



EFFECT OF FISH OIL AND VARIOUS PREPARATIONS OF COCONUT OIL ON BODY WEIGHT AND SERUM LIPID PROFILE IN RATS*

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Abstract

Effect of fish oil (FO) and various preparations of coconut oil viz., copra oil/RBD coconut oil (CO), seasoned coconut oil (SCO) and virgin coconut oil (VCO) was evaluated on body weight and serum lipid profile in rats. Significant decrease and increase in body weight was observed in VCO and FO fed rats respectively whereas, CO and SCO did not show any significant variation, when administered for a period of three months. Coconut oil administered rats showed significant increase in serum triacylglycerol (TAG) and VLDL, significant decrease in LDL and no change in total lipids (TL), total cholesterol (TC) and HDL levels. All the lipid parameters under study were significantly increased in SCO fed rats whereas, VCO significantly increased HDL and decreased LDL, with little change in the levels of TL, TC, TAG and VLDL. Fish oil administration significantly increased TAG, while TL and LDL decreased with no significant variation in the levels of TC, HDL and VLDL. Except SCO, all other oils significantly increased HDL/LDL ratio, being highest in VCO fed group. Comparison between various oil fed rats, revealed a desirable effect with VCO and the effect was comparable to or even superior to that of FO administration. The present study recommends the consumption of VCO due to its favorable effects on serum lipid profile and body weight.

Keywords: Copra oil, Fish oil, Rats, Seasoned coconut oil, Virgin coconut oil

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Consumption of small amount of saturated fats is essential and a high correlation has been suggested between consumption of excess saturated fat and **coronary heart disease (CHD)** (Clarke *et al.*, 1997). Among the vegetable oils, coconut oil has a very high content (90%) of saturated fatty acids. Coconut kernel and oil are consumed mainly by the people of Philippines, Indonesia, India, Brazil, Thailand, Vietnam, Sri Lanka, Malaysia and other tropical countries, for thousands of years. In India, southern states such as, Kerala, Tamil Nadu, Karnataka and Andhra Pradesh are well known for cultivation and production of coconut. Usually people in the western coastal region of India use coconut oil for curried dishes after seasoning it with mustard seeds, onion, curry leaves and turmeric. Coconut features in a lot of South Indian dishes, not just the coconut milk, but the oil and grated coconut are used as well.

Coconut oil has provided the primary source of fat in the diets of millions of people for generations throughout the tropics. Though, there are studies that indicate a positive correlation between consumption of coconut oil and development of CHD, many other investigations conducted in animals and human beings contradicts claims that coconut oil increases the risk of atherosclerosis and heart disease (Awad, 1981; Sabitha *et al.*, 2009). Thus, there exists a controversy among the scientific community regarding the negative effects of coconut oil. The present

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study was carried out to evaluate the role of various preparations of coconut oil viz., copra oil/RBD coconut oil, virgin coconut oil and seasoned coconut oil on body weight and lipid metabolism in rats in comparison with fish oil.

Materials and Methods

Commercial coconut oil (Copra oil/RBD coconut oil) was procured from Kerala Agricultural University. Commercial virgin coconut oil (RUBCO Nutri-ko) and shark liver oil were procured from local market. Seasoned coconut oil was prepared in the laboratory as follows: About 50 ml of copra oil was heated in a pan and added mustard seeds (10% w/v). As soon as the seeds sizzled, added chopped small onion/ red onion (*Allium oschaninii*) (5% w/v) and fried until the colour became golden brown, followed by the addition of the remaining oil, turmeric powder (2.5% w/v) and curry leaves (10% w/v). Mixed well and allowed to cool to room temperature. Then the oil was strained through a muslin cloth. About 250 ml of seasoned coconut oil was prepared each week afresh and used for the study. All other chemicals were procured from Merck India Ltd, Mumbai.

