The effects of vitamin E deficiency on the total fatty acids and the phospholipid fatty acids of rat tissues

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1. A vitamin E-low diet containing 7% stripped lard was given to hooded rats for periods up to 14 months. Control rats were given the same diet with a vitamin E supplement (1 i.u./rat per day).

2. No consistent pattern of changes was found in the total fatty acids of testis, lung, spleen, pancreas, heart, kidney, liver, brain, skeletal muscle and small intestine from rats given the deficient diet for 5, 6 or 7 months when compared with control rats.

3. The fatty acids of the total phospholipid from the same tissues were examined after 4, 5, 8 or 14 months. In the rats deficient in vitamin E the polyunsaturated fatty acids of the linoleic series (ω6) decreased, except for 20:4ω6, which in some tissues tended to increase. After 14 months there were considerable decreases in the percentages of all the ω6 series including 20:4ω6 with increases in the percentages of 18:1ω9 and 20:3ω9; the pattern was similar to that found in essential fatty acid deficiency.

The tocopherols and tocotrienols are known to be effective in vitro antioxidants (Lea, 1960), and it has been assumed that their biological role is connected with this property, in part at least (see Scott, 1962). Various cellular constituents are liable to be destroyed by peroxidation, including the polyunsaturated fatty acids (PUFA). Bieri & Andrews (1964) and Bieri & Prival (1966) found significant changes in the PUFA in the testes of rats given diets deficient in vitamin E. The present work is a more complete survey of the tissues of rats given diets deficient in vitamin E to discover if similar changes occur generally as a sign of vitamin E deficiency.

EXPERIMENTAL

Weanling male Norwegian hooded rats (Animal Suppliers London, Ltd) were given a diet low in vitamin E containing 20% casein (low vitamin content; Fisons Pharmaceuticals, Loughborough), 7% stripped lard (Distillation Products Industries, Rochester, NY) and sucrose, supplemented with vitamins B, vitamin K and minerals. Vitamin A acetate (5000 i.u./100 g diet) and vitamin D (150 i.u./100 g diet) in ethanolic solution were mixed into the diet twice weekly. Control rats were given the same diet and in addition each animal was given 3·5 mg DL-α-tocopheryl acetate in 0·1 ml arachis oil orally twice weekly.

For the estimation of tissue total fatty acids, two rats from each dietary group were killed after receiving the diets for 5, 6 and 7 months, and the tissues from each pair of rats were combined for analysis. For the determination of phospholipid fatty acids, the rats were killed after receiving the diet for 4, 5, 8 and 14 months. The tissues were removed immediately after death and were rinsed in water, blotted dry and

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weighed. The stomach and intestinal contents were washed out, and this tissue was blotted dry and weighed. All tissues were stored at −20° until required for analysis.

For the estimation of total fatty acids, lipids were extracted into chloroform by the procedure of Albrink (1959–60). The chloroform was removed by distillation and the lipid was saponified for 1 h with 1 ml 40% sodium hydroxide and 14 ml methanol for each g lipid. Unsaponified material was removed by washing the saponification mixture three times with diethyl ether. The aqueous phase was acidified with HCl (pH 1–2) and twice washed with light petroleum (b.p. 40–60°). The light petroleum washings, containing the free fatty acids, were combined and the solvent was removed by distillation. Up to 100 mg of the free fatty acids were transferred to a boiling tube and methylated by heating in a boiling water bath with 3 ml boron trifluoride–methanol complex ('about 14% BF_3'; BDH Chemicals Ltd) for 2 min; 20 ml water were added and the methyl esters were extracted in 3 × 15 ml light petroleum. The extracts were combined and dried over anhydrous sodium sulphate, and the solvent was removed by distillation (Metcalfe & Schmitz, 1961). The fatty acid methyl esters so produced were separated by gas–liquid chromatography. A Pye Panchromatograph fitted with a flame ionization detector was used throughout; the column packing was 10% polyethylene glycol adipate on celite, argon (30 ml/min) was used as the carrier gas and the column temperature was 180°. The percentage compositions of the fatty acid methyl ester mixtures was calculated by triangulation.

