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Journal of Nutritional Biochemistry 16 (2005) 633–640

Journal of Nutritional Biochemistry

Different palm oil preparations reduce plasma cholesterol concentrations and aortic cholesterol accumulation compared to coconut oil in hypercholesterolemic hamsters☆

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Received 21 March 2005; received in revised form 21 March 2005; accepted 21 March 2005

Abstract

Several studies have reported on the effect of refined, bleached and deodorized palm oil (RBD-PO) incorporation into the diet on blood cholesterol concentrations and on the development of atherosclerosis. However, very little work has been reported on the influence of red palm oil (RPO), which is higher in carotenoid and tocopherol content than RBD-PO. Thus, we studied the influence of RPO, RBD-PO and a RBD-PO plus red palm oil extract (reconstituted RBD-PO) on plasma cholesterol concentrations and aortic accumulation vs. hamsters fed coconut oil. Forty-eight F_1B Golden Syrian hamsters (*Mesocricetus auratus*) (BioBreeders, Watertown, MA) were group housed (three/cage) in hanging polystyrene cages with bedding in an air-conditioned facility maintained on a 12-h light/dark cycle. The hamsters were fed a chow-based hypercholesterolemic diet (HCD) containing 10% coconut oil and 0.1% cholesterol for 2 weeks at which time they were bled after an overnight fast and segregated into four groups of 12 with similar plasma cholesterol concentrations. Group 1 continued on the HCD, Group 2 was fed the HCD containing 10% RPO in place of coconut oil, Group 3 was fed the HCD containing 10% RBD-PO in place of coconut oil and Group 4 was fed the HCD with 10% reconstituted RBD-PO for an additional 10 weeks. Plasma total cholesterol (TC) and non-high-density lipoprotein-cholesterol (HDL-C) (very low- and low-density lipoprotein) concentrations were significantly lower in the hamsters fed the RPO (−42% and −48%), RBD-PO (−32% and −36%) and the reconstituted RBD-PO (−37% and −41%) compared to the coconut oil-fed hamsters. Plasma HDL-C concentrations were significantly higher by 14% and 31% in hamsters fed the RBD-PO and RPO compared to the coconut oil-fed hamsters. Plasma triglyceride (TG) concentrations were significantly lower in hamsters fed RBD-PO (−32%) and the reconstituted RBD-PO (−31%) compared to the coconut oil-fed hamsters. The plasma γ-tocopherol concentrations were higher in the coconut oil-fed hamsters compared to the hamsters fed the RPO (60%), RBD-PO (42%) and the reconstituted RBD-PO (49%), while for plasma α-tocopherol concentrations, the coconut oil-fed hamsters were significantly higher than only the RPO-fed hamsters (21%). The coconut oil-fed hamsters also had significantly higher plasma lipid hydroperoxide concentrations compared to RBD-PO (112%) and the reconstituted RBD-PO (485%). The hamsters fed the coconut oil diet excreted significantly more fecal total neutral sterols and cholesterol compared to the hamsters fed the RBD-PO (158% and 167%, respectively). The coconut oil-fed hamsters had significantly higher levels of aortic total, free and esterified cholesterol compared to the hamsters fed the RPO (74%, 50% and 225%, respectively), RBD-PO (57%, 48% and 92%, respectively) and the reconstituted RBD-PO (111%, 94% and 94%, respectively). Also, aortic free/ester cholesterol ratio in the aortas of hamsters fed RPO was significantly higher than in those fed the coconut oil (124%). In conclusion, hamsters fed the three palm oil preparations had lower plasma TC and non-HDL-C and higher HDL-C concentrations while accumulating less aortic cholesterol concentrations compared to hamsters fed coconut oil.

Keywords: Atherosclerosis; Plasma cholesterol; Palm oil; Red palm oil; Carotenoids; Tocopherols

1. Introduction

The oil obtained initially upon harvesting the fruit of the oil palm is red due to its content of carotene, tocopherol and tocotrienols. The palm oil generally available for use

☆ The work was supported, in part, by a Research Career Award (HL 00734) to Dr. David Kritchevsky from the National Institute of Health (USA).

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doi:10.1016/j.jnutbio.2005.03.007

(refined, bleached and deodorized or RBD-PO) has been processed to remove the carotene and tocopherols. This fractionation brings about an increase in monounsaturated oleic acid with the concomittant reduction of palmitic acid, the major saturated fatty acid [1,2]. Palm oil contains a higher proportion of palmitic acid as well as considerable quantities of oleic and linoleic acids, which give it a higher unsaturated fatty acid content than coconut oil and palm kernel oil [3–6]. Throughout the world, 90% of palm oil is used for edible purposes (e.g., margarine, deep fat frying, shortening, ice creams, cocoa butter substitutes in chocolate) [7,8].

Red palm oil (RPO) contains 50% saturated, 40% monounsaturated and 10% polyunsaturated fatty acids [9]. Red palm oil is the richest food source of carotenoids (500–750 mg/L) [4] and a very good source of vitamin E (tocopherols and tocotrienols) (560–100 ppm) [2,10,11]. The carotenoids, together with tocopherols and tocotrienols, ascorbic acid, enzymes and proteins, are members of the biological antioxidant network [12,13] converting highly reactive radicals and free peroxy radicals to less active species, thus protecting against oxidative damage to cells [14].

