The influence of selenium-enriched milk proteins and selenium yeast on plasma selenium levels and rectal selenoprotein gene expression in human subjects

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Abstract

Certain forms of dietary Se may have advantages for improving human Se status and regulating the risk for disease, such as cancers, including colorectal cancer (CRC). The present study compared the effects of a Se-enriched milk protein (dairy-Se) with a Se-rich yeast (yeast-Se) on plasma Se levels and rectal selenoprotein gene expression since we reasoned that if these genes were not regulated, there was little potential for regulating the risk for CRC in this organ. A total of twenty-three healthy volunteers with plasma Se in the lower half of the population range were supplemented with dairy-Se (150 µg/d) or yeast-Se (150 µg/d) for 6 weeks, followed by 6 weeks of washout period. Blood was sampled every 2 weeks, and rectal biopsies were obtained before and after Se supplementation and after the washout period. Plasma Se levels and glutathione peroxidase (GPx) activity, and rectal mRNA of selenoprotein P (SeP), cytosolic GPx-1 (GPx-1), gastrointestinal GPx-2 (GPx-2) and thioredoxin reductase-1 (TrxR-1) were measured. Plasma Se levels increased rapidly in both Se groups ($P < 0.001$); plasma GPx activity was not significantly changed. Rectal SeP mRNA increased at 6 weeks compared with baseline in both Se groups ($P < 0.05$); only dairy-Se resulted in a sustained elevation of SeP after the washout period ($P < 0.05$). Rectal GPx-1 and GPx-2 mRNA were higher with dairy-Se ($P < 0.05$) than with yeast-Se at 6 weeks. In conclusion, three rectal selenoprotein mRNA were differentially regulated by dairy-Se and yeast-Se. Changes in rectal selenoproteins are not predicted by changes in plasma Se; dairy-Se effectively regulates the expression of several rectal selenoproteins of relevance to the risk for CRC.

Key words: Selenium: Selenoproteins: Rectal biopsy: Health benefits: Colorectal cancer prevention: Colorectal cancer

Se is an essential trace element for human health. Current average Se intake in most countries is sufficient to prevent overt Se-deficiency diseases but may be suboptimal for protection against a number of adverse health conditions[1]. Studies have reported that most of the Australian population have adequate Se intake as determined by dietary intake surveys and plasma Se surveys[2,3]. Plasma Se levels of 95–100 µg/l are considered to reflect nutritional adequacy. However, since the average plasma Se levels in healthy South Australians were reported as 103 µg/l[4], about one half of the population does not reach desirable Se status. Some groups, in particular, are susceptible to Se deficiency, such as cancer patients, the elderly and smokers. Therefore, a substantial part of the Australian population may have a suboptimal Se status and be potentially responsive to dietary Se supplementation with respect to certain chronic degenerative diseases[5].

There is evidence that low Se status is associated with an increased risk of colorectal cancer (CRC)[6], whereas a higher Se intake may lower CRC mortality[7,8], and a higher Se intake is usually associated with a reduced risk of colonic adenoma recurrence[7,9,10]. Thus, Se supplementation may benefit health, including cancer prevention, beyond correction of Se deficiency. However, increasing Se intake is complicated because a number of recent clinical trials have indicated that Se supplementation may have adverse effects on human health[11,12], including a potentially increased risk for type 2 diabetes[13]. A recent large Se and Vitamin E Cancer Prevention Trial has also failed to show that Se affects prostate cancer risk[14], but the trials did not take account of the initial Se status, nor the chemical form of the Se supplement. While synthetic or naturally occurring forms of Se have shown protection against CRC[15], the question of which is the most

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effective, bioavailable and functional Se form still needs to be established. Improving Se status through Se from food sources is generally considered preferable to supplementation by pharmaceutical Se products, and interest in foods containing high amounts of Se is increasing[1,16].

Se-enriched milk proteins (dairy-Se) have recently been developed as a novel dairy food by Tatura Milk Industries to provide a significant daily intake of Se for adults[17,18]; it contains a high concentration of Se (5 parts per million) compared with 0·34 parts per million in normal milk proteins. We have shown in an animal CRC model that dairy-Se was significantly more effective at reducing CRC incidence than Se supplied as Se-rich yeast (yeast-Se)[19]. Its protection was associated with significant improvements in relevant biomarkers of CRC risk, namely aberrant crypt foci, *K-ras* mutations[19] and regulation of colonic epithelial expression of several selenoprotein genes[20].

Se exerts its wide range of biological roles through twenty-five selenoproteins in humans[21]. Some are particularly relevant to anticancer function and have received considerable attention for their important roles in chemoprevention[22], including selenoprotein P (SeP), cytosolic glutathione peroxidase-1 (GPx-1), gastrointestinal GPx-2 (GPx-2) and thioredoxin reductase-1 (TrxR-1). Selenoprotein levels are dependent on dietary Se intake; currently, dietary reference values for optimal Se intake have mostly relied on blood/plasma Se, or SeP concentrations and GPx activity[23,24], but the changes in these Se status biomarkers do not necessarily reflect expression patterns of selenoproteins in the target tissues, and not all selenoproteins change equally with Se supplementation[23,25]. As far as Se supplementation and selenoproteins are concerned, only a small proportion of studies have examined the effect of Se supplementation on selenoprotein expression in the human gastrointestinal tract[22,26]. Whether selenoproteins specific to different human tissues, for example the colon, are regulated by Se supplementation irrespective of the form is as yet not well determined.

