Altered lipoprotein metabolism in spontaneous vitamin E deficiency of owl monkeys[1-4]

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ABSTRACT Certain owl monkeys (AOT) develop spontaneous hemolytic anemia that responds to vitamin E. The anemia is associated with red blood cell lipid peroxidation and altered red blood cell membrane lipid composition. To investigate these changes, plasma lipid and lipoprotein profiles were characterized in anemic, anemia-susceptible, and anemia-resistant AOT. The plasma vitamin E and vitamin A concentrations were assessed as an index of fat absorption and the effect of corn oil supplementation and vitamin E-selenium injection were measured. Anemia-susceptible AOT had depressed plasma levels of vitamin E and A and an altered lipoprotein metabolism characterized by elevated ratios of low/high density lipoprotein cholesterol and free to esterified cholesterol in these lipoproteins. Vitamin E-selenium injection in anemia-susceptible AOT increased the plasma vitamin E, and vitamin E and corn oil supplements reduced the high density lipoprotein free to esterified cholesterol ratio. The data suggest that the AOT suffer from fat malabsorption and that the consequences (including tocopherol deficiency) result in altered cholesterol metabolism. *Am J Clin Nutr* 1983;38:888–894.

KEY WORDS Vitamin E, lipoproteins, monkeys

Introduction

The owl monkey [*Aotus trivirgatus* (AOT)], a New World primate represented by several karyotypes, spontaneously develops a vitamin E-responsive hemolytic anemia in captivity (1). Susceptibility to anemia varies as a function of karyotype (2), ie, karyotypes 2, 3, 4, 5, 7 are susceptible and karyotypes 1 and 6 resist developing the anemia. Clinical and pathological features of the anemia are consistent with tocopherol deficiency, although initial estimates suggested that plasma tocopherol values were not depressed (3). Nonetheless, intramuscular injection of vitamin E and selenium, but not selenium alone or the oral supplementation of either of these nutrients, proved effective in controlling the anemia (1).

Red blood cell (RBC) glutathione peroxidase, glutathione reductase, and glucose 6-phosphate dehydrogenase levels were within the range of normal values reported for most mammalian species (3), suggesting that the selenium-dependent metabolism of peroxides is probably not the etiological factor underlying the pathogenesis of the disease.

Analysis of RBC membrane lipids from anemia-susceptible and resistant AOT, compared with three other primate species (squirrel, marmoset, and rhesus), revealed major differences in membrane phospholipids and cholesterol (4). AOT RBC (all

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[3] Animals used in this study were maintained in accordance with the guidelines of the Committee on animals of the Harvard Medical School and those prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council [DHEW Publication no (NIH) 78/23, revised 1978].

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karyotypes) had lower percentages of phosphatidylserine and phosphatidylethanolamine and a higher percentage of phosphatidylcholine and sphingomyelin than RBC from other primate species, and anemia-susceptible AOT had a strikingly lower percentage of polyunsaturated fatty acids (PUFA), specifically 18:2ω6 and 20:4ω6, in their phospholipids when compared to anemia-resistant AOT and other primate species. The AOT RBC also had an elevated free cholesterol-to-phospholipid ratio which tended to be even higher in overtly anemic monkeys (4).

Since a likely origin of the peculiar lipid profile in AOT RBC would be plasma lipoproteins (LPs), the present study tested the hypothesis that the plasma LP profile and composition were abnormal in susceptible AOT. The spontaneous development of gallstones (5) and atherosclerosis (Hayes KC, unpublished observations) in AOT made the study of LPs even more relevant since both diseases involve LPs and cholesterol metabolism. The low percentage of PUFA in the RBC membrane phospholipids of susceptible AOT as well as the documented association between essential fatty acid deficiency and hemolytic anemia (6) prompted us to evaluate the plasma lipid profile in an additional group of susceptible AOT supplemented with dietary corn oil. In addition, the tocopherol and vitamin A status in plasma was assessed using the more sensitive and specific HPLC assay (7). The results indicate that susceptible AOT had depressed levels of vitamin A and vitamin E and an abnormal lipoprotein profile and composition which presumably contribute to the development of anemia, gallstones, and atherosclerosis.