Male Wistar rats weighing 180 to 220 g were housed in appropriate cages in a well ventilated experimental animal room under 12 : 12 hr LD cycle at 22 to 28°C and 45 to 55 per cent relative humidity with free access to standard rat pellet diet and drinking water. Experiments were conducted with the approval of Institutional Animal Ethics Committee. Rats were randomly divided into five groups, G1 to G5, each comprising 6 animals. Except G1, rats under all other groups were administered with various oils for a period of 90 days as follows:

- G1 – Normal control (NC)
- G2 – Copra oil/ RBD coconut oil (CO)
- G3 – Seasoned coconut oil (SCO)
- G4 – Virgin coconut oil (VCO)
- G5 – Fish oil (FO)

Dose was fixed based on per capita world average oil consumption level (17.8 kg/head/year), consumption level of developed western world (44 to 48 kg/head/year) and the total coconut oil consumption in Kerala (free oil + oil derived from kernel), which comes around 14 kg/head/year (Rajamohan, 2004). A dose of 30 kg/head/year was fixed, which

comes to a rat dose of 16.4 g/kg body weight. The dose fixed was an average value of per capita world average consumption and consumption of developed western world. Moreover, it was nearly double the per capita coconut oil consumption in Kerala. Oils were administered every day orally using an oesophageal tube in two divided doses, at morning and evening.

Blood samples were collected from the retro orbital plexus under mild ether anesthesia, using heparinised capillary tubes, into sterile microfuge tubes on days, 0 (just before administration of oils), 45 and 90 (45 and 90 days after oil administration) and separated serum by centrifuging at 1000 x g for 10 min. at 15°C. Weight of the animals was also recorded on the above days. Serum samples were analysed for total lipids (TL), total cholesterol (TC) and triacylglycerol (TAG) using commercially available kits (Ecoline Kits, M/s E. Merck India, Ltd, Mumbai). HDL cholesterol was estimated by Heparin–Manganese precipitation procedure (Warnick and Albers, 1978). LDL and VLDL cholesterol were estimated by the conventional Friedewald Equation (Warnick *et al.*, 1990). Data obtained were compared by analysis of co-variance (ANCOVA) followed by Duncan multiple range test to determine the level of significance. The value of $P < 0.05$ was considered statistically significant (Snedecor and Cochran, 1985).

Results and Discussion

Administration of CO and SCO showed a transient increase in body weight, which decreased to that of control rats whereas, VCO significantly decreased the body weight, when administered for a period of three months (Table 1). This could be attributed to the high content of medium chain triacylglycerols (MCTs) in coconut oil, which are readily hydrolyzed by lingual and gastric lipases to release medium chain fatty acids (MCFAs) (Bezard and Bugaut, 1986). Free MCFAs mainly, lauric acid (~ 43 % in CO and 50-57% in VCO), are absorbed readily from gastro intestinal tract and transported to hepatocytes, where these enter the mitochondria without the assistance of carnitine acyltransferase and are oxidized as a rapid source of energy. Thus, MCFAs are considered to be less available for deposition in body fat (Tsuji *et al.*, 2001).

Table 1. Effect of various preparations of coconut oil and fish oil on body weight (g) in rats (Mean \pm SE, n = 6)

Groups	Days		
	0	45	90
Normal control (G1)	202.17 ^a \pm 2.95	205.20 ^a \pm 5.74	241.56 ^{a,b} \pm 3.86
Coconut oil (G2)	201.83 ^a \pm 1.64	260.68 ^b \pm 5.76	251.06 ^a \pm 3.88
Seasoned coconut oil (G3)	202.67 ^a \pm 1.67	224.15 ^c \pm 5.71	232.31 ^b \pm 3.84
Virgin coconut oil (G4)	202.67 ^a \pm 1.71	199.48 ^a \pm 5.71	213.81 ^c \pm 3.84
Fish oil (G5)	207.50 ^a \pm 1.59	249.16 ^b \pm 6.17	287.09 ^d \pm 4.15

In FO fed rats, body weight increased significantly compared to the rats fed with coconut oil on day 90 (Table 1). The increase was about 40% compared to day 0 as against 5% in VCO fed rats, which could be due to the high content of long chain polyunsaturated fatty acids (PUFAs - 65-70%). Long chain fatty acids (LCFAs) require the detergent action of bile to enter the intestinal cells, re-esterified, packaged and secreted into lymph, which are transported to peripheral tissues for storage in fat depots (Tsuji *et al.*, 2001).

