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Selenium-enriched milk proteins and selenium yeast affect selenoprotein activity and expression differently in mouse colon

Ying Hu*, Graeme H. McIntosh, Richard K. Le Leu and Graeme P. Young

Flinders Centre for Cancer Prevention and Control, Flinders University of South Australia, Bedford Park 5042, Australia

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Certain forms of dietary Se may have an advantage in improving Se status and reducing cancer risk. The present study compared the effects of an Se-enriched milk protein product (dairy-Se) with an Se yeast (yeast-Se) on selenoprotein activity and expression in the mouse colon. Mice were fed four diets for 4 weeks: a control milk protein diet (Se at 0·068 parts per million (ppm)), dairy-Se diets with Se at 0·5 and 1 ppm, and a yeast-Se diet with Se at 1 ppm. Cytosolic glutathione peroxidase-1 (GPx-1) activity, mRNA of selenoprotein P (*SeP*), *GPx-1*, gastrointestinal glutathione peroxidase-2 (*GPx-2*) and thioredoxin reductase-1 (*TrxR-1*) were examined in the mouse colon. Dairy-Se diets did not significantly affect *GPx-1* mRNA and GPx-1 activity but produced a dose-dependent increase in *SeP* and *GPx-2* mRNA, with a significantly higher level achieved at 1 ppm Se ($P<0·05$). Yeast-Se at 1 ppm significantly increased *GPx-1* mRNA and GPx-1 activity ($P<0·01$) but not *GPx-2* mRNA. Neither Se supplement had any effect on *TrxR-1*. The present study indicates that selenoprotein levels in the mouse colon are regulated differently depending on the Se supplement. As we have previously shown that dairy-Se at 1 ppm was protective against colorectal cancer (CRC) in an azoxymethane-induced CRC mouse model, this up-regulation of colonic *GPx-2* and *SeP* with Se supplementation may be crucial to its chemopreventive action.

Selenium: Selenoproteins: Colon cancer prevention

Se, as an essential micronutrient, is required for a number of metabolically important enzymes, and its importance for human health and prevention of disease is well established[1]. Se deficiency predisposes to a variety of major human diseases including cancer[2]; conversely, intakes of Se above the normal recommended nutritional intake (supra-nutritional) are associated with reduced risk for a range of cancers[3]. In fact, Se intakes in many parts of the world are below the present dietary reference values because commonly consumed foods are often poor Se sources[4]. Consequently, Se-enriched foods are likely to be beneficial for increasing human Se intake, and perhaps, reducing cancer risk. For instance, Se-enriched plant foods have been shown to significantly protect against colorectal cancer (CRC) in animal models[5,6].

An Se-enriched milk protein product (dairy-Se) has recently been developed as a novel food product by Tatura Milk Industries of Australia. Milk is not normally a major dietary source of Se, but Se concentration in milk proteins can be readily increased to 5 parts per million (ppm) by feeding appropriate Se sources to cows[7,8]. We have shown that such a dairy source of Se produced higher plasma Se levels and significantly suppressed colon cancer incidence and relevant biomarkers of CRC risk, for example, aberrant crypt foci relative to equivalent Se amounts as Se yeast (yeast-Se)[9]. This suggests that delivery of Se through dairy products potentially provides a good opportunity for safely improving human Se status and in the longer term reducing the risk of CRC.

