COMPARATIVE EFFECTS OF LOW ERUCIC ACID RAPESEED OIL AND SOYBEAN OIL ON WHOLE BLOOD HEMATOLOGY AND SERUM LIPIDS IN YOUNG MEN

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ABSTRACT

The effects of low erucic acid rapeseed oil (Brassica napus cv. Tower) and soybean oil on serum lipid patterns and whole blood hematology were investigated with eight healthy male subjects. The diet was composed of conventional low fat foods and added fat, in which soy protein was substituted for meat. Fat contributed 40% of total calories in a 3000 kcal. daily diet. Approximately 93% of the total fat was added fat. The 31 day study was divided into four periods: (1) An eight-day pre-experimental stabilization period during which the added fat was a mixture of fats formulated to simulate the composition of fat in the average North American diet; (2) An eight-day experimental period during which half the subjects were fed rapeseed oil and the other half soybean oil as the added fat; (3) A second eight-day experimental period during which the subjects received the alternate oil (crossover design); and (4) A seven-day post-experimental period during which the diet containing mixed fat again was fed to all subjects. Fasting blood samples were drawn on days 2, 9, 17, 25 and 32. Serum cholesterol and serum lipid phosphorus levels decreased during the pre-experimental mixed fat period, and were lower when vegetable oil was fed as the fat source than when mixed fat was fed; but there was no statistical difference between the two oils, rapeseed and

soybean, in their effect on serum lipids. Changes in serum phospholipid fatty acid patterns reflected changes in dietary fatty acid patterns. Red blood cell count, reticulocyte count, platelet count, hemoglobin levels and hematocrit levels did not change in response to changes in fat source. Leucocyte counts when the test diet was fed (days 9, 17, 25 and 32) were lower than initial counts (day 2). Leucocyte counts were lower when vegetable oils were fed than when mixed fat was fed, and were lower when rapeseed oil was fed than when soybean oil was fed. Nevertheless, all values observed during this study for six hematological parameters, including leucocyte counts, remained within levels considered normal in the human. Therefore inclusion of a high level of low erucic acid rapeseed oil in the diets of healthy young men appears to have no detrimental effect on serum lipid patterns and whole blood hematology.

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INTRODUCTION

Until 1971, soybean oil was the main edible oil used in Canada. In 1971, the use of rapeseed oil (Brassica napus and Brassica campestris) surpassed that of soybean oil for the first time. In 1973 there was 1.37 times as much rapeseed oil as soybean oil used in Canada (Teasdale, 1975). Although soybean oil regained the lead in 1974, the combined usage of these two oils continues to increase. Together they accounted for 78% of the total domestic oil consumption in 1973 and 83% in 1974 (Statistics Canada, 1974).

Since 1971, all rapeseed oil for edible use in Canada has been of the low erucic acid type (less than 5% erucic acid). Erucic acid, a monounsaturated 22 carbon fatty acid, occurs in traditional rapeseed oil at levels of 22-45% of the total fatty acids, (Teasdale, 1975) but may rise to as much as 65% in special high-erucic varieties developed for industrial applications (Downey et al., 1975). Breeding of varieties with low erucic acid content was undertaken because of observations that very long chain monoenoic fatty acids, such as erucic and cetoleic acids, produced cardiopathological changes and detrimental changes in skeletal tissues (Abdellatif and Vles, 1973; Rocquelin et al., 1973; Beare-Rogers et al., 1974). The erucic acid

in traditional rapeseed oil is replaced by its 18 carbon progenitor oleic acid in the low erucic acid varieties. Traditional rapeseed oil and low erucic acid rapeseed oil resemble soybean oil in their linolenic acid content (considered undesirably high in all three oils because linolenic acid deteriorates readily during storage). High and low erucic acid rapeseed oils are both characterized by relatively low levels of linoleic acid compared to soybean oil, and relatively low levels of the saturated fatty acids, palmitic and stearic acids (Downey et al., 1975).

Rape is raised not only for its oil but for the high-protein residue that remains after oil extraction. Traditional rapeseed meal has been used as a supplement for animal feeding even though it contains high levels of glucosinolates, which have growth-depressing and goitrogenic effects. Plant geneticists therefore have been working toward the elimination of these compounds. This goal was achieved in 1974 with the licensing of the Brassica napus cultivar Tower, the first Canadian low glucosinolate, low erucic acid cultivar. Tower is the result of backcrosses of Liho, a low erucic acid variety, and Brownowski, a low glucosinolate variety, to Turret. Plant breeders are now attempting to increase the linoleic acid content to 40% and decrease the linolenic acid content to 3% (Downey et al.,

1975).

The pathogenic effects of traditional rapeseed oil have been attributed to the high level of erucic acid and the low level (4 to 6%) of saturated fatty acids. Erucic acid alone cannot be the entire answer, since Rocquelin and Cluzan (1968) reported pathological changes in rats fed low erucic acid rapeseed oil, a finding that was confirmed by Rocquelin et al (1970) and has been observed repeatedly by other researchers (Kramer et al., 1973; Beare-Rogers et al., 1974).

Although animal studies of rapeseed oil are quite numerous, little work has been done on the metabolism of rapeseed oil in man. Rapeseed and soybean oil, as mentioned previously, are the major edible oils used in Canada. Therefore it was thought meaningful to compare the effect of low erucic acid rapeseed oil from the new cultivar Tower with the effect of soybean oil on whole blood hematology and lipid patterns in the human.

REVIEW OF LITERATURE

A. HISTORY OF RAPESEED OIL IN CANADA

Rapeseed oil became a product of Canadian agriculture in 1942 when it was introduced as a lubricant for reciprocating steam engines. Later rapeseed oil was sold on the domestic market as a replacement for some of the imported edible oils.

Rapeseed oil is extracted from the seeds of two traditional species; Brassica napus or Argentine rape, a high-yielding long-season variety, and Brassica campestris or Polish rape, a somewhat lower-yielding, earlier-maturing variety.

Traditional rapeseed oil is characterized by a high content of the long chain monounsaturated fatty acids erucic acid (C22:1n9) and eicosenoic acid (C20:1n9), members of the oleic acid series. The oil from the two species are similar, although the oil from the traditional B. napus varieties contains more erucic acid than that from the traditional B. campestris varieties. Seventy-five to eighty percent of the commercial crop over the last ten years has been of the B. campestris type, due to the short growing season on the Canadian prairies (Craig et al., 1973).

In 1961, Stefansson and coworkers in Canada succeeded in isolating a true-breeding low erucic acid strain of rapeseed (Stefansson et al., 1961). They used as starting material a variety of Brassica napus called Liho, from Limberger Hof, Germany; a variety which exhibited great variability in the erucic acid content of its seeds. Since then there has been considerable work on genetic selection with rapeseed, and low erucic acid variants of both B. napus and B. campestris have been isolated. These new low erucic acid varieties lack the enzymes necessary for the elongation of the C18 monoenoic fatty acid oleic acid to produce eicosenoic and erucic acids. The new oil from these selected strains therefore is characterized by a high content (53-60%) of oleic acid, the C18:1n9 fatty acid. The new oil was initially termed canbra oil, a contraction of Canada Brassica, and this name is encountered in the early literature.

B. A COMPARISON OF SOYBEAN OIL WITH HIGH AND LOW ERUCIC ACID RAPESEED OIL

In 1973, in accordance with a request from National Health and Welfare, Canadian producers switched over to low erucic acid varieties of rapeseed. This decision was based on reports of deleterious effects of erucic acid.

Rapeseed oil and soybean oil are the most widely used edible oils in Canada. The disappearance data for the leading vegetable oils is presented in Table 1. Prior to 1971, soybean oil was used most widely in Canada. From 1971

Table 1

Domestic Disappearance Statistics for Edible Oils in Canada[1]

	Millions of Pounds			
	Margarine Oil	Shortening Oil	Salad Oil	Total Oils
1973				
Rapeseed oil	75.8	89.3	72.6	237.7
Soybean oil	59.2	84.6	29.5	173.3
Palm oil	8.8	35.5	0.3	44.3
Coconut oil	0.8	38.8	1.5	41.0
Sunflower seed oil	0.1	3.3	23.0	26.5
1974				
Soybean oil	90.4	120.0	49.7	260.0
Rapeseed oil	63.4	65.3	72.3	201.1
Palm oil	8.9	20.0	0.3	29.2
Coconut oil	0.6	23.2	-	23.8
Sunflower seed oil	0.1	4.1	16.3	20.6

[1]Data from Statistics Canada Fats and Oils.

to 1973, rapeseed oil usage was greater than that of soybean oil, but in 1974, soybean oil again assumed the lead in Canada.

Soybean oil differs in composition from rapeseed oil in that soybean oil contains more of the polyunsaturated fatty acid linoleic and of the saturated fatty acids palmitic and stearic acids (Table 2). Low erucic acid rapeseed oil differs from traditional rapeseed oil in that oleic acid largely replaces eicosenoic and erucic acids (Table 2).

There are also differences between rapeseed oil and soybean oil in positional conformation of the triglyceride. The linoleic acid in soybean oil seems to be randomly distributed among the three positions of the glycerol moiety of the triglycerides (Figure 1) whereas linoleic acid appears to be located primarily in the two position in rapeseed oil (Figure 2). Erucic acid in rapeseed oil is located almost exclusively in the one and three position of the triglyceride and is found only in trace amounts in the two position (Appelqvist, 1971; Grynberg and Szczepanska, 1966; Jacquot et al., 1969). It is not known whether these positional differences affect the metabolism of these oils by the body. However, free erucic acid, when ingested in conjunction with oleic acid, is incorporated into the one and three positions rather than the two position of lymph triglycerides in the rat, indicating a similar positional

Table 2

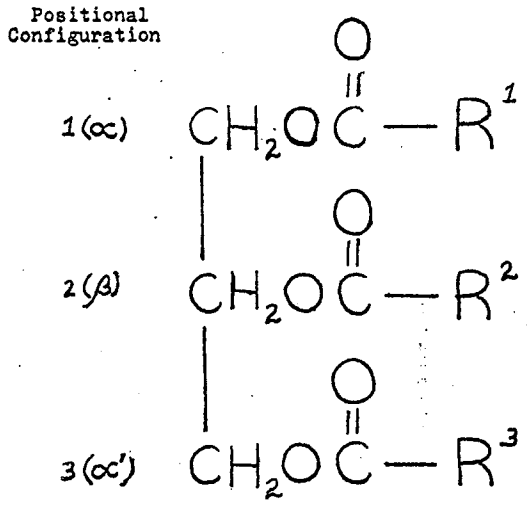
Fatty Acid Compositions of Soybean and Rapeseed Oils[1]

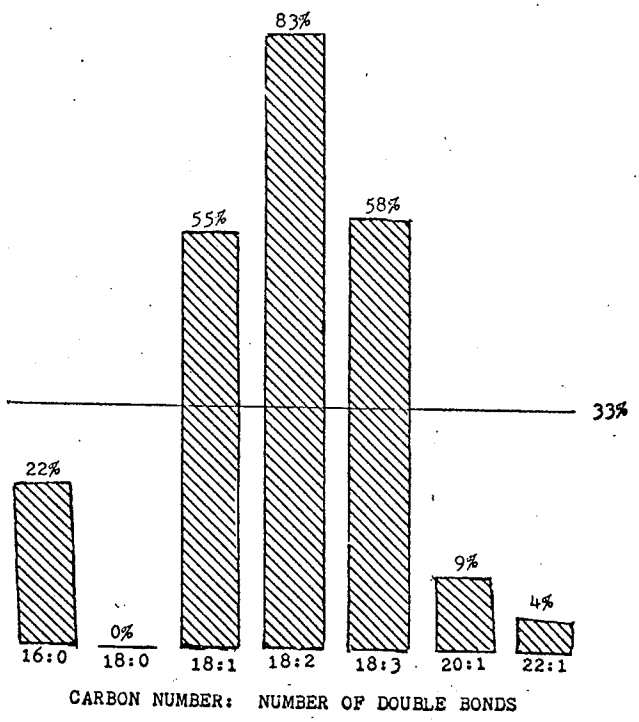
	Rapeseed Oil		Soybean Oil
	Low Erucic Acid	High Erucic Acid	
C16:0 Palmitic	1.5-5.0	2.4	10-11
C16:1 Palmitoleic	0.3-0.9	<0.5	tr
C18:0 Stearic	0.9-2.0	1.2	4
C18:1 Oleic	53-60	13-36	23-26
C18:2 Linoleic	19-25	12-23	50-54
C18:3 Linolenic	8-12	7-11	7-9
C20:0 Arichidic	0.5-1.0	0.5-1.5	tr
C20:1 Eicosenoic	1.5-2.5	8-15	-
C22:0 Behenic	<1.0	tr	-
C22:1 Erucic	tr -5.0	22-45	-

[1]Teasdale, 1975.

Figure 1

Structure of a Typical Triglyceride





[1]Jacquot et al., 1969.

Figure 2

Proportion of Various Fatty Acids in the 2 Position in Triglycerides of High Erucic Acid Rapeseed Oil[1]

incorporation as in the rape plant (Savary and Constantin, 1966).

C. RAPESEED OIL AND CARDIOPATHOLOGY

Concern about the content of erucic acid in rapeseed oil stemmed from reports of pathological changes associated with the feeding of high levels of rapeseed oil. A major concern with traditional high erucic acid rapeseed oil was the high incidence of cardiac lesions in experimental animals fed the oil. All ten animal species studied to date have exhibited cardiac changes in response to a diet containing erucic acid, either as a component of rapeseed oil or as ethyl erucate or glyceryl trierucate (Beare-Rogers et al., 1972 a, b; Beare-Rogers and Nera, 1972; Beare-Rogers, et al., 1971; Houtsmuller, 1972).

The development of cardiopathology in response to rapeseed oil has been reviewed by LeBlanc (1973), King (1974) and Lake (1975). However, a few additional comments deserve mention.

Development of cardiopathology in the rat follows a characteristic pattern. Triglycerides rich in erucic acid accumulate in the heart muscle within 24 hours of ingestion of a diet containing erucic acid. Erucic acid concentration reaches a peak in three to six days after the introduction of erucic acid, and then tapers off gradually

(Abdellatif and Vles, 1973; Abdellatif and Vles, 1970a; Lall et al., 1972).

The reason for this lipid accumulation has not been established. However, two biochemical changes have been reported in response to feeding of erucic acid. Houtsmuller et al (1970) reported a decrease in the rate of ATP synthesis by isolated rat heart mitochondria, although Kramer et al. (1973) disputed this finding and attributed the decrease in ATP production to improper isolation of the mitochondria. Feeding of erucic acid has also been reported to cause a decrease in the rate of β-oxidation in isolated rat heart mitochondria, which may account for the fat accumulation in the heart muscle (Swarttouw, 1974). There appears to be an improvement over time in the ability of heart tissue to metabolize erucic acid to oleic acid through β-oxidation, as evidenced by an increase in the oleic acid in the heart (Christopherson and Bremer, 1972; Houtsmuller et al., 1972; Craig et al., 1963a; Craig and Beare, 1967). This improved β-oxidation may account for the gradual clearing of fat deposits in the heart (Jaillard et al., 1973).

Necrosis and fibrosis of cardiac tissue has been observed in several species fed diets containing erucic acid over an extended period. However there is question as to whether erucic acid alone is responsible for all cardiac lesions observed. Low erucic acid rapeseed oil also produces

cardiac lesions in the rat (Rocquelin et al., 1973; Beare-Rogers, 1975), although development of lesions in response to low erucic acid rapeseed oil is of a lower frequency and severity than that caused by high erucic acid rapeseed oil (Rocquelin et al., 1970; Rocquelin et Cluzan, 1968; Abdellatif and Vles, 1970a). This observation suggests that although erucic acid clearly contributes to the occurrence of cardiopathology, it is not the only factor.

Beare-Rogers (1975) has suggested another factor which may cause abnormalities when rapeseed oil is fed. When the deodorizer condensate from rapeseed oil (obtained during steam-deodorization of the crude oil) was mixed with olive oil and fed to rats, the incidence of cardiac lesions increased.

Kramer et al (1973) however, have suggested that the imbalance in fatty acid composition as a result of the high content of oleic and linolenic acids and low content of the saturated fatty acids in rapeseed oil might be responsible for the cardiac lesions observed with low erucic acid oils. This theory may also explain in part the incidence of cardiac lesions observed in response to feeding of high erucic acid rapeseed oil.

Partial hydrogenation of traditional rapeseed oil was found to reduce the incidence of cardiac lesions, while partial hydrogenation of the low erucic acid rapeseed oils,

Span oil and Zephyr oil, eliminated cardiopathology completely (Beare-Rogers, 1975). Since hydrogenation increases the level of saturated fatty acids threefold, while virtually eliminating the polyunsaturated fatty acids linoleic and linolenic acids, (Table 8) the experimental evidence is compatible with the Kramer theory.

Another change that occurs during the hydrogenation of rapeseed oil is the conversion of cis-dodecanoic acid (erucic acid) to the trans form (brassidic acid). Beare-Rogers et al. (1971) suggested that brassidic acid may be metabolized differently from erucic acid, and that a difference of this nature may explain the reduction in incidence of cardiac lesions with the hydrogenated oils. No experimental work on this theory has been reported.

None of these theories adequately explain why hydrogenation of both types of rapeseed oil results in a decrease in cardiac changes. The deodorizer condensate theory does not explain why the incidence of cardiac lesions decreases with decreasing erucic acid content. Conversely, the brassidic acid theory does not explain the occurrence of cardiac changes when the isolated deodorizer condensate is fed (although the fatty acid imbalance theory may do so). Further research is essential to identify the factor or factors responsible for the cardiopathology associated with rapeseed oil. It is entirely possible that the observed

cardiac lesions are caused by two or more factors.

D. OTHER PATHOLOGICAL EFFECTS OF RAPESEED OIL

Abnormality of tissues other than the heart have been reported in animals ingesting traditional rapeseed oil. Some changes also have been reported in animals consuming low erucic acid oil. The reported effects include changes in skeletal tissue (Abdellatif and Vles, 1970a; Vles and Abdellatif, 1970a), change in the composition of body fat (Hopkins et al., 1957) changes in the cholesterol composition of the adrenals (Carroll and Noble, 1956; Carroll, 1953; Carroll, 1951; Walker, 1972; Walker et al., 1972), abnormalities in liver and kidney (Kramer et al., 1973; Manchon et al., 1973; Abdellatif and Vles, 1970b; Vles and Abdellatif, 1970a), change in the composition of ovarian lipids (Carroll and Noble, 1952), alteration in reproductive capacity (Beare et al., 1961b; Carroll, 1959b; Carroll and Noble, 1957), and change in resistance of rats to cold (Beare-Rogers and Nera, 1974). Usually these changes were attributed to the erucic acid content of the oil.

These topics have been dealt with previously by LeBlanc (1973), Lake (1975) and King (1974), and therefore will not be treated in detail here. However, a few comments are in order.

There is considerable controversy in the literature

concerning the validity of many of the above observations. Attempts to duplicate many of the original findings reported in response to the feeding of rapeseed oil indicated that these abnormalities often were caused by poor experimental design, characteristic weaknesses peculiar to the experimental animal or strain of animal chosen, or deficiencies in the experimental diet in respect to one or more nutrients. Erucic acid, which initially had been blamed in most cases, was often not a factor.

In addition, much of the recent evidence suggests that many of the changes produced by rapeseed oil may be due, not to the erucic acid content, but to the peculiar distribution of fatty acids in both the high and low erucic acid rapeseed oils, especially its low content of saturated fatty acids. This possibility has already been discussed in relationship to cardiopathology, but may apply to other abnormalities as well.

Therefore, it is meaningful to re-examine much of the earlier research on rapeseed oil, in an attempt to explain some of the contradictions in the literature. Selected examples will be given to illustrate how one or more of the above factors may offer a more adequate explanation than erucic acid for the changes observed in response to the feeding of rapeseed oil.

Poor experimental design was a factor in the

experiments reported by Thomasson (1955a) in which death in rats occurred within 17 days of the introduction of a diet containing 73% of calories from rapeseed oil. Since rats fed lower levels (50% of calories) of rapeseed oil lived longer than butter-fed controls, Thomasson, (1955b) concluded that erucic acid was toxic at extremely high levels, but acceptable at moderate levels. However, Alexander and Mattson (1966) demonstrated that the mortality observed when rapeseed oil was fed at 73% of calories was due to separation of the test oil from the solid ingredients in the diet to produce a food form which the rats would not eat. When the fat was gelatinized with ethyl cellulose before it was mixed into the diet, the animals were willing to eat the diet, and lived as long as controls. The erucic acid content of the oil was not responsible for the mortality observed.

