The Influence of Linoleate and Vitamin E from Sunflower Seed Oil on Platelet Function and Prostaglandin Production in the Common Marmoset Monkey

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Summary Vitamin E and linoleate, both of which are found in high concentrations in sunflower seed oil, were examined independently for their influence on general and blood-vascular parameters in vitamin E-deficient common marmosets. A vitamin E-deficient diet (–E, 4 µg/g) was supplemented with either 40 µg/g vitamin E (+E), vitamin E stripped sunflower oil (+10% SSO–E), or SSO (+10% SSO w/w) in a 2×2 factorial designed experiment, and the diets fed for 9 months to 4 even groups of common marmosets. Vitamin E deficiency was associated in marmosets with a loss of skeletal muscle mass and of body weight, enhanced peroxidative haemolysis of erythrocytes, increased white blood cell counts, and in the SSO–E group a relative neutrophilia. Platelet reactivity was increased with vitamin E deficiency, and to a greater degree with the SSO–E group. Aortic prostacyclin production was significantly increased by the addition of vitamin E, linoleate and both as SSO to the deficient diet, the effects being additive. Fatty acid changes associated with the different treatments reflected the influence of high linoleate and vitamin E treatments. The platelet and aortic arachidonate value in the SSO–E group showed the lowest and most variable value, and this was associated with greatest platelet aggregability. An adequate vitamin E intake is essential for stabilising high PUFA diets and biomembranes and enhancing the protective role of prostacyclin in blood vessels against thrombogenesis.

Key Words sunflower seed oil, vitamin E deficiency, platelet aggregation, peroxidation, prostaglandin production, common marmosets

There is increasing evidence for a significant role of vitamin E in the maintenance of normal function of the blood and vascular system (1-6). As well as its antioxidant role in the prevention of lipid peroxidation, vitamin E has a stabilising role for phospholipids in biomembranes and is a modulator of prostacyclin and thromboxane production *via* its influence on phospholipase and the oxygenases acting on arachidonic acid. These prostanoids could have a significant role in the pathogenesis of cardiovascular disease (7-10) *via* their influence on the platelet and vascular endothelium. The clinical consequences of inadequate vitamin E in adults as it affects thromboembolism have been reported previously (11).

The beneficial effects of sunflower seed oil when added to the diet have been generally attributed to the high linoleic acid content of the oil (12, 13). However, sunflower seed oil (SSO) is also a very rich source of vitamin E, the addition of 10% by weight of oil to the diet boosting its vitamin E content by as much as 40 µg/g, a level considered adequate to overcome any biological vitamin E deficiency effects (14, 15). We have examined the influence of such SSO dietary supplements on platelet and cardiac function in the marmoset (16, 17) but to this stage have not attempted to separate the vitamin E influence from the linoleic acid effect.

This study reports the separate and combined influences of vitamin E and linoleic acid in SSO on parameters indicative of blood and cardiovascular and skeletal muscle function. A vitamin E-deficient diet has been used as the base diet to examine these influences and the common cotton-eared marmoset monkey (*Callithrix jacchus*) has been used because it is a relevant primate model for man, and is currently under active investigation for the influence of dietary fats on cardiac health and disease (16-19).

EXPERIMENTAL

Animals/diets. Mature female common marmosets weighing 306 ± 6 g ($\pm$ SEM) and aged 10-14 months were used in this study. Marmosets at this age are 80-90% of mature size with a life expectancy of 12-15 years. They were separated into 4 treatment groups of 6 matched for age and weight and were fed 4 experimental diets for a period of 9 months. They were housed in pairs in aluminium marmoset cages in an environment maintained at $26 \pm 2^\circ\text{C}$ with a humidity of 50% and 12/12 h light/dark cycle with a half hour supplement of UV light daily. The diets and water were provided *ad libitum*. The marmosets were weighed fortnightly and a close check kept on their health and welfare.

