Dietary α-Tocopherol Attenuates the Impact of γ-Tocotrienol on Hepatic 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase Activity in Chickens[1,2]

ASAF A. QURESHI,*[3] BRADLEY C. PEARCE,† ROSNAH M. NOR,** A. GAPOR,** DAVID M. PETERSON‡ AND CHARLES E. ELSON††

*Advanced Medical Research, Madison, WI 53719, †Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, CT 06492, **Palm Oil Research Institute, Kuala Lumpur, Malaysia, ‡Cereal Crops Research, Agriculture Research Service, U.S. Department of Agriculture, Madison, WI 53705 and ††Department of Nutritional Sciences, University of Wisconsin, Madison, WI 53706

ABSTRACT The concentration-dependent impact of γ-tocotrienol on serum cholesterol can be traced to the posttranscriptional down-regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity. γ-Tocotrienol also suppresses tumor growth. Palmvitee, the tocopherol and tocotrienol-rich fraction of palm oil, is the sole commercial source of γ-tocotrienol. Contrary to the universal findings of the efficacy of γ-tocotrienol there are conflicting reports of the impact of Palmvitee on 3-hydroxy-3-methylglutaryl coenzyme A reductase activity, serum cholesterol concentrations and tumor development. These conflicting reports led us to examine the impact of α-tocopherol on the cholesterol-suppressive action of γ-tocotrienol. Control and experimental diets were fed to groups of White Leghorn chickens (n = 10) for 26 d. The control diet was supplemented with 21 nmol α-tocopherol/g. All experimental diets provided 141 nmol of blended tocols/g diet. The α-tocopherol and γ-tocotrienol concentrations of the experimental diets ranged from 21 to 141 and 0 to 120 nmol/g, respectively. We now report that including α-tocopherol in tocol blends containing adequate γ-tocotrienol to suppress 3-hydroxy-3-methylglutaryl coenzyme A reductase activity results in an attenuation of the tocotrienol action ($P < 0.001$). A summary of results from studies utilizing different Palmvitee preparations shows that effective preparations consist of 15–20% α-tocopherol and ~60% γ- (and δ-) tocotrienol, whereas less effective preparations consist of ≥30% α-tocopherol and 45% γ- (and δ-) tocotrienol. J. Nutr. 126: 389–394, 1996.

INDEXING KEY WORDS:

- *γ-tocotrienol* • *α-tocopherol*
- *3-hydroxy-3-methylglutaryl coenzyme A reductase*
- *cholesterol* • *chickens*

The tocotrienols modulate the intracellular mechanism for the controlled degradation of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (EC 1.1.1.34) (Parker et al. 1993), the enzyme considered to be rate limiting for cholesterol synthesis (Goldstein and Brown 1990). The action of the tocotrienols resembles that of the the nonsterol component, believed to be farnesol (Correll et al. 1994), of the multivalent feedback regulation of mevalonate synthesis (Goldstein and Brown 1990). The tocols differ in their mevalonate-suppressive potency; d-γ- and d-δ-tocotrienol are fourfold more potent than d-α-tocotrienol in suppressing cholesterol synthesis in rat hepatocytes, whereas dietary d-α-tocopherol increases avian hepatic HMG-CoA reductase activity (Pearce et al. 1992). The inhibitory actions of the tocotrienol-rich fraction of palm oil (TRF and Palmvitee) prepared and distributed to researchers by the Palm Oil Research Institute of Malaysia and the binary mixtures of tocotrienols tested by Pearce et al. (1992) reflected the additive actions of the individual constituents. The tocotrienols produce a dose-dependent suppression of HMG-CoA reductase activity in hepatocytes. On the other hand, animal studies show that the impact of the tocotrienols on HMG-CoA reductase activity and cholesterol level reaches a plateau (Pearce et al. 1992, Qureshi et al. 1986) and then wanes as the dose is further increased (Khor et al. 1995).

