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ORIGINAL ARTICLE

Influence of virgin coconut oil on blood coagulation factors, lipid levels and LDL oxidation in cholesterol fed Sprague–Dawley rats

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Summary
Background & aims: Experimental and epidemiological studies indicate an association between dietary saturated fatty acids and thrombosis, but the effects of individual fatty acids on haemostasis are still controversial. The purpose of this research is to evaluate the effect of feeding virgin coconut oil (VCO) on blood coagulation factors, lipid levels and in vitro oxidation of LDL in comparison with copra oil (CO) and sunflower oil (SFO) in cholesterol (1%) and oil (10% w/w) fed rats.
Methods: Rats were given the test oils along with cholesterol for 45 days. After the experimental period, serum cholesterol and triglyceride levels, thrombotic risk factor levels *viz*. fibrin, fibrinogen, factor V, 6-ketoPGF1α and prothrombin time were measured. In vitro Cu^{2+} induced oxidation of LDL, erythrocyte membrane and LDL TBARS content and plasma antioxidant vitamins (A and E) were also evaluated.
Results: Administration of VCO showed significant antithrombotic effect compared to copra oil and the effects were comparable with sunflower oil fed animals. The antioxidant vitamin levels were found to be higher in VCO fed animals than other groups. LDL isolated from VCO fed animals when subjected to oxidant (Cu^{2+}) in vitro showed significant resistance to oxidation as compared to the LDL isolated from other two groups. Dietary administration of VCO reduced the cholesterol and triglyceride levels and maintained the levels of blood coagulation factors. Results also indicate that VCO feeding can prevent the oxidation of LDL from oxidants. These properties of VCO may be attributed to the presence of biologically active unsaponifiable components *viz*. vitamin E, provitamin A, polyphenols and phytosterols.

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Introduction

The formation of thrombus in the coronary or cerebral arteries is one of the major causes of morbidity and mortality throughout the world. The role of coagulation system in the process of coronary heart disease is increasingly recognized.[1] Identified risk factors for thrombosis include genetics, diet, life style, smoking, lipids and cholesterol levels, molecular and circulating signals of chronic vascular inflammation.[2] Animal models of thrombosis are critical to mimic the human diseases and have been widely implemented in antithrombotic drug development.[3,4]

Numerous studies have examined the relationship between dietary fat and cardiovascular disease (CVD), but the effects of individual fatty acids on haemostasis are still controversial.[5,6] Experimental and epidemiological studies indicate an association between dietary saturated fatty acids and venous thrombosis, but the chain of evidences lack documentation from prospective clinical studies.[7] However, it is now thought that some saturated fatty acids may not deserve this reputation.[8] Previous reports suggest that saturated fatty acids and cholesterol each independently elevate blood cholesterol and low-density lipoprotein (LDL) concentrations.[9] Hypercholesterolemia and atherosclerotic disorders have been associated with a low-grade inflammation that involves not only the intrinsic cells of the artery wall, but also circulating cells, *viz* platelet, WBC, Hb and LDL.[10]

Free radical mediated oxidation of circulating LDL also plays a critical role in the progression of atherosclerosis.[11,12] Oxidized LDL stimulates platelet adhesion and aggregation by decreasing endothelial production of nitric oxide (NO) and increasing prostacyclin (PGI2) production.[13] It also stimulates coagulation, reduces fibrinolytic activity of endothelium and may also contribute to its dysfunction and plaque disruption.[14] During the course of normal metabolism, reactive oxygen species (ROS) and free radicals are formed, which induce oxidative damage to biomolecules and play an important role in the pathological conditions such as atherosclerosis, aging and inflammatory diseases and variety of other disorders.[15] Oxidatively modified lipids cause both direct and indirect toxic effects on the vascular endothelium. The antioxidant system (AOS) that includes the enzymatic and non-enzymatic mechanisms for lipid peroxide (LPO) product inactivation confers protection from endothelial dysfunction.[16]