The aim of the present study was to investigate the efficacy of dairy-Se (150 µg Se/d) compared with yeast-Se (150 µg Se/d) on plasma Se levels and, specifically, rectal selenoprotein gene expression in twenty-three healthy volunteers, whose plasma Se levels were in the lower half of the population. A sequential design was followed, where all subjects provided baseline measures, followed by random allocation to either dairy-Se or yeast-Se for 6 weeks, with observations extending throughout the 6-week treatment and 6-week washout period. This design enabled us to assess whether Se supplementation resulted in the regulation of rectal selenoprotein gene expression; we reasoned that if such were not to occur, the capacity to alter the risk for colorectal disease would be low.

Materials and methods

Subjects and recruitment

A group of sixteen male and seven female healthy volunteers aged 52–79 years, considered at risk for CRC by virtue of their age and/or other standard risk factors, were recruited by advertisement. Inclusion criteria were plasma Se concentration at or below 103 µg/l, no evidence of Se supplements or other drug regimens, and with no active bowel disease or previous history of colorectal polyp removal in the last 6 months. This plasma Se level was chosen as it represented the median point of our population and because it has previously been shown that reduced cancer risk is observed only in subjects where plasma Se was at or below 106 µg/l[27]. Exclusion criteria were any allergy or intolerance to milk/dairy products, evidence of any active mucosal bowel disease, e.g. colitis or malabsorption, and evidence of any other active clinical disease precluding participation in the study. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects/patients were approved by the Flinders Clinical Research Ethics Committee (reference no. 214/067; Flinders Medical Centre, Bedford Park, SA, Australia) and the trial registration number is ACTRN012607000345482. Written informed consent was obtained from all subjects for the biopsies to be used exclusively for the present study.

Selenium supplements

A dairy-Se (Tatura-Bio®Se) was provided by Tatura Milk Industries Limited, Tatura, VIC, Australia. The protein isolate containing this highly bioavailable form of Se was then prepared for human use by Ozscientific Limited (Hoppers Crossing, VIC, Australia) as a water-miscible powder. It contained 70 % protein, 5 % lactose, 4 % moisture, 1·5 % Ca, 1·2 % fat and 5 parts per million Se. A 30 g sachet/d provided 150 µg Se and 21 g milk proteins. Yeast-Se for human use was provided by Alltech Biotech Private Limited (Dandenong South, VIC, Australia) as compressed yeast tablets, with three tablets providing 150 µg Se/d (each tablet contained 50 µg Se, 100 mg lactose and 65 mg microcrystalline cellulose). Both Se products have been analysed for Se compounds: dairy-Se contained 83 % selenomethionine, 5 % selenocysteine and 4 % unknown (R Lobinski, unpublished results); yeast-Se contained 83 % selenomethionine, 5 % selenocysteine and 3 % selenite[27].

Study protocol

The present study was a 3-month, double-blind and non-cross-over human intervention study, in which subjects were randomly assigned to 6-week dietary supplementation with either dairy-Se or yeast-Se (Fig. 1); all subjects were then observed for a 6-week washout period. Our interest was to assess whether each intervention increased endpoints relative to baseline in these subjects with below average plasma Se and to assess whether any increases were sustained beyond the cessation of supplementation. The dietary approach was pragmatic and allowed participants to readjust the remainder of their diet. Subjects taking dairy-Se were encouraged to substitute this milk protein product in place of two daily dairy serves. Of the twenty-three participants, twelve received dairy-Se (150 µg Se/d) and eleven received yeast-Se (150 µg

Se/d) for 6 weeks. Peripheral blood samples were taken every 2 weeks, including 2 or 4 weeks before the study. Rectal biopsies were obtained before Se supplementation (baseline), at the end of Se supplementation and during the washout period. To examine for any potential for intoxication with Se supplements, plasma markers for liver and renal function were assessed. Examination of fasting blood glucose was used to account for any changes in glucose homeostasis. C-reactive protein was also assessed as a measure of acute or chronic inflammation.

Blood sampling, plasma selenium and glutathione peroxidase activity measurements

Blood samples were collected according to a standardised protocol, placed in 10 ml EDTA-coated tubes, centrifuged for 10 min (3000 g) at 4°C, and plasma samples were stored at −80°C until analysis. The plasma Se level was measured by atomic absorption spectroscopy using a Perkin Elmer Analyst 800 (PerkinElmer Life and Analytical Sciences, Shelton, CT, USA) with furnace. Plasma GPx activity was determined spectrophotometrically using a glutathione peroxidase assay kit (Cayman Chemical Company, Ann Arbour, MI, USA). GPx activity of 1 unit (U) is defined as 1 μmol NADPH oxidised/min and expressed as U/mg protein.

Processing of biopsies

Rectal biopsies were taken by a gastroenterologist using oversized rectal biopsy forceps; one biopsy was first placed in RNAlater (Applied Biosystems/Ambion, Ambion, Inc., The RNA Company, Austin, TX, USA) solution at −4°C for 24 h and stored at −80°C for rectal selenoprotein mRNA expression, and another was formalin fixed and embedded in paraffin for immunohistochemical studies.

Rectal biopsies for RNA isolation and complementary DNA synthesis

Total RNA (30 mg) was extracted from rectal biopsies 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.