Feeding CO and SCO significantly increased the levels of TL and TAG by day 45. On day 90 the level of TL decreased to that of NC while TAG remained significantly high in CO fed rats. However, both TL and TAG remained at a significantly higher level in SCO fed rats (Table 2). Increased hepatic expression of apo C-III by MCTs in coconut oil (both CO and SCO) and apo C-III-mediated

inhibition of catabolism of TAG-rich lipoproteins might have contributed to a rise in serum lipid and TAG concentrations (Wang *et al.*, 1998). Administration of VCO transiently increased the levels of TL and TAG, which decreased to that of control rats by day 90 (Table 2). Similar observations were also reported by earlier workers and suggested that this could be due to the quality difference in CO and VCO. High content of polyphenols and minor constituents such as vitamin A and E might have contributed to the lipid lowering effect of VCO (Nevin and Rajamohan, 2004).

Rats fed with FO showed a significant increase in the levels of serum TL and TAG by day 45 and later by day 90, the level of TL decreased significantly whereas, the level of TAG was significantly higher than VCO and lower than CO and SCO fed rats (Table 2). Lipid lowering effect of FO is in accordance with earlier reports (Frenoux *et al.*, 2001).

Table 2. Effect of various preparations of coconut oil and fish oil on serum total lipids and triacylglycerol (mg/dl) levels in rats (Mean \pm SE, n = 6)

Groups	Total lipids			Triacylglycerol		
	Days			Days		
	0	45	90	0	45	90
Normal control (G1)	337.68 ^a \pm 8.72	333.16 ^a \pm 8.51	334.33 ^a \pm 5.94	41.33 ^a \pm 1.98	43.22 ^a \pm 3.89	44.35 ^a \pm 2.37
Coconut oil (G2)	356.88 ^a \pm 7.83	647.38 ^b \pm 8.33	353.72 ^a \pm 5.81	49.00 ^a \pm 5.37	60.12 ^b \pm 3.91	68.81 ^b \pm 2.38
Seasoned coconut oil (G3)	345.22 ^a \pm 7.87	620.64 ^c \pm 8.27	402.24 ^c \pm 5.77	46.17 ^a \pm 2.01	71.65 ^c \pm 3.82	74.35 ^b \pm 2.33
Virgin coconut oil (G4)	356.82 ^a \pm 10.68	366.37 ^d \pm 8.33	323.15 ^a \pm 5.81	45.83 ^a \pm 3.39	54.24 ^{a,b} \pm 3.82	43.15 ^a \pm 2.32
Fish oil (G5)	352.12 ^a \pm 10.75	363.67 ^d \pm 8.24	275.62 ^d \pm 5.75	43.00 ^a \pm 3.59	77.10 ^c \pm 3.84	53.52 ^c \pm 2.34

Reduction in the level of TL could be attributed to the EPA and DHA components of PUFA present in FO, which are known to reduce both plasma lipid levels and platelet aggregation (Goodnight *et al.*, 1982). Feeding rats with diets containing marine hilsa FO decreased the levels of serum cholesterol, TAG and phospholipids while, chital oil (oil from fresh water fish) elevated plasma cholesterol level while TAG and phospholipid levels remained unaltered (Banerjee *et al.*, 1992), which suggested that rather than the total quantity of PUFA, the composition of PUFA and an interaction between EPA and DHA is important in lipid metabolism.

In both CO and SCO fed groups, the levels of TC and HDL also showed a significant increase by day 45 but after 90 days, the levels of both, decreased to that of NC in CO fed rats while these continued to be at a significantly higher level in SCO fed group. Virgin coconut oil fed rats showed a transient increase in the level of TC, which decreased to that of control rats by day 90 whereas, HDL remained at a significantly high level throughout the period of study (Table 3).

