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Strain Differences in Testes Degeneration, Myopathy, and the Lymphocyte Mitogen Response in Vitamin E-Deficient Rats (41886)

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Abstract. The differences in sensitivity to vitamin E deficiency were examined in two genetically related inbred strains of rat, the spontaneously hypertensive rat and its genetic ancestor, the Wistar–Kyoto rat, as well as in the outbred Sprague–Dawley strain. The three strains showed differences in growth rate, myopathy, testes degeneration, and immunological responses in response to vitamin E deficiency with the spontaneously hypertensive rat showing the greatest sensitivity to the deficiency.

We have previously reported that tissue vitamin E levels in the spontaneously hypertensive rat (SHR) fed a commercial rodent diet were lower than in the ancestral, normotensive Wistar–Kyoto (W/K) strain (1). This was also the case when vitamin E was administered *per os* to SHR and W/K fed vitamin E-deficient diets (2). These findings suggested that there may be a difference in absorption, depletion, utilization, and/or requirement for vitamin E between these two strains. Furthermore, the data raised the questions as to whether the hypertension and depressed immune responses (3) in the SHR were related to the physiological consequences of lower tissue vitamin E and whether the SHR would be more sensitive to vitamin E deficiency than other rat strains.

In the study reported here, we compare the responses of the SHR, W/K, and the well-characterized Sprague–Dawley (SD) rat to vitamin E deficiency or supplementation by measuring several classical symptoms of vitamin E deficiency (growth rate, myopathy, and testes degeneration) as well as blood pressure and immune responses.

Materials and Methods. *Animals.* Weanling male SHR, W/K, and SD[2] were randomly separated into two groups. One group received a vitamin E-deficient casein-based semipurified diet (4) modified by substituting 10% stripped lard for corn oil.[3] The second group received the identical diet supplemented with 200 mg/kg of *all-rac-α*-tocopheryl acetate, hereafter referred to as vitamin E.

All animals were given food and water *ad libitum* and kept on a 12-hr light/dark cycle. Animals were caged in plastic containers until 6 weeks of age and then placed in stainless-steel wire-floored cages with six animals/cage. All animals remained on their respective diets until 20 weeks of age.

Body weights were measured bimonthly. Blood pressures were measured by indirect tail cuff method (5). After 5, 8, and 17 weeks on the diet, 6–12 animals from each dietary group were anesthetized with Metofane[4] and blood collected by cardiac puncture using 5% EDTA as anticoagulant. The plasma was separated and pyruvate kinase (PK) levels determined (6, 7). At the termination of the experiment, plasma, liver, testes, and gastrocnemius muscles were removed from the supplemented groups for vitamin E analysis (2).

Spleens were removed aseptically and prepared for measurement of *in vitro* mitogen activity (8). Specifically, individual spleens were evaluated for activity against the T-cell mitogens Concanavalin A (Con A), and phytohemagglutinin (PHA), and the B-cell mitogen, lipopolysaccharide from *Escherichia coli* (LPS). Single cell suspensions were prepared in RPMI 1640 tissue culture medium with 25 mM Hepes buffer supplemented with 5×10^{-5} *M* 2-mercaptoethanol, 2 m*M* glutamine, and 5.0 μg/ml gentamicin. Spleen cells (5×10^6 cells/ml) were placed in 96-well mi-

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[3] Prepared by Dyets, Bethlehem, Pa.

[4] Pitman-Moore Inc., Washington Crossing, N.J.

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crotiter plates, incubated at 37°C in a humidified 5% CO_2 incubator for 24 hr, pulsed with 1.0 μCi of [^{3}H]thymidine (sp act 2 Ci/mmole)[5] and harvested 24 hr later using a Ph.D. cell harvester.[6] The glass fiber filter disks were suspended in Liquifluor[5] and counted in a liquid scintillation counter. Data are presented as counts/minute (cpm).

In an effort to determine the reason for the more rapid onset of vitamin E deficiency symptoms of the SHR, a comparison was made of the depletion rates of the vitamin between SHR and W/K. Twenty male weanling SHR and W/K were fed the vitamin E-deficient diet for 4 weeks. At 0, 1, 2, and 4 weeks, 5 animals from each strain were sacrificed and testes and leg muscles were analyzed for vitamin E content.