Virgin coconut oil (VCO), unlike the coconut oil obtained from dried copra, is extracted directly from coconut meat under mild temperature. This extraction process avoids the loss of minor components like provitamin A and vitamin E and polyphenols due to UV irradiation from sunlight during drying of copra. Sunflower oil is reported to have beneficial effect which is associated with coronary heart disease (CHD).[17] We recently reported that VCO is more beneficial than copra oil (CO) and groundnut oil in lowering lipid levels and preventing the oxidation of LDL by physiological oxidants.[18] In this context we proposed to examine the effect of virgin coconut oil on blood coagulation factors that contribute towards thrombosis, lipid levels and LDL oxidation compared to copra oil (CO) and sunflower oil (SFO) in cholesterol fed rats.

Material and methods

Chemicals

α-Tocopherol, retinol, 6-ketoPGF1α, and prothrombin were purchased from Sigma Chemicals SA. All other chemicals used were of high analytical grade.

Test oils

Mature coconuts (West Coast Tall variety), grown at the Kerala University Campus were used for the extraction of copra oil and virgin coconut oil. (a) *Virgin coconut oil*: the solid endosperm of mature coconut was crushed, made into viscous slurry and squeezed through cheesecloth to obtain coconut milk which was refrigerated for 48 h to separate fat and water layer. Fat layer was carefully removed and subjected to mild heating (50 °C) in a thermostat oven. The obtained virgin oil was filtered through cheesecloth and was used for the present study.[18] (b) *Copra oil*: coconut meat was dried in sunlight continuously for 4 days to remove moisture and the resulted copra was pressed in the mill to obtain copra oil. (c) *Sunflower oil*: sunflower oil (Gold Winner brand, Chennai) was purchased from the local market.

Fatty acid analysis of VCO and CO

The fatty acid composition of VCO and CO was analyzed by gas chromatography. Fats were methylated with trimethylsulfonium hydroxide.[19] Fatty acid methyl esters were separated by gas chromatography using a system (HP 5890, Hewlett Packard GmbH, Waldbronn, Germany) equipped with an automatic on-column injector, a polar capillary column (30 m FFAP, 0.53 mm I.D., Macherey and Nagel, Düren, Germany) and a flame ionisation detector.[20] Helium was used as carrier gas at a flow rate of 5.4 ml/min. Fatty acid methyl esters were identified by comparing their retention times with those of individually purified standards (Table 1).

Table 1 Fatty acid composition of the test oils

Fatty acids	VCO	CO	SFO[45]
8:0	8.05	8.15	<0.1
10:0	5.42	5.56	0.0
12:0	45.51	43.55	0.0
14:0	19.74	18.38	0.1
16:0	7.83	8.25	6.5
18:0	3.14	2.65	4.2
18:1	4.70	6.70	18.7
18:2	1.88	1.49	68.6
20:0	0.086	0.086	0.3
20:1	0.027	0.042	0.3
22:0	0.016	0.018	0.7
24:0	0.032	0.065	0.2

Values are mean of three estimations and are expressed as percentage amount.

Animals

All the animal cares and procedures were according to the guidelines of the Institutional Animal Ethical Committee (IAEC). One-month-old male Sprague—Dawley rats (100—130 g) bred in our department animal house was used for the study. The animals (6/group) were housed individually in polypropylene cages in a room maintained at 25 ± 1 °C with a 12 h light and 12 h dark cycle.

Experimental protocol

A total of 18 rats were used to perform the present study. Rats were divided into three groups with six animals each as follows. (I) Copra oil (10% w/w) + 1% cholesterol, (II) virgin coconut oil (10% w/w) + 1% cholesterol, and (III) sunflower oil (10% w/w) + 1% cholesterol. Oils were fed along with the normal laboratory diet (10 g/rat) (Amrut Laboratory Animal Feed, Mumbai) for 45 days. Gain in body weight was recorded weekly. After 45 days, animals were fasted overnight and sacrificed by sodium pentothal injection, blood and tissues were collected for various estimations.