The species chosen can also have a marked effect on the biochemical and histological changes observed in response to the feeding of rapeseed oil. Although cardiac changes have been reported in response to the feeding of rapeseed oil in all ten species studied, the severity and duration of these changes vary markedly. Rats and gerbils appear to be unusually susceptible to the rapid accumulation of high levels both of lipid and of erucic acid in cardiac tissues, and both species develop extensive and longterm

necrosis of heart muscle (Abdellatif, 1973; Abdellatif and Vles, 1973). In contrast, miniature and regular baby pigs exhibit microscopic lipid droplets in cardiac tissue, but do not accumulate excessive levels of erucic acid, whereas squirrel monkeys show an increase in erucic acid but no histological changes (Beare-Rogers and Nera, 1972). Some changes have been observed in one species only, such as cirrhosis of the liver in ducklings (Abdellatif and Vles, 1970b). Even strain may have an effect. The adrenals of Sprague-Dawley rats enlarged in response to a diet containing rapeseed oil, while the adrenals of Wistar rats on the same diet remained normal (Beare-Rogers, 1975).

Other nutrients in the test diet must also be present in appropriate quantities. Digestibility co-efficients reported for rats fed a diet containing rapeseed oil have been shown to be affected by the relative and absolute amounts of calcium and phosphorus in the diet (Cheng et al., 1949). Depressed growth and appetite in response to rapeseed oil have been reported by many researchers, but some of these changes may have been caused by other factors. For instance, Carroll (1959b) reported that addition of vitamin A acetate improved both growth rate and final weight gain, when he repeated some of his earlier experiments.

The fatty acid composition of both high and low erucic acid rapeseed oils may affect growth and appetite.

Some evidence has been advanced to implicate erucic acid in growth retardation and appetite depression. For example, chickens fed diet containing high erucic acid rapeseed oil ate less and gained weight less rapidly than chickens fed similar diets containing low erucic acid rapeseed oil, soybean oil, or lard (Vogtman et al., 1973). However, in 1955, Hopkins et al suggested that the low saturated fatty acid content of rapeseed oil might also be implicated. This was confirmed in 1963 by Beare et al., who showed that the addition of saturated fat to a rapeseed oil diet improved growth. In 1973, Craig et al reported that the level of saturated fatty acids had more effect on weight gain than the erucic acid content. Partial hydrogenation of rapeseed oil, which increases the level of saturation, was shown to improve both growth rate and final weight gain, further supporting this hypothesis (Beare et al., 1961a).

These examples are by no means exhaustive, but they serve to illustrate the inadequacy of the simplistic erucic acid theory to explain the wide range of abnormalities reported in response to the feeding of rapeseed oil.

E. DIGESTIBILITY OF RAPESEED OIL

The digestibility of rapeseed oil has been reviewed by LeBlanc (1973), King (1974) and Lake (1975). However, it may be worth re-emphasizing that there is a species

difference in the utilization of rapeseed oil. The rat utilizies rapeseed oil very inefficiently. Deuel (1948) reported digestibility co-efficients of 77% and 82% for male and female rats, respectively. It has repeatedly been confirmed since then that the rat is a poor model for man (Rocquelin et LeClerc, 1969; Carroll, 1958; Carroll and Richards, 1958). Holmes in 1918 found the digestibility co-efficient for rapeseed oil in the human to be 98.8%, a finding which was confirmed by Deuel (1949) and by Vaisey et al (1973). Vaisey et al. (1973) also found that low erucic acid rapeseed oil was as well absorbed as traditional high erucic acid oil.

F. EFFECT OF RAPESEED OIL ON BLOOD LIPID PATTERNS

Reports on research in this area are very limited, both in animals and in man. This is unfortunate, since serum lipid patterns and whole blood hematology (and to a lesser extent fat biopsy) are the only readily accessible tissue specimens in the human.

Vles and Abdellatif (1970b) found that the serum cholesterol of rabbits fed high erucic acid rapeseed oil was significantly higher than that of rabbits fed soybean oil. On the other hand, rats fed high erucic acid rapeseed oil and soybean oil showed similar levels of cholesterol in the liver and blood serum, but the rats fed rapeseed oil had higher levels of adrenal

cholesterol (Alexander and Mattson, 1966). Feeding of erucic acid to rats at a level of 10 to 15 calories per cent did not affect plasma cholesterol level, although adrenal cholesterol rose, largely due to an increase in cholesterol erucate (Carroll and Noble, 1956; Carroll, 1962).

Grande et al. (1962) found that there was no difference in mean serum cholesterol and phospholipid levels of humans consuming a diet containing 32% of total caloric intake from high erucic acid rapeseed oil or from a corn and olive oil mixture, although both parameters increased when the diet contained 32% of total caloric intake from butter. Malmros and Wigand (1957) reported a study conducted with human subjects in which various fats and oils were fed at a level of 40% of calories. A diet containing rapeseed oil, (50% erucic acid) caused a moderate decrease in serum cholesterol compared to levels observed for the subjects' normal diet. In comparison, safflower oil caused a marked decrease in serum cholesterol, olive oil produced only a slight decrease and milk fat produced a slight increase. These findings are consistent with the general observation that an increase in polyunsaturated fatty acids and/or a decrease in saturated fatty acids results in a decrease in cholesterol levels in the human.

Changes in serum cholesterol, lipid phosphorus and serum triglycerides in response to either high or low erucic

acid rapeseed oil have been studied by LeBlanc (1973), King (1974) and Lake (1975) from this laboratory. The response of the serum lipids after eight days of feeding of a diet containing rapeseed oil are summarized in Table 3.

G. EFFECT OF RAPESEED OIL ON WHOLE BLOOD HEMATOLOGY

Rapeseed oil, especially high erucic acid rapeseed oil, has been reported to influence whole blood hematology.

Rapeseed oil was reported by Abdellatif et al.(1972) to result in an increase in the hematocrit of ducklings, while hemoglobin levels remained normal. In guinea pigs, rapeseed oil was found to increase the number of immature red blood cells (reticulocytes) and to decrease hemoglobin content, packed cell volume and red blood cell count, and was suspected of inducing hemolytic anemia (Vles and Abdellatif, 1970a).

Reports on hematological changes in the human in response to rapeseed oil are limited. King (1974) reported that the leucocyte count for humans was significantly lower when the diet contained high erucic acid rapeseed oil (39% erucic acid) than when the diet contained mixed fat. Many of the values observed by King (1974) were below the levels generally considered normal for the human, thus complicating the interpretation of these results. LeBlanc (1973) found no consistent effect of a diet containing low erucic acid

Table 3

Comparison of Changes in Serum Lipids When Rapeseed Oil Was Fed for Eight Days Following a Similar Diet Containing Mixed Fat

Study	Rapeseed Oil Used	% of calories contributed by fat	CHANGE AFTER 8 DAYS ON RSO		
			Serum Cholesterol	Lipid Phosphorus	Serum Triglyceride
LeBlanc (1973)	2.6% erucic acid(Span)	38%	decrease	decrease	decrease
King (1974)	38% erucic acid	38%	decrease	decrease	decrease
Lake (1975)	0.4% erucic acid (Zephyr)	38%	decrease	decrease	decrease

rapeseed oil (2.6% erucic acid) on the number of leucocytes. Lake (1975) reported that ingestion of either soybean oil or low erucic acid rapeseed oil resulted in an appreciable decrease in leucocyte counts, although mean leucocyte counts remained within the normal range and there were no significant differences between the experimental oils.

King (1974) reported a decrease in the number of platelets in response to the feeding of high erucic acid rapeseed oil. Other researchers, using similar diets containing low erucic acid rapeseed oil or soybean oil, did not observe any decrease in platelets. Changes in platelets in response to fat source is of considerable interest. Investigation into the clotting mechanism in atherosclerosis suggests that platelet aggregation may play a key role in the formation of thrombi or clots (Hoak et al., 1967; Mickel and Horbar, 1973). In addition, intravenous injection of arachidonic acid into rabbits was found to provoke the formation of a fatal clot within three minutes (Silver et al., 1974). Fatty acids are involved in platelet function in two other ways. The phospholipid component of platelets is essential for the formation of Autoprothrombin C, and for the acceleration of function of this clotting factor (Seegers et al., 1968; Seegers, 1969). The prostoglandins, derived from arachidonic acid, are also intimately involved in the clotting process (Stryer, 1975).

In general, however, there is very little data available on the relationship of minor fluctuations in whole blood hematology to human health. This makes assessment of the meaning of changes in such parameters in humans very difficult.

H. UNRESOLVED ASPECTS OF THE EFFECTS OF RAPESEED OIL IN THE HUMAN DIET

It is now generally accepted that erucic acid is undesirable in the human diet because of the abnormalities in the cardiac tissue of experimental animals attributed to erucic acid. However, the erucic acid content of rapeseed oil is no longer a practical nutritional problem in Canada since only low erucic acid oils have entered the edible oil market since 1973.

Nutritionists are now concerned about the suitability of the particular pattern of fatty acids found in rapeseed oil when fed as the sole source of dietary fat. Feeding of unhydrogenated low erucic acid rapeseed oil as the sole source of dietary fat also has been found to produce cardiac lesions and growth abnormalities in experimental animals (Beare-Rogers et al., 1974; Abdellatif and Vles, 1973).

In the human diet, liquid rapeseed oil is rarely the sole source of dietary fat, except in isolated experimental or therapeutic situations. Although few Canadians

have fat intake patterns resembling the pattern of domestic disappearance of edible fats, most probably consume more than one processed fat (e.g. as a table spread, in salad dressings, or in cooking and baking). In addition, much of the fat in a typical diet is present as a constituent of foods such as meat, eggs and dairy products. Furthermore only a portion of vegetable oils is consumed in the liquid form; two-thirds of the rapeseed oil marketed in Canada is hydrogenated to make shortenings and margarines (Table 1).

Single-fat source studies yield much valuable information about that specific fat. However, such studies cannot always predict the effects of the same fat in the context of a mixed fat intake, especially in respect to changes which are caused by specific fatty acid patterns. Little is known about ideal fatty acid intake patterns in the human. Recently there has been growing suspicion that an intake of a balanced variety of fatty acids may be optimal for the human and that this balance may most easily be achieved through a diet which provides fat from several sources. Rapeseed oil, and fats in general, deserve investigation within the context of a mixed fat intake (which more closely resembles actual dietary practices than do single-fat source diets) as well as within the traditional contexts of sole-fat source studies.

Very little research about the effects of dietary

rapeseed oil in the human has been reported. Animal research can provide preliminary data only, since species differences in response to rapeseed oil are common. Conclusive investigation on the effects of rapeseed oil in the human diet must be carried out with human subjects (Beare-Rogers, 1975).

OBJECT OF RESEARCH

The present study was designed

1. To compare the effects of low erucic acid rapeseed oil (Brassica napus cultivar Tower) and soybean oil on whole blood hematology and serum lipid patterns in the human. These oils were chosen because together they supply over eighty percent of the edible oils consumed in Canada.
2. To compare the effects on whole blood hematology and serum lipid patterns of a diet containing a single vegetable oil as the sole source of added fat with the effects of a diet containing a mixture of fats simulating a conventional Canadian dietary fat intake.

EXPERIMENTAL METHODS

A. EXPERIMENTAL DESIGN

The study consisted of a 31 day metabolic trial involving eight healthy male subjects. The experimental design is illustrated in Figure 3. The first eight days of the study served as a stabilization period (Pre-Experimental Period) during which the subjects were fed a mixed fat diet. During this period the subjects were accustomed to the routine of the study and caloric requirements were adjusted to meet the needs of each subject. Following the stabilization period four of the subjects were placed on a diet in which low erucic acid rapeseed oil (cv. Tower) provided 92 to 94% of the fat for eight days. The other four subjects were placed on the same diet except that soybean oil supplied the fat for eight days. This regimen constituted Experimental Period I. The subjects were then switched in a crossover fashion such that the four subjects fed low erucic acid rapeseed oil during the first period received soybean oil during the next eight days (Experimental Period II) and those fed soybean oil in Period I received rapeseed oil during Period II. Period II was followed by a final seven-day period (Post-Experimental Period) during which all eight subjects again received the diet containing mixed fat.

Figure 3

Experimental Design

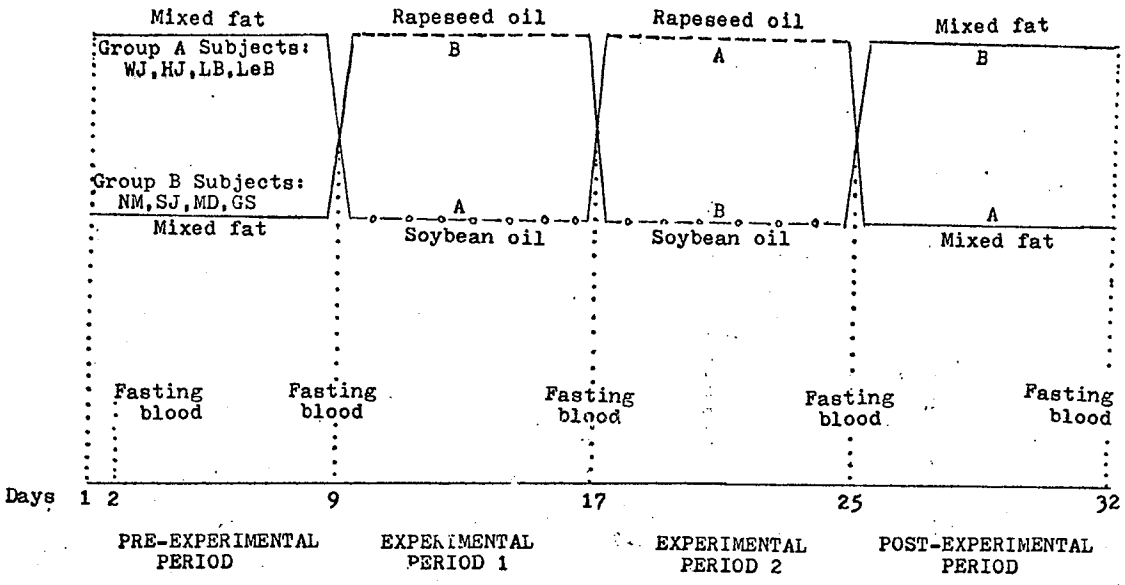


Table 4

Physical Data for Subjects

	Age(yr)	Height(cm)	Weight (Kg) Initial[1]	Rapeseed Oil Diet[2]	Soybean Oil Diet[2]	Final[1]
Group A						
WJ	23	183.4	66.7	66.7	66.8	66.8
HJ	22	177.6	74.7	73.9	73.0	73.3
LB	27	189.7	94.2	93.5	93.3	92.3
LeB	21	187.5	81.8	81.2	80.7	81.1
Group B						
NM	29	175.5	78.4	77.1	77.6	76.6
SJ	25	181.2	72.9	72.3	72.6	71.4
MD	26	174.7	74.8	74.9	74.7	74.8
GS	24	181.2	61.4	60.8	61.1	60.9

[1]Single weighing.

[2]Mean of eight daily weighings.

On the seventh and eighth day of each experimental period the subjects were exercised on a bicycle ergometer, as described by Parker (1975).

Fasting blood samples were taken before breakfast on the second day of the experiment, on the first day of each subsequent dietary period, and on the last day of the study.

B. SUBJECTS

The eight subjects who participated in the study were male college students or University of Manitoba employees between the ages of 21 and 29 years (average age 24.6) selected from respondents to posted notices. The subjects were selected on the basis of an interview with the project director, a physical examination, and a positive response to a detailed outline of the protocol of the study and the subjects' responsibilities. Before starting the study all subjects were carefully instructed as to the importance of adhering to a strict dietary regimen for the duration of the study.

Subjects maintained their normal activity patterns. They resided in their own homes for the entire study, but took all weekday meals as well as weekend breakfasts in the

Home Economics Building, University of Manitoba. The noon and evening meals on Saturday and Sunday were either consumed in the Home Economics Building or packaged for home preparation by the subjects, depending on their personal preferences.

The subjects weighed themselves daily before breakfast. Body weight was maintained constant for each individual by adjusting his caloric intake in response to any consistent change in body weight.

The subjects were without diagnosed metabolic disorders or recent history of ill health. Physical data for the subjects are presented in Table 4.

C. TEST FATS

The fat sources used in the mixed fat diet (pre-experimental and post-experimental periods) were beef tallow,[1] vegetable shortening,[2] lard,[3] butter[4] and corn oil.[5] The proportion of each of these fats in the mixed fat diet

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[1]Bleached, clarified, deodorized, Canada Packers Ltd., Winnipeg.

[2]Crisco, Proctor and Gamble, Toronto.

[3]Tenderflake, Canada Packers Ltd., Toronto.

[4]Modern Dairies, Winnipeg.

[5]Mazola, Best Foods Division, Canada Starch Co., Montreal.

was based on domestic disappearance statistics for Canada.[1] Butter and corn oil were used for spread and salad dressing respectively, while a mixture (Fat Mix) of tallow, shortening and lard was used in the entrees, cereals, scrambled egg albumin, potatoes and rice, and baked products for the mixed fat diet. The fatty acid composition of the Fat Mix and salad oil are presented in Table 5.

Tower rapeseed oil,[2] (a low erucic acid rapeseed oil), specially prepared Tower rapeseed oil margarine,[3] soybean oil[4] and soybean oil margarine[5] were the sole sources of added fat to the rapeseed oil and soybean oil diets. The fatty acid composition of the low erucic acid rapeseed oil and the margarine prepared from this oil are given in Table 6. The fatty acid composition of the soybean oil and soybean oil margarine are presented in Table 7.

D. DIET COMPOSITION

The test diet used in this study was similar to the

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[1]Paul Simms, Food Research Institute, Ottawa.

[2]Agra Industries, Nipiwin, Saskatchewan.

[3]Canada Packers, Toronto. Prepared through the courtesy of Mr. B.F. Teasdale.

[4]Crisco Oil, Proctor and Gamble, Toronto.

[5]Canada Packers, Toronto.

Table 5

Fatty Acid Composition of Fat Mix[1] and Corn Oil

Fatty Acid	% of Total Fatty Acids	
	Fat Mix	Corn Oil
Decanoic C10:0[1]	0.13	-
Lauric C12:0	0.11	-
Myristic C14:0	1.46	0.11
Palmitic C16:0	20.88	10.99
Palmitoleic C16:1	1.96	-
Stearic C18:0	15.74	1.79
Oleic C18:1	43.48	26.25
Linoleic C18:2	13.88	58.82
Linolenic[3] C18:3	0.84	1.69
Eicosenoic C20:1	0.26	-

[1]Fat mix used in the preparation of the entrees, cereals egg albumin and snacks contained lard:tallow:shortening in the ratio of 4:5:6 (W:W:W)

[2]Carbon number:number of double bonds

[3]Not distinguished from C20:0 with the column used to resolve fatty acids.

Table 6

Fatty Acid Composition
of Low Erucic Acid Rapeseed Oil and Margarine[1]

Fatty Acid	% of Total Fatty Acids	
	Oil	Margarine
Lauric C12:0[2]	-	0.34
Myristic C14:0	-	0.09
Palmitic C16:0	4.64	4.93
Palmitoleic C16:1	tr	tr
Stearic C18:0	1.73	12.28
Oleic C18:1	58.25	76.28
Linoleic C18:2	22.23	3.67
Linolenic[3] C18:3	9.70	0.64
Eicosenoic C20:1	2.42	1.14
Behenic C22:0	tr	tr
Erucic C22:1	0.79	0.59

[1]Oil from B. napus cv. Tower, Agra Industries, Nipiwin, Saskatchewan. Margarine specially prepared from Tower oil, Canada Packers, Toronto.

[2]Carbon number:number of double bonds.

[3]Not distinguished from C20:0 with the column used to resolve fatty acids.

Table 7

Fatty Acid Composition of Soybean Oil and Margarine[1]

Fatty Acid	% of Total Fatty Acids	
	Oil	Margarine
Lauric C12:0[2]	0.03	2.33
Myristic C14:0	0.06	1.54
Palmitic C16:0	9.27	11.66
Palmitoleic C16:1	-	tr
Stearic C18:0	3.80	7.95
Oleic C18:1	46.81	35.29
Linoleic C18:2	36.35	36.15
Linolenic[3] C18:3	3.37	4.92
Eicosenoic C20:1	0.08	-
Behenic C22:0	0.04	-

[1]Crisco Oil, Proctor and Gamble, Toronto, Ontario. Margarine prepared by Canada Packers Ltd., Toronto.

