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RESEARCH PAPER

Gene expression profiling reveals differential effects of sodium selenite, selenomethionine, and yeast-derived selenium in the mouse

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Abstract The essential trace mineral selenium is an important determinant of oxidative stress susceptibility, with several studies showing an inverse relationship between selenium intake and cancer. Because different chemical forms of selenium have been reported to have varying bioactivity, there is a need for nutrigenomic studies that can comprehensively assess whether there are divergent effects at the molecular level. We examined the gene expression profiles associated with selenomethionine (SM), sodium selenite (SS), and yeast-derived selenium (YS) in the intestine, gastrocnemius, cerebral cortex, and liver of mice. Weanling mice were fed either a selenium-deficient (SD) diet (<0.01 mg/kg diet) or a diet supplemented with one of three selenium sources (1 mg/kg diet, as either SM, SS or YS) for 100 days. All forms of selenium were equally effective in activating standard measures of selenium status, including tissue selenium levels, expression of genes encoding selenoproteins (Gpx1 and Txnrd2), and increasing GPX1 enzyme activity. However, gene expression profiling revealed that SS and YS were similar (and distinct from SM) in both the expression pattern of individual genes and gene functional categories. Furthermore, only YS significantly reduced the expression of Gadd45b in all four tissues and also reduced GADD45B protein levels in liver. Taken together, these results show that gene expression profiling is a powerful technique capable of elucidating differences in the bioactivity of different forms of selenium.

Keywords Selenium · Genomics · Nutrigenomics · DNA damage

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Introduction

The trace mineral selenium plays a key role in several biological processes, including the response to oxidative stress (Brenneisen et al. 2005), DNA damage and repair (Seo et al. 2002a, b), cancer susceptibility (Rayman 2005), and viral pathogenicity (Beck 2007). Selenium is fed to animals and humans either as inorganic salts, such as sodium selenite (SS) and selenate, as selenomethionine (SM) or as yeast-derived selenium (YS) that contains selenium as protein-bound SM and other less characterized selenium organic compounds (McSheehy et al. 2005; Ip et al. 2000). While some studies suggest that inorganic selenium (selenite or selenate) is less bioavailable or less bioactive than SM or YS (Rider et al. 2010; Mahmoud and Edens 2003; Qin et al. 2007), other studies suggest that the source of selenium does not differentially affect parameters such as growth or expression and activity of selenoproteins (Qin et al. 2007, 2009; Wang et al. 2010). A human study that addressed the effects of different forms of selenium on

selenium-replete subjects suggests that plasma selenium reflects the SM content of yeast and that selenium in the form of SM is better absorbed than SS based on urinary selenium excretion (Burk et al. 2006).

The selection of an appropriate source of selenium for supplementation studies is relevant to human health because extensive epidemiological data suggest a link between selenium status and cancer at various sites, and a number of trials testing the effects of selenium supplementation in cancer chemoprevention have led to positive results (Reid et al. 2006; Duffield-Lillico et al. 2003). One study in humans that involved selenium supplementation in the form of YS was associated with a marked reduction in cancer incidence and mortality (Clark et al. 1996). These and other observations led to the design of the SELECT study, a phase III randomized, placebo-controlled trial testing the role of SM and/or vitamin E supplementation on prostate cancer incidence. The trial was terminated early due to observations suggesting negative effects of selenium and/or vitamin E intake. However, a major concern in the design of this study was the selection of SM as the chemical form of selenium to be used; SM was selected as it is the most abundant selenium chemical form in YS, and also because the chemical composition of independent batches of YS was thought to be variable (Lippman et al. 2005, 2009). Nevertheless, the use of SM in a study designed to confirm a previous study performed with a different chemical form of selenium seems problematic and could yield contradictory results. In the absence of detailed knowledge of the biological properties of different selenium chemical forms, rational choice of chemical source of selenium for chemoprevention studies is not possible.

Because mechanistic studies of different forms of selenium at the molecular level are lacking, we investigated the effects of SS, SM, and YS on several parameters in the mouse, including global gene expression profiles in multiple tissues, the effects on key selenoproteins, and oxidative DNA damage. Our findings suggest striking differences regarding the biological activities of different chemical forms of selenium.

Materials and methods

Animals and diets

Male and female C57BL6/J mice were purchased from Jackson Labs (Bar Harbor, Maine), maintained as breeding pairs, and received LabDiet 5001 ad libitum. Immediately after weaning at 21 days, male mice were randomly assigned to *a* selenium-deficient diet (SD) or a diet containing 1 mg selenium/kg diet from one of three sources: L-selenomethionine (SM, Sigma-Aldrich, St. Louis, Missouri), sodium selenite (SS, Sigma-Aldrich, St. Louis, Missouri), or yeast selenium (YS, Sel-Plex®, Alltech Inc., Nicholasville, Kentucky).

Experimental diets were torula yeast-based diets, prepared by Harlan-Teklad (Madison, WI) and described in detail elsewhere (Rao et al. 2001). We supplemented the SD, SS, and SM diets with an equal amount of non-selenium-enriched yeast (selenium < 0.5 ppm on a product basis) to control for the effects of non-selenium-related yeast components. Selenium levels in dietary premixes were evaluated by atomic absorption spectroscopy (Connolly et al. 2003); selenium level in the SD diet was confirmed to be <0.03 ppm, whereas levels in the supplemented diets were YS $= 1.05$ ppm, SS $= 0.99$ ppm, and SM $= 1.02$ ppm. Mice were housed two to three per cage, and food and water were provided ad libitum. At 100 days of age, mice were euthanized by cervical dislocation, and tissues were rapidly dissected, flash-frozen in liquid nitrogen, and stored at $-80°C$ for later analysis. All procedures were approved by the Animal Care Committee at the William S. Middleton Veterans Administration Hospital.

Selenium analysis in tissues

We focused our studies on four tissues: cerebral cortex, small intestine (3-cm section corresponding to the jejunum), gastrocnemius muscle, and liver. Tissues from seven mice per diet were used for measurement of selenium content using molecular fluorescence spectrometry following wet digestion and reaction with 2,3-diaminonapthalene [described previously (Koh and Benson 1983)]. Bovine liver Standard Reference Material from the National Institute of Standards and Technology was used as a standard. Dietary effects were analyzed by one-way analysis of variance; if the overall treatment effect was statistically significant ($P < 0.05$), differences between individual diets were determined using Tukey's post hoc tests.