Materials and methods

Animals

Fifteen AOT, five of karyotypes 2 and 3 (anemia-susceptible), five karyotype 6 (anemia-resistant), and five susceptible monkeys fed 5% corn oil added to the Purina Chow were part of 150 AOT maintained at the New England Regional Primate Research Center for the past 12 yr. Monkeys of both sexes between the ages of 2 and 15 yr had been fed high-protein Purina Monkey Chow when studied. The rhesus monkeys and marmosets used for comparison of plasma tocopherol and vitamin A levels were all kept at New England Regional Primate Research Center and were fed Purina Monkey Chow. The animals were fasted overnight and anesthetized with Ketalar (ketamine HCl) for bleeding. Blood was collected from the femoral vein in EDTA.

To study the effect of tocopherol on plasma lipid and HDL lipid classes, five AOT were injected with Seletoc (0.22 mg sodium selenite plus 10.2 IU *dl*-tocopherol) and blood was collected before and 1 wk after injection for lipid analysis.

Analytical methods

A complete blood count was obtained for all the animals using Clay Adams HA-4 Hematology Analyzer (Parsippany, NJ) and Wright staining of blood smears. Anemic monkeys were defined as those with Hb values less than 10 mg/dl and with circulating nucleated RBC in their peripheral blood smear. Plasma was obtained by centrifugation at 1500 × *g* for 20 min at 4°C and a portion frozen at −20°C for further analysis. Very low-density (VLDL) plus low-density (LDL) lipoproteins were separated from high-density lipoproteins (HDL) by heparin-manganese precipitation of fresh plasma as described by Warnick and Albers (8). HDL_2 was separated from HDL_3 by the method of Kekish et al (9) using polyacrylamide gel electrophoresis, and the relative concentration of each determined by scanning densitometry. VLDL + LDL cholesterol was obtained by subtracting the HDL value from the total plasma cholesterol. Plasma total cholesterol (TC) was quantitated by an enzymatic method (10) and unesterified cholesterol (FC) was measured using the same method except that cholesterol esterase was omitted and the aliquot volume was increased. Phospholipids were measured by the procedure of Bartlett (11) and triglycerides by an enzymatic method incorporated in the Sigma triglyceride kit (12). Plasma α-tocopherol and retinol levels were measured by the HPLC method of Bieri et al (7).

Statistical analysis

Results were analyzed using "Student's" *t* test except for the data on vitamin E supplementation where the "paired" *t* test was used.

Results

Hematology

Hematological evaluation (**Table 1**) revealed that all AOT, except for the anemic monkeys, had Hb values between 14 to 16 mg/dl and no circulating nucleated RBC. Susceptible and anemic monkeys had elevated white blood cell counts, and the number of eosinophils was remarkably elevated in resistant AOT. The latter findings have been noted previously (2).

Circulating lipids

An abnormal cholesterol metabolism was evidenced by the significant depression in

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the plasma cholesterol concentration of susceptible AOT, whereas the highest values occurred in anemic animals (**Table 2**). The free cholesterol concentration (FC) did not differ between groups, but esterified cholesterol (EC) was markedly depressed in susceptible monkeys resulting in a modest, but significant, elevation of the FC/EC in that group (Table 2). In susceptible monkeys, a depressed total cholesterol concentration resulted in a low TC/PL ratio, whereas this ratio was markedly elevated in overtly anemic monkeys. Susceptible AOT tended to have the highest triglyceride values and anemic monkeys the lowest phospholipid concentrations, but these differences were not statistically significant. Corn oil supplementation of susceptible AOT increased TC and lowered the FC/CE ratio to values similar to that of resistant AOT.

