

Sparing effects of selenium and ascorbic acid on vitamin C and E in guinea pig tissues

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Abstract

Background: Selenium (Se), vitamin C and vitamin E function as antioxidants within the body. In this study, we investigated the effects of reduced dietary Se and L-ascorbic acid (AA) on vitamin C and α -tocopherol (AT) status in guinea pig tissues.

Methods: Male Hartley guinea pigs were orally dosed with a marginal amount of AA and fed a diet deficient (Se-D/MC), marginal (Se-M/MC) or normal (Se-N/MC) in Se. An additional diet group (Se-N/NC) was fed normal Se and dosed with a normal amount of AA. Guinea pigs were killed after 5 or 12 weeks on the experimental diets at 24 and 48 hours post AA dosing.

Results: Liver Se-dependent glutathione peroxidase activity was decreased ($P < 0.05$) in guinea pigs fed Se or AA restricted diets. Plasma total glutathione concentrations were unaffected ($P > 0.05$) by reduction in dietary Se or AA. All tissues examined showed a decrease ($P < 0.05$) in AA content in Se-N/MC compared to Se-N/NC guinea pigs.

Kidney, testis, muscle and spleen showed a decreasing trend ($P < 0.05$) in AA content with decreasing Se in the diet. Dehydroascorbic acid concentrations were decreased ($P < 0.05$) in several tissues with reduction in dietary Se (heart and spleen) or AA (liver, heart, kidney, muscle and spleen). At week 12, AT in liver, heart and spleen was lower ($P < 0.05$) in Se-D/MC compared to Se-M/MC guinea pigs. Further, AT in liver, kidney and spleen was lower ($P < 0.05$) in Se-N/MC compared to Se-N/NC guinea pigs.

Conclusions: Together, these data demonstrate sparing effects of Se and AA on vitamin C and AT in guinea pig tissues.

Background

Vitamin C is a water soluble antioxidant. In contrast to many mammals, humans (and guinea pigs) are unable to synthesise vitamin C due to the lack of the enzyme L-gulonogamma-lactone oxidase [1] and therefore must rely on diet for maintaining adequate levels of the vitamin. In tissues, the active form of vitamin C, L-ascorbic acid (AA), can be regenerated by the reduction of its oxidised forms, dehydroascorbic acid (DHAA) and the ascorbate free radical (AFR) in a process mediated by glutathione (GSH) [2-6]. Notably, however, other systems have also been implicated in the regeneration of AA [7,8].

Selenium (Se) and vitamin E also function as important antioxidants within the body. Se is an essential trace element that functions in oxidant defence as a component of selenoproteins [9,10]. Vitamin E is a lipid soluble antioxidant present in cell membranes where it plays a vital role in protecting against lipid peroxidation [11-13]. Vitamin E refers to several structurally related compounds; however, α -tocopherol (AT) is the predominant form found in animal tissues. Like vitamin C, vitamin E must be obtained from the diet.

The importance of maintaining adequate levels of Se, vitamin C and vitamin E is underscored by studies indicating that low antioxidant status may be associated with increased risk of developing various diseases [14-16]. Se has been shown to spare both AA [7,8] and AT [17]. Further, sparing effects of AA on AT have also been reported [18-20]. Given that Se, vitamin C and vitamin E activities are interconnected, it is

important to understand how deficiency in one of these antioxidants influences another.

In this study we sought to explore the sparing effects of Se and AA on vitamin C and AT in guinea pig, an *in vivo* model that cannot synthesise vitamin C.

Methods

Animals and Test Diets

On arrival, male Hartley guinea pigs (~10 days old) (Elm Hill Breeding Labs, Inc., Chelmsford, MA) were subjected to a 2 week adaptation period. Following the adaptation period, guinea pigs (n = 22/diet group) had free access to one of 4 test diets (Table 1) and demineralised drinking water. Normal or marginal amounts of AA were given to each guinea pig in a 0.5 mL aqueous solution via oral dosing three times per week (i.e. Monday, Wednesday and Friday). Amount of AA was calculated from the previous day's mean body weight for the diet group [2.4 (normal) or 0.3 (marginal) mg AA/100 g body weight]. Normal and marginal AA levels were chosen based on the AA requirement for growing guinea pigs and previous studies demonstrating suboptimal dietary AA levels [21-23]. Test diets were torula yeast-based diets deficient in Se and similar to diets previously used to induce Se deficiency in guinea pigs [3,24]. Test diets were supplemented with 0 (deficient), 0.05 (marginal) or 0.20 (normal) mg Se/kg diet.

Guinea pigs were killed following an overnight fast by exsanguination while anaesthetised with 3% isoflurane. Half the guinea pigs per diet group were killed after 5 weeks and the remainder after 12 weeks on the experimental diets at 24 or 48 hrs post AA dosing. Blood was withdrawn from the abdominal aorta and collected in heparinised tubes. Plasma was separated from cells by centrifugation ($1000 \times g$, 20 min, 4°C). Skeletal muscle (from quadriceps) and soft tissues were extracted and immediately frozen in liquid nitrogen. Plasma and tissues were stored at -80°C until analysis. The Health

Canada Animal Care Committee approved the experimental protocol. Guinea pigs were treated in accordance with the guidelines of the Canadian Council on Animal Care.

