EFFECT OF RAPESEED OIL ON SERUM LIPID PATTERNS AND BLOOD HEMATOLOGY OF YOUNG MEN

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Abstract

The effect of rapeseed oil and canbra oil on serum lipid patterns and blood hematology was investigated in two 39-day metabolic trials each involving 7 healthy male subjects. Each trial consisted of: 1) a 9-day preliminary period when a mixed fat formulated to simulate the average Canadian fat intake was fed; 2) a 22-day experimental period when canbra oil or RSO was fed; and 3) an 8-day post-experimental period when the mixed fat again was fed. The diet consisted of ordinary foods except that textured soy protein was substituted for meat. The mixed fat, canbra oil (2.6 % erucic acid) and rapeseed oil (39.0 % erucic acid) supplied approximately 38 % of the total calories (i.e. 95 % of the fat calories). Mean serum cholesterol levels (mg/100 ml) on Days 1, 10, 18, 25, 32, and 39 were 203, 174, 159, 151, 144, and 183, respectively, on the canbra oil study and 200, 187, 167, 165, 177, and 195, respectively, on the RSO study. Serum lipid phosphorus followed similar patterns to those of cholesterol. Serum triglycerides also decreased to Day 18 and then increased again from Day 25 to 39 on the RSO study. Only small changes were observed in serum triglycerides in the canbra oil study. Blood platelet counts decreased to levels below 120,000/cu.mm for 5 of 7 subjects fed rapeseed oil. Similar decreases did not occur on the canbra oil. All other hematological measures were within normal ranges for both studies. The results of these studies suggest that the physiological and metabolic responses to canbra oil and RSO may differ in the human.

Rapeseed oil (RSO) production and consumption in Canada has increased substantially during the past 5 years to where RSO has replaced soybean oil as the major edible oil consumed by Canadians. As of January 1973, only canbra oil (low erucic acid rapeseed oil) has been processed for domestic consumption in Canada. This change from the traditional high erucic RSO to canbra oil stemmed from observations that RSO caused necrotic lesions and changes in lipids of heart and skeletal muscles of experimental animals (ABDELLATIF and VLES, 1970; BEARE-ROGERS et al., 1972, ROCQUELIN and CLUZAN, 1968). However, doubt has been expressed that erucic acid is the sole pathological factor in RSO because canbra oil also has been reported to produce myocardial changes (ROCQUELIN and CLUZAN, 1968).

Although RSO has been found to produce physiological and pathological changes in several species, differences in the metabolism of rapeseed oil have been observed among species. Man (DEUEL et al., 1949; VAISEY et al., 1973) and the dog (CRAMPTON et al., 1960) appear to handle RSO well while relatively poor digestibilities were found with the rat, pig, and guinea pig (CRAMPTON et al., 1960; ROCQUELIN and LECLERC, 1969). Digestibility of canbra oil was appreciably higher than RSO in the rat whereas no

difference in digestibilities of these oils was found for man (VAISEY et al., 1973). The object of the study herein reported was to investigate the effect of canbra oil and RSO as the sole source of added dietary fat on serum lipid patterns and blood hematology of young men.

Experimental Methods

The study involved two 39-day metabolic trials in which 7 healthy young males were fed fat-control diets comprised of customary foods except that textured soybean protein was substituted for meat. Each trial was divided into:

1) a 9-day preliminary period, during which dietary fat was supplied by a mixture comprised of 39.3 % butter, 21.5 % corn oil, 7.1 % edible tallow, 10.7 % margarine (Parkay, Kraft Foods Ltd., Montreal, Canada), 14.3 % lard and 7.1 % shortening (Crisco, Proctor & Gamble Ltd., Toronto, Canada);
2) a 22-day experimental period when either canbra oil and margarine or rapeseed oil and margarine supplied 95 % of the fat and approximately 38 % of the calories in the diet; and
3) an 8-day post-experimental period when the subjects again were fed the mixed fat.

The subjects were healthy male college students selected from among volunteers. During the conduct of the study they reported for meals but otherwise were encouraged to maintain normal activities. The subjects were weighed daily before breakfast. Individual caloric intakes were adjusted periodically to maintain constant body weight.