Lipoprotein lipids

LDL. The LDL cholesterol reflected the plasma cholesterol value, being markedly elevated in anemic monkeys and lower in susceptible AOT when compared to anemia-resistant monkeys (**Table 3**). These increases were balanced between the FC and EC so that LDL FC/EC ratio was similar for all groups (Table 3). Phospholipid and triglyceride values for the LDL fraction were unremarkable.

HDL. In contrast to plasma and LDL, the HDL total cholesterol concentration in anemic AOT was low by comparison to the value for resistant and corn oil-fed monkeys. Most striking was the depressed EC and the doubled FC/EC ratio in susceptible monkeys (**Table 4**). Similar to plasma values corn oil feeding in susceptible monkeys increased

TABLE 1
Summary of hematological data from AOT under different conditions (mean ± SD, n = 5)

Criterion	Anemic AOT	Susceptible AOT	Resistant AOT	Susceptible AOT fed corn oil
Hg (mg/dl)	9.6 ± 0.83	15.48 ± 0.91	15.18 ± 0.66	14.72 ± 1.11
Hematocrit (%)	28.6 ± 1.73	45.2 ± 2.98	43.54 ± 1.45	43.72 ± 1.9
RBC $10^6/mm^3$	3.5 ± 0.19	5.94 + 0.22	5.36 ± 0.21	5.56 ± 0.28
Nucleated RBC/10^2 white blood cells	100.0 ± 49	0	0	0
White blood cells 10^3	8.2 ± 1.5*	12.39 ± 2.77*	7.94 ± 2.6	7.62 ± 1.38
Seg (%)	25.0 ± 22	32.4 ± 14.1	26.8 ± 9.1	37.2 ± 9.2
Bands (%)	1.3 ± 1.5	0	0	0
Lymphs (%)	68.3 ± 24.5	52.2 ± 15.8	44.6 ± 11.3	51.2 ± 12.8
Prolymphs (%)	0	0	0	0
Monos (%)	3.0 ± 3.5	2.4 ± 1.5	2.0 ± 2.3	3.2 ± 1.8
Eos (%)	2.0 ± 1	3.6 ± 3.8	26.0 ± 14.9	7.8 ± 4.7
Basos (%)	1.3 ± 1.5	0.8 ± 1.7	0.6 ± 0.5	0.4 ± 0.5
Blasts (%)	0	0	0	0

* Significantly different from resistant AOT at $p < 0.05$.

TABLE 2
Plasma lipid composition in AOT (mean ± SD, n = 5)

	TC	FC	EC	FC/EC	TG	PL	TC/PL
				mg/dl			
Anemic AOT	143 ± 34*	NA†	NA	NA	NA	155 ± 47	0.96 ± 0.33
Susceptible AOT	86 ± 1*	30 ± 0.9*	56 ± 1*	0.54 ± 0.02*	71 ± 13	181 ± 13	0.48 ± 0.07*
Resistant AOT	128 ± 13	38 ± 2	91 ± 11	0.42 ± 0.03	62 ± 18	208 ± 39	0.67 ± 0.16
Susceptible AOT fed corn oil	118 ± 21	35 ± 4	84 ± 18	0.42 ± 0.06	56 ± 18	198 ± 24	0.69 ± 0.09

* Significantly different from resistant AOT at $p < 0.05$.
† Not available.

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the TC and decreased FC/EC values to that of resistant monkeys.

LDL and HDL lipid classes. **Table 5** reveals that the various changes in LP lipids altered the character of the lipoproteins such that anemic monkeys developed a marked rise (2 to 3-fold) in their LDL/HDL cholesterol ratio. The ratio of esterified cholesterol to phospholipid in HDL was lowest in susceptible monkeys. Analysis of HDL_2/HDL_3 ratio revealed that AOT, unlike most other primate species (13), have markedly more HDL_2 than HDL_3. The resistant monkeys tended to have a smaller HDL_2/HDL_3 ratio than susceptible monkeys, but this difference was not statistically significant due to the extraordinary variation in susceptible monkeys (Table 5).

