High-selenium lentil diet protects against arsenic-induced atherosclerosis in a mouse model

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Abstract

Background: Cardiovascular disease (CVD) is a major cause of death worldwide, and arsenic (As) intake, mainly through drinking water, is a well-known risk factor for CVD as well as other health problems. Selenium (Se) is a known antagonist to As toxicity. Objective: We tested the potential of high-Se lentils from the Canadian prairies as a therapeutic food to alter the outcome of As-enhanced atherosclerosis. Materials and Methods: Male ApoE<sup>-/-</sup> mice exposed to a moderate level of As (200 ppb) in their drinking water, and control mice on tap water received one of three lentil diets: Se-deficient (0.009 mg/kg), Se-adequate (0.16 mg/kg) or Se-high (0.3 mg/kg). After 13 weeks, lesion formation in the aortic arch and sinus were assessed. Intralesional cellular composition, serum lipid levels and hepatic oxidative stress were assessed as well. Results: Arsenic-exacerbated plaque formation was reduced in the sinus and completely abolished in the aortic arch of mice on the Se-fortified lentil diet, whereas lesions were increased in As-exposed mice on both the Se-deficient and Se-adequate diets. Notably, Se deficiency contributed to proatherogenic composition of serum lipids in As-exposed mice as indicated by high-density lipoprotein:low-density lipoprotein. At least adequate Se status was crucial for counteracting As-induced oxidative stress. Conclusion: This study is the first to show the potential of high-Se lentils to protect against As-triggered atherosclerosis, and this invites further investigations in human populations at risk from As contamination of their drinking water. Crown Copyright © 2015 Published by Elsevier Inc. All rights reserved.

Keywords: Arsenic toxicity; Atherosclerosis; Selenium; Biofortification; Lentils; Oxidative stress

1. Introduction

Contamination of drinking water with naturally occurring arsenic (As) is a global problem, and long-term exposure to As has numerous adverse health effects, including being a risk factor for developing cardiovascular disease (CVD), peripheral vascular disease, hypertension, neurological disorders and cancers [1–4]. While it is established that chronic exposure is associated with vascular disease in humans, the risk for CVD from low to moderate As exposure (<300 ppb) is difficult to evaluate since epidemiological studies have revealed inconsistent findings [5]. More recent studies have emerged, however, that link even low As exposure with CVD [6,7]. Experimental mouse studies confirmed a strong correlation between atherosclerosis and environmentally relevant As exposure (≥1 ppm) [8–10]. Another study demonstrated that not only did even lower As concentration (200 ppb) in drinking water increase formation of atherosclerotic lesions, but also the plaque composition changed into a presumably less stable form [11]. The exacerbation of atherosclerosis from As exposure was observed in mice on normal chow diets, suggesting that exposure to low environmental As may trigger atherosclerosis even in populations not considered to be high CVD risk based on their diet.

The trace element selenium (Se) has antioxidative properties that may provide protection from As-induced CVD. Selenium is necessary for the expression of enzymes involved in antioxidant defense, e.g. glutathione peroxidase (GPX) [12], which protects organisms from oxidative damage by using GSH (glutathione) as hydrogen donor to neutralize peroxides. The recommended daily allowance (RDA) for Se in Canada and the USA is currently 55 μg per person per day, but especially for populations exposed to toxicological stressors such as As, the current RDA may not be adequate [13]. Indeed, Se has been shown to counteract As toxicity and vice versa. Followed by many animal studies, Moxon was the first to report that arsenic reduced toxicity from consumption of seleniferous grains [14]. Selenium and As promote the biliary excretion of each other [15], thought to be in
the form of seleno-s-glutathionyl-arsenium ion ([GS2AsSe]−), which was shown to be excreted in the bile after injection of rabbits with arsenite plus selenite and selenate [16,17]. Se-biofortified rodent chow and chow formulated with lentils as the Se source decrease chronic As toxicity in rats exposed to environmentally relevant levels of As [18,19]. The authors observed reduced arsenic toxicity expressed as reduced hepatic peroxidative damage, lower renal arsenic residues, reversal of antibody mediated immunosuppression and reestablished beneficial levels of antioxidants in circulation [18].