Textured vegetable protein (TVP, Archer Daniels Midlands, Minneapolis, Minn.), skim milk and spray-dried egg white were the primary protein sources in the diet. Three physical forms of TVP were incorporated into four main entrees: meatballs with tomato sauce; hamburger patties; sweet and sour pork; and beef stew. Two of these entrees supplied approximately 50 % of the daily fat intake. Fat was added to cooked breakfast cereal, scrambled egg whites, and pastries to provide another 35 % of the total daily fat intake. The remaining fat was provided as a spread. Energy intake was standardized for each subject with the proportion of calories from fat being maintained constant by adjusting the amount of spread provided each subject. Meals were served at the customary hours. In addition the subjects reveived three snack items daily. Fatty acid composition of the fats used in these studies (Table 1) was determined by methods previously described (VAISEY et al., 1973).

Twelve-hour fasting blood samples were taken on Days 1, 10, 18, 25, 32, and 39 of each study. A few drops of blood were used immediately for red cell fragility determinations by the hypotonic saline methodology (EMERSON et al., 1956). Another 3 ml was used for hematological determinations (hemoglobin, hematocrit, red cell count, reticulocyte count, and platelet count). The remaining blood was allowed to clot and the sera re-

Table 1: Major fatty acids in fat mixture and canbra and rapeseed oils and margarines used in studies

Fatty acid[1]	Levels of fatty acids (%)				
	Fat Mixture	Canbra		Rapeseed	
		Oil	Margarine	Oil	Margarine
C14:0	3.9	-	-	-	-
C16:0	21.4	4.0	5.0	3.5	3.4
C18:0	11.0	2.0	12.4	1.8	6.6
C18:1	37.3	57.2	71.1	21.4	29.3
C18:2	21.1	21.2	5.6	12.4	4.8
C18:3[2]	-	10.3	tr	6.4	3.0
C20:1	-	2.5	2.9	14.1	12.6
C22:0	-	-	-	-	4.5
C22:1	-	2.6	1.7	39.0	35.4

[1] carbon number: number of double bonds
[2] C18:3 and C20:0 not completely resolved by columns used in these studies

moved, frozen and stored for later determination of cholesterol, lipid phosphorus, triglycerides and fatty acid composition of phospholipids. Total serum cholesterol was quantitated by the method of PEARSON et al. (1953), serum lipid phosphorus by the method of CHEN et al. (1956) and serum triglycerides by the method of van HANDEL and ZILVERSMIT (1957) following extraction according to RYAN and RASHO (1967). The phospholipids in lipid extracts (BLIGH and DYER (1959) of serum were precipitated from acetone by the method of BEARE-ROGERS (1969). Methyl esters of the fatty acids in phospholipids were prepared by the method of BARNES and HALLODAY (1972) and resolved by gas-liquid chromatography using EGSS-Y organosilicone (Applied Science Laboratories, State College, Pa., U.S.A.) as the liquid phase. Blood hematology was carried out by the General Hospital, Health Sciences Center, Winnipeg.

Fat biopsies were obtained from two subjects on Day 10 and again on Day 32 of the RSO study. The biopsies were taken from the mid-dorsal abdomen. Fatty acid composition of the biopsies was determined by the same methods that were used for dietary fat sources (VAISEY et al., 1973).

Results and discussion

The subjects remained in good health throughout both the canbra oil and RSO studies. Body weights remained essentially constant during both studies. Thus changes in serum lipid patterns were attributed to dietary fat sources and not to sickness or changes in energy balance.

No digestive upsets were reported during the course of these studies even though the subjects consumed approximately 130 g of canbra or rapeseed oil and margarine daily on the experimental regimen. These observations

contrast with those of TREMOLIERES et al. (1971) who reported diarrhoea with humans given 0.5 g RSO/kg body weight on an empty stomach. Reason for the apparent difference between the studies reported here and those of TREMOLIERES et al. (1971) appears related to method of administering the fat; ingestion in a meal versus oral dosing with pure oil.