For the estimation of phospholipid fatty acids, the total lipid obtained by the procedure of Albrink (1959–60) was subjected to silicic acid–celite column chromatography, based on the method of Borgström (1952). The phospholipid obtained was saponified and methyl esters were prepared as above, or the phospholipid was transmethylated by heating under reflux with 3 ml 14% BF_3–MeOH for 20 min. No significant differences were found when control samples were methylated by these two methods. The fatty acid methyl esters were separated and estimated by gas–liquid chromatography as above.

RESULTS

Table 1 lists the percentages of the major fatty acids in the total lipid isolated from the tissues of rats given a diet deficient in vitamin E (−E) or the same diet supplemented with vitamin E (+E) for 5–7 months. There were no significant trends apparent in the levels of the various fatty acids with increasing time, and the values obtained at 5, 6 and 7 months have been averaged. Some differences were found between +E and −E groups, but no consistent pattern of change was obvious. The percentage of $\Delta^{8,11,14}$ eicosotrienoic acid (20:3ω6) appeared to fall in all tissues taken from the rats of the −E group, with the exception of lung; however, the magnitude of these changes was small.

The testis and heart from rats of the −E group exhibited more marked changes from +E controls than did the other tissues. In testis the percentage of Δ^{9} hexadecenoic acid (16:1ω7) and Δ^{9} octadecenoic acid (18:1ω9) increased, whereas the percentage of $\Delta^{5,8,11,14}$ eicosotetraenoic acid (20:4ω6), $\Delta^{7,10,13,16}$ docosotetraenoic acid

(22:4ω6) and $\Delta^{4,7,10,13,16}$ docospentaenoic acid (22:5ω6) decreased, when compared with the +E group. In heart, the percentage of octadecanoic acid (18:0) fell markedly from 23·4% in +E rats to 18·7% in −E rats. The percentage of 20:4ω6, 22:5ω6 and $\Delta^{4,7,10,13,16,19}$ docosohexaenoic acid (22:6ω3) in the hearts from −E rats showed an increase when compared with the +E group.

Table 1. *Percentages of the major fatty acids of the total lipid of the tissues of rats given a diet deficient in vitamin E with (+E) or without (−E) a vitamin E supplement for 5–7 months*

Tissue	Diet	16:0	16:1ω7	18:0	18:1ω9	18:2ω6	20:3ω9	20:3ω6	20:4ω6	22:4ω6	22:5ω6	22:6ω3
Testis	−E	28·26	7·06	4·99	27·45	4·01	0·49	0·60	10·08	1·53	13·07	0·49
	+E	29·93	4·14	4·53	24·78	3·77	0·61	0·71	12·54	1·78	15·17	0·39
Lung	−E	27·70	6·97	6·21	43·31	4·66	0·35	0·20	5·33	1·21	0·47	0·39
	+E	26·76	6·27	6·07	46·24	4·98	0·36	0·18	4·39	0·99	0·32	0·32
Pancreas	−E	24·43	6·33	6·31	42·14	7·41	0·65	0·21	8·56	0·52	0·43	0·59
	+E	24·46	7·71	6·35	43·95	7·09	0·63	0·24	5·51	0·46	0·37	0·29
Spleen	−E	25·83	3·72	14·51	28·92	4·63	1·01	0·42	14·43	2·36	0·79	0·62
	+E	22·61	4·21	11·94	29·69	4·61	1·13	0·46	15·61	2·22	0·83	0·85
Heart	−E	14·51	2·84	18·66	21·53	9·04	0·76	0·32	21·92	0·81	2·40	4·24
	+E	15·84	2·78	23·40	20·38	8·70	0·71	0·89	17·82	0·86	1·89	3·35
Kidney	−E	19·35	4·92	13·06	31·15	6·50	0·56	0·47	20·27	0·50	0·45	0·82
	+E	20·20	5·10	12·27	32·35	6·40	0·56	0·51	18·98	0·43	0·43	0·86
Liver	−E	19·54	5·89	12·98	32·00	6·42	1·25	0·85	15·81	0·28	1·31	2·66
	+E	19·67	5·39	14·68	30·07	6·63	1·29	1·03	15·89	0·34	1·10	2·47
Brain	−E	19·71	1·21	19·04	28·73	0·85	0·25	0·12	10·07	2·77	1·92	11·16
	+E	19·52	1·45	19·01	28·97	0·59	0·41	0·21	10·43	2·75	1·96	12·24
Muscle	−E	21·95	6·84	7·53	41·89	8·46	0·58	0·26	6·73	0·47	0·86	2·02
	+E	21·89	8·00	7·84	37·27	9·10	0·72	0·36	7·26	0·47	1·01	2·42
Gut	−E	21·02	5·76	9·52	46·79	7·94	0·59	0·23	4·43	0·56	0·36	0·31
	+E	22·07	6·09	8·26	46·34	7·93	0·40	0·43	4·36	0·55	0·31	0·39

