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Vitamin E levels in superficial and intra-abdominal locations of white adipose tissue in the rat

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Concentrations of RRR-α-tocopherol (vitamin E) were measured in white adipose tissue (WAT) from three superficial and three intra-abdominal locations of rats fed diets using low-, normal-, or high-vitamin E content for 3 months. Vitamin E levels in WAT were responsive ($P < 0.05$) to the amount of the vitamin in the diet. Irrespective of treatment, superficial locations of adipose tissue accumulated four to six times more vitamin E than the intra-abdominal locations. Some of the rats in the low and high vitamin E diet were switched to the opposite diet and the repletion and depletion of the vitamin were monitored for 10, 20, and 30 days after the diet change. Thirty days after the change in diet, WAT from superficial and intra-abdominal locations did not respond during depletion. The deposition of vitamin E in all superficial locations was more rapid than in the intra-abdominal locations after the diet change from low vitamin E to high vitamin E. (J. Nutr. Biochem. 8: 392–396, 1997) © *Elsevier Science Inc. 1997*

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Introduction

Vitamin E as RRR-α-tocopherol (αT) is distributed widely in the tissues of humans and animals. White adipose tissue (WAT) contains approximatcly 90% of the total body vitamin E, where it is localized in the lipid droplets of adipocytes.[1] Although the αT concentration of WAT can be modified by long-term dietary manipulations, it is certain that this quantitatively important deposit of vitamin E is relatively unavailable for exchange, as demonstrated in rats[2–4] and guinea pigs.[5,6] In spite of this, and because of the unreliable data obtained in measuring plasma αT,[7] the vitamin E content of WAT has been used to assess the vitamin E status in epidemiologic studies of diet and disease in humans.[8–10] Several authors have reported on the vitamin E content in WAT obtained from different anatomical locations. Traber and Kayden[1] reported the αT content of the subcutaneous WAT from the chest. Schäfer and Overvad[11] described no differences in αT content of the subcutaneous WAT from the waist and buttock in humans. Parker[12] reported values from WAT removed from the abdominal region of patients undergoing corrective surgery, but the exact location was not indicated. It has been proposed that WAT vitamin E concentration can be used to measure the long-term nutritional status of the vitamin in the organism.[8–11]

Several studies have documented differences between WAT deposits with respect to adipocyte morphology, metabolism, and composition.[13] However, it is generally assumed that the composition of all WAT within an individual is the same. We, therefore, decided to investigate whether the vitamin E levels were similar in WAT obtained from different locations in the rat. We selected three subcutaneous and three intraabdominal sites to determine the differences in the vitamin E content between the "inside" and "outside" locations.

Methods and materials

Animal and diets

Male Wistar rats (Charles River, Canada, St. Constant, Quebec) weighing 100 gm ± 10% were used in all experiments. They were fed a modified AIN-76 diet[14] for 3 months and allocated to three

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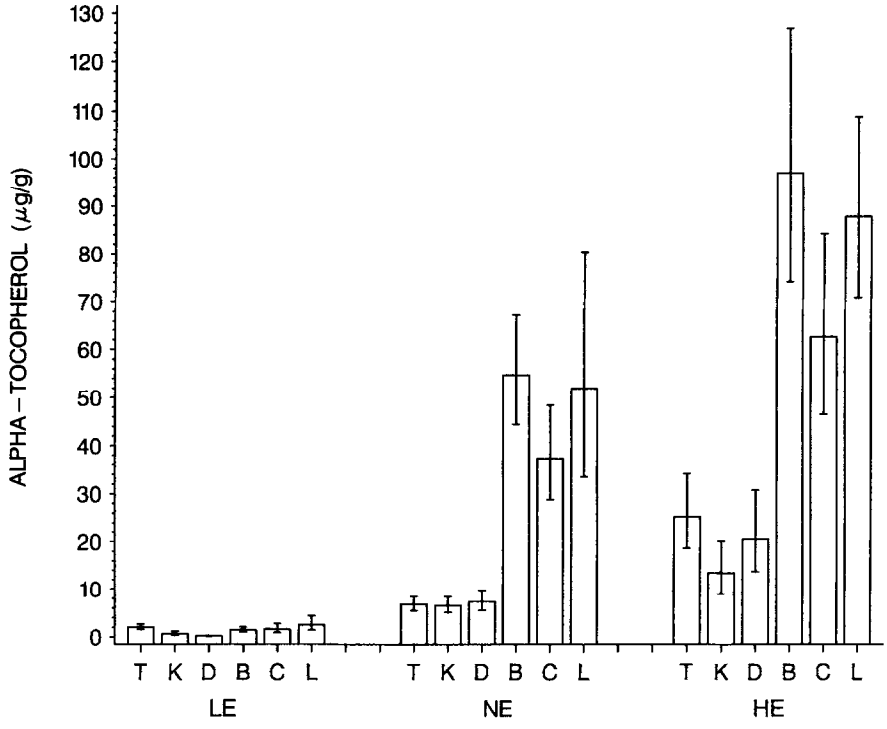

