Kinetics of tissue RRR-α-tocopherol depletion and repletion. Effect of cold exposure

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Vitamin E was estimated in plasma and tissues of rats kept for three months on a low vitamin E diet or a high vitamin E diet. Some of the animals from each group were switched to the opposite diet, and the kinetics of uptake and depletion of vitamin E were followed 3, 8, and 15 days after the diet change. Some rats were also submitted to cold exposure (6°C) for three days. During repletion plasma, red blood cells, liver, spleen, and adrenal gland were the only tissues that responded rapidly to the diet change; after three days, their vitamin E levels corresponded to that of the new diet. Heart, brain, lung, muscle, and thymus were slow in reacting to diet change. Fifteen days after the change in diet, white adipose tissue did not respond. The rate of repletion for all tissues was more rapid than the rate of depletion, but liver was the only tissue that after three days had vitamin E levels corresponding to the low-vitamin diet. Cold exposure for three days did not produce any significant change in the vitamin E content of any tissue, indicating that despite high oxygen consumption by the animal, vitamin E was not consumed or mobilized.

Keywords: Vitamin E; d-α-tocopherol; cold exposure; depletion; repletion

Introduction

It is known that different tissues have different kinetics of α-tocopherol depletion and repletion. Draper and Csallany[1] reported that in rats and rabbits fed a vitamin E deficient diet, liver α-tocopherol decreased rapidly for several weeks, then reached a plateau, whereas the muscle concentration of α-tocopherol declined much more slowly. Studies of vitamin E accumulation in the body have generally been restricted to one tissue or have considered only one or two points in time.[2,3] Two more extensive studies have been performed.[4,5] The first one studied effects of the addition of low levels of vitamin E to a diet deficient in vitamin E over a 25-week period in rats.[4] The second one studied the effect of long-term administration of high levels of vitamin E.[5] However, the rates of depletion and repletion were measured at different ages in the two studies. Both investigations used an interval of one or more weeks. In the experiments described in this paper, measurements were taken 3, 8, and 15 days after starting depletion or repletion. In addition, kinetics of α-tocopherol depletion and repletion were estimated in animals at the same age by using rats fed a low- or a high-vitamin E diet and then switching diets in these two groups. This study was also extended to some important tissues, such as adrenal gland, spleen, and thymus, that were not included in previous studies.[4,5]

A secondary aim of this experiment was to determine if the tissue uptake and depletion of α-tocopherol could be modified by subjecting the animal to an increased oxidative stimulus. Instead of using toxic drugs such as CCl_4 or an iron-overload, the animal was submitted to a cold exposure in a chamber at 6°C for three days. It is well known that in this condition, the animal maintains its body temperature by "shivering thermogenesis" in which its oxygen consumption rate is increased. This increase could be up to a maximum value of 5 times the basal metabolic rate, depending on the species and the experimental conditions.[6,7] The rationale was that under this physiological stimulus, the chance to produce more "free radicals" in vivo increases markedly; therefore, a substantial catabolism or use of vitamin E could potentially be observed.

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An increase in the rate of depletion of α-tocopherol and a decrease in the rate of uptake of the vitamin were expected.

Materials and methods

Animals and diets

Thirty-nine male Wistar rats (Charles River Canada, St. Constant, Quebec) weighing 100 g ± 10% were used in all the experiments and for three months were fed a modified AIN-76 diet[8] as follows. The diet for the first group (18 rats) (Group LE) contained the AIN-76 diet in which vitamin E was omitted from the vitamin mixture. In the second group (21 rats) (Group HE), d-α-tocopherol acetate (RRR-α-tocopherol-acetate; ICN Nutritional Biochemicals, Cleveland, OH) was added to the vitamin mixture to give 1.0 g d-α-tocopherol acetate (1360 IU)/kg diet. Twelve rats from each LE and HE groups were switched to the opposite diet and fed the new diet for 3, 8, or 15 days. In addition, three of these rats from each group also were transferred to a cold room (6°C ± 1°C) for three days.

Blood and tissue collection

Blood and tissues were obtained from animals anesthetized with halothane (2% in oxygen) (Fluothane, Ayerst Laboratory, Montreal). Plasma was obtained from heparinized blood after centrifugation at 3000 × g. Red blood cells (RBC) were obtained after washing the pellet with 0.9% cold saline followed by centrifugation.

