Minor Constitutents of Palm Oil — Nutritional Considerations**

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INTRODUCTION

Vitamin E acting as an antioxidant or radical scavenger arrests the oxidative deterioration of cellular membranes. Apart from this action, there is little definitive evidence to explain other postulated functions for the vitamin, one of which is a possible action on cholesterol metabolism (Vogelsang and Shute 1946; Hermann *et al.*, 1979; Hirahara 1987).

As with other fat-soluble vitamins, vitamin E activity is not limited to a single compound. It is associated with eight derivatives of chroman-6-ol, four of which have a saturated phytyl side chain and the others an unsaturated prenyl side chain. The biosynthesis of the saturated tocopherols and the unsaturated tocotrienols is shown in *Figure 1* (Pennock 1983). While all these compounds act as biological antioxidants, α, β, and γ tocopherol and α-tocotrienol account for nearly all the biological activity in foods (Bieri and Farrell, 1976).

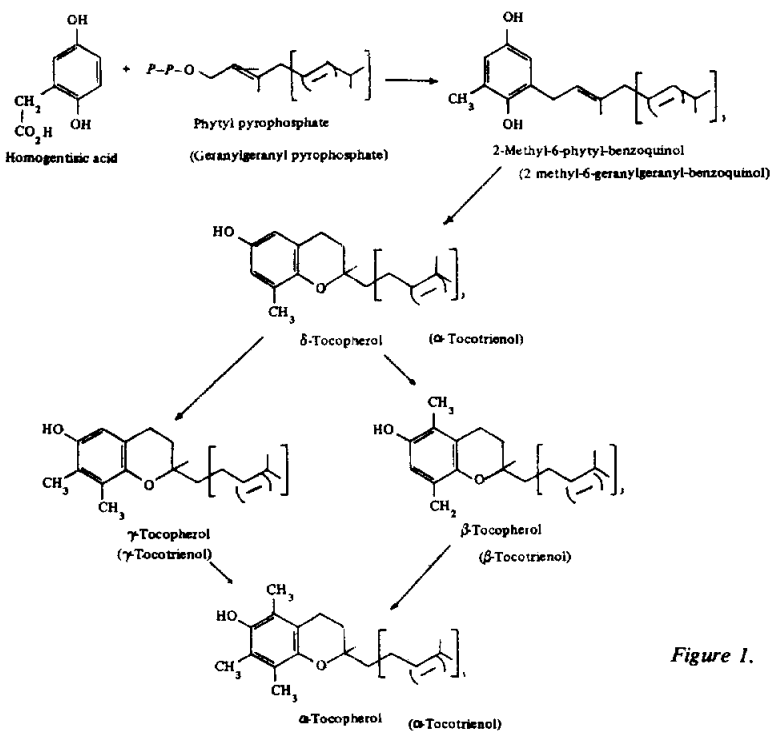

Figure 1.

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Cholesterol Metabolism. In a classic paper, Brown and Goldstein (1980) proposed regulatory mechanisms through which serum cholesterol homeostasis might be maintained. Elegant studies at the forefront of molecular biology have proven their insight. Briefly, the enzymic activity considered to be rate-limiting for the synthesis of cholesterol, 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) is controlled by multivalent feedback regulations. What this means is that serum cholesterol levels are maintained relatively constant over extended periods despite variations in cholesterol consumption. This constancy is manifested in an increased HMGR activity when serum cholesterol falls and a decreased activity when serum cholesterol increases. Therefore, if the dietary intake of cholesterol decreases or the body's loss of cholesterol increases, there is a compensatory increase in cholesterol synthesis. If dietary cholesterol increases, there is a compensatory decrease in its synthesis. Brown and Goldstein (1980) also postulated a second regulatory action, that of a non-sterol, post-mevalonate product which acts in concert with elevated serum cholesterol to control HMGR activity. The failure of this multivalent regulatory sequence is associated with an extremely high risk of cardiovascular disease.

