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Effects of Seven Dietary Oils on Blood Serum Lipid Patterns in Rats

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The objective of this study was to examine the effects of seven dietary oils on the serum lipid patterns of rats. Seventy weanling Wistar Kyoto rats were divided into seven groups of ten rats each. Walnut oil (rich in PUFA), wheat germ oil (rich in PUFA), corn oil (rich in PUFA), canola oil (rich in monounsaturated fatty acids), fish oil (rich in PUFA), primrose oil (rich in PUFA), and palm oil (rich in saturated fatty acids) were employed for 21 days. Serum total cholesterol concentrations for rats fed palm oil, walnut oil, and wheat germ oil were significantly higher than were concentrations for rats receiving corn oil, fish oil, and primrose oil. The mean serum LDL cholesterol values for rats fed fish oil, primrose oil, and corn oil were significantly lower than those for rats fed walnut oil, wheat germ oil, canola oil, and palm oil. HDL cholesterol concentrations were the highest when wheat germ oil was fed and the lowest when fish oil was fed. The feeding of wheat germ oil and palm oil to rats resulted in considerably higher serum triglyceride levels than did all other treatments. The feeding of wheat germ oil to rats resulted in considerably higher serum phospholipid levels. Serum phospholipid concentrations were significantly lower in rats fed the canola oil, fish oil, and primrose oil diets, when compared to concentrations achieved with the feeding of walnut oil, wheat germ oil, corn oil, and palm oil. Palm oil, which has a high ratio of saturated to polyunsaturated fatty acids, resulted in the highest serum total cholesterol and highest LDL cholesterol levels, while fish oil, primrose oil, and corn oil produced the lowest total cholesterol and LDL cholesterol. Wheat germ oil produced the highest values for HDL cholesterol, triglycerides, and phospholipids. In general, feeding oils rich in polyunsaturated fatty acids produced more favorable responses than feeding oils containing large amounts of monounsaturated or saturated fatty acids.

Key words : seven oils, cholesterol, triglyceride, phospholipid, rat.

INTRODUCTION

Coronary heart disease (CHD) is one of the most frequent causes of death in the developed and developing nations. Despite substantial success in reducing CHD mortality in the past two decades, the disease is still responsible for more than 50,000 deaths annually in the United States. About 20% of hospital discharges for acute CHD are for premature disease, i.e., in patients under 55 years of age[1]. The incidence of cardiovascular disease (CVD) has been increasing rapidly in Korea[2] and the mortality rate from this disease reached 123.2 per 100,000 people in 2000[3].

Coronary heart disease is a multifactorial disease. The following inherited and lifestyle factors appear to be the most significant risk factors for this disease: genetic background, cigarette smoking, sex, obesity, hypercholesterolemia, hypertension, diabetes mellitus, physical inactivity, and, of course, diet[4-6]. Dietary changes have been stressed in prevention programs because of their theoretical importance and their relative ease of application.

Epidemiological studies have identified various factors which are associated with both the increased and decreased incidence of coronary heart disease. Among these factors, dietary practices have been among the first interventions to be considered. Both positive and negative significant correlations between coronary heart disease incidence and the intake of fat, carbohydrate, and protein have been found. Because the principal component of plaque is lipid, the kinds and levels of dietary fat and cholesterol were the first, and still remain, the most frequently investigated dietary factors.

Consumer awareness of the quality and quantity of dietary fats has increased many fold. However, the availability of nutritional information for many vegetable oils is limited. Palm oil represents the second largest volume of vegetable oil produced in the world[7]. Consumption of canola oil is being encouraged on the basis of its health promotion characteristics thought to be related to its high concentrations of oleic acid, a monounsaturated fatty acid. Concurrently, the health value of plant oils that are rich sources of ω-6 fatty acids are being either dismissed or downgraded. Fish oils are

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unique polyunsaturated fats since they are rich in the ω-3 fatty acids as opposed to those of the ω-6 family such as linoleic acid which characterize vegetable oils. The ω-3 fatty acids have a wide variety of metabolic effects. The most widely recognized effects of these fatty acids are on serum lipid levels[8]. The objective of this project was to study the effects of various dietary oils on the lipid patterns of the blood serum and of selected organs of rats.

