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Tissue specificity of selenoprotein gene expression in rats*

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To investigate the tissue-specific effects of inadequate, adequate, and high selenium intake on selenoprotein gene expression and enzyme activity, weanling rats were fed a selenium-deficient diet or the same diet supplemented with 0.1 or 2.0 mg of selenium/kg of diet for 91 days. No significant differences in growth were observed. In liver, transcription of genes for cellular glutathione peroxidase, type I iodothyronine 5'-deiodinase, and selenoprotein P was unaffected by selenium intake. Steady-state levels of mRNA for glutathione peroxidase and selenoprotein P were higher in liver than in kidney. For iodothyronine 5' deiodinase in the opposite was true. In liver, selenium deficiency reduced glutathione peroxidase mRNA by 89% and virtually abolished enzyme activity. For iodothyronine 5' deiodinase, mRNA and enzyme activity were reduced 69 and 70%, respectively. In kidney, selenium deprivation decreased glutathione peroxidase mRNA by 91% and reduced enzyme activity to nearly zero. For iodothyronine 5'-deiodinase, decreases in mRNA and enzyme activity were 19 and 62%, respectively. Reductions in selenoprotein P mRNA were 50% in kidney but only 14% in liver. The only difference in the effects between the two supplements was in liver, where iodothyronine 5'-deiodinase activity was reduced by increasing the selenium supplement above a nutritionally adequate level. Hence, for these selenoproteins, mRNA turnover appears to be the pretranslational process most sensitive to selenium intake. In addition, selenoprotein mRNAs are stabilized differentially in selenium deficiency, depending upon the tissue examined. Percentage changes in the activity of selenoenzymes were not always the same as the changes in their mRNA levels. This suggests that other processes, including translation and protein turnover, may determine the ultimate level of enzyme activity attained in response to dietary selenium intake. (J. Nutr. Biochem. 6:367–372, 1995.)

Keywords: selenium; selenoproteins; gene expression; rat

Introduction

The functions and effects attributed to the dietary essential trace element selenium (Se) are increasing in number. By regulating activity of glutathione peroxidase (GPX) (EC 1.11.1.9) family enzymes,[1] Se plays an integral role in the enzymatic defense against oxidative damage. Selenium supplementation enhances cellular and humoral immune response.[2] Studies in experimental animals show that high levels of Se intake protect against chemically or virally induced tumorigenesis.[3] In humans, low intakes of Se have been correlated with increased risk of cancer at certain sites[3] and with increased risk of heart disease.[4] Normal metabolism of thyroid hormones depends on adequate Se intake.[5] Decreased serum Se is seen in several disease states.[6–9]

The effects of Se are mediated in many cases by selenoproteins, in which Se is incorporated into the primary structure of the proteins as selenocysteine, encoded by a TGA opal codon. Selenoproteins include the four members of the GPX family[10] and the type I iodothyronine 5' deiodinase (5'-ID),[11] which converts the prohormone thyroxine (T4) to its active form, 3,5,3'-triiodothyronine (T3). Activity of these enzymes rises and falls with corresponding changes in dietary intake of Se.[1] Other selenoproteins, some of which show similar responses to Se intake, have been identified. These include the plasma glycoprotein selenoprotein P (SeP),[12] a rat muscle protein termed selenoprotein W,[13] and

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a mouse epididymal protein.[14] The functions of these other selenoproteins is, at present, uncertain.

The RDA for Se is based on balance studies and on the plateauing of GPX activity in blood.[15] With the techniques of modern biology it is now possible to begin to explore the molecular basis for nutrient function and requirements. A first step in that exploration is to measure the effect of changes in dietary intake of a nutrient on the expression of genes for enzymes dependent on that nutrient for activity. We have shown that transcription of the gene for GPX1, the classic cellular form of the enzyme, is the same in livers of rats fed Se-adequate or Se-deficient diets.[16] However, Se deficiency markedly decreases the stability of the mRNA produced for this protein.[16–18] Others have shown that steady-state levels of mRNA for 5′-ID and for SeP are reduced in rat liver in Se deficiency, compared with Se adequacy.[18]