Plant Metabolites. It has been observed that populations which consume diets rich in cereal grains tend to have a low incidence of cardiovascular disease (Gould *et al.*, 1980). The cholesterol-lowering action of whole grains has long been associated with their fibre constituents which, in the intestinal tract, bind cholesterol and its metabolites thereby increasing their excretion. An animal which is fed a fibre-rich, cholesterol-free, semi-purified diet accordingly has an elevated capacity to synthesize cholesterol. Cholestyramine, a drug which has a fibre-like action also stimulates cholesterol synthesis.

We found that barley was the most effective of the cereal grains in lowering the serum cholesterol levels of experimental animals (Qureshi *et al.* 1986). Contrary to our expectation, we failed to find an adaptive increase in cholesterol synthesis when we fed barley or its fibre-rich milling fractions to chicks. Indeed, their capacity to synthesize cholesterol was somewhat lower than that of birds which were fed standard rations. We soon found that washing the fibre fraction with organic solvents removed the substance that suppressed cholesterol synthesis (Qureshi *et al.* 1985). This led to the isolation of the cholesterol-suppressive metabolite which, on mass spectrometric analysis, was identified as α-tocotrienol (Qureshi *et al.*, 1986).

Under the conditions of the experiment recorded in *Table 1*, we demonstrated a concentration-dependent reduction in induced HMGR activity by dietary α-tocotrienol. (The birds were fasted for 48 hr and re-fed for 72 hr prior to sacrifice, in order to elevate HMGR activity). Concomitantly, the birds' serum total and LDL cholesterol levels fell while the HDL cholesterol remained unchanged. Unlike some inhibitors of HMGR activity, *e.g.*, lovastatin, compactin and mevinolin, the tocotrienol does not inhibit the enzyme directly; instead, it appears to act more like the oxidized sterols which decrease the quantity of the enzyme present in the liver. In other words, the tocotrienol dampens the adaptive response of the enzymic machinery to a low-cholesterol diet.

Under the conditions of the above experiment, we found that dietary α-tocopherol at levels 10 times those employed in the tocotrienol study elicited a modest but significant increase in HMGR activity *(Table 2)* and consistent with the human responses others report, a modest increase in HDL cholesterol (cf. Hermann *et al.*, 1979).

In summary, two compounds, differing only in the unsaturation of their side chains, exert opposite effects on the putative rate-limiting enzyme for cholesterol synthesis.

TABLE 1. CONCENTRATION-DEPENDENT EFFECTS OF DIETARY d-α-TOCOTRIENOL ON CHOLESTEROL METABOLISM IN BROILER COCKERELS[a]

d-α-Tocotrienol	HMGR[b]	Total Cholesterol	HDL-Cholesterol mg/100ml	LDL-Cholesterol
ppm				
0	198 ± 15[c]	161 ± 13[c]	54 ± 5[c]	81 ± 7[c]
2.5	172 ± 12[c, d]	152 ± 10[c]	54 ± 6[c]	73 ± 5[c]
5.0	161 ± 12[d]	144 ± 8[c]	51 ± 4[c]	60 ± 4[d]
10.0	144 ± 9[d, e]	125 ± 7[d]	52 ± 3[c]	54 ± 4[d]
15.0	135 ± 8[e]	122 ± 6[d]	50 ± 6[c]	52 ± 5[d]
20.0	130 ± 6[e]	105 ± 8[e]	48 ± 5[c]	38 ± 5[e]

[a] The feeding period was 21 days, followed by a 2-day fast and 3 days of refeeding; n=9.
[b] pmol of mevalonic acid synthesized/min/mg of microsomal protein.
[c-e] Values not sharing a common superscript letter are different at $p < 0.01$.