Gene expression profiling and pathway analysis

For each of the four tissues described above, we performed gene expression profiling on five mice from each diet. Affymetrix Mouse Genome 430A arrays were used to measure gene expression in the intestine, and Mouse Genome 430 2.0 arrays were used for gastrocnemius muscle, cerebral cortex, and liver. At the time of the analysis, the Mouse Genome 430A array represented 12,445 unique genes, and the Mouse Genome 430 2.0 array represented 20,318 unique genes. Details regarding sample preparation and array hybridization are described elsewhere (Lee et al. 1999). Briefly, total RNA was isolated from individual tissues using TRIzol (Life Technologies) and was processed to biotin-labeled cRNA according to

Affymetrix protocols. Microarrays were scanned with the Affymetrix GeneArray Scanner (Affymetrix), and the value for each RNA abundance was automatically calculated with the Affymetrix GeneChip Analysis Suite version 3.3 after scanning. When a gene was represented by multiple probe sets on an array, only the probe set having the greatest signal intensity (averaged across all 20 arrays within a tissue) was included for analysis. A gene was considered to be significantly changed in expression when the P value for a two-tailed t-test was <0.01.

We performed a pathway analysis using parametric analysis of gene set enrichment (PAGE) to identify gene functional classes that were affected by selenium supplementation (Kim and Volsky 2005). Gene expression data were annotated with functional data from the Gene Ontology (GO) consortium (http://www.geneontology.org). We only analyzed GO terms that were annotated at Level 3 or greater and were represented by at least 10 but not more than 1,000 genes. A GO term was considered to be significantly changed by treatment if the P value was <0.01.

Real-time RT-PCR confirmation of DNA microarray results

To confirm the DNA microarray findings, we used gastrocnemius muscle and liver from the microarray study to measure the expression of two genes changed by all diets using reverse transcriptase quantitative PCR (RT-qPCR; glutathione peroxidase 1, Gpx1, and thioredoxin reductase 2, Txnrd2). The RT-qPCR assay was performed using primers from Applied Biosystems on an Eppendorf realplex^2 instrument using the δ-δC_t method as described previously (Barger et al. 2008a). Beta-2-microglobulin (B2m) and mitochondrial ribosomal protein L13 (Mrpl13) were used as normalizing genes in liver and muscle, respectively, because the microarray data revealed that they were abundantly expressed and unchanged by any selenium treatment. Dietary effects were analyzed by one-way analysis of variance; if the overall treatment effect was statistically significant ($P < 0.05$), differences between individual diets were determined using Tukey's post hoc tests.

Western blot analysis and selenoprotein activity

Microarray analysis also revealed that the growth arrest and DNA-damage-inducible 45 beta gene (Gadd45b) was decreased in expression by one or more selenium sources in every tissue studied. To confirm this finding at the protein level, we quantified the abundance of the protein encoded by this gene by western blotting in liver. Tissues were homogenized on ice in seven volumes of cold protein extraction buffer that consisted of 20 mM HEPES pH 7.9, 125 mM NaCl, 0.1% Igepal (NP-40), 0.1% Triton X-100, and 1 mM EDTA. Homogenates were clarified by centrifugation at 4°C for 10 min at 18,000g. Supernatants were aliquoted and stored at −80°C. Samples for western blotting were electrophoresed in NuPAGE 7% polyacrylamide Tris–acetate gels (Invitrogen) and transferred to nitrocellulose membranes using the iBlot Dry Blotting System (Invitrogen). Membranes were blocked in 0.5% gelatin in TBST (137 mM NaCl, 0.1% Tween-20, 20 mM Tris, pH 7.6) for 1 h. Primary and secondary antibodies were diluted in 0.5% gelatin in TBST. Rabbit polyclonal anti-GADD45β (H-70) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). HRP-linked goat anti-rabbit IgG was purchased from Pierce (Rockford, IL) as part of the Dura SuperSignal West chemiluminescent substrate kit, which was used to detect antibodies. Chemiluminescent bands were visualized and analyzed using a UVP Bioimaging Systems (Upland, CA). MemCode^{TM} Reversible Protein Stain Kit was purchased from Pierce and used to control for protein loading. A loading correction factor based on the memcode protein band intensity data was used to adjust the GADD45β band intensity data.

Glutathione peroxidase and thioredoxin reductase enzyme activities in liver were measured using commercially available kits (Cayman Chemical); protein homogenates were prepared from the same source material used in the microarray analysis, and enzyme activity was measured according to the manufacturer's instructions. Dietary effects were analyzed by one-way analysis of variance; if the overall treatment effect was statistically significant ($P < 0.05$), differences between individual diets were determined using Tukey's post hoc tests.

Oxidative damage to DNA

DNA damage was quantified from DNA isolated from liver tissue of mice using a highly sensitive HPLC/EC/UV system as described previously (Barger et al. 2008b). Samples were compared against a calibration curve with known standards to quantify the levels of the oxidative products 8-hydroxy-2′-deoxyguanosine/10^6 2′-deoxyguanosine (8-oxo-dG). Data were analyzed by one-way analysis of variance; if the overall treatment effect was statistically significant ($P < 0.05$), differences between individual diets were determined using Tukey's post hoc tests.

Results

Effects of experimental diets on tissue selenium

Mice consuming the SD diets had low selenium levels in all tissues examined, below 0.1 μg selenium/g tissue (Fig. 1). When compared to the SD diet, SM and YS

significantly elevated selenium levels in all four tissues; SS elevated tissue selenium levels compared to SD in all tissues except for gastrocnemius. When comparing the different sources of selenium to one another, the effect of diet was different depending on the tissue studied: In cerebral cortex and intestine, levels of selenium were highest in SM, intermediate in YS, and lowest in the SS diet (SM > YS > SS); in gastrocnemius, selenium levels were similar between SM and YS diets, but higher than the SS diet (SM = YS > SS). In liver, selenium levels were highest in the SM, intermediate in the YS, and lowest in the SS diet (SM ≥ YS ≥ SS).