From the results obtained in this experiment, the tissues from rats of the −E group can be divided into three groups, when compared with the +E group:

(1) those tissues in which the percentage of PUFA in the total lipid showed an overall decrease (testis and skeletal muscle, and, to a lesser extent, brain);

(2) those tissues in which the percentage of PUFA in the total lipid showed an overall increase (lung, pancreas, heart and kidney);

(3) those tissues in which the percentage of PUFA in the total lipid showed no consistent pattern of change (spleen, liver and small intestine).

Table 2 lists the percentages of the major fatty acids in the total phospholipid of the tissues from rats given a diet deficient in vitamin E for 4, 5, 8 and 14 months, or the same diet supplemented with vitamin E. The results obtained from rats supplemented with vitamin E generally showed little variation during the course of this experiment. For this reason the control figures given in Table 2 are the averaged results for supplemented rats killed after 4, 5, 8 and 14 months.

Comparing the pattern of fatty acid distribution obtained from rats given a diet deficient in vitamin E for 4 or 5 months with that from supplemented rats, few major differences were found. In both testis and spleen at 5 months there appeared

Table 2. *Percentages of the major fatty acids of the phospholipids of the tissues of rats given a diet deficient in vitamin E*

Tissue	Months on diet	16:0	16:1ω7	18:0	18:1ω9	18:2ω6	20:3ω9	20:3ω6	20:4ω6	22:4ω6	22:5ω6	22:6ω3
Testis	4	33·13	1·59	7·46	13·92	2·52	0·39	0·74	17·00	1·37	19·09	0·56
	5	38·84	tr	8·75	13·59	2·83	tr	0·98	14·73	1·16	15·62	0·67
	8	34·73	tr	6·82	16·49	2·73	tr	1·17	18·27	1·58	15·23	0·38
	14	35·71	2·15	9·47	23·02	2·07	2·24	tr	12·54	1·13	10·21	tr
	C	35·11	1·36	6·48	15·93	2·89	0·55	0·85	16·64	1·35	17·19	0·57
Lung	4	33·41	8·21	11·87	16·82	2·84	0·80	0·50	13·54	3·06	1·14	0·70
	5	34·10	3·67	12·00	18·81	3·16	0·91	0·55	14·70	2·92	0·96	0·72
	8	37·67	4·57	8·87	19·37	3·25	1·00	0·26	12·63	2·54	1·70	0·80
	14	37·81	5·86	12·28	22·02	1·88	1·52	0·25	9·66	2·54	1·41	tr
	C	34·45	4·09	11·12	19·09	3·08	0·74	0·46	14·10	3·41	1·26	0·81
Pancreas	4	21·06	2·57	9·64	16·10	11·24	2·34	0·44	22·13	0·52	0·76	0·71
	5	26·36	tr	10·29	19·60	10·66	1·70	0·48	21·80	0·66	1·22	0·97
	8	23·88	1·80	14·82	14·70	9·49	1·47	0·99	21·24	0·72	1·82	0·81