The transcription rates for selenoprotein genes other than GPX1, and their dependence on dietary Se intake, have received little attention. Examination of selenoprotein gene expression in tissues other than liver is also required, since certain selenoproteins are specific to certain tissues. Finally, selenoprotein gene expression in animals consuming high levels of dietary Se has not been considered. Some of the effects of Se are seen only at dietary intakes that far exceed the nutritional requirement. Hence, the aims of this work were: (1) to compare the transcription rates of the genes for GPX1, 5′-ID, and SeP, in livers of rats fed diets deficient, adequate, and high in Se; (2) to determine the stability of mRNAs produced for these selenoproteins in liver and kidney under the same dietary conditions; and (3) to measure the effects of these dietary manipulations on the activities of the enzymes for which these genes code.

Methods and materials

Animals

All procedures related to animal use were approved by the Brigham Young University Animal Care and Use Committee. Weanling male Sprague–Dawley rats (SASCO, Inc., Omaha, NE) were housed in pairs in stainless steel hanging wire cages in a temperature- and light-controlled room (12 hr light, 12 hr dark). Animals were given free access to food and tap water.

Diets

A Torula yeast-based Se-deficient diet[16] was used as the basal diet. Such formulations provide <0.02 mg of Se/kg of diet. Animals received this basal diet or the same diet supplemented with 0.1 or 2.0 mg of Se/kg of diet as sodium selenite. These mixtures provided deficient, adequate, and high levels of dietary Se, respectively. All nutrients, except Se, were provided in the basal diet at levels equal to or exceeding those recommended by the American Institute of Nutrition Ad Hoc Committee on Standards for Nutritional Studies.[19,20] There were 6 rats in each of the three dietary groups. Animals were fed their respective diets for 91 days.

Animal processing

All animals were weighed weekly. At the end of the feeding period, animals were sacrificed by exsanguination via the abdominal aorta under ether anesthesia. Blood was quickly transferred to Vacutainer tubes containing disodium EDTA (10.5 mg/7 ml tube). Plasma was separated from cellular components by centrifugation at 1500*g* for 15 min. Livers and kidneys were excised. Livers were perfused via the portal vein with cold Kcl (150 mmol/L). Portions of each tissue were aliquoted for subsequent isolation of total RNA, for enzyme assays, and—in the case of liver—for isolation of nuclei. Blood was removed from kidney samples by repeated fine mincing with surgical scissors in cold GPX assay buffer or in cold 5′-ID homogenization buffer.

Measurement of glutathione peroxidase activity

To assay GPX1 activity in each individual sample, each aliquot (0.5–1.0 g) of liver or kidney was homogenized with a Tissumizer (Tekmar Company, Cincinnati, OH) in 3 vol ice cold GPX assay buffer prepared according to the method of Lawrence and Burk.[21] Homogenates were centrifuged at 105,000*g* for 1 hr. Supernates were assayed for cytosolic enzyme activity.

Activity of GPX1 in liver and kidney cytosol, and of GPX3 (the plasma form of the enzyme), was assayed by the coupled method of Lawrence and Burk[21] using 2.0 mmol/L of GSH, and 0.25 mmol/L of H_2O_2 as substrate. Protein concentration of cytosols was determined by the method of Lowry et al.[22] One unit of enzyme activity was defined as 1 μmol NADPH oxidized/min.

