristics of participants by study group and by treatment group

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>SeHeart (n = 130)</th>
<th>SeCath (n = 125)</th>
<th>Placebo (n = 126)</th>
<th>Selenium (n = 129)</th>
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<tbody>
<tr>
<td>Age (y)</td>
<td>70 (43–90)</td>
<td>63 (38–88)</td>
<td>66 (38–88)</td>
<td>68 (41–90)</td>
</tr>
<tr>
<td>Male sex [n (%)]</td>
<td>92 (71)</td>
<td>79 (63)</td>
<td>80 (63)</td>
<td>58 (45)</td>
</tr>
<tr>
<td>BMI ≥30 kg/m² [n (%)]</td>
<td>35 (27)</td>
<td>43 (34)</td>
<td>38 (30)</td>
<td>40 (31)</td>
</tr>
<tr>
<td>Current smoker [n (%)]</td>
<td>13 (10)</td>
<td>32 (26)</td>
<td>24 (19)</td>
<td>21 (16)</td>
</tr>
<tr>
<td>Medical history [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>20 (15)</td>
<td>17 (14)</td>
<td>18 (14)</td>
<td>19 (15)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>113 (87)</td>
<td>88 (70)</td>
<td>102 (81)</td>
<td>99 (76)</td>
</tr>
<tr>
<td>Family history of IHD</td>
<td>28 (22)</td>
<td>45 (37)</td>
<td>32 (26)</td>
<td>41 (32)</td>
</tr>
<tr>
<td>3-Vessel disease</td>
<td>84 (64)</td>
<td>23 (18)</td>
<td>53 (42)</td>
<td>54 (42)</td>
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<tr>
<td>Diagnosed ACS</td>
<td>106 (81)</td>
<td>114 (91)</td>
<td>111 (88)</td>
<td>109 (84)</td>
</tr>
<tr>
<td>Medications [n (%)]</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Statins</td>
<td>126 (97)</td>
<td>124 (99)</td>
<td>123 (98)</td>
<td>127 (98)</td>
</tr>
<tr>
<td>β-Blockers</td>
<td>113 (87)</td>
<td>125 (100)</td>
<td>119 (94)</td>
<td>119 (92)</td>
</tr>
<tr>
<td>Aspirin</td>
<td>126 (97)</td>
<td>123 (98)</td>
<td>122 (97)</td>
<td>127 (98)</td>
</tr>
<tr>
<td>GPx1 Pro200Leu genotype</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Pro/Pro</td>
<td>70 (54)</td>
<td>67 (54)</td>
<td>67 (53)</td>
<td>70 (54)</td>
</tr>
<tr>
<td>Pro/Leu</td>
<td>56 (43)</td>
<td>48 (38)</td>
<td>51 (40)</td>
<td>53 (41)</td>
</tr>
<tr>
<td>Leu/Leu</td>
<td>4 (3)</td>
<td>10 (8)</td>
<td>8 (6)</td>
<td>6 (5)</td>
</tr>
<tr>
<td>Selenium status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma selenium (μmol/L)</td>
<td>1.36 ± 0.34</td>
<td>1.34 ± 0.39</td>
<td>1.34 ± 0.38</td>
<td>1.33 ± 0.35</td>
</tr>
<tr>
<td>Whole blood GPx (U/g Hb)</td>
<td>41 ± 10</td>
<td>35 ± 10</td>
<td>38 ± 10</td>
<td>38 ± 11</td>
</tr>
<tr>
<td>C-reactive protein (g/L)²</td>
<td>1.3 (0.6, 3.5)</td>
<td>1.7 (0.8, 3.5)</td>
<td>1.8 (0.7, 3.5)</td>
<td>1.4 (0.7, 3.6)</td>
</tr>
</tbody>
</table>

¹ACS, acute coronary syndrome; GPx, glutathione peroxidase; GPx1, glutathione peroxidase 1; Hb, hemoglobin; IHD, ischemic heart disease; SeCath, Selenium and Catheterization Study; SeHeart, Selenium and Heart Study.