[2]Carbon number:number of double bonds.

[3]Not distinguished from C20:0 with the columns used to resolve fatty acids.

diet used by Lake (1974). The diet was comprised of conventional low fat foods and added fats.

A two-day menu (Tables 8 and 9) was used throughout the study, with each menu designed to provide approximately 40 percent of the calories from fat. The experimental diets were similar to the mixed fat diet except that the sources of fat in the diet were replaced by rapeseed oil and rapeseed oil margarine (rapeseed oil diet) or soybean oil and soybean oil margarine (soybean oil diet).

Bread was included at each meal to serve as a base for the spread (butter, rapeseed oil margarine and soybean oil margarine) and to wipe up any visible fat remaining on serving dishes.

The daily menus were designed to include all food groups, and to provide a variety of textures and flavors. All nutrient intakes recommended in the revised Canadian Dietary Standard (1974) were met except for vitamin A and thiamin on Menu 1 and niacin on both menus (Table 10). Niacin probably was not deficient in these diets since they contained generous quantities (a total of 85 grams per day) of high quality protein. It is generally assumed that 60 mg. of tryptophan are equivalent to one mg. niacin. Since vitamin A is readily stored and since the two menus supplied an average of 1437 Retinol Equivalents per day, the overall intake of this vitamin was more than adequate.

Table 8

Composition of Meals

Menu 1	Menu 2
Breakfast[1]	
Apple juice 120 g.	Orange juice 120 g.
Rolled oats[2] 30 g.	Cream of wheat[2] 30 g.
Scrambled egg albumin[2] 14.3 g.	Scrambled egg albumin[2] 14.3 g.
Bread[3] 1 slice	Bread[3] 1 slice
Strawberry jam or marmalade 14 g.	Strawberry jam or marmalade 14 g.
Sugar, white or brown 11 g.	Sugar, white or brown 11 g.
Skim milk 280 ml.	Skim milk 280 ml.
Butter or margarine[4] 25 g.	Butter or margarine[4] 25 g.
Lunch[1]	
Scalloped potatoes[2]	Spaghetti with tomato sauce[2]
Salad - tomatoes 30 g.	Salad - leaf lettuce 50 g.
leaf lettuce 50 g.	cucumber 10 g.
radish 10 gm.	green onion 5 g.
green onion 5 g.	Piquant dressing[2,5]
Piquant dressing[2,5]	Bread[3] 1 slice
Bread[3] 1 slice	Nectarine 150 g.
Watermelon 250 g.	Skim milk 280 ml.
Skim milk 280 ml.	
Dinner[1]	
Chili[2]	Beef Stew[2]
White rice[2] 30 g.	Instant mashed potato 30 g.
Coleslaw - cabbage 50 g.	Salad - iceberg lettuce 50 g.
green pepper 5 g.	tomatoes 50 g.
carrots 10 g.	Bread[3] 1 slice
Bread[3] 1 slice	Canned plums 120 g.
Canned peaches 100 g.	Skim milk 280 ml.
Skim milk 280 ml.	

[1]Coffee and tea allowed ad lib. Alcohol and other beverages forbidden.

[2]Prepared according to recipes in Appendix Tables 1-13.

[3]Wholewheat or white.

[4]25 grams butter as spread per day for mixed fat diet
25 grams soybean margarine for soybean oil diet and
25 grams Tower rapeseed oil margarine for rapeseed oil diet.

[5]Daily allotment of salad dressing was divided between lunch and supper at the discretion of each individual.

Table 9

Composition of Snacks
(Menus 1 and 2)

I Snacks containing fat

2 raisin-oatmeal cookies[1]
1 spicy fruit square[1]

II Non-fat snacks[2] - subjects selected one of the following daily

A. 7-UP - 10 oz.

B. 1 apple
raisins - 15 grams
3 caramels[3]

C. 1 apple
raisins - 30 grams

D. apple juice - 6 oz.
raisins - 15 grams
1 caramel[3]

E. applecot juice - 6 oz.
raisins - 15 grams
1 caramel[3]

F. applelime juice - 6 oz.
raisins - 15 grams

G. 1 banana
4 caramels[3]

H. 1 banana
raisins - 15 grams

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[1]Prepared according to recipes in Appendix Table 12 and 13.

[2]Snacks A to H were essentially isocaloric.

[3]Kraft caramels.

Table 10

Calorie and Nutrient Composition of Test Diet[1]

Composition	Menu 1	Menu 2	Recommended[2]
Calories	3004	3000	3000
Protein (g.)	85.3	85.0	56.0
Fat (g.)	133.86	133.22	-
Carbohydrate (g.)	368	379	-
Calcium (mg)	1191	1169	800
Phosphorus (mg)	1328	1236	800
Iron (mg)	14.6	25.8	10.0
Vitamin A (ug RE)	979	1894	1000
Thiamin (mg)	1.4	1.5	1.5
Riboflavin (mg)	2.3	2.6	1.8
Niacin (mg)	14	14	20
Vitamin C (mg)	170	212	30

[1]Calculated values using U.S.D.A. Handbook #8 Composition of Foods. (Watt and Merrill, 1963). Detailed nutrient values for each food item served may be found in Appendix Tables 17 and 18

[2]Recommended daily nutrient intakes. Bureau of Nutritional Sciences, Health and Welfare Canada. (Committee for Revision of the Canadian Dietary Standards, 1974). Recommendations are for activity pattern A, which is considered characteristic for males 19 to 35 years of age.

In addition to breakfast, lunch and dinner served at customary hours during the day, each subject received two fixed snack items incorporating the fat under study (rapeseed oil, soybean oil or Fat Mix) and a free-choice non-fat snack selected from the list in Table 9. These items were consumed as between-meal and evening snacks, distributed according to the preferences of the individual subjects. All food items were prepared from standardized recipes (Appendix Tables 1 to 13).

Fluid skim milk, spray dried egg albumin,[1] TVP,[2] Bontrae[3] and cereal products were the primary sources of protein in the diets. The relatively fat-free nature of these products resulted in diets in which added fat contributed over 92% of the total fat present in the diet.

Four entrees were used in the two-day menu- Scalloped Potatoes, "Beef" Stew, Spaghetti with Tomato Sauce and Chili. The TVP was hydrated with water, the appropriate fat added, and the mixture incorporated into the "Beef" Stew. Frozen Bontrae was weighed and incorporated into the Chili. The Scalloped Potatoes and Spaghetti with Tomato Sauce did not contain soy protein. Three of the entrees, "Beef" Stew,

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[1]Export Packers, Jarvis St., Winnipeg.

[2]Textured Vegetable Protein (soy protein) Archer Daniels Midlands purchased from British Canadian Importers, Vancouver.

[3]Bontrae frozen soy protein isolate, General Mills, Minneapolis.

Chili and Spaghetti with Tomato Sauce were prepared in advance in individual foil containers,[1] frozen and stored as described by LeBlanc (1973). For meal service the entrees were heated as described in the recipes (Appendix Tables 6, 7 and 8). The Raisin Oatmeal Cookies and Spicy Fruit Squares also were made in advance and stored frozen. The fourth entree, Scalloped Potatoes, was made on the day it was eaten according to the recipe in Appendix Table 5.

E. MEAL ANALYSIS

Composites of each daily menu were made for the mixed fat, rapeseed oil and soybean oil diets. The portion sizes for individual food items were determined weighing the items to the nearest gram on a Sartorius top loading balance.[2] The composites of each daily menu were homogenized with approximately 200 ml. of hot distilled water in a tared one-gallon Waring commercial blender,[3] and the total weight of the homogenate recorded. Two aliquots, each containing 150 to 200 grams of the homogenate, were lyophilized in a Virtis Freeze-Drier[4] and stored in #8992

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[1]EKCO Foil Containers with Lids, Price Wilson Ltd., 830 King Edward St., Winnipeg, Manitoba Sizes 685LL and 705-35.

[2]Model 2254 Sartorius-Werke AG, Gottingen, Germany.

[3]Model CB-5 Waring Products Co., Winsted, Connecticutt.

[4]Model 10-140 MR-BA Virtis Co. Inc., Gardiner, New York.

plastic Whirl-Pak bags[1] at -10°C for later analysis.

Total lipid was extracted from the lyophilized food samples using $CHCl_3/CH_3OH/H_2O$ according to the method of Bligh and Dyer (1959). Methyl esters of the fatty acids were prepared by the method of Metcalfe et al. (1966). The fatty acid methyl esters were dissolved in petroleum ether and stored for later analysis by gas-liquid chromatography as described by LeBlanc (1973). The individual fatty acids were identified by plots of the log of the retention time against carbon number, with individual lines for saturated, monoenoic, dienoic and trienoic fatty acids.

Protein content of the diet composites was determined using the Kjeldahl nitrogen determination procedure with the boric acid modification (AOAC 1960).[2] Total calories were determined by use of a Parr Adiabatic Calorimeter Model #U30M equipped with a Parr #1241 oxygen bomb calorimeter and a Parr #1541 waterheater.[3]

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[1]Canlab Laboratory Equipment, Winnipeg, Manitoba.

[2]Official Methods of Analysis of the Association of Official Agricultural Chemists, 1960.

[3]Parr Instrument Co., 211 Fifty-third Street, Moline, Illinois 61625.

F. BLOOD COLLECTION PROCEDURES

All blood samples were drawn from the antecubital vein. Fasting blood samples were drawn on days 2, 9, 17, 25 and 32. Subjects were instructed to have nothing to eat or drink except water after midnight prior to taking the blood samples. Three samples of blood were drawn from each subject on each collection day. One seven ml sample and one three ml sample taken in B.D. Vacutainer tubes treated with EDTA 18 (No 4759 and No 4854, respectively)[1] were sent to the Health Sciences Centre Hematology Laboratory, Winnipeg, Manitoba, for whole blood hematology. The blood samples were refrigerated prior to being transported to the laboratory on the same day they were obtained. One 15 ml blood sample taken in a B.D. Vacutainer tube (No 4796)[2] was obtained for serum lipid analysis. The serum was separated and stored as described by King (1974).

G. CHEMICAL ANALYSIS OF BLOOD

G.1. Whole Blood

Whole blood samples were analyzed for leucocytes, total red blood cell count, platelets, reticulocytes, hematocrit and hemoglobin by the Health Sciences Centre Hematology Laboratory.

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[1]Canlab Laboratory Equipment, Winnipeg.

[2]Canlab Laboratory Equipment, Winnipeg.

G.2. Serum

Serum samples were analyzed for cholesterol, lipid phosphorus, and phospholipid fatty acid patterns.

G.2.a. Total Cholesterol. Total serum cholesterol was determined by the method of Pearson et al. (1953). Serum was treated with glacial acetic acid, paratoluene sulfonic acid and acetic anyhydride, and the resulting color complex was compared to that of a cholesterol standard.

G.2.b. Lipid Phosphorus. Extraction of the lipid from the serum was carried out as described by Fiske and Subbarow (1925) with two modifications. Shaking of the solvent-serum mixture was found to be critical. Best results were obtained by covering each test tube with a piece of pliofilm and shaking the hot contents gently for 15 seconds, immediately following removal of the test tube from the water-bath. Caution in shaking was necessary to keep the hot solvent from volatilizing and spurting from the test-tube. After centrifugation for ten minutes at 12,500 G., the extract was pipetted into a second set of centrifuge tubes and recentrifuged at 12,500 G. for 12 minutes to ensure that no fragments of the extremely flocculent precipitate remained in the extract.

Ashing and colorimetric determinations were carried out as described by Chen et al. (1956). The color complex

was developed using a mixture of sulfuric acid, ammonium molybdenate and ascorbic acid. Spectrophotometric readings for serum samples were compared to those of a phosphorus standard prepared with reagent grade KH_2PO_4.

G.2.c. Phospholipid Fatty Acid Pattern. Phospholipid fatty acid patterns were determined as described by LeBlanc (1973).

H. STATISTICAL ANALYSIS

All data were subjected to analysis of variance of a two-way classification for a completely randomized block design according to Snedecor and Cochran (1957) with modification made to include a comparison of the effects of rapeseed oil versus soybean oil. The sum of squares attributed to days was partitioned in order to make appropriate orthogonal comparisons to determine significant differences between time periods.

RESULTS AND DISCUSSION

A. SUBJECTS

All eight subjects remained in good health for the duration of the 31 day study. Body weight was essentially constant for each subject during the entire experiment (Table 4). To accomplish this, it was necessary to increase the caloric intake of some of the subjects; LB received extra sugar and bread, WJ additional bread, and HJ bread and margarine. All changes in serum lipid patterns were attributed to dietary fat sources, and not to illness or changes in energy metabolism.

The subjects reported no digestive upsets even though they consumed 106 to 109 grams of liquid oil daily during the experimental periods. This contrasts to the report of Trémolières et al (1971) that a single oral dose of high erucic acid rapeseed oil (0.5 ml./kg.b.w.) following an overnight fast produced diarrhoea in 5 of 8 experimental subjects. The absence of digestive problems coincides with previous results from our laboratory (LeBlanc, 1973; King, 1974; Lake, 1975). Oil fed as an ingredient of a solid test diet may affect the gastrointestinal tract differently from liquid oil fed alone as was the case in the study reported by Trémolières et al (1971).

LeBlanc (1973) and King (1974) had reported flatulence in subjects consuming a test diet containing 28.5 to 37.5 grams soy protein per day. Flatulence was not a problem in the present study or in the study reported by Lake (1975), probably because soybean protein was restricted to a level of 11.1 to 12.3 grams daily.

The subjects maintained their normal activity patterns throughout the study. In addition, they participated in fitness testing (ergometer) at the end of each dietary period, as reported by Parker (1975). Since there were no major variations in exercise patterns from period to period during the study, all serum lipid changes were attributed to dietary fat source.

Subject NM objected to having fasting blood samples taken, claiming they made him dizzy. No evidence of dizziness was observed, and he routinely underwent the withdrawal of three 30-ml samples of blood during the 30 minutes of exercise on days 7, 15, 23 and 31 with no apparent difficulties. However, permission was granted him to omit the fasting sample on day 32. The missing values for subject NM were estimated using the formula of Snedecor and Cochran (1963).

B. DIETS

The two test menus were well accepted by all

subjects. Occasionally an egg nog was made from the raw egg albumin and part of the daily sugar and skim milk allotment, to provide variety in the diet.

B.1. Caloric Composition

The two menus were designed to be isocaloric, based on information from USDA Handbook No. 8 Composition of Foods (Watt and Merrill, 1963). Analyses of a composite of each daily menu incorporating each fat source (two menus, three fat sources) were very similar in total caloric content (Table 11). Total calories as determined by bomb calorimetry generally were higher than the calculated values; as would be expected, since bomb calorimetry measures gross energy whereas the values given in Handbook No. 8 are Atwater physiological fuel values (i.e. utilizible energy).

B.2. Protein Content

Protein contents as determined by the Kjeldahl method were essentially the same in all six composites and were remarkably similar to the calculated values based on Handbook No. 8 (Table 12).

B.3. Fat Content

Total fat, analysed by the Bligh and Dyer method, was similar for all six composites. However, recovered fat was appreciably lower than the calculated values based on the quantity of fat known to be added to the diet (Table 13).

Table 11

Daily Energy Intakes[1]

DIET	CALCULATED[2]	ANALYSED[3]	PERCENT DIFFERENCE[4]
Mixed Fat			
Menu 1	3004	2959	1.5
Menu 2	3000	3011	0.3
Soybean Oil			
Menu 1	3004	3171	5.5
Menu 2	3000	3081	2.7
Low Erucic Acid Rapeseed Oil			
Menu 1	3004	3146	4.7
Menu 2	3000	3033	1.1

[1]Mean of duplicate analyses.

[2]Calculations based on Watt and Merrill, U.S.D.A. Handbook No. 8 Composition of Foods, Agriculture Research Service, Washington, D.C. 1963.

[3]Results obtained by bomb calorimetry.

[4]Percent difference between analysed and calculated values.

Table 12

Daily Protein Intakes[1]

DIET	CALCULATED[2] (g)	ANALYSED[3] (g)	PERCENT DIFFERENCE[4]
Mixed Fat			
Menu 1	85.2	85.3	0.1
Menu 2	84.9	84.6	0.3
Soybean Oil			
Menu 1	85.2	86.7	1.7
Menu 2	84.9	86.3	1.7
Low Erucic Acid Rapeseed Oil			
Menu 1	85.2	85.2	
Menu 2	84.9	83.1	2.0

[1]Means of duplicate analyses.

[2]Calculations based on Watt and Merrill, U.S.D.A. Handbook No. 8 Composition of Foods, Agriculture Research Service, Washington, D.C. 1963.

[3]Results obtained by the Kjeldahl method.

[4]Percent difference between analysed and calculated values.

Table 13

Daily Fat Intakes[1]

DIET	CALCULATED[2] (g)	ANALYZED[3] (g)	PERCENT DIFFERENCE[4]
Mixed Fat			
Menu 1	133.9	112.5	15.9
Menu 2	133.2	106.2	20.2
Soybean Oil			
Menu 1	133.9	127.5	4.7
Menu 2	133.2	116.5	12.5
Low Erucic Acid Rapeseed Oil			
Menu 1	133.9	121.6	9.1
Menu 2	133.2	116.7	12.3

[1]Results of duplicate analyses.

[2]Calculations based on Watt and Merrill, U.S.D.A. Handbook No. 8 Composition of Foods, Agriculture Research Service, Washington, D.C. 1963.

[3]Results obtained by the Bligh and Dyer method.

[4]Percent difference between analysed and calculated values.

This discrepancy may have been due in part to the tendency of the added fat to adhere to the corrugated walls of the aluminum containers used for freezing and serving the entrees.

B.4. Fatty Acid Composition

Fatty acid patterns were essentially similar for both menus for each of the three test fats; an expected finding, since added test fat accounts for 92 and 94 percent of the total fat in menus 1 and 2, respectively. Thus the results for menus 1 and 2 were averaged for each test fat prior to tabulation (Table 14).

The composites for LoRSO menus 1 and 2 were inadvertently made up using high erucic acid rapeseed oil margarine from a previous study (King, 1974) instead of Tower rapeseed oil margarine. Since the error was discovered too late to assemble correct replacement composites, it was necessary to estimate the fatty acid composition of the LoRSO diet through calculations based on available chromatographic data for the fatty acid composition of the incorrect composites, the high erucic acid margarine, and the Tower margarine (Appendix Table 32).

Of the three diets, the diet containing mixed fat had the highest percentage (37%) of saturated fatty acids; the diet containing soybean oil had the highest percentage (39%) of polyunsaturated fatty acids, and the diet containing rpaeseed oil had the highest percentage (62%) of monounsaturated fatty acids (Figure 4).

Table 14

Fatty Acid Composition of Mixed Fat, Soybean Oil, and Low Erucic Acid Rapeseed Oil Diets

FATTY ACID	% of Total Fatty Acids		
	Mixed Fat Diet[1]	Soybean Oil Diet[1]	Low Erucic Acid Rapeseed Oil Diet[2]
Lauric C12:0[3]	0.71	0.20	0.60
Myristic C14:0	1.68	0.58	0.39
Palmitic C16:0	21.54	10.95	6.01
Stearic C18:0	12.93	4.54	3.81
Oleic C18:1	40.27	43.91	58.98
Linoleic C18:2	20.74	34.82	19.20
Linolenic C18:3[4]	1.54	3.82	7.65
Eicosenoic C20:1	0.32	0.10	2.34
Behenic C22:0	--	--	0.49
Erucic C22:1	--	--	0.46
Saturated			
Total	36.86	16.27	11.30
C12:0 to C16:0	23.93	11.73	7.00
Monounsaturated	40.91	43.93	61.83
Polyunsaturated	22.28	38.64	26.85

[1]As determined by gas chromatography (mean of menus 1 and 2, several duplicate observations per menu).