MATERIALS AND METHODS

1. Experimental animals and protocol

Seventy male weanling Wistar-Kyoto rats (Harlan-Sprague Dawley Co., Madison, WI), ranging in weight from 40 to 50 grams, were used. Upon arrival, the rats were housed in wire bottomed stainless steel cages, and fed a standard rat diet (Purina Rat Chow Co., St. Louis, MO) and water ad libitum for a period of three days to allow for adjustments to surroundings and recovery from any stress involved with travel. The environmental temperature was kept between 20 and 25℃. Light was provided from 0700-1900 hours, with alternating twelve hours of light and twelve hours of darkness. The relative humidity was maintained between 50 and 55 percent.

After three days of adjustment, the rats were randomly assigned to individual cages and to one of seven dietary treatment groups. Ten rats were assigned to each treatment, in a completely randomized treatment design, so that the initial weights of each group were similar. One level of dietary fat (5 percent of the diet by weight) and seven different test oils were used in this experiment. Test oils were walnut oil, wheat germ oil, corn oil, canola oil, fish oil, primrose oil, and palm oil. The composition of the test diets is shown in Table 1, and treatment descriptions are shown in Table 2. Fatty acid compositions of the test oils are listed in Table 3[9]. All ingredients were purchased from Teklad (Madison, WI) except for the test oils, the corn starch and the sucrose which were purchased from a local supermarket. Constant amounts of casein, fat, fiber, sucrose, vitamin mix, and mineral mix were included in all the diets.

Table 1. Composition of experimental diets

Ingredients	g/kg
Casein (vitamin-free)	200.0
DL methionine	3.0
Sucrose	500.0
Corn starch	152.0
Test oil (varied)	50.0
Cellulose (fiber)	50.0
Mineral Mix, AIN-76	35.0
Vitamin Mix, AIN-76	10.0

Table 2. Treatment

Treatment number	Number of rats	Basic diet	Test oil
1	10	AIN76A	Walnut
2	10	AIN76A	Wheat germ
3	10	AIN76A	Corn
4	10	AIN76A	Canola
5	10	AIN76A	Fish
6	10	AIN76A	Primrose
7	10	AIN76A	Palm

2. Data collection

The animals were fed daily and were allowed to consume feed and water ad libitum for the duration of the study (21 days). Feed intakes were recorded on a

Table 3. Fatty acid composition of test oils (wt%)

Fatty Acid	Walnut	Wheat germ	Corn	Canola	Fish	Primrose	Palm
12:0			0.1				0.1
14:0		0.1	0.1	0.1	7.0		1.0
16:0	7.0	16.6	12.1	4.6	18.2	5.7	43.8
18:0	2.0	0.5	2.4	1.5	4.8	1.2	4.8
20.0			0.5	0.6	2.2		
22:0			0.2	0.3	3.4		
24:0			0.2	0.1			
16:1	0.1	0.5	0.2	0.3	8.7		
18:1	22.2	14.6	32.1	59.4	12.8	10.6	38.9
20:1	0.4		0.3	1.5	1.6		
22:1				0.7	0.7		
24:1				0.2			
18:2	52.9	54.8	50.9	20.7	1.8	71.6	10.6
18:3 ω-6						9.0	
18:3 ω-3	10.4	6.9	0.9	10.1	0.5		0.3
20:5 ω-3					18.0		
22:5 ω-3					3.2		
22:6 ω-3					17.4		

weekly basis for each animal by determining the difference between feed weighed at the beginning and end of each week. Spillage was collected, weighed and subtracted from the initial feed weight. Body weights were also recorded throughout the study on a weekly basis using an Ohaus Autogram 1000 balance (Ohaus Scale Co., Union, NJ).

At the end of the experimental period, animals were fasted for twelve hours, lightly anesthetized with carbon dioxide, and sacrificed. Blood (via open-cavity cardiac puncture), livers, and hearts were removed. The blood was allowed to clot, then centrifuged, and the serum collected and frozen. Livers and hearts were also frozen for later analysis.