The purpose of the current study was to examine the potential of naturally Se-rich lentils from the prairie regions of Canada to counter As-induced atherosclerosis in apoE−/− mice with moderate As exposure, as well as to identify the concurrent cost of selenium deficiency.

2. Materials and Methods

2.1. Lentil diet formulation

Low-Se lentils (Cultivar 1=Se content-0.03 mg/kg) from the northwestern US and high-Se lentils (Cultivar 2=0.67 mg/kg Se), grown on naturally Se-rich soil in Saskatchewan, were provided by Dr. Albert Vandenberg, University of Saskatchewan, Saskatoon, SK. Other than the Se content, both lentil cultivars had similar nutritional profiles (Table 1) and very low levels of As, as determined by atomic absorption spectrometry [20]. Phytic acid, an antinutritional factor in legumes and cereals, binds nutrient cations such as Zn, Fe and Ca, making them less bioavailable. The phytochemical concentrations of the lentils used in this study were determined as described by Talamond et al.[21]. The final phytic acid content of the complete diets was between 1 and 1.6 mg/g (Table 1), which is considered low [22].

The diets containing 50% (w/w) lentils were produced by Harlan Laboratories (Indianapolis, IN). The Se-deficient diet formulated using only Cultivar 1, the Se-fortified diet was based on only Cultivar 2 and the Se-adequate diet was equal parts of the two lentil types. Other than Se content, ingredients of all diets were the same as in commercial rodent chow (2018; Harlan Laboratories Inc), ensuring that all other nutrients met or exceeded the requirements for standard rodent nutrition (Table 1).

2.2. Experimental exposure protocol

B6.129P2-ApoE<sup>−/−</sup>/Japo<sup>−/−</sup>/J male mice were obtained from Jackson laboratory (Bar Harbor, ME). Mice were housed and exposed to 200 ppb As (0.35 mg/L NaAsO<sub>2</sub>) in their drinking water as described before [11]. At weaning, all mice were introduced to their respective lentil diet with 2 weeks to acclimate (n=10 per group). Then mice on the three different diets were subdivided into As-exposed or control groups (n=5 animals per group). Sample size was based on the previous As exposure study in this type of mouse [11] and in compliance with the Animal Care Committee requirements to use the minimum number of animals necessary to gather scientific evidence. Mice received food and water ad <i>libitum</i> for 13 weeks. Low levels of arsenic were detected in the tap water (0.75 ppb±S.D. of 5%) [inductively coupled plasma mass spectrometry (ICP-MS) McGill Geochemistry Department][11].

Food consumption and weight gain of the mice were monitored weekly. Arsenic water consumption was monitored for 1 week to ensure that mice drank similar amounts. The McGill Animal Use Committee approved the experimental protocol, and animals were handled in accordance with guidelines.

2.3. Total arsenic residues in kidneys and urine

Mouse kidneys were flash frozen and urine samples per diet group were collected before and after As exposure. Arsenic content was determined by ICP-MS technique at Prairie Diagnostic Services (Saskatoon, SK).

2.4. Serum lipid analysis

Mouse serum separation was described earlier [11]. Serum was analyzed for high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol and for triglyceride levels by IDEXX Laboratories (Toronto, ON).

2.5. Assessment of atherosclerotic lesion formation

Analyses of atherosclerotic lesions of the aortic arch and the aortic sinus were conducted as detailed in Lemaire et al.[11]. Pictures of the aortic arch, defined as the region from ascending arch to bifurcation of the first intercostal arteries, were taken at original magnification ×20 and were evaluated using the ImageJ software (National Institutes of Health, USA). Lesion areas were quantified as percentage of the total arch area.

Paraformaldehyde-fixed mouse hearts were frozen in Tissue Tek OCT (Sakura, CA) and 6-μm crosssections (3-5 sections per animal) were cut from the aortic root throughout the aortic sinus and stained with oil red O. Lesion areas were quantified as percentage of the total sinus area (ImageJ). All analyses were done blinded to treatment group.