Se is essential for a wide range of biological functions, which are mediated by at least twenty-five selenoproteins[10]; some selenoproteins are particularly relevant to anticancer function in the gastrointestinal tract[11,12], such as cytosolic glutathione peroxidase (GPx-1), gastrointestinal glutathione peroxidase (GPx-2), selenoprotein P (SeP) and thioredoxin reductase-1 (TrxR-1). For instance, a link between selenoproteins and colon cancer risk has been reported by genetic data and animal models[13–16] and functional polymorphisms in selenoprotein genes have also been linked to human cancer risk[17]. It has been proposed that genetic variation in selenoprotein genes could affect their function(s), their response to dietary Se intake and cancer risk[14]. As far as Se and selenoproteins are concerned, studies so far have mostly relied on the assessment of blood or plasma SeP concentration and GPx-1 activity, or focused on Se deficiency and alteration in selenoprotein level[18], or compared selenoprotein expression pattern between cancers and normal tissues[11,19–21]. How dietary supplementation of Se may influence selenoprotein activity and expression in the colon has been examined only in a few animal studies[22]; those studies did not include giving animals diets with higher Se levels than the normal recommended nutritional range. Activity or expression of specific selenoproteins in target tissues is likely to provide considerable insights into the possible involvement of those selenoproteins in health benefits including cancer prevention[19]. The purpose of the present study was to compare a dairy-Se with a yeast-Se for

Abbreviations: CRC, colorectal cancer; dairy-Se, Se-enriched milk proteins; GPx-1, cytosolic glutathione peroxidase-1; GPx-2, gastrointestinal glutathione peroxidase-2; ppm, parts per million; SeP, selenoprotein P; TrxR-1, thioredoxin reductase-1; yeast-Se, Se yeast.

* **Corresponding author:** Dr Ying Hu, fax +61 8 8204 3943, email ying.hu@flinders.edu.au

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their effects on *SeP*, *GPx-1*, *GPx-2* and *TrxR-1* expression and GPx-1 activity in the mouse colon.

Materials and methods

Selenium supplements

Dairy-Se (TaturaBio®Se) was produced by Tatura Milk Industries (Tatura, VIC, Australia). It is a milk protein isolate with a high Se concentration (about 5 ppm), compared with control milk proteins (0·34 ppm); Yeast-Se (Sel-Plex®; 1800 μg Se/g dry weight) was provided by Alltech Biotechnology P/L (Dandenong South, VIC, Australia).

Animals

A total of forty-eight wild-type male mice of the C57BL/6J strain were obtained from the Animal Resource Centre, Perth, Australia. Animal protocols were approved by the Animal Welfare Committee at Flinders University of South Australia (reference 593/04). Mice were divided randomly into four equal experimental groups, housed in cages (four per cage) and maintained in a temperature- and humidity-controlled animal facility with a 12 h light–dark cycle at 22 ± 2°C temperature and 80 ± 10 % humidity. Mice were given free access to water.

Diets

The experimental diets fed to the mice were based on a modified form of the American Institute of Nutrition (AIN)-76A diet for rodents[23] and have been described by us previously[9]. Control milk proteins and dairy-Se were used as protein sources; however, because the dairy protein sources have relatively high Ca concentrations, Ca was not included in the diets. The four diet groups were: (1) milk protein control diet (Se at 0·068 ppm); (2) dairy-Se diet (Se at 0·5 ppm); (3) dairy-Se diet (Se at 1 ppm); (4) milk protein control + yeast-Se diet (Se at 1 ppm). Details of the diets are provided in Table 1.

Experimental procedures

Mice, aged 10 weeks, were assigned to each of the four diets (twelve mice per group). After 4 weeks on the diet, mice were killed by cardiac puncture after ketamine–xylazine anaesthesia. Part of the colon was placed in RNAlater® (Ambion, Austin, TX, USA) solution at 4°C for 24 h, and stored at −80°C until real-time PCR analysis; the remaining colon was fresh frozen immediately in liquid N_2 and stored at −80°C for assay of GPx-1 activity.

