Adipose Tissues and Vitamin E

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Summary 1. Vitamin E content in the adipose tissue was examined in rats with and without vitamin E deficiency. With the progression of vitamin E depletion, the more rapid decrease in tocopherol concentration was observed in brown adipose tissue (BAT) than in white adipose tissue (WAT), and the rate of decrease of tocopherol was approximately three times faster in BAT than in WAT. After the intramuscular administration of 10mg/kg of all-rac-tocopheryl acetate twice a week for two weeks to vitamin E-deficient rats, a similar pattern of increase was observed in the tocopherol concentrations of BAT and WAT, although the rate of increase was slower in WAT than in BAT. 2. Changes of tocopherol concentration in BAT and WAT were investigated in normo-nourished rats with hyperlipemia produced by the intramuscular injection of Triton WR-1339 for 7 days. A marked increase in tocopherol concentration was observed in both BAT and WAT in the late period of hyperlipemia, with the increase being greater in WAT. 3. The fatty acid composition of adipose tissue was compared between rats with and without vitamin E deficiency. No significant differences were observed in BAT and WAT between the two groups. 4. The glucose uptake of WAT was not altered in vitamin E-deficient rats when compared with control rats.

Key Words vitamin E, adipose tissue, white adipose tissue, brown adipose tissue, fatty acids, glucose-uptake function

The body lipid mass including both adipose tissue and plasma lipids has been proposed to affect the vitamin E status in available sites, specifically in biomembranes (1-4). Bieri et al. (1) examined hyperlipemic obese rats who had three-fold higher plasma lipid and tocopherol levels than normo-nourished rats, and reported that their tissue tocopherol concentrations were far lower than those in normal rats. Machlin et al. (2) have proposed that adipose tissue tocopherol represents a tissue
depot with an extremely low turnover and may not be available to supply the vitamin to rats with myopathy due to vitamin E deficiency. Mino et al. (3) have reported that cellular organelles, such as mitochondria and microsomes, obtained from hepatocytes in hyperlipemic rats had lower tocopherol concentrations than those from control rat livers. Mino et al. (5) also examined erythrocyte tocopherol levels in obese children, which reflect in part the tissue tocopherol levels (5), and reported that children with higher grade obesity had lower erythrocyte tocopherol levels (4). These findings indicate that the various lipid pools in the body act as a relatively nonexchangeable depot for tocopherol, and can drastically affect the uptake of the vitamin by other tissues. In mammals, there are two types of adipose tissues, white adipose tissue (WAT) and brown adipose tissue (BAT). These differ from each other both morphologically and functionally. To our knowledge, there have been few reports on BAT with regard to vitamin E nutrition.

In this study, we examined BAT and WAT with respect to changes in the content of vitamin E. Membrane function was also investigated in WAT with respect to glucose uptake.

EXPERIMENTALS

Animals. Wistar rats weighing 100 g were used after weaning. After being fed a standard diet prepared by the addition of 2 mg of all-rac-tocopheryl acetate to 100 g of a vitamin E-deficient diet, the study was commenced.

1) Vitamin E-deficient rats. Vitamin E-deficient rats were produced as reported previously (6). The vitamin E-deficient diet consisted of 36% corn starch, 25% vitamin-free casein, 10% alpha wheat starch, 8% powdered filter paper, 6% salt mixture, 5% granulated sugar, 2% vitamin mixture, and 8% stripped corn oil (from Tama Seikagaku Co., Ltd., Tokyo). The salt mixture consisted of the following salts (per 100 g of diet): K, 692 mg; P, 579 mg; Ca, 411 mg; Na, 270 mg; Mg, 86 mg; Fe, 41 mg; Zn, 0.4 mg; Mn, 1.3 mg; and I, 7.7 mg. The vitamin mixture consisted of the following vitamins (per 100 g of diet): vitamin A, 1,000 IU; D2, 200 IU; B1, 2.4 mg; B2, 8 mg; B6, 1.6 mg; B12, 0.001 mg; C, 60 mg; K, 10.4 mg; biotin, 0.04 mg; folic acid, 0.4 mg; Ca-panthenate, 10 mg; para-aminobenzoic acid, 10 mg; niacin, 12 mg; inositol, 12 mg; and choline-Cl, 4,000 mg. Vitamin E-deficient rats were produced after being fed the above basal vitamin E-deficient diet for 12 weeks.