First-strand complementary DNA (20 μl) was synthesised from 0·3 μg total RNA for each sample using a QIAGEN QuantiTect Reverse Transcription Kit (QIAGEN).

Quantitative real-time RT-PCR

Quantitative real-time RT-PCR was performed in triplicate on a Rotor Gene 3000 Cycler (Corbett, Sydney, NSW, Australia). Oligonucleotide primers were designed using Primer Express software version 1.5 (Applied Biosystems, Inc., Foster City, CA, USA), based on sequences from the GenBank database (Table 1). The PCR was determined in a 20 μl final volume containing 6 μl of diluted 1:30 complementary DNA using the 2× QuantiTect SYBR Green PCR kit (QIAGEN). The primer concentration for each gene was 10 μM (forward and reverse primers). The cycling protocol was similar as described previously[20]. The specificity of PCR was confirmed by melting curve analysis of the amplified PCR products. For each PCR run, a non-template reaction was included as the negative control.

Cycle thresholds were determined using the relative quantification analysis module in the Rotorgene 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 complementary DNA. Quantitative real-time PCR analysis was then performed using Q-Gene software (Integrated DNA Technologies, Inc., Coralville, IA, USA)[28], with the amplification efficiency applied to the relative concentration analyses of both the genes of interest and the housekeeping gene (β-actin). Gene-of-interest expression data were normalised by dividing by the corresponding levels of β-actin for each sample.

Rectal epithelial histopathological analysis

Rectal biopsies were formalin fixed, embedded in paraffin and cut into 5 μm sections for haematoxylin and eosin and immunohistochemistry staining. A total of twenty full-length, non-bifid crypts were selected from each volunteer and counted for the rate of Ki-67-positive cells and crypt height. The tissue was orientated to optimise the longitudinal crypt axis. Ki67 immunohistochemistry was performed using a mouse anti-Ki67 monoclonal primary antibody (M7248; DakoCytomation,
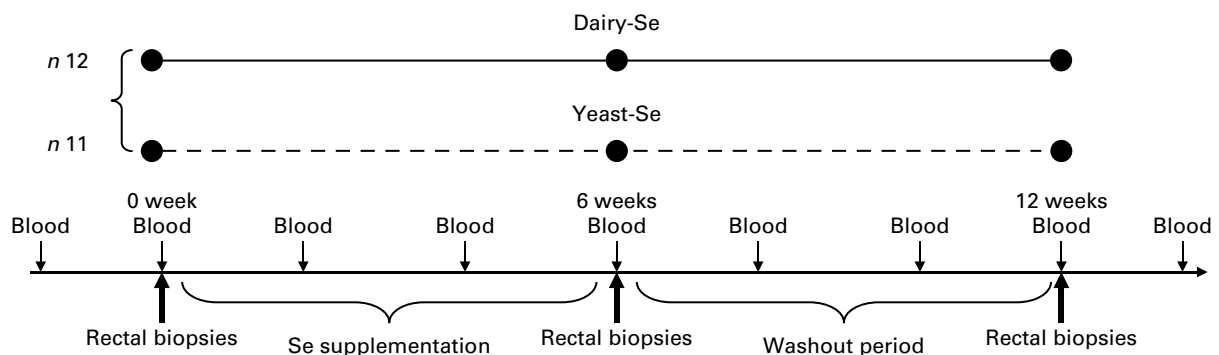

Fig. 1. Overview of blood and tissue sampling and timing of interventions in normal volunteers. Dairy-Se, Se-enriched milk proteins; yeast-Se, Se-rich yeast.

Table 1. Oligonucleotide primers used for real-time RT-PCR

Gene	Gene accession no.	Primers	Primer sequences 5′–3′
SeP	NM_005410	Sense	TTACTGTGAAAAGAAATGTGGAAACTG
		Antisense	AAAGCTCACTGCTGCCAAGGG
GPx-1	NM_000581	Sense	TTCCCGTGCAACCAGTTTG
		Antisense	GAGACCAGGTGATGAGCTTG
GPx-2	NM_002083	Sense	TGGTCCTTGGCTTCCCTTGC
		Antisense	AAGACAGGATGCTCGTTCTG
TrxR-1	NM_003330	Sense	GAAGAACATGGCATCAAGTTTATAAGA
		Antisense	TCTTCCTATTGCCAGCATCAC
β-Actin	NM_001101	Sense	TTCTACAATGAGCTGCGTGTG
		Antisense	ATAGCAACGTACATGGCTGG

Copenhagen, Denmark) and a level 2 Ultra Streptavidin detection system (Signet Laboratories, Inc., Dedham, MA, USA). The proliferation index was calculated as the percentage of Ki67-stained cells per crypt column divided by the total number of cells within the crypt column, reported as a mean proliferation index (%). Cell heights are reported as the mean number of cells per crypt column.

Statistical analysis

All analyses were provided using STATA software (Stata Corporation, College Station, TX, USA) version 10.1. Results are expressed as means with their standard errors. Comparisons of standard blood markers between dairy-Se and yeast-Se at baseline, after Se intervention and during the washout period were performed using the Mann–Whitney test. Within-group comparisons (plasma biomarkers, rectal selenoprotein genes and cell kinetics) between baseline and each time point were performed using a random intercept linear mixed model, with subject as a random effect. Fixed effects for each model consisted of time, Se treatment group and time-by-treatment interactions, with time included as a categorical variable. Between-group comparisons at each time point were assessed within the same model using the time-by-treatment group interaction terms. Pearson's correlations were used to examine associations for variables within each group and associations for variables as combined group. Statistical analyses were two-sided, and a P value of <0.05 was considered to be statistically significant for within-group and between-group comparisons and for all Pearson's correlations.