They were fed a vitamin E-deficient base diet (-E) composed of the following: Rolled dehusked oats 59%, meat meal 10%, cottonseed meal 2%, blood meal 2%, soyabean meal 20%, skim milk powder 5%, vitamins, minerals and NaCl 0.8% (excluding vitamin E) and stripped SSO 1.2%. This diet was found on analysis to contain 4 µg/g vitamin E and 0.11 µg/g selenium. To this diet (1) supplements were added to provide 3 of the 4 dietary treatments: (2) +E, vitamin E added as pure *d*- α -tocopherol acetate (Henkel Corporation, Minneapolis, USA) to provide 40 µg/g

vitamin E; (3) sunflower seed oil stripped of vitamin E (SSO-E) (10% w/w). The SSO was stripped using activated charcoal after first mixing the oil with redistilled petroleum ether. The mixture was filtered and the solvent removed by rotary evaporation. By this means vitamin E content of the oil was reduced to about one-twentieth the initial concentration. This approach was a modification of the method by Mohri *et al.* (20); (4) unstripped SSO containing 400 µg/g vitamin E added at 10% (w/w). The 4 diets were analysed for vitamin E content by GLC with the following result: -E 4 µg/g, +E 48 µg/g, +SSO -E 5 µg/g, +SSO 41 µg/g. More than 90% of vitamin E in SSO is present as the *d*- α -tocopherol form. The base diet contained 8.0% fat with the following fatty acids: 24% 16:0, 11% 18:0, 32.5% 18:1, and 24.3% 18:2. There was no measurable 20:4 in the diet. The SSO contained 6.4% 16:0, 3.5% 18:0, 21% 18:1, and 67% 18:2. This composition of fatty acids was not changed by the vitamin E stripping process.

Haematology. Blood was taken from the marmosets *via* the femoral artery on three separate occasions throughout the experiment. It was collected into EDTA tubes and analysed using a Coulter counter model 'S' for packed cell volume (PCV) haemoglobin concentration, platelet count, white blood cell count and lymphocyte to neutrophil ratio. For platelet aggregation and thromboxane production, blood was taken into citrate (0.129 M trisodium citrate) and platelet rich plasma harvested by centrifuging at $180 \times g$ for 8 min. The methodology of platelet aggregation and thromboxane measurement using radioimmunoassay have been described previously (17,21). Acid soluble collagen (ASC) was used at 3 concentrations 1.6, 3.1 and 6.3 µg/ml for platelet aggregation using platelet rich plasma diluted to 250,000 platelets per µl with autologous platelet poor plasma and the percentage of maximum response calculated for each animal at each concentration of agonist.

Vitamin E, lipid and fatty acid assays. The plasma and platelets were prepared from blood taken into EDTA tubes by methods which have been described previously (17). Vitamin E was assayed using HPLC with fluorimetric detection by a method previously described (22). An internal standard of δ -tocopherol was used in the assay (supplied by Dr. K. Abe, Eisai Research Labs., Tokyo, Japan). The plasma value was expressed relative to plasma cholesterol which was measured by gas-liquid chromatography (23) using stigmasterol as internal standard and an OV17 column as liquid phase. The platelet tocopherol was expressed as µg per 10^9 platelets, the platelets being counted by Coulter counter (model F, Coulter Electronics, Bedfordshire, UK).

For phospholipid fatty acid analyses, samples of plasma, platelets and aorta (from sacrificed animals) were homogenised with 4 ml methanol 8 ml chloroform and 2 ml water, with the addition of 5 mg butylated hydroxytoluene. The two layers were separated by centrifugation, the chloroform layer drawn off and the aqueous phase re-extracted with chloroform. The chloroform extract was evaporated to dryness under vacuum and the residue run on TLC plates of silica Gel H in a solvent of acetone to petroleum spirit (40–60°C boiling point) 1:3. The phospholipids remaining at the origin were methylated with 2 ml boron trifluoride-methanol and

the methyl esters extracted and analysed by GLC using a 2 m $\times$ 2 mm I.D. column packed with Supelco SP 2310 and a Hewlett Packard 5710A gas chromatograph equipped with a 3390A recording integrator (24).

Marmosets were sacrificed at the end of the experiment by anaesthetising with saffan (Glaxo Laboratories, Middlesex, UK) and exsanguinating the marmosets *via* the abdominal aorta. Blood was collected into heparin and EDTA containers for haematology, platelet and plasma analyses. Erythrocytes from heparinised samples were assayed for erythrocyte fragility using 2.0% hydrogen peroxide (25). The rectus femoris muscle from the right hind limb was cut at its attachments and weighed for each animal. The liver was collected into liquid nitrogen and stored at -80°C for subsequent malondialdehyde assay (26). The aorta was removed along its abdominal length, cleaned up and discs (2 mm diameter) prepared. These were incubated for 30 min in Tyrodes buffer containing glucose, the reaction stopped with 1 N HCl and the stable hydrolysis product of prostacyclin, 6-keto $\text{PGF}_{1\alpha}$ assayed by RIA (17).