TABLE 2. CONCENTRATION-DEPENDENT EFFECTS OF DIETARY α-TOCOPHEROL ON CHOLESTEROL METABOLISM IN 6-WEEK-OLD COCKERELS[a]

α-Tocopherol	HMGR[b]	Total Cholesterol	HDL-Cholesterol mg/100ml	LDL-Cholesterol
ppm				
0	210 ± 17[c]	145 ± 10[c]	50 ± 5[c]	65 ± 8[c]
25	260 ± 14[d]	146 ± 9[c]	54 ± 6[c]	59 ± 3[c]
50	250 ± 15[d]	136 ± 8[c]	59 ± 5[c]	62 ± 6[c]
100	270 ± 13[d]	138 ± 8[c]	62 ± 7[c]	64 ± 5[c]
150	280 ± 17[d]	145 ± 11[c]	61 ± 10[c]	60 ± 6[c]
200	278 ± 21[d]	147 ± 12[c]	60 ± 8[c]	65 ± 7[c]

[a] The feeding period was 23 days, followed by a 2-day fast and 3 days of refeeding; n = 6, value x ± SD.
[b] pmol mevalonic acid synthesized/min/mg microsomal protein.
[c-d] Values not sharing a common superscript are different at $p < 0.05$.

Our search of the literature reveals that other compounds having the prenyl chain, *e.g.*, ubiquinone, squalene and ascofurone, also exert a cholesterol-static action (Krishnaiah and Ramasarma 1970; Brown and Goldstein 1980; Saskai *et al.*, 1973). With this in mind, we fed geranial-rich lemon grass oil to chicks and to hypercholesterolemic humans.

A diet containing 50 ppm lemon grass oil elicited a 30% decrease in induced HMG-CoA reductase activity and a 15% decrease in serum cholesterol *(Table 3)*.

Less dramatic results were forthcoming from the human study where hypercholesterolemic males were given 140 mg lemon grass oil daily for a 3-month period. Nearly one-third of the subjects responded with a greater than 10% decrease in cholesterol level. Dietary records were available for 11 subjects, five of whom responded to the treatment *(Table 4)*.

TABLE 3. INFLUENCE OF LEMON GRASS OIL ON CHOLESTEROL METABOLISM IN BROILER COCKERELS

Lemon grass Oil ppm	HMGR[a]	Serum Cholesterol[b]
0	618 ± 156	158 ± 9
50	435 ± 106	134 ± 13

[a] pmol mevalonic acid synthesized per min/mg of microsomal protein.
[b] mg/100 ml.

TABLE 4. INFLUENCE OF LEMON GRASS OIL ON HUMAN CHOLESTEROL LEVELS

	Responder	Non-responder
n	5	6
Weight (kg)	94.2 ± 7.9	84.9 ± 2.2
Energy Intake (Kcal)	1888 ± 113	2216 ± 273
Fat (g)	80 ± 6	92 ± 13
Fat (% energy)	38 ± 1	37 ± 4
Cholesterol	199 ± 26	280 ± 49
Cholesterol (mg/100ml)		
Initial	315 ± 21	313 ± 25
4 wk	287 ± 24	298 ± 23
8 wk	294 ± 25	319 ± 29
12 wk	281 ± 15	316 ± 23
3 months after test	328 ± 15	

Whether the differential response is due to genetic traits, diet, adherence to protocol or other factors cannot be determined. Non-responders consumed 30% more energy and 56% more cholesterol per unit body weight. Other studies (Hirahara, 1987) including our unpublished results (Qureshi *et al.*, 1987) suggest that a high cholesterol intake masks the action of the prenylated compounds on cholesterol synthesis.

Rationale for a cholesterol-suppressive action of α-tocotrienol. Brown and Goldstein (1980) propose that the full suppression of cholesterol biosynthesis requires two regulators, cholesterol derived from LDL and a non-sterol, post-mevalonate product, both of which modulate HMGR activity. Plants, presenting a complex series of pathways branching from mevalonic acid *(Figure 2)* provide a broad variety of non-sterol metabolites. Since these products, as well as cholesterol, are formed through a common sequence of reactions, an end-product inhibition by an excess of one product might be expected to decrease not only its own production but also that of other products derived from a common precursor (Qureshi *et al.* 1985).

Such evidence is forthcoming from our studies as well as from the work of Clegg *et al.* (1982) who reported that both HMGR mass and activity are decreased in rat liver following the administration of monoterpenes.