Results

Baseline characteristics of subjects and compliance with treatment

A total of twenty-three participants were randomised for the study; all were healthy at the time of commencement of the study. Their mean age was 64 years, ranging from 52 to 79 years. Of these twenty-three participants, twenty-one completed the entire intervention study (fourteen males and seven females) and one withdrew after week 3 due to an inability to tolerate the size of the dairy-Se portion; all other participants tolerated both diary and yeast products. Rectal biopsies of one participant could not be used for PCR due to inadequate quantity of mucosal tissues. There were no significant differences in baseline characteristics between treatment groups (data not shown). There was 100% compliance with the Se products for those completing the study as estimated by counting the returned sachets at the end of the supplementation.

There were no significant disturbances in plasma glucose or C-reactive protein, or in liver and renal function tests for the duration of the study in any of the participants (Table 2). Consumption of dairy-Se was associated with a non-significant trend towards higher plasma urea in the supplemental period, consistent with compliance with the dairy protein supplement.

Table 2. Effect of selenium supplementation on human plasma glucose, C-reactive protein (CRP), Hb and urea
(Mean values with their standard errors)

	Dairy-Se						Yeast-Se						
	Baseline		6-Week Se intake		6-Week washout		Baseline		6-Week Se intake		6-Week washout		
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	P^*
Glucose (mmo/l)	5·07	0·09	5·20	0·14	5·36	0·19	5·05	0·09	5·12	0·15	5·15	0·18	NS†
CRP (mmo/l)	1·97	0·57	2·33	0·75	2·45	1·02	1·96	0·49	2·28	0·58	2·25	0·63	NS†
Hb (g/l)	145·5	3·59	145·6	3·05	148·8	3·32	144·4	3·16	144·8	4·02	146·8	4·53	NS†
Urea (mmo/l)	5·67	0·36	6·16	0·25	5·93	0·35	6·33	0·47	5·85	0·40	5·60	0·24	NS†

Dairy-Se, Se-enriched milk proteins; yeast-Se, Se-rich yeast.
* Using the Mann–Whitney test.
† Observed values were not significant between dairy-Se and yeast-Se at baseline, after Se supplementation and during the washout period.

Table 3. Effect of selenium-enriched milk proteins (dairy-Se) and selenium-rich yeast (yeast-Se) intervention on plasma selenium concentration and plasma glutathione peroxidase (GPx) activity in human subjects‡

(Mean values with their standard errors)

| | Baseline | | Week of Se supplementation | | | | | | | | Week of washout | | | | | | Follow-up | |
| | | | 2 | | 4 | | 6 | | 8 | | 10 | | 12 | | | | | |
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Plasma Se level (μg/l)																
Dairy-Se (n 11)	99·6	2·7	117***	3·7	130***	4·7	126***	4·0	116***	3·4	114**	3·7	112**·††	3·6	111***	5·9
Yeast-Se (n 10)	97·7	3·1	117***	3·8	128***	4·8	135***	5·2	120***	3·3	118***	3·2	114***·††	3·3	104	3·2
Plasma GPx activity (nmol/min per ml)																
Dairy-Se (n 11)	74·9	5·6	–		–		73·6	6·1	–		–		70·9	4·1	–	
Yeast-Se (n 10)	70·9	4·7	–		–		76·2	6·0	–		–		69·8	4·5	–	

Mean values were significantly different from those of baseline: ** $P < 0.01$, *** $P < 0.001$.
†† Mean values were significantly different for week 12 from those of week 6 ($P < 0.01$).
‡ All comparisons performed using a random-intercept linear mixed model.

Table 4. Effect of sex on plasma selenium concentration and plasma glutathione peroxidase (GPx) activity in human subjects

(Mean values with their standard errors)

| | Baseline | | Week of Se supplementation | | | | | | | | Week of washout | | | | | | Follow-up | | |
| | | | 2 | | 4 | | 6 | | 8 | | 10 | | 12 | | | | | | |
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	P*
Plasma Se level (μg/l)																	
Male (n 14)	97·6	2·8	114·9	2·9	124·9	3·6	128·7	4·0	116·4	2·7	113·5	3·0	111·4	3·3	109·1	4·9	NS
Female (n 7)	100·9	2·1	120·8	5·2	130·7	6·3	133·4	6·2	121·8	4·4	119·7	4·0	116·5	2·8	104·8	2·2	NS
Plasma GPx activity (nmol/min per ml)																	
Male (n 14)	76·1	4·5	–		–		77·4	5·3	–		–		69·5	3·5	–		NS
Female (n 7)	66·8	5·7	–		–		69·6	6·8	–		–		65·5	5·0	–		NS

* Observed values were not significant between men and women at baseline, after Se supplementation and during the washout period.