Blood lipid patterns followed slightly different trends during the canbra oil and RSO studies (Table 2). There was a substantial decrease in serum cholesterol for all subjects during the pre-experimental period (Day 1 vs. Day 10) on canbra oil. Serum cholesterol also decreased during the pre-experimental period on RSO, although the change was less pronounced. Serum cholesterol continued to decrease in a fairly consistent manner on the canbra oil diet which coincides with the low levels of saturated fatty acids in canbra oil. Mean serum cholesterol level also decreased during the first week on the RSO diet, which like canbra oil contains a relatively low level of saturated fatty acid, but increased again during the third week on this regimen. The increase in mean serum cholesterol level between Day 25 and Day 32 was particularly pronounced for one subject (48 mg/100 ml) although increases were observed for 6 of the 7 subjects. KEYS et al. (1957) stated that the major change in serum cholesterol occurs during the first week following a change in dietary fat. A further small change may occur during the second week but no further changes should be expected over the next one to two months. Reversion to the mixed fat diet was accompanied by a concomitant increase in mean serum cholesterol level in both the canbra oil and RSO studies. Thus responses during the experimental period were attributed to the canbra oil and RSO because the only difference between the experimental diets and the mixed fat diet was the source of fat.

Table 2: Summary of serum lipid levels during canbra oil and rapeseed oil studies

	Initial Obser. Day	Mixed Fat Day 10	Test Fat Day 18	Test Fat Day 25	Test Fat Day 32	Mixed Fat Day 39
			mg/100 ml			
Canbra oil study						
Total serum cholesterol	203[1]	174	159	151	144	183
Serum lipid phosphorus	11.7	10.1	9.2	7.9	6.7	10.5
Serum triglyceride	84	80	80	80	78	81
Rapeseed oil study						
Total serum cholesterol	200	187	167	165	177	195
Serum lipid phosphorus	9.9	7.8	7.4	6.6	7.1	8.9
Serum triglycerides	138	115	85	82	99	107

[1] All values are mean for 7 subjects

The decrease in serum cholesterol on the canbra oil diet coincided with similar decreases reported by MALMROS and WIGAND (1957) and GRANDE et al. (1962) for subjects fed RSO over a similar period of time. No explanation can be offered for the pattern observed on the rapeseed oil diet in the present studies. However, the fact that serum lipid phosphorus followed a similar pattern to serum cholesterol in the present studies (Table 2) is consistent with reports (ERICKSON et al., 1964; McGANDY et al., 1970) that the pattern of response in serum lipid phosphorus to changes in diet composition is similar to that of serum cholesterol.

Mean serum triglyceride patterns differed appreciably between the two studies reported here (Table 2). Very little change in serum triglyceride levels occurred in response to diet manipulation in the canbra oil study. By contrast, mean serum triglyceride level, which was much higher at the start of the study, decreased during the pre-experimental period and the first week on the RSO diet. The decrease in serum triglyceride during the first week on the experimental diet was followed by a small increase during the third week (Day 25 to 32) and a further substantial increase on reversion to the mixed fat diet. The pattern of change in mean serum triglyceride level during the RSO study was similar to that of serum cholesterol. However, considerably more variation occurred among subjects for serum triglycerides than for either serum cholesterol of serum lipid phosphorus.

Fatty acid patterns of the serum phospholipids precipitated from acetone are shown in Table 3. There was an appreciable decrease in palmitic acid in response to canbra oil and RSO (Day 32 vs. Day 10). Stearic and linoleic acids also decreased slightly on the experimental diets. These changes were compensated by a marked increase in oleic acid. With the exception of linoleic acid in the canbra oil study, these fatty acids returned to pre-experimental levels upon return to the mixed fat diet for 8 days (Day 32 to Day 39). Very little erucic acid was incorporated into serum phospholipids even though it comprised 35 to 40 % of the dietary fatty acids in the RSO study. These observations coincide with low levels of erucic and eicosenoic acids deposited in the plasma lipids of rats fed 30 calories percent rapeseed oil for 18 weeks (WALKER, 1972). A small amount (0.4 and 2.0 % of the total fatty acids) of erucic acid was incorporated into the adipose tissue from two of the subjects on the RSO study. However, it would appear that erucic acid is preferentially converted to other fatty acid, presumably oleic acid, in man as in other species.