Statistical analyses by Student's *t* test were performed to determine the effect of the dietary vitamin E level on the body weight, organ weight, and mitogenic responses within strains. Comparisons among strains were determined for the plasma PK and tissue vitamin E levels.

Results. *1. Effect of vitamin E deficiency on body weight.* After 17 weeks on the vitamin E-deficient diet, all three strains had lower body weights compared to their vitamin E-replete controls. However, this difference did not become manifest in the SD until 14 weeks on the diet (Fig. 1). In contrast, the W/K showed consistent weight differences after 3 weeks, and the SHR showed consistent weight differences after 9 weeks on the deficient diet. The SD was heavier than either SHR or W/K in both the deficient and replete groups throughout the experiment.

2. Myopathy. Following 5 weeks on the vitamin E-deficient diet, the PK of SHR was more than two times higher than that of W/K ($P < 0.01$), indicating the SHR's earlier onset of myopathy (Fig. 2). However, the degree of myopathy which eventually developed in all three strains was equivalent, as shown by PK levels following 8 or 17 weeks on the vitamin E-deficient diet.

3. Testicular degeneration and splenomegaly. The testes of SHR showed macroscopic degeneration (cyanotic appearance, decreased

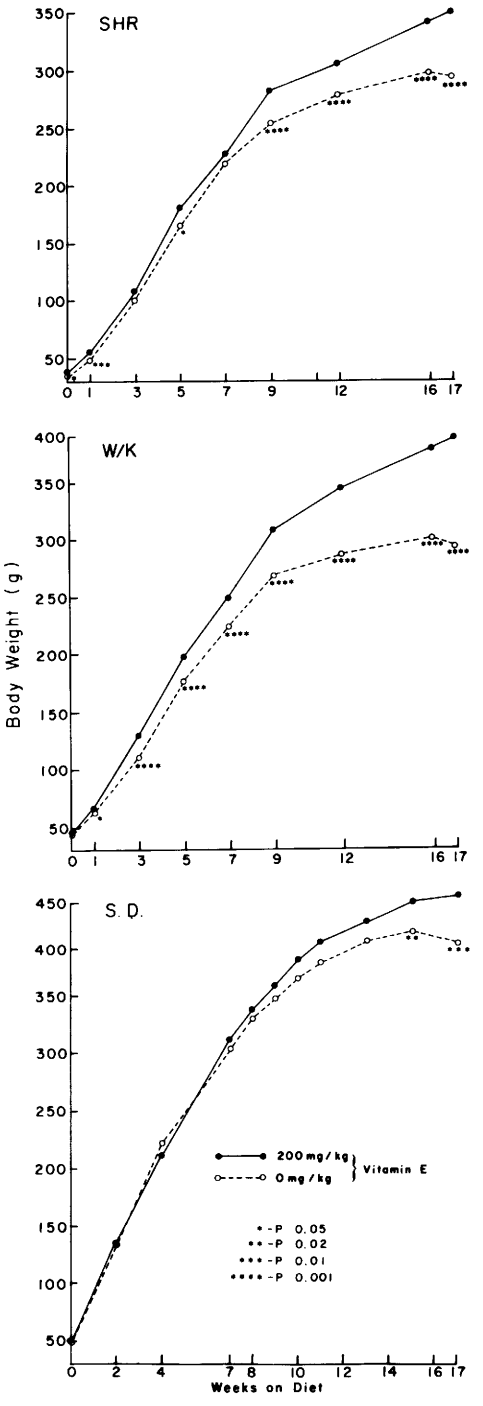

FIG. 1. Growth curves of SHR, W/K, and SD rats fed a vitamin E-deficient diet or the deficient diet supplemented with 200 mg/kg all-rac-α-tocopheryl acetate from weanling for 17 weeks. Statistical comparisons are between the vitamin E-deficient and vitamin E-supplemented animals of each strain.

[5] New England Nuclear, Boston, Mass.

[6] Cambridge Technology, Cambridge, Mass.

