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EFFECT OF RAPESEED OIL ON WHOLE BLOOD HEMATOLOGY AND SERUM LIPIDS IN YOUNG MEN

by

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ABSTRACT

The effect of rapeseed oil on serum lipid patterns and whole blood hematology was investigated in a 39-day metabolic trial involving 7 healthy male subjects. The study consisted of: 1) a 9-day stabilization period when a mixed fat diet was fed; 2) a 22-day experimental period when rapeseed oil supplied the dietary fat; and 3) an 8-day post experimental period when the mixed fat diet was again fed. The diet, which contained 36 percent of the calories as fat, consisted of ordinary foods except that textured vegetable protein was substituted for meat. Fasting blood samples were taken on Days 1,10,18,25,32 and 39. Sera were analyzed for total cholesterol, lipid phosphorous, triglyceride and phospholipid fatty acid patterns. Mean serum cholesterol levels decreased by 13 mg/100 ml during the initial mixed fat diet, by another 20 mg/100 ml during the first week of the rapeseed oil diet, tended to plateau during the second week of the rapeseed oil period and increased during the last week of the rapeseed oil period and the post-experimental mixed fat diet. The same general pattern was followed by lipid phosphorous and triglycerides. Eicosenoic and erucic acids made up very little of the serum phospholipid fraction (5.4 percent) of subjects after 22 days on the rapeseed oil diet even though these fatty acids comprised 52 percent of the dietary fatty acids. The decrease in saturated fatty acids and increase in oleic acid observed in the phospholipids reflect the low level of saturated and high level of monounsaturated fatty acids in the rapeseed oil diet. Hemoglobin, hematocrit, red cell fragility and red cell, reticulocyte, leucocyte and platelet counts were determined on whole blood. Except for leucocyte and platelet counts which decreased to below normal levels on

the rapeseed oil diet for most subjects, hematological parameters were within normal ranges at all times. Fat biopsies were taken on two subjects on the first and last day of the rapeseed oil period. Small amounts of erucic acid were found in biopsy samples taken after 22 days on the rapeseed oil diet. Other than the decrease in platelet count there was no evidence of any marked or deleterious effect of rapeseed oil in the human.

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INTRODUCTION

Rape is a unique plant in that it produces an oil which has a low content (2 to 7 percent) of saturated fatty acids and a high content of long chain monounsaturated fatty acids, particularly erucic acid (C22:1), which comprises 20 to 55 percent of the oil (Rocquelin and Potteau, 1968). Rape is widely cultivated in European countries such as Poland, Sweden, Germany and France and, since 1948, in Canada as well (Porteous and Johnson, 1970). A great deal of research on rapeseed oil has been undertaken in the past 15 to 20 years partly because of the economic importance of the crop and partly because of the observation that physiologic changes occurred in animals fed rapeseed oil but did not occur in animals fed oils such as corn and soya (Rocquelin et al., 1973)

Feeding relatively high levels of rapeseed oil to young rats was found to depress growth and produce changes in liver lipid composition (Thomasson, 1955). Digestibility of rapeseed oil for the rat was lower than that of other common oils (Deuel et al., 1948) although differences in the metabolism of rapeseed oil have been observed among species. Man, for example, absorbs rapeseed oil very efficiently (Vaisey et al., 1973). Accumulation of lipid in heart muscle of the rat appeared after 3 to 4 days following the inclusion of rapeseed oil in the diet, and in 2 to 6 months histiocyte infiltration, myocardial necrosis and fibrosis were observed (Rocquelin and Potteau, 1968).

While the pathological effects associated with the ingestion of rapeseed oil are undeniable researchers are beginning to question whether erucic acid alone is responsible for the changes observed since low erucic acid rapeseed oil (canbra oil) also has been reported to

produce myocardial changes in experimental animals.

Little work has been reported on the metabolism of rapeseed oil in man. It has been observed that the ratio of erucic acid to other fatty acids in the blood and other tissues of humans is considerably less than that of the diet (Tremolieres et al., 1972). Serum cholesterol and phospholipid levels in man have been reported to be lower when rapeseed oil is fed than when other fats and oils such as coconut oil (Malmros and Wigand, 1957) and butter fat (Grande et al., 1962) were fed. As rapeseed oil is the major edible oil consumed in Canada, it was of interest to examine its effect on serum parameters and whole blood hematology in humans.

REVIEW OF LITERATURE

A. INTRODUCTION

Rape is an oil producing plant found mainly in parts of Asia, Europe and North America (Rocquelin and Potteau, 1968). Two species of rapeseed oil are grown in Canada; Brassica napus or Argentine-type rape, and Brassica campestris or Polish-type rape. Oil from older varieties of these two species is characterized by containing, as compared to other oils such as soya or corn, a large quantity of long chain monounsaturated fatty acids such as erucic acid with 22 carbon atoms and eicosenoic acid with 20 carbon atoms (Table 1). As well,rapeseed oil contains relatively low amounts of saturated fatty acids. The other major fatty acids in rapeseed oil are linolenic, linoleic and oleic acids. Since 1961 Canadian workers have developed varieties of rape with oil containing very low levels of erucic acid and eicosenoic acid and high levels of oleic acid (Stefansson et al., 1961). A comparison of fatty acid composition of rapeseed oil and this new type of oil, commonly called canbra oil, is shown in Table 1.

Rapeseed oil, as it will be discussed here, refers to traditional rapeseed oils sold in Canada until 1973 and not to canbra oil. Canadian rapeseed oils differed from those produced in Europe in that erucic acid made up only about 30 to 40 percent by weight of the fatty acids (Craig, 1970) whereas European oils contain about 50 percent by weight erucic acid (Abdellatif, 1972).

Fats of animal origin usually contain approximately 50 percent saturated fatty acids whereas most vegetable oils contain about 90

Table 1

Comparison of Canadian Rapeseed Oils with Canbra and Other Vegetable Oils[1]

Fatty Acid	Rapeseed Oils		Canbra	Soya	Corn
	B. napus	B. campestris			
Palmitic, C16:0[2]	4.0[3]	3.0	4.7	11.5	12.1
Stearic, C18:0	1.0	2.0	1.8	3.9	2.3
Oleic, C18:1	19.0	27.0	63.8	24.6	28.7
Linoleic, C18:2	14.0	18.0	20.0	52.0	56.2
Linolenic, C18:3	8.0	9.0	8.9	9.0	0.7
Eicosenoic, C20:1	14.0	12.0	1.3	-	-
Erucic, C22:1	40.0	31.0	-	-	-

[1]Data from Craig, 1970.