Overview of gene expression patterns

Selenium supplementation with SM, SS, or YS resulted in the differential expression of many (hundreds to thousands) of genes, with some tissues being more responsive than others (Fig. 2). Gastrocnemius and cerebral cortex were the most responsive to selenium supplementation, with 21 and 17%, respectively, of the genes represented on the array being changed in expression by at least one diet. In liver, 12% of the genes represented on the array were changed in expression by at least one diet, and in the intestine, only 4% of the genes represented on the array were changed in expression by at least one diet. Despite the fact that tissue selenium levels were highest in response to the SM diet, SM supplementation affected the expression of the least number of genes (compared to SS and YS). In contrast, tissue selenium levels were lowest in response to the SS diet, but SS affected the expression of more genes than either SM or YS.

For each tissue, there were groups of genes changed in expression by only one selenium source, groups of genes changed by two selenium sources, and a group of genes changed in expression by all three treatments (Fig. 2). In general, if a gene was significantly changed in expression by more than one selenium source, the direction of the change in expression (increased or decreased) was similar for the other treatment(s); this similarity is represented by the fractions within the intersecting regions of Fig. 2. A remarkable finding was that the overlap between SS and YS was higher than that of any other pair of treatments. For example, in the small intestine, 86 genes were significantly changed in expression by both the SS and YS diets,

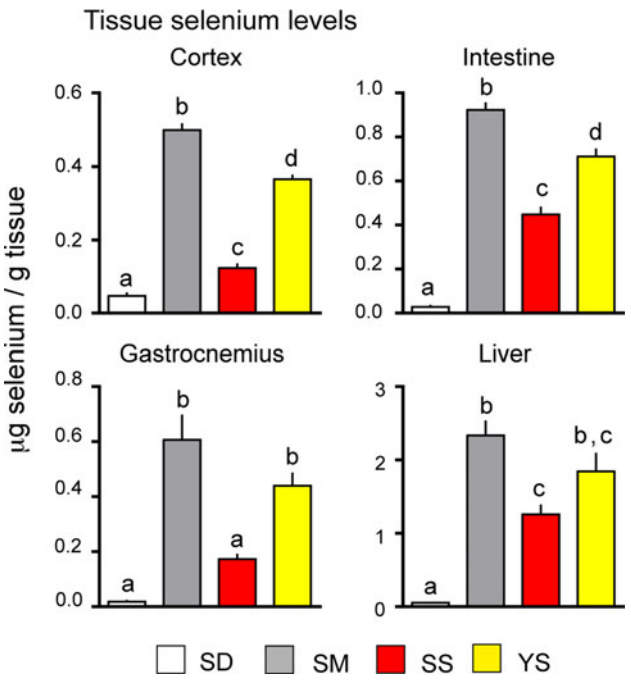


Fig. 1 Tissue levels of selenium (mean + SEM) in four tissues from mice fed a selenium-deficient (*SD*) or selenium-supplemented diet (*SM* selenomethionine, *SS* sodium selenite, and *YS* yeast selenium). Within a tissue, *different letters* indicate a statistically significant difference between treatments ($P < 0.05$)

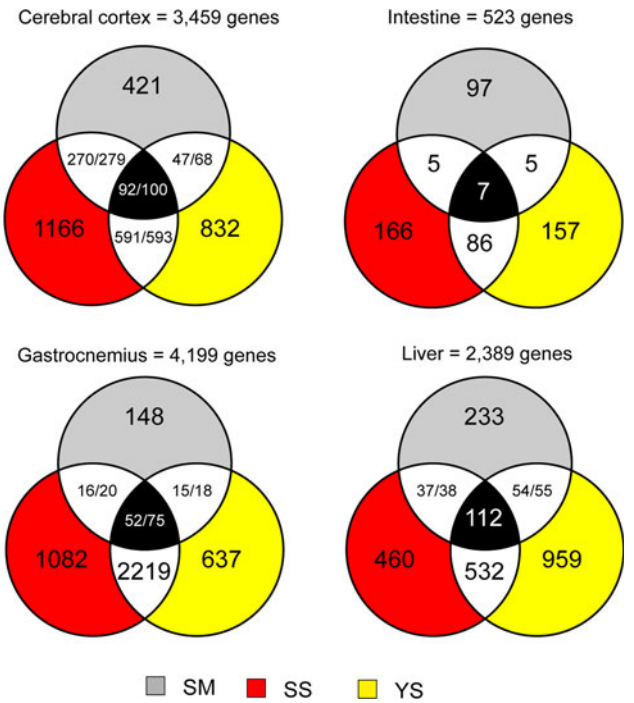


Fig. 2 Effect of selenium supplementation on the number of differentially expressed ($P < 0.01$) genes in four tissues of mice. When genes were changed by more than one treatment (shown in intersecting regions), the *denominator* indicates the total number of genes changed and the *numerator* indicates how many of those genes were changed in the same direction by all treatments. If a ratio is not shown for a given intersection, all genes were changed in the same direction by all treatments. As described in the "Materials and methods," there were fewer genes represented on the array used for the intestine which explains, in part, the lower number of differentially expressed genes in this tissue; the percentage of differentially expressed genes (relative to the total number of genes represented on the array) for each tissue is stated in the "Results"

whereas only five genes were significantly changed by both SM and SS diets and SM and SY diets (an abbreviated list of genes changed in expression by more than one diet in small intestine is shown in Table 1). This large similarity between SS and YS diets was also observed in the other three tissues as shown in the overlap in the Venn diagrams in Fig. 2 (indicated by the greatest number of genes in the overlap between SS and YS circles). A complete list of the individual genes changed in expression by more than one diet for all four tissues is shown in Online Resources 1–4. Thus, the gene expression profile of YS is much more similar to that of SS, despite the fact that SM is a major component of YS. Perhaps surprisingly, there was no gene that was significantly changed in expression at $P < 0.01$ in all tissues by all selenium diets. However, selenoprotein W was increased in expression in all tissues and diets as compared to SD at a lower level of statistical significance ($P < 0.05$, data not shown). As discussed below, Gadd45b was the only gene not related to selenium biology that was changed in expression by multiple diets in every tissue.