Determination of Vitamin C and α -tocopherol in Tissues

To prevent oxidation of vitamin C, aliquots of plasma were preserved by treating with metaphosphoric acid to a final concentration of 0.85% w/v and excised tissues were immediately frozen in liquid nitrogen [25]. AA and total vitamin C (following reduction of sample) were measured by reverse-phase HPLC with electrochemical detection as described [26,27]. DHAA was calculated as the difference between total vitamin C and AA. AT content was determined by reverse-phase HPLC with fluorescence detection [28].

Enzyme and Other Assays

Se-dependent glutathione peroxidase (Se-GSHPx) activity was measured essentially as described [29] using a SPECTRAMax PLUS microplate spectrophotometer (Molecular Devices, Sunnyvale, CA). Liver extracts were prepared by homogenising in 0.2% triton-X-100. Se-GSHPx activity is expressed as U/g protein, where one unit of activity catalyses the oxidation of 1.0 mmol of reduced NADPH/minute. Total plasma GSH was determined by HPLC using a manual adaptation of the automated NaBH₄ reduction and monobromobimane derivatization procedures described previously [30,31]. Liver cytosolic and plasma protein carbonyls were determined by slot-blot immunoassay using reagents from an OxyBlot Protein Oxidation Detection Kit (Intergen, NY, USA) as previously described [32]. Lipid peroxide concentrations in liver homogenates and

plasma were determined using a commercially available kit (LPO-CC Lipid Peroxides, Kamiya Biomedical, Seattle, WA, USA). Protein concentration was determined by the bicinchoninic acid method [33].

Statistical Analyses

Data were analysed by one-way ANOVA and differences between means were determined by Fisher's least significant difference test. For tissue vitamin C, data were analysed using univariate ANOVA with diet as the main effect. Since the variability of tissue AA levels increased with an increase in mean, AA data were transformed using the square root transformation. Two contrasts were included in the analyses to test for an effect of Se or AA intake on tissue AA, DHAA and total vitamin C concentrations. The Se-D/MC, Se-M/MC and Se-N/MC diet groups were used to test for an effect of Se intake. These diet groups were also used to determine whether a decreasing or increasing trend was present as the amount of Se decreased in the diet. Trend here refers to an overall increasing or decreasing response to a decrease in dose. To test for an effect of AA intake, the Se-N/MC and Se-N/NC diet groups were compared. For vitamin C analyses, data from guinea pigs killed at week 5, 48 hrs post AA dosing and week 12, 24 hrs post AA dosing were combined in order to increase the power of the statistical comparisons. The ability to combine these data is predicated on the similarity of the response (i.e. the lack of an interaction to Se level between each age group). Data are shown as means \pm SEM. Statistical significance was set at $P < 0.05$. Data were analysed using Statistica 7 (StatSoft, Tulsa, OK) and SAS (SAS Canada, Ottawa, Canada) software.

Results

To investigate the sparing effects of Se on vitamin C and AT when intake of AA is low, guinea pigs were orally dosed with a marginal amount of AA and fed a diet deficient (Se-D/MC), marginal (Se-M/MC) or normal (Se-N/MC) in Se. An additional diet group (Se-N/NC) dosed with a normal amount of AA and fed a normal Se diet was included in the experimental protocol to allow investigation of the sparing effects of AA on vitamin C and AT (comparison with diet group Se-N/MC). Approximately one third of the guinea pigs fed the Se-D/MC diet developed paralysis of their hind limbs and showed poor mobility beginning as early as 4 weeks on the diet. Three Se-D/MC guinea pigs died or were euthanised prior to the week 5 necropsy and two prior to the week 12 necropsy.

Liver Se-GSHPx activity of guinea pigs killed at week 5 showed a dose-dependent decrease ($P < 0.05$) with decreasing amounts of Se in the diet, confirming induction of graded levels of Se status (Table 2). At week 12, Se-D/MC guinea pigs had lower ($P < 0.05$) Se-GSHPx activity compared to Se-M/MC or Se-N/MC guinea pigs. Notably, the lower activity of Se-M/MC compared to Se-N/MC guinea pigs was approaching statistical significance ($P = 0.058$). Se-N/MC guinea pigs had lower ($P < 0.05$) activity compared to Se-N/NC guinea pigs at week 12. At week 5, the difference in activity between Se-N/MC and Se-N/NC guinea pigs was approaching statistical significance ($P = 0.064$). Plasma total GSH concentrations were similar ($P > 0.05$) in guinea pigs fed the different test diets, consistent with a previous study showing no change in GSH levels with decreased Se and vitamin C status [7]. However, plasma GSH concentrations were lower ($P < 0.05$) in week 12 compared to week 5 guinea pigs for all diet groups.