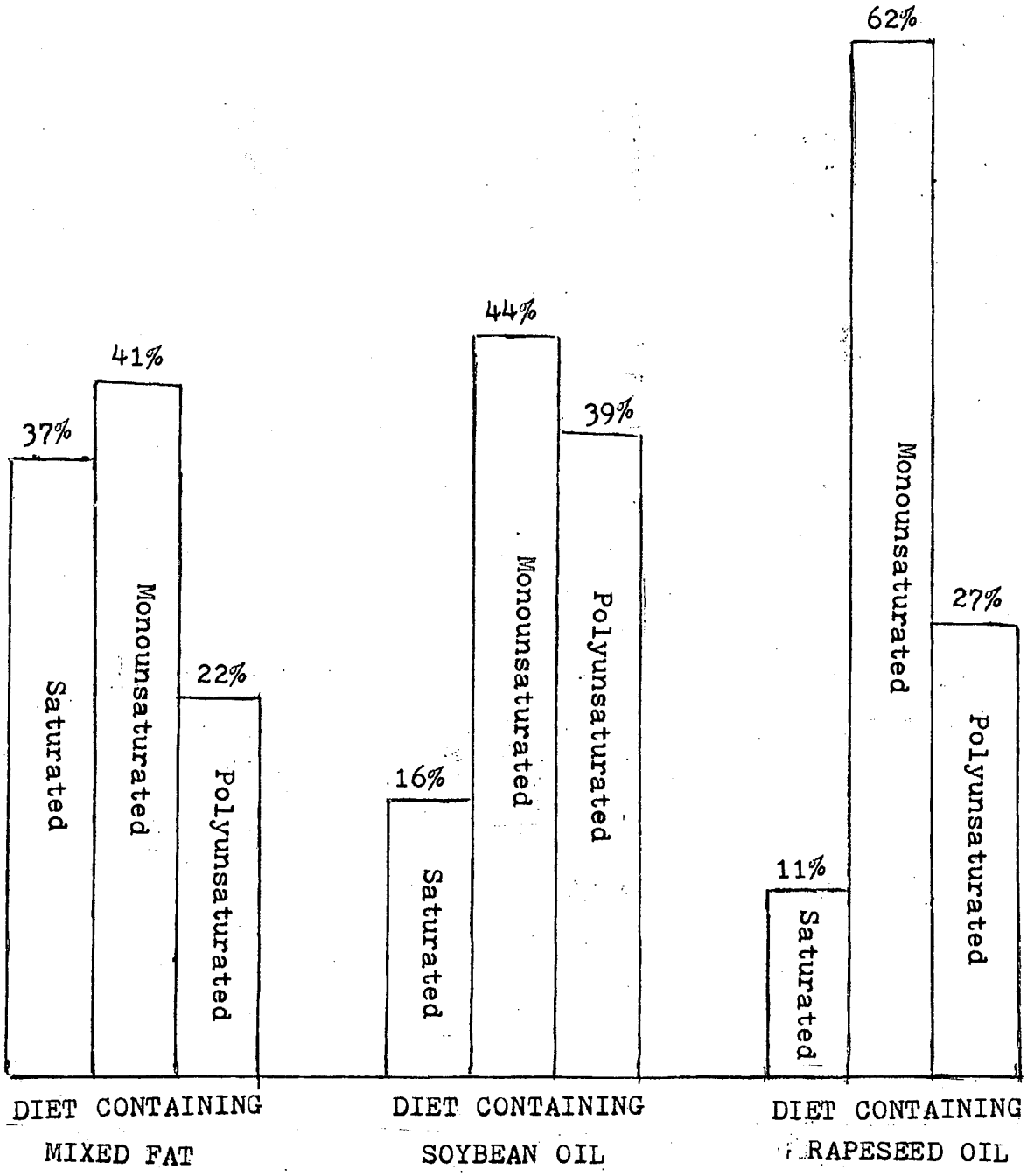
[2]As estimated by calculation from values determined by gas chromatography (See Appendix Table 32).

[3]Carbon number:number of double bonds.

[4]Not distinguished from C20:0 with the column used to resolve fatty acids.

Figure 4

Proportion of Saturated: Monunsaturated: Polyunsaturated Fatty Acids for Various Fat Sources



C. SERUM LIPIDS

C.1. Effect of Diet on Serum Cholesterol

Individual and mean serum cholesterol levels (initial levels and levels at the end of each dietary period) in the present study are reported in Table 15 and Figure 5.

Mean serum cholesterol levels decreased 13 mg./100 ml. of serum during the first eight days of the experiment. Orthogonal comparison of the means of observations during the experiment (days 9, 17, 25 and 32) with initial levels (day 2) indicated that serum cholesterol levels were significantly ($P < 0.05$) lower during the experiment than at the beginning of the study (Appendix Tables 19 and 20). Lower serum cholesterol levels after the first few days of the experiment have been observed in several previous studies from this laboratory (Initial vs. After Pre-Experimental Mixed Fat - Table 16). The most likely explanation for the drop in serum cholesterol in response to the test diet is the lower dietary cholesterol intake when the test diet replaced the customary diet of the subjects. The mixed fat diet supplied approximately 145 mg. of cholesterol per day, which was nearly 400 mg. per day less than the estimated daily per capita intake in Canada (Table 17). Mattson *et al.* (1972) found there is a linear relationship

Table 15

Serum Cholesterol Levels[1] (mg./100 ml. serum) During the Experiment

SUBJECTS	INITIAL	DIETARY TREATMENT			
		MIXED FAT		SOYBEAN OIL[4]	LOW ERUCIC ACID RAPE-SEED OIL[4]
		I[2]	II[3]		
GROUP A[5]					
WJ	195	155	157	144	142
HJ	177	162	159	145	155
LB	211	199	179	150	150
LeB	123	118	117	108	99
GROUP B[5]					
NM	200	198	181[6]	157	151
SJ	176	162	156	149	135
MD	177	160	173	149	139
GS	147	149	146	138	118
GROUP A MEAN	177	159	153	137	137
GROUP B MEAN	175	167	164	148	136
OVERALL MEAN	176	163	159	143	142

[1]As determined by the method of Pearson et al (1953).

[2]Pre-experimental period (samples drawn after eight days).

[3]Post-experimental period (samples drawn after seven days).

[4]Samples drawn after eight days.

[5]During the experimental period, Group A was fed soybean oil for the first eight days and rapeseed oil during the next eight days, and Group B received rapeseed oil first and soybean oil second.

[6]Calculated value according to Snedecor, G.W. and Cochran, W.G. 1967. Statistical Methods. 6th Ed. Iowa State University Press, Ames, Iowa. P. 317.

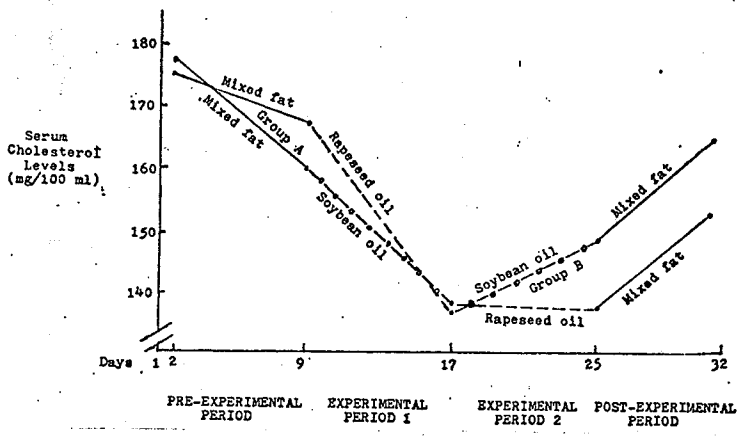


Figure 5

Mean Serum Cholesterol Levels During the Experiment

Table 16

Comparison of Serum Cholesterol Levels in Normal Young Men Fed Diets Containing High or Low Erucic Acid Rapeseed Oil

	TYPE OF TEST FAT[1]					
	High Erucic Acid Rapeseed Oil	Low Erucic Acid Rapeseed Oil	Low Erucic Acid Rapeseed Oil and Soybean Oil[2]			
	King (1974)	LeBlanc (1973)	Lake (1975) A	Lake (1975) B	Masniuk (1976) A	Masniuk (1976) B
NUMBER OF SUBJECTS	7	7	2	2	4	4
CHOLESTEROL LEVELS (mg./100 ml. of serum)						
Initial	200	203	222	207	177	175
After pre-experimental mixed fat[3]	187	174	194	171	159	167
After 8 days test fat	167	159	184[4]	148[4]	137	136
After 15 days test fat	165	151	--	--	137[5]	148[5]
After 22 days test fat	177	144	163	149	--	--
After 8 days post-experimental mixed fat	195	183	--	--	153	164

[1]Test fats provided 122 to 132 grams of fat daily, of which 20 to 36 grams was hydrogenated to make margarine.

[2]The two test fats were fed in a crossover design during experimental periods 1 and 2. Group A subjects were fed soybean oil first. Group B subjects were fed rapeseed oil first. Subjects were fed each test fats for eleven days in the Lake study and eight days in the Masniuk study.

[3]The pre-experimental mixed fat period lasted nine days in the King and LeBlanc studies, ten days in the Lake study, and eight days in the Masniuk study.

[4]Samples taken after ten days instead of eight days on the test fat.

[5]Samples taken after 16 days instead of 15 days on the test fat.

Table 17

Comparison of Test Diet with Average Canadian Diet

	Average[1] Canadian Diet	TEST DIET	
		Using Mixed Fat	Using LaRSO or Soybean Oil
Protein per day	95 grams	85 grams	85 grams
Animal protein			
Meat	35%	0%	0%
Milk products	28%	36%	36%
Egg	5%	13%[2]	13%[2]
Vegetable protein	32%	51%	51%
Cholesterol per day	540 mg.	145 mg.	25 mg.

[1]Based on consumption and disappearance statistics (Appendix Table 33).

[2]Egg white only.

between serum cholesterol and dietary cholesterol intake over the range 0 to 317 mg. of cholesterol/1000 kcal of energy, and that this relationship can be expressed by the following equation:

$$\Delta \text{serum chol}_{\text{mg/100ml}} = 1.60 + 0.118\ (\Delta \text{dietary chol}_{\text{mg/1000 kcal}})$$

Assuming that 540 mg. per day is a valid estimate of the cholesterol content of the average diet providing 3000 kcal, the Mattson equation would predict a decrease of 17 mg./100 ml. in serum cholesterol when the subjects were fed the mixed fat diet providing only 145 mg. of cholesterol daily. This predicted increase is similar to the 13 mg./100 ml. decrease observed after the first eight days (Table 18). However, factors other than dietary cholesterol also must be taken into consideration. The mixed fat diets used during the stabilization period may not have reflected the true fatty acid pattern of the fat in the subjects' customary diet. In addition, fat may have provided more than 40% of calories in the subjects' customary diets. The entire protocol of the experiment — including frequency of meals, a fixed routine, a standardized daily caloric intake, and abstinence from alcohol — also may have had an effect on cholesterol levels. The test diet contained slightly less protein than would be predicted for a diet based on Canadian per capita disappearance statistics

Table 18

Comparison Between Predicted and Observed Changes in Serum Cholesterol for Various Changes in Fat Source

CHANGE IN FAT SOURCE		SERUM CHOLESTEROL LEVELS(mg)	
From	To	Observed	Expected[1]
Subject's usual diet	Mixed fat[2]	-13	--
Mixed fat	Soybean oil[3]	-22	-20
Mixed fat	Rapeseed oil[4]	-31	-18
Soybean Oil	Rapeseed oil[3]	0	+1
Rapeseed oil	Soybean oil[4]	+12	-1
Rapeseed oil	Mixed fat[3]	+16	+18
Soybean oil	Mixed fat[4]	+16	+20

[1]Based on Keys et al., 1965 equation $\Delta C = 1.2 (2\Delta S' - \Delta P)$
where ΔC = change in serum cholesterol in mg/100 ml.
$\Delta S'$ = change in total C12 to C16 fatty acids expressed as a percent of total dietary calories.
ΔP = change in total polyunsaturated fatty acids expressed as a percent of total dietary calories.

[2]All eight subjects.

[3]Group A subjects only (four subjects).

[4]Group B subjects only (four subjects).

(Table 17) and a greater proportion of the protein in the test diet was of vegetable origin than in the average Canadian diet. Carroll and Hamilton (1975) found that the feeding of diets based on vegetable proteins resulted in lower serum cholesterol levels in rabbits than the feeding of diets based on animal proteins. In contrast, Cobden (1975) found no difference in the serum cholesterol levels of human subjects when lean beef was substituted for soy protein in a test diet similar to the one used in the present study.

Substitution of vegetable oil, either low erucic acid rapeseed oil (LoRSO) or soybean oil (SBO), for mixed fat resulted in significantly ($P<0.05$) lower serum cholesterol levels after only eight days (Tables 15 and 18). It is important to note that substitution of vegetable oils for the mixed fat changed two dietary parameters known to affect serum cholesterol levels; fatty acid composition and cholesterol intake. Not only were the diets containing vegetable oil higher in unsaturated fatty acids than the diet containing mixed fat, they also contained 120 mg./day less cholesterol. On the basis of the observations by Mattson et al. (1972), one would expect the lower cholesterol intake to result in a decrease in serum cholesterol of 6 mg./100 ml. when vegetable oils were substituted for mixed fat. Similarly, an increase of 6 mg./100 ml. would

be expected when mixed fat was re-introduced. It is perhaps worth noting, however, that researchers at the University of Minnesota, (Keys et al., 1965; Keys et al., 1968; Grande et al., 1968; Grande et al., 1972) have suggested that dietary cholesterol intake is of secondary importance to the fatty acid composition of dietary fat in determining serum cholesterol levels in the human. Keys et al.(1965) proposed an equation (Table 18, Footnote 1) which described the relationship they found between dietary fatty acid composition and observed serum cholesterol levels. This equation predicted a decrease in serum cholesterol of 18 mg./100 ml. when rapeseed oil was substituted for mixed fat, and a decrease of 20 mg./100 ml. when soybean oil was substituted for mixed fat.

No differences ($P>0.05$) were found in the effects of LoRSO and SBO on serum cholesterol in the present study. Although the polyunsaturated fatty acid content of LoRSO is much lower than that of SBO, the LoRSO diet provided a much lower content of saturated fatty acids than the SBO diet. Neither oil contained cholesterol. Thus the expected effects of these dietary fats on serum cholesterol are similar, based on either the Keys or the Mattson equation.

Other factors related to dietary fat source may affect serum cholesterol levels. Hegsted et al. (1965) have suggested that not only the percentage of dietary

calories contributed by each fatty acid but also the ratios of the various fatty acids may influence serum cholesterol levels. In addition, McGandy et al. (1970), working with semisynthetic fat mixtures, found that interesterified tallow was hypercholesteremic; whereas unaltered tallow, rich in stearic acid, did not elevate serum cholesterol. McGandy et al. (1970) attributed this difference between interesterified and unaltered tallow to the spatial arrangement of the stearic acid on the triglycerides.

In a study similar to the present study, Lake (1975) compared the effects of LoRSO and SBO on serum cholesterol. Although the changes in cholesterol levels for the four groups of subjects fed SBO and LoRSO in the Lake study and the present study were not significantly different statistically ($P > 0.05$), mean serum cholesterol levels for the twelve subjects in the two studies were nearly 8 mg./100 ml. lower when LoRSO was fed than when SBO was fed. Only two subjects had lower serum cholesterol levels when SBO was fed, whereas eight had lower serum cholesterol levels when LoRSO was fed. This difference perhaps may be explained on the basis of differences in triglyceride composition and individual fatty acid ratios.

Three previous studies involving rapeseed oil have been carried out in the Department of Foods and Nutrition at the University of Manitoba. The salient features of

Table 19

Experimental Design of Four Studies on the Response of Young Adult Males to Dietary Rapeseed Oil

	STUDY			
	King (1974)	LeBlanc (1973)	Lake (1975)	Masniuk (1976)
TYPICAL DAILY CALORIC INTAKE	3000	3000	3000	3000
PERCENT OF TOTAL CALORIES FROM FAT	38%	38%	38%	40%
CONTROL FAT[1]	Mixed fat	Mixed fat	Mixed fat	Mixed fat
TEST OILS	HiRSO[1]	LoRSO[3]	LoRSO/SBO[3]	LoRSO/SBO[4]
RAPESEED OIL USED				
Erucic acid content	38 %	2.6%	0.4 %	0.79%
Cultivar		Span	Zephyr	Tower
NUMBER OF SUBJECTS	7	7	2+2=4[5]	4+4=8[5]
DURATION OF STUDY (days)	39	39	32	31
DURATION OF EACH PERIOD (days)				
Pre-ex perimental (mixed fat)	9	9	10	8
Experimental (test oils)	22	22	11=11=22[5]	8=8=16[5]
Post experimental (mixed fat)	8	8	--	7
NUMBER OF FASTING BLOOD SAMPLES TAKEN PER SUBJECT	6	6	4	5
SERUM LIPID PARAMETERS				
Serum Cholesterol	X	X	X	X
Serum Lipid phosphorus	X	X	X	X
Serum Phospholipid Fatty Acid Patterns	X	X	--	X
Serum Triglycerides	X	X	X	--
Serum Lipoproteins	--	X	--	--
WHOLE BLOOD HEMATOLOGY				
Red Blood Cell Counts	X	X	X	X
Red Blood Cell Fragility	X	X	--	--
Reticulocyte Counts	X	X	X	X
Hemoglobin Levels	X	X	X	X
Hematocrit Levels	X	X	X	X
Platelet Counts	X	X	X	X
Leucocyte Counts	X	X	X	X

[1]Fat mixtures used in the mixed fat control diet were similar but not identical for the various studies.

[2]HiRSO - high erucic acid rapeseed oil.

[3]LoRSO - low erucic acid rapeseed oil.

[4]LoRSO/SBO - low erucic acid rapeseed oil or soybean oil (crossover design).

[5]The two test oils were fed in a crossover design during the experimental period. Half the subjects were fed soybean oil first; the other half were fed soybean oil first. Subjects were fed each test oil for eleven days in the Lake study and eight days in the Masniuk study.

each study are summarized in Table 19.

The responses of serum cholesterol observed in the present study are compatible with previous observations from this laboratory (Table 16). Mean serum cholesterol levels decreased ($P < 0.05$) in response to the introduction of the test diet in all four studies. Mean serum cholesterol levels also decreased in response to the feeding either of LoRSO (LeBlanc, 1973) or of LoRSO and SBO in a crossover design (Lake, 1953; Masniuk, 1976). In contrast, when high erucic acid rapeseed oil was fed as the sole source of added dietary fat, mean serum cholesterol levels decreased during the first week, plateaued during the second week and increased during the third week, resulting in a slight but non-significant ($P > 0.05$) overall decrease during the 22-day period.

C.2. Effect of Diet on Serum Lipid Phosphorus

Individual and mean serum lipid phosphorus levels (initial levels and levels at the end of each dietary period) in the present study are shown in Table 20 and Figure 6.

Serum lipid phosphorus levels responded to dietary changes similarly to serum cholesterol levels, but the magnitude of change tended to be larger. Lipid phosphorus levels dropped ($P < 0.05$) during the initial eight days of

Table 20

Serum Lipid Phosphorus Levels[1] (mg./100 ml. serum) During the Experiment

SUBJECTS	INITIAL	DIETARY TREATMENT			
		MIXED FAT		SOYBEAN OIL[4]	LOW ERUCIC ACID RAPE-SEED OIL[4]
		I[2]	II[3]		
GROUP A[5]					
WJ	7.0	5.8	5.4	5.5	5.5
HJ	7.9	6.8	5.4	6.7	6.4
LB	7.3	6.7	5.7	4.7	4.5
LeB	5.2	4.9	4.4	4.5	5.1
GROUP B[5]					
NM	8.4	7.2	6.8[6]	6.0	6.7
SJ	6.4	5.5	5.3	4.9	4.7
MD	5.6	5.1	5.3	4.6	4.7
GS	5.8	5.8	5.9	5.3	5.1
GROUP A MEAN	6.9	6.1	5.2	5.4	5.4
GROUP B MEAN	6.6	5.9	5.8	5.2	5.3
OVERALL MEAN	6.7	6.0	5.5	5.3	5.3

[1]As determined by the method of Chen et al (1956). ciences Centre, Winnipeg, Manitoba.

[2]Pre-experimental period (samples drawn after eight days).

[3]Post-experimental period (samples drawn after seven days).

[4]Samples drawn after eight days.

[5]During the experimental period, Group A was fed soybean oil for the first eight days and rapeseed oil during the next eight days, and Group B received rapeseed oil first and soybean oil second.

[6]Calculated value according to Snedecor, G.W. and Cochran, W.G. 1967. Statistical Methods. 6th Ed. Iowa State University Press, Ames, Iowa. P. 317.

