

# Abnormalities of lipid metabolism in the vitamin E-deficient monkey

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**ABSTRACT** A soybean protein diet was used to induce vitamin E deficiency in rhesus monkeys. The deficient monkeys had reduced triglyceride concentrations in liver and skeletal muscle, but the cholesterol concentration in their skeletal muscle was increased. A constant amount of radioactively labeled  $^3\text{H}$ -cholesterol- $7\alpha\text{-}{}^3\text{H}$  was fed daily for 48–114 days to control and vitamin E-deficient monkeys to study the relationship between plasma, liver, and skeletal muscle cholesterol. Plasma cholesterol reached constant, maximum specific activity by the 42nd day both in control and in vitamin E-deficient monkeys. In control and previously deficient vitamin E-treated monkeys the specific activity of cholesterol in liver and skeletal muscle was approximately equal to that of plasma. In vitamin E-deficient monkeys the liver cholesterol specific activity was equal to that of plasma cholesterol, but the ratio of skeletal muscle cholesterol specific activity to plasma cholesterol specific activity was reduced. It is concluded from these studies that there is a specific defect(s) in cholesterol metabolism in the skeletal muscle of vitamin E-deficient monkeys.

**KEY WORDS** primate · rhesus monkey · vitamin E deficiency · cholesterol · lipids · plasma · muscle · liver · metabolism · soybean protein · choline · methionine · cystine

SEVERAL ABNORMALITIES of lipid metabolism have been described in vitamin E-deficient animals. An increased concentration of cholesterol in skeletal muscle is an invariable finding in vitamin E-deficient calves (1), rabbits (2, 3), chicks (4), guinea pigs (5), and rats (6). Vitamin E-deficient rabbits (2) and guinea pigs (5) also have increased plasma cholesterol concentrations, and increased concentrations of total lipid have been found in the muscles of vitamin E-deficient calves (1), rabbits

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(2), guinea pigs (5), and rats (6). Neither the mechanisms responsible for these abnormalities nor the normal functions of vitamin E in lipid metabolism are known.

Since a complete understanding of the functions of vitamin E may require knowledge of the effects of its deficiency in various species, we have studied lipid metabolism in vitamin E-deficient rhesus monkeys, a species hitherto not used for such studies. In these studies, we have used control and vitamin E-deficient monkeys that had been part of another study (7). These monkeys had been fed a soybean protein basal diet with and without supplemental choline, methionine, and cystine. Thus, we had the opportunity to study the effects on lipid metabolism of a diet low in three important dietary constituents other than vitamin E.

## MATERIAL AND METHODS

A soybean protein basal diet which has been described in detail elsewhere (8) was used. Direct measurement showed this diet to contain 2.6 mg of cholesterol per 100 g. Food consumption was measured during a 5 wk period; it was, on the average, 80–110 g per day for each animal. The cholesterol intake did not exceed 3 mg of cholesterol per kg of body weight per day for any of the monkeys, even when tritiated cholesterol was added to the diet. For some of the monkeys the diet was supplemented with 100 mg of choline per 100 g of diet or with 100 mg of choline, 630 mg of methionine, and 73 mg of cystine per 100 g of diet (Table 1). These supplements did not affect the time of appearance or the characteristics of the vitamin E deficiency syndrome (7).

All the vitamin E-deficient monkeys developed anemia and muscular dystrophy (Table 1). Postmortem examination confirmed the presence of muscular dystrophy in each of the vitamin E-deficient monkeys. Control monkeys received 80 mg of vitamin E (*dl*- $\alpha$ -tocopherol)

TABLE 1 SOME CHARACTERISTICS OF VITAMIN E DEFICIENCY IN THE MONKEY

Monkey	Sex	Dietary Conditions	Days on Diet	Body Wt.*	Hemo-globin	Urinary Creatine:Creatinine	Muscular Weakness†
2-31	M	Basal plus choline, cystine, methionine, and vitamin E (control)	721	4420	12.7	0.3	0
2-33	M	Basal plus choline and vitamin E (control)	747	3585	11.8	0.2	0
2-45	F	Basal plus vitamin E (control)	747	3975	13.7	0.4	0
2-34	M	Basal plus choline, cystine, and methionine; treated with vitamin E‡	727	4080	15.7	0.4	0
2-46	M	Basal plus choline; treated with vitamin E‡	801	3375	13.8	0.5	0
2-35	F	Basal§	740	3010-2030	4.7	3.1	+++
2-36	M	Basal plus choline, cystine, and methionine§	802	3265-2760	8.3	2.6	+++
2-39	M	Basal plus choline	721	3700-2585	7.0	2.5	++
2-41	F	Basal§	696	2900-1915	7.6	3.6	+++
2-42	M	Basal plus choline§	683	2300-1840	6.8	3.9	+++

\* Maximal and (or) terminal body weights of animals.