3. Laboratory analysis

Serum total cholesterol, HDL cholesterol, and triglyceride content were analyzed using a kit purchased from Sigma Diagnostics based on the methods of Fletcher[10], Lopes-Virella et al.[11] and Cho[12]. The phospholipid content of the serum was analyzed by methods described by Bloor[13] and Bouman[14]. LDL cholesterol values were obtained by subtracting HDL cholesterol from total cholesterol. Total liver lipid was extracted and total cholesterol quantified utilizing a method described in Folch et al.[15], and Cho[12]. Total heart lipid and cholesterol contents were analyzed by the same method. In addition, total weight gain was determined and feed efficiency ratios were calculated.

4. Statistical analysis

Statistical analyses of the data for serum total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, phospholipids, liver total lipids and cholesterol, heart total lipids and cholesterol, and weight gain and feed efficiency ratios, were completed using the Statistical Analysis System (SAS). Response criteria were measured using contrast evaluations and the Analysis of Variance was used to detect possible differences due to treatment variation. Duncan's Multiple Range Test was used to detect differences in mean responses.

RESULTS AND DISCUSSION

1. Weight gain and feed efficiency

Mean weight gain and feed efficiency results are presented in Table 4. Although mean weight gain values were not statistically different, the feeding of fish oil resulted in the highest weight gain and the feeding of palm oil resulted in the lowest weight gain. Contrast analysis of weight gain data indicated that there were no significant differences in weight gain between the test oils high in unsaturated fatty acids and the test oil high in saturated fatty acids, and the test oils high in polyunsaturated fatty acids and the test oil high in monounsaturated fatty acids.

The feed efficiency of the corn oil diet was greater than the palm oil diet. Contrast analysis of feed efficiency data indicated that the test oils high in unsaturated fatty acids had a higher feed efficiency than did the test oil high in saturated fatty acids ($P<0.0095$). There was no significant difference in feed efficiency between the test oils high in polyunsaturated fatty acids and the test oil high in monounsaturated fatty acids.

2. Serum lipids

Mean serum lipid concentrations are shown in Table 5. Serum total cholesterol concentrations were the highest when palm oil was fed and the lowest when fish oil was fed. Serum total cholesterol concentrations for rats fed palm oil, walnut oil, and wheat germ oil were significantly higher than for rats fed corn oil, fish oil, and primrose oil ($P<0.0001$). Considering the relatively high concentrations of palmitic acid (16:0, 43.8%) in palm oil, the high blood serum total cholesterol levels produced by feeding this oil are not surprising. However, the fact that walnut oil and wheat germ oil (both oils containing high amounts of linoleic acid, 52.9% and 54.8%, respectively) produced relatively high levels of blood serum cholesterol is more difficult to explain.

Contrast analysis indicated that diets containing higher amounts of unsaturated fatty acids, in comparison to diets containing higher amounts of saturated fatty acids, produced significantly lower total cholesterol concentrations ($P<0.0010$). The Sugano and Imaizumi study[16] showed that palm oil increased serum cholesterol concentrations compared with safflower oil.

Diets containing higher amounts of polyunsaturated

Table 4. Weight gain and feed efficiency

Parameter	Walnut	Wheat germ	Corn	Canola	Fish	Primrose	Palm
Weight gain	102.7±10.5[1)a]	103.6±9.8[2)a]	101.2±8.6[a]	100.6±8.2[a]	106.2±9.0[a]	103.1±11.2[a]	100.2±7.0[a]
Feed efficiency	0.39±0.04[ab]	0.38±0.03[ab]	0.41±0.07[a]	0.39±0.01[ab]	0.37±0.03[ab]	0.39±0.04[ab]	0.35±0.02[b]

1) Mean ± SD of 10 rats per group
2) Values with different superscripts are different from one another at $p<0.05$

fatty acids (walnut oil, wheat germ oil, corn oil, fish oil, and primrose oil) in comparison with the diet containing higher amounts of monounsaturated fatty acids (canola oil) did not produce significantly different total cholesterol concentrations (P<0.2388).