Tissue vitamin C and AT concentrations are only presented for guinea pigs killed at week 5, 48 hrs post AA dosing and at week 12, 24 hrs post dosing (see Discussion). Total vitamin C, AA and DHAA concentrations were determined in liver, heart, kidney, testis, muscle, spleen and plasma of guinea pigs fed the experimental diets (Fig. 1). Proportion of AA to DHAA in each tissue was relatively similar between guinea pigs killed at week 5 or 12 (Fig. 1). The only exception was in plasma, where most of the vitamin C was present as AA at week 5, but as DHAA at week 12 (Fig. 1G). The ratio of DHAA to AA varied markedly between tissues. In testis, the majority of vitamin C was AA (Fig. 1D), whereas in heart and muscle most of the vitamin C was DHAA (Fig. 1B and E).

Tissue vitamin C concentrations for guinea pigs fed different levels of Se did not differ ($P > 0.05$) when guinea pigs killed at the same age and times post AA dosing were compared (data not shown) which is likely due to the large variability in vitamin C concentrations between individual guinea pigs. Therefore, vitamin C data from week 5 and 12 guinea pigs killed at 48 and 24 hrs post AA dosing, respectively, were combined (see Methods and Discussion). Effects of Se or AA intake on tissue AA, DHAA and total vitamin C were determined by univariate ANOVA (Table 3). Differences between the Se-D/MC, Se-M/MC and Se-N/MC diet groups were determined to test for sparing effects of Se (Se Effect). Additional statistical analyses were performed to test whether there was a decreasing or increasing trend in vitamin C with decreasing Se in the diet [Se Effect (Trend)]. Differences between the Se-N/MC and Se-N/NC diet groups were determined to test for sparing effects of AA (AA Effect).

Different levels of dietary Se affected ($P < 0.05$) AA concentrations in kidney and muscle (Table 3, Se Effect). Trend analyses confirmed that the differences detected reflected a decrease in AA concentrations with decreasing Se in the diet [Table 3, Se Effect (Trend)]. Significant differences in AA concentrations were not detected ($P > 0.05$) in liver, heart, testis, spleen and plasma with changes in dietary Se. However, testis and spleen showed a decreasing trend with reduction in dietary Se. Total vitamin C concentrations were affected by dietary Se in liver, heart, testis, muscle and spleen and showed a decreasing trend with decreasing dietary Se. A Se effect and similar decreasing trend was observed for DHAA in heart and spleen. In contrast, the Se effect on liver DHAA reflected an increasing trend with decreasing Se in the diet.

Guinea pigs dosed with marginal AA had reduced ($P < 0.05$) AA in all tissues compared to guinea pigs dosed with a normal amount of AA [Table 3, AA Effect]. DHAA was reduced ($P < 0.0001$) in liver, heart, kidney, muscle and spleen, but not significantly ($P > 0.05$) decreased in testis and plasma. All tissues from guinea pigs dosed with marginal AA had decreased ($P < 0.0001$) concentrations of total vitamin C compared to guinea pigs dosed with normal AA.

At week 5, there were no significant ($P > 0.05$) differences in AT concentrations between diet groups for any of the tissues analysed (Table 4). At week 12, Se-D/MC guinea pigs had lower ($P < 0.05$) AT concentrations in liver, heart and spleen compared to Se-M/MC guinea pigs (Table 4). Difference in muscle AT concentrations between week 12 guinea

pigs fed the Se-D/MC or Se-M/MC diets was approaching statistical significance ($P = 0.09$). Significant differences in tissue AT concentrations were not observed ($P > 0.05$) between guinea pigs fed the Se-D/MC or Se-M/MC diets compared to guinea pigs fed the Se-N/MC diet. However, the lower AT concentration in heart of Se-D/MC compared to Se-N/MC guinea pigs was approaching significance ($P = 0.067$). AT was lower ($P < 0.05$) in liver, kidney and spleen of Se-N/MC compared to Se-N/NC guinea pigs. Differences in AT concentrations in testis ($P = 0.075$) and plasma ($P = 0.061$) between Se-N/MC and Se-N/NC guinea pigs were approaching significance. Collectively, these data indicate that reductions in dietary Se or AA decrease vitamin C and AT concentrations in guinea pig tissues.

Given that Se, vitamin C and AT function as antioxidants, it prompted us to examine tissues for oxidative damage. Liver cytosol and plasma protein carbonyl and lipid peroxide concentrations were similar ($P > 0.05$) in guinea pigs fed the different experimental diets (Table 5).

Discussion

The primary objective of this study was to investigate the sparing effects of dietary Se and AA on tissue vitamin C and AT concentrations. Guinea pigs were chosen for these studies as they are similar to humans in their inability to make vitamin C and therefore likely provide a more relevant model system for these studies compared to previously used cell culture systems [17,18,20] or animal models that have the ability to make vitamin C [7]. Further, we chose to investigate the sparing effects of Se under conditions of marginal AA intake, given that Se may play a more biologically significant role in sparing vitamin C and AT when intake of AA is low.