Assay of glutathione peroxide-1 activity

GPx-1 activity in the mouse colon was measured by a commercially available Glutathione Peroxidase Cellular Activity Assay Kit (Sigma, Sydney, NSW, Australia), using cumene hydroperoxide as a substrate. The colon was cut open, the mucosa scraped off and homogenised in a buffer containing 1 M-2-amino-2-hydroxymethyl-propane-1,3-diol (Tris) (pH 7·6) and 0·5 M-EDTA and centrifuged at 9391 ***g*** for 20 min at 4°C. The protein concentration was quantified using a Protein Assay Kit (Bio-Rad, Hercules, CA, USA). GPx-1 activity was determined in duplicate using 2·5–5 μl of the supernatant fraction (15–30 μg of proteins), assayed in a 100 μl reaction volume containing 5 mM-NADPH, 30 mM-H_2O_2 and 42 mM-reduced glutathione. The oxidation of NADPH to NADP was monitored at 340 nm on a UV-Vis spectrophotometer. A quantity of 1 unit of glutathione peroxidase will cause the formation of 1 μmol of NADP from NADPH per min in the presence of reduced glutathione, glutathione reductase, and *tert*-butyl hydroperoxide. GPx-1 activity was expressed as U/mg protein.

RNA isolation and cDNA synthesis

Total RNA was extracted from the mouse colon (30 mg) using a QIAGEN RNeasy Mini Kit (QIAGEN, Hilden, Germany). The concentration and purity of the total RNA was estimated using a NanoDrop® ND-1000 UV-Vis spectrophotometer by measuring the absorbance at 260 and 280 nm. All RNA samples had a 260:280 absorbance ratio between 1·9 and 2·1. First-strand cDNA (20 μl) was synthesised from 0·3 μg

Table 1. Composition of experimental diets (g/100 g diet)

Ingredient	Control diet (Se at 0·1 ppm)	Dairy-Se diet (Se at 0·5 ppm)	Dairy-Se diet (Se at 1 ppm)	Yeast-Se diet (Se at 1 ppm)
Casein*	0	0	0	0
Milk protein*	20	10	0	20
Tatura-Bio®Se*†	0	10	20	0
Sucrose	20	20	20	20
Maize starch	31·3	31·3	31·3	31·2
Fibre (α cell)	5	5	5	5
Sunflower-seed oil	19	19	19	19
Choline	0·2	0·2	0·2	0·2
Mineral mix	3·5	3·5	3·5	3·5
Vitamin mix	1	1	1	1
Sel-Plex®†	0	0	0	0·1

ppm, Parts per million; dairy-Se, Se-enriched milk proteins; yeast-Se, Se yeast.

* Milk protein was used as the protein source for the control diet and the yeast-Se diet; Tatura-Bio®Se (Tatura Milk Industries, Tatura, VIC, Australia) was used as the protein source for the dairy-Se diets (Se at 0·5 and 1 ppm).

† Tatura-Bio®Se was used as the Se source for the dairy-Se diets (Se at 0·5 and 1 ppm); Sel-Plex® (Alltech Biotechnology P/L, Dandenong South, VIC, Australia) was used as the Se source for the yeast-Se diet.

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Table 2. Oligonucleotide primers used for real-time PCR

Gene	Gene accession no.	Primers	Primer sequence 5′–3′
SeP	NM_009155	Sense	TTGGTTTGCCTTACTCCTTCCT
		Antisense	TTGTGGTGGCTATGAGCCTCT
GPx-1	NM_008160	Sense	ATCAGTTCGGACACCAGGAG
		Antisense	TCACCATTCACTTCGCACTTC
GPx-2	NM_030677	Sense	TAGTTCTCGGCTTCCCTTGC
		Antisense	AAGACAGGATGCTCGTTCTGC
TrxR	NM_015762	Sense	TATGTCGCCTTGGAATGTGCAG
		Antisense	ATGGTCTCCTCGCTGTTTGTG
GAPDH	NM_008935	Sense	AACATCATCCCTGCATCCAC
		Antisense	TTGAAGTCRCAGGAGACAAC

SeP, selenoprotein P; *GPx-1*, cytosolic glutathione peroxidase-1; *GPx-2*, gastrointestinal glutathione peroxidase-2; *TrxR*, thioredoxin reductase; *GAPDH*, glyceraldehyde 3-phosphate dehydrogenase.

total RNA for each sample using a QIAGEN QuantiTect Reverse Transcription Kit (QIAGEN). cDNA was diluted 1:30 with nuclease-free water and used for real-time PCR.