Walnut oil, wheat germ oil, corn oil, and primrose oil provided diets containing a high content of ω-6 fatty acids, and these were compared with canola oil, a diet containing high ω-9 fatty acids; no significant differences in total cholesterol concentrations were found as a result of feeding any of these oils (P<0.6671). Similarly, Mensink and Katan[17] and Mata et al.[18] reported that oleic acid was as effective as linoleic acid in lowering total cholesterol. However, the Chang and Huang study[19] showed that large amounts of dietary monounsaturated fat may increase plasma cholesterol.

The mean serum LDL cholesterol values for rats fed fish oil, primrose oil, and corn oil were significantly lower than those for rats fed walnut oil, wheat germ oil, canola oil, and palm oil (P<0.0001).

Contrast analysis indicated that rats receiving test oils high in unsaturated fats had significant reductions in serum LDL cholesterol compared to rats receiving palm oil (P<0.0003) which is high in saturated fat. Studies have shown that palmitic acid, the major saturated fatty acid in palm oil, increased LDL cholesterol levels in parallel with total cholesterol concentrations in the diets[20,21].

Rats receiving the test oils high in polyunsaturated fats had lower serum HDL cholesterol, compared to values for rats receiving the high monounsaturated fat treatment (P<0.04321). If fish oil is excluded, polyunsaturated fatty acid rich diets (i.e.ω-6 fatty acid rich diets) did not result in significant reductions in serum LDL cholesterol when compared to values achieved with monounsaturated fat diets (P<0.1245). Similarly, Mattson and Grundy[22] and Chan et al.[23] reported that the experimental diets were equally effective in lowering total and LDL cholesterol and apolipoprotein B concentration in plasma. This indicated that dietary oleic acid and linoleic acid were equally hypocholesterolemic in normolipidemic men.

HDL cholesterol concentrations were found to be the highest when wheat germ oil was fed and the lowest when fish oil was fed. It has been suggested that alpha tocopherol has the ability to raise HDL cholesterol concentrations while other forms of tocopherol do not. Wheat germ oil is noted for its high concentrations of alpha tocopherol. Contrast analysis indicated that there were no significant differences in serum HDL cholesterol levels produced by feeding oils rich in unsaturated and saturated fatty acids (P<0.7979). Saturated fatty acids did not appear to reduce HDL cholesterol levels.

In populations consuming diets high in saturated fatty acids, levels of both LDL and HDL cholesterol tend to be high[24]. Dietary monounsaturated fatty acids do not lower HDL cholesterol levels when substituted for saturated fatty acids[25]. In contrast to monounsaturated fatty acids, high intakes of ω-6 polyunsaturates reduced HDL cholesterol concentration[26,27]. Unlike the results obtained by Vega et al.[26], Jackson et al.[27], and Grundy et al.[25], in the present study those rats fed ω-6 polyunsaturated fats (walnut, wheat germ, corn, and primrose oil) were found to have significantly higher HDL cholesterol levels (P<0.029) than rats fed monounsaturated fat (canola oil). Rats fed ω-3 polyunsaturated fats had significantly lower blood serum HDL cholesterol levels than rats fed a ω-9 monounsaturated fat diet (P<0.0510).

A strong, inverse, correlation exists between levels of HDL cholesterol and rates of CHD in high risk populations[28,29]. Although low HDL levels may be inherited in some people, lifestyle factors appear to be the predominant determinants of reduced HDL cholesterol concentrations. Certain populations that habitually consume low-fat diets have low levels of both LDL and HDL, and also have low rates of CHD. This finding has led to the conclusion that the diet-induced lowering of HDL levels does not enhance coronary risk.

Feeding of wheat germ oil to rats resulted in considerably higher serum triglyceride levels than did all other treatments in this study. Contrast analysis indicated that feeding of a saturated fat (palm oil) diet causes significantly higher serum triglyceride levels as compared to levels achieved with the feeding of an

Table 5. Mean serum lipid concentrations for rats consuming diets containing fats from varied sources