Selenium intervention and plasma selenium levels

Plasma Se concentration increased significantly from baseline to between 126 and 135 µg/l ($P < 0.001$) over the 6-week Se intervention in the two Se groups (Table 3). Plasma Se levels were slightly greater in the yeast-Se group (135 (SEM 5·2) µg/l) than in the dairy-Se group (126 (SEM 4·0) µg/l) after 6 weeks of Se supplementation ($P < 0.05$). At the end of the washout period (week 12), plasma Se levels remained significantly elevated, relative to the baseline level ($P < 0.01$ for dairy-Se and $P < 0.001$ for yeast-Se), but a steady decline over 6 weeks up to week 12 was apparent. There was no sex effect on plasma Se levels at baseline, after the Se intervention and during the washout period, but women had a slightly higher plasma Se levels than men (Table 4).

Selenium intervention and plasma glutathione peroxidase activity

Neither dairy-Se nor yeast-Se had significant effects on plasma GPx activity over the 6-week Se intervention (Table 3). Again, there was no sex effect on plasma GPx activity at baseline, after Se supplementation and at the end of the washout period, but men had a trend towards higher plasma GPx activity (Table 4).

Selenium intervention and rectal selenoprotein expression

Changes in rectal selenoprotein gene expression obtained at baseline, after Se intervention and during the washout period were examined in twenty-one participants (Fig. 2). The expression levels of selenoprotein genes differed from each other; the highest level was found in SeP, followed by GPx-2 and GPx-1. TrxR-1 was the least expressed.

Following 6 weeks of Se supplementation, the level of SeP mRNA was statistically significantly higher than baseline levels in both Se groups ($P < 0.05$; Fig. 2(a)). This significant elevation was more sustained in the dairy-Se group compared with the yeast-Se group at the end of the washout period (week 12; $P < 0.05$). The level of GPx-1 and GPx-2 mRNA expressions at the end of the Se intervention (week 6) was also significantly higher in the dairy-Se group ($P < 0.05$), but not in the yeast-Se group (Fig. 2(b) and (c)). At week 6, the increase in GPx-2 was greater than that in GPx-1, and at week 12, the level of GPx-2 did not return to the baseline level as rapidly as the level of GPx-1 mRNA. Neither dairy-Se nor yeast-Se significantly affected the TrxR-1 mRNA expression (Fig. 2(d)). Further comparison between the two Se groups showed that the changes in GPx-1 and GPx-2 mRNA at week 6 depended on the Se supplementation (significant $P < 0.05$ for treatment-by-time interaction), but there was no difference in SeP mRNA at week 12 between
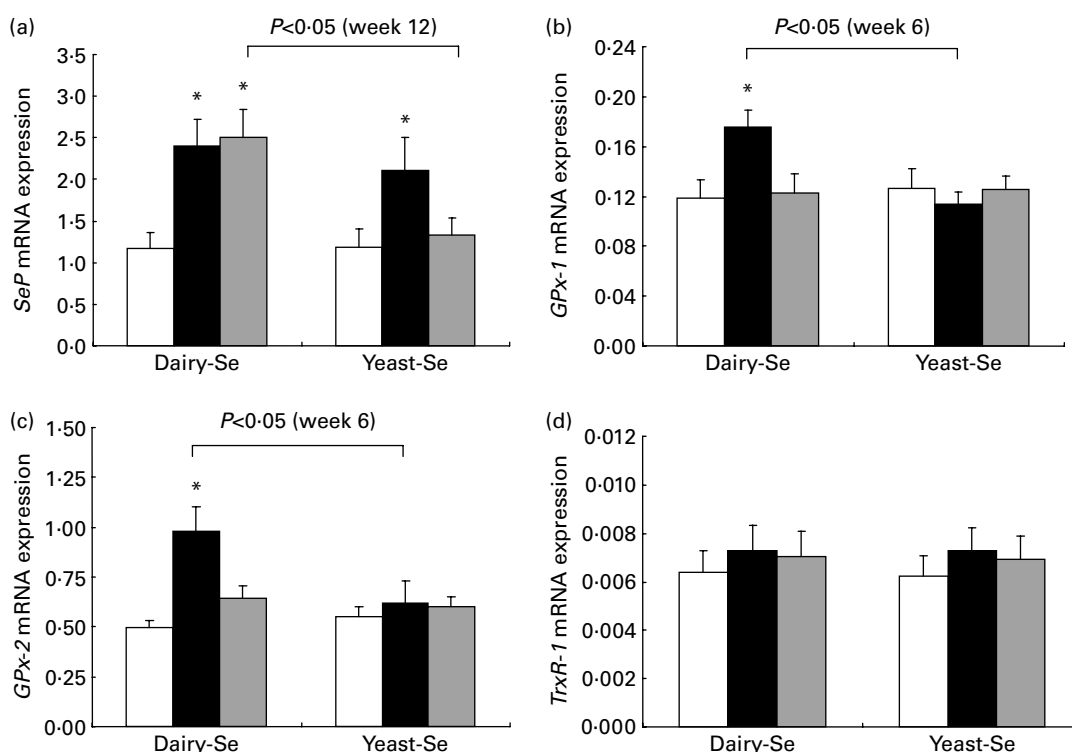
Fig. 2. Quantitative real-time RT-PCR analysis of (a) selenoprotein (SeP), (b) cytosolic glutathione peroxidase-1 (GPx-1), (c) gastrointestinal glutathione peroxidase-2 (GPx-2) and (d) thioredoxin reductase-1 (TrxR-1) mRNA expression in rectal biopsies for Se-enriched milk proteins (dairy-Se, n 11) and Se-rich yeast (yeast-Se, n 10) before Se supplementation (baseline, week 0), after Se supplementation (week 6) and at the end of the washout period (week 12). Gene expression values have been normalised against the reference gene β-actin. Values are means, with their standard errors represented by vertical bars. * Mean values were significantly different compared with the data at week 0 within groups, and brackets indicate differences in data at week 6 or at week 12 between groups ($P < 0.05$). □, Week 0; ■, week 6; ▨, week 12.

the two Se groups. There was no significant sex-specific difference in rectal selenoprotein expression. Rectal *SeP* and *GPx-2* mRNA levels at baseline, after Se supplementation and at the end of the washout period were slightly higher in men than those in women. But the response of *SeP*, *GPx-2* and *GPx-1* to Se supplementation and Se withdrawal was similar in men and women (Table 5).