[2]Carbon number: number of double bonds.

[3]Percentage of total fatty acids.

percent mono- and polyunsaturated fatty acids. Prolonged feeding of vegetable oils to animals at 20 percent by weight of a balanced diet was found to be satisfactory until it appeared that there were physiopathological changes such as depressed growth rate, myocardial alterations and modifications in adrenal and liver lipid composition associated with feeding rapeseed oil at 20 percent by weight of the diet (Thomasson and Boldingh, 1955; Rocquelin and Cluzan, 1968; Abdellatif and Vles, 1970; Beare-Rogers et al., 1971). Extensive reviews of the literature into these changes have recently been written by Rocquelin and Cluzan, (1968); Abdellatif, (1972); LeBlanc, (1973) and Rocquelin et al., (1973).

B. EFFECTS OF RAPESEED OIL ON ANIMAL GROWTH

In general, growth has been found to be depressed in experimental animals fed RSO as compared to those fed other fats and oils. Two main theories have been proposed to explain the depression in growth:
(1) depressed growth is due solely to the presence of erucic acid and
(2) depressed growth is the result of an imbalance of saturated to unsaturated fatty acids.

An early study in support of the first postulate showed that a diet containing nasturtium oil with erucic acid making up 30 percent of the total calories was as effective in depressing growth as a diet containing rapeseed oil where erucic acid made up 29 percent of the total caloric value (Thomasson and Boldingh, 1955). Beare et al. (1959) found that weight gain of rats fed ad libitum diets containing 20 percent rapeseed oil or corn oil supplemented with ethyl erucate was inversely proportional to the erucic acid content of the diets. Canbra oil, which contains little erucic acid, did not depress growth when studied in several animal species (Craig and Beare, 1968; Rocquelin

and Cluzan, 1968). Since canbra has almost the same saturated/unsaturated ratio as regular rapeseed oil, erucic acid has been proposed to be the causative factor in growth depression. There is however some evidence to suggest that growth depression in response to rapeseed oil also may be related to the very high ratio of unsaturated, particularly monounsaturated, to saturated fatty acids (Murray et al., 1958; Beare et al., 1963). Murray et al. (1958) compared the effects of diets containing 5 per cent corn oil, methyl oleate, methyl 11-eicosenoate or methyl erucate on the growth of rats. All 3 of the monoenoic fatty acids reduced growth rate in females in comparison to corn oil but none had any effect on the growth of male rats. Beare et al. (1963) were able to overcome the growth depressing effect of rapeseed oil in rats by increasing the saturated fatty acid content of the diet. They found more rapid growth of rats on a mixture of palm oil and rapeseed oil than on a diet with only rapeseed oil although both diets contained the same amount of erucic acid.

Although both erucic acid and the low ratio of saturated/unsaturated fatty acids in rapeseed oil affect growth, Rocquelin and Potteau (1968) concluded that the effect of erucic acid is predominant.

C. DIGESTIBILITY OF RAPESEED OIL

Fat digestibility is a term which describes the overall availability of dietary fat to an organism (Deuel, 1955). Many studies

with the rat have indicated that the digestibility of rapeseed oil is lower than that of many other common vegetable oils. The main cause of the lowered digestibility is thought to be the high content of erucic acid (Deuel et al., 1948; Carroll, 1958; Rocquelin and Potteau, 1968). Deuel et al. (1948) found the coefficient of digestibility for rapeseed oil for the rat was 77 percent. This low value was believed to be related to poor absorption of the erucic acid fraction. Carroll (1958) demonstrated that the coefficient of digestibility of long chain fatty acids by male rats diminished very rapidly with increasing molecular weight; the digestibility of long chain monoeonic fatty acids such as erucic acid was lower than that of shorter chain monoenoic fatty acids such as oleic acid. Deuel's results were subsequently confirmed by Rocquelin and Potteau (1968) who found the coefficient of digestibility of traditional rapeseed oil was 77.6 percent whereas the coefficient of digestibility of canbra oil was 95.5 percent. As discussed previously the primary difference between these two oils is that the erucic acid of rapeseed oil is replaced by oleic acid in canbra oil.

There are, however, very distinct differences between the results obtained from digestibility studies with the rat and with man. Digestibility of rapeseed oil by the human was found to be excellent (99 percent) when it provided 25 percent of dietary calories (Deuel, 1949). Similar observations have been reported by Vaisey et al. (1973). These results would suggest that species differences exist with respect to utilization of rapeseed oil.

D. PHYSIOLOGICAL EFFECTS OF RAPESEED OIL

D.1. Heart High levels of rapeseed oil in the diet of experimental animals have been found to produce specific pathological effects in

certain organs, especially the heart. In addition, the oil caused modifications in myocardial lipid patterns of several animal species (Abdellatif and Vles, 1970; Beare-Rogers, 1970; Beare-Rogers et al., 1971; Beare-Rogers et al., 1972a). In the rat there was a buildup of heart lipid which was followed by histiocyte cell infiltration that was maximal at 16 weeks. This was followed by interstitial fibrosis of the myocardium, an irritation due to the continued supply of erucic acid through the interstitium, which was maximal at 64 weeks (Abdellatif and Vles, 1970, 1970a; Beare-Rogers, 1970). However, Dallocchio et al. (cited by Rocquelin et al., 1973) have found that a diet containing a high level of rapeseed oil will cause immediate but temporary lipid infiltration of the myocardium followed by a histiocyte reaction visible on the fifteenth day. Peanut and canbra oils had no effect during the same period. Considerable evidence points to erucic acid being responsible for the pathogenicity of rapeseed oil (Abdellatif and Vles, 1970a; Beare-Rogers, 1970; Rocquelin et al., 1973). Lesions similar to those produced by rapeseed oil were found in rats and ducklings fed isocaloric diets containing the same amounts of erucic acid and glyceryl trierucate (Abdellatif and Vles, 1970a). In fact, Beare-Rogers (1970) has shown that other long chain docosanoic acids such as that of herring oil produced cardiac lipidosis in the rat and that severity of lipidosis was proportional to the amount of oil in the diet.