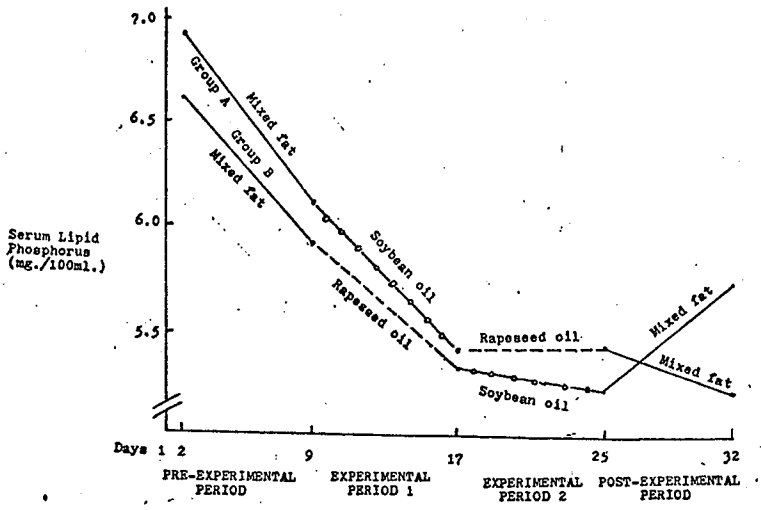


Figure 6

Mean Serum Lipid Phosphorus Levels During the Experiment

Table 21

Comparison of Serum Lipid Phosphorus Levels in Normal Young Men Fed Diets Containing High or Low Erucic Acid Rapeseed Oil

	TYPE OF TEST FAT[1]					
	High Erucic Acid Rapeseed Oil	Low Erucic Acid Rapeseed Oil	Low Erucic Acid Rapeseed Oil and Soybean Oil[2]			
	King (1974)	LeBlanc (1973)	Lake (1975)		Masniuk (1976)	
			A	B	A	B
NUMBER OF SUBJECTS	7	7	2	2	4	4
SERUM LIPID PHOSPHORUS (mg./100 ml. of serum)						
Initial	9.9	11.7	6.8	7.2	6.9	6.6
After pre-experimental mixed fat[3]	7.8	10.1	6.1	6.3	6.1	5.9
After 8 days test fat	7.4	9.2	5.3[4]	5.3[4]	5.4	5.3
After 15 days test fat	6.6	7.9	--	--	5.4[5]	5.2[5]
After 22 days test fat	7.1	6.7	5.2	4.7	--	--
After 8 days post-experimental mixed fat	8.9	10.5	--	--	5.2	5.8

[1]Test fats provided 122 to 132 grams of fat daily, of which 20 to 36 grams was hydrogenated to make margarine.

[2]The two test fats were fed in a crossover design during experimental periods 1 and 2. Group A subjects were fed soybean oil first. Group B subjects were fed rapeseed oil first. Subjects were fed each test fats for eleven days in the Lake study and eight days in the Masniuk study.

[3]The pre-experimental mixed fat period lasted nine days in the King and LeBlanc studies, ten days in the Lake study, and eight days in the Masniuk study.

[4]Samples taken after ten days instead of eight days on the test fat.

[5]Samples taken after 16 days instead of 15 days on the test fat.

the study (Appendix Tables 19 and 20), indicating that some factor in the experimental regimen affected serum lipid levels. As in the Lake study (1975), the introduction of vegetable oil as the fat source resulted in a further decrease ($P < 0.05$) in serum lipid phosphorus, but there was no difference ($P > 0.05$) in serum lipid phosphorus levels between the two groups fed the two oils.

The finding in the present study that serum lipid phosphorus responds to changes in diet composition in a manner similar to the response of serum cholesterol is compatible with the findings of earlier studies (McGandy et al., 1970; LeBlanc, 1973; King, 1974; Lake, 1975). LeBlanc, King and Lake (Table 21) all found that the response of serum lipid phosphorus to diet manipulation was greater in magnitude and somewhat slower than the response of serum cholesterol.

C.3. Effect of Diet on Serum Phospholipid Fatty Acid Patterns

The serum phospholipid fatty acid patterns (Table 22) responded, to some degree, to changes in the fatty acid composition of the dietary fat (Table 14). Higher levels of oleic acid in the diet resulted in slightly higher levels of oleic acid in the phospholipids. Similarly, lower levels of palmitic and stearic acids in the diet resulted in lower contents of these acids in the

Table 22

Percent Fatty Acid Composition of Serum Phospholipids

		DIETARY FAT SOURCE			
	INITIAL	MIXED FAT		SOYBEAN	RAPESEED
		I[1]	II[2]	OIL	OIL
Palmitic C16:0[5]	30.6	32.6	31.5	30.7	30.7
Stearic C18:0	16.2	16.6	17.3	15.1	15.5
Oleic C18:1	17.4	17.3	16.3	18.2	19.5
Linoleic C18:2	23.1	22.4	24.6	25.7	23.5
Eicosetrienoic C20:3	5.5	5.1	9.1	1.2	1.4
Arachidonic C20:4	5.5	5.3	4.9	5.0	4.7

[1]Days 1 to 8 inclusive.

[2]Days 25 to 31 inclusive.

[3]Four subjects were fed soybean oil on days 9 to 16 and the other four subjects were fed this oil on days 17 to 24.

[4]Four subjects were fed rapeseed oil on days 9 to 16 and the other four subjects were fed this oil on days 17 to 24.

[5]Carbon number: Number of double bonds.

phospholipids.

These changes coincided with those observed by LeBlanc (1973) and King (1974), who replaced diets containing mixed fat by diets containing low erucic acid and high erucic acid rapeseed oil (LoRSO and HiRSO) respectively.

D. WHOLE BLOOD HEMATOLOGY

There are several advantages to measurement of hematological parameters. Blood is a fast, easy and relatively painless tissue specimen to sample, compared to fat biopsy and organ biopsy. Because blood analyses are standard laboratory procedures, they are relatively cheap and accurate, using automated and centralized facilities. Because there are well-established normal ranges for these parameters, hematological measurements may be used to assess the nutritional adequacy of the diet in respect to non-lipid factors and to monitor the presence of infections as signalled by leucocyte count.

Most reports on the effect of nutrition on whole blood hematology have focussed on the effect of vitamins (e.g. folic acid, B_{12}) and minerals (e.g. copper, iron) on these parameters. Little work has been reported on the effects of lipids on whole blood hematology, although there is some evidence that rapeseed oil, especially high erucic acid rapeseed oil, may affect blood cells.

In evaluating the results of hematological analyses in studies of this type, it is necessary to distinguish very carefully between the various effects that may be observed. It is important to recognize that the test diet differs from the subjects' customary diet in many non-lipid ways. Especially important are factors such as the type of iron (all the iron in the test diet is nonheme iron), the level and type of protein, and the level of micronutrients (vitamins and minerals) all of which have been found to affect whole blood hematology. In an examination of the effects of lipids on whole blood hematology it is also necessary to identify those effects which are due to vegetable oils (as contrasted to mixed fat sources), effects due to rapeseed oil in general (as contrasted to other vegetable oils), and effects due to the erucic acid content of rapeseed oil. One must be careful to distinguish between effects that are statistically significant ($P < 0.05$) but remain within the normal range for the human and are therefore probably not significant from a functional or physiological point of view, and changes which fall outside the normal range.

D.1. Effect of Diet on the Red Blood Cell Fraction

Red blood cells are the most common type of blood cell (over 95% of all blood cells). In addition, they

contain the vital oxygen - transporting compound hemoglobin. Red blood cell counts, hemoglobin levels, hematocrit levels and reticulocyte or immature red blood cell counts are often considered simultaneously in diagnosing functional abnormalities such as anemia. Abnormalities in the red blood cell fraction have been reported in animals fed diets containing high levels of high erucic acid rapeseed oil (HiRSO) compared to controls fed soybean oil or hardened palm oil. Abdellatif et al. (1972) found that the hematocrits of ducklings fed HiRSO were elevated, while hemoglobin levels remained normal. In contrast, Vles and Abdellatif (1970a) observed lower hemoglobin levels in guinea pigs fed HiRSO, while reticulocyte counts and red blood cell fragility were higher in these animals than in control guinea pigs.

D.1.a. Red Blood Cell Counts. Individual and mean red blood cell counts (initial levels and levels at the end of each dietary period) in the present study are reported in Table 33 and Figure 7.

No significant changes ($P > 0.05$) in red blood cell counts were observed in the present study (Appendix Table 23). These results coincide with those reported by Lake (1975) in a study similar to the present study (Table 24). In the King (1974) study, red blood cell counts decreased during the first week that HiRSO was fed, increased during the second week and decreased during the third week,

Table 23

Red Blood Cell Counts[1] (millions/cu. mm.) During the Experiment

SUBJECTS	INITIAL	DIETARY TREATMENT			
		MIXED FAT		SOYBEAN OIL[4]	LOW ERUCIC ACID RAPESEED OIL[4]
		I[2]	II[3]		
GROUP A[5]					
WJ	5.3	5.0	5.3	5.2	5.2
HJ	4.7	4.6	4.3	4.6	4.8
LB	4.9	4.9	5.0	4.7	4.8
LeB	5.4	5.2	5.1	5.2	5.1
GROUP B[5]					
NM	4.9	4.9	4.8[6]	4.8	4.9
SJ	5.1	5.1	5.1	5.2	4.8
MD	5.2	5.2	5.3	5.2	5.2
GS	4.9	4.5	4.6	4.8	5.3
GROUP A MEAN	5.1	4.9	4.9	4.9	4.9
GROUP B MEAN	5.0	4.9	5.0	5.0	5.1
OVERALL MEAN	5.1	4.9	4.9	5.0	5.0

NORMAL RANGE: 4.5 - 6.5 million/cu. mm. (Davie and Lewis, 1963)

[1] As determined by Hematological Laboratories, Health Sciences Centre, Winnipeg, Manitoba.

[2] Pre-experimental period (samples drawn after eight days).

[3] Post-experimental period (samples drawn after seven days).

[4] Samples drawn after eight days.

[5] During the experimental period, Group A was fed soybean oil for the first eight days and rapeseed oil during the next eight days, and Group B received rapeseed oil first and soybean oil second.

[6] Calculated value according to Snedecor, G.W. and Cochran, W.G. 1967. Statistical Methods. 6th Ed. Iowa State University Press, Ames, Iowa. P. 317.

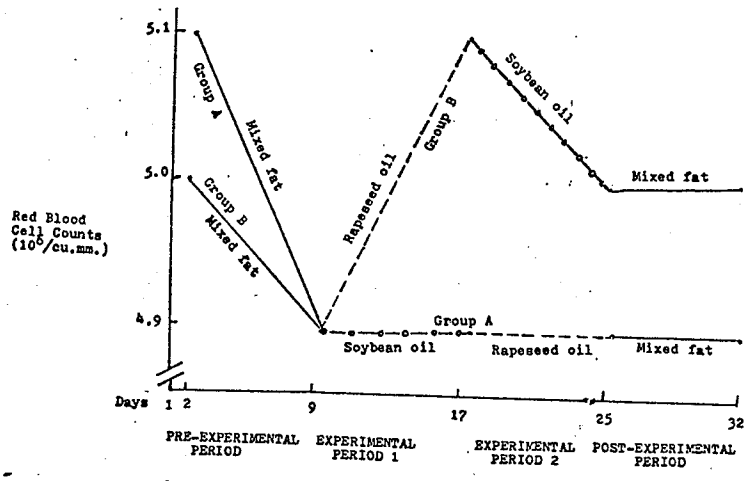


Figure 7

Mean Red Blood Cell Counts During the Experiment

Table 24

Comparison of Red Blood Cell Counts in Normal Young Men Fed Diets Containing High or Low Erucic Acid Rapeseed Oil

	TYPE OF TEST FAT[1]					
	High Erucic Acid Rapeseed Oil	Low Erucic Acid Rapeseed Oil	Low Erucic Acid Rapeseed Oil and Soybean Oil[2]			
	King (1974)	LeBlanc (1973)	Lake (1975) A	Lake (1975) B	Masniuk (1976) A	Masniuk (1976) B
NUMBER OF SUBJECTS	7	7	2	2	4	4
RED BLOOD CELL COUNT (millions/cu.mm.)						
Initial	5.0	5.0	5.1	5.4	5.1	5.0
After pre-experimental mixed fat[3]	5.0	4.8	4.9	4.4	4.9	4.9
After 8 days test fat	4.8	4.9	5.1[4]	5.4[4]	4.9	5.0
After 15 days test fat	5.4	5.0	--	--	4.9[5]	5.0[5]
After 22 days test fat	4.8	4.9	5.0	5.4	--	--
After 8 days post-experimental mixed fat	4.9	5.0	--	--	4.9	5.0

[1]Test fats provided 122 to 132 grams of fat daily, of which 20 to 36 grams was hydrogenated to make margarine.

[2]The two test fats were fed in a crossover design during experimental periods 1 and 2. Group A subjects were fed soybean oil first. Group B subjects were fed rapeseed oil first. Subjects were fed each test fats for eleven days in the Lake study and eight days in the Masniuk study.

[3]The pre-experimental mixed fat period lasted nine days in the King and LeBlanc studies, ten days in the Lake study, and eight days in the Masniuk study.

[4]Samples taken after ten days instead of eight days on the test fat.

[5]Samples taken after 16 days instead of 15 days on the test fat.

resulting in a statistically significant ($P < 0.05$) overall decrease during the 22 days that HiRSO was fed. In contrast, LeBlanc (1973) reported that red blood cell count increased during the first nine days that the test diet was fed, but that no further changes occurred in response to the test oil, LoRSO, introduced on day 10 of the study.

D.1.b. Reticulocyte Counts. Individual and mean reticulocyte counts (initial levels and levels at the end of each dietary period) in the present study are reported in Table 25 and Figure 8. Reticulocyte counts in the present study and the Lake study (1975) are reported as actual counts, whereas the reticulocytes in the LeBlanc (1973) and King (1974) studies were reported as percentages of red blood cell counts. The Merck Manual of Diagnosis and Therapy cautions that absolute reticulocyte levels are important in diagnosis of functional abnormalities. For ease of comparison, the actual reticulocyte counts obtained from the original laboratory reports for the King and LeBlanc studies are summarized in Table 26.

There were no significant changes ($P > 0.05$) in reticulocyte count due to diet in the present study. Similarly, Lake (1975) reported no significant changes ($P > 0.05$) in mean reticulocyte count. King (1974), on the other hand, found reticulocyte counts were higher during the experiment than at the start of the study (initial

Table 25

Reticulocyte Counts[1] (thousands/cu. mm.) During the Experiment

SUBJECTS	INITIAL	DIETARY TREATMENT: MIXED FAT I[2]	MIXED FAT II[3]	SOYBEAN OIL[4]	LOW ERUCIC ACID RAPESEED OIL[4]
GROUP A[5]					
WJ	42.4	45.0	53.0	36.4	57.2
HJ	9.4	41.4	34.4	13.8	25.8
LB	19.6	34.0	25.0	28.2	28.8
LeB	27.0	20.8	30.6	31.2	15.3
GROUP B[5]					
NM	53.7	44.1	58.7[6]	33.6	57.6
SJ	10.2	25.5	61.2	26.0	26.0
MD	67.6	36.4	58.3	52.0	58.3
GS	9.8	27.0	32.3	28.8	13.8
GROUP A MEAN	24.6	35.3	35.8	27.4	31.8
GROUP B MEAN	35.3	33.3	52.6	35.1	38.9
OVERALL MEAN	30.0	34.3	44.2	31.3	35.4
NORMAL RANGE: (adults)	0.2-2.0 percent of total red blood cells (Dacie and Lewis, 1963).				

[1] As determined by Hematological Laboratories, Health Sciences Centre, Winnipeg, Manitoba.

[2] Pre-experimental period (samples drawn after eight days).

[3] Post-experimental period (samples drawn after seven days).

[4] Samples drawn after eight days.

[5] During the experimental period, Group A was fed soybean oil for the first eight days and rapeseed oil during the next eight days, and Group B received rapeseed oil first and soybean oil second.

[6] Calculated value according to Snedecor, G.W. and Cochran, W.G. 1967. Statistical Methods. 6th Ed. Iowa State University Press, Ames, Iowa. P. 317.

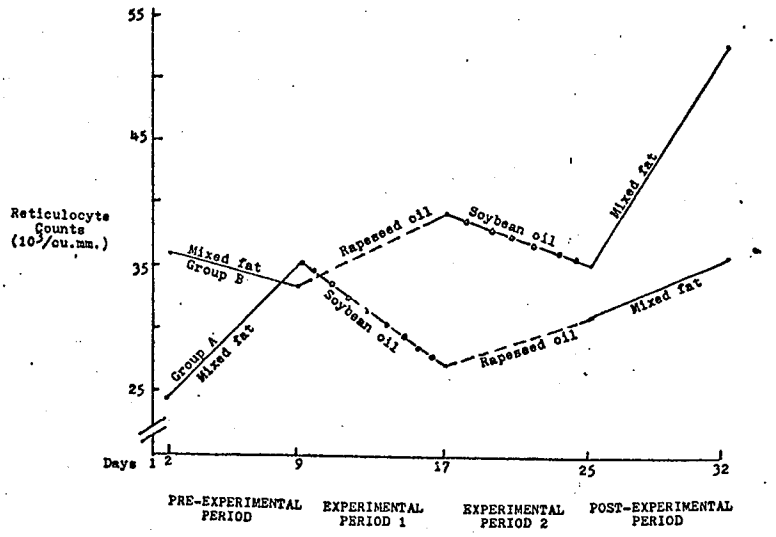


Figure 8

Mean Reticulocyte Counts During the Experiment

Table 26

Comparison of Reticulocyte Counts in Normal Young Men Fed Diets Containing High or Low Erucic Acid Rapeseed Oil

	TYPE OF TEST FAT[1]					
	High Erucic Acid Rapeseed Oil	Low Erucic Acid Rapeseed Oil	Low Erucic Acid Rapeseed Oil and Soybean Oil[2]			
	King (1974)	LeBlanc (1973)	Lake (1975)		Masniuk (1976)	
			A	B	A	B
NUMBER OF SUBJECTS	7	7	2	2	4	4
RETICULOCYTE LEVELS (thousands/cu.mm.)						
Initial	27.4	61.1	17.7	30.0	24.6	35.3
After pre-experimental mixed fat	42.6	18.8	51.1	37.3	35.3	33.3
After 8 days test fat	44.0	43.0	35.4[4]	40.2[4]	27.4	38.9
After 15 days test fat	35.3	54.7	--	--	31.8[5]	35.1[5]
After 22 days test fat	40.7	57.3	52.5	40.0	--	--
After 8 days post-experimental mixed fat	59.3	60.0	--	--	35.8	52.6

[1]Test fats provided 122 to 132 grams of fat daily, of which 20 to 36 grams was hydrogenated to make margarine.

[2]The two test fats were fed in a crossover design during experimental periods 1 and 2. Group A subjects were fed soybean oil first. Group B subjects were fed rapeseed oil first. Subjects were fed each test fats for eleven days in the Lake study and eight days in the Masniuk study.

[3]The pre-experimental mixed fat period lasted nine days in the King and LeBlanc studies, ten days in the Lake study, and eight days in the Masniuk study.

[4]Samples taken after ten days instead of eight days on the test fat.

[5]Samples taken after 16 days instead of 15 days on the test fat.

levels). In contrast, LeBlanc (1973) reported a significant ($P<0.05$) decrease in reticulocyte count during the first nine days of the experiment, with recovery during the rapeseed oil period to levels near those at the start of the study. Nevertheless all reticulocyte counts in the four studies were within the range of values considered normal for the adult human.

D.1.c. Hemoglobin Levels. Individual and mean hemoglobin levels (initial levels and levels at the end of each dietary period) observed in the present study are reported in Table 27 and Figure 9.

Initial hemoglobin levels were higher ($P<0.05$) than levels during the study, with gradual recovery during the second mixed fat period (Appendix Tables 25 and 26). There is no obvious explanation for these results when account is taken of the fact the lifespan of the red blood cell is 120 days, and therefore it is unlikely that changes in hemoglobin synthesis would be reflected in a time period of only 32 days. There were no changes in hemoglobin due to fat source.