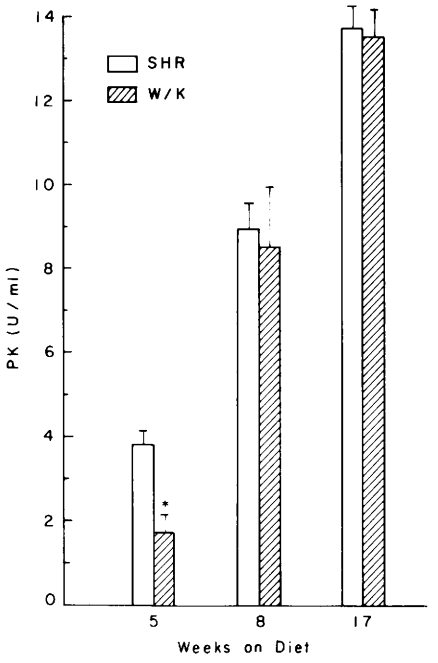

FIG. 2. Plasma pyruvate kinase levels of vitamin E-deficient SHR and W/K measured after 5, 8, and 17 weeks on the diet (*$P < 0.01$).

size and weight) after 17 weeks on a vitamin E-deficient diet. The W/K and SD showed none of these testicular changes following 17 weeks on the deficient diet (Table I). The higher testes/BW ratio seen in the W/K fed the vitamin E-deficient diet compared to the W/K fed the diet with vitamin E was due to the lower body weight of the vitamin E-deficient W/K. All three strains exhibited statistically significant splenomegaly on the deficient diet. Splenomegaly was more pronounced in the SHR and W/K than in the SD.

4. Blood pressure. The presence or absence of vitamin E in the diet had no effect on the systolic blood pressure of the SHR which was approximately 210 mm Hg at 20 weeks of age for both dietary groups. Blood pressures were not measured in the normotensive W/K and SD.

5. Mitogenic responses. All three rat strains fed the vitamin E-deficient diet had depressed splenic mitogen responses compared to animals fed the diet containing vitamin E (Fig. 3). SD splenocytes showed consistently higher mitogenic responses to both T- and B-cell mitogens than splenocytes from either SHR or W/K on either diet. Both the SHR and W/K mitogenic responses to Con A and PHA were significantly enhanced when animals were fed the diet with vitamin E compared to SHR and W/K fed the vitamin E-deficient diet. Mitogenic responses of SD were increased, but to a lesser extent, by addition of vitamin E to the diet. In all three strains there was an increase in splenic mitogen response to LPS with the addition of vitamin E to the diet.

6. Tissue vitamin E levels and depletion rates. When both SHR and W/K were fed the semipurified diet containing 200 mg/kg vitamin E, the plasma, liver, and testes of the

TABLE I. TESTES AND SPLEEN WEIGHTS OF RATS FED VITAMIN E-DEFICIENT OR -SUPPLEMENTED DIETS FOR 17 WEEKS

Rat strain[a]	Dietary vitamin E (mg/kg)	Testes		Spleen	
		(g)	(mg/kg body wt)	(g)	(mg/g body wt)
SHR	0	1.6 ± 0.2[b]	0.56 ± 0.07	0.79 ± 0.04	0.27 ± 0.01
	200	2.9 ± 0.1***[c]	0.82 ± 0.03*	0.63 ± 0.02**	0.18 ± 0.01***
W/K	0	2.5 ± 0.1	0.87 ± 0.01	0.96 ± 0.06	0.33 ± 0.02
	200	2.7 ± 0.1*	0.70 ± 0.01***	0.79 ± 0.04*	0.20 ± 0.01***
SD	0	3.5 ± 0.1	0.88 ± 0.02	0.92 ± 0.04	0.23 ± 0.01
	200	3.6 ± 0.1	0.84 ± 0.04	0.78 ± 0.03**	0.18 ± 0.01***

[a] SHR = spontaneously hypertensive rat; W/K = Wistar-Kyoto; SD = Sprague-Dawley.
[b] Mean ± SE, from eight animals/strain.
[c] Statistical comparisons are between the vitamin E-deficient and -replete animals within each strain.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