Effect of vitamin E-selenium injection on lipid profiles

As seen in **Tables 6 and 7** Seletoc injection of anemia-susceptible monkeys tended to increase total cholesterol, particularly the esterified cholesterol fraction. Thus, using each monkey as its own control, Seletoc

TABLE 3
Lipid classes of AOT LDL* (mean ± SD, n = 5)

	TC	FC	EC	FC/EC	TG	PL
			mg/dl			
Anemic AOT	110 ± 32	NA†	NA	NA	NA	NA
Susceptible AOT	50 ± 3	11 ± 1	39 ± 3‡	0.27 ± 0.01	34 ± 12	80 ± 21
Resistant AOT	70 ± 21	16 ± 1	63 ± 5	0.27 ± 0.01	25 ± 11	69 ± 30
Susceptible AOT fed corn oil	62 ± 18	13 ± 4	50 ± 16	0.25 ± 0.02	20 ± 7	68 ± 20

* Heparin-manganese precipitable lipoproteins includes all apo B (VLDL + LDL). Monkeys typically have minimal VLDL.
† Not available.
‡ Significantly different from resistant AOT at $p < 0.05$.

TABLE 4
HDL lipid classes of AOT (mean ± SD, n = 5)

	TC	FC	EC	FC/EC	TG	PL	TC/PL
				mg/dl			
Anemic AOT	33 ± 2*	NA†	NA	NA	NA	NA	NA
Susceptible AOT	37 ± 9*	20 ± 0.8	17 ± 3*	1.20 ± 0.15*	37 ± 4	103 ± 8	0.36 ± 0.02*
Resistant AOT	58 ± 11	23 ± 3.0	36 ± 9	0.65 ± 0.11	37 ± 13	139 ± 34	0.42 ± 0.13
Susceptible AOT fed corn oil	56 ± 12	22 ± 4.0	34 ± 8	0.66 ± 0.06	37 ± 17	130 ± 28	0.43 ± 0.07

* Significantly different from resistant AOT at $p < 0.05$.
† Not available.

TABLE 5
LDL and HDL cholesterol and phospholipid ratios

	LDL-C/HDL-C	HDL-FC/HDL-PL	HDL-EC/HDL-PL	HDL_2/HDL_3
Anemic AOT	3.33 ± 0.86*	NA†	NA	NA
Susceptible AOT	1.39 ± 0.17	0.19 ± 0.02	0.17 ± 0.02*	9.3 ± 12.1
Resistant AOT	1.29 ± 0.51	0.17 ± 0.04	0.27 ± 0.09	2.4 ± 1.2
Susceptible AOT fed corn coil	1.09 ± 0.46	0.17 ± 0.02	0.26 ± 0.05	11.7 ± 15.1

* Significantly different from resistant AOT at $p < 0.01$.
† Not available.

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injection significantly increased HDL total and esterified cholesterol and significantly lowered the FC/EC ratio.

Vitamin E and A status

The HPLC analysis for tocopherol indicated a marked depression of tocopherol in plasma of susceptible and anemic AOT, with resistant monkeys having levels representative of borderline deficiency (**Table 8**). Seletoc injection of susceptible monkeys raised the plasma tocopherol to the level of resistant monkeys. Vitamin A levels were low in all AOT regardless of their karyotype (Table 8).

TABLE 6
Effect of Seletoc* injection on plasma lipid profile in AOT (mean ± SD, n = 5)

Seletoc status	TC	FC	EC	FC/EC
		mg/dl		
Before	109 ±15	23 ±3	86 ±13	0.27 ±0.32
After	122 ±30	23 ±4†	99 ±27	0.24 ±0.04

* 0.22 mg sodium selenite plus 10.2 international unit *dl*/α-tocopherol/kg body wt injected intramuscularly.
† Significant effect due to Seletoc injection ($p < 0.05$, paired *t* test).

TABLE 7
Effect of Seletoc injection on HDL lipid profile

Seletoc status	TC	FC	EC	FC/EC
		mg/dl		
Before	48 ±12	10.8 ±2	37 ±10	0.30 ±0.03
After	58 ±16*	10.8 ±2	48 ±14*	0.24 ±0.05*

* Significant effect due to Seletoc injection ($p < 0.05$, paired *t* test).