Analytical methods

Alpha- and γ-tocopherols (vitamin E) were determined in plasma and tissues with the high performance liquid chromatography (HPLC) method of Thompson and Hatina[9] as previously described.[10]

Statistical analysis

Student's t-test[11] was used for statistical evaluation of the results.

Results

Table 1 shows vitamin E levels in plasma and tissues of rats fed LE and HE diets for 3 months, and after they were switched to the opposite diet. The effect of cold exposure at 6°C for three days is also included. As expected, very low levels of vitamin E were observed in all tissues from rats fed the LE diet; of the studied tissues, only adrenal gland and brain retained more than 4.0 μg/g of tissue. Adrenal gland was the tissue that accumulated the most vitamin E in rats fed the HE diet; second in this regard was liver. At the other extreme, red blood cell was the rat tissue that retained the least vitamin E.

Changing animals to the opposite diet produced in each tissue a different result. Rates of repletion and depletion were calculated for the different time intervals and are shown in *Table 2*. Adrenal gland and liver, followed closely by spleen, were the tissues that accumulated α-tocopherol at a faster rate than any of the other tissues. After only three days on the HE diet, these organs had vitamin E levels comparable to those from rats fed the HE diet for 3 months. They may have reached these levels even earlier. The same tissues were also the ones that lost α-tocopherol the fastest. Other tissues maintained relatively unchanged rates of repletion and depletion during the intervals of 3 to 8 and 8 to 15 days. In general, in all tissues, rates of repletion were higher than rates of depletion, the exception being white adipose tissue in the interval 0 to 3 days, but there was a great variability in this tissue (*Table 1*). It seems that liver, as indicated in *Table 2*, is the only tissue that accumulates and loses vitamin E at a similar rate. The rate of repletion in lung was approximately twice that of depletion during the first 8 days of the experiment (*Figure 1*). Plasma had a relatively high repletion rate but a very slow depletion rate. All the other tissues examined had slow repletion and depletion rates. Muscle, white adipose tissue, and brain showed a very small change or no change at all, even after 15 days of diet change.

Cold exposure at 6°C for three days failed to decrease the rate of repletion and to accelerate the rate of depletion in any significant way in any of the tissues investigated (*Tables 1 and 2*). *Figure 1* shows this graphically for lung, the tissue in which the high oxygen uptake during cold exposure takes place first. In general, cold exposure produced higher levels of vitamin E than in the corresponding control tissues at three days. This was translated into a higher rate of repletion (*Table 2*), which was the inverse of what was expected. The only exception was plasma in which the rate of repletion was slightly smaller but not significantly different than that at 3 days at room temperature. *Table 2* shows that rate of depletion was smaller or did not change with the cold exposure in comparison with the rate of depletion at room temperature. Once again, the exception was plasma, which was the only site where the rate of depletion under cold exposure was higher than the control at room temperature.

Discussion

In the present study, tocopherol apparently reached saturation levels after rats were fed the HE diet (1,360 IU/kg) for three months, because no further increases were observed after an extra 15 days. In other experiments in which higher doses of dietary vitamin E were given (10,000 IU/kg), tocopherol levels continued to increase in all tissues examined for the duration of the supplementation.[5] Also, Yang and Desai reported that there was a continued increase in both liver and plasma after 8 and 16 months of feeding high levels of vitamin E.[12] These observations suggest that saturation of tissues with tocopherol depends on the level in the diet.

Adrenal glands and liver followed closely by spleen

Table 1 Vitamin E levels in plasma and tissues of rats fed LE and HE diets and after switching to the opposite diet. Effect of cold exposure (6°C)