Using RT-PCR, we examined the expression of seven genes that showed diet-specific changes in expression in the microarray analysis (Table 2). Three of these genes (Cyp7b1, Sucnr1, and Tsc22d1) showed an identical pattern of gene expression for both the microarray and RT-PCR analyses. For the remaining genes, the direction of the fold change in expression was similar between the two techniques, but the number of significantly different genes varied.

Pathway analysis

Similar to what was observed in the analysis of individual genes (above), pathway analysis revealed that different selenium sources had both individual and overlapping effects on functional classes of genes in each tissue (Online Resource 5–8). Figure 3 represents GO terms changed by at least 2/3 diets and shows that all three diets tended to have similar effects on both gastrocnemius and liver (Fig. 3a, c, respectively), with SM having a less pronounced effect in gastrocnemius. In the intestine and cerebral cortex, however, the overall effect of the SM diet tended to be the opposite of both the SS and YS diets (Fig. 3b, d, respectively).

Because GO term "mitochondrial inner membrane" (GO:0005743) was significantly regulated by at least two treatments in each tissue (Fig. 3a–d), this pathway provides a useful parameter for comparison among the different selenium sources. In gastrocnemius, the class of genes representing the mitochondrial inner membrane was upregulated by all diets; in the liver, this gene class was upregulated by SM and YS; in the intestine, this gene class was upregulated by SM but downregulated by both SS and YS. In cerebral cortex, SM and YS downregulated this class of genes, but SS upregulated this gene class overall. Thus, different sources of dietary selenium clearly have different effects at the gene expression level.

Analysis of selenoproteins

The functional category that was most consistently and robustly modulated by all selenium diets was the GO term "*selenium binding*" (GO:0008430), which is defined as a class of genes that "interact selectively and non-covalently with selenium." This GO term was significantly upregulated by all three diets in every tissue except for cortex (Fig. 4a). The expression of two key genes in this GO term was confirmed by RT-qPCR in liver: Gpx1 and Txnrd2 expression was increased approximately five- and twofold in expression, respectively, by all three diets relative to the SD group (Fig. 4b). Total glutathione peroxidase enzymatic activity in liver was also significantly increased nearly eightfold by all three selenium diets, whereas thioredoxin reductase activity in liver was significantly increased threefold by the SM diet, with an intermediate level of activity in the SS and YS diets (Fig. 4c).

Gadd45b and DNA oxidation

In a search for genes unrelated to selenium biology that were consistently regulated by selenium supplementation across tissues, we observed that the gene Gadd45b was decreased in expression by all selenium diets in cerebral cortex, decreased by SS and YS in gastrocnemius, and decreased by YS only in intestine and liver (Fig. 5a). Gadd45b is thought to be an important mediator of the DNA damage response, and its expression is induced with DNA damage or aging (Liu et al. 2009; Hoffman and Liebermann 2009). Because this gene was decreased in expression by selenomethionine in a previous study (Rao et al. 2001), we expanded upon the microarray findings by measuring the abundance of the protein encoded by this gene. GADD45B protein was not different in SM and SS diets compared to the SD diet, but was significantly reduced by 44% in liver of the YS diet (Fig. 5b). Finally, we measured levels of DNA oxidation (8-oxo-dG) in the liver of mice fed the four diets. The amount of oxidized DNA was not significantly affected by selenium supplementation, though there was a trend toward a decreased level of oxidized DNA (27%) in the YS diet (Fig. 5c).

Discussion

We used a nutrigenomic approach to assess the effects of three different forms of selenium in four tissues from mice. Because all forms of selenium corrected the alterations in

Table 1 Selected microarray data from intestine for genes changed in expression by at least two diets ($P < 0.01$)

Gene symbol	Gene name	FC (SM)	FC (SS)	FC (YS)
Fasl	Fas ligand (TNF superfamily, member 6)	3.63	5.35	nsd
Dolpp1	Dolichyl pyrophosphate phosphatase 1	1.37	1.46	nsd
Srsf2ip	Serine/arginine-rich splicing factor 2, interacting protein	−1.73	−1.82	nsd
Pla1a	Phospholipase A1 member A	1.84	nsd	2.26
Txnrd1	Thioredoxin reductase 1	1.7	nsd	1.86
Odc1	Ornithine decarboxylase, structural 1	1.44	nsd	1.64
Aadat	Aminoadipate aminotransferase	−2.84	nsd	−2.91
Lrp5	Low-density lipoprotein receptor-related protein 5	nsd	4.25	3.92
Dmpk	Dystrophia myotonica-protein kinase	nsd	2.99	3.32
Anpep	Alanyl (membrane) aminopeptidase	nsd	2.45	2.62
Polr2a	Polymerase (RNA) II (DNA directed) polypeptide A	nsd	2.09	2.52
Rasa2	RAS p21 protein activator 2	nsd	2.65	2.33
Scly	Selenocysteine lyase	nsd	1.89	2.33
Leng8	Leukocyte receptor cluster (LRC) member 8	nsd	2.22	2.29
Oxct1	3-Oxoacid CoA transferase 1	nsd	2.24	2.23
Dak	Dihydroxyacetone kinase 2 homolog (yeast)	nsd	1.83	2.23
2210023G05Rik	RIKEN cDNA 2210023G05 gene	nsd	2.52	2.2
Syvn1	Synovial apoptosis inhibitor 1, synoviolin	nsd	1.93	2.18
Ncstn	Nicastrin	nsd	1.9	2.18
Brwd1	Bromodomain and WD repeat domain containing 1	nsd	2.38	2.1
Fmo5	Flavin containing monooxygenase 5	nsd	2.27	2.06
Cic	Capicua homolog (*Drosophila*)	nsd	2.06	2.05
Tra2a	Transformer 2 alpha homolog (*Drosophila*)	nsd	2.21	2.04
Mical2	Microtubule associated monoxygenase, calponin and LIM domain containing	nsd	1.98	2.03
Src	Rous sarcoma oncogene	nsd	2.01	2
Gbf1	Golgi-specific brefeldin A-resistance factor 1	nsd	1.98	2
Slc30a9	Solute carrier family 30 (zinc transporter), member 9	nsd	−1.66	−1.75
Tprkb	Tp53rk-binding protein	nsd	−1.5	−1.79
Ppp1cb	Protein phosphatase 1, catalytic subunit, beta isoform	nsd	−1.9	−1.82
Fzd4	Frizzled homolog 4 (*Drosophila*)	nsd	−1.99	−1.89
Nans	*N*-acetylneuraminic acid synthase (sialic acid synthase)	nsd	−1.99	−1.97
Cpd	Carboxypeptidase D	nsd	−2.02	−2.04
Homer2	Homer homolog 2 (*Drosophila*)	nsd	−1.95	−2.25
Ndufab1	NADH dehydrogenase (ubiquinone) 1, alpha/beta subcomplex, 1	nsd	−2.45	−3.07
Sepw1	Selenoprotein W, muscle 1	4.86	8.15	7.15
2700094K13Rik	RIKEN cDNA 2700094K13 gene	5.25	3.82	4.73
Gpx1	Glutathione peroxidase 1	4.34	4.06	4.69
Gpx3	Glutathione peroxidase 3	3.63	2.74	2.95
Dio1	Deiodinase, iodothyronine, type I	1.95	2.87	2.28
Pias1	Protein inhibitor of activated STAT 1	1.27	1.61	1.63
Pnpo	Pyridoxine 5′-phosphate oxidase	1.25	1.2	1.31

