

The Effects of Oil Enriched Diet on Plasma Lipid Profile, Glucose and Coagulation Time in Rats

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Purpose: the effects of chronic intake of olive, canola, or coconut oil rich diet on blood lipid profile, glucose, and coagulation time in rats were studied. **Methods:** animals were divided into four groups control, canola, coconut and olive oil groups. All groups received an isocaloric rat chow diet, at the time where the oil group diets contained an extra 5 % by weight of the corresponding oil. **Results:** after 13 weeks of dietary intervention, groups on oil-rich diets showed a significant decrease in coagulation time compared with the control group ($p < 0.05$). There was no difference in fasting blood sugar among all groups. Coconut oil increased plasma total cholesterol by 10 % ($p = 0.06$) and LDL cholesterol by 24% ($p = 0.03$) compared with the control group. Enrichment of the diet with either olive or canola oil reduced plasma triacylglycerol concentrations ($p = 0.008$ and $p = 0.02$ respectively) and increased HDL cholesterol concentrations ($p = 0.02$ and $p = 0.002$ respectively) with respect to the control group. Determination of plasma total apolipoprotein B100 concentration revealed a significant increase in coconut oil group compared with all other groups ($p < 0.05$).

Conclusion, a diet rich in canola or olive oil had a much more favorable effects on blood lipid profile, and plasma lipoproteins compared with coconut oil. However, irrespective of the oil type, blood coagulation time seems to be inversely related to the percentage of fat content in the diet.

Background

Cardiovascular disease (CVD) has been the number one killer in the United States in the last century. Dietary fatty acid composition influences plasma lipids and lipoproteins associated with the development of atherosclerosis and ischemic heart disease. The effect of dietary fatty acids on plasma total cho-

lesterol, low density lipoprotein (LDL) and high density lipoprotein (HDL) cholesterol, has been subject to many studies, and prediction algorithms have been developed (1-4). Adequate levels of cholesterol have crucial effects on survival. Cholesterol is an essential component of the cell membrane and a precursor of steroid hormones. It is derived from the diet or synthesized in the liver.

The liver export lipids as very low density lipoprotein (VLDL). As VLDL passes past tissues, lipoprotein lipase (LPL), activated by apoC II, hydrolyses the TAG core. ApoC II is then lost, and lipoprotein particle (LP) released as intermediate density lipoprotein (IDL) that will change to LDL. LDL contains some TAG, lots of cholesterol esters (CE) and cholesterol (C) and one apolipoprotein B100 (apoB100). Recent studies have revealed that apoB100 concentration in the blood is a better indicator of potential myocardial infarction than total cholesterol or LDL cholesterol (5), especially in individuals with low or normal LDL-cholesterol (6). LDL, the major circulating pool of cholesterol in the blood, provide cholesterol to the peripheral tissues, whereas, HDL is used to remove cholesterol from cells (reverse cholesterol transport).

Monounsaturated and saturated fatty acids are not essential fatty acids since they can be synthesized in the body (7). The primary monounsaturated fatty acids in the diet are oleic (C18:1, n-9) and palmitoleic (C16:1, n-9) acids. Olive oil, rapeseed oil (canola oil), cocoa butter, and beef are excellent sources of

Samples containing the lipoprotein fractions were delipidated in a methanol-diethyl ether solvent system

oleic acid. Conventional wisdom held that monounsaturated fatty acids had a neutral effect on fasting plasma cholesterol (8), or triacylglycerol concentrations in humans (9,10). Later, Grundy (11) reported that monounsaturated fatty acids, when substituted for saturated fatty acids in the diet, effectively reduce plasma LDL cholesterol concentrations and might be utilized in dietary modifications to lower plasma chole-