Sparing effects of Se on vitamin C were detected in most tissues when vitamin C data from guinea pigs killed at week 5 and 12, 48 and 24 hrs post AA dosing, respectively, were combined (see Methods). In contrast, preliminary analyses failed to reveal sparing effects of Se on vitamin C in guinea pigs killed at week 5 and 12, 24 and 48 hrs post AA dosing, respectively (data not shown). The reason for the observed sparing effects at different times post dosing (i.e. 48 vs. 24 hrs) for week 5 and 12 guinea pigs is not clear. Notably, however, vitamin C concentrations in tissues were higher for guinea pigs killed at 24 compared to 48 hrs post dosing for both week 5 and 12 guinea pigs (data not shown) indicating that vitamin C concentrations rise in tissues following dosing and then fall over time as the vitamin is consumed. Increases in tissue vitamin C concentrations at early times post dosing or low concentrations after an extended time post dosing may mask sparing effects of Se. It is therefore possible that differences in metabolism (e.g. absorption of AA or rate of AA consumption by tissues) of the dosed AA between

younger (week 5) and older (week 12) guinea pigs may account for the discrepancy in time post AA dosing for the observed sparing effects of Se on vitamin C.

In vivo, AA is oxidised to DHAA. We show here that dietary Se and AA restriction decreased both the reduced (AA) and oxidised (DHAA) forms of vitamin C.

Interestingly, liver was the only tissue that showed an increasing trend in DHAA with decreasing Se in the diet. Impaired regeneration of AA from DHAA with Se restriction may have resulted in accumulation of DHAA in liver, perhaps due to slower elimination of DHAA in liver compared to other tissues.

The observed sparing effects of Se on vitamin C may be explained by Se's role as a component of selenoproteins. It has been reported that the Se-dependent enzyme thioredoxin reductase (TR) can regenerate AA from DHAA [7] and the ascorbyl free radical [8]. Although we were unsuccessful in developing an assay to measure TR activity in guinea pig tissues, it is possible that the low Se diets reduced TR activity which may have contributed to lower concentrations of vitamin C. Decreased antioxidant activity due to decreased activity of Se-dependent enzymes may also have contributed to the lower vitamin C and AT concentrations in tissues, since demand for their antioxidant activity may have been increased. The observed sparing effects of Se on AT may also be partly explained by a secondary effect of Se on AT given that vitamin C may play a role in the regeneration of vitamin E [34-35]. In this regard, marginal AA intake reduced AT concentrations in liver, kidney and spleen.

A reduction in AT with decreased Se or AA intake was only observed in week 12 guinea pigs suggesting that longer-term Se or AA deficiency is more detrimental to tissue AT status than short-term deficiency. Previous studies with guinea pigs failed to observe reductions in AT in tissues with Se [24] or vitamin C [36] deficiency, including liver, which was depleted in AT in this study. However, in contrast to these previous studies, this study was of longer duration and guinea pigs were fed a combined Se-deficient and marginal AA diet.

AT was lower in tissues of Se-D/MC compared to Se-M/MC guinea pigs, but not Se-N/MC guinea pigs. Given the absence of significant differences between guinea pigs fed the Se-M/MC or Se-N/MC diets, these data are likely explained by the large variability in tissue AT concentrations between individual guinea pigs. However, these data suggest that marginal amounts of Se are sufficient to maintain tissue AT concentrations.

In most tissues, a large proportion of vitamin C was detected as DHAA, consistent with an earlier report by Hidioglou et al that measured vitamin C in guinea pig tissues [27]. Another study by Martensson et al [37] reported that most of the total vitamin C in liver, lung, kidney and brain of control guinea pigs fed a standard guinea pig chow (Purina) diet was present as AA. It is highly unlikely, however, that the large DHAA/AA ratios reported here and in the study by Hidioglou et al reflect oxidation of AA to DHAA following extraction of the tissues as tissues were immediately frozen, a procedure that has been shown to prevent oxidation of vitamin C [25]. Further, we show here that a

large proportion of vitamin C was detected as DHAA in plasma (for week 12 guinea pigs) that was preserved with metaphosphoric acid.

Differences in vitamin C status of guinea pigs may account for the discrepancies in DHAA/AA ratios reported in tissues. We note that tissue vitamin C concentrations were lower for guinea pigs in this study and that by Hidioglou et al compared to control guinea pigs in the study by Martensson et al, most likely due to lower vitamin C intakes. Further, Martensson et al also report a large decrease in total vitamin C and a significant accumulation of DHAA in liver and kidney of guinea pigs fed an ascorbate-deficient diet. Given these data, it is conceivable that reduced vitamin C intakes and consequently tissue vitamin C concentrations promote an increase in the DHAA/AA ratio in guinea pig tissues.

Se-GSHPx activity decreases with a reduction in Se status and is often used as a measure of Se nutriture in experimental animals, including guinea pigs [24,38,39] Interestingly, guinea pigs killed at week 12 and dosed with marginal AA had lower Se-GSHPx activity compared to guinea pigs dosed with normal AA. These data demonstrate a sparing effect of AA on Se-GSHPx activity suggesting that AA intake influences Se status.

Lastly, since decreased antioxidant status can lead to oxidation of cellular components, we examined liver and plasma for oxidative modifications of proteins and lipids. We failed to detect any differences in protein carbonyl and lipid peroxide concentrations in liver cytosols or plasma between guinea pigs fed the different diets. Although these data

indicate the absence of severe oxidative modifications to proteins and lipids in these tissues, we cannot rule out the presence of subtle changes that may be detected with more sensitive assays or the presence of differences in other markers of oxidative stress.