Estimation of hematological parameters

Blood was drawn in WBC pipette followed by WBC diluting fluid (Turke's fluid). Mixture was mixed well and transferred into a counting chamber. WBC was counted uniformly in four large corner squares. For counting platelets, blood was drawn in an RBC pipette and diluted with 1% ammonium oxalate and charged the chamber and platelet counted using 40× objective lens in the 80 smaller squares of the chamber. Hemoglobin was estimated using cyanomethemoglobin. For RBC count blood was mixed with RBC diluting fluid and counted as described earlier.[21]

Thrombotic risk factor levels

Fibrinogen was estimated as described by Fearnley and Chakrabarti[22] using a Fibroquant kit from Tulip Diagnostics (P) Ltd, Goa, India. Fibrin was estimated as described by King and Wootten.[23] Plasma (0.05 ml) was diluted with 2 ml of isotonic saline and 0.2 ml of 2.5% calcium chloride solution was added. The mixture was kept at 37 °C for overnight until a clot was formed. The fibrin was carefully collected, pressed to remove liquid and washed with water. Sixty percent of perchloric acid (0.2 ml) was added and digested until it become colorless. Solution is cooled, mixed with 5 ml of water and 1 ml of Nesslers reagent. The color developed was read against reagent blank at 620 nm. Pure dry ammonium chloride (4 mg/100 ml water) was used as the standard.

Prothrombin time was determined using liquiplastin kit from Tulip Diagnostics (P) Ltd, Goa, India. To 25 μl plasma at 37 °C forcibly added 0.2 ml of liquiplastin reagent (prewarmed at 37 °C for 3 min), simultaneously started a stopwatch and stopped as soon as the first fibrin strand was visible and the clot formation begins. Time is recorded in seconds and was taken as prothrombin time. Factor V was assayed by the method of Daniel.[24] The following cold reagents were taken in a small tube, 0.2 ml of prothrombin (200 U/ml), 0.2 ml of fibrinogen solution (0.5%) and 0.2 ml plasma diluted to 5% and allowed to stand at 37 °C for 4 min. Added 0.2 ml of $CaCl_2$ solution (25 mM) and the coagulation time was recorded.

Isolation and estimation of 6-ketoPGF1α by HPLC

Lipids were extracted from the serum with chloroform:methanol (1:1). It was then filtered through a Whatman No 1 filter paper. The residue collected was evaporated to dryness under N_2, after protein estimation. Residue was redissolved in 5 ml of ethanol and 15 ml of water was added, acidified to pH 3 with formic acid and kept for 24 h at 4 °C with constant shaking at 2 h interval. Sample was applied to a C18 Seppak catridge, and washed with 5 ml of water followed by 5 ml of 15% ethanol and 5 ml of hexane. The eicosanoids were eluted with 2.5 ml ethyl acetate.[25] 6-ketoPGF1α was detected using a C18 column (isocratically with acetonitrile:water:H3P04, 30:70:0.01, v/v, pH 2.95, flow rate 0.5 ml/min detection at 192 nm) in a Shimadzu S PDA 10 chromatograph.[26]

Serum vitamins A and E

Vitamins A and E content of serum were determined simultaneously by HPLC.[27] Briefly, 100 μl serum was added to 10 ml methanol containing 0.01% butylated hydroxytoluene (BHT) and hexane. Mixture was centrifuged and hexane layer collected, dissolved to dryness with N_2 and added 2 ml 10% methanolic KOH. After keeping for 2 h at room temperature 4 ml hexane was added. Hexane layer was collected, evaporated under N_2 and dissolved in 100 μl methanol and injected into HPLC column (C18 silica column, 100% methanol as mobile phase with 1.5 ml/min flow rate) and detected at 292 nm.