Previous work has shown that tocopherol and tocotrienols may be anti-atherogenic, although they may have no effect on plasma cholesterol concentrations. Tocopherols have been reported to inhibit atherosclerosis in rabbits [15,16] and in monkeys [17]. Antioxidants have been shown to reduce the risk of atherosclerosis in human populations [18,19]. Tocotrienols have been reported to be natural inhibitors of cholesterol synthesis [20,21]. The tocopherols and tocotrienols promote an antithrombotic state by reducing platelet aggregation and modulating prostanoid synthesis [21–24]. Based on this information, one might then expect RPO to be less atherogenic than RBD-PO. Kritchevsky et al. [25] have shown this to be the case in studies of experimental atherosclerosis in rabbits. To further investigate the role that the carotenoids and tocopherols of RPO may play in its comparative protective action, Kritchevsky et al. [26] have compared atherogenicity in cholesterol-fed (0.1%) rabbits of RPO, RBD-PO and RBD-PO to which the antioxidant components of RPO have been added (reconstituted RPO). RBD-PO was shown to be 58% more atherogenic than RPO and 13% more atherogenic than the reconstituted RBO.

In this manuscript, we describe the atherogenic effects of RPO, RBD-PO and reconstituted RBD-PO when substituted for coconut oil in the hamster model, which has been previously described [27].

2. Materials and methods

2.1. Animals and experimental design

Forty-eight F.B. Golden Syrian hamsters (*Mesocricetus auratus*) (BioBreeders, Watertown, MA) were used. They were group housed (three/cage) in hanging polystyrene cages with bedding in a temperature-controlled room (25°C) maintained on a 12-h light/dark cycle. Hamsters were given food and water ad libitum. Hamsters were fed Purina Hamster Chow (Ralston Purina, St. Louis, MO) for 1 week in order to acclimate them to the facility. The hamsters were then fed a nonpurified hypercholesterolemic diet (HCD) containing 10% coconut oil and 0.1% cholesterol for 2 weeks at which time they were bled after an overnight fast (16 h) and segregated into four groups of 12 with similar plasma cholesterol concentrations. A nonpurified diet, rather than a semipurified diet, was used because published data from our laboratory [27] and those from another [28] indicate that hamsters on the nonpurified diet are more responsive to various cholesterolemic interventions and a resultant lipoprotein profile [non-high-density lipoprotein cholesterol (HDL-C)>HDL-C] which is similar to that of humans. Group 1 was continued on the HCD, Group 2 was fed the HCD containing 10% RPO in place of the coconut oil, Group 3 was fed the HCD containing 10% RBD-PO in place of the coconut oil and Group 4 was fed the HCD containing 10% reconstituted RBD-PO. Treatment diets were fed for 10 weeks. The characteristics of the treatment oils are given in Table 1 and were measured for each treatment oil as stated below. Food disappearance and body weights were monitored on a weekly basis. The animals were maintained in accordance with the guidelines of the Committee on Animal Care of the University of Massachusetts Lowell Research Foundation, and the guidelines prepared by the Committee on Care in Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (DHEW publication no. 85-23, revised 1985).

Table 1
Characteristics of treatment oils fed to hamsters for 10 weeks

Fatty acid (%)	Coconut oil	RPO	RBD-PO	Reconstituted RBD-PO
12:0	44.6	0.27	0.29	0.29
14:0	16.8	0.96	0.95	0.96
16:0	8.2	33.68	39.12	39.15
16:1(n9)	–	0.20	0.16	0.16
18:0	2.8	3.30	3.85	3.91
18:1(n9)	5.8	47.05	43.62	44.33
18:2(n6)	1.8	13.55	11.32	10.86
18:3	–	0.56	–	–
20:0	–	0.31	0.33	0.33
22:4	–	0.13	–	–
Other	14.1	0.58	–	–
Total vitamin E (mg/100 g)[a]	3.6	73.2	62.0	97.2
α-Tocopherol (mg/100 g)	0.4	16.2	15.8	26.6
Phytosterols (mg/100 g)	86.0	57.0	47.0	53.0
Carotenoids (mg/100 g)	–	70.6	1.2	78.1

[a] Total vitamin E includes tocopherols plus tocotrienols.

2.2. Plasma lipid determinations

Blood samples were taken at 8 and 10 weeks from food-deprived hamsters (12 h) and collected via the retro-orbital sinus into heparinized capillary tubes under ultrapure CO_2/O_2 (50/50) gas (Northeast Airgas, Salem, NH) anesthesia. Plasma was harvested after centrifugation at 1500×*g* at room temperature for 20 min, and plasma total cholesterol (TC) [29] and triglyceride (TG) [30] concentrations were measured enzymatically. Plasma very low- and low-density lipoprotein cholesterol, which we combined and termed non-HDL-C, were precipitated with phosphotungstate reagent [31], and HDL-C was measured in the supernatant. The concentration of non-HDL-C was calculated as the difference between plasma TC and HDL-C. The accuracy of the procedures used for the measurement of plasma TC, HDL-C and TG concentrations is maintained by participation in the Lipid Standardization Program of the Center for Disease Control and the National Heart, Blood and Lung Institute.

