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Selenium persistency and speciation in the tissues of lambs following the withdrawal of dietary high-dose selenium-enriched yeast

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The objective was to determine the concentration of total selenium (Se) and the proportion of total Se comprised as selenomethionine (SeMet) and selenocysteine (SeCys) in post mortem *tissues of lambs in the 6 weeks period following the withdrawal of a diet containing high-dose selenised yeast (HSY), derived from a specific strain of* Saccharomyces cerevisae CNCM (Collection Nationale de Culture de Micro-organism) I-3060. Thirty Texel × Suffolk lambs used in this study had previously received diets (91 days) containing either HSY (6.30 mg Se per kg dry matter (DM)) or an unsupplemented control (C; 0.13 mg Se per kg DM). Following the period of supplementation, all lambs were then offered a complete pelleted diet, without additional Se (0.15 mg Se per kg DM), for 42 days. At enrolment and 21 and 42 days later, five lambs from each treatment were blood sampled, euthanased and samples of heart, liver, kidney and skeletal muscle (*longissimus dorsi and* psoas major*) tissue were retained. Total Se concentration in whole blood and tissues was significantly (P < 0.001) higher in HSY lambs at all time points that had previously received long-term exposure to high dietary concentrations of SY. The distribution of total Se and the proportions of total Se comprised as SeMet and SeCys differed between tissues, treatment and time points. Total Se was greatest in HSY liver and kidney (22.64 and 18.96 mg Se per kg DM, respectively) and SeCys comprised the greatest proportion of total Se. Conversely, cardiac and skeletal muscle (*longissimus dorsi and* psoas major*) tissues had lower total Se concentration (10.80, 7.02 and 7.82 mg Se per kg DM, respectively) and SeMet was the predominant selenised amino acid. Rates of Se clearance in HSY liver (307 μg Se per day) and kidney (238 μg Se per day) were higher compared with HSY cardiac tissue (120 μg Se per day) and skeletal muscle (20 μg Se per day). In conclusion, differences in Se clearance rates were different between tissue types, reflecting the relative metabolic activity of each tissue, and appear to be dependent on the proportions of total Se comprised as either SeMet or SeCys.*

Introduction

During the latter part of the 1950s, it was discovered that selenium (Se) played an important physiological role in animal nutrition and that suboptimal concentrations often resulted in the appearance of myopathies (Schubert *et al*., 1961).

Diets for ruminants are typically of plant origin, and as plant Se concentrations can be extremely variable, dietary Se supplements are generally required for ruminant diets. Selenium supplements are in two forms: inorganic mineral salts, such as sodium selenite (SS; Na_2SeO_3) or selenate (Na_2SeO_4), or organic forms, such as Se-enriched yeast (SY),

in which selenomethionine (SeMet) is the predominant form of Se (Korhola *et al*., 1986). However, the use of SY has only recently been authorised within the European Union (EU) after a series of tolerance studies established that animal health was not adversely affected by the administration of at least 10 times the maximum permitted concentration, a level thought to represent an accidental over dosage (Commission Directive 87/153/EC amended by 2001/79/EC). However, at the completion of such studies there are no such requirements to investigate the persistency of Se within the blood, visceral, and in particular edible tissues, of animals that could be intended for human consumption.

Studies with both dairy cows and beef cattle have shown that SY is more efficiently absorbed and assimilated than

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inorganic Se, as SeMet can be taken up via methionine transporter mechanisms within the gastro-intestinal tract and non-specifically incorporated into body proteins in place of methionine (Weiss, 2003), resulting in elevated concentrations of Se in both milk (Juniper *et al.*, 2006) and body tissues (Qin *et al.*, 2007). Surai (2006) reported that SeMet is retained in tissue proteins to a greater extent than seleno-cysteine (SeCys) or Se derived from inorganic forms. A number of studies have detailed the accumulation of Se within the tissues of animals exposed to supranutritional concentrations of organic Se (Lawler *et al.*, 2004; Taylor, 2005) but very little information is available on either the persistency of Se or the distribution of selenised amino acids within the tissues of animals following the withdrawal of diets containing high doses of organic Se. This paper aims to redress this shortfall in information and reports on the concentration of total Se and the proportion of total Se comprised as either SeMet or SeCys in *post mortem* tissues of lambs following the withdrawal of high-dose SY (HSY) diets.