	14	33·35	2·81	18·36	24·24	4·33	2·51	tr	8·39	0·45	1·60	nd
	C	25·38	2·18	15·31	18·10	9·60	1·75	0·43	18·61	0·58	1·00	0·83
Spleen	4	25·44	tr	16·84	17·11	4·07	1·93	0·54	22·11	3·23	1·03	0·75
	5	35·25	tr	19·34	20·36	2·44	0·70	0·43	12·85	2·37	0·98	0·58
	8	31·56	1·73	21·76	15·01	3·00	0·91	0·91	16·57	2·52	1·60	1·06
	14	26·15	2·18	18·50	20·85	2·73	2·10	0·71	18·25	3·15	1·11	0·95
	C	28·95	2·53	16·35	18·46	3·20	0·61	0·86	18·73	3·09	1·31	0·83
Heart	4	14·68	1·55	27·79	14·42	10·45	1·02	0·37	20·03	0·74	2·56	3·42
	5	12·01	1·53	25·65	13·13	10·15	0·64	0·44	22·86	0·96	2·81	5·29
	8	13·49	1·10	27·20	14·38	6·88	0·73	tr	20·23	0·65	4·14	4·29
	14	15·29	2·04	21·98	21·25	8·99	1·45	0·62	17·86	1·11	3·35	2·16
	C	12·27	1·17	24·38	14·18	11·01	0·66	0·50	23·41	0·84	3·21	4·51
Kidney	4	17·81	1·53	19·43	18·71	7·20	0·86	0·79	29·61	0·20	1·12	1·26
	5	17·51	3·05	19·54	16·54	7·00	0·75	0·90	29·62	0·60	0·61	1·03
	8	19·08	1·12	19·40	19·29	7·01	0·68	0·34	28·80	0·50	1·07	0·86
	14	15·66	1·70	15·54	24·42	7·49	2·48	tr	29·29	0·56	1·36	0·80
	C	18·79	1·96	18·64	18·84	6·91	0·72	0·71	29·26	0·62	0·82	1·48
Brain	4	21·89	1·28	18·32	27·76	tr	0·24	tr	8·32	2·81	1·75	10·45
	5	18·34	1·85	19·14	27·67	0·72	0·21	0·15	7·98	2·83	1·80	10·52
	8	21·73	1·08	19·05	28·29	0·77	0·22	0·12	8·33	2·37	1·73	8·80
	14	21·44	tr	16·70	32·86	tr	0·25	tr	9·47	2·54	3·13	10·62
	C	20·57	1·39	17·88	29·62	0·69	0·22	0·16	8·14	3·10	2·00	9·78
Liver	4	14·48	3·43	22·85	17·08	8·00	2·56	1·28	24·35	0·31	1·20	3·84
	5	17·80	3·30	23·93	15·58	6·20	2·15	1·32	21·45	0·35	2·60	4·04
	8	19·70	1·90	24·39	15·35	5·29	1·86	1·05	19·05	0·41	4·42	2·83
	14	18·84	4·18	22·26	18·32	3·60	7·23	1·45	15·83	0·38	3·95	2·70
	C	19·01	3·60	21·75	16·34	6·15	2·38	1·44	21·35	0·42	2·80	3·63
Muscle	4	23·47	1·40	14·82	15·35	11·26	1·40	0·71	18·98	0·90	2·75	5·87
	5	24·05	1·92	16·55	14·93	12·22	1·30	0·84	15·87	0·92	1·52	5·19
	8	22·75	2·17	15·56	16·12	11·09	1·27	0·77	18·57	0·87	2·31	4·06
	14	21·03	5·55	14·91	24·06	9·31	1·59	0·78	13·49	1·18	2·63	3·49
	C	24·31	2·31	13·51	15·72	11·95	1·19	0·81	17·46	1·06	2·18	5·82
Gut	4	20·01	1·38	23·18	22·66	9·52	1·21	0·61	13·17	2·20	1·20	1·16
	5	20·89	2·23	16·71	25·33	11·96	1·05	0·84	13·19	1·58	1·39	1·01
	8	25·61	2·16	26·87	23·78	6·98	0·72	tr	6·13	0·92	0·90	0·58
	14	20·64	3·35	25·01	25·42	6·04	2·18	0·39	9·13	1·13	0·98	0·42
	C	21·92	2·35	21·12	23·60	10·28	0·83	0·68	11·06	1·75	1·16	0·98