Real-time PCR

Real-time quantitative PCR of the four genes was performed in triplicate on a Rotor-Gene 3000 Cycler (Corbett, Sydney, NSW, Australia). Oligonucleotide primers were designed using Primer Express software v. 1.5 (Applied Biosystems, Inc., Foster City, CA, USA), based on sequences from the Genbank database (Table 2). All PCR reagents were purchased from QIAGEN. The PCR reaction was determined in a 20 μl final volume containing 6 μl of diluted cDNA and 2 × QuantiTect SYBR Green PCR Kit. The primer concentration for each gene was 10 μM (forward and reverse primer). The cycling protocol started with an initial hot-start at 95°C for 15 min, followed by forty-five cycles at 94°C for 15 s, 60°C for 30 s and 72°C for 30 s, and finished with a final extension at 72°C for 4 min. The specificity of PCR was confirmed by melting-curve analysis with only one peak being present for PCR products of *SeP*, *GPx-1*, *GPx-2* and *TrxR-1* genes, and of the housekeeping gene of glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*). For each PCR run, a non-template reaction was included as negative controls.

Cycle thresholds were determined using the relative quantification analysis module in the Rotor-Gene 3000 Series software (Corbett). The amplification efficiency of each primer pair was estimated from a real-time PCR dilution curve generated using serial dilutions of cDNA. Real-time quantitative PCR analysis was then performed using Q-Gene software[24]; with the amplification efficiency applied to the relative concentration analyses of both the genes of interest and the housekeeping gene (*GAPDH*). Gene of interest expression data were normalised by dividing the corresponding levels of *GAPDH* for each sample.

Statistical analyses

Statistical analyses were performed using SPSS for Windows, version 14.0 (SPSS Inc., Chicago, IL, USA). Data were expressed as mean values with their standard errors. Between-group comparisons for each gene were assessed using one-way ANOVA with correction for multiple comparisons by Tukey's *post hoc* test. Differences between groups were considered significant when $P<0{\cdot}05$.

Results

Effects of dietary selenium intake on cytosolic glutathione peroxidase-1 activity in mouse colon

The effects of dietary Se intake on mouse colon GPx-1 activity are shown in Table 3. After 4 weeks on the diets with different Se forms and concentrations, GPx-1 activity differed in mouse colon across the four diets. GPx-1 activity in mice fed the yeast-Se diet with Se at 1 ppm was significantly higher (8·12 (SEM 0·63) U/mg protein) than in those on the control diet (5·85 (SEM 0·63) U/mg protein) ($P<0{\cdot}01$). However, GPx-1 activity in mice fed dairy-Se diets did not differ significantly across the doses tested; it was 6·15 (SEM 0·57) U/mg protein in mice fed at 0·5 ppm and 5·95 (SEM 0·75) U/mg protein in mice fed at 1 ppm.

Expression of selenoprotein P, cytosolic glutathione peroxidase-1, gastrointestinal glutathione peroxidase-2 and thioredoxin reductase-1 mRNA in mouse colon

Selenoprotein gene expression was analysed by quantitative real-time PCR. The expression pattern of *SeP*, *GPx-1*, *GPx-2* and *TrxR-1* in the mouse colon was comparable with those reported in previous studies in human subjects and rodents[25,26]. Our data showed that *SeP* was the major selenoprotein expressed in mouse colon with a relative expression level of 2·56 (SEM 0·33), followed by *GPx-1* and *GPx-2*, with a relative expression level of 0·93 (SEM 0·21) and 0·88 (SEM 0·22), respectively. *TrxR-1* was also observed in

Table 3. Effects of dietary supplementation of selenium on cytosolic glutathione peroxidase-1 (GPx-1) activity in mouse colon

(Mean values with their standard errors)

	Control diet (Se at 0·1 ppm) (*n* 12)		Dairy-Se diet (Se at 0·5 ppm) (*n* 12)		Dairy-Se diet (Se at 1 ppm) (*n* 12)		Yeast-Se diet (Se at 1 ppm) (*n* 12)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
GPx-1 activity (U/mg protein)	5·85	0·63	6·15	0·57	5·95	0·75	8·12*	0·63

ppm, Parts per million; dairy-Se, Se-enriched milk proteins; yeast-Se, Se yeast.
* Mean value was significantly different from that of the control diet ($P<0{\cdot}01$; ANOVA).