2) Administration of tocopherol to the vitamin E-deficient rats. All-rac-alpha-tocopherol was emulsified with 1% HCO 60, which was a detergent (polyoxyethylene hydrogenated caster oil derivatives 60 mol ester, Nikko Chemicals, Tokyo), and given intramuscularly at a dose of 10 mg/kg.

3) Production of hyperlipemic rats. Hyperlipemic rats were produced as described in the previous report (3). They were given once-daily intramuscular injections of 300 mg/kg of Triton WR-1339 dissolved in 1 ml of normal saline for 7 consecutive days. Control rats were given the same volume of normal saline. There

were no significant changes in body weight and plasma lipid levels in the control.

Collection of samples. Heparinized blood samples were collected by heart puncture after a 24-h fast and immediately centrifuged at 3,000 rpm for 10 min to separate erythrocytes (RBCs) from plasma. WAT and BAT were obtained from the periepididymal fat pad and the interscapular brown fat pad, respectively, after rats were killed by ethyl ether anesthesia.

Assay methods.

1) Tocopherol assay. We used a slightly modified high-performance liquid chromatography (HPLC) method of Ishibashi to determine plasma, RBC, and adipose tissue tocopherol concentrations, as described in our previous report (7).

2) Separation of phospholipids in BAT. Total lipid extraction and purification were performed by a slight modification of Bligh and Dyer's method (8). Lipids were extracted from BAT with chloroform–methanol (1:2, v/v) and chromatographed on a Silica Gel H plate developed with a solvent system of chloroform/methanol/H2O/ammonia (85/50/4/0.2). The areas corresponding to phospholipids were scraped off and eluted with chloroform.

3) Fatty acid analysis. Fatty acid methyl esters were prepared by a simple method described by Shimazaki and Privett (9), eliminating the extraction step using 2,2'-dimethoxypropane. The fatty acid composition of the acetone solution of the extract was analyzed by gas-liquid chromatography (Shimadzu GC-7A, Shimadzu Co., Ltd., Kyoto) performed under the following conditions: detector, FID; column, glass column (3.1 m × 3 mm i.d.) packed with 5% Shinchrom E-71 (Shimalite); oven temperature, 230°C; carrier gas, N2; flow rate, 30 ml/min.

4) Glucose-transport function of adipose cells. This was determined only in WAT by the method described in our previous paper (11). Adipose cells were separated using collagenase by the method of Roddbell (10) as follows: Incubation for cell separation was carried out for 75 min in a solution with a final concentration of 0.1% collagenase (products), 35% albumin, and 1.1 mM glucose, which was added to 4 g of wet WAT. The isolated adipose cells were filtered through mesh gauze, washed three times with 1% albumin in a Ringer-phosphate-KRPH buffer containing 131.2 mM NaCl, 4.41 mM KCl, 2.47 mM CaCl2·2H2O, 1.24 mM MgSO4·7H2O, 2.48 mM NaH2PO4, and 10.0 mM HEPES. They were then prepared in a concentration of 20–30% lipocrit. Subsequently, a 50 μl aliquot of the cell suspension was diluted with 270 μl of the KRPH-1% albumin buffer (mentioned above) with appropriate amounts of insulin (in a final concentration of 25, 50, 100, 200, 800, or 8,000 pM), and then incubated at 37°C for 30 min. After the incubation, a tracer amount (0.3 μM) of [U-14C]-D-glucose (NEN, 341.2 mCi/mmol) was added to the suspensions, and incubation was performed for an additional 30 min. The radioactivity in the cell pellets was measured with a scintillation counter (Packard Model 320C). The glucose clearance was determined by measurement of cpm of radioactivity in the cell pellets, and expressed as glucose transportation (pl/cell/s). The extracellular radioactivity was corrected for by subtracting the amount of radioactivity in the cells at zero time.