See Online Resource 3 for complete list

expression of genes encoding selenium-binding proteins and also corrected the reduced glutathione peroxidase activity in the SD diet, we conclude that the different forms of selenium (fed a 1 ppm of the diet) are equivalent in their ability to correct a selenium deficiency. Nonetheless, there appear to be distinct effects of the different forms of selenium: We observed that yeast-derived selenium (YS) results in overall gene expression profiles that are similar to those of SS, despite the fact that YS is thought to contain selenomethionine as the major selenium source (McSheehy

Table 2 Comparison between microarray and RT-PCR analyses for selected genes

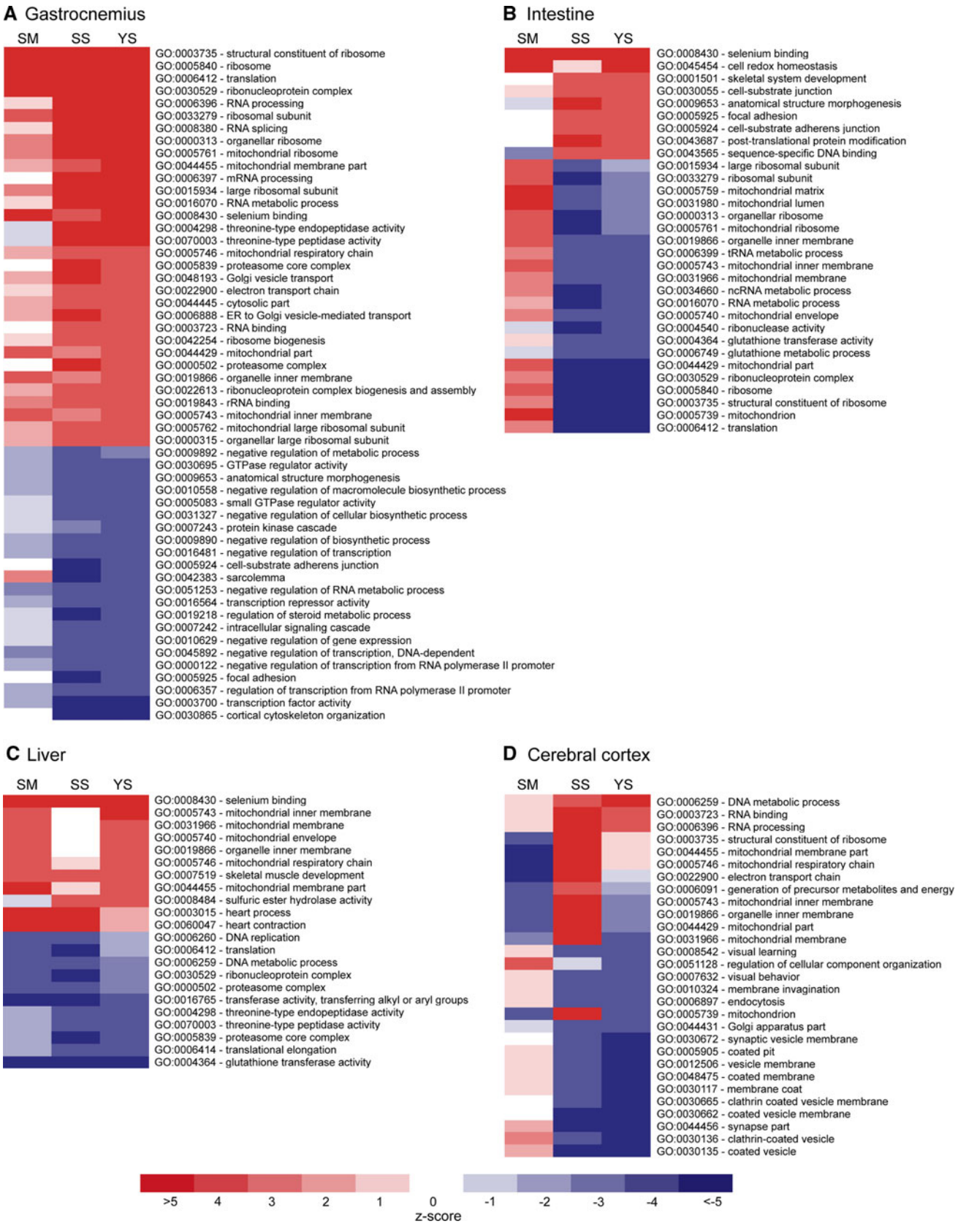
Gene	Tissue	Microarray fold change (*P* value)			RT-PCR fold change (*P* value)		
		SM	SS	YS	SM	SS	YS
Gadd45b	Gastrocnemius	1.02 (0.934)	−2.42 (0.011)	−2.16 (0.011)	−1.16 (0.407)	−1.48 (0.183)	−1.87 (0.039)
Crkl	Gastrocnemius	−1.02 (0.801)	−1.29 (0.002)	−1.27 (0.001)	−1.25 (0.000)	−1.30 (0.000)	−1.81 (0.000)
Tsc22d1	Liver	1.2 (0.591)	1.63 (0.136)	2.24 (0.002)	−1.48 (0.136)	1.29 (0.271)	2.43 (0.030)
Sucnr1	Liver	1.12 (0.713)	1.25 (0.361)	1.75 (0.008)	1.03 (0.919)	1.03 (0.151)	2.17 (0.029)
Mapre2	Liver	−1.30 (0.258)	−3.10 (0.002)	−3.71 (0.001)	−1.33 (0.001)	−1.33 (0.001)	−1.84 (0.001)
Cyp7b1	Liver	1.12 (0.758)	1.62 (0.181)	1.47 (0.002)	1.24 (0.701)	1.61 (0.201)	1.60 (0.009)
Nfe2l2	Liver	1.00 (0.986)	1.48 (0.007)	1.59 (0.002)	-1.28 (0.081)	1.30 (0.357)	1.43 (0.027)