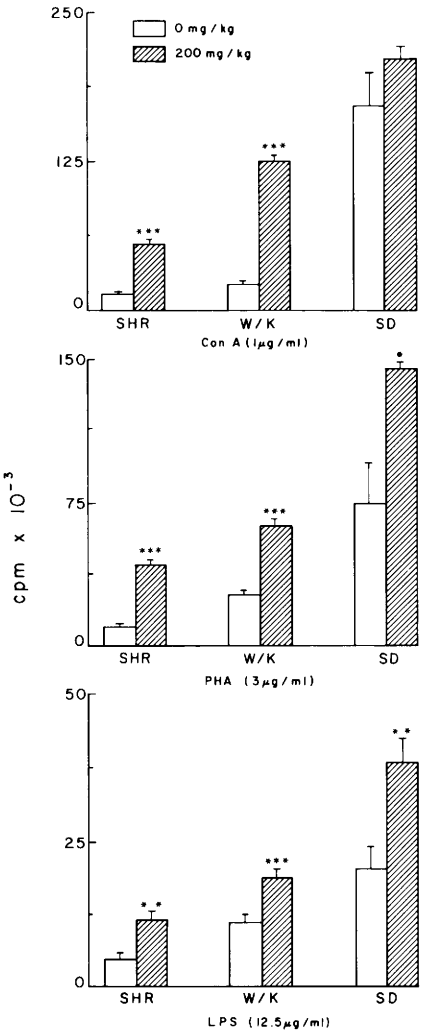

FIG. 3. Mitogen responses of SHR, W/K, and SD rat splenocytes fed either the vitamin E-deficient diet or the identical diet supplemented with 200 mg/kg all-rac-α-tocopheryl acetate for 17 weeks. Data represent responses to the optimal concentration of each mitogen. Details of culturing procedure are discussed under the Materials and Methods section. Data represent means ± SE from three experiments. Comparisons for statistical significance are between the two dietary groups in each strain for each mitogen examined (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

SHR still had significantly lower vitamin E levels than the W/K (Table II). There was no difference in the vitamin E level of skeletal muscle. Plasma of the SD had a level of vitamin E intermediate between the SHR and W/K which was significantly different from both. The level in the testes of the SHR and SD was identical and significantly lower than the W/K. The level of vitamin E in the muscle of SD was not different from the SHR, but was significantly higher than the W/K. The depletion rates of vitamin E from testes and muscle of the SHR and W/K fed a vitamin-E deficient diet were the same over a 4-week period (Fig. 4).

Discussion. Based on testes degeneration, body weight, and plasma PK, the SHR fed a vitamin E-deficient diet from weaning developed vitamin E deficiency signs more rapidly than the W/K or SD rats fed the identical vitamin E-deficient diet. Both of the inbred strains showed weight reduction earlier than the SD. In addition, the vitamin E-deficient SHR had the lowest response of all three strains to mitogen stimulation of splenocytes. The SHR has been shown to have lower mitogen responses than the W/K (3). In this study, we have demonstrated that all three rat strains had responses to mitogens which were modulated by dietary vitamin E, and that even on a vitamin E-deficient diet, the strain difference in mitogen responses between the SHR and W/K remained.

The lymphocyte mitogen response was enhanced to a greater extent by vitamin E in both the SHR and W/K rats than in the SD (Fig. 3). The SD is an outbred strain and splenocytes from this strain demonstrated the strongest responses to the mitogens tested. B-cell responses to the mitogen LPS were enhanced equally in all three strains by dietary vitamin E when compared to animals fed a vitamin E-deficient diet (Fig. 3).