Measurement of 5′-iodothyronine deiodinase activity

To assay 5′-ID activity in each individual sample, each aliquot (0.5–1.0 g) of liver or kidney was homogenized with a Tissumizer in 2 vol of 5′-ID potassium phosphate buffer, prepared according to the method of Beckett et al.[23] The assay for 5′-ID activity was adapted from the procedures of Beckett et al.[24] and Chopra,[25] which measure T3 produced from added T4. Duplicate aliquots (0.9 ml) of each crude homogenate were incubated in glass tubes at 37°C for 5 min. For each pair, at the end of the incubation, 100 μL of T4 solution (50 μg/mL) (L-thyroxine, sodium salt, pentahydrate; T-2501, Sigma Chemical Co., St. Louis, MO) were added to one tube (sample), and 100 μL of deionized water was added to the other (blank). Duplicate 100 μL aliquots from each tube were then immediately extracted into 200 μL of ethanol (zero time). Additional duplicate aliquots were taken from each tube at 20 min for liver samples and at 45 min for kidney samples. Ethanolic extracts of all aliquots were centrifuged for 5 min at 12,000*g*. Supernates were assayed for total T3. Blank values were subtracted from sample values to determine the amount of T3 produced from added T4. Protein content of homogenates was measured by the method of Lowry et al.[22] Enzyme activity was expressed as femtomoles of T3 produced from added T4 min/mg of protein. Total T3 in ethanolic extracts was measured by radioimmunoassay (Canine T3 Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA).

Isolation of total RNA

Equal aliqots of liver from each animal within a dietary group were combined for isolation of total RNA by the method of Chirgwin et al.[26] as modified by Freeman et al.[27] The same procedure was followed for kidney samples. The concentration of RNA in the finished preparation was determined by optical density measurement at 260 nm. There were no significant differences in RNA yield or purity due to diet. RNA samples were stored frozen at −70°C.

Isolation of nuclei

Liver nuclei were isolated by the method of Kasper[28] as modified by Simmons et al.[29] Nuclei suspended in storage buffer were flash frozen in liquid nitrogen and stored at −70°C until they were used in run-on transcription assays.

Measurement of transcription rate

The effects of different Se intakes on the relative rates of transcription of the genes for selenoproteins was determined in nuclear run-on transcription assays performed as described by Celano et al.[30] using α-^{32}P-UTP (E.I. DuPont de Nemours & Co., NEN Products, Boston, MA) to label nascent transcripts. Plasmids containing cDNA probes for various genes were denatured by boiling for 10 min, then slot blotted (5 μg/slot) to charge-modified nylon membranes (MagnaGraph, Micron Separations Inc., Westboro, MA) using a slot blot apparatus (Bio-Dot SF, Bio-Rad Laboratories, Richmond, CA). Vacuum baking for 30 min at 80°C, followed by UV crosslinking at 120 mJ/cm^2 (Spectrolinker, Spectronics Corporation, Westbury, NY) fixed the plasmids to the membranes. Membranes were prehybridized in Church–Gilbert buffer (10 g/L of bovine serum albumin, 1 mmol/L of EDTA, 0.5 mol/L of $NaHPO_4$, pH 7.2, 70 g/L of SDS)[31] at 65°C for 2–6 hr. Following isolation of nuclear RNA containing the labeled transcripts, equal quantities of incorporated radioactivity from each assay were added to prehybridized nylon filters containing immobilized plasmids and hybridized overnight in fresh Church–Gilbert buffer at 65°C. Membranes were then washed three times (5 min, 5 min, then 15 min) in 0.2× SSC, 4 g/L of SDS at 65°C (20× SSC is 3 mol/L of NaCl, 0.3 mol/L of sodium citrate, pH 7.0), air dried, and exposed to x-ray film using an intensifying screen at −70°C for up to 96 hr, depending on the intensity of the signal detected. Signal intensity on the x-ray film was quantitated by laser densitometry (LKB UltroScan XL Enhanced Laser Densitometer, with GelScan XL Software, Version 2.0, Pharmacia LKB Biotechnology, Uppsala, Sweden).

Probes

The cDNAs for rat selenoproteins were generous gifts from the authors who first reported their isolation and sequence. The cDNA for GPX1 was the same probe used previously in our work[16] and was provided originally by Dr. Ye-Shih Ho of Duke University.[32] The cDNA probe for 5′-ID was a generous gift of Dr. Marla Berry of Harvard University,[11] and the SeP cDNA was kindly supplied by Dr. Kristina Hill of Vanderbilt University.[12] A cDNA for glyceraldehyde 3-phosphate dehydrogenase (G3PDH)[33] was used as a control probe in Northern blot analysis to ensure equal loading of RNA.

