# FATTY ACIDS IN RAT TESTES AS AFFECTED BY VITAMIN E

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In the course of studies of a possible relationship between tissue polyunsaturated fatty acids and vitamin E, analyses of testes from rats deficient in the vitamin revealed marked changes in several acids derived from linoleic acid.

Methods. Weanling rats were fed a purified diet containing isolated soy protein, adequate minerals (including selenium) and vitamins (except E), sucrose and 4% stripped lard. Control rats received 200 mg dl,$\alpha$-tocopheryl acetate/kg diet. Growth of control and deficient animals was equally good with the only gross evidence of vitamin E deficiency in the latter being a decrease in testicular weight after 16-20 weeks. Tissue extracts in chloroform-methanol (2:1) were prepared according to Folch et al. (1957). Aliquots of the extracts were evaporated under nitrogen and the residue dissolved in methanol-benzene (2:1). Transesterification was effected by refluxing for 1 hour with $BF_3$ as catalyst. After extracting into hexane, the methyl esters were analyzed by gas liquid chromatography (GLC) on a 15% ethylene-glycol succinate column in a Barber Colman Model 15 instrument with conditions as described previously (Bieri and Andrews, 1963). Identification of peaks was made by comparing carbon numbers (Woodford and van Gent, 1960) with known standards, or in cases where standards were not available, by comparing with carbon numbers found by Dr. R. L. Holman of the Hormel Institute (personnel communication). Peaks of less than 1% of the total area are not reported.

Separation and determination of individual phosphatides were accomplished by the paper chromatographic system of Marinetti et al. (1957) with minor modifications. The spots corresponding to lecithin, cephalin and sphingomyelin were eluted from the paper and methyl esters of the fatty acids prepared and analyzed by GLC as described above.

Results. Testes of rats receiving adequate dietary linoleic acid are characterized by a relatively high content of docosapentaenoic acid (22:5ω6) (Aaes-Jørgensen, 1958). The most striking change in vitamin E-deficient testes was a decrease in the 22:5ω6 to about one seventh that of the control rats (Table I). This difference was reflected in an increase of palmitoleic and oleic acids. Linoleic (18:2ω6) and arachidonic (20:4ω6) acids increased slightly.

TABLE I. Fatty Acids in Testes of Normal and Vitamin E-Deficient Rats*

| Fatty Acid | 16:0 + 18:0 | 16:1 + 18:1 | 18:2ω6** | 20:3ω6 | 20:4ω6 | 22:4ω6† | 22:5ω6 |
|---|---|---|---|---|---|---|---|
| +Vit. E | 36.2±1.1 | 29.2±1.3 | 3.3±0.2 | 0.6±0.1 | 11.1±0.7 | 1.2±0.1 | 15.0±0.8 |
| -Vit. E | 30.6±1.2 | 43.3±2.1 | 4.6±0.2 | 0.7±0.1 | 13.9±1.7 | 2.2±0.3 | 1.9±0.2 |

*Values are means of area per cents with standard errors. Six rats per group; 28-week period.

**The number following ω is the number of terminal carbon atoms after the last double bond.

†This peak also contains 24:1.

Analyses of the lipid extracts revealed decreases in the total lipids of deficient testes which were accounted for by a loss of almost one half the phospholipids. Chromatographic separation of the major phosphatides (Table II) showed that there was a slight decrease in cephalins and an equivalent increase in sphingomyelins. Otherwise, the loss of lipid phosphorus by deficient testes was uniformly distributed among the classes of phosphatides.

The fatty acid composition of the lecithins, cephalins and sphingomyelins after elution from the filter paper is shown in Table III. Major differences between normal and vitamin E-deficient tissues were found

TABLE II. Phosphatide Composition of Normal and Vitamin E Deficient Rats' Testes

| Phosphatide | Per cent of total lipid phosphorus* | |
|---|---|---|
| | + Vitamin E | - Vitamin E |
| Inositol | 0.8 ± 0.1 | 0.7 ± 0.1 |
| Lysolecithin | 3.3 ± 0.3 | 3.8 ± 0.2 |
| Sphingomyelin | 17.1 ± 0.5 | 23.7 ± 1.1 |
| Lecithin | 47.7 ± 0.9 | 46.3 ± 1.0 |
| Serine | 3.0 ± 0.4 | 3.1 ± 0.6 |
| Cephalin | 25.2 ± 0.6 | 20.5 ± 1.0 |
| Unidentified | 2.9 ± 0.6 | 1.9 ± 0.2 |

*Six rats per group; 28-week period. Values are means with standard errors.