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RESULTS

1. Changes of tocopherol concentration in adipose tissues in relation to vitamin E status

1) Vitamin E deficiency. Rats developed almost complete depletion of vitamin E stores after 12 weeks on the basal vitamin E-deficient diet, as shown by their low plasma and RBC tocopherol levels (Fig. 1), and by the increased RBC hemolysis induced by dialuric acid (data not shown). The tocopherol concentration decreased in BAT in parallel with the plasma and RBC concentrations, while the decrease in the WAT tocopherol concentration was only minor. The rate of decrease was thus greater in BAT (1.29 µg/g tissue/day) than in WAT (0.48 µg/g tissue/day), as shown in Fig. 1. After 12 weeks of vitamin E depletion, the WAT tocopherol concentration remained at a level of 10.4±3.6 µg/g tissue, while the BAT concentration was 2.4±0.7 µg/g tissue. The pattern of decline of tocopherol concentration in the plasma and RBCs was the same as described in a previous paper (6).

2) Administration of tocopherol to vitamin E-deficient rats. All-rac-
Fig. 2. Changes in tocopherol concentrations in adipose tissue, plasma, and RBCs after administration of tocopherol. Vitamin E-deficient rats were given 10mg of alpha-tocopherol/kg body weight by intramuscular injection twice a week for two weeks. The points on each curve show the means±SD (n=5).

tocopheryl acetate (10 mg/kg) was given intramuscularly to vitamin E-deficient rats twice a week for 2 weeks. As shown in Fig. 2, the tocopherol concentrations in BAT and WAT increased similarly during the two weeks of tocopherol administration. However, the rate of increase was greater in BAT than in WAT. For plasma and RBC tocopherol levels, the rate of increase was linear in plasma throughout the two weeks, while that in RBCs was slower during the second week.

2. Influence of hyperlipemia on adipose tissue tocopherol concentrations

In the rats treated with Triton WR-1339, no changes occurred in body weight. However, the plasma lipid level on the 3rd and the 7th days of treatment were increased 1.7-fold and 3.1-fold for cholesterol, 30.2-fold and 96.6-fold for triglycerides, and 3.9-fold and 7.1-fold for phospholipids, respectively. Elevation of plasma tocopherol levels and a reduction of RBC tocopherol levels were induced by hyperlipemia (Fig. 3), as described previously (3). No significant changes in
Fig. 3. Changes in plasma total lipids, plasma tocopherol, and RBC tocopherol in rats after intramuscular administration of Triton WR-1339 for 7 days. Each bar represents the mean ± SD (n = 5).

tocopherol concentrations occurred in the first 3 days in both BAT and WAT. However, both BAT and WAT showed marked increases on the 7th day, indicating that tocopherol was transferred from lipoprotein molecules into the adipose tissue together with cholesterol, triglycerides, and phospholipids. The increase was greater in WAT (2.4 times the level before Triton WR-1339) than in BAT (1.4

times), as shown in Fig. 4.

3. Fatty acid compositions in BAT and WAT in rats with and without vitamin E deficiency

There were no differences in fatty acid composition in both BAT and WAT between rats with and without vitamin E deficiency (Table 1). BAT is much richer in phospholipids (as biomembrane constituents) than is WAT, because BAT contains larger amounts of mitochondria and other cellular organelles than WAT does. Therefore, fatty acid compositions in phospholipids were compared for BAT alone between vitamin E-deficient and non-deficient rats. No differences were shown between the phospholipid components in BAT from rats with and without vitamin E deficiency (Table 2).

4. Glucose uptake by WAT in rats with and without vitamin E deficiency

In the vitamin E-deficient rats that were used, the tocopherol concentrations in

Table 1. Fatty acid compositions of the total fat from BAT and WAT in rats with and without vitamin E deficiency (M ± SD (n = 5)).