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Table 4. Relative expression of selenoprotein P (*SeP*), cytosolic glutathione peroxidase-1 (*GPx-1*), gastrointestinal glutathione peroxidase-2 (*GPx-2*) and thioredoxin reductase-1 (*TrxR-1*) mRNA in mouse colon*

(Mean values with their standard errors)

	SeP (*n* 12)		*GPx-1* (*n* 12)		*GPx-2* (*n* 12)		*TrxR-1* (*n* 12)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Colon tissues	2·56	0·33	0·93	0·21	0·88	0·22	0·062	0·04

*The gene expression of the four selenoprotein genes is related to the expression of glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) as a reference gene.

mouse colon but expressed at a relative lower level of 0·062 (SEM 0·04) (Table 4).

Effects of dietary selenium on selenoprotein P, cytosolic glutathione peroxidase-1, gastrointestinal glutathione peroxidase-2 and thioredoxin reductase-1 mRNA in mouse colon

Fold changes of colonic *SeP*, *GPx-1*, *GPx-2* and *TrxR* mRNA in response to dietary Se supplementation relative to control are shown in Fig. 1. After 4 weeks of Se supplementation, selenoprotein gene expression in the mouse colon responded differently depending on the Se supplement. *SeP* mRNA level increased in a dose-dependent manner in response to dairy-Se diets, being significantly higher (> 2-fold) in mice fed dairy-Se with Se at 1 ppm than those on the control diet ($P<0·05$) (Fig. 1(a)). A trend to increased *SeP* mRNA was also found in mice fed equivalent 1 ppm Se as yeast-Se, but it was not significantly different compared with that of the control diet ($P=0·068$). Increases in *GPx-2* mRNA levels in response to dairy-Se diets were also dose-dependent, with a significantly higher level of *GPx-2* mRNA (1·9-fold) found in mice fed dairy-Se with Se at 1 ppm, compared with mice fed the control diet ($P<0·05$) (Fig. 1(b)); however, yeast-Se did not significantly affect *GPx-2* mRNA expression in the mouse colon. In the case of *GPx-1*, a significantly higher