2.3. Plasma and oil vitamin E analyses

Plasma and treatment oil vitamin E concentrations were determined by treating 200 μl of plasma or oil with 200 μl of ethanol containing butylated hydroxytoluene (BHT) (10 mg/L) and 1.0 ml hexane followed by vortex mixing. The samples were centrifuged at 500×*g* for 5 min, and the organic layer transferred to a 7.0-ml brown borosilicate screw top vial. The sample residues were re-extracted with 1.0 ml of hexane, and the organic layers were combined. The organic layer was evaporated under N_2 and reconstituted with 200 μl of ethanol containing BHT (10 mg/dl) and injected into an HPLC. The HPLC system is a Model 5600 CoulArray 8-channel system with two Model 580 pumps, a high-pressure gradient mixer, a PEEK pulse damper, a Model 540 autoinjector, a CoulArray Thermostatic Chamber and a serial array of eight coulometric electrodes (ESA Laboratories, Chelmsford, MA). The column is a 3.0×150-mm, 3 μM, Supelcosil LC-18 (Supelco, Bellefonte, PA). The mobile phase consisted of methanol/1 propanol/1 M ammonium acetate (78:20:2 v/v/v) at a flow rate of 0.8 ml/min. The concentration of vitamin E was determined by external standardization using purified solutions (Sigma, St. Louis, MO). Accuracy and precision of measurements are monitored by participation in the National Institute of Standards and Technology (NIST) Lipid Soluble Vitamin Quality Assurance Program.

2.4. Plasma lipid hydroperoxide analyses

Cayman Chemical Company's Lipid Hydroperoxide Assay Kit was used to measure plasma lipid hydroperoxides by ELISA [32]. This assay measures the hydroperoxides directly utilizing the redox reactions with ferrous ion. The resulting ferric ions are detected using thiocyanate ion as the chromogen.

2.5. Aortic tissue collection, aortic cholesterol extractions and measurements

At the end of the exposure period (week 10), hamsters were anesthetized with an intraperitoneal injection of sodium pentobarbital, and aortic tissue was obtained for determination of cholesterol concentration. The heart and thoracic aorta were removed and stored in phosphate-buffered saline (PBS) at 4°C for subsequent analysis. To measure cholesterol concentrations in the aortic arch, a piece of aortic tissue extending from as close to the heart as possible to the branch of the left subclavian artery was used (approximately 20–40 mg). The tissue was cleaned, weighed and placed in a vial containing 4 ml of methanol and 10 ml of chloroform. The sample was mixed vigorously and left at room temperature for 48 h prior to extraction. The solution was then placed in a 37°C water bath, under N_2. When approximately half of the solution was evaporated, 1 ml of chloroform with 1% Triton-100 was added, mixed and evaporated to dryness at 37°C under N_2. Two hundred and fifty microliters of distilled water was added to the samples, vortexed and placed in a shaking water bath at 37°C for 20 min to solubilize the lipid. After incubation, aortic total and free cholesterol concentrations were determined in triplicate using 25 μl of sample enzymatically (Wako, Richmond, VA) using an ELISA assay. Aortic cholesteryl ester concentration was determined as the difference between the total and the free cholesterol concentrations. A pilot study was conducted to evaluate the extent to which this procedure removed tissue cholesterol. Aortic cholesterol concentrations were determined after the tissue was placed in solvent (4 ml of methanol and 10 ml of chloroform) overnight with frequent vigorous mixing and compared with the concentrations obtained following tissue mincing or homogenization as reported previously [33]. No significant differences in aortic cholesterol content were observed between the different cholesterol extraction procedures.

2.6. Fecal neutral sterol measurements

Fecal samples were collected over the final 3 days of the exposure period, freeze-dried (lyophilized) and ground prior to analysis [34]. Dry feces (200 mg) were extracted with 4 ml of methanol/chloroform (50:50) for 1 h at 100°C in a 5-ml Reacti-vial fitted with a Mininert cap. Samples were then allowed to come to room temperature and centrifuged at 500×*g* at room temperature for 10 min. The supernatant was removed from the fecal pellet and transferred to an 8-ml borosilicate vial. Supernatants were evaporated to dryness at 50°C under N_2. Four milliliters of 0.1N NaOH/ethanol (10:90 by volume) was added to each sample, over-layered with N_2, capped and heated at 100°C for 30 min. The samples were allowed to cool to room temperature followed by the removal of the solvent and transferred to 16×150-mm borosilicate test tubes. Five milliliters of water and 3 ml of hexane were added to the solvent followed by

vortexing and centrifugation at 500×*g* for 2 min. The top hexane layer was removed and placed in vials. The hexane extraction was repeated two more times and pooled. The hexane extracts were stored at −80°C until analysis of neutral sterols. To the hexane portion, 1 ml of 5-α-cholestane (240 μg/ml) (Sigma-Aldrich, St. Louis, MO) was added, and the solution was brought up to 10 ml with hexane in a volumetric flask. Two milliliters was removed and evaporated to dryness at 100°C under N_2. One hundred microliters of Tri-Sil reagent was added, and the samples were heated at 85°C for 20 min, followed by evaporation and reconstitution in 100 μl of methylene chloride. One microliter was then injected and analyzed by capillary GC. Neutral sterols were analyzed using a Shimadzu (Shimadzu Corporation, Kyoto, Japan) GC-14A gas chromatograph with a flame ionization detector and a 50-m×0.2-mm HP-1 capillary column (Hewlett Packard, Andover, MA). The injector and detector temperatures were set at 300°C. The initial column temperature was 220°C and was increased to 300°C at a rate of 2°C/min. The final temperature was held for 10 min. Column flow rate was 1.5 ml/min. Peak areas were quantitated using a Shimadzu CR501 integrator. Quantification and identification of neutral sterols were performed based on the appropriate purified external standards supplied by Sigma-Aldrich. Extraction efficiency for neutral sterols following this protocol was approximately 94%.