Other evidence suggestive of the cholesterol-suppressive action of plant metabolites can be found in the work of O'Brien and Reiser (1979, 1982). They report that dietary factors which elevate plasma cholesterol are more potent when tested using a purified diet than when added to real food diets. It is perhaps pertinent to note that basal cholesterol-free purified diets support higher cholesterol levels than do laboratory animal or human-type diets.

Palm Oil. Hornstra (Anon, 1987) reviewed the anomalous effects of palm oil on blood lipids. Contrary to the predictions of the Keys-Anderson equation, incorporation of palm oil into *ad libitum* control diets does nc elevate plasma cholesterol levels of experimental animals.

We examined hepatic HMGR activities and serum cholesterol levels in chicks which had been fed a basal diet augmented with either 5% corn oil or 5% palm oil for one month. The respective cholesterol levels were 134 ± 4 and 138 ± 10 mg/dl; the HMGR activities were 255 ± 53 and 203 ± 49 pmol mevalonate/mg microsomal protein/min. Palm oil contains about 50% saturated fatty acids and only 10% linoleic acid whereas the proportions in corn oil are reversed, with >50% linoleic acid and <10% saturated fatty acid. Our interpretation is that the claimed cholesterol-elevating action of the

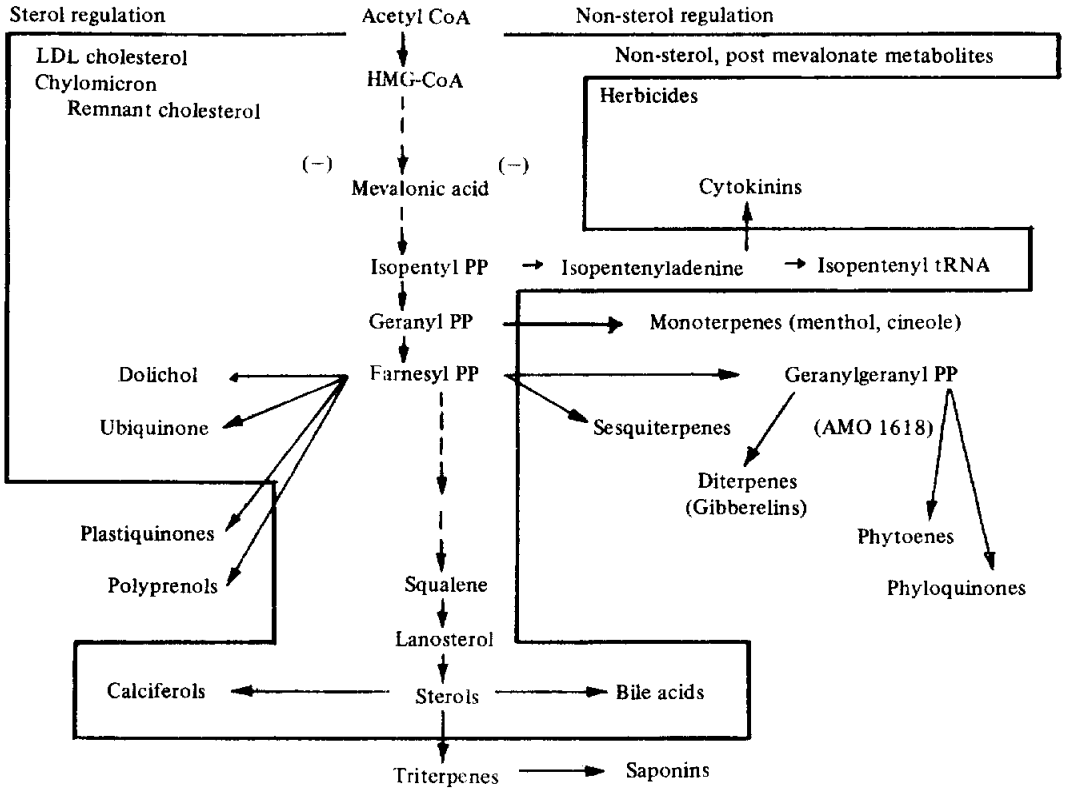

Figure 2. Biosynthesis of Sterols and Other Products Derived from Mevalonate. As far as we can determine, all metabolites within the enclosed space (animal), except LDL and chylomicron remnant cholesterol and the bile acids, are present in plant tissues. We propose that sterol regulation is limited to animal systems and non-sterol regulation is shared by both plants and animals (Qureshi et al., 1985).

palm oil saturated fatty acid is countered by a palm oil constituent which suppresses cholesterol biosynthesis.