Northern blot analysis

Northern blot analysis of total RNA isolated from liver and kidney pools from rats fed three levels of dietary Se was performed according to standard procedures.[34] Following electrophoresis in formaldehyde-agarose gels, RNA was pressure blotted to nylon membranes using a PosiBlot Pressure Blotter (Stratagene, La Jolla, CA). Membranes were subsequently vacuum-baked and UV-crosslinked as described earlier. Probes used were coding sequences from the cDNAs described earlier, radioactively labeled with α-^{32}P-dCTP (E.I. DuPont de Nemours & Co., NEN Products, Boston, MA) by random primed labeling.[35] Prehybridization, hybridization, and subsequent washes were carried out according to the membrane manufacturer's directions. Autoradiography and quantitation were as described before. Signal intensities for each sample were normalized to the intensity of signal for G3PDH from that sample.

Statistics

Effects due to different Se supplements were analyzed using one-way analysis of variance, followed by Student's *t*-tests. Differences occurring at a probability level $P < 0.05$ were accepted as statistically significant.

Results

Figure 1 shows the average growth of rats fed three levels of dietary Se for 13 weeks. No significant differences were seen at any time point.

Figure 2 shows that the Se deficiency reduced GPX1 activity in liver and kidney to barely detectable levels. In plasma, GPX activity was also markedly reduced, to 3% of the Se-adequate (0.1 mg of Se/kg of diet) group. In both tissues and in plasma, increasing the dietary supplement of Se from 0.1 to 2.0 mg of Se/kg of diet did not further increase GPX activity.

Activity of 5′-ID is shown in *Figure 3*. Compared with the Se-adequate group, mean enzyme activity was reduced 70% in liver and 62% in kidney by Se deficiency. Mean values for 5′-ID activity in rats fed a Se supplement of 2.0 mg of Se/kg of diet were intermediate between those of rats fed diets deficient and nutritionally adequate in Se.

In *Figure 4* the results of a run-on transcription assay performed with liver nuclei from rats fed the three levels of dietary Se are shown. Transcription of the genes for GPX1, 5′-ID, and SeP was unaffected either by Se deficiency or by a supplement providing 20 times the amount of Se known to be nutritionally adequate.

The results of Northern blot analysis of total RNA from livers and kidneys of rats fed three levels of dietary Se are

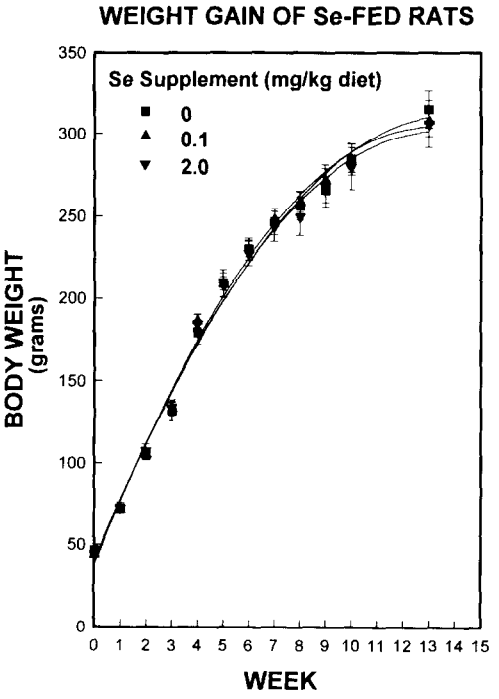

Figure 1 Weight gain of rats fed Torula-yeast diets supplemented with selenium as sodium selenite. Values shown are means ± SEM for each dietary group at each time point. There were no statistically significant differences between groups at any time point.

