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Dietary selenium and vitamin E affect adrenal and brain dehydroepiandrosterone levels in young rats

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This study tested the hypothesis that dietary selenium (Se) and vitamin E can affect dehydroepiandrosterone (DHEA) levels in the adrenal glands and the brain in young rats. Male Sprague-Dawley rats (4-weeks-old) were fed Se-deficient or Se-supplemented (0.2 mg Se/kg) diets containing 0, 30, or 200 mg dl-α-tocopheryl acetate/kg diet for 6 weeks. The results showed that neither dietary Se nor vitamin E affected body weight or relative adrenal weights (expressed as per 100 g body weight). As expected, serum glutathione peroxidase activity in Se-deficient rats was low, only approximately 6% of that in Se-supplemented rats. Dietary vitamin E markedly ($P < 0.001$) and dose-dependently inhibited peroxide-induced hepatic lipid peroxidation, suggesting that vitamin E content in rat tissues reflected those in the diets. The adrenal DHEA levels were significantly decreased by both Se and vitamin E deficiency. Brain DHEA levels were significantly decreased by vitamin E deficiency but not by Se deficiency. Supplementation with 200 mg dl-α-tocopheryl acetate/kg diet did not increase DHEA levels in either the adrenal glands or the brain, and there were no interactions on DHEA levels between the two dietary factors. Thus, this study in young rats demonstrates that dietary antioxidants affect adrenal gland and brain DHEA levels and that vitamin E appears to play a more important role than does Se. (J. Nutr. Biochem. 9:339–343, 1998)
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Introduction

The aging process is controlled by environmental, genetic, and hormonal factors. In humans, the adrenal androgen dehydroepiandrosterone (DHEA) peaks at approximately 25 years of age and dramatically declines to approximately 10% at age 70 or 80 years.[1,2] Animal studies have shown that DHEA protects against obesity,[3–5] cancer,[6,7] atherosclerosis,[8,9] and brain aging.[10] In addition, it has been suggested that DHEA delays and even reverses the aging process[11] and thus it has been advocated as the "fountain of youth."[12] Although a variety of theories has been proposed,[13] the mechanisms by which DHEA exerts its physiologic functions remain unclear.

Oxidative damage to biological membranes and cellular components is thought to contribute to human aging and disease and antioxidants may protect against such damage.[14,15] The adrenal gland is rich in polyunsaturated fatty acids[16] and thus is susceptible to lipid peroxidation. It has been suggested that accumulation of lipofuscin, a highly fluorescent product derived from lipid peroxidation,[17,18] may affect the overall lysosomal processing of lipoprotein cholesterol, therefore limiting the availability of cholesterol for steroidogenesis.[19] Indeed, the zona reticularis of the aging human adrenal cortex exhibits a remarkable accumulation of lipofuscin granules[20] that may be associated with diminished production of DHEA and its sulfate conjugate.[21] Thus, it is not surprising that the adrenal gland has high contents of glutathione (reduced form, GSH),[16] vitamin C, and vitamin E.[22] Vitamin C, which has important roles in several hydroxylases involved in the metabolism of neurotransmitters, steroids, drugs, and lipids, is concentrated at the site of catecholamine formation in the adrenal.[23] Kitabchi et al.[24] have shown that vitamin E deficiency in rats

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Table 1 Composition of the vitamin E- and selenium-deficient basal diet

Ingredient	Amount (g/100 g diet)
Sucrose	53.7
Torula yeast[1]	30.0
Lard (tocopherol-stripped)[1]	5.0
Corn oil (tocopherol-stripped)[1]	5.0
Mineral mix (selenium-free)[2]	5.0
Vitamin mix (vitamin E-free)[3]	1.0
dl-Methionine	0.3

[1]Supplied by Teklad Test Diet, Madison, WI.
[2]William-Briggs Modified mix (TD 170911) g/kg of basal diet: $CaCO_3$, 8.1; $CaHOP_4$, 12.9; $MgSO_4$, 2.6; KCl, 8.3; $NaHPO_4$, 7.4; $CuSO_4$, 0.015; ferric citrate USP, 0.173; $MnSO_4$, 0.176; KI, 0.001; $ZnCO_3$, 0.024.
[3]AIN 76 vitamin mix without vitamin E.

attenuates steroidogenesis in isolated adrenal cells induced by adrenocorticotropic hormone. However, it is not known whether dietary antioxidants may affect the adrenal DHEA in vivo.