2.6. Smooth muscle cell and macrophage content of plaques

Smooth muscle cell (SMC) and macrophage content of the lesions in the aortic sinus were identified by immunofluorescence, using anti-smooth muscle actin (α-SMA) or MOMA-2 monoclonal antibodies, as described previously [11]. Images were acquired using Infinity Capture software and camera (Lumenera, Ottawa, ON) and analyzed using ImageJ software. Positively stained areas were expressed as a percentage of the total lesion area.

2.7. Analysis of hepatic lipid peroxidation

Thiobarbituric acid-reactive substances (TBARS) were measured as an estimate of hepatic lipid peroxidative damage, following a protocol by Reglero et al.[24]. The original protocol was slightly modified and validated for measurement with a microplate reader: 0.05–0.5 g of liver was homogenized with 0.5–5 ml of ice-cold 1.15% KCl in 0.01 M Na<sub>K</sub> phosphate buffer (pH 7.4) with EDTA (0.02 M). An aliquot of the homogenate was then mixed with 1:3 of a solution containing 15% trichloroacetic acid, 0.375% thiobarbituric acid, hydrochloric acid (0.25 N) and 1% butyldihydroxytoluene in deionized water. The samples were heated for 30 min at 85°C in a heatblock (VWR, Radnor, PA). Then, samples were cooled on ice to stop the reaction and were centrifuged for 15 min at 2000 rpm. The supernatants along with standards made with 1,1,3,3-tetramethoxypropane were transferred to a 96-well plate and the absorbance was measured at 535 nm in a SpectraMax plus 384 reader (Molecular Devices, Sunnyvale, CA). Each sample was read in duplicate.

2.8. Assessment of glutathione levels

Reduced glutathione (GSH) and oxidized GSH (GSSG) levels were measured in liver homogenates using the protocol described by Reglero et al.[24] with modifications for analysis in a microplate reader: 0.5 ml of the same homogenate used for TBARS assessment was vortexed with 0.5 ml of diluted trichloroacetic acid (10% in H<sub>2</sub>O) three times, for 5 s each time, within a 15-min period. Next, the mixture was centrifuged at 3500g for 15 min at 4°C and the supernatant was recovered. We prepared 300 μM NADPH (solution I), 6 mM DTNB (solution II) and 50 μM of glutathione reductase (solution III) in homogenization buffer. Solutions I and II were mixed at 7:1 volume,
respectively. 160 ml of this new mixture was then added to 40 μl of sample in a 96-well plate. After 15 s, 20 μl of solution III was added and the absorbance was determined at 405 nm after 30 s. The change in absorbance was used to determine the total GSH concentration by comparing the output with the results from a standard curve generated by serial dilution of GSH. The GSSG was measured by the same method but after the incubation of 400 μl of the supernatant with 8 μl of 2-vinyl pyridine and 17 μl of NaOH (6 N) for 1 h at room temperature. After centrifugation at 3500g for 15 min at room temperature, the supernatant was analyzed for the content of GSH using a calibration curve made with GSH. Samples were measured in duplicates.

2.9. Statistical analysis and data presentation

Data were tested for normal distribution with the Shapiro–Wilk normality test, and variables that did not display normal distribution were log-transformed (only HDL cholesterol). Differences in each variable among treatment groups were tested with generalized linear models (GLMs) adjusted for a linear response, including the treatments (As exposure, plus three diets) and their interaction as fixed factors. GLMs were chosen as a flexible statistical technique that allows the specification of multivariate models in which the response variable may not perfectly fit the assumptions of linearity, normality of the residuals and equality of residual variances because of a relative low sample size. Full models were calculated from the saturated models by iteratively removing nonsignificant terms to find the best-fit model including only significant terms (P<.05). Wald χ² values and the degrees of freedom are given for the GLMs. Specific differences between- or within-subjects factors were studied by pair-wise comparison of estimated marginal means (EMMs) with least-significant difference tests. Significance was set at P<.05 and marginal significance was set at P<.10. Data are presented as original means± the standard error of the mean (S.E.M.) or, for the kidney As levels, as means+ range. Graphs show individual data per animal and the mean. Wherever the GLM showed no effect of As exposure on the examined biomarker, results from combined treatment groups were presented. All tests were performed using IBM SPSS Statistics 20.0.