An opposite trend was observed in the levels of LDL and VLDL in CO fed rats where, LDL decreased and VLDL increased significantly throughout the period of study. In SCO fed group the levels of both LDL and VLDL showed an increasing trend which became significant by day 90 whereas, VCO fed group showed a decreasing trend in the level of LDL and by day 90 the level decreased

to nearly half the value than that observed on day 0. However, there was a transient increase in the level of VLDL, which decreased to that of NC rats by day 90 (Table 4). There was no significant variation in HDL/LDL ratio of SCO fed rats while it increased significantly in CO and VCO fed groups (Table 5).

Transient increase and then a decrease observed in the level of TC on CO administration might be due to the increase and then a decrease in the level of other cholesterol fractions especially, HDL. Coconut oil administered rats showed a very high increase, from 18 mg/dl to 31 mg/dl, in the level of HDL by day 45, which returned to that of basal level (20 mg/dl) by day 90, might have contributed to the increase and subsequent decrease in the level of TC. Studies in human beings also showed that compared to butter fat, coconut oil had a lesser effect in increasing serum cholesterol (Cox *et al.*, 1995). However, significantly high level of TC in SCO fed rats is substantiated by the increased levels of all other cholesterol fractions, such as, HDL, LDL and VLDL during the course of experiment.

Coconut oil and SCO differs only in the fat soluble components of seasoning ingredients and the reason for this varying effect between the two is not clear. It might be due to the presence of phytosterols and squalene in SCO ingredients, which could upregulate cholesterologenesis either by indirect suppression of cholesterol absorption from small intestine or directly by serving as a precursor for cholesterol synthesis (Jones *et al.*, 1994).

Table 3. Effect of various preparations of coconut oil and fish oil on serum total cholesterol and HDL (mg/dl) levels in rats (Mean \pm SE, n = 6)

Groups	Total cholesterol			HDL		
	Days			Days		
	0	45	90	0	45	90
Normal control (G1)	47.33 ^a ± 1.45	47.74 ^a ± 2.45	48.08 ^a ± 1.82	19.17 ^a ± 0.54	19.09 ^a ± 1.19	19.65 ^a ± 1.07
Coconut oil (G2)	45.67 ^a ± 2.26	59.70 ^b ± 2.36	47.86 ^a ± 1.75	18.17 ^a ± 1.05	31.04 ^b ± 1.19	20.34 ^a ± 1.07
Seasoned coconut oil (G3)	43.17 ^a ± 1.14	60.16 ^b ± 2.40	68.44 ^b ± 1.78	17.50 ^a ± 0.81	26.90 ^c ± 1.19	28.53 ^b ± 1.08
Virgin coconut oil (G4)	43.67 ^a ± 1.23	56.06 ^b ± 2.38	47.46 ^a ± 1.76	18.67 ^a ± 0.88	27.15 ^c ± 1.19	27.49 ^b ± 1.07
Fish oil (G5)	44.83 ^a ± 1.70	41.52 ^a ± 2.35	43.49 ^a ± 1.74	18.67 ^a ± 2.09	17.48 ^a ± 1.19	20.99 ^a ± 1.07

Table 4. Effect of various preparations of coconut oil and fish oil on serum LDL and VLDL (mg/dl) levels in rats (Mean \pm SE, n = 6)

Groups	LDL			VLDL		
	Days			Days		
	0	45	90	0	45	90
Normal control (G1)	15.37 ^a ± 0.75	15.84 ^{a,c} ± 1.38	15.50 ^a ± 1.41	12.73 ^a ± 0.56	12.93 ^a ± 0.75	12.66 ^a ± 0.53
Coconut oil (G2)	14.93 ^a ± 1.68	13.58 ^b ± 1.34	11.45 ^b ± 1.37	12.67 ^a ± 0.71	14.77 ^b ± 0.83	15.29 ^b ± 0.58
Seasoned coconut oil (G3)	15.33 ^a ± 1.12	21.13 ^c ± 1.34	25.05 ^c ± 1.3	12.30 ^a ± 0.29	12.85 ^a ± 0.78	15.53 ^b ± 0.54
Virgin coconut oil (G4)	15.10 ^a ± 1.84	14.74 ^{a,b} ± 1.34	7.64 ^b ± 0.37	11.67 ^a ± 0.41	13.57 ^b ± 0.75	12.24 ^a ± 0.53
Fish oil (G5)	15.50 ^a ± 0.85	10.98 ^b ± 1.34	10.94 ^b ± 1.36	11.63 ^a ± 0.81	12.95 ^a ± 0.76	11.02 ^a ± 0.53