Statistical analysis. The experimental data were analysed by regression analysis of a 2×2 factorial experiment. Because of the nature of the platelet aggregation data, a non-parametric technique, the Kruskal-Wallis procedure for 4 independent samples, was used for each concentration of collagen.

RESULTS

The influence of 9 months feeding of experimental diets $-E$, $+E$, SSO $-E$ and SSO on body weights and organ weights is shown in Table 1. There was a gain in body weight of all groups, with means of 15 g for $-E$, 40 g for $+E$, 3.5 g for SSO $-E$ and 21.5 g for SSO for the 9 month period. In this respect the poorest gains were to be seen in the vitamin E-deficient groups, with the SSO $-E$ group showing virtually no gain. These changes were similar for the skeletal muscle mass with the lowest weight in the SSO $-E$ group. Heart weights however were not significantly affected. Adrenals were heavier in the SSO supplemented marmosets, while for other organs there were no significant changes. The poor gains in the SSO and SSO $-E$ diet groups were associated with the death of 2 animals, with sudden and significant loss of body weight prior to death. One other animal died from fighting in the SSO $-E$ group, such that there were at the end of the experiment 4 marmosets in the SSO $-E$ group and 5 in the SSO group.

The effect of the diets on plasma tocopherol, plasma lipids and platelet tocopherols is shown in Table 2. There were significantly lower tocopherol concentrations in the plasma and platelets of vitamin E-deficient groups. The stripped SSO group showed the lowest plasma tocopherol concentration, being less than half of the vitamin E supplemented group (SSO). The platelets reflected these differences to a degree, although the $-E$ group showed the lowest platelet tocopherol values. The triglyceride concentration was significantly lower in the SSO supplemented marmosets being lowest in the stripped SSO group. Although slightly

Table 1. The influence of vitamin E and SSO supplements on marmoset body and organ wet weights.

Treatments (n)	Body weight (g)	Heart (g)	Liver (g)	Spleen (g)	Skeletal muscle (g)	Kidneys (g)	Adrenals (g)
-E	322 ± 3*	1.91 ± 0.11	10.7 ± 0.8	0.54 ± 0.04	1.36 ± 0.05 ^a	1.88 ± 0.08	0.123 ± 0.009 ^a
+E	344 ± 11 ^a	2.09 ± 0.09	12.1 ± 0.8	0.57 ± 0.10	1.54 ± 0.08 ^b	2.04 ± 0.10	0.127 ± 0.020
SSO - E	306 ± 16 ^b	1.85 ± 0.16	10.4 ± 1.1	0.74 ± 0.22	1.28 ± 0.10 ^a	1.92 ± 0.11	0.160 ± 0.014 ^b
SSO	333 ± 22	1.87 ± 0.11	10.4 ± 1.0	0.59 ± 0.12	1.48 ± 0.13 ^b	1.77 ± 0.19	0.150 ± 0.006 ^b

*Mean ± SEM. The following comparison (*F* test) indicates a significant difference ($p < 0.05$): ^aversus^b. Other means were not different from each other. - E is vitamin E-deficient, + E is vitamin E replete, SSO - E is added stripped SSO and SSO, vitamin E replete SSO added.

Table 2. Plasma and platelet tocopherol concentrations and plasma lipid concentrations of marmosets on vitamin E-deficient and supplemented diets.

Dietary treatments	(n)	Plasma			Platelets	
		α -Tocopherol ($\mu\text{g}/\text{dl}$)	Triglyceride (mg/dl)	Cholesterol (mg/dl)	$\frac{\alpha\text{-Tocopherol}}{\text{Cholesterol}}$ ($\mu\text{g}/\text{mg}$)	α -Tocopherol ($\mu\text{g}/10^9$ platelets)
-E	(6)	$254 \pm 31^{*,a}$	47.8 ± 4.4^a	114 ± 6	2.21 ± 0.20^a	0.10 ± 0.03^a
+E	(6)	578 ± 72^b	50.0 ± 5.1^a	137 ± 8	4.17 ± 0.32^b	0.31 ± 0.02^b
SSO-E	(6)	223 ± 32^a	26.3 ± 4.8^b	115 ± 12	2.10 ± 0.36^a	0.16 ± 0.03^a
SSO	(6)	615 ± 78^b	36.4 ± 1.3^b	111 ± 8	5.59 ± 0.75^b	0.35 ± 0.07^b

*Mean $\pm$ SEM. The following comparison (*F* test) indicates a significant difference ($p < 0.05$): ^a*versus* ^b. Other means were not different from each other.