The tocotrienols also suppress the growth of tumors (Guthrie et al. 1994, Komiyama et al. 1989) and inhibit

[1] In part supported by Bristol-Myers Squibb Pharmaceutical Research Institute and the College of Agricultural and Life Sciences, University of Wisconsin, Madison, WI.

[2] The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

[3] To whom correspondence should be addressed.

Manuscript received 17 May 1995. Initial review completed 6 June 1995. Revision accepted 17 October 1995.

TABLE 1

Diet composition

Component	Amount
	g
Ground yellow corn	665
Soybean meal (44% protein)	250
Meat scraps (50% protein)	50
Alfalfa meal (17% protein)	10
Dicalcium phosphate	10
Calcium carbonate	5
Vitamin and mineral mix[1]	10

[1] Provides per kg diet: retinyl acetate, 4 mg; cholecalciferol, 5 μg; *all-rac*-α-tocopheryl acetate, 10 mg; menadione, 5 mg; choline, 1.3 g; thiamin, 1.8 mg; niacin, 27 mg; riboflavin, 3.6 mg; pyridoxine, 3 mg; calcium pantothenate, 10 mg; vitamin B-12, 10 μg; NaCl, 2 g; $ZnSO_4$, 50 mg; and MnO_2, 50 mg.

tumor promotion (Goh et al. 1994). α-Tocopherol has no impact on cholesterol concentration (Pearce et al. 1992), tumor growth (Guthrie et al. 1994, Komiyama et al. 1989) or tumor promotion (Goh et al. 1994, Tan 1992). We postulate that the suppression of HMG-CoA reductase activity underlies the hypocholesterolemic and tumor-suppressive actions of the tocotrienols (Elson 1995, Elson and Qureshi 1995).

In our initial report we suggested that the ratio of the individual tocol constituents of a cereal grain, as well as its tocotrienol content, plays a role in determining the impact of that grain on cholesterol levels (Qureshi et al. 1986). These considerations prompted us to evaluate the impact of dietary α-tocopherol on the cholesterol-suppressive action of γ-tocotrienol.

MATERIALS AND METHODS

One group (n = 10) of 2-wk-old White Leghorn females (Poultry Research Laboratory, University of Wisconsin-Madison) was fed a commercial corn-soybean meal diet formulated with 21 nmol α-tocopherol/g (**Table 1**). The six experimental groups were fed the control diet supplemented with 120 nmol tocols/g: α-tocopherol, γ-tocotrienol or one of four binary blends of γ-tocotrienol and α-tocopherol. The experimental diets provided a total of 141 nmol tocols/g (**Table 2**). The birds were housed by group in brooders at the UW Poultry Research Laboratory. Following a 24-d feeding period the birds were deprived of food for 40 h, refed for 48 h and deprived of food for 10 h before killing by CO_2 overdose. The food deprivation and refeeding segment of this regimen was performed to induce hepatic HMG CoA reductase activity, and the terminal food deprivation was to facilitate chylomicron and VLDL clearance. Blood was collected and processed for serum. Livers were removed, rinsed and weighed, and microsomal fractions were obtained by centrifugation as previously described (Qureshi et al. 1986). The serums and microsomal fractions were held at −20°C for analysis.

Serum cholesterol concentrations were evaluated with a diagnostic kit (Catalog #352) purchased from Sigma Chemical, St. Louis, MO. The LDL and VLDL lipoproteins were precipitated from the serum as described by Assman et al. (1983), and the supernatant was analyzed for HDL cholesterol. All serum assays were performed in triplicate, and average values for each bird were used in calculating group means and standard errors. Microsomal fractions were assayed in triplicate for HMG-CoA reductase

TABLE 2

Impact of changes in γ-tocotrienol and α-tocopherol blend on avian hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase activity and serum cholesterol concentration[1]