TABLE III. Fatty Acids in Phosphatides of Normal and Vitamin E Deficient Rats' Testes

| Fatty Acid* | Lecithin | | Cephalin | | Sphingomyelin | |
|---|---|---|---|---|---|---|
| | + Vit. E | - Vit. E | + Vit. E | - Vit. E | + Vit. E | - Vit. E |
| 16:0+ 18:0 | 33.7±3.6 | 41.3±2.2 | 38.5±5.8 | 33.4±4.4 | 37.2±5.3 | 34.0±5.2 |
| 18:1 | 21.8±3.1 | 12.9±0.6 | 10.5±3.4 | 9.4±0.6 | 7.8±1.2 | 7.1±1.3 |
| 18:2 | 2.1±0.2 | 3.2±0.3 | 0.8±0.4 | 2.2±0.3 | T | 2.6±0.4 |
| 20:3 | 2.5±0.6 | 2.7±0.1 | 4.0±0.9 | 3.8±0.4 | 8.0±4.1 | 7.4±1.0 |
| 20:4 | 12.7±1.7 | 27.6±1.6 | 13.5±1.8 | 26.8±1.7 | 12.6±4.8 | 22.3±3.5 |
| 22:2** | 2.3±0.9 | 1.1±0.2 | 3.9±0.9 | 1.6±0.6 | T | 7.7±1.9 |
| 22:4 | 1.3±0.4 | 5.4±0.4 | 1.8±0.7 | 8.9±1.1 | 11.2±4.1 | 12.8±1.7 |
| 22:5 | 21.4±3.3 | 3.7±0.4 | 25.0±5.0 | 8.4±1.3 | 20.6±3.3 | 2.2±0.9 |

*See Table I for explanation and characterization of fatty acids. T = trace.
**Tentative identification.

primarily in 20:4ω6, 22:4ω6, and 22:5ω6. Arachidonic acid (20:4ω6) was doubled in all three phosphatides from deficient testes, while 22:4ω6 increased fourfold in the lecithins and cephalins. The content of 22:5ω6, which was over 20% in the phosphatides from normal testes, fell to 2-8% in the vitamin E-deficient samples. There was a marked increase in a peak,

tentatively identified as 22:2, in the sphingomyelins. Slight increases were noted in the amounts of linoleic acid but there was no change in 20:3ω6 in the three phosphatides.

Since Bernhard et al. (1963) reported significant increases in arachidonic acid of liver from vitamin E-deficient rats, we analyzed several other organs from rats in a second depletion experiment (Table IV). In the deficient testes, the large decrease in 22:5ω6 and slight increase in 20:4ω6 confirm the results of the first experiment. There were no differences in the polyunsaturated fatty acids of liver or kidneys, but a slightly lower content of arachidonic acid was present in the hearts of vitamin E-deficient rats. Differences in nutritional design, plus the fact Bernhard et al. used female rats, may be responsible for this apparent discrepancy. In an earlier study, we found decreased levels of arachidonic acid in plasma and liver from vitamin E depleted chicks (Bieri and Andrews, 1963).

TABLE IV. Polyunsaturated Fatty Acids of Testes, Liver, Heart and Kidney Total Lipids from Normal and Vitamin E-Deficient Rats*

| | 18:2ω6 | 20:3ω6 | 20:4ω6 | 22:4ω6 | 22:5ω6 | 22:6ω3 |
|---|---|---|---|---|---|---|
| Testes | | | | | | |
| +Vit. E | 3.6±0.3 | 0.8±0.0 | 12.6±0.7 | 1.3±0.0 | 16.4±0.9 | T |
| -Vit. E | 4.5±0.1 | 0.8±0.4 | 13.8±0.7 | 2.2±0.1 | 2.5±0.3 | T |
| Liver | | | | | | |
| +Vit. E | 7.5±0.9 | 1.2±0.1 | 16.9±0.5 | T | 0.7±0.1 | 2.5±0.1 |
| -Vit. E | 7.0±0.4 | 1.3±0.1 | 16.7±0.9 | T | 0.9±0.1 | 2.2±0.2 |
| Heart | | | | | | |
| +Vit. E | 17.2±0.5 | 0.6±0.1 | 22.7±0.8 | 0.5±0.1 | 1.3±0.1 | 3.2±0.2 |
| -Vit. E | 17.6±0.7 | 0.6±0.0 | 20.7±0.5 | 0.5±0.0 | 1.3±0.1 | 3.0±0.3 |
| Kidney | | | | | | |
| +Vit. E | 7.5±0.1 | 0.8±0.1 | 25.4±0.2 | T | T | T |
| -Vit. E | 7.4±0.4 | 1.0±0.1 | 26.5±0.5 | T | T | T |

*Five rats per group; 32-week period. See footnotes of Table I for other explanations.

In summary, the vitamin E-deficient rat testis has increased proportions of 18:2ω6, 20:4ω6 and 22:4ω6 and a marked decrease in 22:5ω6 but no change in the proportion of 20:3ω6. Considering available information on

the transformation of 18:2ω6 to 22:5ω6, the data suggest that vitamin E deficiency inhibits the conversion of 20:4 to 22:5. An increased synthesis of 20:4 in vitamin E-deficient rat liver was postulated recently by Bernhard, Lindlar, Schwed, Vuilleumier and Wagner (1963).

## References

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