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>BAT</th>
<th>WAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>4.1±0.3</td>
<td>3.9±0.4</td>
</tr>
<tr>
<td>16:0</td>
<td>32.3±1.2</td>
<td>31.4±1.2</td>
</tr>
<tr>
<td>16:1</td>
<td>7.7±0.9</td>
<td>7.4±0.7</td>
</tr>
<tr>
<td>18:0</td>
<td>4.8±0.5</td>
<td>4.3±0.7</td>
</tr>
<tr>
<td>18:1</td>
<td>35.6±1.5</td>
<td>35.6±1.9</td>
</tr>
<tr>
<td>18:2</td>
<td>13.9±1.0</td>
<td>15.3±2.2</td>
</tr>
<tr>
<td>18:3</td>
<td>0.2±0.1</td>
<td>0.2±0.1</td>
</tr>
<tr>
<td>20:4</td>
<td>1.4±0.6</td>
<td>1.8±0.5</td>
</tr>
</tbody>
</table>

Table 2. Fatty acid composition of BAT phospholipids from rats with and without vitamin E deficiency (M ± SD (n = 5)).

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Deficient (%)</th>
<th>Non-deficient (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>1.4±0.1</td>
<td>1.7±0.3</td>
</tr>
<tr>
<td>16:0</td>
<td>12.1±0.9</td>
<td>13.1±1.3</td>
</tr>
<tr>
<td>16:1</td>
<td>2.8±0.3</td>
<td>3.4±0.7</td>
</tr>
<tr>
<td>18:0</td>
<td>14.9±0.1</td>
<td>15.1±0.4</td>
</tr>
<tr>
<td>18:1</td>
<td>23.0±0.5</td>
<td>22.8±0.3</td>
</tr>
<tr>
<td>18:2</td>
<td>23.6±1.0</td>
<td>21.8±0.3</td>
</tr>
<tr>
<td>18:3</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>20:4</td>
<td>19.4±0.4</td>
<td>18.8±0.3</td>
</tr>
<tr>
<td>22:6</td>
<td>1.7±0.3</td>
<td>1.3±0.7</td>
</tr>
</tbody>
</table>

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Table 3. Vitamin E status in rats whose WAT cells were examined for glucose-uptake function (M±SD (n=4)).

<table>
<thead>
<tr>
<th>Tocopherol concentration</th>
<th>Deficient</th>
<th>Non-deficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma (µg/100 ml)</td>
<td>80.8±8.3</td>
<td>722.0±40.3</td>
</tr>
<tr>
<td>RBCs (µg/100 ml packed cells)</td>
<td>42.0±1.9</td>
<td>416.2±40.0</td>
</tr>
<tr>
<td>WAT (µg/g wet wt.)</td>
<td>3.8±0.2</td>
<td>26.5±0.4</td>
</tr>
</tbody>
</table>

Fig. 5. Trace glucose uptake corresponded to insulin levels in isolated rat WAT cells from rats with and without vitamin E deficiency. Each point represents the mean of triplicated determinations.

plasma, RBCs, and WAT were almost one tenth of those in control rats, as shown in Table 3. Figure 5 shows glucose uptake corresponding to insulin levels in vitamin E-deficient and non-deficient rat WAT adipocytes. While the glucose uptake increased dose-dependently with increasing levels of insulin, there were no differences in glucose uptake with and without insulin between vitamin E-deficient and non-deficient rat adipocytes.

DISCUSSION

Machlin et al. (2) have proposed that the tocopherol in adipose tissues cannot be mobilized and cannot decrease in guinea pigs with myopathy due to vitamin E deficiency to supply the affected muscles with tocopherol. In our study (Fig. 1), the rate of decrease in tocopherol levels was very much slower in WAT during tocopherol depletion compared with the rate in BAT. Since the total amount of WAT in the body may increase during periods of ongoing tocopherol depletion in parallel with the body weight gain, the depletion of tocopherol in the whole adipose