Material and methods

Animal, experimental design and diets
The work was conducted under the authority of the UK Animals (Scientific procedures) Act 1986 (Home office, 1986) and procedures were undertaken by staff holding appropriate licences authorities under the Act. All lambs were housed on straw bedding with sufficient space as prescribed by Home Office guidelines. Fresh potable water was available at all times.

Thirty Texel $\times$ Suffolk lambs, that had either been previously exposed to a diet supplemented with high dose of selenised yeast (Sel-Plex$^{\circledR}$ (*Saccharomyces cerevisiae* CNCM, Collection Nationale de Culture de Micro-organism, I-3060), Alltech, Nicholasville, KY, USA) (HSY; 6.30 ± 0.18 mg Se per kg dry matter (DM)) or an unsupplemented control (C; 0.13 ± 0.01 mg Se per kg DM) during a 91-day period, were enrolled into the study. Lambs, with a mean age of 97 days and body weight of 43.8 ± 1.40 kg, were offered a complete pelleted diet that contained no additional Se over a period of 42 days. The complete pelleted ration (Dairy Direct, Bury St Edmunds, UK) contained 360, 340, 165, 90, 25 and 20 g/kg DM of ground barley, wheat bran, ground wheat, soya-bean meal, molasses and vitamin and mineral supplement, respectively, was offered fresh daily *ad libitum* (refusal maintained at 100 g/kg DM intake) following the removal of the previous day's refusal.

Sampling procedures and measurements
Feed analyses. Representative samples of unsupplemented feed were taken weekly and frozen ($-20°$C). At the end of the study, feed samples were bulked on a weekly basis, sub-sampled and then analysed for nutritional composition and total Se content.

Oven DM concentration was determined by drying samples in a forced draught oven at 100°C for 24 h. Ash was obtained by incineration in a muffle furnace at 550°C for 16 h. Neutral-detergent fibre (NDF) concentration was determined by an ANKOM-Fibre analyser 200 (ANKOM-Technology, Fairpoint, NY, USA) by procedures described by Ministry of Agriculture Fisheries and Food (MAFF; 1993) using SDS and α-amylase solutions and corrected for residual ash (MAFF, 1982). Starch was determined by polarimetry (MAFF, 1982) with random samples analysed using the enzyme technique (MacRae and Armstrong, 1968) to verify the calibration of the polarimeter. Nitrogen concentration was measured by the Kjeldahl technique and water-soluble carbohydrate (WSC) concentration was measured spectrophotometrically. Oil-B was determined and metabolisable energy (ME) was estimated, using the E_3 method, by MAFF (1993).

Blood and tissue sampling. At enrolment (PT_0), and 21 and 42 days later (PT_{21} and PT_{42}, respectively), five lambs from each treatment group were blood sampled and euthanased and a range of tissues sampled. Blood samples were taken via jugular venepuncture using the Vacutainer system (BD Diagnostics, Oxford, UK) using a 21-Gauge needle into a 5-ml lithium heparin-pretreated tube, for subsequent determination of total Se and Se species. Following euthanasia, samples of heart, liver, kidney and skeletal muscle (*longissimus dorsi* and *psoas major*) were retained to determine total Se and Se species.

Selenium analyses. Total Se for pelleted diets, blood samples and tissues (heart, liver, kidney and skeletal muscle tissue) was determined by mineralising 1 g of each sample in 4-ml HNO_3 and 2-ml H_2O_2 within a closed-vessel heating block system. The solution was further diluted with water and Se was subsequently determined using inductively coupled plasma mass spectrometry (ICP-MS) (Elan 6100 ICPMS; Perkin Elmer, MA, USA).