Changes in hemoglobin levels in response to dietary fat have been observed in other studies in our laboratory (Table 28). LeBlanc (1973) found there was a significant ($P<0.05$) decrease in hemoglobin levels during the first nine days of the study, but no further changes over the succeeding 30 days. In contrast, King (1974) reported

Table 27

Hemeglobin Levels[1] (g./100 ml.) During the Experiment

SUBJECTS	INITIAL	DIETARY TREATMENT: MIXED FAT I[2]	MIXED FAT II[3]	SOYBEAN OIL[4]	LOW ERUCIC ACID RAPESEED OIL[4]
GROUP A[5]					
WJ	15.5	14.4	15.0	14.8	14.9
HJ	14.6	14.1	13.7	14.1	13.3
LB	15.0	15.1	15.8	14.5	15.0
LeB	15.3	14.9	14.5	14.6	14.5
GROUP B[5]					
NM	14.5	14.9	14.5[6]	14.4	14.1
SJ	13.9	13.7	13.9	13.9	13.7
MD	15.9	15.1	15.6	15.3	15.2
GS	14.7	13.8	14.1	14.5	13.7
GROUP A MEAN	15.1	14.6	14.8	14.5	14.4
GROUP B MEAN	14.8	14.4	14.5	14.5	14.2
OVERALL MEAN	14.9	14.5	14.5	14.5	14.3

NORMAL RANGE: 13.5-18.0 grams/100 ml blood (Dacie and Lewis, 1963)

[1]As determined by Hematological Laboratories, Health Sciences Centre, Winnipeg, Manitoba.

[2]Pre-experimental period (samples drawn after eight days).

[3]Post-experimental period (samples drawn after seven days).

[4]Samples drawn after eight days.

[5]During the experimental period, Group A was fed soybean oil for the first eight days and rapeseed oil during the next eight days, and Group B received rapeseed oil first and soybean oil second.

[6]Calculated value according to Snedecor, G.W. and Cochran, W.G. 1967. Statistical Methods. 6th Ed. Iowa State University Press, Ames, Iowa. P. 317.

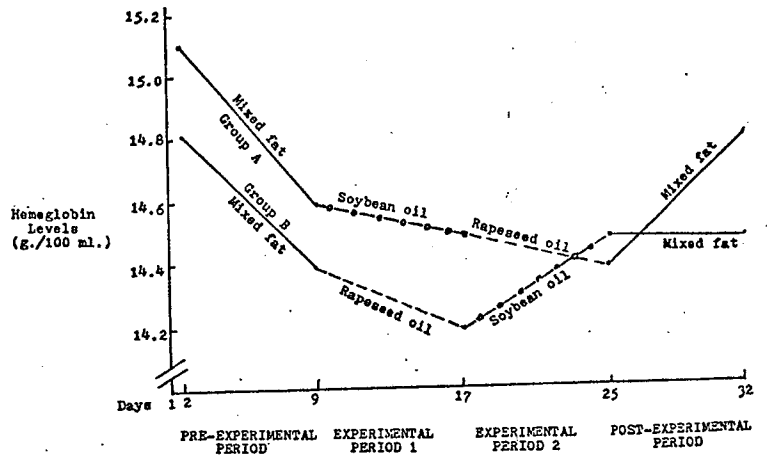


Figure 9

Mean Hemeglobin Levels During the Experiment

Table 28

Comparison of Hemeglobin Levels in Normal Young Men Fed Diets Containing High or Low Erucic Acid Rapeseed Oil

	TYPE OF TEST FAT[1]					
	High Erucic Acid Rapeseed Oil	Low Erucic Acid Rapeseed Oil	Low Erucic Acid Rapeseed Oil and Soybean Oil[2]			
	King (1974)	LeBlanc (1973)	Lake (1975)		Masniuk (1976)	
			A	B	A	B
NUMBER OF SUBJECTS	7	7	2	2	4	4
HEMEGLOBIN LEVELS (g./100 ml.)						
Initial	14.6	15.3	15.4	16.5	15.1	14.8
After pre-experimental mixed fat[3]	14.9	14.5	15.1	16.6	14.6	14.4
After 8 days test fat	14.2	14.8	15.3[4]	16.0[4]	14.5	14.2
After 15 days test fat	14.6	14.7	--	--	14.4[5]	14.5[5]
After 22 days test fat	14.4	14.4	14.8	16.1	--	--
After 8 days post-experimental mixed fat	14.5	14.6	--	--	14.8	14.5

[1]Test fats provided 122 to 132 grams of fat daily, of which 20 to 36 grams was hydrogenated to make margarine.

[2]The two test fats were fed in a crossover design during experimental periods 1 and 2. Group A subjects were fed soybean oil first. Group B subjects were fed rapeseed oil first. Subjects were fed each test fats for eleven days in the Lake study and eight days in the Masniuk study.

[3]The pre-experimental mixed fat period lasted nine days in the King and LeBlanc studies, ten days in the Lake study, and eight days in the Masniuk study.

[4]Samples taken after ten days instead of eight days on the test fat.

[5]Samples taken after 16 days instead of 15 days on the test fat.

higher hemoglobin levels after the first nine days of the study, followed by a decrease over the next 22 days. No changes in hemoglobin levels in response to diet were observed by Lake (1975). There appeared to be no consistent effect of dietary fat source on hemoglobin. Hemoglobin levels in all four studies remained within the range considered normal for the human.

D.1.d. Hematocrit Levels. Individual and mean hematocrit levels (initial levels and levels at the end of each dietary period) in the present study are reported in Table 29 and Figure 10.

Hematocrit decreased when the test diet was introduced ($P<0.05$). There was a further small decrease ($P<0.05$) over the remainder of the study. There was no difference in the effect of LoRSO and that of SBO on hematocrit (Appendix Tables 27 and 28). This gradual drop in hematocrit during the study is difficult to explain, since all of the blood cells (red blood cells, reticulocytes, leucocytes or platelets) either did not change or had recovered to near initial values by the end of the study.

Changes in hematocrit levels in previous studies in our laboratory are varied (Tables 8 and 30). Lake (1975) reported no change in hematocrit during a study involving the same fat sources as the present study i.e. LoRSO and SBO. LeBlanc (1973), on the other hand, reported a

Table 29

Hematocrit Levels[1] (percent) During the Experiment

SUBJECTS	INITIAL	DIETARY TREATMENT			
		MIXED FAT		SOYBEAN OIL[4]	LOW ERUCIC ACID RAPE-SEED OIL[4]
		I[2]	II[3]		
GROUP A[5]					
WJ	47.0	43.0	43.5	44.0	44.0
HJ	44.5	41.5	39.0	42.5	38.0
LB	45.5	44.5	44.5	42.5	43.0
LeB	47.5	44.0	42.5	43.5	42.5
GROUP B[5]					
NM	43.5	43.0	41.0[6]	41.0	42.0
SJ	43.5	43.5	41.0	42.0	42.5
MD	47.0	44.5	44.0	44.0	45.0
GS	44.5	40.0	39.5	41.0	39.5
GROUP A MEAN	46.1	43.3	42.4	43.1	41.9
GROUP B MEAN	44.6	42.8	41.4	42.0	42.3
OVERALL MEAN	45.4	43.0	41.9	42.6	42.1

NORMAL RANGE: 40-65 Percent (Dacie and Lewis, 1963)

[1]As determined by Hematological Laboratories, Health Sciences Centre, Winnipeg, Manitoba.

[2]Pre-experimental period (samples drawn after eight days).

[3]Post-experimental period (samples drawn after seven days).

[4]Samples drawn after eight days.

[5]During the experimental period, Group A was fed soybean oil for the first eight days and rapeseed oil during the next eight days, and Group B received rapeseed oil first and soybean oil second.

[6]Calculated value according to Snedecor, G.W. and Cochran, W.G. 1967. Statistical Methods. 6th Ed. Iowa State University Press, Ames, Iowa. P. 317.

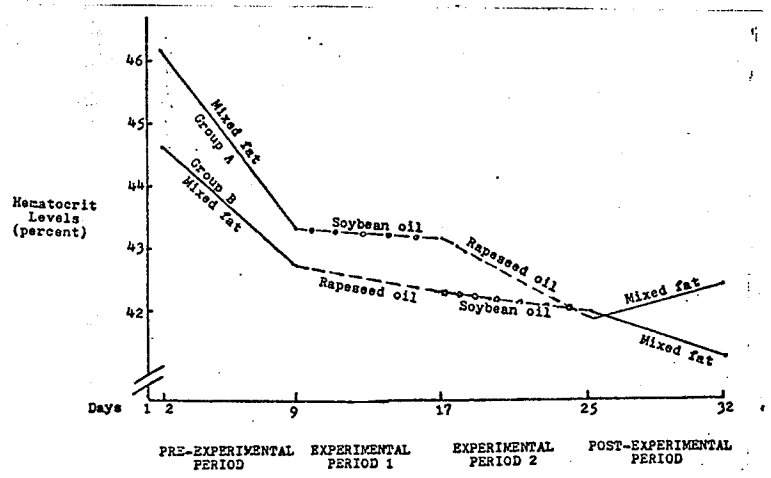


Figure 10

Mean Hematocrit Levels During the Experiment

Table 30

Comparison of Hematocrit Levels in Normal Young Men Fed Diets Containing High or Low Erucic Acid Rapeseed Oil

	TYPE OF TEST FAT[1]					
	High Erucic Acid Rapeseed Oil	Low Erucic Acid Rapeseed Oil	Low Erucic Acid Rapeseed Oil and Soybean Oil[2]			
	King (1974)	LeBlanc (1973)	Lake (1975)		Masniuk (1976)	
			A	B	A	B
NUMBER OF SUBJECTS	7	7	2	2	4	4
HEMATOCRIT LEVELS (percent)						
Initial	43.3	44.6	43.8	47.3	46.1	44.6
After pre-experimental mixed fat[3]	43.1	42.5	42.9	46.8	43.3	42.8
After 8 days test fat	41.3	42.9	43.5[4]	45.8[4]	43.1	42.3
After 15 days test fat	45.1	43.8	--	--	41.9[5]	42.0[5]
After 22 days test fat	41.8	44.1	43.5	41.5	--	--
After 8 days post-experimental mixed fat	42.4	45.1	--	--	42.4	41.4

[1]Test fats provided 122 to 132 grams of fat daily, of which 20 to 36 grams was hydrogenated to make margarine.

[2]The two test fats were fed in a crossover design during experimental periods 1 and 2. Group A subjects were fed soybean oil first. Group B subjects were fed rapeseed oil first. Subjects were fed each test fats for eleven days in the Lake study and eight days in the Masniuk study.

[3]The pre-experimental mixed fat period lasted nine days in the King and LeBlanc studies, ten days in the Lake study, and eight days in the Masniuk study.

[4]Samples taken after ten days instead of eight days on the test fat.

[5]Samples taken after 16 days instead of 15 days on the test fat.

significant ($P < 0.05$) decrease in hematocrit during the first nine days of her study, followed by a gradual increase in hematocrit levels over the remainder of the study. King (1975) observed a gradual decrease during the first 17 days of her study, followed by higher levels on day 25 and lower levels on day 32. None of the changes in hematocrit observed on the King or LeBlanc studies appeared to be related to fat sources. Furthermore, all values observed in all four studies were within the range considered normal for hematocrit levels in the human.

D.2. Effect of Diet on Platelet Counts

Individual and mean platelet counts (initial levels and levels at the end of each dietary period) in the present study are reported in Table 31 and Figure 11.

There were no significant changes ($P > 0.05$) in platelet count during the present study (Appendix Table 29), which agrees with the studies previously reported by LeBlanc (1973) and Lake (1975) (Table 32). All values for platelet count remained with the range considered normal for the human in all three of these studies. However, King (1974) reported that the ingestion of diets containing HiRSO resulted in a marked drop in platelet count to levels below the normal range. Four of seven subjects had platelet counts below 105,000 per cu. mm. after consuming the diet

Table 31

Platelet Counts[1] (thousands/cu. mm.) During the Experiment

SUBJECTS	INITIAL	DIETARY TREATMENT: MIXED FAT I[2]	MIXED FAT II[3]	SOYBEAN OIL[4]	LOW ERUCIC ACID RAPESEED OIL[4]
GROUP A[5]					
WJ	230	248	182	240	176
HJ	152	160	176	162	142
LB	252	280	267	160	230
LeB	245	295	264	297	235
GROUP B[5]					
NM	215	208	229[6]	249	196
SJ	195	176	194	150	155
MD	192	188	205	151	192
GS	340	304	310	253	251
GROUP A MEAN	220	246	222	215	195
GROUP B MEAN	236	219	235	201	199
OVERALL MEAN	228	232	228	208	197

NORMAL RANGE: > 150 thousand/cu. mm. (Guyton, 1971)

[1]As determined by Hematological Laboratories, Health Sciences Centre, Winnipeg, Manitoba.

[2]Pre-experimental period (samples drawn after eight days).

[3]Post-experimental period (samples drawn after seven days).

[4]Samples drawn after eight days.

[5]During the experimental period, Group A was fed soybean oil for the first eight days and rapeseed oil during the next eight days, and Group B received rapeseed oil first and soybean oil second.

[6]Calculated value according to Snedecor, G.W. and Cochran, W.G. 1967. Statistical Methods. 6th Ed. Iowa State University Press, Ames, Iowa. P. 317.

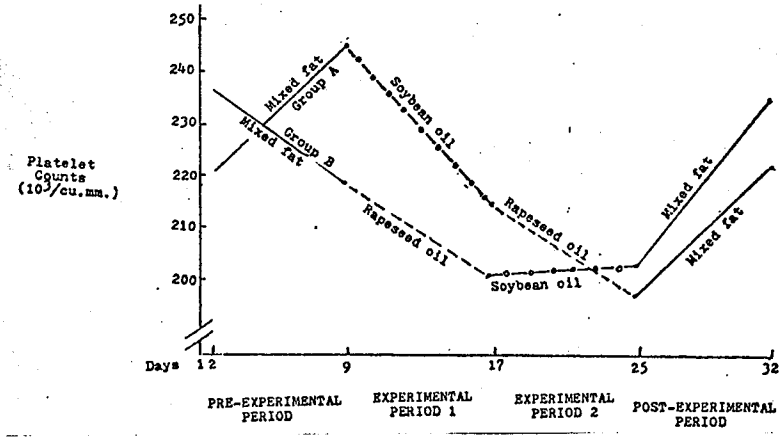


Figure 11

Mean Platelet Counts During the Experiment

Table 32

Comparison of Platelet Counts in Normal Young Men Fed Diets Containing High or Low Erucic Acid Rapeseed Oil

	TYPE OF TEST FAT[1]					
	High Erucic Acid Rapeseed Oil	Low Erucic Acid Rapeseed Oil	Low Erucic Acid Rapeseed Oil and Soybean Oil[2]			
	King (1974)	LeBlanc (1973)	Lake (1975) A	Lake (1975) B	Masniuk (1976) A	Masniuk (1976) B
NUMBER OF SUBJECTS	7	7	2	2	4	4
PLATELET COUNTS (thousands/cu.mm.)						
Initial	N	6N,1LN	N	N	N	N
After pre-experimental mixed fat[3]	N	N	N	N	N	N
After 8 days test fat	6LN,1L	3N,4LN	N[4]	N[4]	N	N
After 15 days test fat	5LN,2L	N	--	--	N[5]	N[5]
After 22 days test fat	2LN,5L	6N,1LN	N	N	--	--
After 8 days post-experimental mixed fat	5N,2LN	4N,3LN	--	--	N	N

N = Normal Range (150,000/cu. mm.) (Guyton, 1971)

LN = Low Normal (120-150 thousand/cu. mm.) L = Low (under 120,000/cu. mm.)

[1]Test fats provided 122 to 132 grams of fat daily, of which 20 to 36 grams was hydrogenated to make margarine.

[2]The two test fats were fed in a crossover design during experimental periods 1 and 2. Group A subjects were fed soybean oil first. Group B subjects were fed rapeseed oil first. Subjects were fed each test fats for eleven days in the Lake study and eight days in the Masniuk study.

[3]The pre-experimental mixed fat period lasted nine days in the King and LeBlanc studies, ten days in the Lake study, and eight days in the Masniuk study.

[4]Samples taken after ten days instead of eight days on the test fat.

[5]Samples taken after 16 days instead of 15 days on the test fat.

containing HiRSO for 22 days. Normal levels for the human generally are considered as counts greater than 150,000 per cu mm. The drop in platelet counts appeared to be related to the erucic acid content of the oil, since platelet counts returned essentially to normal within a week after HiRSO was removed from the diet and since no abnormalities in platelet count have been observed in response to dietary LoRSO in this laboratory.

D.3. Effect of Diet on Leucocyte Counts

Individual and mean leucocyte counts (initial levels and levels at the end of each dietary period) in the present study are reported in Table 33 and Figure 12.

In the present study, leucocyte counts were significantly ($P < 0.05$) lower than initial levels after the test diet was fed for eight days, and were lower ($P < 0.05$) during the two experimental periods when oils were fed as the source of dietary fat than during the two periods when mixed fat was fed (Appendix Tables 31 and 32). There was a marginal difference ($P < 0.05$) in the effects of the LoRSO and the SBO on leucocyte counts, primarily due to a sharp drop when LoRSO was fed during Experimental Period 2.

The same pattern of response, namely an initial decrease in leucocyte counts followed by recovery during the fifth week, was observed in the studies by King (1974)

Table 33

Leucocyte Count[1] (thousands/cu. mm.) During the Experiment

SUBJECTS	INITIAL	DIETARY TREATMENT			
		MIXED FAT		SOYBEAN OIL[4]	LOW ERUCIC ACID RAPE-SEED OIL[4]
		I[2]	II[3]		
GROUP A[5]					
WJ	7.9	7.4	7.6	7.1	5.5
HJ	8.2	7.1	8.3	7.4	6.2
LB	5.6	6.3	6.1	5.6	4.1
LeB	6.7	5.8	5.6	5.2	3.9
GROUP B[5]					
NM	6.1	5.6	6.2[6]	5.2	5.2
SJ	4.9	4.2	4.7	3.1	4.1
MD	7.0	4.9	6.1	4.3	5.2
GS	4.8	4.3	5.3	4.2	4.4
GROUP A MEAN	7.1	6.7	6.9	6.3	4.9
GROUP B MEAN	5.7	4.8	5.6	4.4	4.7
OVERALL MEAN	6.4	5.7	6.2	5.3	4.8

NORMAL RANGE: 4-10 Thousand/mm.^{3} (Dacie and Lewis, 1963)

[1]As determined by Hematological Laboratories, Health Sciences Centre, Winnipeg, Manitoba.

[2]Pre-experimental period (samples drawn after eight days).

[3]Post-experimental period (samples drawn after seven days).

[4]Samples drawn after eight days.

[5]During the experimental period, Group A was fed soybean oil for the first eight days and rapeseed oil during the next eight days, and Group B received rapeseed oil first and soybean oil second.

[6]Calculated value according to Snedecor, G.W. and Cochran, W.G. 1967. Statistical Methods. 6th Ed. Iowa State University Press, Ames, Iowa. P. 317.

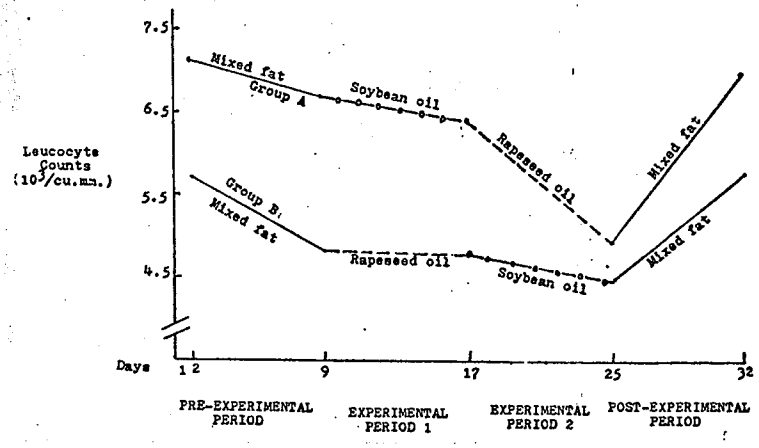


Figure 12

Mean Leucocyte Counts During the Experiment

Table 34

Comparison of Leucocyte Counts in Normal Young Men Fed Diets Containing High or Low Erucic Acid Rapeseed Oil

	TYPE OF TEST FAT[1]					
	High Erucic Acid Rapeseed Oil	Low Erucic Acid Rapeseed Oil	Low Erucic Acid Rapeseed Oil and Soybean Oil[2]			
	King (1974)	LeBlanc (1973)	Lake (1975)		Masniuk (1976)	
			A	B	A	B
NUMBER OF SUBJECTS	7	7	2	2	4	4
LEUCOCYTE COUNTS (thousands/cu.mm.)						
Initial	4.9	5.1	6.8	9.5	7.1	5.7
After pre-experimental mixed fat[3]	4.1	4.4	6.7	11.0	6.7	4.8
After 8 days test fat	4.3	4.6	5.7[4]	5.4[4]	6.3	4.7
After 15 days test fat	3.4	4.7	--	--	4.9[5]	4.4[5]
After 22 days test fat	3.3	4.0	4.9	6.4	--	--
After 8 days post-experimental mixed fat	4.7	4.4	--	--	6.9	5.6

[1]Test fats provided 122 to 132 grams of fat daily, of which 20 to 36 grams was hydrogenated to make margarine.