Blend			HMG-CoA	Cholesterol	
γ-tocotrienol	α-tocopherol	Total tocols	reductase activity	Total	HDL
	nmol/g		*pmol mevalonate/(mg microsomal protein · min)*	mmol/L	
Control		21	327[b]	4.71[b]	2.92[ab]
0	120	141	351[a]	4.86[a]	2.97[a]
60	60	141	275[c]	4.52[c]	2.82[c]
72	48	141	271[c]	4.50[c]	2.84[bc]
84	36	141	275[c]	4.11[d]	2.84[bc]
96	24	141	270[c]	3.98[de]	2.87[bc]
120	0	141	223[d]	3.90[e]	2.82[c]
Pooled SEM			6.11	0.045	0.070
ANOVA (P value)			0.0001	0.0001	0.009

[1] Values are means, n = 10.
[a–e] Values in columns with different superscripts are significantly different, $P < 0.025$.

activity (Phillipp and Shapiro 1979) as previously described (Fitch et al. 1989). Aquasol scintillation solution and DL-3-hydroxy-3-methyl-(3-^{14}C)glutaryl-CoA were purchased from New England Nuclear, Boston, MA. Other chemicals were of analytical grade. Pure preparations of α-tocopherol and γ-tocotrienol were generously provided by Bristol-Myers Squibb, Wallingford, CT.

The protocol was reviewed and approved by the University of Wisconsin-Madison College of Agricultural and Life Sciences Animal Care Committee.

StatView software (Abacus Concepts, Berkeley, CA) was used for the analysis of treatment-mediated effects. Treatment-mediated differences in HMG-CoA reductase activity and serum lipid constituents were identified with ANOVA and Fisher's Protected Least Significant Difference test (Abacus Concepts 1992). Differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Treatment effects on hepatic HMG-CoA reductase activity and serum total and HDL cholesterol concentrations (Table 2) were significant ($P < 0.009$). Increasing dietary tocols to 141 nmol/g with the addition of 120 nmol α-tocopherol/g modestly increased hepatic HMG-CoA reductase activity ($P < 0.001$) and serum total cholesterol concentration ($P < 0.002$). Increasing dietary tocols to 141 nmol/g with 120 nmol γ-tocotrienol produced 35% lower ($P < 0.001$) HMG-CoA reductase activity and concomitantly lower total ($P < 0.001$) and HDL ($P < 0.03$) cholesterol concentrations compared with the values recorded for the group that was fed the diet supplemented with 141 nmol α-tocopherol/g. These results confirm reports that under the conditions of this study, tocopherols increase and tocotrienols decrease HMG-CoA reductase activity (Parker et al. 1993, Pearce et al. 1992, Qureshi et al. 1989). Increasing the dietary tocols to 141 nmol/g with the binary treatment blends (50–80% γ-tocotrienol; 50–20% α-tocopherol, see Table 2) uniformly suppressed HMG-CoA reductase activity by 22%. Hepatic HMG-CoA reductase activities in each of the groups receiving a binary treatment blend were lower than the activities recorded for the control and high tocopherol groups and higher than the activity in the high tocotrienol group (all $P < 0.001$). This response pattern is shown in **Figure 1**. Also plotted in Figure 1 are data from our earlier study (Pearce et al. 1992).

Both studies evaluated the concentration-dependent impact of γ-tocotrienol on hepatic HMG-CoA reductase activities in White Leghorns. In the first study (Pearce et al. 1992) the birds were exposed to a food deprivation and refeeding regimen immediately before killing, a protocol designed to maximally induce HMG-CoA reductase activity, whereas the protocol in this study employed a terminal food deprivation. Figure 1 shows that the control HMG-CoA reductase activity under the deprivation and refeed regimen (Pearce et al. 1992) is double that observed under the deprivation, refeed and deprivation regimen (Fig. 1 and Table 2). Despite this difference in the protocols, both studies showed significant γ-tocotrienol-mediated decreases in HMG-CoA reductase activity. In our first study (Pearce et al. 1992) we maintained a constant level of dietary α-tocopherol (21 nmol/g) while providing γ-tocotrienol in increments of 0, 36, 73 and 109 nmol γ-tocotrienol/g diet (0, 63, 78 and 84% of the tocols). In the current study (Table 2) we maintained a constant level of dietary tocols (141 nmol/g) with γ-tocotrienol (0, 60, 72, 84, 96 and 120 nmol/g diet; 0, 42, 51, 60, 68, and 85% of the tocols) displacing an equivalent amount of α-tocopherol. γ-Tocotrienol provided 0, 43, 51, 60, 68 and 85% of the dietary tocols. The earlier study (Pearce et al. 1992) showed that increasing dietary γ-tocotrienol from 36 to 73 nmol/g diet (63 to 78% of tocols) caused a reduction in HMG-CoA reductase activity, whereas in the present report we show that increasing dietary γ-tocotrienol from 60 to 96 nmol/g diet (43 to 68% of tocols) had no significant impact on reductase activity (Fig. 1). The two plots are consistent in showing that tocol blends comprised of more than 70% γ-tocotrienol were effective in lowering HMG-CoA reductase activity relative to control activities.