Selenium speciation was determined using the method described by Palacios *et al.* (2005). Briefly, samples were initially incubated for 5 h with DL-dithiothreitol and iodoacetamide to reduce and alkylate SeCys. Samples were then spiked with SeMet[77] (SEMET Ltd, Rypin, Poland) and subsequently incubated for 24 h at 37°C with a mixture of protease and lipase maintained at a pH 7.5. Following incubation, the mixture was centrifuged and the supernatant separated and purified by cell exclusion liquid chromatography. Aliquots of the supernatant were analysed by reversed-phase HPLC-ICPMS using an ICP MS equipped with a collision cell (Elan 6100 ICPMS).

Statistical analysis
Statistically significant differences between the two treatment groups, C and HSY, for whole-blood Se content and *post mortem* tissue total Se content were determined by ANOVA using the GLM of SAS (v. 8.2; SAS Institute, Inc., Cary, NC, USA). Sources of variation within the model included treatment groups (1 d.f.) and time of sampling (1 d.f.). Statistical tests were undertaken for main effects and treatment $\times$ time interactions. Results are presented as least square means with the s.e. and level of significance.

Results and discussion

Feed analyses

The nutritional composition of the pelleted diet is shown in Table 1. Mean values for DM, crude protein, starch, WSC, ADF, NDF, ash, oil-B and ME were 877 g/kg fresh weight, 176, 421, 56, 71, 195, 64, 35 g/kg DM and 12.3 MJ/kg DM, respectively. The mean total Se content of the diet was 0.15 ± 0.01 mg/kg DM.

Selenium content of blood and tissues

Total Se concentrations of whole blood, heart, liver, kidney and skeletal muscle (*longissimus dorsi* and *psoas major*) at PT_0, PT_{21} and PT_{42} are shown in Table 2.
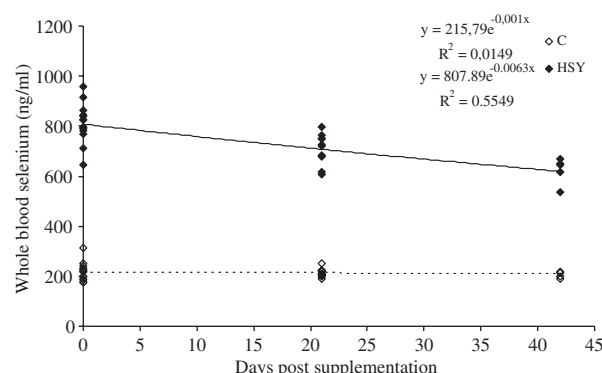
Selenium concentration in whole blood

There were significant differences ($P < 0.001$) in whole-blood total Se concentration between treatment groups. At enrolment, HSY lambs had higher ($P < 0.001$) whole-blood Se concentrations than C (766.4 v. 216.4 ng Se per ml), as would be expected from animals that had been previously exposed to high doses of Se. These values were similar to those found in pregnant ewes (Davis *et al.*, 2006) and

wether lambs (Cristaldi *et al.*, 2005) that had received comparable Se doses in the form of SS. Similarly, whole-blood Se concentrations were still significantly higher ($P < 0.001$) in HSY animals at PT_{21} and PT_{42} when compared with C, although HSY whole-blood total Se values were seen to decline between successive time points ($P < 0.001$), whereas those of C remained relatively constant.

Trend line analysis of whole-blood total Se content over time is shown in Figure 1. Extrapolation of the resultant curve for whole-blood total Se concentration at PT_0, PT_{21} and PT_{42} indicated that it would require approximately 210 days for whole-blood Se concentrations in HSY lambs to achieve similar concentrations to those of C lambs.

Selenium concentration in tissues

Total Se concentrations in heart, liver, kidney and skeletal muscle (*longissimus dorsi* and *psoas major*) were significantly ($P < 0.001$) higher in HSY animals, irrespective of time, when compared with C animals. Tissue total Se concentrations of C animals did not differ appreciably between successive time points, whereas those of HSY animals declined, the rate of decrease varying between differing tissue types.