Table 3. The comparative proportions of phospholipid linoleic (18:2 ω 6) and arachidonic (20:4 ω 6) fatty acids of 3 tissues from marmosets on the vitamin E and SSO supplemented diets.

Dietary treatments	18:2 ω 6 (%)	20:4 ω 6 (%)
Plasma		
-E	36.2 ± 0.6^a	4.4 ± 0.3
+E	37.3 ± 1.1^a	3.3 ± 0.3
SSO-E	47.0 ± 2.1^b	4.1 ± 0.7
SSO	49.8 ± 1.2^b	2.9 ± 0.5
Platelets		
-E	6.0 ± 0.2^a	19.5 ± 0.1
+E	6.2 ± 0.4^a	17.8 ± 0.6
SSO-E	8.4 ± 0.9^b	17.7 ± 0.9
SSO	10.1 ± 0.3^b	18.1 ± 0.3
Aorta		
-E	12.1 ± 1.1^a	17.3 ± 1.0
+E	10.9 ± 0.9^a	18.3 ± 1.1
SSO-E	14.9 ± 2.1^b	16.5 ± 1.2
SSO	15.1 ± 1.4^b	16.6 ± 1.1

Each value is the mean $\pm$ SEM of 4 marmosets per treatment. The following comparison (*F* test) indicates a significant difference ($p < 0.05$): ^a*versus* ^b. Other means were not different from each other.

elevated in the +E group, plasma cholesterol concentrations were not significantly different between groups.

The proportional concentration of phospholipid fatty acids linoleic acid (18:2) and arachidonic acid (20:4) in plasma, platelet and aortas are shown in Table 3.

Table 4. Haematological parameters measured in blood from marmosets on the vitamin E and SSO supplemented diets.

Dietary treatments (n)	White blood cell		Platelet count ($\times 10^3/\mu\text{l}$)	Erythrocytes		
	Count ($\times 10^3/\mu\text{l}$)	Ratio (Neutrophils/ Lymphocytes)		Packed cell volume (%)	Haemoglobin (g/dl)	% Haemolysis (2% H_2O_2)
-E	7.3 $\pm$ 0.5 ^{a,*}	0.67 $\pm$ 0.15	463 $\pm$ 40	40.7 $\pm$ 2.3 ^a	13.3 $\pm$ 0.6	84 $\pm$ 8.4 ^a
+E	5.2 $\pm$ 0.4 ^b	0.80 $\pm$ 0.20	454 $\pm$ 18	47.0 $\pm$ 0.4 ^b	14.8 $\pm$ 0.9	9.5 $\pm$ 3.0 ^b
SSO-E	7.0 $\pm$ 1.5 ^a	1.09 $\pm$ 0.39	421 $\pm$ 55	41.0 $\pm$ 2.3 ^a	13.5 $\pm$ 1.0	63.6 $\pm$ 15.0 ^a
SSO	5.9 $\pm$ 0.8 ^b	0.51 $\pm$ 0.12	481 $\pm$ 51	44.9 $\pm$ 1.2 ^b	15.3 $\pm$ 0.4	5.3 $\pm$ 2.3 ^b

*Mean $\pm$ SEM. The following comparisons (*F* test) indicated a significant difference ($n < 0.05$): ^aversus^b.

Table 5. Platelet aggregation and thromboxane generation from platelet rich plasma using acid soluble collagen at 3 concentrations of agonist.

Dietary treatments	(n)	Platelet aggregation (% of max)			TXB ₂ generated at 6.3µg/ml ASC (µg/10 ⁹ platelets)	
		Acid soluble collagen (µg/ml)				
		1.6	3.1	6.3		
–E	(6)	26±16	59±19	88±5	}*	146±32
+E	(5)	17±13	61±8	72±3		226±30
SSO–E	(5)	22±13	71±32	94±4		161±23
SSO	(5)	0	49±15	82±5		150±38

*Since the assumption that aggregation data are normally distributed is somewhat dubious a non-parametric technique (*viz.* the Kruskal-Wallis procedure for 4 independent samples) was used at each concentration of collagen. This test was significant at $p < 0.05$, indicating a probable systematic difference between groups at 6.3 µg/ml collagen.