et al. 2005; Ip et al. 2000). In addition, pathway analysis suggests that SM, SS, and YS differentially impact key cellular functions, including mitochondrial function and metabolism. Finally, we found that only YS is associated with a pattern of decreased DNA damage.

We note that all selenium sources resulted in a large number of significant changes in gene expression, ranging from 4 to 21% of all genes represented in the array for intestine and gastrocnemius, respectively. This finding is in agreement with our previous study of the effects of selenium in mouse intestine, which showed changes in the expression of a large number of genes in response to different selenium sources (Rao et al. 2001). Given the central role of selenium in the cellular antioxidant system, it appears likely that most changes in gene expression in response to alterations in selenium status are secondary to alterations in redox status. Indeed, many transcriptional factors are redox-regulated, including NF-kB (Kabe et al. 2005), NRF2 (Giudice et al. 2010), and the FOXO family of transcriptional factors (Keizer et al. 2011). As an example of such effects, we note that the selenium-dependent thioredoxin reductase modulates thioredoxin activity, which directly regulates the activity of NF-kB and AP-1 (Hirota et al. 1997). Thus, changes in the activity of multiple redox-sensitive transcription factors may lead to alterations in the expression of a large number of genes in response to changes in selenium status.

The observation that gene expression patterns and gene functional categories are highly similar between SS and YS diets was surprising, given that selenomethionine is thought to be the principal selenium form in yeast-derived selenium (McSheehy et al. 2005; Ip et al. 2000). We note that we observed similar effects of SM on the mouse intestine as previously reported by Kipp et al. (2009), including increases in the expression of pathways linked to translation, ribosomal proteins, and RNA processing. One explanation for the differential effects at the gene expression level between selenomethionine and YS in our study is that most selenomethionine found in YS is bound to proteins, whereas selenomethionine in our study was provided as the free amino acid. Differences in metabolism of free and protein-bound selenomethionine may thus account for some of the differences that we observed. A second explanation is that other as yet uncharacterized selenium compounds present in YS account for the differential effects. Yeast-derived selenium contains several organic selenium compounds in addition to SM, as well as the putative cancer chemopreventive compound Se-methyl-Se-cysteine (CH_3SeCys) (McSheehy et al. 2005; Ip et al. 2000, 2002). Yet, another possibility is that free selenomethionine is more readily oxidized than methionine, forming a selenoxide as the reaction product (Zainal et al. 1998). Therefore, dietary SM as a free amino acid may not absorbed in this form but instead converted to a biologically less active derivative. Dietary SM can be incorporated nonspecifically into proteins or trans-selenated into SeCys and subsequently H_2Se, a compound that plays a central role in selenium metabolism and serves as a precursor of selenophosphate. Selenophosphate serves as a precursor to selenoprotein synthesis, as well as a precursor to methylselenol, a putative cancer chemopreventive form of selenium (Ip et al. 2002). In contrast, inorganic forms such as SS undergo reductive metabolism, also yielding H_2Se. Because our data suggest striking similarities between SS and YS at the gene expression level, it is possible that similar to SS, the SM found in YS is preferentially metabolized to H_2Se as compared to free SM. Poor selenium absorption from SM is unlikely to account for our observations, given that SM resulted in high tissue levels of selenium in our study, and recent findings in humans showing higher selenium absorption when provided as SM when compared to YS and SS (Reid et al. 2006).

There is an interest in the relationship among selenium status, DNA damage, and cancer chemoprevention (Rayman 2005). A study that examined individuals in a high-risk group for prostate cancer development identified a significant inverse correlation between DNA damage in leukocytes and serum selenium levels (Waters et al. 2005). Carriers of a BRCA1 mutation are at high risk for breast cancer development, and this mutation is associated with