²Represents participants recruited from the Dunedin Public Hospital coronary artery bypass graft waiting list (SeHeart) or from the cardiac catheterization register (SeCath) who completed the study and were genotyped.

³Represents all participants from the SeHeart and SeCath studies randomly assigned into either the placebo or the selenium group who completed the study and were genotyped.

⁴Values are means (minimum–maximum).

⁵Significantly different from the SeHeart group: χ²(1 df) = 9.7, P = 0.001.

⁶Significantly different from the SeHeart group: χ²(1 df) = 53.6, P < 0.001.

⁷Mean ± SD (all such values).

⁸Significantly different from the SeHeart group, P < 0.001 (ANOVA).

⁹Values are medians (25th, 75th percentiles).
The change in whole-blood GPx activity with selenium supplementation increased with decreasing baseline plasma selenium concentrations (Table 3). Given this inverse association, we conducted an exploratory analysis of the influence of genotype and baseline selenium status on the magnitude of the increase in GPx activity with selenium supplementation. The results showed a significant nutrient-gene interaction; the increase in GPx activity in Pro homozygotes with baseline plasma selenium concentrations 1.15 μmol/L or lower was 2-fold higher than the increase observed in Leu participants at similar baseline selenium concentrations, whereas at the highest baseline plasma selenium concentrations the trend was reversed. Interestingly, the change in whole-blood GPx activity with selenium supplementation decreased with increasing baseline plasma selenium concentrations in Pro/Pro participants, whereas the change in Leu participants was largely unaffected by baseline selenium concentrations. This suggests that enzymes coded with a Leu allele are less influenced by differing plasma selenium concentrations than are enzymes coded with Pro. These results are consistent with the findings from a cross-sectional study by Jablonska et al (32), who reported weaker correlations between plasma selenium concentration and erythrocyte GPx1 activity in individuals carrying a Leu allele compared with Pro homozygotes. Indeed, on the basis of their results, Jablonska et al (32) hypothesized that CI includes a meaningful effect size (~5 U/g hemoglobin), it may be premature to discount completely a lower response of GPx to selenium in participants carrying a Leu allele. The power to detect a nutrient-gene interaction in this study may have been attenuated because participants had higher baseline selenium status than expected, which resulted in a lower mean increase in whole-blood GPx activity with selenium supplementation. One might expect the influence of the GPx1 Pro200Leu variant, if one exists, to be observed in participants whose whole-blood GPx activity is more responsive to selenium supplementation (ie, in those with lower baseline selenium status). Indeed, this is exactly what our results showed in an exploratory analysis.

The mean difference (95% CI) between genotype groups (difference between week-12 values for the Pro/Pro and Leu/Leu groups minus difference between week-12 values for the Pro/Leu group). The mean difference (95% CI) between week-12 values (selenium placebo) in participants who were homozygous for the GPx1 Pro200 allele (n = 70 in the selenium group and n = 59 in the placebo group).

The mean difference (95% CI) between week-12 values (selenium placebo) in participants who had one or more copies of the GPx1 Pro200Leu allele (n = 6 7 in the placebo group).

The mean difference (95% CI) between week-12 values (selenium placebo) in participants who were homozygous for the GPx1 Pro200 allele (n = 130) and from the cardiac catheterization register (n = 125). GPx, glutathione peroxidase; Hb, hemoglobin.

The difference (95% CI) in whole-blood GPx activity between the placebo and selenium groups at week 12 for specific baseline plasma selenium concentrations; estimated from multiple linear regression models that adjusted for baseline plasma selenium concentration and baseline GPx activity and included interaction terms for treatment-by-study group and treatment-by-genotype.
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The response of GPx1 to increasing selenium intake would be greatest in individuals carrying a Pro allele, because enzyme activity in the Pro/Pro participants appeared to be more influenced by differing selenium concentrations; our findings provide empirical evidence in support of this hypothesis.