[2]The two test fats were fed in a crossover design during experimental periods 1 and 2. Group A subjects were fed soybean oil first. Group B subjects were fed rapeseed oil first. Subjects were fed each test fats for eleven days in the Lake study and eight days in the Masniuk study.

[3]The pre-experimental mixed fat period lasted nine days in the King and LeBlanc studies, ten days in the Lake study, and eight days in the Masniuk study.

[4]Samples taken after ten days instead of eight days on the test fat.

[5]Samples taken after 16 days instead of 15 days on the test fat.

and LeBlanc (1973) (Table 34). The Lake (1975) study, on the other hand, found no change in leucocyte counts. The drop in leucocyte levels observed in these studies does not appear to be due entirely to changes in dietary fat source, since leucocyte counts for many subjects at the end of the second mixed fat period resembled the initial leucocyte levels more closely than they resembled the levels at the end of the first mixed fat period.

E. EFFECT OF FAT SOURCE ON SERUM LIPIDS AND WHOLE BLOOD HEMATOLOGY

Before discussing the general effect of diet and, in particular, fat source on serum lipids and whole blood hematology that have been observed in our laboratory, it is important to recognize the format used in these studies and the differences among the four studies (Table 19). Although a total of 26 subjects were involved in the four studies, response to specific fat sources were determined with as few as two subjects per treatment group in one study (a cross-over design involving a total of four subjects). The studies were of relatively short duration; the longest studies lasted 39 days, with the longest period of feeding of a single fat source being 22 days. Often a given fat source was fed for only eight to ten days. These feeding periods may not have been long enough to allow for complete change

in some parameters. The composition of the mixed fat varied somewhat from one study to another. The three studies of low erucic acid rapeseed oil (LoRSO) each used a different cultivar (Span, Zephyr or Tower) of LoRSO.

It is also important to note that of the six parameters measured to determine the effect of fat source (HiRSO, LoRSO, mixed fat and soybean oil) on blood hematology, only platelet counts and leucocyte counts in response to HiRSO were outside the ranges considered normal for the human. Although statistically significant ($P < 0.05$) changes were observed in other hematological parameters, there was no evidence that those changes were functionally or physiologically meaningful.

Two kinds of effects were observed in these studies: effects due to the test diet itself, or more precisely to the entire experimental protocol; and effects due to dietary fat source.

Serum cholesterol and serum lipid phosphorus levels decreased in response to the test diet. Serum lipid phosphorus levels were lower when vegetable oils (HiRSO, LoRSO or SBO) were fed than when mixed fat was fed. Serum cholesterol levels were lower when SBO or LoRSO was fed than when mixed fat was fed. In contrast, substitution of HiRSO for mixed fat did not result in an overall decrease in serum cholesterol, even though the levels of saturated,

Table 33

Changes in Whole Blood Hematology and Serum Lipids Observed in Various Rapeseed Oil Studies

STUDY	KING 1974		LEBLANC 1973		LAKE 1975		MASNIUK 1976	
TYPE OF TEST OIL	HiRSO		LoRSO		LoRSO/SBO		LoRSO/SBO	
	T	R	T	R	T	V	T	V
SERUM LIPIDS								
Serum cholesterol	↓	-	↓	↓	↓	↓	↓	↓
Serum lipid phosphorus	↓	↓	↓	↓	↓	↓	↓	↓
WHOLE BLOOD HEMATOLOGY								
Red blood cells	-	↓	↓	-	-	-	-	-
Reticulocytes	↑	-	↓	↑	-	-	-	-
Hemoglobin	-	↓	↓	-	-	-	↓↑	-
Hematocrit	-	↓	↓↑	-	-	-	↓	-
Platelets	-	(↓)	-	-	-	-	-	-
Leucocytes	↓↑	(↓)	↓↑	↓	-	-	↓↑	↓

KEY
HiRSO - high erucic acid rapeseed oil
LoRSO - low erucic acid rapeseed oil
SBO - soybean oil

T - change in response to feeding of the test diet
R - change in response to feeding of rapeseed oil
V - change in response to feeding of vegetable oils (SBO and LoRSO were statistically indistinguishible in their effect except for leucocytes)

↑ - increase
- - no change
↓ - decrease
↓↑ - inital decrease followed by an increase by the end of the study.
(↓) - decrease to values below the normal range.

monounsaturated and polyunsaturated fatty acids were similar to the levels in the LoRSO diet. Red blood cell count, hemoglobin levels and hematocrit levels decreased in response to HiRSO, but did not change in response to LoRSO or SBO. Platelet counts decreased to levels below the range considered normal for the human when HiRSO was substituted for mixed fat in the diet. This decrease in platelet count appeared to be due to the erucic acid content of HiRSO, since platelet levels recovered when the mixed fat diet again was fed. Both the test diet and the type of dietary fat appeared to affect leucocyte count. Leucocyte count decreased in response to the test diet and decreased further in response to the feeding of vegetable oils. Leucocyte count was the only parameter of all eight parameters examined in the present study in which the values obsered when LoRSO was fed differed from the values observed when SBO was fed; and in the Lake (1975) study, which also compared the effects of LoRSO and SBO, even this difference was not observed.

Several other statistically significant changes in whole blood hematology were observed during the four studies (e.g. an increase in reticulocyte count when the test diet was introduced in the King study and a decrease in reticulocyte count in the LeBlanc study - Table 35) but these changes did not follow any consistent pattern.

F. SUITABILITY OF LOW ERUCIC ACID RAPESEED OIL IN THE HUMAN DIET

The use of HiRSO as a dietary oil has been discouraged in Canada since 1973, on the grounds of evidence of cardiac changes in experimental animals. Since that time, King (1974) observed a drop in platelet counts to levels below the range considered normal for the human.

In contrast, platelet counts did not decrease to undesirable levels in humans fed LoRSO. Furthermore, LoRSO was at least as effective as soybean oil in lowering serum cholesterol levels, even though the polyunsaturated fatty acid content of LoRSO was lower than that of soybean oil. Lowering of serum cholesterol levels currently is considered desirable by many cardiac specialists, although it has not been established conclusively whether the serum cholesterol that disappears when vegetable oils are substituted for more saturated dietary fats is excreted or simply redistributed to other body tissues. It does not appear necessary for the plant geneticists to increase the linoleic acid content of rapeseed oil from the levels currently found in LoRSO cultivar Tower. Since high levels of dietary polyunsaturates may enhance the requirement for vitamin E, there may in fact be an advantage in the utilization of a dietary vegetable oil as low in polyunsaturates as is compatible with effective cholesterol control.

The studies in our laboratory therefore indicate that LoRSO is a satisfactory dietary oil. Rape has the further advantage of being a suitable crop for areas too cold for traditional oilseed crops such as soybeans. The Prairie Provinces have considerable acreage suited to the growing of rapeseed.

SUMMARY AND CONCLUSIONS

The present study compared the effects of low erucic acid rapeseed oil (Brassica napus cv. Tower) and soybean oil on serum lipid patterns and whole blood hematology of eight healthy young men fed a diet in which added fat contributed 40% of total calories (i.e. 92 to 94% of the total dietary fat). The diet was composed of conventional low fat foods and added fat, in which soy protein (TVP and Bontrae) was substituted for meat. The 31 day study was divided into four periods: (1) An 8-day pre-experimental stabilization period during which the fat source was a mixture of fats formulated to simulate the composition of fat in the average North American diet; (2) An 8-day experimental period during which half the subjects were fed rapeseed oil and the other half soybean oil as their dietary fat source; (3) A second 8-day experimental period during which the subjects received the alternate oil (crossover design); and (4) A 7-day post-experimental period when the diet containing mixed fat was again fed. The primary differences between the three diets was in the amount of palmitic, stearic, oleic, linoleic and linolenic acids. The mixed fat diet contained 22, 13, 40, 21 and 2% of these acids, respectively; the soybean oil diet contained 11, 5, 44, 34 and 4% of these acids, respectively; and the rapeseed

oil contained 6, 3, 59, 19 and 8% of these acids, respectively. The ratio of saturated to unsaturated fatty acids was 1:1.7 for the diet containing mixed fat; 1:5.3 for the diet containing soybean oil and 1:8.1 for the diet containing rapeseed oil.

Fasting blood samples were drawn for the determination of serum lipid patterns and whole blood hematology on days 2, 9, 17, 25 and 32. Serum cholesterol for all subjects decreased during the pre-experimental mixed fat period by an average of 13 mg./100 ml. of serum. Serum cholesterol levels were lower when oil was fed as the fat source than when mixed fat was fed, but there was no statistical difference between the two oils (soybean and rapeseed) in their effect on cholesterol. Lipid phosphorus followed a pattern similar to that of serum cholesterol. Serum lipid phosphorus levels decreased during the preliminary mixed fat period, decreased further when oil was fed, and increased when mixed fat again was fed. There was no difference in the effects of the two oils (soybean and rapeseed). The percent change in lipid phosphorus was greater in magnitude than the changes observed in serum cholesterol. The fatty acid patterns of serum phospholipids reflected the fatty acid composition of the various fat sources.

No consistent effect of diet on blood hematology

was observed. Red blood cell count, reticulocyte count and platelet levels remained constant during the experiment. Platelet counts had been found to decrease to undesirable low levels when high erucic acid rapeseed oil was fed (King, 1974). Hemoglobin, hematocrit and leucocyte levels dropped when the test diet was introduced, possibly due to some nonlipid factor in the diet. Leucocytes also decreased when oil was fed as the fat source, and leucocyte levels were lower when rapeseed oil was fed than when soybean oil was fed. However, since leucocyte levels increased sharply during the last week of the study, to levels close to the levels at the start of the study, the main influence on leucocytes may have been some non-lipid aspect of the test diet. All values for all six hematological parameters studied remained within normal levels for all subjects.

The results of the present study indicate no consistent effect of low erucic acid rapeseed oil or soybean oil on the whole blood hematology of young men, However, inclusion of rapeseed oil or soybean oil at a level providing 40% of calories (103 to 106 g. liquid oil and 25 g. margarine a day in a diet containing conventional low fat foods) had an appreciable lowering effect on serum lipid patterns. Although the rapeseed oil was substantially lower in polyunsaturates than the oils currently encouraged for use in control of serum cholesterol levels, its

correspondingly lower levels of saturated fatty acids gives it a similar or perhaps even more desirable effect on serum cholesterol. This inclusion of a high level of low erucic acid rapeseed oil in the diets of healthy young men had no detrimental effect on metabolism, as reflected by changes in serum lipid patterns and whole blood hematology. In fact the lipid changes observed might be considered desirable, in the light of current theories on cardiovascular health. Low erucic acid rapeseed oil appears to be a satisfactory dietary oil.

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Appendix Table 1

ROLLED OATS (1 serving)

Utensil: 1 cereal bowl

Method:
1. Measure into a large cereal bowl
   150 ml. water
   1/8 tsp. salt
   20 grams fat[1]
2. Sprinkle in
   30 grams rolled oats
3. Stir.
4. Place in microwave oven for 30 seconds
5. Remove and stir.
6. Place in oven for another 60-70 seconds.
7. Serve.

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[1]Fat Mix (Appendix Table 16) - mixed fat diet
Tower rapeseed oil - rapeseed oil diet
Soybean oil - soybean oil diet

Appendix Table 2

CREAM OF WHEAT (1 serving)

Utensil: 1 cereal bowl

Method: 1. Measure into a large cereal bowl
256 ml. water
1/8 tsp. salt
20 grams fat[1]

2. Sprinkle in
30 grams rolled oats

3. Stir.

4. Placd in microwave oven for 90 seconds.

5. Remove and stir immediately.

6. Place in microwave oven again for 90 seconds.

7. Stir.

8. Place in microwave oven again for 15 seconds.

9. Stir.

10. Serve immediately.

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[1]Fat Mix (Appendix Table 16) - mixed fat diet
Tower rapeseed oil - rapeseed oil diet
Soybean oil - soybean oil diet

Appendix Table 3

SCRAMBLED EGG ALBUMIN MIXTURE (yields 9 servings)

Utensil: Blender

Method:
1. Pour 720 ml. cold water into blender.
2. Dust in 20 grams egg albumin powder.
3. Mix in blender for a short time.
4. Stir in 6 drops yellow food coloring.

Appendix Table 4

SCRAMBLED EGG ALBUMIN (1 serving)

Utensil: 1 porcelain individual frypan

Method:
1. Melt in frypan
   10 grams fat[1]
2. Add
   100 grams reconstituted egg albumin.
3. Stir occasionally while cooking.
4. Serve in frypan.

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[1]Fat Mix (Appendix Table 16) - mixed fat diet
Tower rapeseed oil - rapeseed oil diet
Soybean oil - soybean oil diet

Appendix Table 5

SCALLOPED POTATOES

1 - 6½ oz. package Idahoan scalloped potatoes is sufficient for 3 servings
(preparation time: 3-5 minutes)

Utensil: 1 foil container 5"x4"x1½" for each serving.

Method:

1. Preheat oven to 325°F (well ahead of actual preparation).
2. Empty contents of paper package containing dry sauce mix into a sieve and sift out dehydrated onions and set aside. Keep the remainder of the dry sauce mix separate.
3. Into a small foil container measure

   10 grams fat.[1]
4. Add 20 grams dry sauce mix and stir into a paste.
5. Add 1 cup boiling water.
6. Add 2.0 grams dehydrated onion (sifted earlier). Stir to disperse fat-starch mixture and dissolve large lumps.
7. Add 40 grams dried potatoes. Stir.
8. Place uncovered in oven for 40 minutes. Serve at once.

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[1]Fat Mix (Appendix Table 16) - mixed fat diet
Tower rapeseed oil - rapeseed oil diet
Soybean oil - soybean oil diet.

Appendix Table 6

SPAGHETTI WITH TOMATO SAUCE

Utensil: 1 foil container 8"x5½"x2 for each serving.

Method:

1. Place the following ingredients in a small saucepan.

   10 grams fat[1]
   2.0 grams starch
   120.0 grams drained AYLMER canned whole tomatoes
   50.0 grams tomato juice (from can of whole tomatoes)
   25.0 grams tomato paste
   20.0 grams sliced mushrooms (canned)
   0.2 grams dehydrated onion flakes
   2.0 grams salt
   0.2 grams ground oregano
   0.2 grams dried sweet basil leaves
   0.5 grams black pepper
   0.2 grams garlic powder

2. Combine ingredients together, chopping large tomatoes if necessary.

3. Place over medium high heat. Slowly bring to a boil while stirring.

4. Reduce heat and simmer, stirring constantly for 10 minutes.

5. Pour contents of saucepan into foil container.

6. While tomato sauce is cooling, cook the pasta.

7. To prepare CATELLI pasta:

   Bring 500 ml. water and 2 grams salt to a rolling boil in a small saucepan.

   Add 50.0 grams (dry weight) CATELLI spaghetti pasta. Cook for 10 minutes (uncovered).

   Empty contents of saucepan into a sieve. Rinse pasta with cold water.

8. Add cooked spaghetti to cooled tomato sauce separating strands and mixing with the sauce.

9. Cool, cover (with cardboard and foil lid). Label. Freeze.

To reheat: Preheat oven to 325°.
Loosen edges of foil container.
Place frozen, covered spaghetti in oven for 30 minutes.

Remove cover and serve.

---

[1]Fat Mix (Appendix Table 16) - mixed fat diet
Tower rapeseed oil - rapeseed oil diet
Soybean oil - soybean oil diet

Appendix Table 7

CHILI

Utensil: 1 small foil container 5"x4"x1½" for each serving.

Method:

1. Place 30 grams fat[1] in a small saucepan and heat.
2. Saute 8 grams chopped onion until soft.
3. Remove saucepan from heat and stir in 4 grams corn starch.
4. Add the following ingredients and replace on heat.

   150 grams drained AYLMER canned tomatoes
   25 grams tomato paste
   25 grams tomato juice
   25 grams water
   50 grams drained canned light red kidney beans
   1.0 grams salt
   0.2 grams coarsely ground pepper
   2.0 grams chili powder
   grams frozen BONTRAE protein crumbles with a flavor like beef[2].

   Bring to a boil slowly, stirring constantly, reduce heat and simmer (stirring constantly) for 5 minutes or until fat has been absorbed.
5. Place in container. Allow to cool, cover, label and freeze.

To reheat: Thaw chili thoroughly.
Preheat oven to 325°F.
Loosen cover slightly.
Heat thawed chili for 30 minutes.

---

[1]Fat Mix (Appendix Table 16) - mixed fat diet
Tower rapeseed oil - rapeseed oil diet
Soybean oil - soybean oil diet

[2]Bontrae frozen textured soy protein, General Mills, Minneapolis.

Appendix Table 8

BEEF STEW

Utensil: 1 small foil container 5"x4"x1½" for each serving.

Method:

1. Place the following ingredients in a small saucepan and allow to hydrate for 30 minutes.

   25.0 grams TVP[1] beef chunks
   100 ml. tomato juice
   150 ml. water
   0.2 grams dehydrated onion flakes
   1 oxo beef cube
   ¼ tsp. kitchen bouquet
   ¼ tsp. worcestershire sauce

2. Mix together:

   30.0 grams fat[2]
   6.0 grams cornstarch until smooth

   Add starch mixture to hot hydrated TVP mixture.

3. Bring to a boil slowly, simmer for an additional 10 minutes, stirring constantly.

4. Empty contents of the saucepan into foil container.

5. Wait until stew is cold, then cover with foil, label and freeze.

To reheat: Preheat oven to 325°F.
Rinse peas and carrots with lukewarm water to get rid of excess ice.
Weigh out 50 grams frozen peas and 50 grams diced carrots.

Heat frozen stew (container covered with foil) for 30 minutes. Add peas, and carrots.
Stir to combine.
Cook (covered) in oven for an additional 20 minutes.

---

[1]Textured Vegetable Protein, British Canadian Importers, Vancouver.

[2]Fat Mix (Appendix Table 16) - mixed fat diet
Tower rapeseed oil - rapeseed oil diet
Soybean oil - soybean oil diet

Appendix Table 9

PIQUANT SALAD DRESSING

Utensil: Small snaptop plastic container

Method:

1. Place in container
   13 grams oil[1]
   1 tsp. vinegar
   1/8 tsp. salt
   herbs as desired
2. Shake to mix.
3. This constitutes a day's allotment of salad dressing (lunch and dinner).

---

[1]Corn oil - mixed fat diet; Tower rapeseed oil - rapeseed oil diet; soybean oil - soybean oil diet.

Appendix Table 10

WHITE RICE (1 serving)

Utensil: 1 covered 8 oz. pyrex casserole.

Method:
1. Preheat oven to 350°.
2. Add to casserole
   - ¼ tsp. salt
   - 100 ml. boiling water
   - 6 grams fat[1]
   - 30 grams raw rice
3. Cook in oven for 25 minutes
4. Serve hot in casserole.

---

[1]Fat Mix (Appendix Table 16) - mixed fat diet
Tower rapeseed oil - rapeseed oil diet
Soybean oil - soybean oil diet

Appendix Table 11

INSTANT MASHED POTATO (1 serving)

Utensil: 1-8 oz. pyrex dish.

Method:
1. Add the following ingredients to pyrex dish
   - 120 ml. boiling water
   - 9 grams fat[1]
   - ½ tsp. salt
2. Stir in 30 grams instant mashed potatoes
3. Serve hot.