Figure 1 Vitamin E levels in WAT in superficial locations (B, back; C, chest; and L, leg) and intraabdominal locations. (T, testis; K, kidney, and D, diaphragm). The values are expressed as μg of α-tocopherol/g tissue and represent the geometric means and 95% confidence intervals. There were 12 rats for diets LE and HE and 7 rats for diet NE for each value. LE, NE, and HE represent diets with low, normal, and high vitamin E content.

different groups in which the diet varied in vitamin E content. The first group (Group LE) of rats were fed the AIN-76 diet without the inclusion of vitamin E in the vitamin mixture. The second group (Group NE) was fed the AIN-76 diet with the addition of 50 IU of vitamin E/kg of diet. Rats in the third group (Group HE), were also fed AIN-76 with vitamin E at a level of 250 IU/kg. In both the NE and HE groups, d-α-tocopherol acetate (RRR-α-tocopherol-acetate; ICN Nutritional Biochemicals, Cleveland, OH) was added to the vitamin mixture to adjust the amount of vitamin E in the diet. To study the rates of depletion and repletion of αT in WAT, some rats from each LE and HE groups were switched to the opposite diet and fed the new diet for 10, 20, or 30 days. The experiment and procedures were approved by the Animal Care Committee and were performed in accordance with the guidelines of the Canadian Council of Animal Care (1993) as specified in the Guide to the Care and Use of Experimental Animals.

Tissue collection

WAT samples were collected from animals anesthetized with halothane (2% in oxygen) (Fluothane, Ayerst Laboratory, Montreal). The samples were rinsed in 0.9% cold saline, dried on filter paper, and stored at −75°C until analysis. The adipose depot samples included three from superficial ("outside") locations: back (B, subcutaneous overlying the scapula region), chest (C, subcutaneous overlying mid lateral thoraic), and leg (L, subcutaneous mid femoral region overlying the vastus lateralis); and three from intra-abdominal ("inside") locations: testis (T, testicular fat), kidney (K, perineal fat), and diaphragm (D, sternopericardial ligament). Depending on the site of white adipose, approximately 2.0 to 3.0 gm of fat were collected, whereas only approximately 0.5 grams from the diaphragm was sampled.

Analytical methods

The α-tocopherol content of the WAT from the various anatomical sites were prepared according to the method of Traber and Kayden[1] before high performance liquid chromatography (HPLC). Vitamin E concentrations were determined in WAT samples using the HPLC method of Thompson and Hatina.[15]

Statistical analysis

The data for all tissue were log transformed before analysis to stabilize the variance and achieve normality in the distribution of the data. For the analysis of the precrossover data, a multivariate analysis[16] of the tissue levels with a main effect for dose was performed. Contrasts of the normal versus low and high versus normal diet groups were performed.

The depletion and repletion data were analyzed separately. The data were analyzed using a multivariate analysis of variance with a main effect for day. Tests of contrasts of subsequent days were performed. For all analyses, linear transformations of the dependent variables, i.e., the log levels of vitamin E in the various tissues were constructed to provide a within subjects test of deep versus superficial sites, and a within subjects tests of the effect of site within the intra-abdominal tissues and of the effect of site within the superficial tissues. All analysis were performed with the Statistical Analysis System (SAS Institute, Cary, NC USA. Version 6.11), using PROC GLM procedure.