In this study young rats were fed diets deficient in or supplemented with vitamin E and selenium (Se), an integral part of glutathione peroxidase (glutathione: H_2O_2 oxidoreductase, EC 1.11.1.9, GPX),[25] to examine the hypothesis that the DHEA content in the adrenal gland may be affected by dietary antioxidants. Brain DHEA content was also measured because DHEA and its sulfate ester are neuroactive and are present at relatively high concentrations in the brain, which is capable of de novo synthesis of steroids independent of gonads and adrenals.[10,13,26]

Materials and methods

Animals and diets

Male Sprague-Dawley rats (4-weeks-old), purchased from the Animal Center of National Science Council (Taipei, Taiwan), were randomly assigned to six groups of a 2 × 3 factorial design; that is, Se-deficient (−Se) and Se-supplemented (+Se) (0.2 mg Se as $Na_2SeO_3 \cdot 5H_2O$) groups with three levels of vitamin E (as dl-α-tocopheryl acetate) in each Se group: 0 (−E), 30 mg/kg diet (+E), and 200 mg/kg diet (high vitamin E, +HE). The composition of the Torula-yeast based diet deficient in Se and vitamin E is shown in *Table 1*. Rats were housed individually in hanging wiremesh cages with controlled temperature (22 ± 2°C), humidity (60–65%), and light (0700–1900) and were given free access to food and deionized water for 6 weeks. Body weights were measured weekly.

Preparation of tissue homogenates

Rats were decapitated after overnight deprivation of food. The adrenal gland was removed carefully and weighed. After being quickly frozen in liquid N_2, adrenal, brain, and serum samples were stored at −35°C until use. For measurement of DHEA, adrenal and brain tissues were homogenized in ice-cold 0.1 M phosphate buffer (pH 7.4) containing 0.15 M KCl at 2% and 5% w/v, respectively, using a teflon pestle/glass homogenizer.

Table 2 Body weights and relative adrenal weights of rats fed Se-deficient (−Se) or Se-supplemented (+Se, 0.2 mg/kg) diet containing 0 (−E), 30 (+E), or 200 (+HE) mg dl-α-tocopheryl acetate/kg for 6 weeks[1]

Group	*n*	Body weight (g)	Relative adrenal weight (mg/100 g body weight)
−Se, −E	7	335 ± 25	18.2 ± 7.8
−Se, +E	8	350 ± 16	13.6 ± 0.4
−Se, +HE	8	339 ± 30	14.3 ± 3.9
+Se, −E	8	340 ± 26	16.3 ± 2.5
+Se, +E	8	336 ± 23	16.2 ± 4.1
+Se, +HE	7	345 ± 26	15.0 ± 3.6
P		NS	NS

[1]Values are means ± SD.
n, number of rats in each group; NS, not statistically significant ($P > 0.05$).

Measurement of DHEA

DHEA was determined by radioimmunoassay using a commercial kit (No. DS-2100, Wien Laboratory, Succasunna, NJ, USA) according to the instructions provided by the supplier. A major modification was made by using only 20 μL (rather than 50 μL as specified by the supplier) of H^3-DHEA (ca. 2,000 counts/min). This modification saved the radioactive reagent and did not affect the detection limit. An aliquot (0.2 mL) of adrenal or brain homogenates was extracted with 5 mL of dichloromethane. Three milliliters of the dichloromethane layer was evaporated under N_2, and the residue was dissolved in 0.01 M phosphate buffer containing 0.06% bovine albumin (pH 7.4) provided by the supplier. The amount of DHEA was calibrated from a standard curve ($r \geq 0.99$). The amount of DHEA required for 50% binding of the antibody was approximately 54 pg, which is the amount of DHEA contained in approximately 9 mg adrenal and 18 mg brain tissue of rats supplemented with 0.2 mg Se/kg and 30 mg dl-α-tocopheryl acetate/kg diet (this group of rats was considered the control group).

Other determinations

Serum GPX activity was determined by the method of Lawrence and Burk[27] using 0.25 mM H_2O_2 as substrate. Lipid peroxidation was determined immediately after rats were sacrificed as thiobarbituric acid-reactive substances (TBARS) in liver homogenates (10%, w/v), as described previously.[28] Peroxide-induced lipid peroxidation was performed by incubation of the liver homogenates with 0.7 mM *t*-butyl hydroperoxide at 37°C for 30 minutes; ex vivo (uninduced) lipid peroxidation was determined without incubation.[29]

Statistical analysis

Data, expressed as means ± SD (standard deviation), were analyzed using Bartlett's test for homogeneity before using two-way analysis of variance (ANOVA) with general linear model of SAS (SAS Institute, Cary, NC USA). Duncan's multiple range test was used for post hoc comparisons ($\alpha = 0.05$).