Neither canbra oil nor rapeseed oil had any effect on whole blood hematology except for the rather pronounced drop in blood platelet count on the RSO diet (Table 4). After 22 days on the RSO diet 5 of the 7 subjects were classed as low-normal. The fact that platelet count returned to normal for 5 of the subjects when the mixed fat diet was reinstated strongly suggests that the decrease in platelet count was related to the fat component of the diet. The meaning and significance of these observations is not clear. RSO has been reported to induce an increase in reticulocytes and an increased permeability of the red blood cell (VLES and ABDELLATIF, 1970). All other blood parameters remained within normal ranges for all subjects

Table 3: Percentage fatty acid composition of serum phospholipids of subjects fed canbra oil and rapeseed oil for 22 days

Fatty acid[1]	Canbra oil study			Rapeseed oil study		
	Day 10	Day 32	Day 39	Day 10	Day 32	Day 39
C16:0	24.6	19.6	22.4	30.2	21.4	31.3
C16:1	2.0	2.0	2.2	1.5	1.4	-
C18:0	14.2	12.9	14.8	14.9	12.0	16.1
C18:1	11.8	19.2	11.2	14.3	23.7	14.2
C18:2[2]	24.0	20.9	20.0	26.9	24.4	26.4
C18:3[2]	-	-	-	-	1.0	tr
C20:1	2.6	1.6	2.0	sl.tr	3.5	1.8
C20:3	2.1	2.4	2.8	2.0	1.6	2.2
C20:4	7.2	8.8	7.5	7.5	6.2	6.4
C22:1[3]	-	-	-	-	1.5	-
Unknown[3]	2.9	2.7	3.1	sl.tr	tr.	sl.tr

[1] Carbon number: number of double bonds
[2] C18:3 and C20:0 not resolved by conditions used in these determinations
[3] Resolved between C22:1 and C20:5

throughout the RSO study while no hematological changes attributable to treatment were observed during the canbra oil study.

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Table 4: Summary of blood hematology for canbra oil and rapeseed oil studies

	Initial Observ. Day 1	Mixed Fat Day 10	Test Fat Day 18	Test Fat Day 25	Test Fat Day 32	Mixed Fat Day 39
Canbra oil study						
Hemoglobin (g/100 ml)	15.3[1]	14.5	14.8	14.7	14.3	14.6
Hematocrit (%)	44.6	43.0	43.0	43.8	44.1	45.1
Red blood cells (million/cu. mm)	5.0	4.8	4.9	5.0	4.9	5.0
Reticulocytes (thous./cu. mm)	61.1	18.8	43.1	54.7	57.3	60.0
Reticulocytes (% RBC)	1.2	0.4	0.9	1.1	1.2	1.2
Platelets [3]	7N	7N	3N;4LN	7N	6N;1LN	4N;3LN
Rapeseed oil study						
Hemoglobin (g/100 ml)	14.6[2]	14.9	14.2	14.6	14.4	14.5
Hematocrit (%)	43.3	43.1	41.3	45.1	41.8	42.4
Red blood cells (million/cu. mm)	5.0	5.0	4.8	5.4	4.8	4.9
Reticulocytes (thous./cu. mm)	27.4	48.8	44.0	35.3	40.7	59.3
Reticulocytes (% RBC)	0.5	0.9	0.9	0.7	0.8	1.2
Platelets [3]	5N	7N	6LN;1L	5LN;2L	2LN;5L	5N;2LN

[1] All values are means for 7 subjects

[2] Two blood samples were lost on Day 1; values are mean for 5 subjects

[3] Platelet counts above 150 thousand per cu. mm were classed as normal (N). Counts between 120 and 150 thousand were classed as low normal (LN). Counts below 120,000 were considered low (L).

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