Results

Vitamin E levels in WAT were responsive to the amount of vitamin in the diet, with a highly significant increases ($P < 0.0001$) from diet LE to diet NE for all tissues and less significant increases from diet NE to diet HE, with no evidence of a significant increase for the kidney ($P = 0.089$) or the leg and chest ($P = 0.14$) (*Figure 1*). The superficial or "outside" locations had higher concentrations of vitamin E than the intraabdominal locations. These differences were highly significant ($P < 0.0001$) for each diet. For diets NE and HE, WAT from the chest (C) had a lower vitamin E content compared with the other superficial

Table 1 Vitamin E levels in different locations of WAT of rats fed LE, NE, and HE diets at day 0 and after switching to the opposite diet for LE and NE diets

WAT location	Diet	Day 0	Day 10	Day 20	Day 30
Testis	LE→HE	2.0 (1.5, 2.7)	3.8 (2.8, 5.1)	14.8 (10.8, 20.4)	21.8(18.3, 26.0)
	NE	6.8 (5.5, 8.4)			
	HE→LE	25.3 (18.7, 34.1)	13.7 (8.8, 21.2)	12.9 (6.3, 26.1)	23.7 (18.6, 30.4)
Kidney	LE→HE	0.6 (0.3, 1.2)	2.0 (0.9, 4.4)	8.6 (5.2, 14.4)	16.9 (11.8, 24.3)
	NE	6.6 (5.1, 8.4)			
	HE→LE	13.5 (9.0, 20.2)	8.2 (5.4, 12.5)	15.6 (9.9, 24.7)	11.6 (7.7, 17.6)
Diaphragm	LE→HE	0.2 (0.1, 0.3)	1.0 (0.6, 1.7)	4.8 (2.4, 9.7)	13.5 (7.7, 23.8)
	NE	7.4 (5.6, 9.8)			
	HE→LE	20.6 (13.7, 30.9)	18.68 (11.6, 30.2)	19.1 (11.0, 33.1)	29.0 (17.2, 48.9)
Back	LE→HE	1.4 (1.0, 2.1)	19.7 (15.2, 25.6)	41.6 (27.7, 62.5)	82.9 (72.9, 94.3)
	NE	54.8 (44.5, 67.4)			
	HE→LE	97.2 (74.3, 127.2)	107.8 (70.6, 164.6)	111.4 (86.3, 143.7)	104.1 (85.1, 127.3)
Chest	LE→HE	1.6 (0.9, 2.8)	14.9 (11.8, 18.7)	35.9 (29.5, 43.8)	57.4 (47.7, 69.0)
	NE	37.4 (28.9, 48.5)			
	HE→LE	62.8 (46.7, 84.4)	58.9 (44.2, 78.4)	65.0 (51.5, 82.1)	61.4 (48.6, 77.6)
Leg	LE→HE	2.5 (1.4, 4.4)	16.9 (11.8, 24.3)	37.2 (28.4, 48.8)	56.0 (42.7, 73.5)
	NE	52.0 (33.6, 80.5)			
	HE→LE	88.0 (71.0, 109.1)	71.1 (59.2, 85.4)	93.7 (83.0, 105.7)	80.4 (67.2, 96.4)

The values represent geometric mean (95% confidence interval), with five rats per value for day >0 and 12, 7, and 12 rats per value for LE, NE, and HE diets, respectively for day 0. The levels are expressed in μg of α-tocopherol/gm of WAT. LE, diet low in vitamin E; HE, diet high in vitamin E; LE→HE represents the diet change for teh repletion study; and HE→LE represents the diet change for the depletion study.

tissues, i.e., back and leg. This difference was not significant for diet NE ($P = 0.090$), but was significant ($P = 0.0038$) for diet HE. The intra-abdominal locations presented more uniformity in values, nevertheless there was significant evidence ($P < 0.0001$) that the diaphragm (D) and to a lesser degree, the kidneys (K) were more depleted in vitamin than the testis (T) for diet LE. Also, the concentration of vitamin E in the kidneys was lower ($P = 0.0025$) than the concentrations for the other intraabdominal sites for diet HE.

When rats in diets LE and HE groups were switched to the opposite diet to study the rate of depletion and repletion of vitamin E, it was observed that αT content of both superficial and intraabdominal WAT locations were not modified from rats in group HE even after 30 days on diet LE which contained only traces of the vitamin (*Table 1*). This is illustrated in *Figure 2*. In contrast, there was significant repletion (LE→HE) for all tissues, as illustrated in *Figure 3* with rates of repletion greater by factors of 4 to 6 in the superficial locations in comparison to the intraabdominal locations (*Table 1*). At 30 days there was no evidence ($P > 0.05$) of a difference between the repletion levels for the LE→HE group (within each "inside" and "outside" location, respectively) and the depletion levels of the HE→LE group for any tissue.