Differences in serum total ($r^2 = 0.7$) and HDL ($r^2 = 0.8$) cholesterol concentrations paralleled treatment-mediated differences in HMG-CoA reductase activity (Table 2).

Our findings may be relevant to the interpretation of the results of human (Qureshi et al. 1991b, 1995, Tan et al. 1991, Walqvist et al. 1992) and experimental animal (Gould et al. 1991, Hood and Sidhu 1992, Khor et al. 1995, Pearce et al. 1992, Qureshi et al. 1991a, Tan 1992) studies that used Palmvitee as the source of tocotrienols. Some investigators report significant Palmvitee-mediated lowerings of HMG-CoA reductase activity (Khor et al. 1995, Pearce et al. 1992, Qureshi et al. 1991a) and serum cholesterol (Hood and Sidhu 1992, Qureshi et al. 1991a, 1991b, Qureshi et al 1995, Tan et al. 1991). Other investigators report that Palmvitee has no impact on either cholesterol (Hood and Sidhu 1992, Wahlqvist et al. 1992) or HMG-CoA reductase activity (Hood and Sidhu 1992). We (Elson 1995, Elson and Qureshi 1995) have postulated that the strong tumor-suppressive action of the tocotrienols (Goh et al. 1994, Guthrie et al. 1994, Komiyama et al. 1989) is coupled to the suppression of HMG-CoA reductase activity. Although Palmvitee had a marginal impact on chemically initiated mammary car-