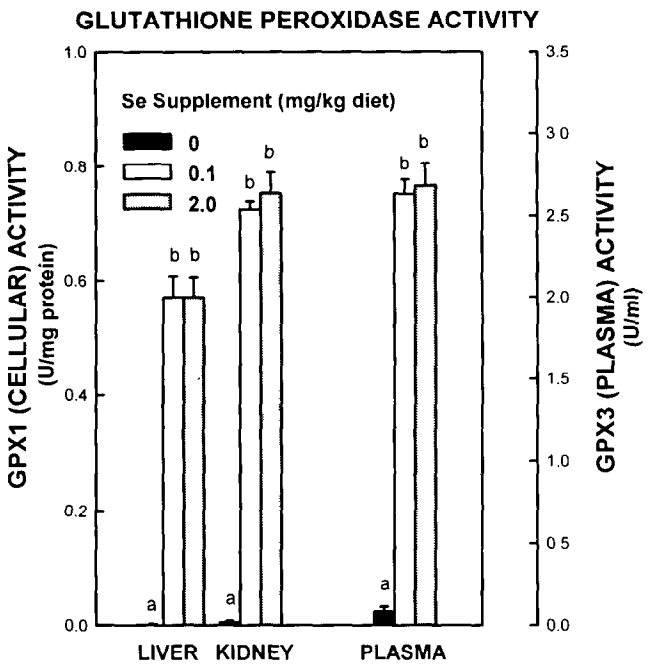

Figure 2 Activity of selenium-dependent glutathione peroxidase in tissues (GPX1) and plasma (GPX3) of rats fed three levels of dietary selenium. Values shown are means + SEM for each dietary group. For each tissue, differences between bars not sharing a common superscript are statistically significant ($P < 0.05$).

known in *Figure 5*. Selenium deficiency markedly reduces steady-state levels of mRNA for GPX1 in both liver and kidney. Intensity of signal in samples from deficient animals, compare with Se-adequate rats, was only 11% in liver and 9% kidney. No further increases were observed in signal intensities for bands in lanes representing samples from rats fed a supplement of 2.0 mg of Se/kg of diet. At each level of Se intake, the intensity of signals from kidney samples was less than from liver samples. When corrected for G3PDH signal intensity, kidney signals ranged from 37–45% of the intensity of liver samples.

As with GPX1, liver levels of SeP mRNA exceeded those in kidney at all dietary intakes. When signal intensity was normalized to that of G3PDH, kidney samples ranged from 25 to 40% of the intensity of liver signals. In addition, turnover of SeP mRNA was less in liver than in kidney during Se deprivation. While inadequate Se intake reduced signal intensity in kidney samples by 50%, there was only a 14% decrease in the intensity of signals from liver samples due to Se deficiency. Signal intensities were the same for both Se supplements.

In contrast to GPX and SeP, mRNA levels for 5′-ID were higher in kidney samples than in liver samples in all dietary groups. Liver signals averaged only 30% the intensity of signals from kidney samples. Interestingly, turnover of 5′-ID mRNA was markedly lower in kidney than in liver in Se deficiency. While liver sample signal intensity was reduced 69% by Se deficiency, the corresponding decrease in kidney signal intensity was only 19%. As with GPX1, high Se intake did not increase the signal intensity of 5′-ID above that achieved with lower but adequate levels of dietary Se.