3. Results

The effects of As exposure (200 ppb) and the three different lentil diets (Se-deficient, Se-adequate and Se-fortified) on developing atherosclerotic lesions and on several molecular markers were examined in ApoE−/− mice after a 13-week trial.

3.1. Dietary acceptance

Mice showed equal acceptance of the three diets demonstrated by similar consumption (mean 20 g/week) and weight gain (Supplementary Fig. 1).

3.2. Arsenic residues in kidneys and urine

Kidneys are the major organ for metal excretion and therefore of As accumulation in rats, which was described previously [19]. Therefore, we analyzed As in kidneys of As-treated animals to determine effects of the Se-manipulated diets.

The mean renal As levels were 91.6 (49.60–140.30) ppb for the Se-deficient group, 64.7 (51.70–98.40) ppb for the Se-adequate group and 109.78 (89.30–212.80) ppb for the Se-fortified group. The effect of the diet on As levels in the kidney was significant in the model (χ² = 9.214, df=2, P=.01). The As level in the Se-adequate group was significantly lower than that in both the Se-deficient (P<.05) and the Se-fortified groups (P=.01). We analyzed the As concentrations in pooled urine samples (necessary because of volume constraints) before As exposure and after. Pooled urine samples of the Se-deficient, Se-adequate and Se-fortified group before As exposure were low with 18.8 ppb. 22 ppb and 18.9 ppb, respectively, confirming that the different lentil diets did not contribute to the body burden of As. Pooled urine samples showed increased As levels after As exposure of 340.1 ppb, 370 ppb and 298 ppb in the Se-deficient, Se-adequate and Se-fortified group, respectively.

3.3. Atherosclerotic lesion formation

Aortic arches of control mice in each dietary group revealed small amorphous plaques (Fig. 1A and B). All treatment factors, including the interaction of As exposure and diet, had a significant effect on atherosclerotic plaque formation (χ² = 25.12, df = 5, P<.001). Arsenic-exposed mice had increased lesion areas when fed the Se-deficient or Se-adequate lentil diet (P=.01) (Fig. 1A and B). Notably, in the Se-fortified, As-exposed group, plaque formation was down to that of the control groups. Different Se levels of the diets did not affect lesions in control mice.

Similar results were observed in the aortic sinus (Fig. 2A and B). Here, diet and As exposure had a significant effect on lesion size (χ² = 26.18, df = 3, P<.001). Se deficiency increased lesion area in the As-exposed and control mice (P<.05). Arsenic exposure alone increased lesions significantly (P<.001) when comparing the respective diet groups.

3.4. Serum lipid profile

Serum lipid levels are directly correlated with cardiovascular risk. Total serum cholesterol was not changed by As exposure, in agreement with previous findings in this mouse model [11] (Table 2). Only the diet had a significant effect on total cholesterol (χ² = 17.778, df = 2, P<.001) and LDL cholesterol (χ² = 16.703, df = 2, P<.001). Se deficiency resulted in higher LDL (from P=0.01 to P=0.066 for the three diets) and higher total cholesterol (from P<.001 to P<.05) regardless of As exposure (Table 2). Arsenic exposure did not affect triglyceride levels. However, Se status did. Selenium-deficient mice had significantly higher triglyceride levels compared to the other dietary groups (P<.01) (GLM: χ² = 13.332, df = 2, P=.001). HDL cholesterol was not affected by As exposure or diet. However, the HDL-to-LDL ratio, arguably the most relevant factor, was significantly affected by the diet, but only in As-treated animals (χ² = 11.286, P=.004). In this group, mice on the Se-deficient diet had a significantly lower HDL-to-LDL ratio than did mice on the Se-adequate or Se-fortified diets (P<.001 and P=.019, respectively).