Palm oil contains a number of constitutents which might suppress HMGR activity. Foremost among these are a number of phytosterols and γ-, δ-, α- and β-tocotrienols (Goh *et al.* 1985). Vitamin E derivatives are present in crude palm oil at levels approaching 1000 ppm, 60% of which is in the tocotrienol form. Assuming a 50% loss during processing, a 5% palm oil diet has a level of tocotrienols in excess of 15 ppm, a quantity sufficient, we believe, to suppress HMGR activity by 20–30 per cent.

DISCUSSION

The advent of the semi-purified diet most certainly ranks as one of the major milestones in the development of nutritional biochemistry. Although associations between dietary practices and diseases had long been recognized, it was only after this milestone that certain diseases could be traced to deficiencies of certain food-borne substances. The subsequent era of explosive growth in the science of nutrition ended with the isolation of vitamin B_{12}. During the past 40 years, a new generation of nutritionists has sought to link the diseases of our society with the presence of food-borne substances in our diet. Raymond Reiser, a highly respected investigator whose career spanned both generations, takes exception to one postulated link. He said that 'the response of serum lipids, especially cholesterol, to dietary fat and cholesterol has been the subject of intense investigation and controversy for more than two decades. Earlier studies showed that human serum cholesterol was virtually independent of diet fat and cholesterol. However, coincidental with the use of purified diets in metabolic studies, significant responses of human serum cholesterol to saturated fat, with and without added cholesterol in the diet, were reported' (O' Brien and Reiser, 1979).

We share the concern implied in this comment. Dietary trials are often designed to examine the impact of a specific nutrient. In order to rule out the effects of other dietary constituents, rigidly defined basal diets consisting of purified ingredients are employed. Under such a regimen, the plasma cholesterol-elevating action of dietary cholesterol is exposed. Yet when eggs are added to normal diets of various populations, the expected plasma cholesterol response is muted. Saturated fatty acids tested under that regimen also elevate plasma cholesterol; yet when palm oil is added to a real food diet, the expected cholesterolemic response fails to materialize.

Food consists of six classes of nutrients: carbohydrate, fat, protein, vitamins, minerals and water. Food also contains a host of other constituents, sometimes labelled collectively as the anutrients. By and large, only investigators concerned with food acceptance and food safety have addressed attention to these anutrients.

There is, we are pleased to report, a growing interest in these anutrients among our colleagues, principally among those studying the diet-cancer link: while some anutrients enhance carcinogenesis, others suppress the process.

It is fairly clear now that the addition of fat to the diet of a carcinogen-treated animal results in the enhanced development of tumours. In studies designed to determine the requirement of essential fatty acid for mammary tumourigenesis, the Ips (Sylvester *et al.* 1986, Ip *et al.*, 1987) found an anomalous response to palm oil. Rats which received a linoleate-adequate palm oil diet developed fewer tumours than did rats which were fed a linoleate-deficient fat or other vegetable fats. Sundram *et al.*, (1987), employing a slightly different protocol, recorded a similar response. The latter study appears to rule out an anticarcino-

genic action of the carotenoids. The report of Kato *et al.* (1985) points to an anticarcinogenic action of tocotrienol. These workers extended the lives of mice bearing transplanted tumours by injecting the mice with α-tocotrienol suppressions.

In this review, we have noted anomalous responses to palm oil in studies oriented towards cancer and cardiovascular disease. These responses cannot be related to the fatty acid profile of palm oil. Our studies and those of others provide evidence that one or more of the minor constituents of palm oil is responsible.

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