† No muscular weakness is indicated by 0. Muscular weakness so severe that the animal could not get up after being placed on its side is indicated by +++.

‡ Monkeys 2-34 and 2-46 were treated with coenzyme Q<sub>10</sub> before being treated with vitamin E. After treatment with vitamin E both made a complete recovery. Monkey 2-34 was started on 100 mg of vitamin E daily on day 658 and on day 668 was changed to the same supplementation schedule as was used for the control monkeys. Monkey 2-46 was started on 100 mg of vitamin E daily on day 692 and on day 704 changed to the same supplementation schedule as was used for the control monkeys.

§ A remission of the deficiency syndrome has been induced in each of these monkeys (2-35, 2-36, 2-41, and 2-42) and they had been permitted to relapse for the present study. The last doses of vitamin E were given on days 377, 458, and 456 for monkeys 2-35, 2-41, and 2-42 respectively. Monkey 2-36 was treated with hexahydrocoenzyme Q<sub>4</sub> and received the last dose on day 775.

|| These two animals died with the deficiency syndrome.

acetate) dissolved in 0.5 ml of 95% ethanol three times per week. They developed none of the manifestations of the deficiency syndrome.

Hemoglobin concentrations were determined by the cyanmethemoglobin method (9) and urinary creatine and creatinine were measured by the method of Folin (10) with minor modifications.

For studies of plasma, fasting blood was drawn into heparinized syringes by femoral vein puncture, red blood cells were separated from plasma after centrifugation, and the plasma was analyzed immediately or stored at -20°C until the time of analysis. Plasma lipids were extracted and washed according to the procedure of Bragdon (11). In the studies of tissue lipids, the monkeys were fasted overnight before being killed by an intravenous or an intraperitoneal injection of sodium pentobarbital. Specimens of liver and of muscle from the posterior aspect of the thigh were blotted, weighed, and stored at -20°C until the time of analysis. They were then homogenized in a Virtis homogenizer and the lipids were extracted by the method of Folch, Lees, and Sloane Stanley (12). The purified lipid extracts were used for the determination of total lipid (11) and for subsequent fractionation into the various lipid fractions.

The lipid fractions were separated on silicic acid by column chromatography (13) and the amount of each

fraction was individually measured. Triglycerides were determined with ferric perchlorate by the method of Snyder and Stephens (14) with the assumption that 1 μmole of fatty acid ester represents 300 μg of triglyceride. Phospholipid phosphorus was measured by the method of Fiske and Subbarow (15) following perchloric acid digestion, and the calculation of phospholipid concentration was based on an assumed phosphorus content of 4%. Free cholesterol was determined by the direct ferric chloride method or was precipitated as the digitonide and measured either with ferric chloride (13) or with <sup>14</sup>C-digitonin (16). Cholesterol esters were saponified and the cholesterol was extracted into redistilled hexane (17) for subsequent measurement by one of the above methods.

To study the relationship between the cholesterol of plasma and tissues, certain monkeys were fed cholesterol-7α-<sup>3</sup>H. A constant amount of the tritiated cholesterol (0.5-3.0 mg: specific activity of 140,000 cpm/mg) was added to a small portion of the diet each day, and precautions were taken to insure that this portion of the diet was ingested. The tritiated cholesterol was fed until the animals were killed, 48-114 days after cholesterol feeding was begun. Determinations of cholesterol specific activity were made only on free cholesterol, either directly (13) or after isolation as the digitonide (13, 16). In an earlier

study it was found that free and ester cholesterol had almost identical specific activities (13).

## RESULTS AND DISCUSSION

### Lack of Effect of Supplemental Choline, Methionine, and Cystine

The dietary supplements of choline, methionine, and cystine did not alter the vitamin E deficiency syndrome (Table 1 of reference 7), and neither did they alter the associated lipid abnormalities. Moreover, the presence or absence of these supplements did not affect the concentration of any of the lipid fractions in plasma, liver, or skeletal muscle (Tables 2 and 3). Three of the monkeys receiving none of these supplements (Nos. 2-35, 2-41, and 2-45) showed slightly high total muscle lipid concentrations, but the significance of this observation is unknown because these three animals were females and all the others were males.