Parameter(mg/dl)	Walnut	Wheat germ	Corn	Canola	Fish	Primrose	Palm
Total cholesterol	165±24[1)a]	171±40[2)a]	142±16[bc]	159±17[ab]	124±23[c]	141±14[bc]	177±24[a]
LDL cholesterol	119±16[a]	105±33[a]	84±16[b]	107±13[a]	81±16[b]	78±14[b]	121±20[a]
HDL cholesterol	52± 7[cd]	66±12[a]	58± 9[abc]	52± 7[cd]	43± 8[d]	63±11[ab]	56±11[bc]
Triglyceride	110±20[c]	212±27[a]	120±31[bc]	124±42[bc]	139±30[bc]	147±23[b]	189±27[a]
Phospholipid	131±21[c]	168±30[a]	159±26[ab]	83± 9[d]	86±19[d]	97±19[d]	141±11[bc]

1) Mean ± SD of 10 rats per group
2) Values with different superscripts are different from one another at p<0.05

unsaturated fat diet ($P<0.0001$). Contrast analysis of blood serum triglyceride levels, comparing values for rats fed polyunsaturated fats with those fed monounsaturated fat, indicated that the feeding of polyunsaturated fats produced higher triglyceride levels ($P<0.0477$).

The feeding of wheat germ oil to rats resulted in considerably higher serum phospholipid levels. Serum phospholipid concentrations were significantly lower in rats fed the canola oil, fish oil, and primrose oil diet, when compared to values achieved with the feeding of walnut oil, wheat germ oil, corn oil, and palm oil ($P<0.0001$). Contrast analysis indicated that the feeding of saturated fats resulted in significantly higher blood serum phospholipid levels than the feeding of unsaturated fats ($P<0.0049$). Comparisons of blood serum phospholipid levels between rats fed monounsaturated fatty acids rich oils and those fed ω-6 polyunsaturated fatty acid rich oils resulted in significantly higher phospholipid levels in rats fed the ω-6 polyunsaturated fatty acid rich fats. Rats fed the ω-3 polyunsaturated fatty acid rich fats diets were found to have significantly higher phospholipid levels compared to rats fed the ω-6 polyunsaturated fats diet ($P<0.0001$).

3. Tissue lipids

Results for the mean liver and heart total lipid and cholesterol concentrations of rats fed the different treatments are shown in Table 6. The mean liver total lipid concentrations for rats fed fish oil and primrose oil were significantly higher than were the concentrations for rats fed walnut oil , wheat germ oil, corn oil, canola oil, and palm oil ($P<0.0001$). No significant differences in total liver lipid concentrations were found between rats receiving the fish oil treatment and those receiving primrose oil. Liver total lipid values were significantly higher in rats fed palm oil compared to those rats fed walnut oil, corn oil, and canola oil. Contrast analysis showed that rats fed high polyunsaturated fatty acid rich diets had increased total liver lipid concentrations compared to rats fed the high monounsaturated fatty acid rich diets ($P<0.0001$).

Total liver cholesterol concentrations were the highest when canola oil was fed and the lowest when primrose oil was fed ($P<0.0001$). The feeding of fish oil and primrose oil caused a significant decrease in total liver cholesterol compared to when wheat germ oil, corn oil, canola oil, or palm oil were fed. Contrast analysis showed that rats fed fats high in monounsaturated fatty acids had higher total liver cholesterol concentrations than rats fed oils high in polyunsaturated fatty acids ($P<0.0001$). The feeding of monounsaturated fats resulted in significantly higher liver cholesterol levels than did the feeding of saturated fat (palm oil). Thus, the logical conclusion is that liver cholesterol deposition was enhanced by the feeding of monounsaturated fats.

Total heart lipid concentrations for rats fed fish oil were significantly lower than for rats receiving wheat germ oil, corn oil, canola oil, primrose oil, and palm oil. The feeding of palm oil to rats also resulted in a lower total heart lipid concentration when compared to rats fed wheat germ oil, corn oil, canola oil, and primrose oil. Total heart lipid concentrations for rats fed wheat germ oil were significantly higher than the concentrations for rats receiving walnut oil, corn oil, canola oil, fish oil, primrose oil, and palm oil ($P<0.0001$). Contrast analysis of heart total lipid concentrations indicated that feeding of test oils high in unsaturated fatty acids resulted in significantly higher concentrations than did the feeding of test oils high in saturated fatty acids ($P<0.0012$). Rats were found to have significantly higher total heart lipid concentrations when fed test oils high in monounsaturated fatty acids, compared to those fed test oils high in polyunsaturated fatty acids ($P<0.0090$).