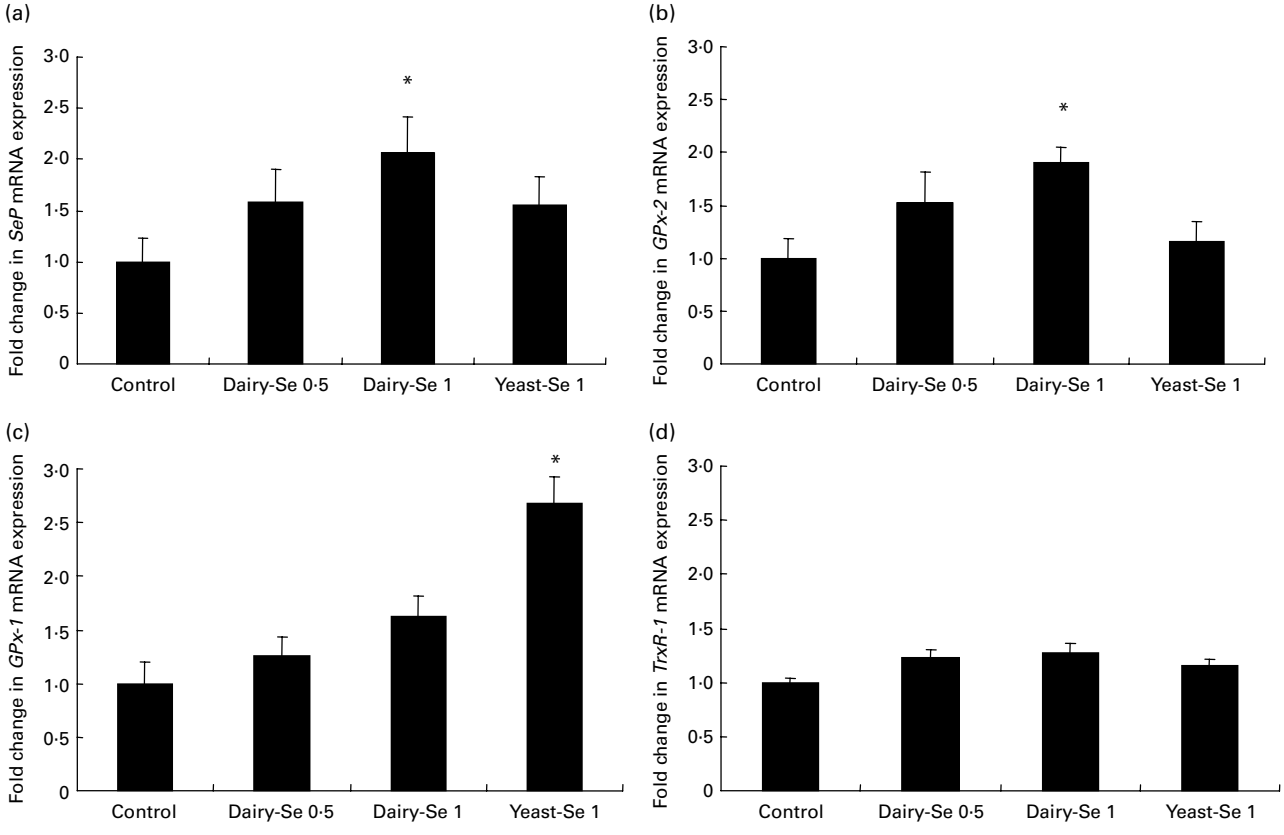

Fig. 1. Effects of dietary supplementation of Se on selenoprotein P (*SeP*) (a), gastrointestinal glutathione peroxidase-2 (*GPx-2*) (b), cytosolic glutathione peroxidase-1 (*GPx-1*) (c) and thioredoxin reductase-1 (*TrxR-1*) (d) mRNA expression in the mouse colon. Data are fold changes of colonic *SeP*, *GPx-2*, *GPx-1* and *TrxR-1* mRNA expression in response to dietary Se supplementation, relative to control, with control expression set at 1. Gene expression values have been normalised against the reference gene of glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*). Dairy-Se 0·5, Se-enriched milk proteins (Se at 0·5 parts per million (ppm)); Dairy-Se 1, Se-enriched milk proteins (Se at 1 ppm); Yeast-Se 1, Se yeast (Se at 1 ppm). Values are means (*n* 12), with standard errors represented by vertical bars. * Mean value was significantly different from that for the control diet ($P<0·05$; ANOVA).

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GPx-1 mRNA level was found in mice fed the yeast-Se diet with Se at 1 ppm; it was 2·7-fold higher than those on the control diet ($P<0·05$) (Fig. 1(c)). A trend of higher *GPx-1* mRNA was also found in mice fed the dairy-Se diet at 1 ppm, but it was not significant compared with those on the control diet ($P=0·060$). Our data indicated that the increased expression of *GPx-1* mRNA in the mouse colon was reflected in that of GPx-1 activity after dietary Se supplementation. However, *TrxR-1* mRNA level was not changed by dietary Se supplementation either from the dairy or yeast source (Fig. 1(d)).