The diet without supplemental choline, methionine, and cystine is similar in composition to the diet used by Wilgram, Lucas, and Best to induce fatty livers in rhesus and *Cebus* monkeys (18). In their experiment each of two

rhesus monkeys had a moderately increased amount of fat in the liver after 1 yr. In our experiment, in which the un-supplemented diet was fed for 2 yr, none of the three monkeys had fatty livers. The reason for the discrepancy between these two studies is not known. Nevertheless, it is evident that the female rhesus monkey, at least, is relatively resistant to the development of a fatty liver as a consequence of being fed a diet low in choline and sulfur amino acids. A similar observation has been made in another primate, the baboon (19).

The diet low in choline and sulfur-containing amino acids also had no effect on the concentration of cholesterol in plasma, liver, or skeletal muscle. The failure of this diet to affect sterol compensation of the rhesus monkey confirms for a more extended experimental period an observation of Mann (20). Mann found that the serum cholesterol of two female rhesus monkeys did not increase when they were fed a soybean protein diet without a cholesterol load.

### Effect of Vitamin E Deficiency

On postmortem examination the most obvious abnormality was an almost complete absence of adipose tissue in the subcutaneous, perirenal, and omental

TABLE 2 SKELETAL MUSCLE LIPID CONCENTRATIONS OF CONTROL AND VITAMIN E-DEFICIENT MONKEYS

Animal No.	Vitamin E Status	Total Lipid	Phospholipid	Triglyceride	Muscle Lipids	
					Total Cholesterol	Ester Cholesterol
2-31	Control	9.4	8.1	0.65	0.53	0.01
2-33	Control	9.6	6.4	1.7	0.41	0.00
2-45	Control	13.3	8.3	3.4	0.49	0.02
2-34	Deficient; treated	49.8	7.6	43.2	0.99	0.05
2-46	Deficient; treated	15.9	8.3	8.2	0.89	0.05
2-35	Deficient	13.4	9.0	0.36	1.6	0.12
2-36	Deficient	8.3	6.5	0.12	1.1	0.03
2-39	Deficient	9.4	7.0	1.5	0.57	0.04
2-41	Deficient	11.1	7.7	0.28	1.5	0.14
2-42	Deficient	8.6	7.0	0.37	1.0	0.04

TABLE 3 PLASMA AND LIVER LIPID CONCENTRATIONS IN CONTROL AND VITAMIN E-DEFICIENT MONKEYS

Animal No.	Vitamin E Status	Plasma Lipids				Liver Lipids			
		Total Lipid	Phospholipid	Triglyceride	Total Chol.	Total Lipid	Phospholipid	Triglyceride	Total Chol.
<i>mg/ml</i>									
2-31	Control	4.14	2.20	0.41	1.17	31.1	26.1	2.9	2.28
2-33	Control	4.23	2.07	0.37	1.10	36.3	33.7	2.0	3.03
2-45	Control	4.68	2.39	0.46	1.30	30.9	28.5	2.5	2.24
2-34	Deficient; treated	3.87	1.76	0.33	1.21	35.8	32.2	1.9	3.04
2-46	Deficient; treated	3.87	2.43	0.43	0.98	27.2	26.1	1.9	2.59
2-35	Deficient	—	—	—	—	26.8	23.9	1.3	2.42
2-36	Deficient	1.26	0.77	0.20	0.37	23.9	20.8	0.61	2.82
2-39	Deficient	4.59	2.34	0.54	1.22	36.4	28.9	4.7	3.02
2-41	Deficient	—	—	—	—	25.3	18.5	0.64	2.75
2-42	Deficient	—	—	—	—	22.2	18.7	0.65	2.26

regions. This finding together with the weight loss (Table 1) suggests that inanition may have been significant terminally, but all the vitamin E-deficient animals continued to eat well, as previously reported (8). One deficient monkey, 2-39, exhibited the same absence of adipose tissue as the other deficient animals although it was not severely dystrophic when killed. Thus, vitamin E deficiency may directly affect adipose tissue. Additional abnormalities were demonstrated by measurement of lipid concentrations in plasma, liver, and skeletal muscle (Tables 2 and 3) and by use of tritiated cholesterol to study the relationships between plasma and tissue cholesterol (Table 4).