Erythrocyte membrane isolation

Blood from rats was drawn into tubes with potassium EDTA as anticoagulant. Plasma and platelets were removed by differential centrifugation (15 min, 1000×*g*). The erythrocytes were dispersed in isotonic phosphate buffer (0.119 mol PO_4/L, pH 7.4) and washed two or three times by centrifugation (20 min, 1000×*g*).[28] Erythrocyte membranes were prepared by hypotonic lysis in 7.6 mmol PO_4/L (pH 7.4) according to the procedure of Dodge.[29] Membrane preparations were washed in the 7.6 mmol PO_4/L until the supernatant was clear to remove hemoglobin and other cytoplasmic components. From this aliquots were removed and TBARS content was measured.[30]

Isolation of LDL

Blood from rats was collected into tubes containing potassium EDTA as anticoagulant. Plasma and platelets were removed by differential centrifugation (15 min, 1000×*g*). A volume of 3 ml plasma was centrifuged at $d = 1.006$ kg/l in an ultracentrifuge (Sorvall Ultra 80) at 40,000 rpm using a T-865 rotor at 14 °C for 10 h.[31] After ultracentrifugation floating VLDL and chylomicrons were removed and LDL was separated by precipitation from

Table 2 Hematological parameters of VCO, CO and SFO fed animals

Groups	Hb (g/100 ml)	WBC ($\times 10^3$/c.mm)	RBC ($\times 10^6$/c.mm)	Platelet count (/c.mm)
Copra oil	14.03 ± 0.12	7900 ± 115.47	6.03 ± 0.09	$26{,}500.00 \pm 288.67$
Virgin coconut oil	13.37 ± 0.18[a]	6600 ± 57.73[a]	5.30 ± 0.06[a]	$24{,}000.00 \pm 57.74$[a]
Sunflower oil	12.23 ± 0.17[a]	6800 ± 57.74[a]	5.17 ± 0.09[a]	$24{,}066.67 \pm 88.19$[a]

Values are mean $\pm$ SEM of six rats.
[a] $p < 0.05$ vs Group I.

the solution.[32] Forty milliliters of 4% phosphotungstic acid in 1 M NaOH was added, stirred and 10 μl of 2 M $MgCl_2 \cdot 6H_2O$ was added and cenrtifuged at $1500 \times g$ for 30 min at 4 °C. The supernatant was discarded and the precipitated LDL was redissolved in 0.4 ml 0.5 M Na_2CO_3, kept in ice overnight and dialyzed against three changes of PBS for 12 h.[18]

Estimation of serum lipid levels

Serum total cholesterol was estimated by the method as described by Abell et al.[33] Triglycerides were estimated by the method of Van Handel and Zilversmit.[34]

Statistical analysis

Analysis was done using SPSS 10. All values are mean $\pm$ SEM calculated by one-way ANOVA. Duncan's variance was applied to assess significant differences of continuous variables among groups.

Results

There were no differences in weight gain pattern of rats between groups. Table 2 represents the levels of hematological parameters. In VCO fed animals the WBC, RBC and Hb levels were lower than CO but the result was similar to that obtained in SFO fed animals. Platelet count was reduced in Groups II and III animals fed VCO and SFO than Group I animals fed CO, which was found to be significantly increased.

Prothrombin time (PT) was significantly lowered in CO treated animals (10.16 ± 0.16). VCO and SFO fed animals showed higher PT (11.25 ± 0.14 and 11.37 ± 0.08, respectively) (Table 3). Fibrin and fibrinogen levels were also found to be lower in VCO and SFO fed animals. In the case of CO fed animals, the levels of these parameters were significantly greater. The levels of factor V were found to be increased in CO fed groups (29.52 ± 0.66) but there were no significant changes in VCO and SFO fed groups (30.28 ± 0.11 and 30.01 ± 0.24, respectively) (Table 3). 6-ketoPGF1α was slightly reduced in VCO fed animals. But their levels were similar in other two groups (Table 3).