Conclusions

In this study, we performed a comprehensive analysis of the sparing effects of Se and AA in guinea pigs, an animal model that is similar to humans and cannot synthesise vitamin C. Dietary restriction of Se and AA decreased both the reduced (AA) and oxidised (DHAA) forms of vitamin C as well as AT in tissues. Given these findings and recent studies indicating inadequate Se intakes in certain population groups [40-42], further studies evaluating the health implications and biological significance of reduced vitamin C and E status attributed to a low Se or AA diet are warranted.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

JB analysed and interpreted the data and wrote the manuscript. NH and RM performed the vitamin C and AT analyses. RP and PJ measured the plasma GSH. KC and GB performed the protein carbonyl and lipid peroxide determinations. KT measured the liver Se-GSHPx activity. AG assisted in the animal phase of the experiment. MI, SH and NG performed the statistical analyses. ML conceived and coordinated the study and was involved in interpreting the data. All authors read and approved the final manuscript.

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References

1. Nishikimi M, Fukuyama R, Minoshima S, Shimizu N, Yagi K: **Cloning and chromosomal mapping of the human nonfunctional gene for L-gulonogamma-lactone oxidase, the enzyme for L-ascorbic acid biosynthesis missing in man.** J Biol Chem 1994, **269**:13685-8.
2. Li X, Qu ZC, May JM: **GSH is required to recycle ascorbic acid in cultured liver cell lines.** Antioxid Redox Signal 2001, **3**:1089-97.
3. Li X, Cobb CE, Hill KE, Burk RF, May JM: **Mitochondrial uptake and recycling of ascorbic acid.** Arch Biochem Biophys 2001, **387**:143-53.
4. Xu DP, Washburn MP, Sun GP, Wells WW: **Purification and characterization of a glutathione dependent dehydroascorbate reductase from human erythrocytes.** Biochem Biophys Res Commun 1996, **221**:117-21.
5. Maellaro E, Del Bello B, Sugherini L, Santucci A, Comporti M, Casini AF: **Purification and characterization of glutathione-dependent dehydroascorbate reductase from rat liver.** Biochem J 1994, **301 (Pt 2)**:471-6.
6. May JM, Qu Z, Li X: **Requirement for GSH in recycling of ascorbic acid in endothelial cells.** Biochem Pharmacol 2001, **62**:873-81.
7. May JM, Mendiratta S, Hill KE, Burk RF: **Reduction of dehydroascorbate to ascorbate by the selenoenzyme thioredoxin reductase.** J Biol Chem 1997, **272**:22607-10.

8. May JM, Cobb CE, Mendiratta S, Hill KE, Burk RF: **Reduction of the ascorbyl free radical to ascorbate by thioredoxin reductase.** J Biol Chem 1998, **273**:23039-45.
9. Burk RF, Hill KE, Motley AK: **Selenoprotein metabolism and function: evidence for more than one function for selenoprotein P.** J Nutr 2003, **133**:1517S-20S.
10. Sun QA, Wu Y, Zappacosta F, Jeang KT, Lee BJ, Hatfield DL, Gladyshev VN: **Redox regulation of cell signaling by selenocysteine in mammalian thioredoxin reductases.** J Biol Chem 1999, **274**:24522-30.
11. Niki E: **Antioxidants in relation to lipid peroxidation.** Chem Phys Lipids 1987, **44**:227-53.
12. Hafeman DG, Hoekstra WG: **Lipid peroxidation in vivo during vitamin E and selenium deficiency in the rat as monitored by ethane evolution.** J Nutr 1977, **107**:666-72.
13. Awad JA, Morrow JD, Hill KE, Roberts LJ 2nd, Burk RF: **Detection and localization of lipid peroxidation in selenium- and vitamin E-deficient rats using F2-isoprostanes.** J Nutr 1994, **124**:810-6.
14. Rimm EB, Stampfer MJ, Ascherio A, Giovannucci E, Colditz GA, Willett WC: **Vitamin E consumption and the risk of coronary heart disease in men.** N Engl J Med 1993, **328**:1450-6.
15. Hansson LE, Nyren O, Bergstrom R, Wolk A, Lindgren A, Baron J, Adami HO:

- Nutrients and gastric cancer risk. A population-based case-control study in Sweden.** *Int J Cancer* 1994, **57**:638-44.
16. Mark SD, Qiao YL, Dawsey SM, Wu YP, Katki H, Gunter EW, Fraumeni JF, Blot WJ, Dong ZW, Taylor PR: **Prospective study of serum selenium levels and incident esophageal and gastric cancers.** *J Natl Cancer Inst* 2000, **92**:1753-63.
 17. Li X, Hill KE, Burk RF, May JM: **Selenium spares ascorbate and alpha-tocopherol in cultured liver cell lines under oxidant stress.** *FEBS Lett* 2001, **508**:489-92.
 18. Huang J, May JM: **Ascorbic acid spares alpha-tocopherol and prevents lipid peroxidation in cultured H4IIE liver cells.** *Mol Cell Biochem* 2003, **247**:171-6.
 19. Liu JF, Lee YW: **Vitamin C supplementation restores the impaired vitamin E status of guinea pigs fed oxidized frying oil.** *J Nutr* 1998, **128**:116-22.
 20. Li X, Huang J, May JM: **Ascorbic acid spares alpha-tocopherol and decreases lipid peroxidation in neuronal cells.** *Biochem Biophys Res Commun* 2003, **305**:656-61.
 21. Suzuki E, Kurata T, Arakawa N: **Effect of erythorbic acid administration on activities of drug metabolic enzyme and phosphatases in guinea pigs administered an adequate amount of ascorbic acid.** *J Nutr Sci Vitaminol (Tokyo)* 1989, **35**:123-31.
 22. Suzuki E, Kurata T, Koda M, Arakawa N: **Effect of graded doses of erythorbic**