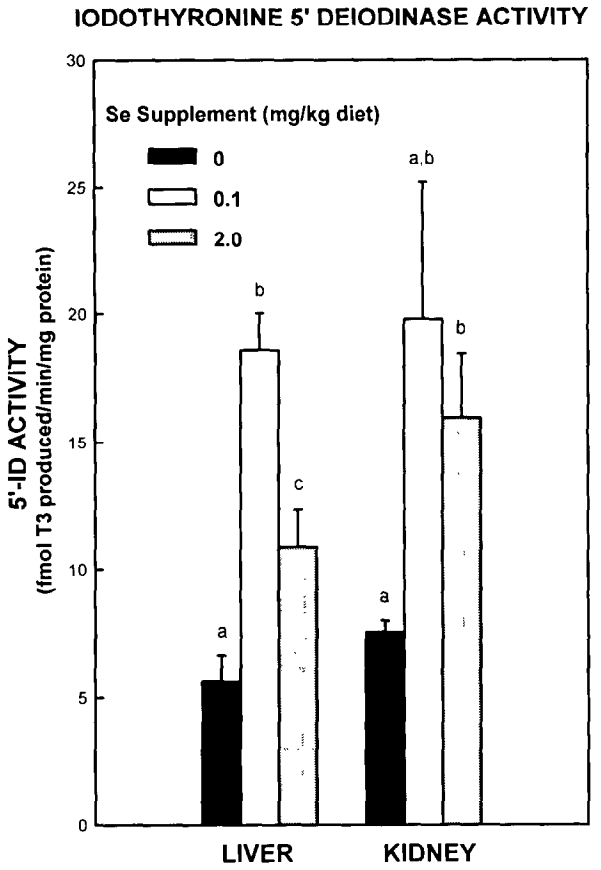

Figure 3 Activity of type I iodothyronine 5′ deiodinase in tissues of rats fed three levels of dietary selenium. Values shown are means + SEM for each dietary group. For each tissue, differences between bars not sharing a common superscript are statistically significant ($P < 0.05$).

Discussion

In this study, growth of weanling male rats was unaffected by Se intake. This result is consistent with those of recent studies showing no effect on growth of a Se-deficient[23,26]

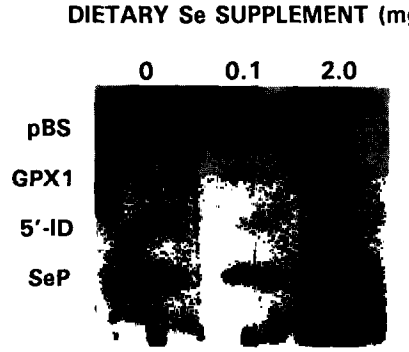

Figure 4 Results of a run-on transcription assay performed with nuclei isolated from livers of rats fed three levels of dietary selenium. Differences between dietary groups in signal intensity, measured by laser densitometry, were less than 10% for each probe. pBS, pBlueScript (Stratagene, La Jolla, CA); GPX1, cellular selenium-dependent glutathione peroxidase; 5′-ID, type I iodothyronine 5′ deiodinase; SeP, selenoprotein P.

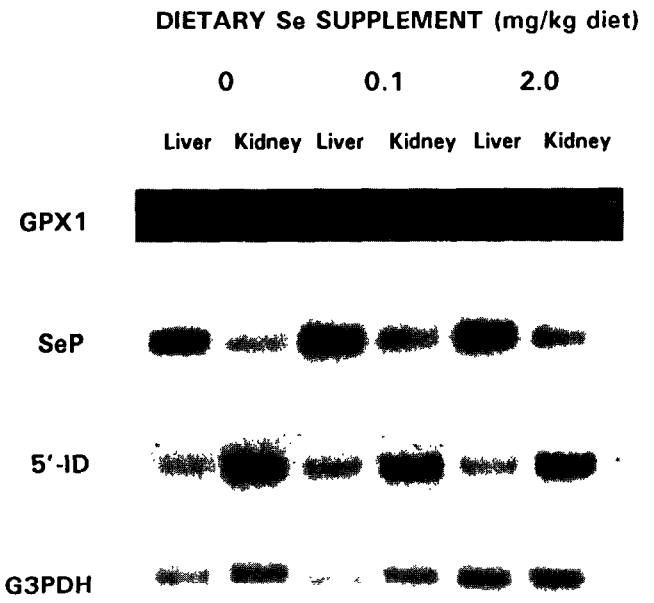

Figure 5 Northern blot analysis of total RNA from livers and kidneys of rats fed three levels of dietary selenium. Signal intensity was measured by laser densitometry. Signal intensities for selenoprotein gene products were normalized to the corresponding signal intensity for G3PDH. GPX1, cellular selenium-dependent glutathione peroxidase; SeP, selenoprotein P; 5'-ID, type I iodothyronine 5' deiodinase; G3PDH, glyceraldehyde 3-phosphate dehydrogenase.

diet or of a diet supplemented with 2.0 mg of Se/kg of diet as sodium selenite.[37] It also suggests that the highest level of Se fed to rats in this study was not toxic. Thus, differences in gene expression in this study were not due to differences in growth or to toxic effects of the high Se supplement.

The effects of different Se intakes on activity of GPX1 and GPX3 are consistent with those previously reported. In almost all cases in rats, Se deficiency reduces GPX activity virtually to zero, while increases in Se intake above the nutritional requirement elicit no further increases in enzyme activity.[38] As noted earlier, this plateauing effect in enzyme activity was used to establish the RDA for Se.