The 18:2 proportions were significantly increased in all three tissues by the SSO supplementation, while 20:4 was elevated (33–41%) in plasma phospholipids of vitamin E-deficient diets (non significant), an effect which was reflected in the platelets of –E marmosets but not of SSO – E marmosets. This latter group showed the lowest and most variable 20:4 level in platelets. The aortic phospholipid fatty acids showed less variable and not significant changes.

The influence of the diets after 9 months on the erythrocyte packed cell volume and erythrocyte peroxidative index assessed by exposure to 2% H₂O₂ is shown in Table 4. There was a significant elevation in peroxidative haemolysis (%) in the vitamin E-deficient groups relative to their controls which was reflected in the PCV. Haemoglobin concentration was also reduced but not significantly in these vitamin E-deficient groups. The white blood cell count was also significantly influenced by vitamin E status, the count being raised in the deficient groups. In the SSO – E group this was associated with a higher neutrophil to lymphocyte ratio (1.09) than in the SSO group (0.51) which is the value normally encountered (21). Platelet counts showed no significant change as a result of the diets.

The influence of dietary treatments on platelet aggregation in marmosets is shown in Table 5. There was a significant increase ($p < 0.05$) in platelet aggregation (expressed as a % of maximum) associated with vitamin E deficiency, the greatest response being observed in the SSO – E marmosets. By comparison the SSO group showed relative resistance of platelets to collagen stimulation. Thromboxane (TXB₂) generated during stimulation of platelet rich plasma at 6.3 µg/ml acid soluble collagen is also shown. There was a reduction in TXB₂ for all treatments –E, SSO, and SSO – E relative to the +E group, the difference between –E and +E being greatest ($0.05 < p < 0.1$).

The influence of diets on prostacyclin (measured as the stable metabolite 6-keto

Fig. 1. The influence of vitamin E and SSO supplements on marmoset aortic prostacyclin generation (expressed as ng 6-keto PGF_{1α}/30 min/2 mm disc of aorta). The means and SEM together with statistical significances (*F* test) for comparisons are shown for each treatment group of marmosets. **p* < 0.05, ****p* < 0.001.

PGF_{1α}) generation from the aorta is shown in Fig. 1. Both E supplemented groups were significantly higher than their E-deficient controls; SSO addition also had a significant effect on prostacyclin production independent of the vitamin E effect, indicating a positive relationship existed between linoleic acid supplementation (as SSO) and prostacyclin production from the marmoset aorta. Compared to the -E group, the effects of linoleic acid and vitamin E supplementation appeared to be additive in effect, albeit the SSO-E group was not significantly greater than the +E group in prostacyclin production.

Malondialdehyde was measured in samples of marmoset liver collected into liquid nitrogen when the animals were killed. The following values were obtained -E, 0.45 ± 0.03 nmol/mg prot; +E, 0.33 ± 0.01 , +SSO, 0.40 ± 0.03 , and +SSO-E, 0.37 ± 0.03 . These results showed a significant decrease (*p* < 0.01) of peroxidised fatty acids in the liver of deficient marmosets when supplemented with vitamin E.

DISCUSSION

The vitamin E-deficient diet used in this study achieved a significant reduction in plasma and platelet α -tocopherol concentrations, but not to levels associated with gross pathology in primates (27-29). The diet composition in other respects was not significantly different from that used for a normal marmoset diet; barley was replaced by oats, because of the latter's lower vitamin E level. The diet was well accepted by the marmosets and there were normal gains in body weight when supplemented with vitamin E to normal dietary level (40 μ g/g vitamin E). This is

consistent with dietary recommendations for the laboratory rat (15, 30). The influence of vitamin E deficiency on the marmoset was unequivocal: a reduction in body weight resulting from lower skeletal muscle weights, as shown by its influence on the rectus femoris muscle. There was no evidence in these marmoset's hearts of loss of weight or pathological change, as has been reported in pigs (1) and baboons (34) with vitamin E deficiency. This may however be a reflection of the level of deficiency achieved or the dietary selenium status which in this case was more than adequate for requirements. Erythrocytes were considerably more fragile, as shown by the peroxidative index, and this was expressed *in vivo* as a fall in PCV, and a lower haemoglobin concentration (not significant). A similar type of pathology has been observed in marmoset wasting disease, which has been attributed by some workers to deficiencies of protein, vitamin E and/or selenium in the diet (31). The lower PCV could have resulted either from reduced erythropoiesis, as has been seen with vitamin E-deficient primates by others (28) or increased erythrocyte breakdown or both. These changes were accompanied by an increased white blood cell count and a relative neutrophilia in the SSO-E group. This would be interpretable clinically as an inflammatory response and could have been indicative of an altered immunological status and/or enhanced risk of infection associated with a low vitamin E state (30, 32, 33). The platelet count however did not vary with deficiency at 9 months. From these observations it must be concluded that the marmoset is vulnerable to pathological changes characteristic of vitamin E deficiency with a lowering of the tocopherol concentration in plasma and platelets to one half or less. The plasma lipid changes were consistent with previous observations (17), the diets supplemented with linoleate producing a significant fall in triglyceride independent of the vitamin E level. The cholesterol in plasma was not significantly influenced by dietary treatments.