A Gastrocnemius
SM SS YS
GO:0003735 - structural constituent of ribosome
GO:0005840 - ribosome
GO:0006412 - translation
GO:0030529 - ribonucleoprotein complex
GO:0006396 - RNA processing
GO:0033279 - ribosomal subunit
GO:0008380 - RNA splicing
GO:0000313 - organellar ribosome
GO:0005761 - mitochondrial ribosome
GO:0044455 - mitochondrial membrane part
GO:0006397 - mRNA processing
GO:0015934 - large ribosomal subunit
GO:0016070 - RNA metabolic process
GO:0008430 - selenium binding
GO:0004298 - threonine-type endopeptidase activity
GO:0070003 - threonine-type peptidase activity
GO:0005746 - mitochondrial respiratory chain
GO:0005839 - proteasome core complex
GO:0048193 - Golgi vesicle transport
GO:0022900 - electron transport chain
GO:0044445 - cytosolic part
GO:0006888 - ER to Golgi vesicle-mediated transport
GO:0003723 - RNA binding
GO:0042254 - ribosome biogenesis
GO:0044429 - mitochondrial part
GO:0000502 - proteasome complex
GO:0019866 - organelle inner membrane
GO:0022613 - ribonucleoprotein complex biogenesis and assembly
GO:0019843 - rRNA binding
GO:0005743 - mitochondrial inner membrane
GO:0005762 - mitochondrial large ribosomal subunit
GO:0000315 - organellar large ribosomal subunit
GO:0009892 - negative regulation of metabolic process
GO:0030695 - GTPase regulator activity
GO:0009653 - anatomical structure morphogenesis
GO:0010558 - negative regulation of macromolecule biosynthetic process
GO:0005083 - small GTPase regulator activity
GO:0031327 - negative regulation of cellular biosynthetic process
GO:0007243 - protein kinase cascade
GO:0009890 - negative regulation of biosynthetic process
GO:0016481 - negative regulation of transcription
GO:0005924 - cell-substrate adherens junction
GO:0042383 - sarcolemma
GO:0051253 - negative regulation of RNA metabolic process
GO:0016564 - transcription repressor activity
GO:0019218 - regulation of steroid metabolic process
GO:0007242 - intracellular signaling cascade
GO:0010629 - negative regulation of gene expression
GO:0045892 - negative regulation of transcription, DNA-dependent
GO:0000122 - negative regulation of transcription from RNA polymerase II promoter
GO:0005925 - focal adhesion
GO:0006357 - regulation of transcription from RNA polymerase II promoter
GO:0003700 - transcription factor activity
GO:0030865 - cortical cytoskeleton organization
B Intestine
SM SS YS
GO:0008430 - selenium binding
GO:0045454 - cell redox homeostasis
GO:0001501 - skeletal system development
GO:0030055 - cell-substrate junction
GO:0009653 - anatomical structure morphogenesis
GO:0005925 - focal adhesion
GO:0005924 - cell-substrate adherens junction
GO:0043687 - post-translational protein modification
GO:0043565 - sequence-specific DNA binding
GO:0015934 - large ribosomal subunit
GO:0033279 - ribosomal subunit
GO:0005759 - mitochondrial matrix
GO:0031980 - mitochondrial lumen
GO:0000313 - organellar ribosome
GO:0005761 - mitochondrial ribosome
GO:0019866 - organelle inner membrane
GO:0006399 - tRNA metabolic process
GO:0005743 - mitochondrial inner membrane
GO:0031966 - mitochondrial membrane
GO:0034660 - ncRNA metabolic process
GO:0016070 - RNA metabolic process
GO:0005740 - mitochondrial envelope
GO:0004540 - ribonuclease activity
GO:0004364 - glutathione transferase activity
GO:0006749 - glutathione metabolic process
GO:0044429 - mitochondrial part
GO:0030529 - ribonucleoprotein complex
GO:0005840 - ribosome
GO:0003735 - structural constituent of ribosome
GO:0005739 - mitochondrion
GO:0006412 - translation
C Liver
SM SS YS
GO:0008430 - selenium binding
GO:0005743 - mitochondrial inner membrane
GO:0031966 - mitochondrial membrane
GO:0005740 - mitochondrial envelope
GO:0019866 - organelle inner membrane
GO:0005746 - mitochondrial respiratory chain
GO:0007519 - skeletal muscle development
GO:0044455 - mitochondrial membrane part
GO:0008484 - sulfuric ester hydrolase activity
GO:0003015 - heart process
GO:0060047 - heart contraction
GO:0006260 - DNA replication
GO:0006412 - translation
GO:0006259 - DNA metabolic process
GO:0030529 - ribonucleoprotein complex
GO:0000502 - proteasome complex
GO:0016765 - transferase activity, transferring alkyl or aryl groups
GO:0004298 - threonine-type endopeptidase activity
GO:0070003 - threonine-type peptidase activity
GO:0005839 - proteasome core complex
GO:0006414 - translational elongation
GO:0004364 - glutathione transferase activity
D Cerebral cortex
SM SS YS
GO:0006259 - DNA metabolic process
GO:0003723 - RNA binding
GO:0006396 - RNA processing
GO:0003735 - structural constituent of ribosome
GO:0044455 - mitochondrial membrane part
GO:0005746 - mitochondrial respiratory chain
GO:0022900 - electron transport chain
GO:0006091 - generation of precursor metabolites and energy
GO:0005743 - mitochondrial inner membrane
GO:0019866 - organelle inner membrane
GO:0044429 - mitochondrial part
GO:0031966 - mitochondrial membrane
GO:0008542 - visual learning
GO:0051128 - regulation of cellular component organization
GO:0007632 - visual behavior
GO:0010324 - membrane invagination
GO:0006897 - endocytosis
GO:0005739 - mitochondrion
GO:0044431 - Golgi apparatus part
GO:0030672 - synaptic vesicle membrane
GO:0005905 - coated pit
GO:0012506 - vesicle membrane
GO:0048475 - coated membrane
GO:0030117 - membrane coat
GO:0030665 - clathrin coated vesicle membrane
GO:0030662 - coated vesicle membrane
GO:0044456 - synapse part
GO:0030136 - clathrin-coated vesicle
GO:0030135 - coated vesicle
>5 4 3 2 1 0 -1 -2 -3 -4 <-5
z-score

◀ **Fig. 3** Analysis of Gene Ontology (*GO*) terms changed by selenium supplementation in four tissues of mice. Terms shown are those significantly changed by at least 2/3 treatments in each tissue ($P < 0.001$). *Red* indicates a GO term that was upregulated by treatment (z-score > 0); *blue* indicates GO terms that were downregulated by treatment (z-score < 0)

increased DNA breakage in response to the oxidant bleomycin (Kowalska et al. 2005). Supplementation with SS normalizes chromosome breakage in this group, supporting a role for selenium in preventing DNA damage or enhancing its repair (Kowalska et al. 2005). However, studies performed in dogs have shown a complex U-shaped relationship between DNA damage in the prostate and selenium levels in response to supplementation (Waters et al. 2003, 2005). We have previously reported, using a similar experimental design, that a selenium-deficient diet is associated with the induction of genes linked to DNA damage and oxidative stress in the intestine, including Gadd45b (Rao et al. 2001). Based on these observations, we suggested that the gene expression profile of low selenium status may be associated with tumorigenesis (Rao et al. 2001). We examined the expression of the DNA damage response gene Gadd45b and found that YS consistently lowered its expression in all tissues tested. SS lowered Gadd45b expression in cortex and gastrocnemius, and SM only reduced its expression in cortex. We also observed that in liver, YS significantly reduced the abundance of the protein encoded by the Gadd45b gene. Finally, we measured the levels of a marker of oxidative DNA