Thus, depending upon the parameter measured, there are distinct strain differences in the sensitivity to a vitamin E-deficient state, with the SHR being most sensitive and the SD the least sensitive. However, it is not possible to correlate tissue levels of vitamin E following ingestion of a diet containing 200 mg/kg of the vitamin for 17 weeks with the variation in sensitivity to vitamin E deficiency. For example, although the SHR developed myopathy (as indicated by plasma PK) earlier than the W/K, the muscle tocopherol levels of SHR and W/K fed diets containing 200 mg/kg vitamin E were the same (Table II). Similarly, although the vitamin E-deficient SHR developed testes degeneration earlier than the vitamin E-deficient SD, the testes

TABLE II. TISSUE α-TOCOPHEROL LEVELS OF SHR, W/K, AND SD AFTER 17 WEEKS ON DIETS CONTAINING 200 mg/kg VITAMIN E

Tissue	Strain		
	SHR	W/K	SD
Plasma (μg/ml)	9.3 ± 0.3^{a}	15.8 ± 0.7^{b}	11.3 ± 0.7^{c}
Liver (μg/g)	43.8 ± 2.0^{a}	55.6 ± 4.0^{b}	$49.8 \pm 2.3^{a,b}$
Testes (μg/g)	24.2 ± 0.4^{a}	31.1 ± 0.5^{b}	23.2 ± 0.7^{a}
Gastrocnemius muscle (μg/g)	$14.3 \pm 0.8^{a,b}$	13.4 ± 0.5^{a}	16.1 ± 1.1^{b}

Note. Data represent the means ± SE of tissue tocopherol levels from eight animals/strain. Means with the same letter superscript are not different statistically ($P > 0.05$).

tocopherol levels were the same when both strains were fed 200 mg/kg vitamin E in the diet.

Measurement of vitamin E content of a tissue at a single time point (after 17 weeks on the diet) does not indicate rate of uptake. In addition, Tengerdy and Brown (9) have indicated that certain tissues may have an upper limit for the capacity for storing vitamin E. This may be the case for the muscle and testes of the rat. Our data (Fig. 4) show that the relative rates of depletion between SHR and W/K strains are the same. Thus, depletion rates cannot account for the difference in time of onset of vitamin E deficiency symptoms between these two strains. Moreover, although at 4 weeks the SHR had elevated plasma PK levels (data not shown), the tocopherol content was not different from skeletal muscle of the W/K whose plasma PK was normal. This suggests that the more rapid onset of myopathy in the SHR may not be directly related to tocopherol status.

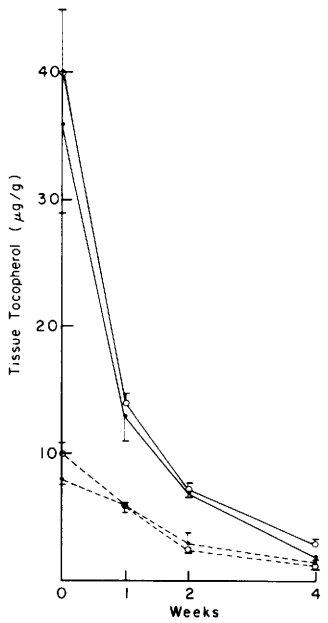

FIG. 4. Depletion of muscle and testes tocopherol from SHR and W/K over 4 weeks. SHR testes (● —— ●); SHR muscle (● -- ●); W/K testes (O —— O); W/K muscle (O - - - O).

It would therefore appear that strain differences in sensitivity to a vitamin E deficiency are not solely related to tocopherol status of the tissue, but may involve other metabolic variables which could influence the onset of pathology in the different tissues. It is likely that such processes are induced by free radicals. Therefore, the relative concentration of other agents involved in protecting the cell against free radical damage, such as superoxide dismutase, catalase, glutathione peroxidase, or ascorbic acid could influence the relative sensitivity to a vitamin E deficiency. Similarly, metabolites which might enhance free radical formation, such as ferrous ion or polyunsaturated fatty acids, might also alter sensitivity of a tissue to vitamin E deficiency. Differences in the utilization of dietary selenium and polyunsaturated fatty acids could certainly influence the sensitivity of the animal to a vitamin E deficiency. Finally, there are diffuse structural alterations in the membranes of cells from the SHR compared to the W/K which could lead to a less stable structure (10). Higher amounts of tocopherol may therefore be needed to stabilize such membranes.

The present study clearly demonstrates that differences in sensitivity to vitamin E defi-

ciency can occur among strains of animals within species. Similar strain differences have been reported for owl monkeys (11). Furthermore, we have developed inbred rats which are sensitive or resistant to a vitamin E deficiency by selective breeding (12). The biochemical basis for these strain differences remains to be established.

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