tr, trace; C, mean results for control rats given the same diet with a vitamin E supplement and killed after 4, 5, 8 and 14 months. nd, not detected.

to be marked decreases in 20:4ω6, while the percentage of 22:5ω6 also decreased in the testis. The percentage of hexadecanoic acid (16:0) increased in both tissues. In the tissues as a whole after 5 months there was a tendency for the percentage of $\Delta^{9,12}$ octadecadienoic acid (18:2ω6) and the other polyunsaturated fatty acids to decrease, with the exception of 20:4ω6, the levels of which were similar to those in the supplemented rats.

The general pattern of changes in the percentage of the PUFA in the phospholipid fraction seen at 8 months was basically the same as that seen at 4 and 5 months, but the percentage of 22:5ω6 increased in all tissues except testis, brain and small

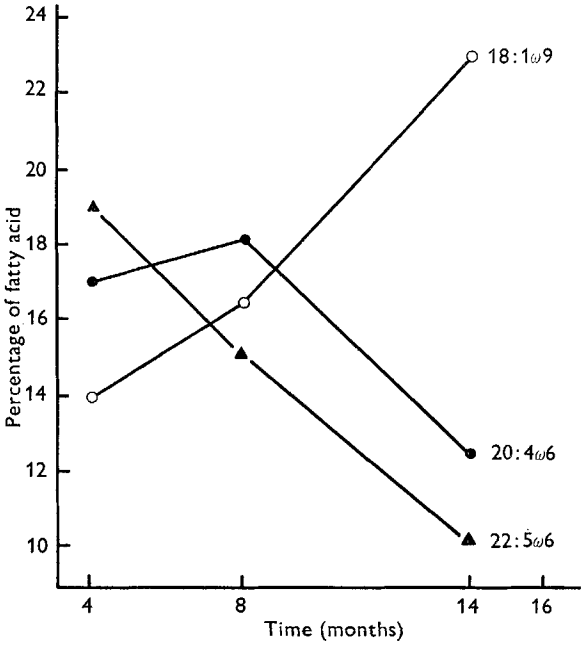

Fig. 1. Percentage of certain fatty acids in testicular phospholipid from rats given a diet deficient in vitamin E for 4, 8 or 14 months.

intestine. In several tissues (lung, heart, liver and small intestine), the percentage of 20:4ω6 was lowered, as was the percentage of 22:6ω3 in seven tissues. In only one tissue, spleen, did the percentage of 22:6ω3 increase. The percentage of 22:4ω6 increased in testis, pancreas and skeletal muscle after 8 months.

The most marked changes observed were found after 14 months. Those rats not receiving vitamin E had smaller percentages of nearly all the PUFA in the phospholipid fraction. The exceptions were $\Delta^{5,8,11}$ eicosotrienoic acid (20:3ω9), which increased in all tissues, and 22:5ω6, which increased in five tissues. The percentage of 18:1ω9 also increased in all tissues and 18:0 increased in seven tissues. The tissue that perhaps exhibited the most significant changes was the testis. In this tissue, the fatty acids that showed the greatest changes in the −E rats were 18:1ω9, 20:4ω6 and 22:5ω6 (Fig. 1). After 4 months there was little difference between the rats not

receiving vitamin E and the supplemented rats. After 8 months the −E rats showed an increase in both 18:1ω9 and 20:4ω6; the percentage of 22:5ω6 was lowered. This pattern of change was not maintained and after 14 months 18:1ω9 and 22:5ω6 exhibited a greater increase and decrease in levels respectively, while the percentage of 20:4ω6 was markedly decreased. The greatest change, relative to control levels, in the testis after 14 months was in the level of 20:3ω9, which was four times that in the control animals. The other fatty acid exhibiting an increase in percentage was 18:0.