- acid on activities of drug metabolic enzyme and phosphatases in guinea pigs.** J Nutr Sci Vitaminol (Tokyo) 1988, **34**:439-47.
23. National Research Council: Nutrient Requirements of Laboratory Animals. Fourth Revised Edition. Washington, D.C.: National Academy Press; 1995.
24. Hill KE, Motley AK, Li X, May JM, Burk RF: **Combined selenium and vitamin E deficiency causes fatal myopathy in guinea pigs.** J Nutr 2001, **131**:1798-802.
25. Schell DA, Bode AM: **Measurement of ascorbic acid and dehydroascorbic acid in mammalian tissue utilizing HPLC and electrochemical detection.** Biomed Chromatogr 1993, **7**:267-72.
26. Behrens WA, Madère R: **A procedure for the separation and quantitative analysis of ascorbic acid, dehydroascorbic acid, isoascorbic acid and dehydroisoascorbic acid in food and animal tissues.** J Liq Chromatogr 1994, **17**:2445-55.
27. Hidioglou N, Madere R, L'Abbé MR: **Influence of oral dosing with D-isoascorbic acid on L-ascorbic acid content in guinea pig tissues.** J Nutr Biochem 1997, **8**:13-8
28. Thompson JN, Hatina G: **Determination of tocopherols and tocotrienols in foods and tissues by high pressure liquid chromatography.** J Liq Chromatogr 1979, **2**:327-44.
29. Hotz CS, Fitzpatrick DW, Trick KD, L'Abbé MR: **Dietary Iodine and selenium**

- interact to affect thyroid hormone metabolism of rats.** J Nutr 1997, **127**:1214-8.
30. Fiskerstrand T, Refsum H, Kvalheim G, Ueland PM: **Homocysteine and other thiols in plasma and urine: automated determination and sample stability.** Clin Chem 1993, **39**:263-71.
31. Pastore A, Massoud R, Motti C, Lo Russo A, Fucci G, Cortese C, Federici G: **Fully automated assay for total homocysteine, cysteine, cysteinylglycine, glutathione, cysteamine, and 2-mercaptopyruvate in plasma and urine.** Clin Chem 1998, **44**:825-32.
32. Cockell KA, Wotherspoon AT, Belonje B, Fritz ME, Madere R, Hidioglou N, Plouffe LJ, Ratnayake WM, Kubow S: **Limited effects of combined dietary copper deficiency/iron overload on oxidative stress parameters in rat liver and plasma.** J Nutr Biochem 2005, **16**:750-6.
33. Stoscheck CM: **Quantitation of protein.** Methods Enzymol 1990, **182**:50-68.
34. Packer JE, Slater TF, Willson RL: **Direct observation of a free radical interaction between vitamin E and vitamin C.** Nature 1979, **278**:737-8.
35. Buettner GR: **The pecking order of free radicals and antioxidants: lipid peroxidation, alpha-tocopherol, and ascorbate.** Arch Biochem Biophys 1993, **300**:535-43.
36. Hill KE, Montine TJ, Motley AK, Li X, May JM, Burk RF: **Combined deficiency of vitamins E and C causes paralysis and death in guinea pigs.** Am J Clin Nutr

2003, **77**:1484-8.

37. Martensson J, Han J, Griffith OW, Meister A: **Glutathione ester delays the onset of scurvy in ascorbate-deficient guinea pigs.** Proc Natl Acad Sci U S A 1993, **90**:317-21.
38. Cammack PM, Zwahlen BA, Christensen MJ: **Selenium deficiency alters thyroid hormone metabolism in guinea pigs.** J Nutr 1995, **125**:302-8.
39. Burk RF, Lane JM, Lawrence RA, Gregory PE: **Effect of selenium deficiency on liver and blood glutathione peroxidase activity in guinea pigs.** J Nutr 1981, **111**:690-3.
40. McLachlan SK, Thomson CD, Ferguson EL, McKenzie JE: **Dietary and biochemical selenium status of urban 6- to 24-month-old South Island New Zealand children and their postpartum mothers.** J Nutr 2004, **134**:3290-5.
41. de Jong N, Gibson RS, Thomson CD, Ferguson EL, McKenzie JE, Green TJ, Horwath CC: **Selenium and zinc status are suboptimal in a sample of older New Zealand women in a community-based study.** J Nutr 2001, **131**:2677-84.
42. Wolters M, Hermann S, Golf S, Katz N, Hahn A: **Selenium and antioxidant vitamin status of elderly German women.** Eur J Clin Nutr 2005.