2.7. Fatty acid and neutral sterol composition of oils

For both fatty acid and neutral sterol analysis of treatment oils, a 300-μl aliquot of oil was extracted using a standard Folch [35] extraction technique as described below. The oil was first mixed with 5 ml of methanol containing 0.2% BHT followed immediately by the addition of 10 ml of chloroform and vortexed vigorously for 30 s. Following the addition of 1.0 ml of 0.09% saline and agitation in a vortex mixer, the mixture was centrifuged at 500×*g* for 10 min. The top aqueous layer was aspirated, and the bottom organic layer was transferred to a glass tube with a Teflon-lined cap and stored at −70°C under N_2. Prior to analyses, samples were evaporated to dryness under N_2 and esterified as we have previously described [36] using an Instant Methanolic HCl Kit (Alltech-Applied Science, Deerfield, IL). Fatty acid methyl esters were analyzed using a Hewlett Packard model 5890 GLC, with a DB-23 (J & W Scientific) column, complete with autosampler and integrator. Neutral sterols were silated and analyzed as stated for fecal neutral sterol measurements above.

2.8. Statistical analysis

SigmaStat software was used for all statistical evaluations (Jandel Scientific, San Rafael, CA) [37]. A repeated measures two-way analysis of variance (RM ANOVA) was used to analyze plasma lipid and lipoprotein cholesterol data between treatment groups and time of measurements. A one-way ANOVA was performed between treatment groups for all other data. When statistical significance was found by ANOVA, the Student–Newman–Keuls separation of means was used to determine group differences. All values were expressed as mean±S.E.M., and statistical significance was set at the minimum $P<.05$.

3. Results

All hamsters in each group survived the entire length of the study. No significant differences were observed between dietary treatments for initial body weight; however, by the end of the 10-week treatment period, the hamsters fed the coconut oil diet and the 50:50 mixture oil diet had gained 10% more body weight than the hamsters fed the RBD-PO ($P<.05$) (data not shown). At the same time, no significant difference for food consumption between the treatment diets was observed (data not shown).

Plasma lipid and lipoprotein cholesterol concentrations between weeks 8 and 10 were not significantly different within dietary treatments and therefore the values were averaged (Table 2). Plasma total cholesterol (TC) and non-HDL-cholesterol (very low- and low-density lipoprotein) concentrations were significantly lower in the hamsters fed the RPO (−42% and −48%, respectively), RBD-PO (−32% and −36%, respectively) and the reconstituted RBD-PO (−37% and −41%, respectively) compared to the coconut oil-fed hamsters (Table 2). None of the dietary treatments containing the palm oil preparations was significantly different from each other for plasma TC or non-HDL-C concentrations (Table 2). Plasma HDL-C concentrations were significantly higher by 14% and 31% in hamsters fed the RBD-PO and RPO compared to the coconut oil-fed hamsters (Table 2). Also, the hamsters fed the RPO had significantly higher plasma HDL-C concentrations compared to the hamsters fed the reconstituted RBD-PO (20%)

Table 2
Plasma lipids and lipoprotein cholesterol concentrations (mmol/L) in hamsters fed treatment diets for 10 weeks (average of weeks 8 and 10 bleeds)

Diet	TC	Non-HDL-C	HDL-C	TC/HDL-C	TG
Coconut oil	16.32±0.49[a]	14.97±0.49[a]	1.35±0.04[a]	12.41±0.47[a]	7.40±0.44[a]
RPO	9.48±0.88[b]	7.72±0.89[b]	1.76±0.05[b]	5.47±0.56[b]	6.72±0.94[ab]
RBD-PO	11.08±1.09[b]	9.55±1.12[b]	1.53±0.07[c]	7.54±0.90[b]	5.06±0.51[b]
Reconstituted RBD-PO	10.36±0.84[b]	8.89±0.86[b]	1.47±0.03[ac]	7.18±0.64[b]	5.08±0.46[b]

Values are means±S.E.M., $n=12$.
[a,b] Values in a column not sharing a superscript are significantly different at $P<.05$.

(Table 2). The hamsters fed the reconstituted RBD-PO were not significantly different from the hamsters fed the coconut oil or the RBD-PO for plasma HDL-C concentrations (Table 2). The plasma TC/HDL-C ratio followed the same pattern as the plasma TC and non-HDL-C. The hamsters fed the coconut oil had a significantly higher plasma TC/HDL-C ratio compared to the hamsters fed the RPO (127%), RBD-PO (65%) and the reconstituted RBD-PO (73%), respectively (Table 2). Plasma triglyceride (TG) concentrations were significantly lower in hamsters fed RBD-PO (−32%) and the reconstituted RBD-PO (−31%) compared to the coconut oil-fed hamsters (Table 2). The hamsters fed the RPO were not significantly compared to any other dietary treatment for plasma TG concentrations, nor were the hamsters fed the RBD-PO or the reconstituted RBD-PO significantly different for plasma TG concentrations (Table 2).