Relationship between selenium status and selenoprotein expression

For subjects treated with dairy-Se, changes in plasma Se levels were not correlated with the changes in rectal *SeP* mRNA at the end of the Se intervention (data not shown), although plasma Se levels did fall within a narrow range. However, the changes in rectal *SeP* mRNA were positively correlated with the changes in *GPx-2* mRNA (r 0·67, $P=0·02$; Fig. 3(a)). A positive trend between the changes in rectal *SeP* mRNA and *GPx-1* mRNA was also found (r 0·46, $P=0·10$; Fig. 3(b)). Significant correlations were not found in subjects treated with the yeast-Se supplement.

Selenium intervention and rectal epithelial biology

Dietary intervention studies in human subjects have shown that 4–6 weeks on a diet is sufficient to stabilise epithelial kinetics[29,30]. Adequate biopsy material, containing a sufficient number of completely sectioned crypts, was available from twenty-one participants before the Se intervention, from twenty participants after the Se intervention and from sixteen participants during the washout period. Neither crypt proliferation nor cell height was significantly affected by Se supplementation (Fig. 4). Occasional spontaneous apoptosis was found in some crypts, but there was no significant difference between dairy-Se and yeast-Se.

Discussion

The present human intervention study investigated the association between organic Se supplementation and selenoprotein gene expression in rectal tissues, because if we are to expect benefit in terms of colorectal disease, we would expect Se supplementation to lead to changes in selenoprotein gene expression in the target tissue. We showed that supplementation of Se (150 μg Se/d) from either dairy or yeast sources resulted in higher rectal *SeP* mRNA expression, but only dairy-Se was able to significantly increase the expression of rectal *GPx-1* and *GPx-2* mRNA, and maintain higher *SeP* mRNA levels during the 6-week washout period. Neither dairy-Se nor yeast-Se regulated rectal *TrxR-1* mRNA. We showed that there was no sex effect on plasma Se levels, plasma GPx activity or rectal selenoprotein expression based on the data of fourteen men and seven women. These results support our previous observation in mice that several selenoprotein genes were regulated differently by dairy-Se compared with yeast-Se, when increasing Se intake tenfold beyond nutritional adequacy[20].

Currently, we are unable to explain why the two Se-rich products are significantly different in their influence. According to the Se speciation data available[27], both products were similar with respect to their predominant components of selenomethionine (83%) and selenocysteine (5%), albeit there was a significant component of dairy-Se unidentified. Given the nature of its preparation, dairy-Se did not contain Se compounds smaller than 10 kDa. The present results support the concept that the chemical form of Se and not Se *per se* is a critical determinant of Se influences on gene expression[31–33]. While it is not clear whether a particular chemical form of Se in dairy protein can significantly influence selenoprotein expression, it is known that the chemical forms of Se in the food will affect Se bioavailability (absorption, retention and utilisation), Se metabolism and the ability to synthesise selenoproteins and to produce methylselenol metabolites[34,35]. It is possible that Se in milk proteins is more

Table 5. Effect of sex on the relative expression of rectal selenoprotein (SeP)* mRNA levels in human subjects
(Mean values with their standard errors)

	Baseline (week 0)		Se supplementation (week 6)		Washout (week 12)		
	Mean	SEM	Mean	SEM	Mean	SEM	P†
Rectal *SeP* mRNA							
Male (*n* 14)	1·21	0·17	2·17	0·29	1·82	0·25	NS
Female (*n* 7)	1·11	0·27	1·96	0·50	1·60	0·45	NS
Rectal *GPx-1* mRNA							
Male (*n* 14)	0·12	0·01	0·15	0·01	0·13	0·01	NS
Female (*n* 7)	0·13	0·02	0·14	0·02	0·11	0·02	NS
Rectal *GPx-2* mRNA							
Male (*n* 14)	0·55	0·04	0·87	0·10	0·67	0·05	NS
Female (*n* 7)	0·47	0·04	0·77	0·17	0·53	0·56	NS
Rectal *TrxR-1* mRNA							
Male (*n* 14)	0·006	0·000	0·007	0·001	0·006	0·001	NS
Female (*n* 7)	0·007	0·001	0·008	0·001	0·008	0·001	NS