Tissue	Diet/ Diet Change	Days 0	3	3 (6°C)	8	15
Plasma	LE	0.4 ± 0.1	—	—	—	0.4 ± 0.1
	LE—HE	—	45.5 ± 5.6	(35.7 ± 10.6)	54.3 ± 13.8	57.5 ± 9.6
	HE	43.5 ± 8.1	48.4 ± 8.7	—	—	50.5 ± 11.6
	HE—LE	—	34.7 ± 2.5	(26.9 ± 5.9)	17.9 ± 1.6	11.4 ± 2.8
Liver	LE	0.9 ± 0.4	—	—	—	0.5 ± 0.3
	LE—HE	—	290.6 ± 133.5	(465.9 ± 37.7)	333.0 ± 110.4	328.6 ± 76.1
	HE	240.2 ± 47.1	249.9 ± 31.4	—	—	247.2 ± 35.6
	HE—LE	—	28.1 ± 12.1	(33.9 ± 10.6)	10.5 ± 1.0	7.6 ± 3.1
Adrenal	LE	4.9 ± 4.9	—	—	—	5.9 ± 4.2
	LE—HE	—	603.3 ± 111.2	(657.6 ± 41.3)	706.7 ± 103.0	719.3 ± 14.1
	HE	710.5 ± 42.8	612.5 ± 69.7	—	—	643.9 ± 78.9
	HE—LE	—	419.5 ± 29.2	(474.1 ± 49.6)	270.4 ± 57.10	228.2 ± 16.3
Lung	LE	0.7 ± 0.2	—	—	—	0.5 ± 0.1
	LE—HE	—	44.6 ± 5.6	(51.8 ± 4.2)	60.7 ± 3.3	61.3 ± 4.9
	HE	79.3 ± 2.1	80.5 ± 5.5	—	—	85.3 ± 5.0
	HE—LE	—	56.0 ± 3.1	(56.9 ± 8.0)	34.2 ± 4.5	24.5 ± 2.1
Thymus	LE	0.5 ± 0.1	—	—	—	0.4 ± 0.0
	LE—HE	—	14.4 ± 2.9	(20.0 ± 3.1)	30.7 ± 4.0	31.7 ± 1.9
	HE	37.4 ± 3.9	40.5 ± 4.4	—	—	38.0 ± 1.6
	HE—LE	—	37.6 ± 4.9	(35.5 ± 8.4)	20.8 ± 3.2	14.4 ± 1.3
Muscle	LE	0.5 ± 0.3	—	—	—	0.5 ± 0.1
	LE—HE	—	5.6 ± 0.8	(7.5 ± 1.5)	16.3 ± 4.4	24.9 ± 3.2
	HE	33.3 ± 13.9	38.7 ± 13.0	—	—	42.5 ± 7.4
	HE—LE	—	37.1 ± 3.2	(38.1 ± 7.9)	32.3 ± 3.2	20.3 ± 0.8

Tissue	Diet/ Diet Change	Days 0	3	3 (6°C)	8	15
Brain	LE	4.1 ± 0.4	—	—	—	4.1 ± 0.3
	LE—HE	—	7.9 ± 0.3	(9.0 ± 1.0)	11.8 ± 1.2	16.4 ± 0.3
	HE	23.2 ± 1.7	23.8 ± 2.7	—	—	26.2 ± 1.8
	HE—LE	—	26.5 ± 2.6	(23.4 ± 3.1)	21.9 ± 0.6	17.1 ± 2.3
RBC	LE	0.0 ± 0.0	—	—	—	0.0 ± 0.0
	LE—HE	—	7.0 ± 0.8	(8.1 ± 0.2)	8.0 ± 1.8	8.3 ± 0.8
	HE	7.5 ± 0.9	7.4 ± 1.1	—	—	6.2 ± 1.2
	HE—LE	—	4.6 ± 0.6	(5.3 ± 0.4)	2.2 ± 0.2	1.6 ± 0.2
Spleen	LE	1.0 ± 0.1	—	—	—	0.8 ± 0.1
	LE—HE	—	89.6 ± 11.4	(88.1 ± 12.0)	87.6 ± 7.8	86.2 ± 14.7
	HE	85.2 ± 8.0	102.2 ± 8.2	—	—	84.5 ± 13.6
	HE—LE	—	57.4 ± 1.4	(65.1 ± 7.8)	34.7 ± 5.5	27.0 ± 2.2
Heart	LE	1.3 ± 0.2	—	—	—	0.8 ± 0.1
	LE—HE	—	19.3 ± 4.5	(27.6 ± 1.2)	49.3 ± 3.1	67.6 ± 4.9
	HE	87.0 ± 4.8	94.1 ± 8.7	—	—	87.0 ± 4.0
	HE—LE	—	89.7 ± 5.1	(75.9 ± 8.6)	67.0 ± 3.1	40.7 ± 5.4
White Adipose	LE	0.7 ± 0.7	—	—	—	0.7 ± 0.1
	LE—HE	—	4.2 ± 2.8	(4.7 ± 0.4)	12.0 ± 1.5	13.0 ± 6.2
	HE	90.0 ± 11.6	84.8 ± 8.5	—	—	75.8 ± 17.7
	HE—LE	—	69.4 ± 6.6	(104.3 ± 2.4)	83.2 ± 25.7	84.3 ± 5.8

The indicated values represent mean ± SD, with 3 rats per value. The levels are expressed in μg/ml for plasma, μg/ml of packed cells for Red Blood Cells (RBC) and in μg/g for all tissues. LE: diet low in vitamin E; HE: diet high in vitamin E; LE—HE represent the diet change for the repletion studies; and HE—LE represent the diet change for the depletion studies.