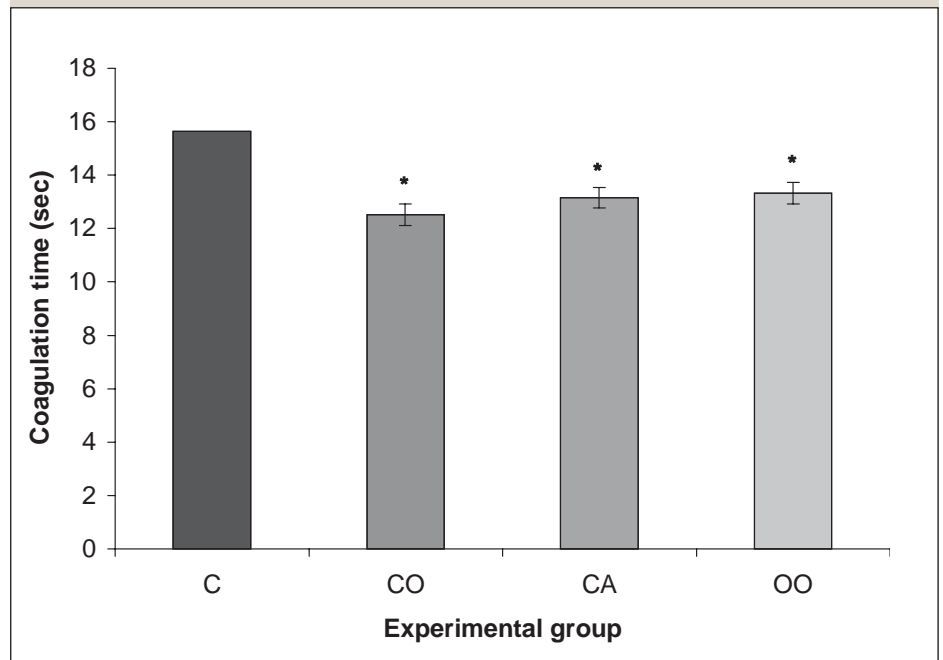


Figure 1 - Fasting plasma coagulation time of rats receiving a regular rat chow diet with (coconut oil, C; canola oil, CA; olive oil, OO) or without (control, C) 5% w/w oil supplementation. Bars denote mean ± S.E.M. (n = 15). * P < 0.05 compared with control group.

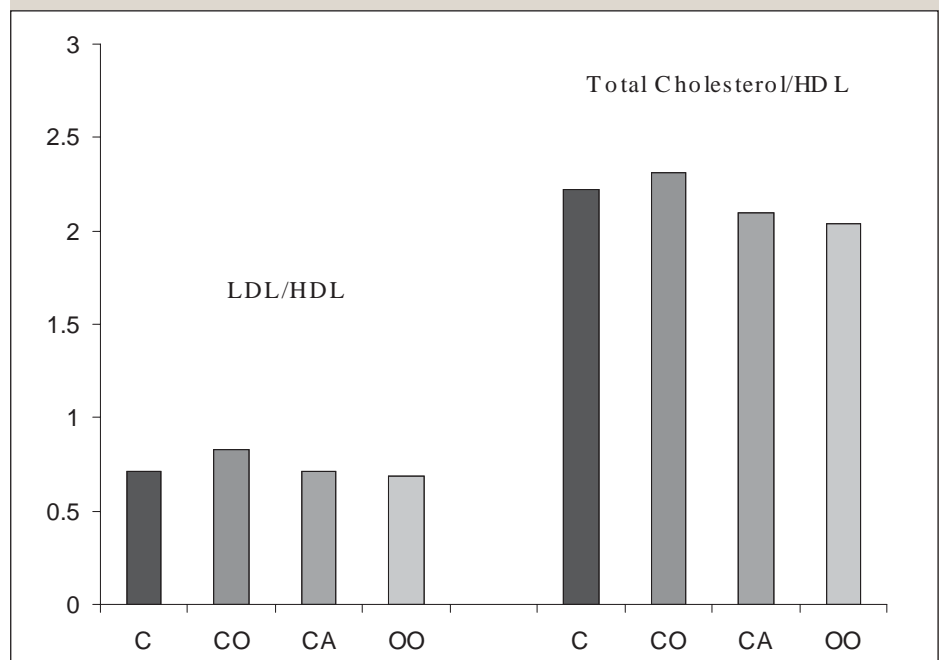


Figure 2 - LDL/HDL and Total cholesterol/HDL cholesterol ratios of rats receiving a regular rat chow diet with (coconut oil, C; canola oil, CA; olive oil, OO) or without (control, C) 5% w/w oil supplementation. Bars denote mean of 15 determinations.