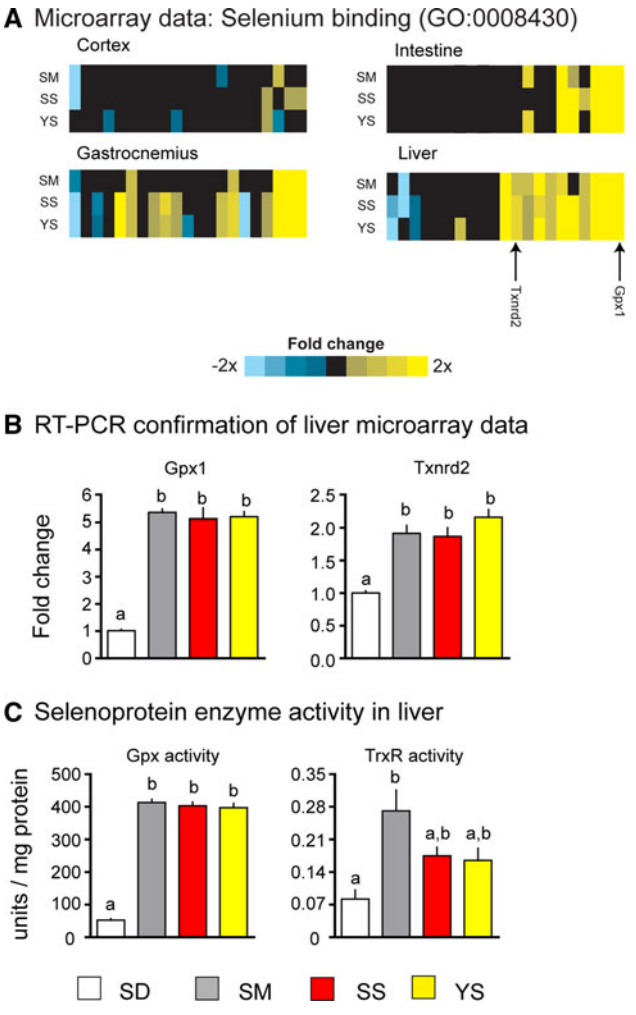


Fig. 4 Effect of selenium supplementation on selenoprotein gene expression and enzyme activity. Values are means ± SEM; values with *different letters* indicate statistically significant differences ($P < 0.05$)

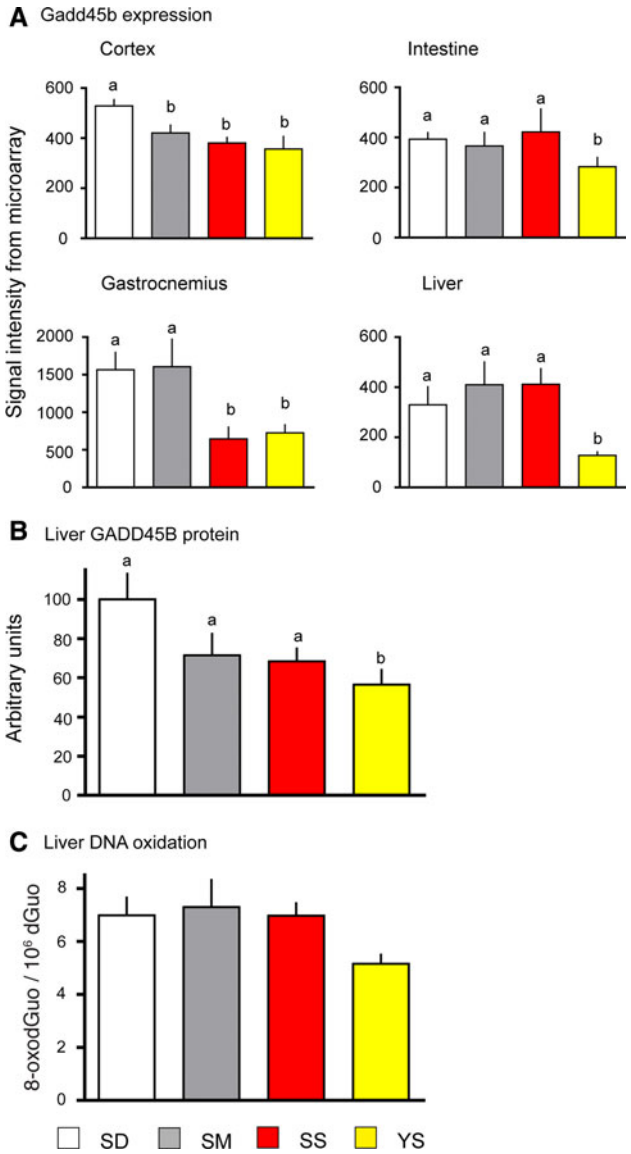


Fig. 5 Effect of selenium supplementation on markers of DNA damage. Values are means ± SEM; values with *different letters* indicate statistically significant differences ($P < 0.05$)

damage (8-oxo-dG) in the liver and found a 27% reduction in 8-oxo-dG in the YS diet, though this was not statistically significant. These findings are consistent with the recent observation that the same source of YS used in our study significantly reduced both RNA and DNA oxidative damage in the brain in the APP1/PS1 mouse model of Alzheimer's disease (Lovell et al. 2009). Thus, if increased Gadd45b observed with SD reflects chronic cellular stress, in our studies, YS appears to be the most effective form of selenium opposing this stress.

Overall, our study suggests that the different forms of selenium had a similar effect on the expression of selenium-dependent genes and selenoenzyme activity; however, the sources of selenium had differential effects on the overall gene expression patterns (as noted by the similarity between SS and YS) as well as on specific functional pathways related to mitochondrial structure and function. Importantly, we observed that YS alone was associated with an enhanced protection against DNA damage. We note that the SELECT cancer chemoprevention trial was designed partly in response to the previous findings of the Nutrition Prevention of Cancer (NPC) trial, which showed a 63% reduction in prostate cancer in individuals receiving 200 μg/day of yeast-derived selenium (Lippman et al. 2005). The divergent gene expression profiles of SM, SS, and YS revealed in this study clearly support the notion of non-equivalency for various chemical forms of selenium and raise the possibility that the choice of selenium source had an impact on the conflicting results of the NPC and SELECT trials. We suggest that the published data regarding the effect of selenium should be re-evaluated with respect to the source of selenium that was administered.

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