Results

During the 6-week feeding period, neither rat body weights nor relative adrenal weights (expressed as per 100 g body weight) were significantly affected by dietary Se and vitamin E (*Table 2*). Serum GPX activity in Se-deficient rats

Table 3 Serum glutathione peroxidase (GPX) activity and hepatic lipid peroxidation in rats fed Se-deficient (−Se) or Se-supplemented (+Se, 0.2 mg/kg) diets containing 0 (−E), 30 (+E), or 200 (+HE) mg dl-α-tocopheryl acetate/kg for 6 weeks[1]

Group	*n*	GPX (μmol/ml serum/min)	TBARS (nmol/g liver)	
			Ex-vivo	Peroxide-induced[2]
−Se, −E	7	0.36 ± 0.09–a	2.17 ± 0.15	18.8 ± 3.58–a
−Se, +E	8	0.33 ± 0.08–a	2.04 ± 0.32	6.32 ± 1.04–b
−Se, +HE	8	0.35 ± 0.09–a	2.05 ± 0.40	2.61 ± 0.53–c
+Se, −E	8	5.80 ± 0.64–b	2.37 ± 0.43	20.5 ± 2.91–a
+Se, +E	8	6.11 ± 0.71–b	2.31 ± 0.60	8.12 ± 1.81–b
+Se, +HE	7	5.98 ± 0.52–b	2.25 ± 0.30	2.77 ± 0.60–c
ANOVA				
Se		0.001	NS	NS
E		NS	NS	0.001
Se × E		NS	NS	NS

[1]Values (means ± SD) in a column not sharing a superscript are significantly different ($P < 0.05$); *n,* number of rats in each group. The +Se, +E group is considered a control group. ANOVA, analysis of variance; NS, not statistically significant ($P > 0.05$).
[2]Peroxide-induced thiobarbituric acid-reactive substances (TBARS) were determined by incubating liver homogenates with 0.7 mM t-butylhydroperoxide at 37°C for 30 minutes.

was low, only approximately 6% of that in Se-supplemented rats (*Table 3*).

Liver was used for measurement of lipid peroxidation because there was insufficient adrenal and brain tissues after the DHEA assay. The results showed that neither dietary Se nor vitamin E significantly affected ex vivo hepatic lipid peroxidation, but supplementation with dietary vitamin E markedly ($P < 0.001$) and dose-dependently reduced peroxide-induced hepatic lipid peroxidation of both Se-deficient and Se-supplemented rats (*Table 3*).

Both dietary Se and vitamin E affected adrenal DHEA levels (*Table 4*). Relative to the control group (+Se, +E; i.e., rats fed diet containing 0.2 mg Se and 30 mg dl-α-tocopheryl acetate/kg diet), the adrenal DHEA level (5.9 ± 2.0 ng/g) was significantly decreased by vitamin E deficiency in both Se-deficient and Se-supplemented groups. The adrenal DHEA level was further decreased ($P < 0.05$) to 1.5 ± 0.6 ng/g by dual deficiencies in Se and vitamin E. However, supplementation with 200 mg dl-α-tocopheryl acetate/kg diet did not increase the adrenal DHEA level. The two dietary factors did not interact with each other.

Table 4 Adrenal and brain DHEA levels in rats fed Se-deficient (−Se) or Se-supplemented (+Se, 0.2 mg/kg) diet containing 0 (−E), 30 (+E), or 200 (+HE) mg dl-α-tocopheryl acetate/kg for 6 weeks[1]

Group	*n*	Adrenal (ng/g)	Brain (ng/g)
−Se, −E	7	1.5 ± 0.6–a	1.3 ± 0.5–a
−Se, +E	8	3.3 ± 1.6–b	2.7 ± 0.8–b
−Se, +HE	8	3.6 ± 1.6–b	2.6 ± 0.6–b
+Se, −E	8	3.6 ± 2.2–b	2.0 ± 0.4–a
+Se, +E[2]	8	5.9 ± 2.0–c	2.8 ± 1.0–b
+Se, +HE	7	5.6 ± 2.2–bc	2.7 ± 0.8–b
ANOVA			
Se		0.02	NS
E		0.002	0.004
Se × E		NS	NS

[1]Values (means ± SD) in a column not sharing a letter are significantly different ($P < 0.05$). *n*, number of rats in each group; ANOVA, analysis of variance; NS, not statistically significant ($P > 0.05$).
[2]This group is considered a control group.