Discussion

This study reports on the content of vitamin E in WAT obtained from three superficial and three intra-abdominal locations. In general, superficial locations accumulated four to six times more αT than the intra-abdominal locations (*Figure 1*). This indicates that mechanisms that regulate the level of the vitamin in WAT are different at both locations. As demonstrated by the depletion experiments, superficial and intra-abdominal WAT do not exchange αT. It seems that once αT enters WAT, there is no active mechanism to exchange the vitamin with other tissues, which are depleted earlier than the 30 days of this study.[4] In the present study it took 3 months of feeding a low-vitamin E diet (LE) to deplete young rats (100 gm) of almost all their αT in WAT; it is more difficult to deplete adult rats after 3 months on a diet high in vitamin E (HE).

The strong affinity for αT to remain in WAT was demonstrated in the study by Shäefer et al.[17] in which an obese patient, fed a severe reducing diet, had a lower content of triglycerides in the adipocytes compared with the relative concentrations of cholesterol and tocopherol in adipocytes. It was concluded that the release of αT by WAT is either a passive mechanism or a process of simple diffusion.[17] Traber and Kayden[1] demonstrated that αT is localized in the lipid droplets of adipocytes and is also unavailable for exchange. In a human study, daily supplementation with 800 mg of vitamin E over a 1 year period, only resulted in a small increase in αT levels in the WAT (buttock region).[18] In addition, these investigators[18] reported that on cessation with αT supplementation, the transfer of αT between adipose tissue and plasma is very slow.

It has been proposed that αT, as well as cholesterol, is delivered to cells via the receptor for low-density lipoprotein (LDL).[19] The LDL are one of the major transport mechanisms for tocopherol in the plasma, and can be taken up by fibroblast via the LDL receptor in vitro, resulting in an increase in the intracellular concentration of αT.[19] The presence of LDL receptor in adipocytes has been reported previously.[20] A dog study performed by Pillai et al.[21] suggests that the adipose tissue αT can be taken up by plasma lipoproteins for delivery to other tissues in the body. Another proposed mechanism for tocopherol transfer to the adipocyte is through the action of lipoprotein lipase.[22] Irrespective of the transfer mechanism for the uptake of αT

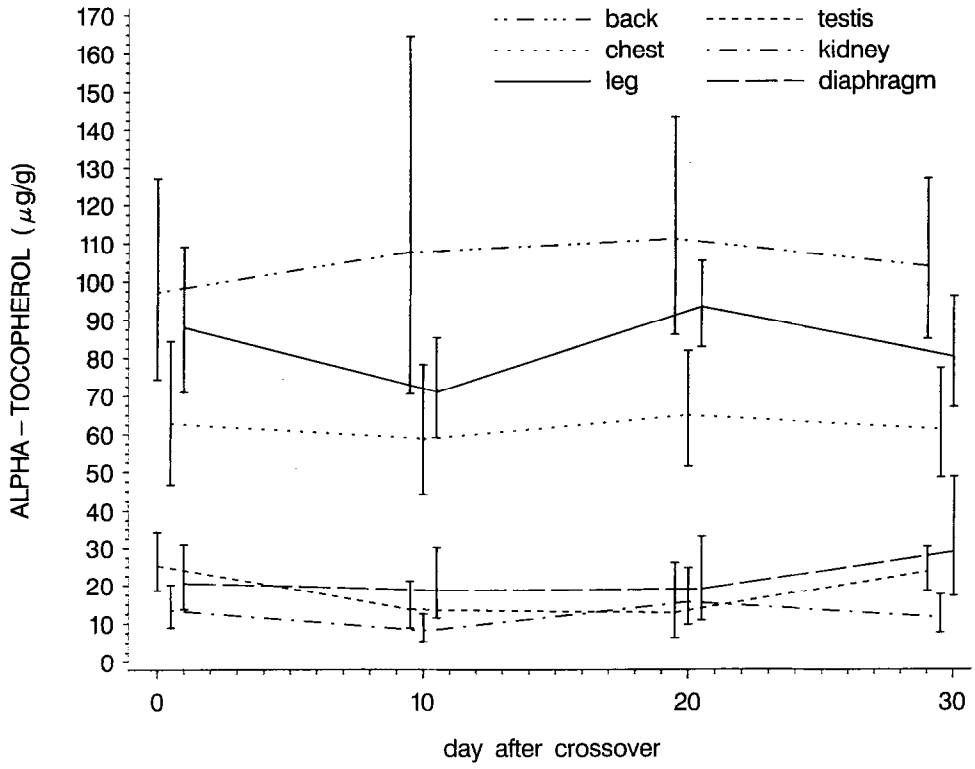

Figure 2 Vitamin E levels in WAT in rats switched from HE to LE at 0, 10, 20, and 30 days after diet change. The values represent geometric means and 95% confidence intervals, with 5 rats per value (12 rats for day 0). The levels are expressed in μg of α-tocopherol/g of WAT.