Vitamin A in the serum of VCO fed animals showed a significant change than SFO and CO fed animals. Vitamin E levels were greater in SFO group than VCO and CO fed animals with CO fed group showed a significantly lower level compared to VCO fed animals (Fig. 1).

Serum cholesterol levels in CO fed animal were significantly greater compared to CO and SFO fed animals. The values were similar in VCO and SFO fed animals. Serum triglyceride levels in CO fed animal were also significantly greater compared to CO and SFO fed animals. The values were similar in VCO and SFO fed animals (Fig. 2).

Erythrocyte membrane and LDL TBARS content of VCO fed animals were significantly lower than CO and SFO fed animals (Fig. 3). In SFO fed animas, LDL and erythrocyte membrane TBARS content were found to be significantly greater than other two oil fed groups.

LDL isolated from VCO fed animals when subjected to oxidation by Cu^{2+} was found to prevent oxidation than the LDL isolated from SFO and CO fed animals. In all the three cases the oxidation of LDL was found to increase with respect to time but the increase was slower in the case of LDL isolated from VCO fed animals compared to SFO and CO fed animals. The oxidation level was higher in LDL from SFO fed animals after 6 h indicated by high TBARS content. Oxidation level was lower in CO but not significant compared to VCO fed animals (Fig. 4).

Discussion

The present study was conducted to evaluate the effect of virgin coconut oil (VCO) on blood coagulation factors, lipid levels and LDL oxidation as compared to copra oil (CO) and sunflower oil (SFO) in cholesterol co-administered rats. Supplementation of VCO diet showed significant beneficial effects on blood coagulation when compared to CO and SFO. The lipid levels, and thrombotic risk factors *viz.*

Table 3 Blood coagulation factor levels of VCO, CO and SFO fed animals

Groups	Fibrin (mg/dl)	Fibrinogen (mg/dl)	Prothrombin time (s)	Factor V (s)	6-ketoPGF1α (pg/dl)
Copra oil	13.57 ± 0.53	296.67 ± 7.26	10.16 ± 0.16	29.52 ± 0.66	16.88 ± 0.25
Virgin coconut oil	10.50 ± 0.50	232.93 ± 4.3[a]	11.25 ± 0.14[a]	30.28 ± 0.11[a]	14.05 ± 0.47[a]
Sunflower oil	11.70 ± 0.82	225.13 ± 4.9[a]	11.37 ± 0.08[a]	30.01 ± 0.24[a]	15.94 ± 0.26

Values are mean $\pm$ SEM of six rats.
[a] $p < 0.05$ vs Group I.

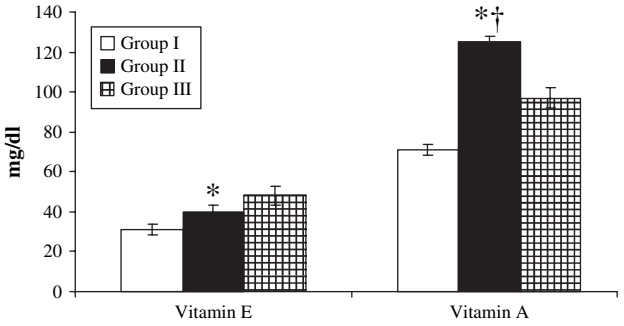

Figure 1 Levels of vitamins A and E levels of serum from test animals. Group I — copra oil (10%) + 1% cholesterol, Group II — virgin coconut oil (10%) + 1% cholesterol, and Group III — sunflower oil (10%) + 1% cholesterol. Bars represent mean values ± SEM of six rats. *$p < 0.05$ vs Group I; and †$p < 0.05$ vs Group III.