Discussion

There is evidence from *in vitro* and *in vivo* studies that dietary supplementation of Se across a significant concentration range may regulate selenoproteins in target tissues[27–30]. In the case of the colon, the expression of several selenoprotein genes is significantly affected in animals fed Se-deficient diets compared with those fed Se-adequate diets[18,22]. However, little is known about whether selenoproteins in the colon are regulated by increasing Se intake beyond what is considered nutritionally adequate (1·5 to 10 times recommended adequate dietary intake), and whether the regulation is dependent on Se form. In the present study, we showed for the first time that colonic selenoprotein levels, namely SeP, GPx-1 and GPx-2 in the mouse colon were regulated differently depending on the Se form. We found that dairy-Se at 1 ppm significantly increased expression of colonic *SeP* and *GPx-2* mRNA but did not affect *GPx-1*, in particular GPx-1 activity, whereas yeast-Se at 1 ppm significantly increased colonic *GPx-1* mRNA and GPx-1 activity without affecting *SeP* and *GPx-2* mRNA. Studies from human clinical trials and animal experiments indicated that the chemical form of Se and not Se *per se* was the critical determinant of Se bioavailability and Se efficacy[31–34]. Our data support this concept, for while Se in both dairy and yeast sources is present as selenomethionine at 83 % and as selenocysteine at about 5 %, dairy-Se does not contain low-molecular-weight Se compounds due to the preparative procedure (filtration at 10 kDa), whereas yeast-Se contains 3 % of selenite. Additionally, dairy-Se also contains 4 % unknown components[35]; thus future studies are needed to identify organic species existing in dairy-Se. It is possible that the different Se forms may account for their different effects by affecting Se metabolism, Se delivery to target tissues, and subsequent selenoprotein synthesis, expression and function.

The beneficial effects of Se are thought to be mediated through the function of selenoproteins. Our particular interest is the potential regulation of *SeP*, *GPx-1*, *GPx-2* and *TrxR-1* in the colon by Se supplementing due to their potential relevance to CRC prevention. Our data support the view that SeP and GPx-2 along with GPx-1 are three selenoproteins of major functional significance in the mouse colon[12,26]. As they responded significantly to supra-nutritional levels of Se intake, they may represent intestinal targets for Se supplementation aimed not at correcting deficiency but at achieving levels thought sufficient to contribute to cancer prevention. A recent human study showed that Se supplementation predominantly affected the genes that function in protein biosynthesis, which were linked to increased selenoprotein expression in the target tissues[11]. Others have proposed that selenoprotein levels in targeted tissues may better reflect the functional selenoprotein activity than the plasma selenoprotein levels[36], and be more relevant to the beneficial anticancer effects of Se.

SeP is a major plasma selenoprotein with a crucial role in Se transport[19]. The presence of SeP is thought to be vital in terms of influencing individual selenoprotein expression in different tissues[25,26,37]. Given its transport function, this might explain the effective influence of SeP on other individual selenoproteins in the colon. In addition, SeP can also function as antioxidative defence and cancer prevention. For instance, *SeP* knock-out mice were linked to increased cancer development[38], a significant reduction or loss of *SeP* mRNA expression was observed in CRC[39] and some genetic variants in *SeP* were associated with human advanced colorectal adenoma[40]. But the potential role of SeP to CRC prevention remains speculative and further studies are needed.

GPx-1 and GPx-2 are the major proteins responsible for 70 % GPx enzyme activity in the gastrointestinal tract. Unlike GPx-1 that is expressed in almost all tissues in the human body[41], GPx-2 is expressed exclusively in the gastrointestinal tract, providing 50 % of GPx activity[37]. GPx-1 and GPx-2 have diverse biological roles that involve antioxidant function, inhibition of hydroperoxide, balance oxidative stress and associated inflammation. Their roles in CRC prevention have also received much attention because *GPx-1*/*GPx-2* double knock-out mice progressively developed colitis and subsequent intestinal cancer[15,16,42]. The present study showed that supra-nutritional intakes of dairy-Se significantly increased colonic *GPx-2* mRNA, and slightly increased *GPx-1* mRNA, but failed to increase colonic GPx-1 activity, whereas yeast-Se significantly increased colonic *GPx-1* mRNA and GPx-1 activity without increasing *GPx-2* mRNA. Since our previous animal studies showed that it was dairy-Se that protected against CRC rather than yeast-Se[9], up-regulation of *GPx-2* may be of importance in terms of CRC prevention, particularly with regard to its tissue specificity and stability in Se deficiency (i.e. selenoprotein hierarchy)[40,43].