by WAT, it must be regulated differently in superficial and intra-abdominal locations. It seems that WAT has the ability to retain αT and even to increase its concentrations in conditions such as hyperlipidemia produced in rats by treatment with Triton WR-1339, a condition in which other tissues such as red blood cells and liver show a decrease in the level of the vitamin.[22]

Our results are in agreement with other studies which, have shown a great interindividual variability in the levels of αT in WAT. In the case of humans, this has been

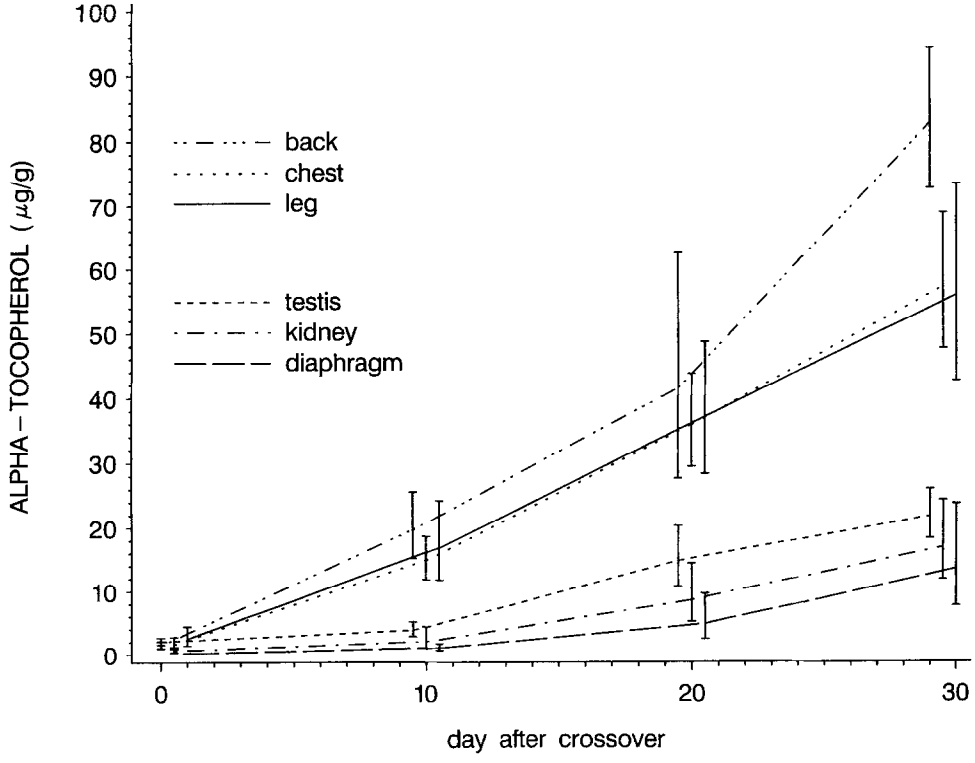

Figure 3 Vitamin E levels in WAT in rats switched from LE to HE at 0, 10, 20, and 30 days after diet change. The values represent geometric means and 95% confidence intervals, with five rats per value 12 rats for day 0). The levels are expressed in μg of α-tocopherol/gm of WAT.

attributed to different diets, or to vitamin supplements. The present study and others in rodents, however, indicate that this is a characteristic of the tissue. This fact, together with the reduced number of subjects used in human studies, could explain some discrepancies in the literature. For example, Handelman et al.[10] have shown that waist fat contained more αT than buttock fat, whereas Schäfer and Overvad[11] found that waist and buttock fat differed with regard to fatty acid composition, but not with respect to vitamin E content. In summary, the data demonstrated differences in vitamin E levels among various WAT sampling sites. During the short-term repletion period from low to high dietary vitamin E (over 30 days), a fairly rapid increase in vitamin E levels occurred in the WAT. However, over the depletion time period (over a 30 day period), WAT vitamin E did not reflect the short-term time change in dietary vitamin E from high to low levels.

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