The influence of vitamin E deficiency on platelet aggregation and prostaglandin synthesis by platelets and aorta was clear. The change in platelet reactivity was significant with vitamin E deficiency, the greatest reactivity being observed in the SSO-E group, while with the SSO diet the platelets were relatively unreactive. These changes however were not large, and were subject to considerable animal to animal variability. The data on thromboxane generation did not support the aggregation observations, and was also subject to considerable variability. This lack of correlation with aggregation data is probably explained by the high % aggregation values observed at the concentration studied. Lands *et al.* (35) have observed a similar lack of association when aggregation values exceeded 70% of maximum response. The phospholipid fatty acids in platelets showed evidence of highest and least variable arachidonate levels in the vitamin E-deficient group and the lowest most variable values in the SSO-E group, a contradictory observation in relation to platelet aggregation observations. However as shown by the vulnerable state of these animals, the marmosets on SSO-E diet showed most variable platelet arachidonates and most reactive platelets suggesting enhanced peroxidation in the presence of high polyunsaturated fats and low vitamin E content in the diet.

Prostacyclin production by the aorta appeared to be enhanced with addition of vitamin E and SSO from a low value in the vitamin E deficiency group to a greatly enhanced value with both linoleic acid and vitamin E supplementation (*via* SSO). The stepwise increase in prostacyclin (PGI_2) associated with vitamin E and linoleic acid supplements suggests that vitamin E supplements and linoleate both contribute together (but not linoleate alone) in improving the blood vessel's protection from thrombosis *via* this antiaggregating prostaglandin. In so far as prostacyclin is generated from specific classes of phospholipids (phosphatidyl inositol and/or phosphatidyl choline), and then is only one end product of arachidonate metabolism, it is unrealistic to expect total phospholipid arachidonate to reflect enhanced prostacyclin generation. The reduced levels of arachidonate in tissues associated with high linoleate diets have been reported previously (20, 22). Other aspects of SSO supplementation at the level used in this study should not be overlooked. The deaths in these SSO supplemented groups were associated with poor body weight gains, an observation which has been seen in some similar experiments with marmosets, particularly when 15% supplements of SSO were used (unpublished data). In this regard 10% (or 12%) of SSO, the levels used in these and related experiments, is probably excessive and should be reduced in future experiments, particularly if the base diet already contains 8% fat. When at the same time vitamin E was low, the peroxidative influence of a high unprotected PUFA intake was clearly manifest in these marmosets.

In summary, these studies have shown that vitamin E deficiency in the marmoset is associated, even at the marginally deficient levels used here, with some significant pathology, and evidence of reduced cardiovascular protection. In this study this was primarily seen as enhanced platelet reactivity and as lowered endothelial prostacyclin production. The altered white blood cell picture suggests an active inflammatory response to E deficiency or a failure to marginate, an observation which deserves further investigation, given the interest in vitamin E and the immunological system (32) and the potential value of white blood cell count as a measure of risk factor in heart disease (36). Diets in which vitamin E levels are low for whatever reason, and which provide high levels of unprotected PUFA represent a significant risk to primates, and may if left unchecked result in the development of some significant vascular pathology. Studies involving the addition of polyunsaturated fats to diets often fail to adequately address the separate influence of vitamin E which accompanies the fat at very variable concentrations. Given its basic role in biological membrane structure and function (37) and as an antioxidant this seems a significant oversight.

