EFFECTS OF DIETARY VITAMIN E ON MICROSOMAL LIPID PEROXIDATION AND GLUTATHIONE PEROXIDASE IN RAT LIVER

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(Received August 11, 1977)

TAPPEL and ZALKIN (1) reported that lipid peroxidation in the tissues of mammals fed a vitamin E-deficient diet shows an increase over that of the control diet. On the other hand, EL-KHATIB et al. (2) did not detect the presence of lipid peroxides in subcellular fractions under vitamin E-deficient conditions. This discrepancy may be partially explained by the difference of the levels of various microsomal enzymes which are modified with a vitamin E-deficient diet. As for influencing factors on lipid peroxidation, it is known that superoxide dismutase inhibits the NADPH-cyt. c reductase catalyzing lipid peroxidation (3) and that catalase and superoxide dismutase inhibit the reaction catalyzed by a xanthine oxidase system (4). However, the in vitro addition of these factors caused no inhibition on NADPH-dependent microsomal lipid peroxidation. GSH was reported to inhibit NADPH-dependent microsomal lipid peroxidation (5). In this paper, the effects of dietary vitamin E on NADPH-dependent microsomal lipid peroxidation and on the enzymes, such as NADPH-cyt. c reductase, catalase, superoxide dismutase and GSH peroxidase, in rat liver have been investigated. In agreement with the report of CHOW et al. (6) who worked with rat adipose tissue, we found that GSH peroxidase activity in liver microsomes of rats fed a vitamin E-deficient diet is enhanced in comparison to that of vitamin E supplemental diet.

Male Wistar strain rats weighing 170 to 200 g were divided into two groups and fed ad libitum for 10 weeks either a vitamin E supplemental diet or a vitamin E-deficient diet purchased from Oriental Co. in Japan (7). The rats were killed by decapitation and livers were homogenized in 3 volumes of 0.15 M KCl. The homogenate was centrifuged at 10,000 × g for 10 min and then the supernatant was ultracentrifuged at 105,000 × g for 60 min. The microsomal pellet was suspended in 0.15 M KCl at a protein concentration of 10 mg per ml. Microsomal

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Abbreviations: GSH, reduced glutathione; GSSG, oxidized glutathione; cyt. c, cytochrome c; MDA, malondialdehyde.
suspension was stored at $-40^\circ C$ until used for enzyme assays. The rate of lipid peroxidation was assayed by both MDA formation (8) and conjugated diene formation (9) after incubating the microsomes in a medium containing 20 mM sodium phosphate buffer, pH 7.4, 0.3 mM NADPH, 0.4 mM ADP and 12 $\mu$M FeCl$_3$. Incubations were carried out at 37°C for 10 min with 1.0 mg of microsomal protein for MDA formation and for 15 min with 2.0 mg of microsomal protein for conjugated diene formation. The activities of NADPH-cyt. c reductase (10), catalase (11), superoxide dismutase (12) and GSH peroxidase (13) were assayed as previously described.

Table 1. Effect of dietary vitamin E on lipid peroxide formation and activities of NADPH-cyt. c reductase, catalase, superoxide dismutase and GSH peroxidase.

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<tr>
<th>Enzyme activities</th>
<th>Vitamin E</th>
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<td>Lipid peroxide formation</td>
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<td>malondialdehyde (nmole MDA/min/mg prot.)</td>
<td>Mc$^b$ 0.87±0.15$^d$</td>
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<tr>
<td>conjugated diene ($\Delta OD_{234nm}$/min/mg prot. × 10$^3$)</td>
<td>Mc 5.5±1.6</td>
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<tr>
<td>NADPH-cyt. c reductase (nmole/min/mg prot.)</td>
<td>Mc 53.3±21.6</td>
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<td>Catalase (units/mg prot.)</td>
<td>S$^105$ 203.6±129.4</td>
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<tr>
<td>Superoxide dismutase (units/mg prot.)</td>
<td>S$^105$ 828.5±140.4</td>
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<tr>
<td>GSH peroxidase (nmole NADPH oxidized/min/mg prot.)</td>
<td>Mc 16.2±4.7</td>
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<td>S$^105$ 48.3±5.6</td>
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$^a$ Three animals were used. $^b$ Microsomes. $^c$ 105,000 g supernatant. $^d$ Mean±S.D. $^e$ N.S. indicates $p>0.05$.

NADPH-dependent microsomal lipid peroxidation in a vitamin E-deficient diet and in a vitamin E supplemental diet measured by either MDA or conjugated diene formation showed no difference (Table 1). There was also no change in the activities of NADPH-cyt. c reductase, catalase and superoxide dismutase between the two groups. However, only the activity of GSH peroxidase significantly increased in vitamin E-deficient liver microsomes. GSH peroxidase catalyzes the following reaction: $2$GSH$+H_2O_2 \rightarrow GSSG+2H_2O$. Since McCAY et al. (14) reported that GSH peroxidase prevents a free radical attack on the polyunsaturated membrane lipid, we tested the in vitro effects of GSH and GSSG on the NADPH-dependent lipid peroxidation. As shown in Fig. 1, the rate of MDA formation in microsomes of control diet was inhibited by the addition of
GSH. GSSG also inhibited MDA formation in proportion to its concentration. The inhibitory effect of GSSG was further intensified by the addition of GSH reductase (Fig. 1). Therefore, the low level of NADPH dependent lipid peroxidation observed in vitamin E-deficient rat microsomes (Table 1) is attributed to the redox states of the glutathione generating system caused by high GSH peroxidase activity.

REFERENCES