Table 2 Repletion and depletion rates of α-tocopherol in rat tissues

Tissue		Time Intervals 0–3 Days	3–8 Days	8–15 Days
Adrenal	(R)	199.5 (217.6)[a]	20.7	1.8
	(D)	97.0 (78.8)	29.8	6.0
Liver	(R)	96.6 (155.0)	8.5	0.6
	(D)	70.7 (68.8)	3.5	0.4
Spleen	(R)	29.5 (29.0)	0.0	0.0
	(D)	9.3 (6.7)	4.5	1.1
Plasma	(R)	15.0 (11.8)[b]	1.8	0.5
	(D)	2.9 (5.5)	3.4	0.9
Lung	(R)	14.6 (17.0)	12.0	0.1
	(D)	7.8 (7.5)	4.4	1.4
Thymus	(R)	4.6 (6.5)	3.3	0.1
	(D)	0.0 (0.6)	3.4	0.9
Heart	(R)	6.0 (8.8)	6.0	2.6
	(D)	0.0 (3.7)	4.5	3.8
RBC	(R)	2.3 (2.7)[c]	0.2	0.0
	(D)	1.0 (0.7)	0.5	0.1
Muscle	(R)	1.7 (2.3)	2.1	1.2
	(D)	0.0 (0.0)	1.0	1.7
White Adipose	(R)	1.2 (1.3)	1.6	0.1
	(D)	6.9 (0.0)	0.0	0.0
Brain	(R)	1.2 (1.6)	0.8	0.7
	(D)	0.0 (0.0)	0.9	0.7

[a] Values expressed in μg/g tissue per day. R: repletion; D: depletion. The numbers in parentheses are those corresponding to the cold exposure at 6°C for three days. [b] μg/ml plasma per day. [c] μg/ml packed cells per day.

were the tissues that accumulated α-tocopherol at a faster rate than any of the other tissues. This could be an indication that an active transport-and-uptake system is operating in these tissues. Machlin and Gabriel[5] reported that adipose tissue accumulated vitamin E at a very rapid rate, but this was not the case in the present experiment. However, their calculated rate of accumulation was for a longer period. It seems that an accelerated uptake occurred only after 4 weeks when the diet was supplemented with 1,000 IU/kg. Therefore, this indicated that uptake mechanisms in this tissue take more time to develop in comparison to liver and other tissues.

The present work demonstrated that tissues accumulate vitamin E at a characteristic rate. It seems that different mechanisms of uptake, depending on the metabolism of vitamin E, operate in each tissue. It is known that this mechanism is specific for α-tocopherol in comparison to γ-tocopherol,[13] and even for RRR-α-tocopherol in comparison to its stereoisomer SRR.[14]

On the other hand, in some tissues, a high rate of depletion could indicate utilization or could be an indirect measurement of the involvement of the vitamin in metabolic processes that take place in the tissue, notably in adrenals, liver, and spleen where the rate of depletion is very high compared to other tissues. It has been suggested that liver is the main storage organ for tocopherol and that liver helps to maintain plasma levels when the intake becomes inadequate.[5] However, the high rate of depletion to levels of LE diet reported here, while plasma and other tissues take a long time to decrease the level of vitamin, argue against this possibility. In contrast, in white adipose tissue the rate of release is so slow that it cannot maintain plasma levels. In fact, it has been documented that in guinea pig, the rate of release or depletion from adipose tissue is so slow that blood levels of vitamin E are not maintained, and animals develop a myopathy even though adipose tissue stores are still high.[15]

It seems that those tissues that lose α-tocopherol rapidly, such as adrenals and liver, lack mechanisms for regeneration of vitamin E. Nevertheless, these tissues also have high levels of vitamin C.[16] This vitamin has been postulated to form part of a system that regenerates vitamin E in vivo, due to the interaction of these two vitamins in vitro.[17] Experiments performed in this laboratory, in which the vitamin C status (ascorbic and dehydroascorbic acids) of rats fed diets varying in vitamin E was measured, indicated that this interaction does not take place in vivo.[18]

Therefore, the high depletion rate of vitamin E in adrenals, liver, and spleen could be attributed to a higher utilization in situ; this could be a strong indication that in these tissues, vitamin E plays a specific and urgent metabolic role that needs to be elucidated.