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REFERENCES

- 1) Nafstad, I. (1974): Endothelial damage and platelet thrombosis associated with PUFA rich, vitamin E deficient diet fed to pig. *Thrombosis Res.*, **5**, 251–258.
- 2) Lake, A. M., Stuart, Mj., and Oski, F. A. (1977): Vitamin E deficiency and enhanced platelet function: Reversal following E supplementation. *J. Pediatr.*, **90**, 722–725.
- 3) Machlin, L. J., Filipski, R., Willis, A. L., Kuhn, D. C., and Brin, M. (1975): Influence of vitamin E on platelet aggregation and thrombosis in the rat. *Proc. Soc. Exp. Biol. Med.*, **149**, 275–277.
- 4) Panganamala, R. V., and Cornwell, D. G. (1982): The effects of vitamin E in arachidonic acid metabolism, in *Vitamin E: Biochemical Haematological and Clinical Aspects*, ed. by Lublin, B., and Machlin, L. J., *Ann. N.Y. Acad. Sci.*, **393**, 376–391.
- 5) Hamelin, S. St. J., and Chan, A. C. (1983): Modulation of platelet thromboxane and malonaldehyde by dietary vitamin E and linoleate. *Lipids*, **18** (3), 267–269.
- 6) Karpen, C. W., Pritchard, K. A., Arnold J. H., Cornwell D. G., and Panganamala, R. V. (1982): Restoration of prostacyclin/thromboxane A₂ balance in the diabetic rat. Influence of dietary vitamin E. *Diabetes*, **31**, 947–951.
- 7) Gryglewski, R. J., Denbinska-Kiec, A., Zmuda, A., and Gryglewska, T. (1978): Prostacyclin and thromboxane A₂ biosynthesis capacities of heart arteries and platelets at various stages of experimental atherosclerosis in rabbits. *Atherosclerosis*, **31**, 385–394.
- 8) Okuma, M., Takayoma, H., and Uchino, H. (1980): Generation of prostacyclin-like substance and lipid peroxidation in vitamin E deficient rats. *Prostaglandins*, **19**, 527–534.
- 9) Jorgensen, K. A., and Dyerberg, J. (1983): Platelets and atherosclerosis, in *Advances in Nutritional Research*, ed by Draper, H. H., Plenum Press, New York and London, pp. 57–75.
- 10) Giani, E., Masi, I., and Galli, C. (1985): Heated fat, vitamin E and vascular eicosanoids. *Lipids*, **20** (7), 439–448.
- 11) Kanofsky, J. D., and Kanofsky, P. B. (1981): Prevention of thromboembolic disease by vitamin E. *New Eng. J. Med.*, **305**, 173–174.
- 12) DeDeckere, E. A. M., Nugteren, D. H., and Ten Hoor, F. (1980): Influence of type of dietary fat on the prostaglandin release from isolated rabbit and rat hearts and from rat aortae. *Prostaglandins*, **17**, 945–955.
- 13) Hornstra, G. (1982): *Dietary Fats, Prostanoids and Arterial Thrombosis*, Martinus Nijhoff Publishers, The Hague.
- 14) Scott, M. L. (1978): Vitamin E. The fat soluble vitamins, in *Handbook of Lipid Research*, Vol. 2., ed. by DeLuca, H. F., University of Wisconsin, Plenum Press, New York and London, pp. 133–209.
- 15) Buckingham, K. W. (1985): Effect of dietary polyunsaturated/saturated fatty acid ratio and dietary vitamin E on lipid peroxidation in the rat. *J. Nutr.*, **115**, 1425–1435.
- 16) McIntosh, G. H., and Looker, J. W. (1982): Development of a marmoset colony in Australia Lab. *Anim. Sci.*, **32**, 677–679.
- 17) McIntosh, G. H., McMurchie, E. J., James, M., Lawson, C. A., Bulman, F. H., and Charnock, J. S. (1987) The influence of dietary fats on platelet aggregation and prostaglandin production in the common marmoset. *Arteriosclerosis*, **7**, 159–165.
- 18) Charnock, J. S., McLennan, P. A., McIntosh, G. H., Barnden, L. R., and Buttfeld, H. (1987) The influence of dietary lipid supplements on cardiac function in the marmoset (*Callithrix jacchus*). *Cardiovascular Res.*, **21**, 369–375.