Discussion

These data support the hypothesis that the AOT fed Purina Chow suffers from depressed plasma vitamin E levels and that this depletion in tocopherol is associated with altered lipoprotein lipids and anemia.

As the anemia-susceptible monkeys become anemic the primary change in lipoproteins is a marked rise in the LDL cholesterol. It appeared from susceptible monkeys that this was associated with a drop in HDL cholesterol ester, such that the HDL FC/EC ratio rose sharply. Increased HDL-free cholesterol may contribute to the rise in the free cholesterol concentration previously described in RBC of anemic monkeys (4). Such an increase leads to RBC membrane fragility and hemolysis (14, 15). A decline in the removal and esterification of free cholesterol from the RBC by HDL would be in keeping with the hypothesis that tocopherol must be present to assure normal esterification of cholesterol by the LCAT enzyme associated with HDL (16).

The beneficial effects of corn oil and tocopherol supplementation suggest that the abnormally elevated FC/CE ratio in susceptible AOT may represent limited availability of PUFA substrate needed for cholesterol esterification and/or direct depression of LCAT activity, respectively. Purina Chow-fed AOT have a decreased level of 18:2ω6 and 20:4ω6 in their RBC phospholipids (4), which was previously linked with evidence of lipid peroxidation (3). It is noteworthy that LCAT deficiency has been demon-

TABLE 8
Plasma α-tocopherol and retinol concentrations in three species of primates (mean ± SD)

Species	n	α-Tocopherol	n	Retinol
		μg/dl		*μg/dl*
AOT				
Susceptible AOT	14	45 ± 33*	5	4 ± 1
Susceptible AOT injected with Seletoc	5	309 ± 77	5	5 ± 1
Resistant AOT	4	375 ± 203	1	6
Rhesus	3	853 ± 98*	3	33 ± 7*
Marmoset	5	1437 ± 700*		NA†

* Significantly different from resistant AOT at $p < 0.01$.
† Not available.

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strated in vitamin E-deficient monkeys (16) and that genetic deficiency of LCAT is associated with hemolytic anemia (17). Normal LCAT activity is dependent on the sulfhydryl groups of the enzyme which are disrupted by peroxidation when tocopherol is absent. The rise in HDL CE and decrease in the FC/CE ratio after injection of Seletoc further supports the hypothesis that tocopherol-dependent LCAT activity was involved.

The marked rise in LDL cholesterol and the decline in HDL cholesterol are in keeping with atherosclerosis (Hayes KC, unpublished observations) and gallstones (5) observed in AOT and parallel the occurrence of these degenerative lesions in humans. Whether these two diseases are correlated with karyotype and susceptibility to anemia has not been determined, but the risk in humans for developing atherosclerosis is increased by a high LDL/HDL ratio (18), and women (HDL_2 profile) experience a higher incidence of gallstone formation than men (HDL_3 profile) (19).

The etiology of tocopherol deficiency and generalized peroxidation evidenced in susceptible karyotypes is not completely understood. However, preliminary observation (Hayes KC, unpublished observations) indicates that AOT (all karyotypes) fed Purina Chow suffer from a chronic enteritis characterized by moderate to severe small bowel disease and colitis. These lesions have certain similarities to celiac disease, or so-called gluten-sensitive enteropathy and milk-protein enteritis of infants, which are considered by some to represent hypersensitivities to dietary protein (20). Low levels of plasma vitamin E and A, as well as low plasma levels of vitamins C (one monkey) and B_{12} (two monkeys) (Meydani SN, unpublished data), support the hypothesis that AOT are malabsorbing, albeit the fat-soluble vitamins worse than water-soluble vitamins. The higher plasma tocopherol level in anemia-resistant karyotypes (Table 8) suggests that anemia-susceptible AOT experience an additional pathological defect that further compromises their tocopherol status.

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