We are not aware of any randomized controlled trials that have investigated the influence of the GPx1 Pro200Leu polymorphism on enzyme activity. Recently, the results of a small, non-randomized trial involving 37 obese women who increased Brazil nut consumption—providing 290 mgSe/d—for 8 wk showed that the mean increase in whole-blood GPx activity did not differ by GPx1 Pro200Leu genotype; however, similar to our primary study, there was a nonsignificant trend for a lower increase in GPx activity in participants (n = 5) carrying a Leu allele (33). Our results support the findings from cross-sectional studies that have reported lower erythrocyte or whole-blood GPx activity in participants carrying a Leu allele with moderately low selenium concentrations [49–60 µg/d (23, 34)] or plasma selenium concentrations [1.05 mmol/L (24)].

The results of the current study may have important implications for understanding selenium requirements and disease risk. A large proportion (0.45) of participants carried one or more Leu alleles, and the difference in the response of whole-blood GPx activity to supplementation between the 2 genetic groups—when baseline selenium status was low—was large. This suggests that the GPx1 Pro200Leu variants may explain a significant proportion of the interindividual variation in the response to supplementation that has been observed in previous trials (35). Moreover, whole-blood GPx activity increased with supplementation at all baseline selenium concentrations in participants carrying a Leu allele, whereas no increase in GPx activity was observed in Pro/Pro participants with higher baseline selenium concentrations. Given that one of the criteria used to define selenium requirements is the level of intake that achieves maximum GPx activity (36), these findings suggest that individuals with a Leu allele have a higher selenium requirement than do Pro homozygotes.

A functional effect of the GPx1 Pro200Leu variants is supported by genotype-disease association studies, which have shown an increased risk of coronary artery disease (37–39), some cancers (40, 41), and diseases associated with selenium deficiency [Keshan disease (21) and Kashin Beck disease (42)] in individuals carrying a Leu allele. Interestingly, a nested case-control study from the EPIC-Heidelberg Study found that participants carrying a Leu allele had a significantly lower risk of prostate cancer with higher serum selenium concentrations, whereas no such interaction was observed in Pro homozygotes (43). It would seem, therefore, that Leu individuals might benefit more from higher selenium intakes, which may help overcome their altered enzyme activity. The finding in the current study that whole-blood GPX activity in Leu participants was responsive to supplementation, even at the highest baseline selenium concentrations, supports this contention.

This study had several strengths that support the validity of our findings. Randomization achieved equal distribution of the GPx1 genotypes—as well as other patient demographics, disease characteristics, and baseline selenium status—between the 2 treatment groups. Very few randomly assigned participants were lost to follow-up, and genotype was available for all but 8 participants. Finally, no differences were observed between the SeHeart and SeCath studies in the responses of GPx activity to treatment by genotype; therefore, combining these patient groups was appropriate.

In terms of the limitations of the study, although the intention to examine the nutrient-gene interaction between selenium supplementation and GPx activity was determined before the inception of the studies, the regression model used to describe the influence of baseline selenium status on the treatment effect was designed post hoc. Therefore, we view the finding of a nutrient-gene interaction with low baseline selenium status as exploratory and in need of confirmation in future studies. Furthermore, because our study involved coronary artery disease patients, the generalizability of our findings to other populations is uncertain; however, because there is little evidence to suggest that any genotype-selenium interaction would be modified by the disease state of these participants, we are unable to completely discount such a possibility.

In summary, the GPx1 Pro200Leu genetic variants did not influence the response of GPx1 activity to selenium supplementation in New Zealand coronary artery disease patients with relatively high baseline selenium status. However, exploratory analyses showed an interaction between genotype and GPx1 enzyme induction in patients with low initial selenium status. If confirmed in future studies, the identification of individuals with the Leu allele and low selenium status may help direct nutritional or therapeutic interventions. It is important that such studies are undertaken to elucidate the influence of the genetic variants on GPx1 enzyme responsiveness, selenium requirements, and ultimately on disease susceptibility and response to treatment.

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