sterol concentration. Lauric acid (C12:0), a major fatty acid found in coconut oil (tropical oil), appears to have a marked hypercholesterolaemic effect (1,12). Compared with carbohydrates, monounsaturated and polyunsaturated fatty acids, saturated fatty acids consistently increased plasma total

and LDL cholesterol (12,13). The present study, investigates the effects of long-term (13weeks) dietary intervention in term of fat type (saturated vs monounsaturated) and quantity (high fat vs low fat) upon blood lipid profile, glucose, apolipoprotein B100 and coagulation time in the rat model.

Materials and Methods

Reagents

Unless stated otherwise, all reagents and kits were obtained from Sigma Chemical Co. (Poole,UK).

Animals

The study was approved and financed by the university research committee. Animals were maintained and experimental protocols complied with the Guide for the Care and Use of Laboratory Animals (14). All animals were sacrificed using diethyl ether, at the end of the procedures described, without recovery from anaesthesia.

Sixty four male Sprague-Dawley rats (150-200g) (Lebanese American University stock), maintained under a 12 h photoperiod (08.00–20.00) at an ambient temperature of 22 °C, were fed the appropriate rat chow diet until 12 h prior to the experiment, when food was withdrawn. Water was available ad lib. Rats were subdivided into four groups Control (C), Coconut oil (CO), Canola oil (CA) and Olive oil (OO), each containing 16 rats. All groups received an isocaloric rat chow diet, where the oil group diets contained an extra 5% by weight of the corresponding oil. Fat saturation of the different supplemented oils is shown in table 1. Following thirteen weeks of dietary intervention, about 10 ml of blood were drawn from the inferior vena cava of anaesthetized rats after 12 hours fasting for lipid profile, total apoB100, coagulation time, and glucose determinations. Blood was appropriately distributed into 3 sets of tubes to have serum, and plasma containing either disodium ethylene diaminetetraacetic acid (Na₂EDTA 1 mg/ml) or sodium citrate. Blood was centrifuged at low speed centrifugation (2000g, 20 min) at a temperature of 4°C. Then the plasma and serum were collected and divided accordingly for the different tests.

Total apoB 100 content in the plasma of fasted animal was estimated in the lipoprotein fraction (d < 1.063 g/ml) as described previously by Daher et al.

(15). Briefly, 2 ml of plasma were put in a 10 ml polycarbonate ultracentrifuge tube (Sorvall, Kendro Laboratory Products) and 140 mg/ml of solid NaCl was added to increase the density to 1.1 g/ml. To minimize proteolytic degradation of apoB100 the following were added: EDTA 1mg/ml plasma, 5 ml/ml plasma of aprotonin (Fluka, Switzerland), 2 mg/liter, and 5 ml/ml plasma of phenylmethylsulfonyl fluoride (PMSF), 5 mM in 2-propanol. The plasma sample was overlaid with 5 ml of NaCl solution (d = 1.063 g/ml) containing 0.01% w/v Na₂EDTA and 0.02% w/v NaN₃ (pH=7.4). The top 0.5 ml lipoprotein layer was collected after 48h of centrifugation at 28,000 rpm at 15°C (Sorvall RC 28S centrifuge; Supraspeed F-28/13 fixed angle rotor).

Preparation of samples for Total apo B100 analysis

Samples containing the lipoprotein fractions were delipidated in a methanol-diethyl ether solvent system (16). The protein material was dissolved in 0.15M sodium phosphate, 12.5% v/v glycerol,

2% w/v sodium dodecyl sulfate (SDS), 5% v/v mercaptoethanol, 0.001% w/v bromomophenol blue, pH=6.8, at room temperature for 30 min, after which it was transferred to an Eppendorf tube, denatured at 80 °C for 10 min and centrifuged for 4 min at 15680g. Samples were frozen (-20 °C) and subjected to SDS-PAGE within 3 day.