Plasma tocopherol and lipid hydroperoxide concentrations are stated in Table 3. The plasma γ-tocopherol concentrations were higher in the coconut oil-fed hamsters compared to the hamsters fed the RPO (60%), RBD-PO (42%) and the reconstituted RBD-PO (49%) (Table 3). None of the hamsters fed the dietary treatments containing the palm oil preparations was significantly different from each other for plasma γ-tocopherol concentrations (Table 3). While for plasma α-tocopherol concentrations, the coconut oil-fed hamsters were significantly higher than only the RPO-fed hamsters (21%) (Table 3). The hamsters fed the RBD-PO and the reconstituted RBD-PO were significantly different from each other or any other dietary treatment for plasma α-tocopherol concentrations (Table 3). Although the coconut oil-fed hamsters had significantly higher plasma tocopherol concentrations, they also had significantly higher plasma lipid hydroperoxide concentrations compared to RBD-PO (112%) and the reconstituted RBD-PO (485%). Also, the hamsters fed the RBD-PO had significantly higher plasma lipid hydroperxide concentrations compared to the hamsters fed the reconstituted RBD-PO (176%) (Table 3). Although the hamsters fed the RPO were not significantly different from the coconut oil- and RBD-PO-fed hamsters, they did have significantly higher plasma lipid hyroperoxide concentrations compared to the hamsters fed the reconstituted RBD-PO (356%) (Table 3).

Table 3
Plasma γ- and α-tocopherol (mmol/L) and lipid hydroperoxide (LHP) (μmol/L) concentrations in hamsters fed the treatment diets for 10 weeks

Diet	γ-Tocopherol	α-Tocopherol	LHP
Coconut oil	4.36±0.52[a]	8.54±0.51[a]	5.09±2.28[a]
RPO	2.72±0.34[b]	7.06±0.68[b]	3.97±1.33[ab]
RBD-PO	3.08±0.28[b]	8.04±0.30[ab]	2.40±1.45[b]
Reconstituted RBD-PO	2.92±0.27[b]	8.02±0.39[ab]	0.87±0.21[c]

Values are mean±S.E.M., $n=12$.
[a,b] Values in a column not sharing a superscript are significantly different at $P<.05$.

Table 4
Fecal neutral sterol concentrations (mg/g dry feces) of hamsters fed the treatment diets for 10 weeks

Fecal sterol	Coconut oil	RPO	RBD-PO	Reconstituted RBD-PO
Coprostanol	1.25±0.14	1.00±0.20	0.64±0.21	1.36±0.25
Coprostanone	0.10±0.02	0.05±0.01	0.02±0.01	0.07±0.03
Cholesterol	0.64±0.04[a]	0.43±0.07[ab]	0.24±0.06[b]	0.52±0.10[a]
Campesterol	1.79±0.17[a]	1.52±0.26[a]	0.67±0.18[b]	1.13±0.21[ab]
Stigmasterol	0.23±0.02[a]	0.19±0.04[ab]	0.08±0.02[b]	0.14±0.03[ab]
Sitosterol	0.74±0.06[a]	0.61±0.15[a]	0.25±0.06[b]	0.41±0.08[ab]
Sitostanol	0.52±0.04[a]	0.38±0.05[ac]	0.16±0.04[b]	0.30±0.07[bc]
Total	5.28±0.46[a]	4.17±0.68[ab]	2.05±0.56[b]	3.94±0.75[ab]

Values are means±S.E.M., $n=4$ pools of three animals each.
[a,b] Values in a row not sharing a superscript are significantly different at $P<.05$.

Fecal neutral sterol excretion data are shown in Table 4. The hamsters fed the coconut oil excreted significantly more fecal total neutral sterols compared to the hamsters fed the RBD-PO (158%) (Table 4). No other dietary treatments were significantly different from each other for total fecal sterol excretion (Table 4). Also, the hamsters fed the coconut oil and the reconstituted RBD-PO diets, which were not different, excreted significantly more fecal cholesterol compared to the hamsters fed the RBD-PO (167% and 117%, respectively) (Table 4). The hamsters fed the RPO diet were not different from any of the other dietary treatments for fecal cholesterol excretion (Table 4).

Despite having significantly higher plasma vitamin E concentrations and excreting significantly more fecal cholesterol, the coconut oil-fed hamsters had significantly higher levels of aortic total, free and esterified cholesterol compared to the hamsters fed the RPO (74%, 50% and 225%, respectively), RBD-PO (57%, 48% and 92%, respectively) and the reconstituted RBD-PO (111%, 94% and 94%, respectively) (Table 5). The hamsters fed the dietary treatments containing the palm oil preparations were not significantly different from each other for aortic total, free or esterifed cholesterol concentrations (Table 5). Also, aortic free/ester cholesterol ratio in the aortas of hamsters fed RPO was significantly higher than in those fed the coconut oil (124%) (Table 5). No other dietary treatments

Table 5
Total cholesterol, free cholesterol (FC) and cholesterol ester (CE) concentration (μg/mg tissue) in ascending aortas of hamsters fed the treatment diets for 10 weeks

Diet	TC	FC	CE	FC/CE
Coconut oil	4.65±0.42[a]	3.48±0.45[a]	1.17±0.29[a]	2.71±0.11[a]
RPO	2.68±0.24[b]	2.32±0.25[b]	0.36±0.13[b]	6.08±3.12[b]
RBD-PO	2.96±0.26[b]	2.35±0.35[b]	0.61±0.11[b]	3.92±0.86[ab]
Reconstituted RBD-PO	2.20±0.16[b]	1.79±0.14[b]	0.42±0.11[b]	4.83±2.93[ab]

Values are mean±S.E.M., $n=12$.
[a,b] Values in a column not sharing a superscript are significantly different at $P<.05$.

were significantly different from each other for aortic free to ester ratio (Table 5).