The data in Table 2 show that four out of five of the vitamin E-deficient monkeys had greatly increased concentrations of cholesterol and reduced concentrations of triglyceride in their skeletal muscle. This combination of abnormalities plus a normal phospholipid concentration resulted in total muscle lipid concentrations within the normal range. The muscle cholesterol concentration was elevated in the two monkeys recovered by treatment with vitamin E, but this may be explained, in part, by fatty replacement of previously degenerated muscle. Fatty replacement is suggested by the unusually high triglyceride concentrations in muscles of the two treated monkeys. One monkey did not show changes in lipid concentrations despite being clearly vitamin E-deficient (No. 2-39). However, this monkey had only moderate muscular weakness whereas the others were severely dystrophic and were almost too weak to move (Table 1).

The terminal plasma and liver lipid concentrations are shown in Table 3. The data on terminal plasma lipid concentrations in the vitamin E-deficient monkeys are insufficient to permit firm conclusions, but measurements of cholesterol concentrations which were made periodically during a 4 month period while the deficiency syndrome was developing provided additional information. The ranges for plasma cholesterol concentration during this period were 110–155 for the control and 80–165 mg/100 ml for the vitamin E-deficient monkeys. Thus, in contrast to the findings in other species (2, 3, 5), these determinations as well as the terminal ones revealed

no increase in plasma cholesterol concentration. The concentration of cholesterol in the liver also was not changed by vitamin E deficiency, although total lipid and triglyceride concentrations were reduced.

The increase in muscle cholesterol without an increase in the concentration in plasma or liver raises the possibility that a specific defect in cholesterol metabolism occurs in skeletal muscle. This possibility is strengthened by the data from the experiment using tritiated cholesterol. In this experiment, a constant, maximum plasma cholesterol specific activity was reached by the 42nd day both in the control and in the vitamin E-deficient monkeys. This time interval is in agreement with similar studies in the rat (21). The specific activity of plasma cholesterol in the five monkeys ranged from 1300 to 5400 cpm/mg, and the ratio of liver to plasma cholesterol specific activity was approximately one in each of the monkeys (Table 4). Vitamin E deficiency apparently did not alter the relationship between liver and plasma cholesterol.

The ratio of the specific activities of cholesterol from skeletal muscle and from plasma was nearly unity in two control and in one recovered monkey; but significantly lower ratios were found in two vitamin E-deficient monkeys (Table 4). One of the latter monkeys (No. 2-39) had only moderate muscular weakness and was the exceptional monkey with a relatively low muscle cholesterol concentration. Although the number of animals available for this experiment was small, the data suggest a defect of cholesterol metabolism in skeletal muscle which may occur before the concentration of cholesterol increases. Furthermore, the finding of a normal ratio of muscle to plasma cholesterol in a vitamin E-treated monkey (No. 2-34) suggests that the defect is reversible.

Assuming that the reduced ratio of muscle to plasma cholesterol specific activity is caused by the same defect that causes the increased concentration of cholesterol in muscle, it may be concluded that the increased concentration is not the result of an increased rate of accumulation of cholesterol from the plasma. On the contrary, the amount of cholesterol in muscle that has been derived from labeled plasma cholesterol is apparently reduced.

TABLE 4 PLASMA CHOLESTEROL SPECIFIC ACTIVITY\* AND RATIO OF SPECIFIC ACTIVITY OF LIVER AND MUSCLE CHOLESTEROL TO TERMINAL PLASMA CHOLESTEROL SPECIFIC ACTIVITY IN CONTROL AND VITAMIN E-DEFICIENT MONKEYS

Animal No.	Vitamin E Status	Days Fed Cholesterol- $7\alpha$ - $^3$ H	Plasma Cholesterol S.A.*	Liver: Plasma S.A.	Muscle: Plasma S.A.
2-31	Control	88	5400	0.87	0.85
2-33	Control	114	3500	0.95	0.95
2-45	Control	114	2400	1.00	0.96
2-34	Deficient; treated	93	2500	1.00	0.92
2-39	Deficient	84	3000	0.97	0.70
2-42	Deficient	48	1300	0.85	0.77

\* Specific Activity = cpm/mg cholesterol.

If vitamin E deficiency does not impair the exit of cholesterol from muscle, then the reduced ratio would indicate greater *in situ* synthesis of cholesterol.

To define further the abnormality in cholesterol metabolism and to determine whether it occurs within the muscle cell or whether it results from abnormal accumulation of other cell types, such as inflammatory cells, other studies will be required. Other studies are also needed to elucidate the mechanisms responsible for the reduced amount of adipose tissue and for the reduced triglyceride concentrations in liver and muscle.

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