Rocquelin et al. (1970) have expressed doubt that erucic acid is the sole pathogenic factor in rapeseed oil as they encountered cases of histocyte cell infiltration in the myocardium of rats fed canbra oil for 2 to 6 months. The lesions were less severe in the group fed canbra

oil than in the group fed rapeseed oil, and no fatty accumulation was observed. Nonetheless, these observations cannot be satisfactorily explained. Lall et al. (1972) observed some vacuolization and lipid infiltration of the cardiac cells of chickens fed diets containing either 20 percent by weight of rapeseed oil (37 percent erucic acid) or 20 percent by weight of canbra oil although the effect was much less marked with the canbra oil.

Of interest is the fact that both rapeseed oil and canbra oil have a low ratio of saturated/unsaturated fatty acids. In some cases increasing the saturation of the diet seemed to have an ameliorating effect on the physiopathological changes accompanying feeding of erucic acid (Beare et al., 1963; Beare-Rogers, 1970). Rocquelin et al. (1970) also tested the hypothesis that the unfavorable ratio might be responsible for the myocardial lesions. They formulated various fat mixtures with the ratio of saturated/unsaturated fatty acids found in rapeseed oil. Myocarditis was observed only in rats fed a triglyceride mixture containing some erucic acid. Thus it would seem that erucic acid was responsible for the rapeseed oil-induced lesions.

It is not possible from research reported thus far to satisfactorily explain metabolic disorders responsible for the myocarditis. Incorporation of eicosenoic, erucic or cetoleic acids into rat diets has been accompanied by an accumulation of lipids in the myocardium (Beare-Rogers et al., 1972b). This cardiac lipidosis is characterized by an accumulation of triglycerides with a high erucic and eicosenoic acid content (Houtsmuller et al., 1972). The extent of incorporation of

erucic and eicosenoic acids into myocardial lipids varied with the quantity of erucic acid in the diet, with the length of the period the diet was fed, and with the animal species being studied. The percentage of these two acids increased in the heart as the percentage of erucic acid increased in the diet (Beare Rogers et al.,1971). These changes were transitory and decreased with time irrespective of the level incorporated into the diet (Beare-Rogers et al., 1972a). While detection of the presence of lipid globules in myocardial cells of the pig is possible only by use of the electron microscope (Rocquelin et al., 1973), the gerbil accumulates lipids in the myocardium comparable to accumulation seen in the rat (Beare-Rogers et al., 1972b). In addition to its presence in the triglyceride fraction, erucic acid is also found in free fatty acids, cholesterol esters and phospholipids of myocardial lipid fractions (Beare-Rogers et.al.,1972a).

D.2. Adrenals and Other Tissues The adrenal glands normally contain a relatively high concentration of lipid, and cholesterol esters constitute a substantial portion of the lipids. This is probably related to the requirement for a reservoir of cholesterol for the biosynthesis of steroid hormones. It has been shown however that diets containing 25 percent of rapeseed oil cause a three-to-four fold increase of the cholesterol content of rat adrenal glands (Carroll 1951; 1953). The administration of nervonic acid led to similar elevation of cholesterol in the adrenals (Table 2)(Carroll, 1953; Walker, 1972). The differences observed among erucic, nervonic and oleic acids was even more significant when it is taken into consideration that digestibility of nervonic acid was 25 percent while the digestibility of erucic acid was 55 percent

Table 2

Effects of Monoenoic Fatty Acids on the Cholesterol Content of the Adrenal Glands in Rats[1]

Fatty acids	Amount of fatty acids in the diet	Weight of the rats	Weight of the adrenal	Total cholesterol in the adrenals
	percent	gm.	mg.	mg.
Oleic acid	20	178	26.2	2.30
Methyl eicosanoate	20	160	27.2	2.28
Methyl erucate	20	137	35.1	5.16
Methyl erucate	10	168	35.1	4.15
Nervonic acid	15	170	38.1	4.65
Nervonic acid	10	162	34.2	3.08

[1] Data from Carroll, 1953

and digestibility of oleic acid was 85 percent. The total quantity of fat in the adrenals was not greatly changed but the composition was altered. Thus the accumulation of cholesterol appears to be at the expense of other lipid fractions. Carroll (1957) also found the feeding of rapeseed oil to mice increased the cholesterol content of the adrenals but had no effect on cholesterol levels in the adrenals of cats, dogs, rabbits, guinea pigs or chicks. The accumulation of cholesterol appears to be related to the marked incorporation of erucic acid into the steroid ester fraction of the adrenals in response to the feeding of rapeseed oil. Thirty-four percent of fatty acids esterified with cholesterol in the adrenal glands of rats were accounted for by erucic acid when a diet containing 15 percent by weight of erucic acid was fed for three weeks (Carroll, 1962a). Thus the accumulation of cholesterol erucate is largely responsible for the high cholesterol content of these glands. Erucic acid also was present in the triglycerides (11 percent) and phospholipids (1.2 percent) of the adrenal lipids. Similar results were reported by Walker et al. (1972).

In the plasma, erucic acid was found mainly in the triglycerides (17.8 percent) with smaller quantities in the cholesterol esters (3.5 percent) and phospholipids (5.7 percent)(Walker et al., 1972). Walker et al. (1972) also noted that the amount of oleic acid was increased.

The liver is another organ in which erucic acid is found in the lipid fraction, the amount incorporated again being proportional to that found in the dietary oil although the concentration of erucic acid in the liver lipid was much lower than in the diet (Kramer, 1973). As with the adrenals the total amount of fat did not change but composition

of the lipids changed. There was significantly lowered saturated fatty acid content and an appreciably elevated unsaturated fatty acid concentration in the liver. Carroll (1953) and Carroll and Noble (1956) found an accumulation of cholesterol in the liver and increased cholesterol in the feces when erucic acid was fed at levels of 10 to 15 percent of the diet. The diets used by Carroll contained essentially no cholesterol. In experiments utilizing acetate-1-^{14}C, Carroll (1959) found that the primary effect of erucic acid in the liver was a stimulation of cholesterol synthesis. Increased fecal excretion of cholesterol was thus likely due to the increased synthesis.