3.5. Plate characterization

Stability of atherosclerotic lesions is indicated by their macrophage and SMC content, as well as other factors. Higher SMC and lower macrophage content suggest increased stability, which is associated with lower health risk and lower risk of fragment emboli in humans [25]. We measured the macrophage and SMC content in lesions of the aortic sinus (Supplementary Fig. 2 and Table 3). Macrophage and SMC content of the plaques was only affected by the lentil diet (χ² = 6.434, df = 2, P=.04) and (χ² = 7.234, df = 2, P=.025). In the As-exposed groups, Se deficiency was associated with an increase in macrophage content, when compared to Se-adequate (P<.01) and Se-fortified (P<.05) diets. Overall, Se deficiency resulted in plaques with higher macrophage content (P<.05).

Dietary Se predicted SMC content in a dose-dependent manner (Table 3).

3.6. Oxidative stress

Selenium-dependent GPx is crucial for the free radical scavenging system, which uses GSH to reduce hydroperoxides in turn producing GSSG (oxidized GSH) [26]. Total hepatic GSH was not altered by As exposure or the different lentil diets (overall P=.364). While As exposure alone had no effect on reduced GSH, it was significantly affected by the lentil diets, (χ² = 7.762, df = 2, P=.021). Adequate or fortified Se decreased reduced GSH in the As-exposed group (P<.001).
Arsenic exposure and the diets plus their interaction had a significant effect on GSSG ($\chi^2=41.412$, df = 5, $P < 0.001$). In As-exposed mice, Se-containing diets increased hepatic GSSG ($P < 0.001$). GSSG in As-exposed mice differed significantly from GSSG in nonexposed mice of the same diet group ($P < 0.05$).

In the present study, TBARS were diet dependent only in the As-exposed group ($\chi^2=25.85$, df = 2, $P < 0.001$) (Table 4) but otherwise not different from controls on the same diets.

4. Discussion

Many people worldwide are exposed to As levels in their drinking water [27,28]. Probability maps show that many more are at risk of exposure than originally thought, especially when one considers the growing demands for groundwater [29]. Studies demonstrate a correlation between moderate As exposure ($<300$ ppb) and mortality from CVD [30–33], although no clear inference about causality could be drawn from their studies.

Naturally Se-rich lentils, as opposed to synthetic pills, may serve as a practical, inexpensive, whole food solution for As-affected people, especially in populations where lentil is already a staple food. In this study with different Se levels, we tested otherwise nutritionally matched lentil diets (Table 1), in their effectiveness to reverse As-triggered atherosclerosis in male mice. We present the caveat that these effects observed in male mice cannot be extrapolated to females without experimental evidence, since expression of selenoproteins is sexually dimorphic in mice and humans [34,35]. Selenium possibly plays a more important role for cardiovascular health in men than women, in accordance with the findings on the importance of Se status on cancer risk [36]. Although we formulated diets with the two different lentil varieties, the resultant nutritional analysis is very similar. Although both diets meet or exceed the nutritional needs of rodents, we cannot completely rule out a dietary effect from the varietal differences.

In accordance with Lemaire et al. [11], As exposure triggered the development of atherosclerotic plaques in the aortas of experimental mice. Although Se deficiency was associated with increased plaque formation even in control animals, Se status became even more important for protecting against atherosclerosis in the As-challenged mice. Se fortification further reduced or completely abolished plaque formation in the As-exposed mice. This result is best explained by the metabolic interaction of As and Se that results in both being excreted together [37]. It points to mice on the Se-adequate diet likely having suboptimal Se status triggered by As exposure, which was restored by Se fortification.

Atherosclerosis and related oxidative stress were the only health measures negatively affected by As. The other biomarkers were not sensitive to this low As challenge; however, Se deficiency alone had negative effects, which supports epidemiological studies proposing Se deficiency as a risk factor for CVD [38]. In contrast to a previous trial on young rats exposed to 40 ppm As [18], 200-fold higher than the As exposure used here, kidney residues did not reflect increasing dietary

Fig. 1. Atherosclerotic lesion formation in the aortic arch. After 13 weeks of As exposure, aortas of control and As-exposed animals were stained with oil red O (A). Lesion size is given as percentage of the whole aortic arch area (B). Control animals are depicted with open symbols, and As-treated animals are depicted with solid symbols (see legend); $^a$ is significantly different from b ($P < 0.05$).
and the high interindividual variability seen in the current study was likely due to the much lower exposure. Pooled urine samples confirmed no effect of the diets alone on As exposure, in accordance to previous findings in rats [18]. Urine samples showed increased As levels after exposure as expected.