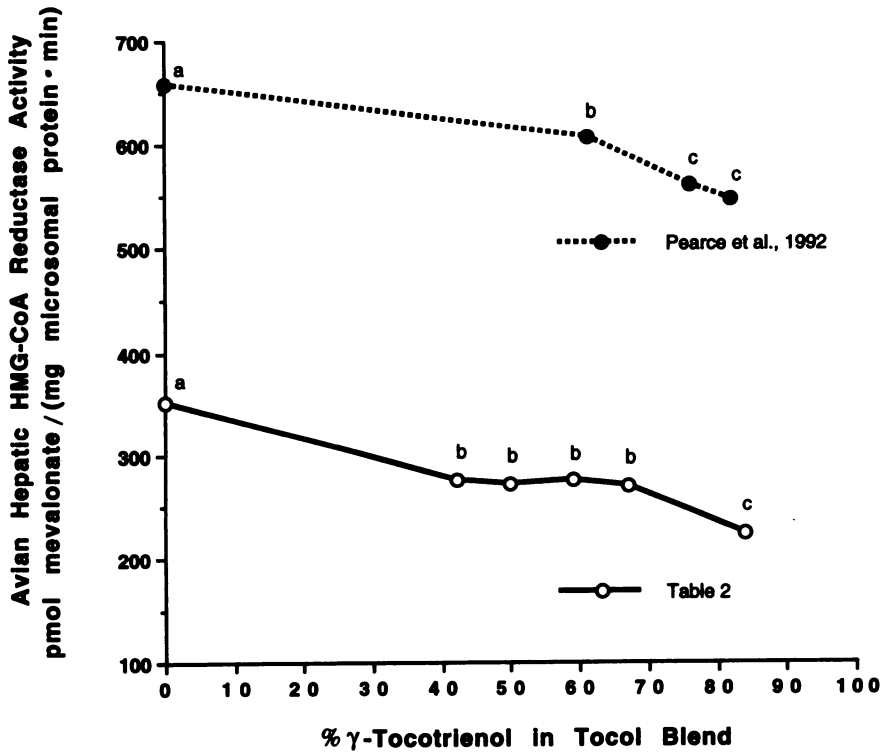

FIGURE 1 The suppression of avian hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-C0A) reductase activity by feeding diets containing blends of α-tocopherol and γ-tocotrienol. Data for the upper curve and statistical analysis are from Pearce et al. 1992 in which chickens (n = 6 per group) were deprived of food and then refed. Those diets contained a constant 21 nmol α-tocopherol/g with increasing amounts of γ-tocotrienol. Data for the lower curve are from Table 2 where the chickens were deprived of food for the terminal 10 h. The tocol concentration of the diets was held to a constant 141 nmol/g, whereas the ratio of γ-tocotrienol to α-tocopherol was varied. Within each study, different letters indicate significant differences, $P < 0.05$.

cinogenesis (Gould et al. 1991), it had a substantial impact on chemically initiated skin carcinogenesis and virally induced lymphoma formation (Tan 1992).

A review of these studies (**Table 3**) shows that Palmvitee preparations low in α-tocopherol concentration were consistently effective in both cholesterol (Khor et al. 1995, Qureshi et al. 1991a, 1991b, and 1995) and tumor (Tan 1992) studies. The Palmvitee preparations relatively high in α-tocopherol concentration (> 30 g/100 g) had no impact on chemically initiated carcinogenesis (Gould et al. 1991). The high α-tocopherol Palmvitee had a significant cholesterol-lowering impact in one of two 4-wk studies with quail. The distinguishing factor appears to be the type of dietary fiber (Hood and Sidhu 1992). The high α-tocopherol Palmvitee had a significant cholesterol-lowering impact in one human study (Tan et al. 1991) but not in a second (Wahlqvist et al. 1992). Gould et al. (1991) fed a very high level of the Palmvitee (3.38 mmol/kg diet) in their studies. Kohr et al. (1995) recently reported that the impact of Palmvitee on HMG-CoA reductase activity is essentially lost when high doses are administered to guinea pigs.

Other factors may be involved. Pearce et al. (1992) reported the dose-dependent impact of a high α-tocopherol Palmvitee preparation on HMG-CoA reductase activity in rat hepatocytes. α-Tocopherol appears to attenuate the tocotrienol effect in vivo but not in isolated liver cells. These findings suggest that transport systems might play a role. Traber and Kayden (1989) report the preferential transport of α-tocopherol in serum lipoproteins. Khor et al. (1995) report the anomalous finding that a tocopherol-free Palmvitee preparation caused a dose-dependent increase in the α-tocopherol content of the guinea pig liver and serum. Tocotrienols were not detected in the serum. Our preliminary analysis found that dietary γ-tocotrienol elevated serum α-tocopherol (data not shown).

The composition of Palmvitee is highly variable. The proportion of α-tocopherol in Palmvitee preparations ranges from 15 g/100 g (Qureshi et al. 1991a) to 44 g/100 g (Hayes et al. 1993). Moreover, the relative proportions of the tocotrienol isomers varies from lot to lot of Palmvitee. The concentration of the low potency α-tocotrienol concentration ranges from 15 to 23 g/100 g and that of the sum of the potent γ- and δ-tocotrienols, from 41 to 68 g/

TABLE 3

Composition of tocol-rich fraction of palm oil (Palmvitee) corrected for nontocol constituents and biological response