E. METABOLISM OF RAPESEED OIL

Recent reviews on the metabolism of rapeseed oil and its effect on various animal tissues are available (Rocquelin and Potteau, 1968; Abdellatif, 1972; LeBlanc, 1973; Rocquelin et al., 1973). In brief, it has been reported that the ratio of erucic acid to other fatty acids in the blood and other tissues of animals fed rapeseed oil is considerably less than that of the diet (Hopkins et al., 1957; Carroll, 1962a; Craig and Beare 1967). These authors have suggested that significant increases in the amounts of oleic acid in the plasma, adrenals, kidney and heart of animals fed rapeseed oil indicates that erucic acid undergoes degradation with elimination of 2-carbon units from the carboxyl end of the chain. Boucrot and Bezard (cited by Rocquelin et al., 1973) and Lecerf (cited by Rocquelin et al., 1973) studied the metabolic conversion of 14-^{14}C erucic acid in the livers of rats that had ingested diets containing 15 percent by weight of rapeseed oil for 8 to 60 days. They found that erucic acid was converted not only to oleic acid but also to longer and shorter homologues.

Bach et al. (1969) demonstrated that oxidation of erucic acid proceeded at the same rate as the oxidation of oleic acid as evidenced by the specific activity of carbon dioxide expiration. On the other hand it has been reported by Abdellatif (1972), Clouet et al. (cited by Rocquelin et al., 1973) and Lemarchal et al. (1973) that erucic acid is not as well oxidized by the mitochondria of the heart and that it is therefore incorporated into triglycerides, the lipid in which most erucic acid is found in the heart during lipidosis. Erucic acid is also found at low levels in free fatty acids and phospholipid fractions in the myocardium (Rocquelin et al., 1973; Houtsmuller, 1970).

Erucic acid may inhibit the oxidation of other fatty acids such as palmitic acid since erucic acid causes other fatty acids to accumulate in cardiac triglycerides as well (Beare-Rogers et al., 1972b; Christopherson and Bremer , 1972). Christopherson and Bremer (1972) have postulated that a general blockage in the mitochondrial oxidation of fatty acids may be caused by erucyl-CoA or the early intermediate products of B-oxidation of erucic acid. Clouet et al. (cited by Rocquelin et al., 1973) have reported that there is a recovery in the ability of the heart mitochondria to oxidize fatty acids during the first two months on a rapeseed oil diet. The heart thus adapts by utilizing the accumulation of lipid in heart muscle of rats fed rapeseed oil. Houtsmuller et al. (1972) noted a decrease in A T P production and a marked decrease in the rate of oxygen consumption with substrates such as glutamate, oxaloglutarate, decanoate, succinate and malate when 50 calories per cent rapeseed oil was incorporated into the diet. In a subsequent experiment they found that values for rate of oxygen uptake were inversely proportional to levels of erucic acid in the heart

and suggested that the ability of rat heart mitochondria to oxidize substrates is decreased when increasing amounts of rapeseed oil are fed. These effects are not specific for erucic acid, but are also caused by its homologues eicosenoic acid (C20:1) and nervonic acid (C24:1). However, Dallochio et al. (cited by Rocquelin et al., 1973) did not find any significant changes in the activity of ATP-ases in the hearts of rats fed rapeseed oil. Houtsmuller et al. (1972) also found a slowdown in the transport of long-chain acylcarnitines at the level of the mitochondrial membrane in rats fed rapeseed oil. It may be at this stage that erucic acid triggers the metabolic disorders of lipidosis, histiocyte infiltration and fibrosis of the cardiac cell. Rocquelin et al. (1970) have also studied the effect of the low saturated/monounsaturated ratio of fatty acids in rapeseed oil on the myocardium. They found that lesions were more frequent and severe in animals fed rapeseed oil or synthetic fat mixtures containing erucic acid than was found in rats fed synthetic fat mixtures containing the same ratio of saturated/monounsaturated fatty acids but no erucic acid. Beare-Rogers et al. (1972a) fed synthetic oils and found that increasing the amount of saturated fatty acids in the diet did not prevent the erucic acid induced fat accumulation in heart tissue. Erucic acid thus appears to have a major effect on myocardial lesions although the effect of saturated to monounsaturated ratios has not been definitively resolved.

Ultimately the concern with rapeseed oil is for its effect on man. Research regarding metabolism of rapeseed oil in man is limited. Tremolieres et al. (1972) gave 0.5 g/kg of body weight of 47.3 percent erucic acid rapeseed oil to 9 subjects. The oil and a control (peanut oil) were fed to fasted subjects. They noted that erucic acid in the

plasma reached a peak 2½ hours after feeding for 4 of 9 subjects but was still rising 3½ hours after ingestion in another 4 subjects. Tremolieres et al. (1971) also found that feeding the same amount of rapeseed oil causes a significant reduction in the respiratory quotient in subjects at rest. More research with animals and man is obviously required to clarify our understanding of the metabolism of rapeseed oil.

F. EFFECTS OF RAPESEED OILS ON BLOOD LIPID PATTERNS IN MAN

Very little research has been reported on the effects of rapeseed oils on blood lipid patterns in either animals or man. Feeding rapeseed oil or erucic acid at 10 to 15 calories percent of the diet had little effect on plasma cholesterol (Carroll and Noble, 1956; Beare et al., 1959a; Carroll, 1962a) or phospholipid levels (Carroll, 1962a) of experimental animals. With human subjects, Grande et al. (1962) reported that mean serum cholesterol and phospholipid levels did not differ when subjects were fed 32 percent of the calories as rapeseed oil (24 percent erucic acid) or a mixture of corn and olive oil but increased when the subjects were fed the same amount of butter. Malmros and Wigand (1957) observed a decrease in serum cholesterol of 40 mg/100 ml in subjects after one week of feeding on a diet containing 40 calories percent rapeseed oil (50 percent erucic acid). LeBlanc (1973) investigated the effect of canbra oil on serum lipid patterns of male subjects fed a diet containing 38 percent of the calories as fat. The substitution of canbra oil for mixed fat in the diet was accompanied by a substantial decrease in serum cholesterol and lipid phosphorous and a slight decrease in serum triglycerides. Reversion to the mixed fat diet was accompanied by an increase in level of all three parameters.