Table 2
Serum lipid levels (mmol/L) of control mice and As-treated mice, plus all animals on each specific diet

<table>
<thead>
<tr>
<th>Variable</th>
<th>Diet</th>
<th>No As mean±S.E.M.</th>
<th>With As mean±S.E.M.</th>
<th>All animals mean±S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>Se-deficient</td>
<td>20.81±1.81</td>
<td>19.01±0.89</td>
<td>19.83±0.94</td>
</tr>
<tr>
<td></td>
<td>Se-adequate</td>
<td>12.23±1.18***</td>
<td>15.7±1.51*</td>
<td>14.16±1.12***</td>
</tr>
<tr>
<td></td>
<td>Se-fortified</td>
<td>15.92±2.41**</td>
<td>14.43±1.61**</td>
<td>15.18±1.29**</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>Se-deficient</td>
<td>2.72±0.46</td>
<td>3.17±0.29</td>
<td>2.96±0.26</td>
</tr>
<tr>
<td></td>
<td>Se-adequate</td>
<td>1.98±0.29</td>
<td>1.76±0.13***</td>
<td>1.86±0.14**</td>
</tr>
<tr>
<td></td>
<td>Se-fortified</td>
<td>1.86±0.61</td>
<td>1.96±0.31***</td>
<td>1.91±0.33***</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>Se-deficient</td>
<td>3.22±0.85</td>
<td>2.45±0.11</td>
<td>2.8±0.39</td>
</tr>
<tr>
<td></td>
<td>Se-adequate</td>
<td>2.58±0.21</td>
<td>2.87±0.08</td>
<td>2.74±0.11</td>
</tr>
<tr>
<td></td>
<td>Se-fortified</td>
<td>2.83±0.97</td>
<td>2.45±0.14</td>
<td>2.64±0.46</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>Se-deficient</td>
<td>16.35±2.04</td>
<td>15.62±0.59</td>
<td>15.95±0.93</td>
</tr>
<tr>
<td></td>
<td>Se-adequate</td>
<td>8.75±2.4**</td>
<td>12.04±1.51*</td>
<td>10.58±1.11***</td>
</tr>
<tr>
<td></td>
<td>Se-fortified</td>
<td>12.25±1.90*</td>
<td>11.09±1.41**</td>
<td>11.67±1.13**</td>
</tr>
<tr>
<td>HDL/LDL</td>
<td>Se-deficient</td>
<td>0.23±0.08</td>
<td>0.16±0.01</td>
<td>0.19±0.04</td>
</tr>
<tr>
<td></td>
<td>Se-adequate</td>
<td>0.32±0.05</td>
<td>0.26±0.04***</td>
<td>0.28±0.03</td>
</tr>
<tr>
<td></td>
<td>Se-fortified</td>
<td>0.27±0.12</td>
<td>0.23±0.02**</td>
<td>0.25±0.06</td>
</tr>
</tbody>
</table>

Note: Values are presented as means±S.E.M., and significance was determined using the EMM±S.E.M.: ***P≤.001, **P≤.01, *P≤.05 and #P>.1, when compared to Se-deficient animals of the same treatment group.

Table 3
Macrophage (MOMA-2) and SMC (α-SMA) content (% of total lesion area) of sinus lesions in control, As-exposed and all animals

<table>
<thead>
<tr>
<th>Variable</th>
<th>Diet</th>
<th>No As mean±S.E.M.</th>
<th>With As mean±S.E.M.</th>
<th>All animals mean±S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOMA-2</td>
<td>Se-deficient</td>
<td>50.65±6.82</td>
<td>50.56±4.20</td>
<td>50.61±3.82</td>
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<td></td>
<td>Se-adequate</td>
<td>46.51±4.09</td>
<td>34.15±3.65**</td>
<td>40.89±3.28*</td>
</tr>
<tr>
<td></td>
<td>Se-fortified</td>
<td>41.58±3.90</td>
<td>39.26±4.74*</td>
<td>40.42±2.92*</td>
</tr>
<tr>
<td>α-SMA</td>
<td>Se-deficient</td>
<td>13.91±5.8</td>
<td>16.61±3.16</td>
<td>15.13±3.34</td>
</tr>
<tr>
<td></td>
<td>Se-adequate</td>
<td>23.26±2.97*</td>
<td>21.64±3.85</td>
<td>22.53±2.27*</td>
</tr>
<tr>
<td></td>
<td>Se-fortified</td>
<td>28.31±3.44*</td>
<td>20.2±2.45</td>
<td>24.62±2.44**</td>
</tr>
</tbody>
</table>