Figure Legend

Figure 1

Vitamin C concentrations in tissues of guinea pigs fed the experimental diets. Bars signify the amount of total vitamin C (DHAA + AA) in tissues of guinea pigs fed the Se-D/MC, Se-M/MC, Se-N/MC or Se-N/NC diets. The proportions of DHAA (white portion of bar) and AA (black portion of bar) are shown. Values are reported as the mean. Number of tissues analysed for each diet group are indicated above the bars. For each tissue, data are shown for guinea pigs killed after 5 or 12 weeks on the experimental diets at 48 or 24 hrs post AA dosing, respectively.

Table 1: Composition of experimental diets

Ingredients	Diets			
	Se-D/MC	Se-M/MC	Se-N/MC	Se-N/NC
	<i>g/kg diet</i>			
Torula Yeast	400	400	400	400
Non-nutritive fibre	150	150	150	150
Cornstarch	142	142	142	142
Mineral mix ¹	90	90	90	90
Sucrose	73.1	73.1	73.1	73.1
Stripped corn oil ²	73	73	73	73
Dextrinised starch	51.9	51.9	51.9	51.9
Vitamin mix ³	10	10	10	10
Choline chloride	4	4	4	4
L-methionine	3	3	3	3
L-arginine	3	3	3	3
DL- α -tocopherol acetate ⁴	40	40	40	40
Se ⁵	0	0.05	0.20	0.20
L-ascorbic acid ⁶	0.3	0.3	0.3	2.4

¹ Dyets no 200151 salt mix for Reid-Briggs guinea pig diet.

² Containing 0.2 mg *Tert*-butylhydroquinone/g stripped corn oil.

³ Dyets no 300151 vitamin mix for Reid-Briggs guinea pig diet (Vitamin E omitted).

⁴ IU/kg diet; added via stripped corn oil.

⁵ mg/kg diet; added as Na₂SeO₄.

⁶ mg L-ascorbic acid/100 g average body weight for each diet group; given by oral dosing 3 times weekly.

Table 2: Liver Se-GSHPx activity and plasma GSH concentration of guinea pigs after 5 or 12 weeks on the experimental diets¹

Diet Group	Liver Se-GSHPx (U/g protein)		Plasma GSH (uM/L)	
	Week 5	Week 12	Week 5	Week 12
Se-D/MC	15.02 ± 1.32 ^a (n = 8)	14.36 ± 1.49 ^a (n = 9)	7.55 ± 0.45 ^a (n = 8)	4.23 ± 0.20 ^a (n = 7)
Se-M/MC	33.97 ± 1.87 ^b (n = 11)	30.84 ± 1.58 ^b (n = 11)	8.44 ± 0.85 ^a (n = 8)	3.86 ± 0.13 ^a (n = 8)
Se-N/MC	46.78 ± 2.70 ^c (n = 11)	35.52 ± 1.90 ^b (n = 10)	7.38 ± 0.49 ^a (n = 8)	4.12 ± 0.31 ^a (n = 8)
Se-N/NC	57.04 ± 6.46 ^c (n = 11)	43.12 ± 1.70 ^c (n = 11)	7.52 ± 0.92 ^a (n = 8)	4.33 ± 0.38 ^a (n = 9)

¹ Data from guinea pigs killed at both 24 and 48 hrs post L-ascorbic acid dosing.

² Values in a column without a common letter differ, $P < 0.05$. Values are means ± SEM.

³ n values are in parentheses.

Table 3: Univariate significance tests for the effects of Se and AA intakes on vitamin C concentrations in guinea pig tissues¹

Tissue	Se Effect ²			AA Effect ³			Se Effect (Trend) ⁴		
	AA	DHAA	Total	AA	DHAA	Total	AA	DHAA	Total
Liver	NSD	< 0.05	< 0.01	< 0.05	< 0.0001	< 0.0001	NSD	< 0.005	< 0.005
Heart	NSD	< 0.05	< 0.05	< 0.01	< 0.0001	< 0.0001	NSD	< 0.01	< 0.01
Kidney	< 0.05	NSD	NSD	< 0.005	< 0.0001	< 0.0001	< 0.05	NSD	NSD
Testis	NSD	NSD	< 0.05	< 0.0001	NSD	< 0.0001	< 0.05	NSD	< 0.005
Muscle	< 0.05	NSD	< 0.05	< 0.001	< 0.0001	< 0.0001	< 0.05	NSD	< 0.05
Spleen	NSD	< 0.05	< 0.01	< 0.0001	< 0.0001	< 0.0001	< 0.05	< 0.05	< 0.005
Plasma	NSD	NSD	NSD	< 0.0005 ⁵	NSD ⁵	< 0.0001 ⁵	NSD	NSD	NSD

¹ Type III *P* values are shown; NSD = no statistical difference.

² Comparison between Se-D/MC, Se-M/MC and Se-N/MC guinea pigs killed at week 5 and 12, 48 and 24 hrs post AA dosing, respectively (n = 31).