In contrast to *GPx-1*, the lack of *GPx-2* was more detrimental because one intact allele was sufficient to prevent intestinal inflammation[44], thereby indicating that it has anti-cancer effects rather than acting as an anti-inflammatory. GPx-2 is involved in cell growth and differentiation, suppression of cyclo-oxygenase-2 expression[45] and activation by the β-catenin–T cell factor (TCF) complex[46]. Its expression is also regulated by Nrf2, a transcription factor that induces enzymes that are cytoprotective and tumour preventive[47]. But GPx-2 may have dual roles in carcinogenesis because GPx-2 was highly expressed in human colorectal adenomas and carcinomas[39,48,49]; some hypothesised that a beneficial role of GPx-2 in carcinogenesis may depend on the stage of tumorigenesis[45]. During the initiation stage, GPx-2 can protect cells from oxidative damage and reduce cyclo-oxygenase-2 expression and PGE2 production. One notable characteristic of Se is that its protective effects are more pronounced in the early stage of carcinogenesis[5,50–52].

Whether GPx-1 has protective effects for cancer prevention remains an interesting topic for future research[53]. There were reports that genetic variants of the *GPx-1* were associated with increased CRC risk, and loss of heterozygosity at the *GPx-1* locus was involved with malignant progression[42].

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These data, along with the differential expression patterns reported for GPx-1 in tumour *v.* normal tissues[54], support the relevance of GPx-1 in cancer prevention[55]. However, increasing evidence has suggested that GPx-1 might not act as the prime mechanism of chemoprevention[16,56] because it reached its maximum with adequate Se intake, and did not change appreciably when Se intake increased to the levels that were 10-fold higher; such levels are necessary to see chemopreventive effects in the animal models[57]. Since Se intake at a supra-nutritional level also reduced the risk of colon cancer in transgenic mice that had reduced *GPx-1* expression, the chemopreventive effect of Se may not be dependent on *GPx-1* expression[16].

TrxR-1, as part of the thioredoxin system, is important in antioxidant defence, but it has dual and contradictory effects on tumour development[54]. Like GPx-2, TrxR-1 levels were highly expressed in a variety of tumour tissues in humans[39]. There are reports that Se may affect TrxR-1 in two ways; increasing with excess of Se intake and declining with continued high levels of Se intake[57]. Since the expression of *TrxR-1* in the mouse colon was very low compared with *SeP*, *GPx-1* and *GPx-2*, and it did not respond to Se supplement from either the dairy or yeast source, this suggests that the function of TrxR-1 in the colon might not be as important as SeP, GPx-1 and GPx-2.

In conclusion, the present study indicates that activity and expression of selenoproteins in the mouse colon is regulated differently by different dietary sources of Se, namely dairy-Se compared with yeast-Se. We have previously shown that dairy-Se at 1 ppm, but not yeast-Se at the same level, was protective against CRC in an azoxymethane-induced CRC mouse model. The present study shows that dairy-Se but not yeast-Se up-regulates colonic *GPx-2* and *SeP* mRNA expression, suggesting that regulation of these genes is important in the prevention of CRC.

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Y. H., G. M. and G. Y. were involved in the design of the study. Y. H. and R. L. L. were responsible for execution of the experimental work and data collection. Y. H. was responsible for the data analysis and writing of the manuscript.

None of the authors has conflicts of interest.

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