The lack of effect of the cold exposure on the levels of α-tocopherol in all tissues, and on the rate of repletion and depletion, is important in view of the proposed antioxidant role of the vitamin.[19] It was expected that when mammals exposed to a cold environment increased their oxygen consumption, the production of free radicals would also increase, thereby resulting in an increased utilization of vitamin E, at least in those tissues that would directly handle this extra oxygen, such as lung, red blood cells, and muscle. However, other tissues under the stress of

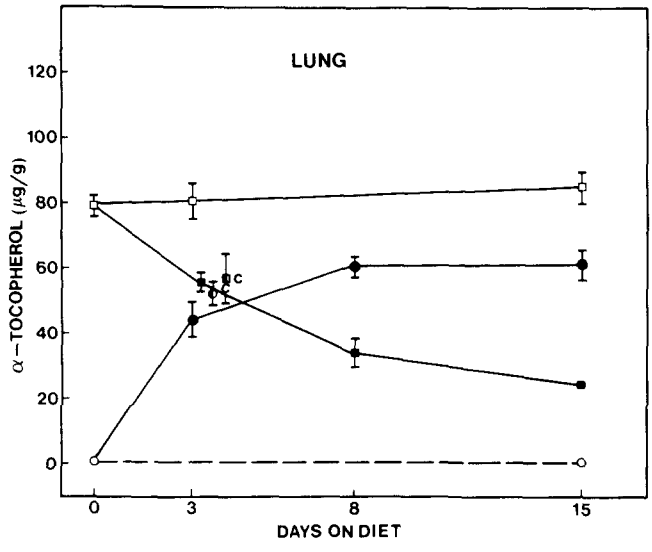

Figure 1 Vitamin E levels in lung of rats fed LE (O---O) and HE diets (□——□) and after switching to the opposite diet (closed symbols). Symbols represent mean ± SD expressed as μg α-tocopherol/g tissue. There were 3 rats for each value. The letter C beside a half-open symbol denotes the levels of tocopherol after the 3 days' cold exposure. (More details in footnote in *Table 1*.)

cold would also respond actively with their normal and physiological responses to the stress; therefore, changes in vitamin E would be expected there also. Nevertheless, none of this occurred under the stimulus of cold during three days.

Several other stimuli characterized by imposing a high oxygen consumption have failed to produce significant decreases in some tissues of α-tocopherol. Kihlström et al.[20] found decreased levels of several cardiac antioxidants in endurance-trained rats (200 hours' swimming), but α-tocopherol levels were decreased only by a small amount, and not correlated with the severity of the training. This agrees with the results of Gohil et al.[21] who found that there was not a significant decrease in the concentration of vitamin E in the skeletal muscle or in the myocardium of rats after a long-lasting running program. Oikawa et al. reported lower levels of vitamin E in muscle of rats undergoing training for 9 weeks.[22] However, as the vitamin content was expressed for g of wet tissue, a small reduction in muscle vitamin E could well result from lipid mobilization during long training, leading to a leaner muscle. No decreases in vitamin E were observed in liver, muscle, or brown adipose tissue of rats submitted to endurance exercise.[23] Even strenuous exercise that induces necrotic myopathy in skeletal muscle did not produce any changes in the concentration of vitamin E in this tissue.[24] Moreover, iron administration, an oxidative stress, failed to produce a significant decrease in the vitamin content in skeletal muscle of rats.[25]

Therefore, it seems that the metabolism of vitamin E is not related or interconnected with oxidative processes. This, of course, casts a shadow of doubt upon the proposed antioxidant role of this vitamin.[19] The other possibility is that free radicals are not produced in vivo in sufficient amounts to consume this vitamin, as suggested several years ago by Green.[26] It could be added, that the amount of α-tocopherol in membranes is so small in comparison to that of the phospholipid to be protected,[27] that the antioxidant action of the vitamin is difficult to conceive in vivo. Hence, the main metabolic role of α-tocopherol needs further elucidation.

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