Table 1 *Mean ($\pm$ s.e.) laboratory-determined nutrient composition of experimental diets*

g/kg dry matter (DM)[†]	Mean $\pm$ s.e.
DM (g/kg fresh weight)	877 ± 0.57
Crude protein	176 ± 2.00
Metabolisable energy (MJ/kg DM)	12.3 ± 0.07
Starch	421 ± 3.71
Water-soluble carbohydrate	56 ± 0.88
Acid-detergent fibre	71 ± 2.33
Neutral-detergent fibre	195 ± 2.08
Ash	64 ± 0.67
Oil-B	35 ± 0.33
Selenium (mg/kg DM)	0.15 ± 0.01

[†]Unless otherwise stated.

Figure 1 Trend line analysis of the relation between whole-blood total selenium content and days of sampling of lambs that had been exposed to a diet supplemented with high dose of selenised yeast (HSY) or unsupplemented (C).
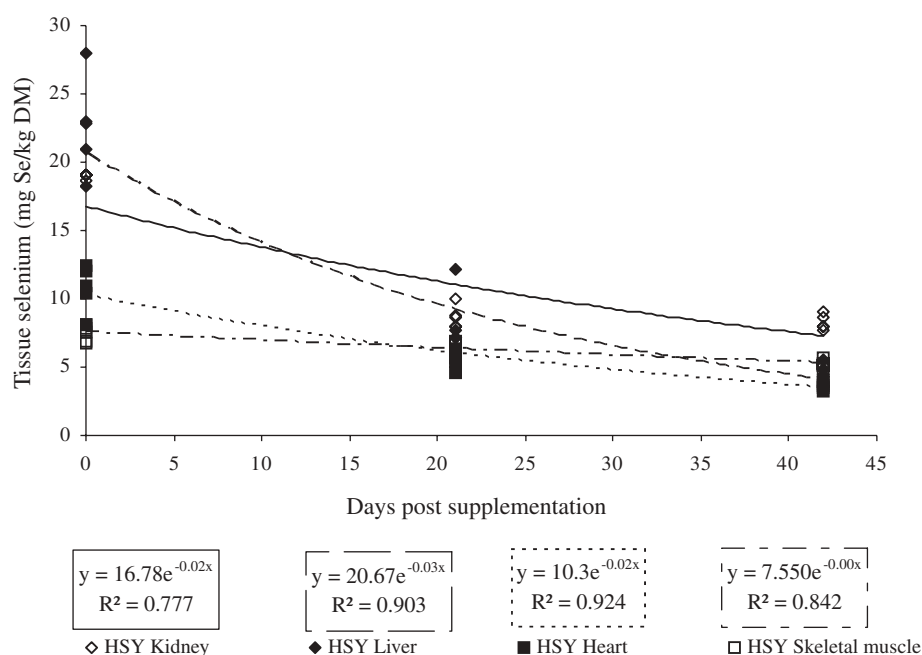
Table 2 *Mean ($\pm$s.e.) for total selenium concentrations in blood (ng/ml) and in heart, liver, kidney and muscle (psoas major and longissimus dorsi) (mg/kg dry matter) of lambs that had been exposed to a diet supplemented with high dose of selenised yeast (HSY) or unsupplemented (C) after 0 (PT_0), 21 (PT_{21}) and 42 (PT_{42}) days of withdrawal*

	Time effect							Significance	
	PT_0		PT_{21}		PT_{42}				
	HSY	C	HSY	C	HSY	C	s.e.	Treatment	Treatment $\times$ Time
Whole blood	766.4^b	216.4^a	712.2^b	212.2^a	622.0^c	205.3^a	25.84	***	ns
Heart tissue	10.80^b	1.28^a	5.62^c	1.46^a	3.64^d	1.38^a	0.35	***	***
Liver tissue	22.64^b	1.35^a	8.06^c	1.49^a	4.50^d	1.16^a	0.81	***	***
Kidney tissue	18.96^b	5.90^a	8.70^c	5.56^a	8.26^c	5.88^a	0.31	***	***
Skeletal muscle tissue									
Longissimus dorsi	7.82^b	0.30^a	6.74^c	0.32^a	5.38^d	0.29^a	0.15	***	***
Psoas major	7.02^b	0.29^a	6.44^b	0.33^a	5.02^c	0.33^a	0.18	***	***

a,b,c,dMean values within a row with different superscripts are significantly different ($P < 0.05$). Significance levels.
***$P < 0.001$; ns = $P > 0.05$.