Linear gradient (4-20%) polyacrylamide gel slabs (1.5mm thickness) were prepared as described by Hames (17). 10 µl of molecular weight marker (High Molecular Weight-SDS Calibration Kit, Amersham Pharmacia Biotech Inc, USA) solution was loaded into the first gel lane, and into the following lanes 2.5, 5, 15 and 30 µl of standard apolipoprotein B100 sample (0.6-7.2 µg) were loaded. The remaining lanes were loaded with the lipoprotein samples. All samples were run in duplicates.

Human apolipoprotein B100 was used as a standard for rat apolipoprotein B48 quantitation. Kotite et al. (18) and Van Beek et al. (19) have shown that human apolipoprotein B100 and rat apolipoprotein B100 and B48 have sim-

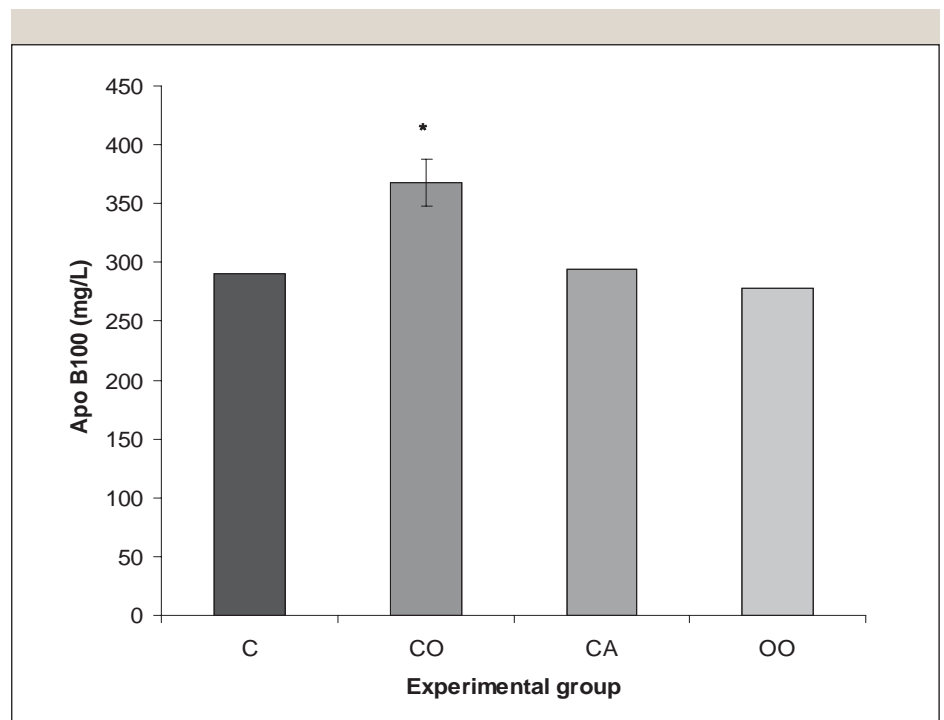


Figure 3 - Fasting plasma apolipoprotein B100 concentrations of rats receiving a regular rat chow diet with (coconut oil, C; canola oil, CA; olive oil, OO) or without (control, C) 5% w/w oil supplementation. Bars denote mean ± S.E.M. (n = 15). * P < 0.05 compared with control group.

ilar chromogenicity when stained with Coomassie Blue R-250, hence validating the use of human apolipoprotein B100 as a standard. Apolipoprotein B100 standards were prepared from human LDL ($1.030 < d < 1.040$ g/ml) isolated from fasting human plasma samples by the density gradient ultracentrifugation procedure described by Karpe et al. (16). Electrophoresis was carried out, and the gels were stained and destained using an automated gel stainer-destainer (Hofer processor plus, Amersham Pharmacia Biotech, USA), and the concentrations of apoB100 were determined as described by Daher et al. (15).