Tremolieres et al. (1972) found that a single dose of 0.5 g/kg of body weight of rapeseed oil was accompanied by a significant elevation in erucic, eicosenoic and linolenic acids in the plasma although the levels of the latter fatty acids were lower that those of erucic acid. Erucic acid and eicosenoic acid were present in the plasma mainly in the form of triglycerides. No erucic acid was found in the plasma phospholipids during the 24 hour observation period.

Abdellatif and Vles (1970b) found an accumulation of erucic acid in the membranes of erythrocytes of guinea pigs that were fed for 8 weeks a diet containing 25 calories percent rapeseed oil. They suggested that the diet might account for hemolytic tendencies observed. On the other hand Tremolieres et al. (1972) analyzed blood only a few hours after feeding the rapeseed oil but found no accumulation of erucic acid in red blood cells. It is difficult to draw conclusions on the effect of rapeseed oil on blood lipid patterns in the human from the limited number of studies that have been reported.

OBJECT OF RESEARCH

Because we cannot investigate the actual consequence of rapeseed oil on organs such as the heart in man, it is essential that exhaustive research with regard to its effect on measurable parameters such as blood and feces be done if we are in any way to extrapolate results from animal studies and apply them even with extreme caution to our own species. At this time hematological and organ studies on experimental animals are far from complete, and in man they have hardly begun.

The objective of the present study was to investigate the effect of rapeseed oil on (a) serum lipid patterns and (b) whole blood constituents of normal male subjects when rapeseed oil provided about 36 percent of the total calories in a mixed diet.

EXPERIMENTAL METHODS

A. EXPERIMENTAL DESIGN

The study consisted of a 39-day metabolic trial conducted in May and June, 1973 and included 3 periods.

a) An initial 9-day stabilization period throughout which a mixed fat diet was fed.

b) A 22-day experimental period when the subjects received a rapeseed oil diet.

c) An 8-day follow up period when subjects were again fed the mixed fat diet.

Subjects resided in their own homes but were served meals in the Home Economics Building, University of Manitoba. If desired by the individual, Saturday and Sunday lunch and dinner were packaged for home preparation. Meals were served at the customary hours but with some flexibility to accomodate individual requirements. In addition to the meals, subjects were given 3 snack items daily and were instructed to consume no other foods.

Two daily menus were alternated during both the mixed fat period and the experimental period. Fixed recipes as described by LeBlanc (1973) were followed in the preparation of all food items except that a high erucic acid rapeseed oil (39 percent erucic acid) was substituted for canbra oil.

Subjects were weighed daily before breakfast. When necessary, individual caloric intake was adjusted to maintain body weight.

Fasting venous blood samples were obtained at 8:00 AM on Days 1,10,18,25,32 and 39. Fat biopsies were taken on two volunteer subjects on the first and last day of the rapeseed oil diet.

B. SUBJECTS

Seven healthy college males, ages 20-27 (median age 25 years), were chosen from volunteers who responded to notices advertising the study. They were of average height and weight (Table 3) with no diagnosed metabolic disorders or recent history of poor health. One subject did not serve successfully and was replaced on the fifth day of the initial stabilization period by subject R.D. As a consequence, subject R.D. was fed the mixed fat diet for only 4 days prior to the experimental period.

C. DIET COMPOSITION

The two menus (Table 4) were planned to utilize textured vegetable protein (T.V.P.)[1], fresh skim milk and spray dried egg albumen as the primary source of protein. Three different forms of T.V.P. (Table 5) were used in place of the meat normally found in the main entrées: hamburger patties, meat balls, sweet and sour pork and beef stew. The entrées and snack items were prepared in advance, frozen and stored at -10°C until required. No changes in flavor, texture or appearance were observed as a result of freezing and storage.

The menus were calculated to provide approximately 3000 calories and were designed to meet all nutrient allowances as recommended in the Canadian Dietary Standard. Calorie and nutrient content of the diet is shown in Table 6. The calculated nutrient content of the individual menus

[1]Trade name for Textured Vegetable Protein. Archer Daniels Midland Co. 733 Marquette Ave., Minneapolis, Minnesota 55440.

Table 3

Height and Weight Data of Subjects

Subject	Age (yr)	Height (cm)	Weight (Kg)	
			Initial	Over 21 - Day Experimental Period
R.U.	26	183	68.6	64.8 ± 0.8[1]
M.Q.	23	170	76.7	76.0 ± 0.8
T.T.	20	180	75.8	74.7 ± 1.2
C.G.	25	173	72.6	72.7 ± 1.0
S.K.	25	183	67.7	67.5 ± 0.5
R.A.	23	170	63.1	63.3 ± 1.2
R.D.	27	180	76.7	76.2 ± 1.1

[1] Mean ± S.D. for 39 daily weighings.

Table 4

Composition of Diets

Menu I	Menu II
Breakfast[1,2]	
Orange juice	Apple juice
Cooked rolled oats[3]	Cooked cream of wheat[3]
Scrambled egg albumen[3]	Scrambled egg albumen[3]
Sliced white bread	Slice white bread
Strawberry jam or orange marmalade	Strawberry jam or orange marmalade
White or brown sugar	White or brown sugar
Butter or margarine[4]	Butter or margarine[4]
Skim milk	Skim milk
Lunch[1,2]	
Hamburger patties[3]	Spaghetti/meatballs/tomato sauce[3]
Hamburger bun	Slice white bread
Coleslaw[3]	Tossed salad[3]
Coleslaw dressing[3]	Piquant salad dressing[3]
Canned fruit with juice[5]	Canned fruit with juice[5]
Skim milk	Skim milk
Dinner[1,2]	
Sweet and sour pork[3]	Beef stew[3]
Rice[3]	Mashed potato[3]
Tossed salad[3]	Coleslaw[3]
Piquant salad dressing[3]	Coleslaw dressing[3]
Slice white bread	Slice white bread
Canned fruit with juice[5]	Canned fruit with juice[5]
Skim milk	Skim milk
Snacks[1,2]	
Spicy fruit square, uniced[3]; white cupcake[3]; raisin oatmeal cookies[3]	

[1] Coffee and tea allowed *ab. lib.*

[2] For quantities of each item see Diet Calculation, Appendix tables 1,2,3,4.