$$y = 16.78e^{-0.02x}$$
$$R^2 = 0.777$$

$$y = 20.67e^{-0.03x}$$
$$R^2 = 0.903$$

$$y = 10.3e^{-0.02x}$$
$$R^2 = 0.924$$

$$y = 7.550e^{-0.00x}$$
$$R^2 = 0.842$$

◇ HSY Kidney   ◆ HSY Liver   ■ HSY Heart   □ HSY Skeletal muscle

Figure 2 Changes in tissue selenium concentrations in the 42 days period following the withdrawal of long-term high-dose administration of selenised yeast (HSY).

Among HSY animals, the concentration of total Se at PT_0 was highest in the liver (22.64 mg/kg DM), which declined significantly to approximately 35% of PT_0 values by PT_{21} and 20% by PT_{42}). Similar Se concentrations to those of HSY animals at PT_0 have been recorded in the liver of pregnant ewes (Davis *et al.*, 2006) and wether lambs (Cristaldi *et al.*, 2005) that had received comparable doses as SS. The total Se content of kidney in HSY animals at PT_0 was 18.96 mg/kg DM; this value was higher than those reported for pregnant ewes (Davis *et al.*, 2006) and wether lambs (Cristaldi *et al.*, 2005), indicating that Se source may influence Se retention between different tissues. The concentration of total Se in kidney declined over time to 46% of PT_0 values by PT_{21} and 43% by PT_{42}. Total Se concentrations in both cardiac and skeletal muscle of HSY animals at PT_0 were 10.8 and 7.42 mg Se per kg DM, respectively. At PT_{21} and PT_{42}, there was a marked reduction in total Se within cardiac tissue (50% and 34% of PT_0 values, respectively). However, skeletal muscle did not show the same rate of Se clearance, with Se values at PT_{21} being approximately 90% of PT_0 and 70% at PT_{42}. These results indicate that Se clearance rates were greatest in glandular visceral tissues, namely the liver and kidney.

Trend line analysis of the relation between whole-blood and tissue total Se is shown in Figure 2. Extrapolation of the resultant curve for whole-blood Se concentration and the concentration of Se within individual tissue types indicated that the Se content of kidney, liver and heart of HSY animals would decline to those of C animals after 55, 70 and 80 days post supplementation, respectively. However, in contrast regression trend line analysis indicated that skeletal muscle tissue would require considerably longer, with an estimate of approximately 380 days, following the
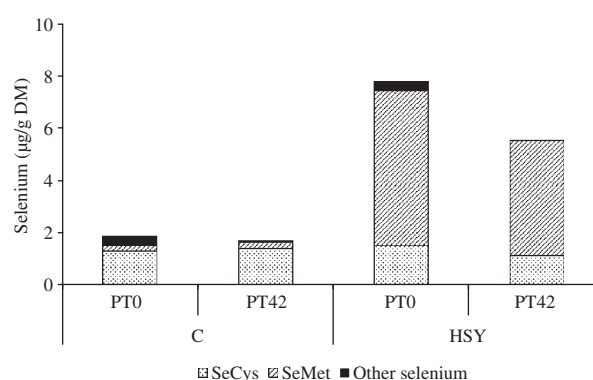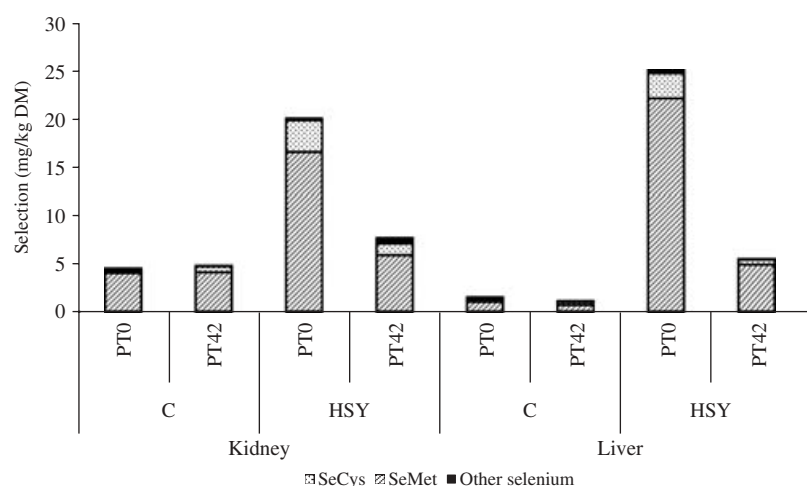