4. Discussion

Palm oil has been stigmatized as a hypercholesterolemic fat because of its palmitic acid (16:0) content. In this study, coconut oil was the most hyperlipidemic of the test fats. Red palm oil was somewhat less cholesterolemic (10–20%) than either RBD-PO or the reconstituted RBD-PO resulting in a lower total to HDL-cholesterol ratio. Plasma triglyceride concentrations in hamsters fed RPO were almost as high as those in hamsters fed coconut oil and about 33% higher than in hamsters fed either RBD-PO or the reconstituted RBD-PO. These results are similar to previous work that showed in healthy volunteers coconut oil increased serum cholesterol concentrations, while palm oil consumption decreased serum cholesterol concentrations after 5 weeks of treatment [38]. The explanation for these blood cholesterol differences can be partially explained by the positional distribution of fatty acids, especially palmitic acid, in these oils. Previous work has shown that the presence of palmitic acid at the SN2 position of a triglyceride renders that triglyceride more cholesterolemic and atherogenic [39–41]. Palm oil contains about 40% palmitic acid, but only about 3% is in the SN2 position. The most interesting observation was that the plasma HDL-C concentrations in hamsters fed the palm oil preparations ranged from 10% (reconstituted RBD-PO) to 31% (RPO) higher than those observed in the coconut oil-fed animals. This finding is similar to previous work which showed an increase in plasma HDL-C concentrations in rats when fed RPO [42]. This difference may be due to the vitamin E (tocopherols plus tocotrienols) content of the palm oil preparations. It is noteworthy, however, that while RPO and the reconstituted RBD-PO contained virtually the same amount of vitamin E, the reconstituted RBD-PO prepared by adding exogenous carotenoids and tocopherol was not as effective as the unfractionated oil (RPO).

The plasma tocopherol levels in the coconut oil-fed hamsters were significantly higher than those of the other groups, while their plasma α-tocopherol levels were only slightly higher. The reason for these findings is unknown, since one would have expected high plasma vitamin E concentrations in the hamsters fed the palm oil preparations due to the significantly more vitamin E in these oils vs. coconut oil. The lower plasma concentrations of tocopherol in the hamsters fed the palm oil preparations are in contradiction to other work that showed the supplementation of a normal diet of RPO to healthy volunteers led to an increase in serum α-tocopherol concentrations compared to baseline [43]. However, in the present study, RPO was substituted for coconut oil and not used as a supplement to a coconut oil diet, which may be a better comparison to this previous work. Nevertheless, while plasma vitamin E concentrations were higher in the coconut oil-fed hamsters, their plasma lipid hydroperoxide concentrations were significantly higher than those of the hamsters fed the palm oil preparations. The lower levels of unsaturated fatty acids and the higher levels of tocopherols in the coconut oil did not combine to reduce circulating plasma lipid hydroperoxides, suggesting either different pathways of antioxidant effect or an intrinsic effect of the dietary fats.

Since palm oil contains small, but measurable, levels of phytosterols, and in view of their known hypolipidemic effects [44], we deemed it of interest to measure fecal sterol concentrations in the hamsters. The major fraction in every case was phytosterols. Hamsters fed coconut oil or RPO excreted a greater amount (80% more) of sterol (62–64% phytosterol) than those fed RBD-PO or the reconstituted RBD-PO (53–55%). The proportion of cholesterol present in the cholesterol plus metabolite portion of the sterols was about the same in all four groups (26–32%). The major fecal phytosterol was campesterol (57% of total phytosterol in all groups). Thus the different fats affected the amount of fecal sterol but not the distribution of individual compounds. The hamsters fed RBD-PO or the reconstituted RBD-PO excreted less sterol than those fed RPO.

In contrast to several reports on the cholesterolemic effect of palm oil, information on its effects on atherosclerosis per se is lacking. Atherosclerosis is a multifactorial disease; its progression is slow and it develops over many decades starting silently with intra- and extracellular accumulations of lipid, derived mainly from LDL, in the intimas of arteries. In 1910, Windaus [45] described the inordinate accumulation of cholesteryl ester in atherosclerotic human aortas. Newman and Zilversmit [46] showed a gradual accumulation of aortic cholesteryl ester in normally fed rabbits and a gross accumulation of ester in cholesterol fed rabbits. Smith [47] found appreciable cholesteryl ester accumulation in normal aging human aortas and even greater levels of cholesteryl ester in human atheromata. These observations have been confirmed in other studies of atherosclerosis in humans and experimental animals [48]. Hence we analyzed the aortic levels of free and ester cholesterol in the animals used in this study. The total cholesterol content of the aortas of the hamsters fed coconut oil was significantly higher than that of the aortas of the other three groups. The levels of free and esterified cholesterol were also higher in the coconut oil-fed hamsters. The ratio of free to esterified cholesterol was highest in aortas of hamsters fed RPO, a reflection of lowered ester deposition and, thus, less severe atherosclerosis. The free/esterified cholesterol ratio in the aortas of the RPO-fed rabbits was 50% higher than those fed RBD-PO, 25% higher than those fed the reconstituted RBD-PO and 160% higher than those fed coconut oil. Thus, consistent with the rabbit data [26], RPO is less atherogenic than either RBD palm oil or reconstituted palm oil. Other work has shown that the substitution of palm olein oil for sunflower oil or lard significantly reduced the development of early atherosclerotic lesions in vervets [49].