³ Comparison between Se-N/MC and Se-N/NC guinea pigs killed at week 5 and 12, 48 and 24 hrs post AA dosing, respectively (n = 22).

⁴ Analyses to determine a decreasing or increasing trend with decreasing Se in the diet. Comparison of Se-D/MC, Se-M/MC and Se-N/MC guinea pigs killed at week 5 and 12, 48 and 24 hrs post AA dosing, respectively (n = 31). All significant values indicate a decreasing trend with decreasing Se in the diet, except for liver DHAA which indicates an increasing trend.

⁵ n = 20.

Table 4: Effects of Se and AA intakes on α -tocopherol concentrations in guinea pig tissues¹

Animals	Liver	Heart	Kidney	Testis	Muscle	Spleen	Plasma ²
Week 5 ³				$\mu\text{g AT/g}$			
Se-D/MC (n = 5)	6.50 \pm 1.57 ^a	2.22 \pm 0.67 ^a	2.68 \pm 0.88 ^a	2.42 \pm 0.65 ^a	1.46 \pm 0.36 ^a	3.71 \pm 0.86 ^a	1.26 \pm 0.28 ^a
Se-M/MC (n = 5)	12.13 \pm 3.42 ^a	3.20 \pm 0.86 ^a	2.77 \pm 0.81 ^a	2.61 \pm 0.84 ^a	1.11 \pm 0.42 ^a	5.53 \pm 1.22 ^a	1.11 \pm 0.18 ^a
Se-N/MC (n = 5)	10.30 \pm 2.88 ^a	2.71 \pm 1.12 ^a	2.92 \pm 1.11 ^a	3.07 \pm 0.92 ^a	1.19 \pm 0.49 ^a	5.53 \pm 1.23 ^a	1.00 \pm 0.31 ^a
Se-N/NC (n = 5)	14.78 \pm 4.11 ^a	3.70 \pm 1.41 ^a	4.91 \pm 1.15 ^a	2.74 \pm 0.63 ^a	1.38 \pm 0.34 ^a	6.75 \pm 1.63 ^a	1.13 \pm 0.33 ^{a4}
Week 12 ³							
Se-D/MC (n = 4)	10.35 \pm 3.58 ^a	2.64 \pm 0.77 ^a	3.90 \pm 0.92 ^a	2.34 \pm 0.59 ^a	0.95 \pm 0.32 ^a	5.32 \pm 1.58 ^a	0.59 \pm 0.27 ^a
Se-M/MC (n = 5)	20.71 \pm 1.54 ^{bc}	6.79 \pm 0.72 ^b	5.46 \pm 0.72 ^a	2.98 \pm 0.35 ^a	1.68 \pm 0.31 ^a	8.75 \pm 1.02 ^{bc}	1.10 \pm 0.15 ^{ab}
Se-N/MC (n = 6)	15.10 \pm 1.81 ^{ab}	5.49 \pm 0.67 ^{ab}	5.12 \pm 0.64 ^a	2.49 \pm 0.21 ^a	1.53 \pm 0.25 ^a	7.37 \pm 0.87 ^{ab}	0.73 \pm 0.11 ^{ab}
Se-N/NC (n = 5)	23.87 \pm 3.86 ^c	6.37 \pm 1.59 ^b	7.98 \pm 1.03 ^b	3.63 \pm 0.62 ^a	1.66 \pm 0.23 ^a	10.76 \pm 0.98 ^c	1.48 \pm 0.47 ^b

¹ AT concentrations in tissues from guinea pigs killed at week 5 and 12, 48 and 24 hrs post AA dosing, respectively.

² $\mu\text{g AT/mL}$.

³ For week 5 and week 12, values in a column without a common letter differ, $P < 0.05$. Values are means \pm SEM.

⁴ n = 4.

⁵ n values are in parentheses.

Table 5: Protein carbonyl and lipid peroxide concentrations in liver cytosol and plasma of guinea pigs after 12 weeks on the experimental diets¹

Diet Group	Protein Carbonyl		Lipid Peroxide	
	Liver cytosol ($\mu\text{mol/g}$ protein)	Plasma ($\mu\text{mol/g}$ protein)	Liver cytosol (nmol/g wet wt)	Plasma (nmol/mL)
Se-D/MC	3.4 \pm 0.1 (n=9)	3.5 \pm 0.3 (n=9)	91 \pm 13 (n=9)	4.1 \pm 0.5 (n=7)
Se-M/MC	3.3 \pm 0.1 (n=11)	3.1 \pm 0.3 (n=11)	87 \pm 13 (n=11)	3.9 \pm 0.6 (n=8)
Se-N/MC	3.4 \pm 0.2 (n=11)	3.3 \pm 0.2 (n=11)	78 \pm 10 (n=11)	2.7 \pm 0.6 (n=8)
Se-N/NC	3.5 \pm 0.2 (n=11)	3.8 \pm 0.2 (n=11)	84 \pm 8 (n=11)	3.3 \pm 0.4 (n=8)

¹ Data from guinea pigs killed at both 24 and 48 hrs post L-ascorbic acid dosing.

² Values within each column were not significantly different at $P < 0.05$. Values are means \pm SEM.

³ n values are in parentheses.