Figure 3 Whole-blood total selenium and proportion of total selenium as selenomethionine (SeMet), selenocysteine (SeCys) and other selenium species of lambs at 0 (PT_0) and 42 days (PT_{42}) days after exposure to a diet supplemented with a high dose of selenised yeast (HSY) or an unsupplemented control (C).

withdrawal of HSY supplementation, to achieve similar Se concentrations to those of C group animals, with average Se clearance rates of 307, 238, 120 and 20 µg Se per day for liver, kidney, heart and skeletal muscle, respectively.

Selenised amino acids contents of whole blood and tissues
Total Se and the proportion of total Se comprised as either SeMet, SeCys or as other unidentified Se fractions, for whole blood, visceral and muscle tissues are shown in Figures 3, 4 and 5, respectively.

Selenised amino acids content of whole blood. Speciation of Se fractions showed that much of the large difference in total blood Se concentrations in HSY animals was due to the greater proportion of total Se comprised as SeMet, probably

Figure 4 Total selenium and proportion of total selenium comprised as selenomethionine (SeMet), selenocysteine (SeCys) and other selenium species in the kidney and liver of lambs that had been exposed to a diet supplemented with high dose of selenised yeast (HSY) or unsupplemented (C) after 0 (PT_0) and 42 (PT_{42}) days of withdrawal.
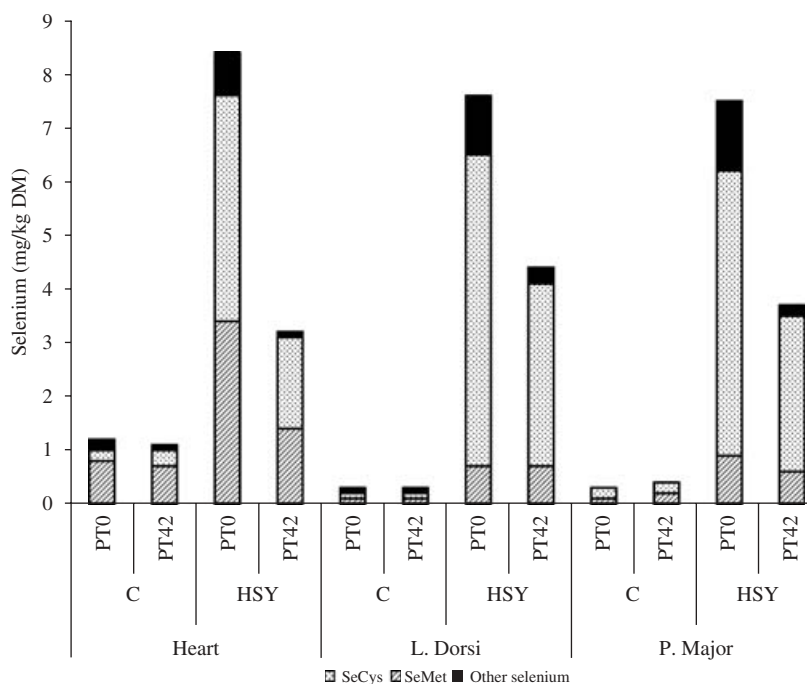
Figure 5 Total selenium and proportion of total selenium comprised as selenomethionine (SeMet), selenocysteine (SeCys) and other selenium species in the muscles of lambs that had been exposed to a diet supplemented with high dose of selenised yeast (HSY) or unsupplemented (C) after 0 (PT_0) and 42 (PT_{42}) days of withdrawal.