Significance was determined using the EMM±S.E.M.: **P≤.01, *P≤.05 and #P>.1, when compared to Se-deficient animals of the same As-treatment group.

* Percentage of total lesion area, presented as mean±S.E.M.

Se, and the high interindividual variability seen in the current study was likely due to the much lower exposure. Pooled urine samples confirmed no effect of the diets alone on As exposure, in accordance to previous findings in rats [18]. Urine samples showed increased As levels after exposure as expected.
In agreement with previous findings [5,11], here cholesterol levels were unchanged by arsenic. However, Se deficiency exaggerated the undesirable cholesterol profile, which was reversed through Se fortification in As-exposed animals. It is noteworthy that the Se concentration of the diets and cholesterol levels in non-As-exposed mice showed a U-shaped association, pointing toward the importance of adequate selenium status for cholesterol homeostasis. A U-shaped association between selenium status and protection from cancer has been reported and has been suggested for cancer mortality and risk of type 2 diabetes as well [39,40].

In contrast to Lemaire’s findings [11], triglycerides were not increased with As exposure, which could be driven by other nutritional benefits of lentils, since lentils have been shown to lower serum lipids, possibly due to their low glycemic index [41]. However, Se status may be pivotal in triglyceride homeostasis, as Se deficiency increased triglycerides in As-exposed mice.

Se deficiency appears to cause less stable plaques based on higher macrophage and lower SMC content. It would be valuable to consider Se and plaque composition in more detail, examining other plaque constituents such as collagen, oxidized LDL (oxLDL) and other inflammatory cells, all of which contribute to plaque vulnerability. In light of our findings from this study, as a valuable next step, especially oxLDL would be of interest because inactivation of the selenium-dependent enzyme GPX leads to oxidation of LDL in mice [42].

Hepatic peroxidation is one manifestation of oxidative stress occurring at a systemic level, which also plays a key role in CVD [43]. Other studies in rodents exposed to arsenite at ≥500 ppb [18,44] report increased hepatic peroxidation, which was not evident in our study possibly because 200 ppb As was too low to cause peroxidative damage. It seems that ApoE−/− mice already have inherently higher lipid peroxidation, but this genetic predisposition is positively affected by moderate As and adequate Se. In order to explain the decrease in lipid peroxidation only in As-exposed, Se-adequate group, further investigation would be needed.

One of the most important free radical scavenging systems in the body is evaluated through the biomarker GSH [24]. The selenoenzyme GPX uses GSH as electron donor to neutralize hydroperoxides, resulting in the formation of GSSG (oxGSH) [12]. Simply put, the protective response to oxidative stress results in increased production of GSSG, a response that was only seen in Se-adequate or Se-fortified animals that were exposed to As. Se deficiency compromised this protective mechanism. We conclude that all As-exposed animals incurred oxidative stress; however, only in the Se-supplemented groups the GPX redox system responded to the stressor. This observation agree with a recent study of As exposure in rats that were deficient or supplemented with Se. GPX activity was substantially suppressed with Se deficiency and increased by As only in the Se-supplemented animals [45].

In conclusion, this experimental study emphasizes the importance of adequate Se status for cardiovascular health and suggests that Se deficiency constitutes a major health risk to As-exposed individuals. Our findings with mice suggest that dietary Se intake above the US Food and Drug Administration RDA may be beneficial to reduce the risk of As-related atherosclerosis and warrants human-specific investigation.

Disclosure

The authors disclose no competing financial interests.

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References


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