Measurement of serum lipids and glucose

Total serum cholesterol levels were measured using a cholesterol ester/oxidase enzymatic procedure. HDL chole-

The increased rate of chylomicron metabolism in polyunsaturated fatty acid-fed animals appear to depend on chronic fat feeding since acute fat feeding failed to show similar effects

sterol concentrations were measured, using cholesterol ester/oxidase enzymatic procedure, following precipitation of LDL, VLDL and chylomicron fractions by buffered polyethylene glycol 6000. LDL cholesterol levels were measured using a direct enzymatic selective protection method. TAG levels were measured using a glycerol kinase-based enzymatic procedure. Glucose levels were measured using a glucose oxidase-based enzymatic procedure.

Statistics

Values are presented as mean \pm S.E.M.

	Saturated fat	MUFA	PUFA
Canola oil	7%	61%	21%
Coconut oil	91%	7%	2%
Olive oil	15%	75%	9%

Table 1 - Percentage fat saturation of coconut, olive and canola oils.

Normal distribution of the data were confirmed using the Kolmogorov Smirnov one-sample goodness of fit test. Unacceptable heteroscedesity was eliminated, where possible, by the logarithmic transformation of the data. Where data were normally distributed comparisons between groups were made using one-way analysis of variance with localization of differences being achieved with Duncan's multiple range test. Where data were not normally distributed and could not be transformed to achieve normal distribution, the Kruskal-Wallis non-parametric analysis was used and differences located using the Mann-Whitney "U" test with appropriate adjustment to the critical value of P. Statistical significance was assumed at $P < 0.05$.

Results

Following 13 weeks of dietary intervention, animals of the control group showed the longest ($p < 0.05$) coagulation time with respect to all oil groups (Fig. 1). However, no significant difference has been observed among all members of the oil groups. Coconut oil group showed the shortest coagulation time among all groups.

Fasting sugar level results are shown in Table 2. It appeared that all groups had similar fasting blood sugar levels irrespective of the dietary habit followed. Consequently, the present data show that neither augmentation of fat intake nor changing the type fat had an impact on blood sugar level. Table 2 also reveals the fasting triacylglycerol concentrations in the blood. Surprisingly, increasing fat content in the diet was associated with a decrease in fasting triacylglycerol serum level. Chronic intake of both canola and olive oil resulted in a signifi-

cant decrease in triacylglycerol concentrations with respect to the control group, feeding relatively on a lower fat diet. Animals of the coconut oil group, however, showed a mild non-significant drop in fasting triacylglycerol concentration relative to the control group. Fasting serum total, LDL and HDL cholesterol concentrations are presented in Table 2. Fasting serum total cholesterol and LDL cholesterol concentrations were highest in the coconut oil group and lowest in the control group. Significant difference between the latter groups was only reached with LDL cholesterol. Both olive oil and canola oil groups were similar in total and LDL cholesterol, and did not show a significant change from either the control or the coconut oil group. HDL cholesterol concentrations (Table 2) of all oil groups were higher than the control group but significance was only reached with both canola oil and olive oil groups. Calculation of the LDL / HDL and total cholesterol / HDL ratios (Fig. 2) revealed that the olive oil group showed the best lipid profile by having the lowest ratios among all groups. Canola oil group exhibited ratios almost equal to that of the olive oil group and better than both of the control and coconut oil groups. By far the coconut oil group showed the highest ratios among all groups. Determination of the fasting serum apoB100 concentrations (Fig. 3) revealed that the coconut oil group had significantly higher apoB100 levels compared with all other groups. No significant changes were observed among the latter groups.

Discussion

The present report extends earlier studies and confirms the positive correlation between dietary intake of saturated fatty