Vitamin E deficiency significantly decreased brain DHEA levels in both Se-deficient rats and Se-supplemented rats (*Table 4*). Dietary Se had no significant effect and there was no interaction between Se and vitamin E.

Discussion

Results obtained from the present study generally support the hypothesis that dietary Se and vitamin E status affect adrenal and brain DHEA levels in young rats. Vitamin E was apparently more effective than Se because deficiency of the former significantly decreased DHEA levels in both adrenal and brain, whereas deficiency of the latter only significantly decreased adrenal DHEA level. However, the high dietary vitamin E (200 mg α-tocopheryl acetate/kg) did not further increase either adrenal or brain DHEA levels.

Dietary vitamin E and Se may exert their effects on DHEA levels through their antioxidant properties. Vitamin E deficiency has been shown to decrease steroidogenic activity of the adrenal gland in rats, possibly due to increased lipid peroxidation in that organ.[22,24] Although we did not determine Se and vitamin E levels and lipid peroxidation in either the adrenal gland or the brain due to limited amounts of these tissues, two lines of evidence suggest that the tissue Se and vitamin E levels reflect the status of the two antioxidants in the diets. First, serum GPX activity in Se-supplemented rats was approximately 16 times that in Se-deficient rats. Second, dietary vitamin E (but not dietary Se) dose-dependently inhibited peroxide-induced hepatic lipid peroxidation. Interestingly, Meydani et al.[30] have shown that the degree of in vitro lipid peroxidation of rat brain tissues is partially reflected by dietary vitamin E but not by Se. In addition, the present study showed that brain DHEA levels were significantly affected by dietary vitamin E but not by Se.

In addition to their antioxidant properties, vitamin E and Se may affect tissue DHEA levels by other functions. In this context, vitamin E is known to regulate de novo synthesis of xanthine oxidase, modulate activities of protein kinase C and microsomal enzymes, and inhibit tumor cell prolifera-

tion.[31] Recently, vitamin E was also shown to modulate generation of inflammatory mediators such as prostaglandins.[32,33] However, the adrenal vitamin E content is not correlated with adrenal steroidogenicity because the former increases, rather than decreases, as the age of rats increases from 12 months to 29 months.[34] A plausible explanation of this discrepancy is the localization of vitamin E in different compartments of the adrenal cortex. It has been shown that the adrenocortical cell membranes have two specific receptor sites for vitamin E with apparent binding constants of 4×10^{-5} and 7×10^{-6}, respectively.[35] In addition, Staats et al.[36] have shown that the content of vitamin E in the inner portion of the adrenal cortex (zona reticularis) is much lower than that in the outer portion (zona fasciculata and glomerulosa), where vitamin E may counter the age-related lipid peroxidation to maintain steroidogenic homeostasis. It is interesting to note that the adrenal gland may only require certain amounts of vitamin E to maintain steroidogenesis, because the present study demonstrated that the adrenal DHEA level was not increased in rats supplemented with a high vitamin E level (200 mg α-tocopheryl acetate/kg).

Similarly, the steroidogenic effect of Se also may be related to functions other than its antioxidant property. However, despite the fact that several forms of selenoproteins have been identified,[37] the antioxidant activity of Se as an integral part of GPX[25] and phospholipid hydroperoxide GPX[38] remains its best-characterized function.[15] Unlike other mitochondria, adrenal cortical mitochondria are highly susceptible to lipid peroxidation, which leads to damage to mitochondrial enzymes.[39] In bovine adrenal cortex, GPX exists predominantly (92%) in the cytoplasm but a small amount of activity (8%) also exists in mitochondria, in which GPX protects against damage to cytochrome P-450 and succinate dehydrogenase induced by nicotinamide adenine dinucleotide phosphate (NADPH)-dependent lipid peroxidation.[40]

In summary, by showing that dietary Se and vitamin E can affect adrenal and brain DHEA levels, the present study demonstrates a functional connection between dietary antioxidants and steroidogenesis in young rats. In future studies, we hope to obtain direct evidence showing that oxidative damage to rat brain and adrenal tissues results in decreased DHEA biosynthesis in vivo and that such damage is prevented by antioxidants.

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