Tocopherol	Tocotrienol	Tocotrienols α-	γ-	δ-	Species	Variable evaluated	Response[1]	Reference
39	61	20	27	14	Quail	Cholesterol[2]	NS	Hood and Sidhu 1992
39	61	20	27	14	Quail	Cholesterol[3]	S	Hood and Sidhu 1992
34	66	21	33	12	Rats	Chemical carcinogenesis	NS	Gould et al. 1991
33	67	23	35	9	Rat hepatocytes	HMG CoA reductase	S	Pearce et al. 1992
30	70	23	31	16	Humans	Cholesterol	NS	Walqvist et al. 1992
30	70				Humans	Cholesterol	S	Tan et al. 1991
18	82	15	40	27	Humans	Cholesterol	S	Qureshi et al. 1991b
17	83	23	49	11	Mice	Chemical carcinogenesis	S	Tan 1992[4]
17	83	23	49	11	Mice	Viral lymphoma	S	Tan 1992[4]
15	85	18	43	24	Humans	Cholesterol	S	Qureshi et al. 1995
15[5]	85	17	45	23	Swine	HMG CoA reductase	S	Qureshi et al. 1991a
0	100	43	50	7	Guinea pigs	HMG CoA reductase	S	Khor et al. 1995

[1] Response: S = significant ($P < 0.05$); NS = nonsignificant.
[2] Diet contained 11% α-cellulose.
[3] Diet contained 11% guar gum.
[4] Composition from Tan 1989.
[5] Average composition of two preparations.

100 g. These factors, we believe, account for the conflicting reports of the responses to Palmvitee in light of the uncontested findings of potent γ-tocotrienol effects.

LITERATURE CITED

Abacus Concepts, StatView. (1992) Abacus Concepts, Inc., Berkeley, CA.

Assman, G., Schriewer, H. & Schnitz, G. (1983) Quantification of high-density lipoprotein cholesterol by precipitation with phosphotungstic acid/MgCl2. Clin. Chem. 29: 2026–2030.

Correll, G. C., Ng, L. & Edwards, P. A. (1994) Identification of farnesol as the non-sterol derivative of mevalonic acid required for the accelerated degradation of 3-hydroxy-3-methylglutaryl coenzyme A reductase. J. Biol. Chem. 269: 17390–17393.

Elson, C. E. (1995) Suppression of mevalonate pathway activities by dietary isoprenoids: protective roles in cancer and cardiovascular disease. J. Nutr. 125: 1666S–1672S.

Elson, C. E. & Qureshi, A. A. (1995) Coupling the cholesterol- and tumor-suppressive actions of palm oil to the impact of its minor constituents on 3-hydroxy-3-methylglutaryl coenzyme A reductase activity. Prostaglandins Leukotrienes Essent. Fatty Acids 52: 205–208.

Fitch, M. E., Mangels, A. R., Altmann, W. A., El Hawary, M., Qureshi, A. A. & Elson, C. E. (1989) Microbiological screening of mevalonate-suppressive minor plant metabolites. J. Agric. Food Chem. 37: 687–691.

Goh, S. H., Norhanom, A. W. & Yadav, M. (1994) Inhibition of tumor promotion by various palm-oil tocotrienols. Int. J. Cancer 57: 529–531.

Goldstein, J. L. & Brown, M. S. (1990) Regulation of the mevalonate pathway. Nature (Lond.) 343: 425–430.

Gould, M. N., Haag, J. D., Kennan, W. S., Tanner, M. A. & Elson, C. E. (1991) A comparison of tocopherol and tocotrienol for the chemoprevention of chemically-induced mammary tumors. Am. J. Clin. Nutr. 53: 1068S–1070S.

Guthrie, N., Gapor, A., Chambers, A. F. & Carroll, K. K. (1994) Inhibition of proliferation of MDA-MB-435 human breast cancer cells by individual tocotrienols from palm oil. Proc. Am. Assoc. Cancer Res. 35: 629 (abs.).

Hayes, K. C., Pronczuk, A. & Liang, J. S. (1993) Differences in the plasma transport and tissue concentrations of tocopherols and tocotrienols: observations in humans and hamsters. Proc. Soc. Exp. Biol. Med. 202: 353–359.

Hood, R. L. & Sidhu, G. S. (1992) Effect of guar gum and tocotrienols on cholesterol metabolism on the Japanese quail. Nutr. Res. 12: S117–S127.