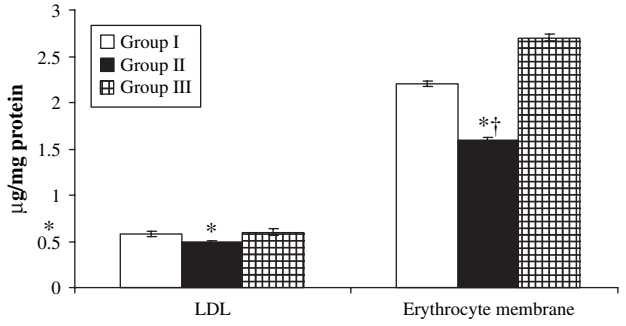

Figure 3 Levels of LDL and erythrocyte membrane TBARS content of test animals. Group I — copra oil (10%) + 1% cholesterol, Group II — virgin coconut oil (10%) + 1% cholesterol, and Group III — sunflower oil (10%) + 1% cholesterol. Bars represent mean values ± SEM of six rats. *$p < 0.05$ vs Group I; and †$p < 0.05$ vs Group III.

platelets, fibrin, fibrinogen, and factor V were lower in rats fed VCO. Hematological factors *viz*. WBC, Hb and RBC were also lower in this group. Blood coagulation is the host mechanism involved in perfecting the integrity of the vascular system in which platelets are known to play an active role. Elevated platelet count also contribute to the circulation and progression of coronary artery narrowing by atherosclerotic plaques resulting in occlusive coronary arterial thrombosis and finally to unstable angina and myocardial infarction. WBC is suggested to promote myocardial ischemia by release of toxic oxygen metabolites. There are substantial evidence that dietary factors, particularly fatty acids may affect platelet function.

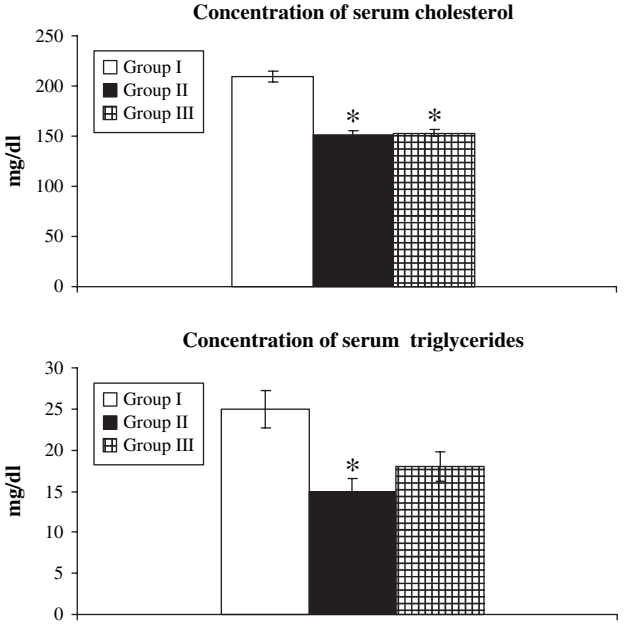

Figure 2 Levels of serum total cholesterol and triglyceride levels of test animals. Group I — copra oil (10%) + 1% cholesterol, Group II — virgin coconut oil (10%) + 1% cholesterol, and Group III — sunflower oil (10%) + 1% cholesterol. Bars represent mean values ± SEM of six rats. *$p < 0.05$ vs Group I.

The lower levels of fibrinogen and fibrin observed in VCO and SFO fed rats reflects the decreased blood-clotting tendency. The rate of conversion of fibrinogen to insoluble product fibrin is a key factor in haemostasis.[35] Fibrinogen, an acute-phase protein, becomes elevated as a consequence of inflammatory reactions that occur during the development of atherosclerotic plaques.[36] Previous studies have identified fibrinogen as a risk factor as powerful as cholesterol in producing ischemic events. Interaction of platelets with fibrinogen mediates a variety of responses including adhesion, platelet aggregation and fibrin clot retraction. We have also found decreased levels of factor V in VCO and SFO fed animals compared to CO fed group. Factor V, a large single chain plasma glycoproteins is an essential component of blood coagulation cascade and also an independent risk factor for myocardial infarction. During coagulation, factor V is converted to active co-factor, factor Va which combines with Xa and assemble to form prothrombinase complex, which converts prothrombin to thrombin.[37]

