PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 149, 500–502 (1975)

Effect of Plasma Lipid Levels and Obesity on Tissue Stores of α-Tocopherol (38836)

J. G. BIERI AND R. POUKKA EVARTS

Laboratory of Nutrition and Endocrinology, National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20014

It is well documented in man that plasma concentration of α-tocopherol (vitamin E) correlates highly with plasma total lipid (1), and a similar relationship has been shown for rats (2). Since it is known that the concentration of α-tocopherol in tissues is related to the plasma concentration (3), it was of interest to explore the effect of altered plasma lipids on tissue storage when intake of the vitamin was held constant. These relationships have been determined in rats whose blood lipids were (a) decreased by feeding orotic acid (4) or (b) increased by genetic obesity (5). The role of excessive adipose stores on tocopherol deposition in the obese rat has also been evaluated.

Methods. Orotic acid-fed rats. Weanling male Sprague–Dawley rats were fed a vitamin E-free diet containing in %: vitamin-free casein, 22; Fox-Briggs salt mix, 6; stripped lard, 5; vitamin mix, 2; cellulose, 4; and sucrose, 61. After partially depleting the vitamin E reserves for 19 days the animals were separated into three groups and the diets of two groups supplemented with 0.5 or 1.0% of orotic acid. After 5 days, all groups received 50 mg of dl-α-tocopheryl acetate/kg diet. Food intake was equalized by restricting the daily allowance, 14–17 g, to the consumption of the smallest eater. After ingesting the diets with vitamin E for 14 days, the nonfasting animals were bled to death from the aorta while under ether anesthesia.

Obese rats. Female Zucker 13 M normal and genetically obese rats, 29 days old, were obtained from the Harriet G. Bird Memorial Laboratory, Stow, MA. They were fed the vitamin E-deficient diet described above ad lib. for 14 days to partially deplete vitamin E reserves. The diet was then supplemented with dl-α-tocopheryl acetate, 40 mg/kg, and all animals were given the same amount of this diet daily, 11–14 g, determined by the consumption of the smallest eater. Obese rats received an additional 4–8 g of diet not containing α-tocopherol, depending on appetite. In this way the intake of α-tocopherol was similar for both obese and nonobese animals. After 42 days of feeding the diet with vitamin E (85 days of age), the rats were fasted overnight and killed as described above. Tissues were frozen at −20° until analyzed.

Plasma total lipids were determined by the procedure of Bragdon (6), and plasma total cholesterol as described by Pearson *et al.* (7). Tissue α-tocopherol was separated from the unsaponifiable fraction by thin-layer chromatography and determined as previously described (8).

Results. Body weights of rats fed either 0.5 or 1.0% of orotic acid were slightly lower than those for control rats (Table I). The two dietary levels of orotic acid produced similar plasma lipids and liver enlargement, and thus will be discussed as one group. As expected, orotic acid reduced plasma total lipids to about one-third normal, and increased liver size (2). The decrease in plasma α-tocopherol concentration from feeding orotic acid paralleled the reduction in plasma total lipid, and was reflected in a decrease in concentration of the vitamin in all tissues except liver. The latter accumulates fat due to inhibition of lipoprotein release by orotic acid (4), thus accounting for the increased α-tocopherol deposition. The size and lipid content of the other organs are not altered by orotic acid feeding.

Obese rats had elevated cholesterol and plasma total lipids (Table II), as previously described (5), and livers were grossly very fatty and considerably enlarged. Plasma α-tocopherol increased in the obese rats to the same degree as the plasma cholesterol and lipids, about three times normal. The elevated plasma α-tocopherol was not reflected in the tissue concentrations; heart and

TABLE I. EFFECT OF FEEDING OROTIC ACID ON TISSUE CONCENTRATIONS OF α-TOCOPHEROL.[a]

Body wt. (g)	Plasma total lipids (mg/ml)	Plasma α-tocopherol (μg/100 ml)	Liver wt. (g)	α-Tocopherol (μg/g)				
				Liver	Heart	Testes	Muscle	Fat
Control								
268	4.47	471	9.39	20.0	24.6	6.9	5.3	17.6
±7	±0.63	±95	±0.50	±0.67	±2.2	±0.7	±0.6	±0.9
0.5% Orotic acid								
248	1.38	177	14.07	19.0	16.2	3.6	2.2	10.4
±16	±0.3	±14	±1.18	±3.3	±2.0	±0.5	±1.0	±3.6
1.0% Orotic acid								
242	1.34	173	14.48	22.3	17.1	3.5	1.7	7.2
±3	±0.23	±20.8	±1.41	±3.2	±2.0	±0.4	±0.6	±1.4

[a] Values are means ± SEM for three rats.