Others have shown that Se deficiency decreases activity of 5'-ID in tissues of rats, in a manner similar to that observed in this study.[23] Likewise, Behne et al.[37] reported that a 0.3 mg of Se/kg of diet supplement significantly increased 5'-ID activity in rat liver, compared with animals receiving no supplement. However, when the Se supplement was increased to 2.0 mg/kg of diet, 5'-ID activity was reduced to a level intermediate between those of the deficient and adequate groups. A similar pattern was seen in this work (*Figure 3*). The mean value for 5'-ID activity was lower in both kidney and liver of animals fed the higher Se supplement than for the 0.1 mg of Se/kg of diet group. However, in this work that difference was significant only in liver.

As is the case for GPX1,[16] transcription of the genes for SeP and for 5'-ID in rat liver appears to be unaffected by Se deficiency. Likewise, a high intake of dietary Se has little measurable effect on selenoprotein gene transcription. Although transcription of these genes does not appear to depend on Se intake, the possibility remains that changes in dietary intake of Se may elicit changes in the transcription of other genes. We have shown that Se deficiency alters binding of nuclear proteins to transcription regulatory elements in rat liver.[39] While these elements have not yet been reported to be present in the flanking regions of selenoprotein genes, they occur in several other genes. Transcription of selenoprotein genes may be altered by means other than changes in dietary Se intake. The recent report of an oxygen-responsive element in the flanking region of the GPX1 gene[40] suggests the possibility of transcriptional regulation in tissues subjected to oxidative stress, such as the lung.

The effect of Se deficiency on steady-state levels of mRNA for selenoproteins appears to be tissue-specific. It is interesting to note that mRNA for GPX1 and for SeP is more abundant in liver than in kidney, while the opposite is true for 5'-ID mRNA. For 5'-ID and SeP, the tissue in which mRNA levels were higher in Se adequacy was also the tissue in which, on a percentage basis, mRNA was more resistant to degradation in Se deficiency. In contrast, mRNA for GPX1 was reduced in Se deficiency to the same degree in both liver and kidney. In no case did the higher level of Se supplementation further augment signal intensity for any of the three gene products measured.

Selenium deficiency did not affect transcription of genes for selenoproteins but it did reduce steady-state levels of selenoprotein mRNA. These observations together suggest that mRNA turnover is the pretranslational step in the expression of these genes most affected by dietary Se intake. However, the relative resistance to mRNA degradation in Se deficiency varied among the three gene products and between tissues. It therefore appears that whatever stabilizing factors are responsible for decreasing selenoprotein mRNA turnover when Se is available are also diet-inducible. Identification and characterization of these stabilizing factors and their regulation by diet will do much to broaden our understanding of the molecular basis for Se nutrient requirement.

In summary, dietary Se deficiency reduced activity of GPX and 5'-ID in all matrices studied. In addition, steady-state levels of mRNA for GPX, 5'-ID, and SeP were also reduced. This reduction in steady-state mRNA occurred in the absence of any demonstrable effects of diet on gene transcription. Hence, as it is with GPX, mRNA turnover for 5'-ID and SeP appears to be the pretranslational process most sensitive to changes in dietary Se intake.

The only statistically significant difference between the effects of a 0.1 and a 2.0 mg of Se/kg of diet supplement on enzyme activity was in liver, where increasing the supplement reduced 5'-ID activity. There were no notable differences between the two supplements on gene expression.

The mechanisms by which stabilization of selenoprotein mRNA varies among tissues remain to be explored. The observation that high levels of Se intake do not increase mRNA levels above those achieved by lower but nutritionally adequate levels of Se suggests that the process by which such stabilization occurs is a saturable one. Further, percentage decreases in steady-state mRNA and enzyme activity were not always the same. This observation leaves open the possibility that message translation and protein turnover

may also play a role in some tissues in determining the ultimate level of selenoenzyme activity achieved. Resolution of these issues awaits the results of future studies.

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