GPx-1, cytosolic glutathione peroxidase-1; GPx-2, gastrointestinal glutathione peroxidase-2; TrxR-1, thioredoxin reductase-1.
* Gene expression of four SeP genes is related to the expression of β-actin as a reference gene.
† Observed values were not significant between men and women at baseline, after Se supplementation and during the washout period.
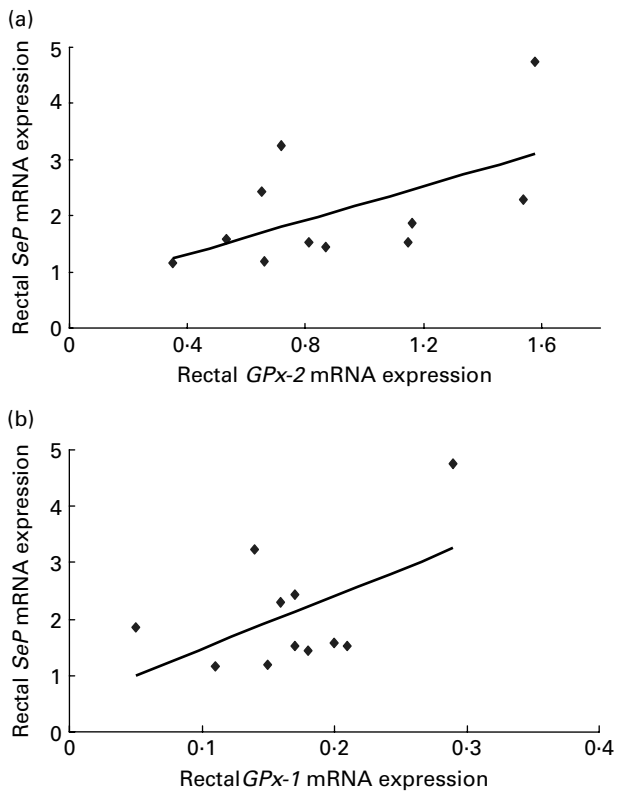
Fig. 3. (a) Correlation between plasma rectal selenoprotein (*SeP*) mRNA and gastrointestinal glutathione peroxidase-2 (*GPx-2*) mRNA (*r* 0·67; *P*=0·02) and (b) rectal *SeP* mRNA and cytosolic glutathione peroxidase-1 (*GPx-1*) mRNA (*r* 0·46; *P*=0·10) after 6 weeks of Se-enriched milk protein supplementation (*n* 11, one participant withdrew). There is a positive correlation between the changes in rectal *SeP* mRNA and *GPx-2* mRNA, and a trend towards positive correlation between rectal *SeP* mRNA and *GPx-1* mRNA.

highly absorbed, differentially metabolised, and/or transported and/or retained by the rectal tissues than with Se from yeast, resulting in an increased expression of rectal molecular markers examined. The present study is limited in the ability to control for protein intake in comparative treatments. Unlike our previous mouse study in which the comparison of dairy-Se and yeast-Se allowed us to balance milk protein concentration (20 %), in the present study, milk protein was substituted into the diet in the place of normal dairy serves. While this is an uncontrolled factor in the study, it represents just a fraction of total protein intake, about one-third to one-fifth of daily protein intake for adult Australians. There has been no evidence that protein concentration in the diet affects Se status, Se metabolism or selenoprotein expression, whereas there has been good evidence in the literature for Se intake influencing selenoproteins[21]. In addition, genetic variants in selenoprotein genes may also account for inter-individual differences in response to Se supplementation[36].

Because Se functions through selenoproteins, plasma Se levels and GPx activity are commonly used as indicators of Se status because they respond rapidly to the changes in dietary Se intake[37]. Selenomethionine is an important factor influencing plasma Se levels, having higher bioavailability and higher retention rates. Since dairy-Se and yeast-Se had a similar selenomethionine concentration and were equally

efficient in raising plasma Se levels, this indicates that selenomethionine from both sources was converted to selenocysteine in SeP (approximately 65 % of plasma Se is SeP)[37]. Our data showed that 6 weeks of supplementation was an adequate time for circulating plasma Se levels to reach a plateau. Interestingly, plasma Se levels remained relatively high during the washout period and had not returned to entry levels at the end of the washout period, particularly for dairy-Se. Similar to our observation, the lack of return of plasma Se to baseline levels during the washout period has also been found after a 6-week supplementation with 100 μg sodium selenite[38]. Whether this is associated with incorporation of Se into protein pools or retention of Se in other tissues needs to be investigated in future.

The lack of response of plasma GPx activity to both Se supplements implies that subjects in the present study had adequate starting Se levels (mean baseline value 98·6 μg/l) for full expression of plasma GPx activity. The important observation from the present study was the lack of correlation between plasma Se status and rectal selenoprotein gene expression, such that neither plasma Se levels nor plasma GPx activity reflected the biological efficacy of Se supplementation on regulating selenoproteins in target tissues[39]. A recent Se supplementation study has suggested that plasma Se biomarkers do not mirror Se intake once the Se requirement has been met[37], thus there are limitations with the use of plasma Se biomarkers.
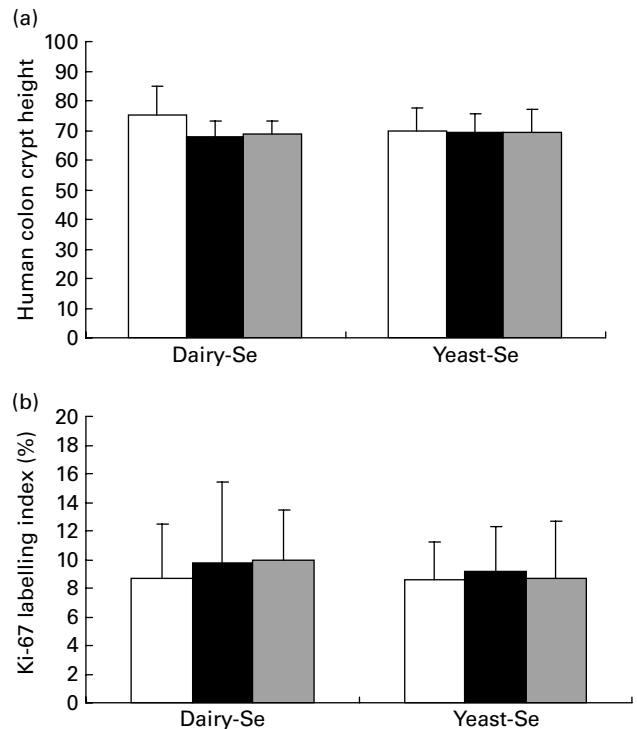
Fig. 4. Effects of dietary Se intervention on (a) crypt height and (b) cell proliferation in rectal biopsies before Se supplementation (baseline, week 0, □), after supplementation (■, week 6) and at the end of the washout period (▣, week 12). Values are means, with their standard errors represented by vertical bars. Se supplementation has no effects on human rectal cell kinetics. Diary-Se, Se-enriched milk proteins; yeast-Se, Se-rich yeast.