[3] Recipes from LeBlanc, 1973.

[4] 11.0 gm butter as spread per day for Menu I Mixed Fat Diet; 20.0 gm butter for Menu II Mixed Fat Diet; 14.0 gm HEAR margarine for Menu I Experimental Diet; 20.0 gm HEAR margarine for Menu II Experimental Diet.

[5] Pears, apricots, pineapple, peaches or plums.

Table 5

Typical Analysis of TVP[1]

Content, %	Beef Strips, #10	Beef Chunks, #15	Pork Chunks, #10
Moisture	5 - 6.5	5 - 6.5	5 - 6.5
Fat	0.7 - 0.9	0.6 - 0.9	0.6 - 0.9
Protein	51 - 53	48 - 50	50 - 53
Carbohydrate	35.3 - 29.6	35.4 - 31.6	33.4 - 26.6
Fiber	2 - 3	2.5 - 2.7	2.5 - 2.7
Minerals	9 - 10	11 - 13	11 - 13
Salt	3.0	5.0	4.5
MSG	3.0	3.5	1.0
Vitamins per 100 grams			
Thiamin	0.21 mg	0.34 mg	0.34 mg
Riboflavin	0.42	1.26	*
Niacin	2.42	2.02	*
Vitamin B_6 (pyridoxol)	0.70	1.29	*
Pantothenic acid	1.30	1.35	*
Folic acid	0.30	0.32	*
Inositol	270	280	*
Vitamin B_{12} (cobalamin)	under 0.50	under 0.50	under 0.50
Minerals, %			
Phosphorous	0.64	*	*
Calcium	0.20	*	*
Iron	60 ppm	*	*
Sodium	1.50	*	*
Potassium	2.45	*	*
Magnesium	2.50	*	*
Copper	19.50 ppm	*	*
Zinc	55.70 ppm	*	*

[1]Analysis data supplied by Archer Daniels Midland Co., 733 Marquette Ave., Minneapolis, Minnesota 55440.

*No values available at present.

Table 6

Calculated Nutrient Composition of Diets[1]

Composition	Diet				
	Mixed Fat		Experimental		
	Menu Day I	Menu Day II	Menu Day I	Menu Day II	Recommended[2]
Calories	2963	2944	2977	3025	2850
Protein(g)	98.3	103.2	95.4	103.5	38.0
Fat(g)	119.6	117.9	119.5	118.9	-
Carbohydrate(g)	383.2	404.6	389.9	389.3	-
Calcium(mg)	1164	969	1166	965	500
Phosphorous(mg)	1132	1095	1170	1091	-
Iron(mg)	8.90	21.00	9.35	21.72	6.0
Vit. A(I.U.)	4495	13341	4041	12404	3700
Thiamin(mg)	1.42	1.77	1.47	1.8	0.9
Riboflavin(mg)	2.66	2.78	2.64	2.72	1.4
Niacin(mg)	8.26	14.88	8.28	14.88	9.0
Vit. C(mg)	140	177	139	174	30

[1]Values determined using USDA Hamdbook #8 Composition of Foods (Watt and Merril, 1963).

[2]Based on Revised Dietary Standard for Canada (1964). Values given for males, 158 lb (70 kg), activity level A.

is presented in Appendix Tables 1,2,3 and 4.

In addition to the prescribed diet, soy sauce, Worchestershire sauce, ketchup and sweet pickle relish were available. When additional calories were required in order to maintain body weight items such as marshmallows, sugar and bread were added to the diet and the fat intake altered to maintain the calories from fat at 36 percent of the total.

D. FAT COMPOSITION OF THE DIET

About 36 percent of the daily calories were derived from fat. Composition of fat in the mixed fat diet was designed to closely resemble the amount and composition of fat in an average North American diet. Details of the development of the formulation have been described by LeBlanc (1973). The proportion of each fat used in the mixed fat diet is shown in Table 7. A major portion of the fat was incorporated into a variety of menu items with butter available as the spread. Fatty acid composition of a composite mixture of these fats is shown in Table 8.

The experimental diet was analogous to the mixed fat diet except that rapeseed oil and rapeseed oil margarine were substituted for the fats contained in the mixed fat diet.

E. SOURCE OF TEST FAT

Source and description of test fats used in the mixed fat diet are listed in the footnotes of Table 7. High erucic acid rapeseed oil[2] and specially prepared rapeseed oil margarine[3] were utilized as the principle fats in the experimental diet.

[2]Capri Vegetable Oil, Canada Packers Ltd., St. Boniface, Manitoba.

[3]Co-op Margarine, made for Interprovincial Co-operatives Ltd., Winnipeg, Manitoba, by Canada Packers Ltd., St. Boniface, Manitoba.

Table 7

Composition of Fat Mixture

Ingredients	Percent
Butter[1]	39.3
Corn oil[2]	21.5
Tallow[3]	7.1
Parkay Margarine[4]	10.7
Lard[5]	14.3
Crisco shortening[6]	7.1

[1]Lucerne Brand, Canada Safeway Ltd., Winnipeg, Manitoba.

[2]Mazola, Best Foods Division, Canada Starch Co. Ltd., Montreal Quebec.

[3]Bleached, clarified, deodorized, Canada Packers Ltd., St. Boniface, Manitoba.

[4]Parkay Brand, Kraft Foods Limited, Montreal 101, Quebec.

[5]Tenderflake Brand, Canada Packers Ltd., Toronto, Ontario.

[6]Crisco, Proctor and Gamble, Toronto, Ontario.

Table 8

Fatty Acid Composition of Fat Mixture

Fatty Acid	% of Total Fatty Acids
Octanoic, C8:0[1]	0.37
Decanoic, C10:0	0.87
Lauric, C12:0	0.88
Myristic, C14:0	3.86
Tetradecenoic, C14:1	0.70
Palmitic, C16:0	21.41
Palmitoleic, C16:1	1.78
Stearic, C18:0	11.02
Oleic, C18:1	37.27
Linoleic, C18:2	21.14

[1]Carbon Number: number of double bonds.