This study has shown that palm oil is less atherogenic for hamsters than coconut oil probably due to the differences in fatty acid saturation. Among the different palm oil preparations, RPO (containing carotenoids and vitamin E) appears to be less atherogenic than RBD-PO. However, adding the carotenoids and vitamin E fraction back to RBD-PO, thus making our reconstituted RBD-PO reduce its atherogenicity somewhat but not to the level of RPO. Can there be an intrinsic distribution of carotenoids and vitamin E in RPO that cannot be duplicated by simply adding these components back to RBD-PO? Is the fine structure of red palm oil different from that of the reconstituted RBD-PO? Epidemiological data [19,20] suggest that dietary vitamin E and/or carotenoids may be protective against coronary heart disease and lung cancer, but experimental studies in which carotenoids and vitamin E were added to human diets have shown no protective effect [50,51]. The natural form in which a micronutrient is presented may provide a more effective delivery system than adding the isolated micronutrient to the diet. The natural packaging of micronutrients requires further attention.

Acknowledgment

The palm oil preparations were a gift of the Malaysian Palm Oil Board, Kuala Lumpur, Malaysia. The authors would like to thank Monica McIntyre, Julie Desjardins, Anthony DeSimone, Catherine Jones and Ben Woolfrey for their technical assistance, and Maureen Faul for her administrative assistance.

References

[1] MacFarlane N, Swetmen AA, Coursey DG. Comparison of traditional and industrial palm oil. Palm Oil News 1984;28:11–7.
[2] Chong YH, Ng TKW. Effects of palm oil on cardiovascular risk. Med J Malaysia 1991;46:41–50.
[3] Gunstone FD, Harwood JL, Padley FB. The lipid handbook. London: Chapman and Hall; 1986.
[4] Cottrell RC. Nutritional aspects of palm oil. Am J Clin Nutr 1991;53: 989S–1009S.
[5] Satchithanandam S, Reiks M, Calvert RJ, Cassidy MM, Kritchevsky D. Coconut oil and sesame oil affect lymphatic absorption of cholesterol and fatty acids in rats. J Nutr 1993;123:1852–8.
[6] Ayre KJ, Hulbert AJ. Dietary fatty acid profile influences the composition of skeletal muscle phospholipids in rats. J Nutr 1996; 126:653–62.
[7] Hassan AH. Palm oil and health. Planter 1988;64:505–19.
[8] Idris NA, Samsuddin S. Developments in food uses of palm oil: a brief review. Palmas 1993;15:66–9.
[9] Rukmini C. Red palm oil to combat vitamin A deficiency in developing countries. Food Nutr Bull 1994;15:126–9.
[10] Sundrum K, Top AGM. Vitamin E from palm oil. Its extraction and nutritional properties. Palmas 1994;15:77–82.
[11] Goh SH, Hew NF, Norhanom AW, Yadav M. Inhibition of tumor promotion by various palm oil tocotrienols. Int J Cancer 1994;57: 529–31.
[12] Krinsky NI. Mechanism of action of biological antioxidants. Proc Soc Exp Biol Med 1992;200:248–54.
[13] Hendrich S, Lee K, Xu X, Wang H, Murphy PA. Defining food components as new nutrients. J Nutr 1994;124:1789S–92S.
[14] Packer L. Interactions among antioxidants in health and disease. Vitamin E and the redox cycle. Proc Soc Exp Biol Med 1992;200: 271–6.
[15] Wilson RB, Middleton CC, Sun GY. Vitamin E, antioxidants and lipid peroxidation in experimental atherosclerosis in rabbits. J Nutr 1978; 108:1858–67.
[16] Brattsand R. Action of vitamin A and E and some nicotinic acid derivatives on plasma lipids and on lipid infiltration of aorta in cholesterol-fed rabbits. Atherosclerosis 1995;13:223–37.
[17] Verlangeri AJ, Bush MJ. Effects of D-α-tocopherol supplementation on experimentally-induced primate atherosclerosis. J Am Coll Nutr 1992;11:131–8.
[18] Gey KF, Moser VK, Jorda P, Stabelin H, Eicholzer M, Luden E. Inverse correlation between plasma vitamin E and mortality from ischemic heart disease in cross cultural epidemiology. Am J Clin Nutr 1991;53:326S–34S.
[19] Stampfer MJ, Hennekeus CH, Manson JE, Colditz GA, Rosner B, Willet WC. Vitamin E consumption and risk of coronary disease in women. N Engl J Med 1993;328:1444–9.
[20] Pearce BC, Parker RA, Deason ME, Qureshi AA, Wright JJ. Hypocholesterolemic activity of synthetic and natural tocotrienols. J Med Chem 1992;35:3595–606.
[21] Qureshi AA, Qureshi N, Wright JJK, Shens S, Kromer G, Gapor A, et al. Lowering of serum cholesterol in hypercholesterolemic humans by tocotrienols (palm vitee). Am J Clin Nutr 1991;53:1021S–6S.
[22] Helub BJ, Sicilia I, Mahadevappa VG. Effect of tocotrienol derivatives on collagen and ADP-induced plasma platelet aggregation, Abstracts, PORIM International Palm Oil Development Conference, September 5–9, Kuala Lumpur (N9), 1989.
[23] Steiner M, Anatasi J. Vitamin E and platelet aggregation. J Clin Invest 1975;57:732–7.
[24] Chan AC, Leith MK. Decreased prostacyclin synthesis in vitamin E deficient rabbit aorta. Am J Clin Nutr 1981;34:2341–7.
[25] Kritchevsky D, Tepper SA, Kuksis A, Wright S, Czarnecki SK. Cholesterol vehicle in experimental atherosclerosis: 22. Refined, bleached, deodorized (RBD) palm oil, randomized palm oil and red palm oil. Nutr Res 2000;20:887–92.
[26] Kritchevsky D, Tepper SA, Czarnecki SK, Sundram K. Red palm oil in experimental atherosclerosis. Asia Pac J Clin Nutr 2002;11: 5433–7.
[27] Terpstra AHM, Holmes JC, Nicolosi RJ. The hypercholesterolemic effect of soybean protein vs. casein in hamsters fed cholesterol-free or cholesterol enriched semipurified diets. J Nutr 1991;121: 944–7.
[28] Krause BR, Bousley RF, Kieft KA, Stanfield RL. Effect of the ACAT inhibitor CI-976 on plasma cholesterol concentrations and distribution in hamsters fed zero- and no-cholesterol diets. Clin Biochem 1992; 25:371–7.
[29] Allain CC, Poon LS, Chen CSG, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem 1974;20:470–5.
[30] Bucolo G, David H. Quantitative determination of serum triglycerides by the use of enzymes. Clin Chem 1973;36:476–82.
[31] Weingand KW, Daggy BP. Quantitation of high-density lipoprotein cholesterol in plasma from hamsters by differential precipitation. Clin Chem 1990;36:575.
[32] Mihaljevic B, Katusin-Razem B, Razem D. The reevaluation of the ferric thiocyanate assay for lipid hydroperoxides with special considerations of the mechanistic aspects of the response. Free Radic Biol Med 1996;21:53–63.
[33] Rudel LL, Kelley K, Sawyer JK, Shah R, Wilson MM. Dietary monosaturated fatty acids promote aortic atherosclerosis in LDL receptor-null, human ApoB100-overexpressing transgenic mice. Arterioscler Thromb Vasc Biol 1998;18:1818–27.
[34] Wilson TA, Rogers EJ, Nicolosi RJ, Sacchiero R, Goldberg DJ. Studies of cholesterol and bile acid metabolism in hamsters fed GT16-239, a novel bile acid sequestrant (BAS). Atherosclerosis 1998;140: 315–24.