---

[1]Fat Mix (Appendix Table 16) - mixed fat diet
Tower rapeseed oil - rapeseed oil diet
Soybean oil - soybean oil diet

Appendix Table 12

SALAD A (1 serving)

Utensil: 1 small pyrex bowl

Method: 1. Combine

30 grams tomato, diced

50 grams leaf lettuce, torn

10 grams radishes, sliced thinly

5 grams green onion, chopped

2. Refrigerate until serving time.

COLESLAW (1 serving)

Utensil: 1 small pyrex bowl

Method: 1. Combine

50 grams cabbage, shredded

5 grams green pepper, chopped

10 grams raw carrot, shredded

2. Refrigerate until serving time.

Appendix Table 13

SALAD B (1 serving)

Utensil: 1 small pyrex bowl

Method: 1. Combine

50 grams leaf lettuce, torn

10 grams cucumber, pared and diced

5 grams green onion, chopped

2. Refrigerate until serving time.

SALAD C (1 serving)

Utensil: 1 small pyrex bowl

Method: 1. Combine

50 grams iceberg lettuce

50 grams tomato, diced

2. Refrigerate until serving time.

Appendix Table 14

SPICY FRUIT SQUARE

Utensil: 1-7½"x7½" cake pan

Method:

1. Preheat oven to 350°F.
2. Cream together

   200 grams brown sugar
   64 grams fat[1]
3. Prepare and add 100 grams reconstituted egg albumin (6 parts water:1 part egg albumin w:w).
4. Add ½ tsp. vanilla essence to fat and sugar mix.
5. Sift together in a separate bowl

   85 grams all purpose flour
   1 tsp. baking powder
   ¼ tsp. salt
   ½ tsp. cinnamon
6. Use a small amount of this flour mixture to toss in a third bowl with

   40 grams seedless raisins
   40 grams chopped candied cherries
7. Gradually add dry ingredients to creamed ingredients. Blend well after each addition.
8. Stir in above fruits.
9. Lightly grease baking pan.
10. Pour batter into pan and bake at 350°F for 30 minutes.
11. Cool. Remove from pan and divide into 16 equal squares.

---

[1]Fat Mix (Appendix Table 16) - mixed fat diet
Tower rapeseed oil - rapeseed oil diet
Soybean oil - soybean oil diet

Appendix Table 15

RAISIN OATMEAL COOKIES

Method:

1. Preheat oven to 350°F.
2. Place in large bowl and mix

   215 grams sifted pastry flour
   3 grams salt
3. Mix in

   190 grams quick cooking rolled oats
4. Combine

   150 grams fat[1]
   150 grams brown sugar
   4 ml. vanilla
5. Dissolve

   4.5 grams baking soda

   in 50 ml. boiling water

   and stir into oil mixture. Mix well.
6. Add wet ingredients to dry ingredients and mix well.
7. Weigh out individual cookies, - 25 grams each
8. Place on ungreased cookie sheet and flatten with a fork into a round cookie.
9. Bake at 350°F for 15 minutes or until golden brown. Yield: 30 cookies at 25 grams each.

---

[1]Fat Mix (Appendix Table 16) - mixed fat diet
Tower rapeseed oil - rapeseed oil diet
Soybean oil - soybean oil diet

Appendix Table 16

FAT MIX

Utensil: Large saucepan

Method:

1. Weigh into saucepan.
   200 grams lard
   250 grams tallow
   300 grams shortening[1]
2. Heat over medium heat until melted
3. Stir well and cool until solidified
4. Use in recipes calling for Fat Mix
   (Weigh in the solid state.)

[1]Crisco

Appendix Table 17

Calculated Nutrient Composition of the Test Diet, Menu 1[1]

Item	Item[2] Number	Grams	Cal.	Pro. (g)	Fat (g)	CHO (g)	Ca (mg)	P (mg)	Fe (mg)	Vitamins A (RE)[3]	Thiamin (mg)	Ribo-flavin	Niacin (mg)	C (mg)
Breakfast														
Apple juice (vit)	27	120	56.4	0.12	-	14.28	7.2	10.8	0.72	-	0.012	0.024	0.12	42.0
Rolled oats	1390	30	117.0	4.26	2.22	20.46	15.9	121.5	1.35	-	0.180	0.042	0.30	0
Scrambled egg albumin	981	14.3	53.2	11.47	0.03	0.82	9.4	15.7	0.14	-	0.006	0.285	0.10	0
Bread	459	30	80.7	2.61	0.96	15.12	21.0	26.1	0.72	-	0.075	0.051	0.69	-
Jam or jelly	1148.-9	14	38.2	0.05	0.01	9.84	2.9	1.1	0.18	0.1	0.001	-	0.03	1.3
Sugar, white or brown	2229-30	14	52.8	-	-	13.69	7.6	1.7	0.01	-	-	-	-	-
Lunch														
Scalloped potato[4]		315	283.5	5.63	10.24	40.30	11.0	74.2	0.78	0.4	0.112	0.024	2.16	12.8
Salad[4]		95	18.7	1.16	0.24	3.80	13.2	25.2	1.02	132.1	0.050	0.059	0.48	21.7
Bread	459	30	80.7	2.61	0.96	15.12	21.0	26.1	0.72	-	0.075	0.051	0.69	-
Watermelon (with rind)	2424	250	31.1	0.54	0.27	7.30	8.1	11.4	0.57	67.8	0.035	0.035	0.19	8.1
Supper														
Chili[4]		381	472.2	16.35	32.68	29.48	98.1	231.1	4.79	370.3	0.226	0.243	5.61	43.1
Rice white	1877	30	108.9	2.01	0.12	24.12	7.2	28.2	0.24	-	0.021	0.009	0.48	-
Coleslaw[4]		65	17.3	0.82	0.13	3.91	28.7	19.2	0.31	118.6	0.035	0.034	0.24	30.7
Bread	459	30	80.7	2.61	0.96	15.12	21.0	26.1	0.72	-	0.075	0.051	0.69	-
Canned peaches	1484	100	97.0	0.40	0.10	25.10	4.0	12.0	0.30	42.0	0.010	0.020	0.50	3.0
Snacks														
Oatmeal cookies[4]		50	223.9	2.81	10.81	29.02	17.6	64.3	1.30	-	0.137	0.057	0.63	-
Fruit square[4]		35	127.9	1.43	4.29	21.38	14.4	11.1	0.72	-	0.029	0.039	0.24	t
Seven-up		305	140.3	-	-	36.60	-	-	-	-	-	-	-	t
Daily Allotments														
Skim milk[4]	1322	840	302.4	30.24	0.84	42.84	877.3	688.8	t	t	0.290	1.305	0.73	7.3
Oil in salad dressing		13	117.0	-	13.00	-	-	-	-	-	-	-	-	-
Spread		25	180.0	0.15	20.00	-	-	-	-	247.5	-	-	-	-
Cooking fat		36	324.0	-	36.00	-	-	-	-	-	-	-	-	-
TOTALS			3003.9	85.27	133.86	368.30	1191.6	1327.82	14.59	978.8	1.369	2.270	13.88	170.0

[1]Calculated values using USDA Handbook #8 Composition of Foods (Watt and Merrill, 1963).

[2]Item number as listed in USDA Handbook #8, ibid.

[3]Retinol Equivalents (10 IU carotene = 3.33 IU preformed Vitamin A = 1 Retinol Equivalent)

[4]Recipes as in Appendix Tables 1 to 15

Appendix Table 18

Calculated Nutrient Composition of the Test Diet, Menu 2[1]

Item	Item[2] Number	Grams	Cal.	Pro. (g)	Fat (g)	CHO (g)	Ca (mg)	P (mg)	Fe (mg)	Vitamins A (R.E.)[3]	Thiamin (mg)	Ribo-flavin	Niacin (mg)	C (mg)
Breakfast														
Orange juice	1437	120	54.0	0.84	0.12	12.84	10.8	19.2	0.12	24.0	0.108	0.012	0.36	54.0
Cream of wheat	993	30	105.9	3.15	0.36	22.32	-	-	12.60	-	-	-	1.05	-
Scrambled egg albumin	981	14.3	53.2	11.47	0.03	0.82	9.4	15.7	0.14	-	0.006	0.285	0.10	-
Bread	459	30	80.7	2.61	0.96	15.12	21.0	26.1	0.72	-	0.075	0.051	0.69	-
Jam or jelly	1148-9	14	38.2	0.05	0.01	9.84	2.9	1.1	0.18	0.1	0.001	-	0.03	1.3
Sugar, white or brown	2239-40	14	52.8	-	-	13.69	7.6	1.7	0.01	-	-	-	-	-
Lunch														
Spaghetti[4]		315	212.4	4.58	10.66	25.75	53.7	143.7	3.15	235.3	0.238	0.214	4.24	41.6
Salad B[4]		65	11.8	0.79	0.18	2.35	38.5	16.3	0.84	105.0	0.032	0.049	0.25	12.7
Bread	459	30	80.7	2.61	0.96	15.12	21.0	26.1	0.72	-	0.075	0.051	0.69	-
Nectarine	1374	150	89.0	1.6	t	27.13	5.7	33.3	0.70	68.9	-	-	-	54.0
Supper														
Beef Stew[4]		265	448.6	16.37	30.59	28.20	32.3	75.5	2.00	1010.0	0.326	0.416	2.61	24.1
Mashed potatoes	1747	30	109.2	2.16	0.18	25.20	10.5	51.9	0.51	-	0.069	0.018	0.62	9.6
Salad C[4]		100	17.5	1.00	0.15	3.80	16.5	24.5	0.50	61.5	0.060	0.050	0.50	4.5
Bread	459	30	80.7	2.61	0.96	15.12	21.0	26.1	0.72	-	0.075	0.051	0.69	-
Canned plums	1646	120	122.4	0.48	0.12	32.04	9.6	10.8	0.90	141.6	0.024	0.024	0.48	2.4
Snacks														
Oatmeal cookies[4]		50	223.9	2.81	10.81	29.02	17.6	64.3	1.30	-	0.137	0.057	0.63	-
Fruit squares[4]		35	127.9	1.43	4.29	21.38	14.4	11.1	0.72	-	0.029	0.039	0.24	t
Seven-Up		305	140.3	-	-	36.60	-	-	-	-	-	-	-	t
Daily Allotments														
Skim milk[4]	1322	840	302.4	30.24	0.84	42.84	877.3	688.8	t	t	0.290	1.305	0.73	7.3
Oil in salad dressing		13	117.0	-	13.00	-	-	-	-	-	-	-	-	-
Spread		25	180.0	0.15	20.00	-	-	-	-	247.5	-	-	-	-
Cooking fat		39	351.0	-	39.00	-	-	-	-	-	-	-	-	-
TOTALS			2999.6	84.95	133.22	379.18	1169.8	1236.2	25.83	1893.9	1.545	2.622	13.91	211.5

[1]Calculated values using USDA Handbook #8 Composition of Foods (Watt and Merrill, 1963).

[2]Item number as listed in USDA Handbook #8, ibid.

[3]Retinol Equivalents (10 IU carotene = 3.33 IU preformed Vitamin A = 1 Retinol Equivalent)

[4]Recipes as in Appendix Tables 1 to 15

Appendix Table 19

Analysis of Variance - Serum Cholesterol≠

Source of Variation	df	SS	MS	F-value	P *
Replications (days)	4	8125.35	2031.31	24.38	0.000
Subjects	7	15391.10	2198.73	25.79	0.000
Soybean vs rapeseed	1	162.56	162.56	1.91	0.190
Residuals (error)	26	2166.59	83.33		
Total	38	25883.10			

* P is the probability of exceeding the given F value by chance.

≠ Days 2, 9, 17, 25, 32.

Appendix Table 20

Orthogonal Comparisons: Serum Cholesterol≠

NO.	COMPARISON	df	SS	F-value	P*
1	Days 9 and 32 vs Days 17 and 25 (mixed fat vs oils)	1	3655.13	42.88	0.000
2	Day 9 vs Day 32 (pre-exp. mixed fat vs post-exp. mixed fat)	1	76.56	0.90	0.352
3	Day 17 vs Day 25 (Exp. Period I vs Exp. Period II)	1	150.06	1.76	0.196
4	Day 2 vs Days 9,17,25 and 32 (Initial vs test diet)	1	4243.60	49.78	0.000
	Residuals (error)	26	8125.35		

* P is the probability of exceeding the given F value by chance

≠ Days 2, 9, 17, 25, 32

Appendix Table 21

Analysis of Variance - Serum Lipid Phosphorus≠

SOURCE OF VARIATION	df	SS	MS	F-value	P*
Replications (days)	4	11.18	2.79	11.46	0.000
Subjects	7	19.67	2.81	11.52	0.000
Soybean vs rapeseed	1	0.02	0.02	0.06	0.808
Residuals (error)	26	6.34	0.24		
TOTAL	38	37.21			

* P is the probability of exceeding the given F value by chance.

≠ Days 2, 9, 17, 25, 32.

Appendix Table 22

Orthogonal Comparisons: Serum Lipid Phosphorus≠

NO.	COMPARISON	df	SS	F-value	P*
1	Days 9 and 32 vs Days 17 and 25 (mixed fat vs oils)	1	1.58	6.46	0.017
2	Days 9 vs Day 32 (pre-exp. mixed fat vs post-exp mixed fat)	1	0.81	3.32	0.080
3	Day 17 vs Day 25 (Exp. Period I vs Exp. Period II)	1	0.01	0.02	0.889
4	Day 2 vs Days 9,17,25 and 32 (Initial vs test diet)	1	8.79	36.02	0.000
	Residuals (error)	26	11.18		

* P is the probability of exceeding the given F value by chance.

≠ Days 2, 9, 17, 25, 32.

Appendix Table 23

Analysis of Variance - Red Blood Cell Count ≠

Source of Variation	df	SS	MS	F-value	P *
Replications (days	4	0.175	0.044	1.27	0.307
Subjects	7	2.322	0.332	9.63	0.000
Soybean vs rapeseed	1	0.023	0.023	0.65	0.427
Residuals (error)	26	0.896	0.034		
Total	38	3.415			

* P is the probability of exceeding the given F value by chance.

≠ Days 2, 9, 17, 25, 32.

Appendix Table 24

Analysis of Variance - Reticulocyte Count ≠

Source of Variation	df	SS	MS	F-value	P *
Replications (days)	4	927.4	231.8	1.94	0.134
Subjects	7	5915.2	845.0	7.09	0.000
Soybean vs rapeseed	1	67.2	67.2	0.56	0.461
Residuals (error)	26	3100.0	119.2		
Total	38	10009.8			

* P is the probability of exceeding the given F value by chance.

≠ Days 2, 9, 17, 25, 32.

Appendix Table 25

Analysis of Variance - Hemoglobin≠

Source of Variation	df	SS	MS	F-value	P *
Replications (days)	4	1.707	0.427	4.70	0.005
Subjects	7	11.410	1.630	17.97	0.000
Soybean vs rapeseed	1	0.181	0.181	1.99	0.170.
Residuals (error)	26	2.358	0.091		
Total	38	15.656			

* P is the probability of exceeding the given F value by chance.

≠ Days 2, 9, 17, 25, 32.

Appendix Table 26

Orthogonal Comparisons: Hemoglobin≠

No.	Comparison	df	SS	F-value	P *
1	Days 9 and 32 vs Days 17 and 25 (mixed fat vs oils)	1	0.1513	1.66	0.209.
2	Day 9 vs Day 32 (pre-exp. mixed fat vs post-exp. mixed fat)	1	0.1406	1.55	0.224.
3	Day 17 vs Day 25 (Exp. Period I vs Exp. Period II)	1	0.0756	0.83	0.371
4	Day 2 vs Days 9,17,25 and 32 (Initial vs test diet)	1	1.2960	14.24	0.000
	Residuals (error)	26	2.358		

* P is the probability of exceeding the given F value by chance.

≠ Days 2, 9, 17, 25, 32.

Appendix Table 27

Analysis of Variance - Hematocrit≠

Source of Variation	df	SS	MS	F-value	P*
Replications (days)	4	65.06	16.27	15.07	0.000
Subjects	7	81.88	11.70	10.84	0.000
Soybean vs rapeseed	1	1.00	1.00	.93	0.344
Residuals (error)	26	28.06	1.08		
Total	38	176.0			

* P is the probability of exceeding the given F value by chance.

≠ Days 2, 9, 17, 25, 32.

Appendix Table 28

Orthogonal Comparisons: Hematocrit≠

NO.	COMPARISON	df	SS	F-value	P*
1	Days 9 and 32 vs Days 17 and 25 (mixed fat vs oils)	1	0.125	0.12	0.732
2	Day 9 vs Day 32 (pre-exp. mixed fat vs post-exp. mixed fat)	1	5.063	4.69	0.039
3	Day 17 vs Day 25 (Exp. Period I vs Exp. Period II)	1	2.250	2.08	0.169
4	Day 2 vs Days 9,17,25 and 32 (Initial vs test diet)	1	57.600	53.36	0.000
	Residuals (error)	26	28.06		

* P is the probability of exceeding the given F value by chance.

≠ Days 2, 9, 17, 25, 32.

Appendix Table 29

Analysis of Variance - Platelet Count ≠

Source of Variation	df	SS	MS	F-value	P *
Replications (days)	4	7935.0	1848.8	2.34	0.082
Subjects	7	73870.0	10552.9	13.34	0.000
Soybean vs rapeseed	1	451.6	451.6	0.57	0.040
Residuals (error)	26	20569.4	791.1		
Total	38	102286.4			

* P is the probability of exceeding the given F value by chance.

≠ Days 2, 9, 17, 25, 32.

Appendix Table 30

Analysis of Variance - Leucocyte Count≠

Source of Variation	df	SS	MS	F-value	P *
Replications (days)	4	16.819	4.205	25.64	0.000
Subjects	7	42.902	6.128	37.37	0.000
Soybean vs rapeseed	1	0.766	0.766	4.67	0.040
Residuals (error)	26	4.264	0.164		
Total	38	64.750			

* P is the probability of exceeding the given F value by chance.

≠ Days 2, 9, 17, 25, 32.

Appendix Table 31

Orthogonal Comparisons: Leucocyte Count≠

No.	Comparison	df	SS	F-value	P *
1	Days 9 and 32 vs Days 17 and 25 (mixed fat vs oils)	1	6.845	41.74	0.000
2	Day 9 vs Day 32 (preexp mixed fat vs postexp. mixed fat)	1	1.556	7.05	0.013
3	Day 17 vs Day 25 (Exp Period I vs. Exp, Period II)	1	3.706	22.60	0.000
4	Day 2 vs Days 9, 17, 25 and 32 (Initial vs test diet)	1	5.112	31.16	0.000
	Residuals (error)	26	16.819		

* P is the probability of exceeding the given F value by chance.

≠ Days 2, 9, 17, 25, 32.

Appendix Table 32

Calculated Fatty Acid Patterns for the Diet Containing Low Erucic Acid Rapeseed Oil

	% OF TOTAL FATTY ACIDS			
	Rapeseed Oil Diet[1]	HiRSO Margarine[2]	LoRSO Margarine	Revised Rapeseed Oil Diet
Lauric C12:0[4]	0.56	--	0.34	0.60
Myristic C14:0	0.36	--	0.09	0.39
Palmitic C16:0	5.86	3.72	4.93	6.01
Stearic C18:0	2.97	6.56	12.28	3.81
Oleic C18:1	52.31	29.27	76.28	58.98
Linoleic C18:2	19.49	4.78	3.67	19.20
Linolenic C18:3[5]	8.05	2.96	0.64	7.65
Eicosenoic C20:1	4.07	12.61	1.14	2.34
Behenic C22:0	0.50	tr	tr	0.49
Erucic C22:1	5.67	35.36	0.59	0.46

[1]As determined by gas chromatography of incorrect composites of menus 1 and 2. These daily composites were correct in all aspects except for the type of margarine used: 25 grams of HiRSO margarine was used; 25 grams of Tower LoRSO margarine should have been used.

[2]High erucic acid rapeseed oil margarine as analysed by King (1974).

[3]Tower low erucic acid rapeseed oil margarine (Table 8).

[4]Carbon number: Number of double bonds.

[5]Not distinguished from C20:0 with the column used to resolve fatty acids.

Appendix Table 33

Canadian Food Usage Patterns

	ANNUAL PER CAPITA USAGE		
	Usage (kg)	Protein(kg)	Cholesterol[1](g)
Meats[2]			
Beef	39.2	5.76	27.6
Pork	23.5	2.47	16.5
Mutton and Lamb	1.8	0.22	1.3
Poultry meat	19.4	3.07	11.3
Canned meat, offal	5.2	0.52	11.5
Eggs[2]	14.8	1.69	81.5
Milk products[2]			
Fluid milk	130.3	4.56	14.3
Other milk solids	12.3	5.16	16.0
Plant protein[2]	--	11.34	0
Fats[3]			
Shortening	8.2	--	0
Margarine	4.5	--	0
Butter	5.7	--	14.1
Lard	2.3	--	2.2
Tallow	0.8	--	.8
Salad Oil	3.2	--	0

[1]Based on Table 4, U.S.D.A. Handbook No. 8, Composition of Foods, Watts and Merrill, 1975.

[2]Based on 1968-69 consumption figures as adapted by Bell, 1975.

[3]Adapted from the Department of Industry, Trade and Commerce Statistics for the 1973 disappearance of fat products in Canada. (Teasdale, 1975).