F. STORAGE OF DIETARY FATS AND OTHER FOOD ITEMS

Fats for both the mixed fat diet and the experimental diet were purchased in single lots and stored in sealed containers. Non-perishable food items were similarly bought in single lots and stored at appropriate temperatures. Fresh skim milk was purchased 3 times weekly, and a weekly supply of both bread (frozen until required) and produce were bought from a single local source. All foods prepared in advance were stored at $-10^{o}C$.

G. ANALYSIS OF MENUS

A daily composite of each menu was made during the mixed fat and experimental periods. Procedures used in preparation of the samples for analysis, extraction of total lipid, and fatty acid analysis were essentially the same as those reported by LeBlanc (1973). In brief, the meals for one day were composited, homogenized in a one-gallon capacity blender, and an aliquot freeze-dried for analysis. Fat was extracted using the method of Bligh and Dyer (1959). Fatty acid methyl esters, prepared using BF_3 as a catalyst (Metcalfe, 1966) were resolved with an Aerograph gas chromatograph[4] fitted with 2.7 m x 3.2 mm steel columns packed with 10 percent EGSS-Y organosilicone on 100/120 mesh GAS CHROM Q[5].

Energy content of the diets was determined using a Parr Adiabatic Bomb Calorimeter equipped with a # 1241 automatic type calorimeter and a # 1541 water heater[6]. Values determined by bomb calorimetry were in

[4]Model 1740-1, Varian Aerograph, 6358 Viscount Rd. Malton, Ontario.

[5]Applied Science Laboratories Inc., P.O. Box 440, State College, Pennsylvania 16801.

[6]Model U30M, Parr Instrument Co., 211 Fifty-third St. Moline, Illinois 61625.

close agreement with calculated values (Table 9).

H. BLOOD INVESTIGATION

Fasting blood samples were taken at 8:00 AM on Days 1,10,18,25, 32 and 39. Subjects were instructed to fast for 10 hours prior to having the blood samples taken. Blood was drawn from the anticubital vein of each person into three 10 ml BD Vacutainer tubes (#4710)[7]. A 3 ml sample (BD Vacutainer tube # 4854)[7] also was drawn. The latter was used for whole blood analysis.

Blood in the 10 ml tubes was allowed to clot at room temperature for one hour and in a refrigerator for a further 30 to 40 minutes. The Vacutainer tubes were then centrifuged[8] at 1400 x g for 10 minutes and the sera removed. The sera was centrifuged at 1400 x g for 5 minutes to remove any contaminating red cells and the clear sera was pipetted into clean 10 ml test tubes. The sera was separated into 2 ml lots and stored at $-4^{o}C$ for further analysis. Each vial was flushed with nitrogen before storage.

Sera from each subject was analyzed in duplicate for total cholesterol, triglyceride content and lipid phosphorous by the methods described below. A standard[9] was run with each set of determinations. Serum phospholipids were precipitated from acetone and the fatty acid composition determined by gas-liquid chromatography.

[7]# 4710 Canlab Laboratory Equipment, Winnipeg, Manitoba.

[8]Model HN-2368 P-2, Centrifuge, International Equipment Co., Needham Hts., Massachusetts.

[9]Moni-trol I, Lot # 112, 135103, Dade Division, American Hospital Supply Corp., Miami, Florida 33152.

Table 9

Total Daily Fat and Energy Intake

	Mixed Fat		Experimental	
	Menu Day 1	Menu Day 2	Menu Day 1	Menu Day 2
Fat (g):				
Calculated	119	118	120	119
Analyzed[1]	114	110	105	110
Energy:				
Calculated[2]	2963	2944	2977	3025
Analyzed[3]	2918	3062	3018	3031

[1]Analyzed using the procedure of Bligh and Dyer (1959).

[2]Calculated values, expressed in Kilocalories, using USDA Handbook #8 Composition of Foods (Watt and Merril, 1963).

[3]Results obtained by bomb calorimetry expressed in kilocalories.

Hemoglobin, hematocrit, red blood cell fragility and red blood cell, reticulocyte, leucocyte and platelet counts were determined on the whole blood.

H.1. Chemical Analysis.

H.1.a. Serum

i. Total Cholesterol. The method of Pearson et al. (1952; 1953) was used to determine total cholesterol. This method utilizes a modified Liebermann-Burchard reaction which consists of treating the serum with acetic acid, p-toluenesulfonic acid, acetic anhydride and sulfuric acid.

ii. Lipid Phosphorous. Phospholipids were extracted according to the method of Chen et al. (1956) except that only 0.2 ml of serum was used for the extraction of lipid. All determinations were made using a Coleman Junior spectrophotometer.[10]

iii. Serum Triglycerides. The method of Ryan and Rasho (1967) was used for extraction of the serum triglycerides except that only 0.1 ml of serum was used for each determination. The saponification and color reaction was done by the method of Van Handel and Zilversmit (1957) with the modification suggested by Van Handel (1961). A Unicam SP 600 Series 2 spectrophotometer[11] was used to read the samples.

[10]Model # 6A-36715, Coleman Instrument Inc., Maywood, Illinois.

[11]Model # 46511, Pye Unicam Ltd., York St., Cambridge, CB12 PX, England.

iv. Phospholipid Fatty Acid Patterns. The procedure of Folch et al. (1956) was used to extract the total lipid from 1.0 ml of serum. The phospholipids were precipitated according to the method of Beare-Rogers (1969). Methyl esters of the fatty acids were prepared for analysis by the method of Barnes and Halloday (1972) except that only 0.50 ml of 0.5 N methanolic NaOH was used. The stoppered vials were heated in a waterbath at 80°C for 5 minutes, cooled, and 0.25 ml of BF_3/CH_3OH solution added directly to each vial. The contents were reheated at 80°C for 3 minutes. After the vials had cooled, NaCl solution (1.5 ml) was added and the methyl esters extracted by shaking with 0.50 ml of n-hexane. The hexane layer was removed, concentrated under nitrogen and injected directly in the gas-liquid chromatograph.

The fatty acid methyl esters were resolved by gas chromatography as previously described under section G for Analysis of Menus. Linear-log plots of carbon number versus retention time and comparison with known standards[12] were used to identify the fatty acid methyl esters.