Concentration of PGF1α was found to be lower in VCO fed animals. 6-ketoPGF1α is a stable metabolite of PGI2, which is a critical local regulation of a variety of cellular processes. 6-ketoPGF1α is found to possess cardioprotective effects in animal model of myocardial infarction. During platelet activation, arachidonate is released from

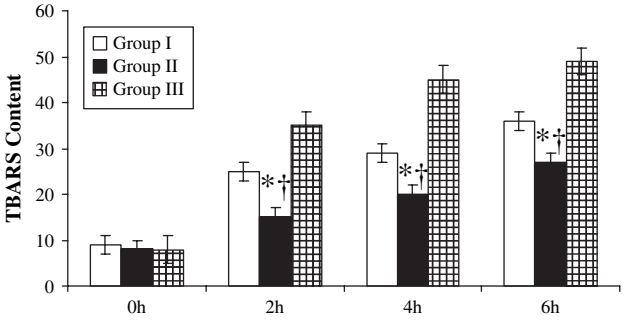

Figure 4 Cu^{2+} induced oxidation pattern of LDL isolated from test animals. Bars represent mean values ± SEM of six rats. TBARS content is expressed as nM/mg protein. *$p < 0.05$ vs Group I; and †$p < 0.05$ vs Group III.

the phospholipids and then converted into prostaglandin H2 and thromboxane A2, which strongly potentiate the activation process.[38] The low levels of 6-ketoPGF1α in VCO fed animals may be due to the lower level of linoleic acid which serves as the substrate for arachidonic acid synthesis.

In the regulation of primary haemostasis, the interaction of blood vessels with platelet plays an essential role and its in vivo evaluation involves bleeding time measurement.[39] Secondary haemostasis is another extremely important factor determining thrombus formation in vessels. Blood clotting can be inhibited by attenuation of plasma prothrombin activation system.[40] Here we have observed an increase in prothrombin time (PT) in VCO and SFO compared to CO fed groups. Serum total cholesterol (TC) was found to be decreased in VCO fed animals and was comparable with SFO fed group. The TBARS content of isolated LDL and erythrocyte membrane of VCO fed animals were also significantly decreased compared to other two groups. The lower levels of LDL and VLDL cholesterol in VCO fed animals may be the reason for the above effect (unpublished report). Feeding cholesterol rich diet caused significant increase in the plasma, erythrocyte and liver TC, plasma TG and TBARS levels. Plasma and erythrocyte TBARS levels are markers of oxidative stress.

Circulating lipid peroxides may promote lipid peroxidation of other circulating lipids and lipoproteins, resulting in disseminated endothelial dysfunction.[41] MDA, a breakdown product of spontaneous fragmentation of peroxides form from polyunsaturated fatty acids (PUFA) mainly from the oxidation of cell membranes.[42] The observed lower level of plasma and erythrocyte TBARS content may be due to the higher amount of antioxidant vitamins in the serum and lower amount of PUFA of VCO. SFO contain very high amount of unsaturated fatty acids (Table 3)[43] and is more susceptible to oxidation. Feeding of oils rich in polyunsaturated fatty acids (PUFA) results in their accumulation in cell membranes and increased the oxidative stress, since PUFAs are highly susceptible to peroxidation compared to monounsaturated and saturated fatty acids. This may be the reason for the higher TBARS formation in the plasma and erythrocyte membrane of SFO fed animals.[44,45]