In skeletal muscle of −E rats, 22:6ω3 progressively decreased from 5·87% to 3·49%, as did 18:2ω6, from 12·0% to 9·3%. The percentage of 20:4ω6 also fell from 19·0% to 13·5%; and 18:1ω9 increased markedly from 15·4% to 24·1% while the percentages of 20:3ω9 and 22:5ω6 rose slightly. In the liver, the percentage of 18:2ω6, 20:4ω6 and 22:6ω3 in the phospholipid fatty acids decreased progressively; the percentage of 20:3ω9 rose to 7·2% after 14 months.

After 14 months, lung, heart, pancreas and small intestine of the rats given a diet deficient in vitamin E showed similar changes to those described in skeletal muscle and liver, whereas in spleen, kidney and brain the levels of the polyunsaturated fatty acids were not appreciably reduced.

DISCUSSION

The results reported above show that there are changes in the fatty acid composition of the tissues of the male rat given a diet deficient in vitamin E and that the changes tend to be more pronounced in the phospholipid fraction than in the tissue total lipid fatty acids. These results also suggest that the type of changes in fatty acid distribution in vitamin E deficiency depends on the length of time the rats are given a diet deficient in vitamin E.

The changes found in the fatty acid content of the testis are perhaps the most interesting and most significant, since the principal sign of vitamin E deficiency in the male rat is testicular degeneration. In the vitamin E-deficient testis there was a fall in 22:5ω6 and slight rises in 20:3ω6, 20:4ω6 and 22:4ω6 up to 8 months. These results are basically the same as those reported by Bieri & Prival (1966). However, after 14 months the pattern of changes had completely altered, and all the fatty acids of the linoleic (ω6) series exhibited decreases to varying degrees. This decrease was reflected in a marked increase in the level of 18:1ω9 and 20:3ω9.

In the pancreas and liver in particular, there was a steady fall in the percentage 18:2ω6 content. The levels of the other acids of the linoleic series were maintained initially but dropped considerably by 14 months. In the tissues as a whole after 14 months there was a general decrease in the level of the linoleic series of acids, with a concomitant increase in the level of 18:1ω9, 20:3ω9 and sometimes 18:0. The general pattern of fatty acid change is very similar to that seen in essential fatty acid deficiency and it is possible that the pattern at 14 months reflects a deficiency in essential fatty acids induced by a lack of vitamin E.

The diet given to our rats contained 7% stripped lard. The diet used by Bieri &

Prival (1966) contained 5% stripped lard, so it probably entailed a lower level of tocopherol than was present in our experiments; perhaps this is why Bieri & Prival (1966) reported changes after a shorter time. They reported changes in fatty acids up to only 8 months. Giving the animals the 7% stripped lard diet for 14 months may have caused a secondary essential fatty acid deficiency. Analysis of the stripped lard showed that it contained 12% 18:2ω6. The complete diet therefore contained 0·84%. This is perhaps low for the rat; a variety of factors affect the requirement for linoleate, but Deuel, Greenberg, Anisfield & Melnick (1951) concluded that the requirement for linoleate for male rats 'would seem to exceed 200 mg/day', which corresponds to about 2% of the diet. However, the +E animals did not show changes in fatty acid composition consistent with essential fatty acid deficiency and Bieri & Prival (1966) found no such changes in supplemented or deficient animals when the lower level of fat was given for 8 months.

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