TABLE II. PLASMA LIPIDS AND TISSUE α-TOCOPHEROL IN NORMAL AND GENETICALLY OBESE RATS.[a]

Body wt. (g)	Plasma total lipids (mg/ml)	Plasma cholesterol (mg/100 ml)	Plasma α-tocopherol (μg/100 ml)	α-Tocopherol (μg/g)				
				Liver	Heart	Muscle	Lung	Fat
Obese								
349	12.54	464	2112	29.6	49.7	16.0	44.3	23.7
±12	±0.8	±16	±236	±3.5	±2.9	±1.4	±2.1	±3.5
Normal								
212	3.46	133	710	47.2	63.9	15.9	55.4	38.0
±5	±0.2	±16	±148	±3.5	±1.5	±1.0	±3.6	±4.0

[a] Values are means ± SEM for five rats.

lung had 75–80% of the concentration in these organs from nonobese rats, while the muscle concentrations were similar. This lack of difference in muscle may have been due to excessive fat between the muscle fibers in obese rats, that could not be removed. (It should be noted that fat-free body mass for obese rats is similar or slightly less than that for nonobese rats of the same age (9).) The lower concentration of α-tocopherol in adipose tissue of obese rats was probably due to their much larger fat deposits, estimated to be about 112 g (32% of body weight) compared to 14 g (6.4%) for the nonobese (calculated from data for female rats supplied by Dr. Patricia R. Johnson, personal communication). Thus, the total storage of α-tocopherol in the adipose depots of obese rats would be approximately 3.41 mg and in the nonobese, 0.38 mg. Similar calculations for the liver yield 0.51 mg for the obese and 0.26 mg for the nonobese. Including the α-tocopherol in the blood, the summation of these three tissues gave 4.23 mg and 0.73 mg for obese and nonobese, respectively.

Discussion. In normal rats after the rapid growth phase, tissue and plasma concentrations of α-tocopherol are proportional to the log of the dietary intake of the vitamin (3). In the two models used in this study, interpretation of results may be complicated by changes in body composition but several conclusions may be warranted as to how plasma lipids affect tissue deposition of α-tocopherol. In rats fed orotic acid, the primary tissue alteration in addition to altered plasma lipoproteins is the excessive fat accumulation in liver. This resulted in an approximately 50% greater amount of total α-tocopherol in this organ compared to controls (295 μg vs 188 μg). Although this accumulation in the liver would have some effect on the α-tocopherol concentration in other tissues, the excess in liver is not great enough to account for the reduction in the rest of the body. It is thus very probable that the lower plasma concentration of α-tocopherol in orotic acid rats is the primary factor in determining deposition in organ and adipose tissues. It is of interest that the concentration of α-tocopherol in plasma lipid was lower in normal than in orotic acid rats (1054 μg/g vs 1283 μg/g, respectively). The lower α-tocopherol concentration found in organ tissues in orotic acid rats is in agreement with the results of Davies *et al.* (2), who used a

single oral dose of labeled α-tocopherol. These workers, however, found higher amounts of α-tocopherol in adipose tissue of orotic acid rats than in controls. Since Davies *et al.* found no effect of orotic acid on intestinal absorption of α-tocopherol, this model shows that tissue concentrations are regulated more by plasma α-tocopherol levels than by absolute intake of the vitamin, at least within a reasonable range of intake.

In the obese rat, the excessive adipose stores, even in the presence of a highly elevated plasma α-tocopherol concentration, appear to take up the vitamin preferentially at the expense of other tissues. At the same intake of the vitamin, the obese animal stored about six times more total tocopherol in the body with most of it in adipose tissue. In this model, the obese state is the primary determinant of tocopherol distribution and deposition, although the lower concentration of α-tocopherol in plasma lipid, 1684 μg/g for obese vs 2052 μg/g for controls, may have been another factor. These experiments indicate that the deposition of α-tocopherol in tissues is significantly affected by the level of plasma lipids and the degree of body adiposity, as well as by the known nutritional factors.

Summary. Experiments were designed to determine how varying levels of plasma lipids affect tissue deposition of α-tocopherol (vitamin E). Hypolipemia was induced by feeding orotic acid, and hyperlipemia was obtained using genetically obese rats. With equal dietary intakes of α-tocopherol, hypolipemic rats had lower plasma and tissue concentrations than rats with normal plasma lipids. An exception was liver, which due to fatty enlargement from orotic acid had more α-tocopherol. Hyperlipemic obese rats had plasma total lipids and α-tocopherol three times those of normal rats with the same intake of α-tocopherol. Tissue concentrations of the vitamin, however, were considerably lower in obese rats. Due to their large adipose mass, obese rats had considerably more total body α-tocopherol than normal rats. It was concluded that both plasma lipid levels and degree of adiposity are important factors in determining tissue deposition of α-tocopherol.

1. Rubinstein, H. M., Dietz, A. A., and Srinavasan, R., Clin. Chim. Acta **23,** 1, 1969.
2. Davies, T., Kelleher, J., Smith, C. L., and Losowsky, M. S., Int. J. Vit. Nutr. Res. **41,** 360, 1971.
3. Bieri, J. G., Ann. N. Y. Acad. Sci. **203,** 181, 1972.
4. Windmueller, H. G., and Levy, R. I., J. Biol. Chem. **242,** 2246, 1967.
5. Zucker, T. H., and Zucker, L. M., Proc. Soc. Exp. Biol. Med. **110,** 165, 1962.
6. Bragdon, J. H., J. Biol. Chem. **190,** 513, 1951.
7. Pearson, S., Stern, S., and McGavack, T. H., Anal. Chem. **25,** 813, 1953.
8. Bieri, J. G., *in* "Lipid Chromatographic Analyses" (G. V. Marinetti, ed.), Vol. 2, p. 459. New York (1969).
9. Johnson, P. R., Zucker, L. M., Cruce, J. A. F., and Hirsch, J., J. Lipid Res. **12,** 706, 1971.

Received Feb. 3, 1975. P.S.E.B.M., 1975, Vol. 149.