[35] Folch J, Lees M, Sloane-Stanley GH. A simple method for the isolation and purification of total lipids from animal tissue. J Biol Chem 1957;226:497–509.
[36] Chong KS, Nicolosi RJ, Rodger RF, Arrigo DA, Juan RW, Mackey JJ, et al. Effect of dietary fat saturation on plasma lipoproteins and high density lipoprotein metabolism of the rhesus monkey. J Clin Invest 1987;79:675–83.
[37] Snedecor GW, Cochran WG. Statistical methods. 6th ed. Ames (Iowa): Iowa State Univ Press; 1980.
[38] Ng TKW, Hassan K, Lim JH, Lye MS, Ishak R. Nonhypercholesterolemic effects of a palm oil diet in Malaysian volunteers. Am J Clin Nutr 1991;53:1015S–20S.
[39] Kritchevsky D, Tepper SA, Wright S, Kuksis Hughes TA. Cholesterol vehicle in experimental atherosclerosis. Cottenseed oil and randomized cottonseed oil. Nutr Res 1998;18:259–64.
[40] Kritchevsky D, Tepper SA, Chen SC, Meijer GW, Krauss RM. Cholesterol vehicle in experimental atherosclerosis. Effects of specific synthetic triglycerides. Lipids 2000;35:621–5.
[41] Renaud SC, Ruf JC, Petithory D. The positional distribution of fatty acids in palm oil and lard influences their biological effects in rats. J Nutr 1995;125:229–37.
[42] Sundaram K, Khor HT, Ong ASH. Effect of palm oil and its fractions on rat plasma high density lipoproteins. Lipids 1990;25:187–91.
[43] Manorama R, Jyothermai RS, Aparna K. Effect of supplementation of red palm olein on serum lipid and antioxidant levels of healthy human subjects and iron absorption in anemic girls. Proceedings of the PORIM International Palm Oil Congress-Nutrition Conference. Kuala Lumpur, Malaysia: Palm Oil Research Institute of Malaysia; 1999. p. 134–46.
[44] Pollak OJ, Kritchevsky D. Sitosterol. Basil: S Karger; 1981.
[45] Windaus A. Uber die Gehalt normaler and Atheromatoser Aorten and Cholesterin und Cholesterinestern. Z Physiol Chem 1910;67:174–6.
[46] Newman HAI, Zilversmit DB. Accumulation of lipid and nonlipid constituents in rabbit atheroma. J Atheroscler Res 1964;4:261–71.
[47] Smith EB. The influence of age and atherosclerosis on the chemistry of aortic intima. Part I. The lipids. J Atheroscler Res 1965;5:224–46.
[48] Kritchevsky D. Current concepts in the genesis of the atherosclerotic plaque. In: Brest AN, Moyer JH editors. Atherosclerotic vascular disease. New York: Appleton-Century Crofts; 1967.
[49] Van Jaarsveld PJ, Smuts CM, Benade AJS. Effect of palm olein oil in a moderate-fat diet on plasma lipoprotein profile and aortic atherosclerosis in non-human primates. Asia Pac J Clin Nutr 2002;11(Suppl):S424–32.
[50] The Alpha Tocopherol/Beta Carotene Cancer Prevention Study Group. The effect of vitamin E and the beta carotene on the incidence of lung cancer and other cancers in male smokers. N Eng J Med 1994;330:1029–35.
[51] Omenn GS, Goodman GE, Thornquist MD, Balmer J, Cullen MR, Glass A, et al. Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. N Engl J Med 1996;334:1150–5.