It is generally believed that the highly saturated nature of coconut fatty acids (Table 3) increases cholesterol synthesis in our body and thus contributes to higher incidence of heart disease. This contention, however, has been refuted scientifically.[46] Available scientific reports showed that it is not hypercholesterolemic and atherogeneic.[47] Medium chain triacyglycerols (MCTs) of coconut oil fed rats reduced storage fat accumulation, serum and tissue cholesterol and linoleate requirement.[48] Compared to CO, VCO contain appreciable amounts of many biologically active minor components viz. polyphenols (80 mg/100 g oil) and antioxidant vitamins (30 μg/100 g oil), which may have an effect on blood coagulation. In vitro studies have proved that polyphenols inhibit platelet aggregation, increases the Ca^{2+} influx and mobilization of Ca^{2+} in endothelial cells.[49] These compounds also reported to suppress adhesion molecules and inhibit experimental atherosclerosis. Diet supplemented with polyphenolic compounds improved the lipid metabolism and increased the plasma antioxidant potential especially in rats fed with added cholesterol.[50] Recently we have reported that VCO polyphenols can prevent the oxidation of LDL in in vitro conditions.[18] They can trap reactive oxygen species from aqueous series such as plasma and interstitial fluid of arterial wall thereby inhibiting oxidation of LDL and showing atherosclerotic activity.[51]

We have observed an increase of serum vitamins A and E in VCO fed animals than CO fed animals and is comparable to SFO fed animals. The lower levels of these vitamins in CO fed animals may be due to the low content in CO (Table 3) that might have lost during the exposure of copra to UV radiation of sunlight during extraction process. Vitamin E has been implicated in the body's protective armory against diseases and is the major chain breaking lipophilic antioxidants in tissue and plasma.[52] Vitamin E decrease platelet aggregation by dephosphorylating protein kinase Cα (PKCα) and might thus affect thrombotic tendencies.[53,54] Plasma vitamin E decreases the TBARS content in hypercholesterolemic rats by trapping the chain-propagating peroxyl radicals.[55] It also suppresses the expression of adhesion molecules and chemokines by endothelial cells/monocytes in culture. Studies showed that antioxidant vitamins A and E were found to be decreased in the plasma of patients with angina and myocardial infarction.[56,57]

LDL isolated from VCO treated animals showed a reduced TBARS content when treated with $CuSO_4$. This may de due to the higher levels of antioxidants *viz*. vitamins E and A and polyphenols in the LDL which protect it from physiological oxidants. Oxidized LDL has been shown to be highly cytotoxic for vascular cells, to activate endothelial recruitment of leukocytes, macrophage cytokine production and stimulate smooth muscle cells (SMCs) proliferation.

Oxysterols are responsible for most of the cytotoxic effects of oxidized LDL.[58] During the oxidation of LDL, the LDL molecule undergoes a large number of structural changes that alter its metabolism.[59] Vascular thrombogenicity is induced by progressive LDL oxidation and that alterations of the antioxidant/oxidant balance of the LDL particle in favor of the antioxidant tone are protective against the thrombotic response triggered by oxidative stress.[60] It is generally accepted that the primary generation of lipid hydroperoxides in our body initiates a reaction cascade leading to rapid propagation and to amplification of the number of reactive oxygen species formed; this ultimately leads to extensive fragmentation of the fatty acid chains,[61] and conversion of the LDL to a more atherogenic form.[62] Peroxidation of LDL with Cu^{2+} produced cholesteryl ester core aldehydes, such as 9-oxononanoylcholesterol (9-ONC) and 5-oxovaleroylcholesterol (5-OVC), as the major oxidized cholesteryl esters.[63] Oxidized LDL reduces the fibrinolytic activity of endothelium by decreasing secretion of tissue-type plasminogen activator (tPA) and increasing release of plasminogen activator inhibitor-1 (PAI-1)[64] also stimulates coagulation by reducing thrombomodulin (TM) transcription.[65]

In conclusion, the results indicate that consumption of VCO supplemented diet exerts a significant antithrombotic effect, which is associated by suppression of platelet aggregation and low levels of cholesterol and triglycerides. VCO also prevented the formation of lipid peroxides in both erythrocyte membrane and LDL of experimental animals.

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