Regarding total heart cholesterol concentrations, rats consuming walnut oil and wheat germ oil had significantly higher values in comparison to rats consuming canola oil, primrose oil, and palm oil ($P<0.0008$). Palm oil gave the lowest values for total heart cholesterol. Contrast analysis indicated that feeding a saturated fat resulted in lower heart cholesterol concentrations than feeding unsaturated fats. There were no significant differences

Table 6. Liver and heart total lipid and cholesterol contents

Parameter	Walnut	Wheat germ	Corn	Canola	Fish	Primrose	Palm
Total lipid (mg/g tissue)							
Liver	$8.0\pm3.2^{1)2)cd}$	10.0 ± 2.7^{bc}	7.0 ± 1.9^{d}	8.7 ± 2.8^{cd}	16.3 ± 2.9^{a}	14.7 ± 2.4^{a}	12.0 ± 1.7^{b}
Heart	7.4 ± 5.5^{e}	32.7 ± 8.8^{a}	24.3 ± 1.9^{b}	22.9 ± 3.9^{bc}	6.6 ± 4.5^{e}	18.5 ± 6.6^{c}	12.5 ± 3.9^{d}
Total cholesterol (mg/100 g tissue)							
Liver	119 ± 13^{cd}	151 ± 34^{ab}	163 ± 39^{a}	171 ± 33^{a}	98 ± 23^{de}	91 ± 9^{e}	136 ± 21^{bc}
Heart	138 ± 23^{a}	136 ± 14^{a}	128 ± 6^{ab}	124 ± 4^{bc}	131 ± 6^{ab}	124 ± 4^{bc}	116 ± 9^{c}

1) Mean ± SD of 10 rats per group
2) Values with different superscripts are different from one another at $p<0.05$

in total heart cholesterol levels between rats fed ω-3, ω-6, or ω-9 unsaturated fatty acid diets.

In general, the feeding of canola oil, which is high in monounsaturated fatty acids, resulted in higher total liver cholesterol values. Similar results were reported by Beynen[30] and Kris-Etherton[31], indicating that olive oil was more hepatocholesterolic in rats than was coconut or corn oil. Thus, it seems that oleic acid (C18:1 n-9) in ingested olive or canola oil may have a specific cholesterol elevating effect in the tissues of rats. Mattson et al.[22], Grundy[21], and Grundy[32] reported that monounsaturated fatty acids decrease serum LDL cholesterol levels by enhancing the activity of LDL-receptors. This may result in the higher uptake of cholesterol by different organs and possibly explains the higher liver cholesterol content found in this study.

The feeding of fish oil resulted in a higher total heart cholesterol content but not a higher total liver cholesterol content. However, Balasubramaniam et al.[33] reported that a diet containing fish oil increased the basal secretion rate of cholesterol into bile in rats, but that the bile acid secretion rate remained unchanged. It was suggested that ω-3 fatty acids increase the liver cholesterol content in rats by increasing the transfer of serum cholesterol into bile in the liver. Grundy and Adrens[34] proposed that the redistribution of cholesterol from plasma to organs is a possible response to dietary polyunsaturated fatty acids. In this study, rats fed test oils high in polyunsaturated fats had a high total heart cholesterol concentration compared to rats fed diets of other test oils.

In conclusion, palm oil, which has a high ratio of saturated to polyunsaturaed fatty acids, resulted in the highest serum total cholesterol and LDL cholesterol levels, while fish oil, primrose oil, and corn oil resulted in the lowest total cholesterol and LDL cholesterol concentrations. Wheat germ oil diets produced the highest values of HDL cholesterol, triglycerides, and phospholipids. Rats fed an oil rich in monounsaturated fatty acids produced higher liver cholesterol concentrations than rats fed oils rich in either saturated fatty acids or polyunsaturated fatty acids. However, rats fed oils rich in polyunsaturated fatty acids had higher heart cholesterol concentrations than those fed oils rich in saturated or monounsaturated fatty acids. In general, the feeding of oils rich in polyunsaturated fatty acids produced more favorable responses than did the feeding of oils containing large amounts of monounsaturated or saturated fatty acids.

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