- 19) Charnock, J. S., McIntosh, G. H., Abeywardena, M. Y., and Russell, G. R. (1985): Changes in the fatty acid composition of the cardiac phospholipids of the marmoset *Callithrix jacchus* after feeding different lipid supplements. *Annu. Nutr. Metab.*, **29**, 83–94.
- 20) Mohri, K., Dohmoto, C., Iresu, H., and Igarashi, O. (1983): A simple elimination method of vitamin E from vegetable and fish oils for the preparation of vitamin E deficient diet. *J. Jpn. Soc. Food Nutr.*, **36**, 122–124.
- 21) McIntosh, G. H., Lawson, C. A., Rodgers, S. E., and Lloyd, J. V. (1985): Haematological characteristics of the common marmoset. *Res. Vet. Sci.*, **38**, 109–114.
- 22) Hatam, L. H., and Kayden, H. J. (1979): A high performance liquid chromatographic method of the determination of tocopherol in plasma and cellular elements of the blood. *J. Lipid Res.*, **20**, 639–645.
- 23) Kaneda, T., Nakajima, A., Fujimoto, K., Kobayashi, T., Kiriyama, S., Ebihara, K., Innami, T., Tsuji, K., Tsuji, E., Kinumaki, T., Shimma, H., and Yoneyama, S. (1980): Quantitative analysis of cholesterol in foods by gas-liquid chromatography. *J. Nutr. Sci. Vitaminol.*, **26**, 497–505.
- 24) McMurchie, E. J., Gibson, R. A., Charnock, J. S., and McIntosh, G. H. (1984): A comparison of mitochondrial respiration and membrane lipid composition in the rat and marmoset following dietary lipid supplementation. *Comp. Biochem. Physiol.*, **78B**, 817–826.
- 25) Kameda, K., Imai, M., and Senjo, M. (1985): The effect of vitamin E deficiency on some erythrocyte membrane properties. *J. Nutr. Sci. Vitaminol.*, **31**, 481–490.
- 26) Satoh, A. (1980): Serum lipid peroxide in cerebrovascular disorders determined by a new calorimetric method. *Clin. Chim. Acta*, **90**, 37–43.
- 27) Hayes, K. C. (1974): Pathophysiology of vitamin E deficiency in monkeys. *Am. J. Clin. Nutr.*, **27**, 1130–1140.
- 28) Fitch, C. D., Brown, G. O., Chou, A. C., and Gallagher, M. I. (1980): Abnormal erythropoiesis in vitamin E-deficient monkeys. *Am. J. Clin. Nutr.*, **33**, 1251–1258.
- 29) Ausman, L. M., and Hayes, K. C. (1974): Vitamin E deficiency anaemia in old and New World monkeys. *Am. J. Clin. Nutr.*, **27**, 1141–1151.
- 30) Bendrich, A., Gabriel, E., and Machlin, L. J. (1986): Dietary vitamin E requirement for optimum immune responses in the rat. *J. Nutr.*, **116**, 675–681.
- 31) Chalmers, D. T., Murgatroyd, L. B., and Wadsworth, P. F. (1983): A survey of the pathology of marmosets (*Callithrix jacchus*) derived from a marmoset breeding unit. *Lab. Anim.*, **17**, 270–279.
- 32) Tengerdy, R. P., Mathias, M. M., and Nockels, C. F. (1984): Effect of vitamin E on immunity and disease resistance, in *Vitamins, Nutrition, and Cancer*, ed. by Prasad, K., Karger, Basel, Switzerland, pp. 123–133.
- 33) Bendich, A., D'Apolito, P., Gabriel, E., and Machlin, L. J. (1984): Interaction of dietary vitamin C and vitamin E on guinea pig immune responses to mitogens. *J. Nutr.*, **114**, 1588–1593.
- 34) Liu, S. K., Dolensek, E. P., Tappe, J. P., Stover, J., and Adams, C. R. C. (1984): Cardiomyopathy associated with vitamin E deficiency in seven gelada baboons. *J. Am. Vet. Med. Assoc.*, **185**, 1347–1350.
- 35) Lands, W. E. M., Culp, B. R., Hirai, A., and Gorman, R. (1985): Relationship of thromboxane generation to the aggregation of platelets from humans. *Prostaglandins*, **30**, 819–825.
- 36) Grimm, R. H., Neaton, J. D., and Ludwig, W. (1985): Prognostic importance of the

- white blood cell count for coronary, cancer, and all-cause mortality. *JAMA* **254**, 1932–1937.
- 37) McMurchie, E. J., and McIntosh, G. H. (1986): Thermotropic interaction of vitamin E with dimyristoyl and dipalmitoyl phosphatidylcholine liposomes. *J. Nutr. Sci. Vitaminol.*, **32**, 551–558.