	Glucose mg/dl	Triacylglycerol mg/dl	Total cholesterol mg/dl	HDL Cholesterol mg/dl	LDL cholesterol mg/dl
Control	148.5 ± 14	73.9 ± 5.2	60.7 ± 2.7	26.9 ± 1.7	19.2 ± 1.4
Coconut oil	166 ± 16	68.1 ± 4.9	66.2 ± 3.1	29 ± 2.1	23.8 ± 1.9a
Canola oil	153 ± 15	54.9 ± 5.4 a	63.3 ± 2.8	30.5 ± 1.9 a	21.7 ± 1.8
Olive oil	159 ± 13	58.8 ± 4.5 a	64.7 ± 2.9	31.9 ± 2.2 a	21.6 ± 2.0

a p<0.05 compared with the control group

Table 2 - Fasting serum glucose, triacylglycerol, total cholesterol, HDL and LDL cholesterol concentrations of rats receiving a regular rat chow diet with (coconut oil, C; canola oil, CA; olive oil, OO) or without (control, C) 5% w/w oil supplementation. Values denote mean ± S.E.M. (n = 15).

acids and atherogenic profile (1,12,13). After 13 weeks of dietary fat intervention serum total and LDL cholesterol concentrations were highest in the coconut oil fed group. This marked hypercholesterolaemic effect is consistent with previous reports (1,12,13). This may reasonably be explained as the result of increased saturated fatty acid intake over the 13 weeks period rather than a temporary increased secretion from the intestine since human (20,21) and animal (22) studies have shown that fat saturation has no effect on cholesterol absorption. Thus, changes in serum

cholesterol concentrations observed are probably the result of the long-term effect of fat saturation on synthesis and catabolism of plasma lipoproteins. This hypothesis is supported by our findings where serum apoB100 concentration in the coconut oil group was the highest among all groups. Having only one apoB100 molecule per VLDL or LDL particle (23,24) indicates that the increase in serum apoB100 concentration is the result of an increased secretion of VLDL particles from the liver and/or a decreased clearance of LDL particles from the blood.

The present report revealed that a diet rich in either olive or canola oils, as good sources of monounsaturated fatty, reduced serum TAG concentrations with respect to low fat diets (control) or diets rich in saturated fatty acids (coconut oil group). Recent studies on postmenopausal women have shown higher fasting plasma TAG, VLDL TAG, and VLDL cholesterol concentrations in women fed high carbohydrate low fat diets compared with those fed low carbohydrate high fat diets (25). Also, Coiffier et al. (26) demonstrated that chylomicrons from rats fed saturated

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fatty acids were hydrolyzed more slowly in vitro compared with chylomicrons from rats fed polyunsaturated fatty acids and this was associated with a higher lipoprotein lipase activity. The increased rate of chylomicron metabolism in polyunsaturated fatty acid-fed animals appear to depend on chronic fat feeding since acute fat feeding failed to show similar effects (27,28). Thus olive and canola oils containing a large fraction of monounsaturated fatty acids and a substantial amount of polyunsaturated fatty acids are expected to promote a better triacylglycerol clearance from the blood. Diets rich in either olive or canola oil appeared to increase serum HDL cholesterol concentrations. Supportive evidence (9) has shown that reducing total fat calories in the diet decreases both plasma LDL and HDL cholesterol concentrations in the blood. Unlike human studies (9), intake of olive or canola oil rich diets in rats did not reduce neither total nor LDL cholesterol concentrations as observed in the present study. Animal studies have shown a fundamental dif-

ference between rodents and human in term of cholesterol response to dietary monounsaturated fatty acids. Intake of monounsaturated fatty acids compared with polyunsaturated fatty acids resulted in increased plasma cholesterol levels in rats (29,30), hamsters (31) and rabbits (30). In spite of this species difference, still the LDL/HDL and Total cholesterol / HDL ratios favor the intake of both olive and canola oils. However, the inconvenience of increasing fat intake, regardless of fat type, is the reduction of coagulation time. Such an effect can possibly be explained through an indirect effect of high fat content in the diet on vitamin K, a key enzyme for blood clotting. It has been shown (4) that a high fat diet affects vitamin K metabolism compared with a normal diet, and results in a 5-fold increase in liver vitamin K. Thus, a shorter coagulation time can be secondary to an increase in vitamin K.

Conclusion, a diet rich in either canola or olive oil, but not coconut oil, has much more favorable effects on blood

lipid profile, and plasma lipoproteins compared with coconut oil. However, irrespective of the oil type, blood coagulation time seems to be inversely related to the percentage of fat content in the diet.



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