Non-specific incorporation of Se into proteins (seleno-methionine) is likely to account for the increase in Se levels in tissues, but we did not measure the Se levels in rectal tissues due to limited availability of tissue, nor can we explain why the changes in plasma Se levels were not reflected in changes in rectal selenoprotein gene expression. Others have also reported that whole-blood cell selenoprotein mRNA, such as *GPx-1* and *SeP*, was not significantly correlated with plasma Se in healthy British adults[23]. It seems likely that specific tissue concentrations of individual selenoproteins can also be used as functional indicators of Se status. In clinical trials, higher Se intake showed protection against cancer without increasing circulating selenoprotein levels[37].

SeP is a major plasma selenoprotein (approximately 70% of plasma Se) and is crucial for Se supply to different organs for the synthesis of other selenoproteins[40,41]. However, the level of Se intake required for maximal expression of SeP is not known. Compared with GPx, SeP requires a greater Se intake to reach its maximum concentration; recent studies have suggested that in addition to the habitual intake (approximately 55 μg Se/d), additional intake of 50 μg Se/d is required to achieve the optimal expression of plasma SeP[24]. Plasma *SeP* has been considered to be a useful bio-marker of Se status in populations, suggesting that rectal *SeP* is also a useful biomarker of Se status. It is likely that subtle alterations in SeP concentration or function would be expected to change Se supply to different tissues and therefore expression of other selenoproteins, such as *GPx-1* and *GPx-2*. Therefore, dietary regulation of rectal *SeP* expression may have benefits beyond its transport functions.

The present results suggest that GPx-1 and GPx-2 are responsible for 70% of GPx activity in the gastrointestinal tract[42]. A selenoprotein hierarchy, resulting from a competition for available Se and for components of the selenoprotein synthetic machinery, is particularly noticeable in the GPx family, with GPx-1 being the last in the ranking order[21]. For instance, *GPx-1* mRNA fell dramatically in Se deficiency, whereas *GPx-2* mRNA was remarkably stable (conserved) and increased rapidly on Se supplementation, indicating the biological importance of GPx-2 in the gastrointestinal tract[22]. To date, most studies have focused on Se deficiency on selenoprotein expression[43,44], whereas there are limited data of Se supplementation on the regulation of selenoproteins in human subjects[45]. In the present study, dairy-Se supplement resulted in a greater increase of rectal *GPx-2* mRNA than *GPx-1* mRNA, which may, in part, be associated with GPx-2 tissue specificity and stability in Se deficiency (i.e. selenoprotein hierarchy). Since GPx-1 and GPx-2 have diverse biological roles and are implicated in the risk and the development of human CRC[46–48], this up-regulation may have health benefits for humans. It is likely that the anti-cancer property of Se is mediated by specific selenoproteins in the gastrointestinal tract.

TrxR-1, as part of the thioredoxin system, is important in antioxidant defence and is relevant to anti-cancer function[49]. Similar to our mouse study, TrxR-1 mRNA did not respond to the Se supplements. Al-Taie *et al*.[50] also reported that Se had no effect on TrxR, so more research is required to demonstrate the roles of TrxR-1 and its regulation in human rectal epithelium.

While diabetes risk was not a primary study endpoint, an additional observation from the present study was that there was no alteration in any markers of diabetes risk, as had been proposed by some researchers[51]. Interestingly, a recent French study has shown the opposite effect: a decreased risk of diabetes in subjects with increasing Se status[52]. Further studies are needed to determine whether any association exists between Se supplementation and diabetes risk.

In conclusion, the present study indicates for the first time that rectal selenoprotein gene expression (i.e. *SeP*, *GPx-1* and *GPx-2*) is significantly regulated by dietary Se supplementation independent of plasma levels of Se and GPx activity; furthermore, regulation depends on the source/form of dietary Se supplementation; dairy-Se had a more sustained effect. Up-regulation of rectal *SeP*, *GPx-1* and *GPx-2* raises the potential for Se supplementation to directly influence the risk for colorectal disease. Therefore, a novel form of Se such as this dairy-sourced Se warrants further investigation to test this possibility, especially in relation to the possibility of reducing the risk of CRC.

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