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Tissue may be less than that expressed as the concentration per wet tissue weight. Therefore, our findings regarding WAT tocopherol may be consistent with Machlin's view. In BAT, on the other hand, the rate of depletion of tocopherol may be greater than that expressed by the wet tissue concentration, because the mass of BAT is known to decrease during development (12). The additional finding that the BAT tocopherol concentration was lower than that in WAT at 12 weeks of depletion indicates that more rapid turnover occurred in BAT. The depleting pattern of tocopherol in BAT seemed to coincide with that in RBCs. Probably, tocopherol in BAT may decrease in a similar pattern to that in general tissue during vitamin E depletion, because RBC tocopherol reflects tissue tocopherol status, as previously reported (13). Tocopherol in BAT may play a role as an antioxidant providing protection against peroxidative reactions which possibly occur during oxidative metabolism for heat production within BAT, while tocopherol deposited in WAT does not seem to act biologically as previously proposed by Machlin et al. (2). On the other hand, after tocopherol administration, the increase in tocopherol concentration was more marked in BAT than in WAT during two weeks of tocopherol administration (Fig. 2). This may be due to the fact that WAT has a much larger pool size for tocopherol deposition compared with BAT, and the tocopherol administered was thus distributed in the larger WAT mass. The rate of increase of the RBC tocopherol concentration slowed during the second week of tocopherol administration when compared with the increase of plasma tocopherol levels. This may have been due to a time lag in tocopherol transportation from plasma lipoproteins to RBC membranes, as described in a previous report (13).

In our previous report (3) regarding hyperlipemia in rats (produced by the Triton WR-1339), it was shown that the plasma tocopherol levels rose markedly with the development of hyperlipemia, whereas the tocopherol content in RBCs and the hepatocyte organelles (mitochondria and microsome) decreased. The same result was obtained in this study with respect to plasma and RBC tocopherol concentrations during hyperlipemia (Fig. 3). The adipose tissue tocopherol concentration increased especially in the late phase of hyperlipemia, but the increase was not marked at 3 days of treatment when hyperlipemia was milder. The finding of an increase in adipose tissue tocopherol levels despite a decrease in RBC tocopherol levels indicates that the increased plasma lipids (including tocopherol) were taken up by both BAT and WAT regardless of the lowered tissue tocopherol concentrations. The uptake seemed to be greater by WAT than by BAT (Fig. 4), because of the greater pool size of WAT.

It is well known that vitamin E deficiency readily induces lipid peroxidation, and there have been many reports (14–16) that the amount of thiobarbituric reactive substances in tissues increases with ongoing vitamin E deficiency. Although WAT acts as a store of triglycerides which can be mobilized and exported for oxidation elsewhere in the body, BAT oxidizes its stored fat itself to produce heat, especially in a cold environment. Enhanced BAT metabolism for heat production is thought to induce increased lipid peroxidation, especially in vitamin...
E deficiency. Since the unsaturated fatty acids in vitamin E-deficient BAT may have been more sensitive to lipid peroxidation, changes in fatty acid composition were examined in vitamin E-deficient BAT and WAT. Unexpectedly, no changes in fatty acid composition, especially in unsaturated fatty acids in BAT phospholipids, were observed in vitamin E-deficient rats. Changes may still appear following enhanced heat production by BAT, a point which deserves to be examined.

The hypothetical reaction of peroxidation in biomembranes is proposed to first occur between lipids (17). Lipid-lipid interactions are likely to dominate due to restriction of chain reaction to the plane of the membrane and due to the larger molar fraction of lipid than protein in biomembranes. If such reaction subsequently affected the proteins in the vitamin E-deficient state, drastic changes in membrane proteins would have a profound effect on the properties of the membrane. Accordingly, membrane function in WAT cells was examined by observing glucose uptake, thus testing the integrity of the insulin-mediated membrane receptors and the postreceptor mechanism for glucose transportation into the cells. BAT was omitted because it comprises only a small part of the adipose tissue and a large amount of tissue was required for performing the test. No significant alteration in the glucose uptake of vitamin E-deficient WAT was observed, a result that could be expected because there were no changes in PUFA in vitamin E-deficient WAT and BAT.

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