Khor, H. T., Chieng, D. Y. & Ong, K. K. (1995) Tocotrienols inhibit HMG CoA reductase activity in the guinea pig. Nutr. Res. 15: 537–544.

Komiyama, K., Iizuka, K., Yamaoka, M., Watanabe, H., Tsuchiya, N. & Umezawa, I. (1989) Studies on the biological activity of tocotrienols. Chem. Pharm. Bull. 37: 1369–1371.

Parker, R. A., Pearce, B. C., Clark, R. W., Gordan, D. A. & Wright, J. J. K. (1993) Tocotrienols regulate cholesterol production in mammalian cells by post-transcriptional suppression of 3-hydroxy-3-methylglutaryl-coenzyme A reductase. J. Biol. Chem. 268: 11230–11238.

Pearce, B. C., Parker, R. A., Deason, M. E., Qureshi, A. A. & Wright J. J. K. (1992) Hypocholesterolemic activity of synthetic and natural tocotrienols. J. Med. Chem. 35: 3595–3606.

Phillipp, B. W. & Shapiro, D. J. (1979) Improved method for the assay and activation of 3-hydroxy-3-methylglutaryl coenzyme A reductase. J. Lipid Res. 20: 588–593.

Qureshi, A. A., Bradlow, B. A., Brace, L., Manganello, J., Peterson, D. M., Pearce, B. C., Wright, J. J. K., Gapor, A. & Elson, C. E. (1995) Response of hypercholesterolemic subjects to administration of tocotrienols. Lipids (in press).

Qureshi, A. A., Burger, W. C., Peterson, D. A. & Elson, C. E. (1986) The structure of an inhibitor of cholesterol biosynthesis isolated from barley. J. Biol. Chem. 261: 10544–10550.

Qureshi, A. A., Peterson, D. M., Elson, C. E., Mangels, A. R. & Din, Z. Z. (1989) Stimulation of avian cholesterol metabolism by α-tocopherol. Nutr. Rep. Int. 40: 993–1001.

Qureshi, A. A., Qureshi, N., Hasler-Rapacz, J., Weber, F. E., Chaudhary, V., Crenshaw, T. D., Gapor, A., Ong, A. S. H., Chong, Y. H., Peterson, D. & Rapacz, J. (1991a) Dietary tocotrienols reduce concentrations of plasma cholesterol, apolipoprotein B, thromboxane B2, and platelet factor 4 in pigs with inherited hyperlipidemias. Am. J. Clin. Nutr. 53: 1042S–1046S.

Qureshi, A. A., Qureshi, N., Wright, J. J. K., Shen, S., Kramer, G., Gabor, A., Chong, Y. H., DeWitt, G., Ong, A. S. H., Peterson, D. & Bradlow, B. A. (1991b) Lowering of serum cholesterol in hypercholesterolemic humans by tocotrienols (Palmvitee). Am. J. Clin. Nutr. 53: 1021S–1026S.

Tan, B. (1989) Palm carotenoids, tocopherols and tocotrienols. J. Am. Oil Chem. Soc. 66: 770–776.

Tan, B. (1992) Antitumor effects of palm carotenoids, tocopherols and tocotrienols. J. Am. Oil Chem. Soc. 66: 770–776.

Tan, D. T. S., Khor, H. T., Low, W. H., Ali, A. & Gapor, A. (1991) Effect of a palm-oil-vitamin E concentrate on the serum and lipoprotein lipids of humans. Am. J. Clin. Nutr. 53: 1027S–1030S.

Traber, M. G. & Kayden, H. J. (1989) Preferential incorporation of α-tocopherol vs γ-tocotrienol in human lipoproteins. Am. J. Clin. Nutr. 49: 517–526.

Wahlqvist, M. L., Krivokuca-Bogetic, Z., Lo, C. H., Hage, B., Smith, R. & Lukito, W. (1992) Differential serum responses to tocopherols and tocotrienols during vitamin E supplementation in hypercholesterolaemic individuals without change in coronary risk factors. Nutr. Res. 12: S181–S201.