Table 5. Effect of various preparations of coconut oil and fish oil on HDL/LDL ratio (Mean \pm SE, n = 6)

Groups	Days		
	0	45	90
Normal control (G1)	1.15 ^a \pm 0.06	1.12 ^a \pm 0.03	1.11 ^a \pm 0.08
Coconut oil (G2)	1.29 ^a \pm 0.17	2.45 ^b \pm 0.32	2.09 ^b \pm 0.31
Seasoned coconut oil (G3)	1.17 ^a \pm 0.11	1.25 ^a \pm 0.11	1.17 ^a \pm 0.09
Virgin coconut oil (G4)	1.33 ^a \pm 0.15	2.04 ^{b,c} \pm 0.34	3.85 ^c \pm 0.41
Fish oil (G5)	1.23 ^a \pm 0.15	1.60 ^{a,c} \pm 0.17	2.04 ^b \pm 0.22

Level of significance was determined column wise between groups. Values not bearing a common superscript letter (a, b, c and d) in a column differ significantly ($P < 0.05$).

Increased hepatic apo A-I expression in animals fed with intermediate chain triglycerides, may contribute to the higher serum HDL (Wang *et al.*, 1998). Lower level of LDL in CO fed rats agrees with earlier findings (Denke and Grundy, 1992., Cox *et al.*, 1995). Elevation in VLDL in CO administration is in accordance with earlier reports and it could be due to an increase in particle number, increased hepatic secretion of VLDL or due to a modest decrease in VLDL TAG clearance capacity (Heek and Zilversmit, 1991; Cox *et al.*, 1995).

High levels of HDL, LDL and VLDL observed in SCO fed rats partly agree with the findings of Beena *et al.* (1996). They reported an increase in HDL whereas, LDL and VLDL were decreased, when rats were fed with a coconut oil diet supplemented with curry leaves and mustard seeds and suggested that polyphenols and flavonoids present in curry leaves and PUFA content of mustard seeds might have contributed to this effect. Seasoned coconut oil used in this study, might contain only very low amount of the above

active principles either might not have exerted any effect or have exerted an antagonistic effect in reducing the levels of LDL and VLDL.

Transient increase and then a decrease in the level of TC in VCO fed rats, correlate with the levels of other cholesterol fractions such as, HDL and LDL, which is in agreement with earlier findings (Nevin and Rajamohan, 2004). High content of flavonoids and other polyphenols might have contributed to this effect (Zhou *et al.*, 2009).

In FO fed rats, the levels of TC, HDL and VLDL did not vary significantly whereas, LDL decreased significantly, showing a significantly high HDL/LDL ratio throughout the period of study (Table 3, 4, 5). Significant reduction in the level of LDL was also reported in rats supplemented with a diet rich in FO, which could be either due to an increased rate of receptor dependent LDL transport or a low level of LDL-cholesterol production (Maree *et al.*, 2009). It has also been reported that PUFA administration decreased the activities of many enzymes associated with lipid synthesis (Toussant *et al.*, 1981).

The above observations suggest that among the various preparations of coconut oil, VCO exhibited desirable effects on body weight and serum lipid profile. The effect was similar to or even superior to that of FO administered rats. Compared to VCO, CO showed adverse effects on the levels of TAG and VLDL while SCO revealed undesirable effect on all serum lipid fractions under study except HDL. In this context, it is worth mentioning that the dose of oil administered was very high, which is an average value of per capita world average consumption and the consumption of developed western world. Moreover, the dose was almost double the per capita coconut oil consumption in Kerala. Since, coconut oil is one of the major components in cooked dishes, the effect of heat and the seasoning ingredients on varying doses of CO and VCO have to be further explored.

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