attributable to a combination of the high concentration of SeMet in the SY product (SeMet comprises 54 to 74% of total Se; Rayman, 2004) and the uptake of SeMet from the gastro-intestinal tract via Methionine transporter mechanisms (Weiss, 2003) during the period of supplementation. Furthermore, decreases seen in whole-blood total Se between successive time points was primarily the result of decreases in the proportion of total Se comprised as SeMet, because the proportion of total Se comprised as SeCys was similar between both treatment groups and sequential time points.

Selenised amino acids contents of tissues. The proportion of total Se comprised as either SeMet or SeCys in the tissues of HSY animals was markedly different, irrespective of time point, from those of controls.

Among C animals, the main form of Se in the kidney, liver and heart was SeCys, which comprised 90%, 60% and 60% of total Se, respectively, while in skeletal muscle tissue, there was little difference in the proportions of SeMet and SeCys, which formed approximately 35% of total Se. The differences between cardiac and skeletal muscles in the proportion of total Se comprised as SeCys reflects the

relative activity of each muscle type. Active tissues, such as cardiac muscle, are much more likely to be subjected to oxidative damage and the higher SeCys content of cardiac tissue could be indicative of greater antioxidant-specific tissue selenoenzyme activity.

In the tissues of HSY animals, the main selenised amino acid in kidney and liver was SeCys, whereas cardiac and skeletal muscle comprised mainly SeMet. At PT_{42}, SeCys still remained the predominant selenised amino acid within kidney and liver, although much of the reduction in total Se contents within these tissues were attributable to reductions in the proportion of total Se comprised as SeCys. Similarly, SeMet was the predominant Se species within heart and skeletal muscle at PT_{42}; however, the reduction in total Se in cardiac tissue was the result of similar decreases in the proportion of total Se comprised as both SeMet and SeCys, whereas reductions in the total Se content of skeletal muscle were largely attributable to changes in the proportion of total Se comprised as SeMet.

It would therefore appear that the rates of Se clearance observed are dependent on the Se species present, as clearance rates are faster when SeCys is the predominant form of selenised amino acid, and that Se species are dependent on tissue type. Glandular visceral tissues, such as kidney and liver, are both metabolically active tissues and exhibited greatest Se clearance rates. In contrast, heart and skeletal muscle are much less metabolically active and have much lower Se clearance rates. The abundance of SeCys in more metabolically active tissues is maybe the result of the conversion of SeMet to SeCys via the actions of selenocystathionine, as a large proportion of dietary Se was SeMet in origin. This pool of endogenous SeCys could then be available for conversion to selenide and either the subsequent incorporation into selenoenzymes via UGA codon or excretion via methylation (Aspila, 1988; Suzuki and Ogra, 2002). However, neither tissue selenoenzyme activity nor the appearance of methylated Se species within urine or exhaled breath was determined during the course of this study and, as such, it is not possible to state the ultimate fate of the high concentrations of endogenous Se.

Conclusions

Selenium clearance rates in the tissues of animals that had received long-term exposure to high dietary concentrations of SY appear to be dependent on Se species. Tissues that are more metabolically active have a larger proportion of total Se comprised as SeCys and faster Se clearance rates when compared with tissues with a lower metabolic rate in which SeMet is the predominant form of Se. Regression analysis indicated that it would require a period well in excess of 300 days for the Se contents of muscle tissue in HSY lambs to achieve those of C animals, a period much longer than the usual finishing time for commercial lambs. Consequently, Se intake in humans consuming such lambs could most probably exceed recommended levels of Se intake.

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