H.1.b. Whole Blood Parameters

Hemoglobin, hematocrit, red blood cell fragility, and red blood cell, reticulocyte, leucocyte and platelet counts were measured. All analyses of whole blood parameters except red cell fragility were carried out by the Hematological Laboratories, Health Sciences Centre, Winnipeg.

i. Red Cell Fragility. Red blood cell fragility was determined according to the method described by Lynch et al. (1969) except that only visual notation was made of the concentration at which hemolysis began and the concentration at which hemolysis appeared complete.

[12]Hormel Institute, Lipids Preparation Laboratory, 801-16th Ave. N.E., Austin, Minnesota 55912.

H.2. Statistical Analysis

Whole blood and serum parameter data were subjected to analysis of variance for a completely randomized block design according to Snedecor and Cochran (1967). The Treatments sum of squares were utilized in a t-test to establish which of the treatments were significantly different from one another. Data for total serum cholesterol and lipid phosphorous were fit to response curves using the method of Snedecor and Cochran (1967) to find the pattern of change that occurred during the rapeseed oil period.

I. FAT BIOPSIES

Fat biopsies were taken on the abdomen of two volunteer subjects on the first and last day of the rapeseed oil diet. Samples were taken at the Health Sciences Centre, Winnipeg, and stored under hexane until analyzed. For analysis the hexane was dried off and the samples saponified and esterified according to the method of Metcalfe et al. (1966). Fatty acid patterns were resolved as in Section G Analysis of Menus.

RESULTS AND DISCUSSION

A. SUBJECTS

All subjects remained in good health throughout the study. No digestive upsets occurred, in contrast to observations of Tremolieres et al. (1971) who noted diarrhea with subjects given a single dose of approximately 30 g of rapeseed oil on an empty stomach. The reason for the difference between this experiment and that of Tremolieres et al. (1971) is probably due to the method of administering the fat; ingestion in a mixed fat diet versus a single oral dose of pure oil.

Flatulence was a problem for most subjects but was not attributed to dietary fat as it was equally prevalent in the mixed fat diet as the rapeseed oil diet. Flatulence was attributed to the relatively high amount (28.5 to 37.5 g/day) of TVP in the diet. This product contains the oligosaccharides raffinose, verbascose and stachyose which ferment in the gut and markedly increase the volume of intestinal gas. The only other subject complaint was a general listnessness. Repetition of the 2-day alteration menu for 39 days may have been a factor.

Body weight remained essentially constant during the experiment (Table 3) and changes observed in serum lipid patterns were attributed to dietary fat sources.

B. DIETARY FAT

Fatty acid patterns were very similar for Menus I and II for both the mixed fat diet and the rapeseed oil diet (Table 10). Saturated fatty acids constituted an average of 38 percent of the total, monounsaturated another 41 percent, and polyunsaturated fatty acids 21 percent in the mixed fat diet. In contrast, the rapeseed oil diet contained only about 7 percent saturated fatty acids but 76 percent monounsaturated. Polyunsaturated fatty acids accounted for the other 17 percent. Both the rapeseed oil and the rapeseed oil margarine contained relatively low levels of palmitic and stearic acids and ligh levels of eicosenoic and erucic acids (Table 11). The major difference in fatty acid composition between the mixed fat and rapeseed oil diets was in the amounts of these four fatty acids that they contained (Table 12). The mixed fat diet contained higher levels of palmitic and stearic acids, little eicosenoic acid and no erucic acid. The ratio of saturated to unsaturated fatty acids was 1:1.6 in the mixed fat diet while the saturated to monounsaturated ratio was 1:1.1. In the rapeseed oil diet the ratios were 1:12.9 saturated to unsaturated fatty acids and 1:10.6 saturated to monounsaturated fatty acids.

C. SERUM PARAMETERS

C.1. Serum Cholesterol

Changes in serum cholesterol are presented in Tables 13 and 14 and Figure 1. Statistical analyses of these changes are presented in Appendix Tables 5 and 7. There was a decrease in serum cholesterol level of 13 mg/100 ml of blood during the pre-experimental mixed fat period and a further significant decrease in serum cholesterol of 20 mg/100 ml of blood.

Table 10

Percent Fatty Acid Composition of Diets

Fatty Acid	Mixed Fat		Rapeseed Oil	
	Menu I	Menu II	Menu I	Menu II
Myristic, C14:0[1]	3.15	3.40	0.11	0.10
Palmitic, C16:0	20.30	19.95	4.12	3.88
Palmitoleic, C16:1	1.82	1.79	0.21	0.21
Stearic, C18:0	13.11	12.17	3.06	2.99
Oleic, C18:1	38.19	37.26	23.34	22.79
Linoleic, C18:2	17.11	19.26	11.64	11.21
Linolenic, C18:3	1.35	1.45	4.92	5.02
Eicosenoic, C20:1	0.50	0.49	12.72	12.98
Erucic, C22:1	-	-	39.05	39.19

[1]Carbon number: number of double bonds.

Table 11

Percent Fatty Acid Composition of Rapeseed Oil and Rapeseed Oil Margarine

Fatty Acid	Oil	Margarine
Palmitic, C16:0[1]	3.33	3.45
Palmitoleic, C16:1	0.20	0.27
Stearic, C18:0	1.80	6.56
Oleic, C18:1	21.24	29.27
Linoleic, C18:2	12.81	4.78
Linolenic, C18:3	6.80	2.96
Eicosenoic, C20:1	14.00	12.61
Erucic, C22:1	38.77	35.36

[1]Carbon number: number of double bonds.

Table 12

Primary Differences in Fatty Acid Composition
Between Mixed Fat and Rapeseed Oil Diets

Fatty Acid	Percent Composition	
	Mixed Fat	Rapeseed Oil
Myristic, C14:0[1]	3.3	0.1
Palmitic, C16:0	20.1	4.0
Stearic, C18:0	12.6	3.0
Oleic, C18:1	37.7	23.1
Linoleic, C18:2	18.2	11.4
Linolenic, C18:3	1.4	4.9
Eicosenoic, C20:1	0.5	12.9
Erucic, C22:1	-	39.1
Ratio, Sat.:Unsat.	1:1.6	1:12.9
Ratio, Sat.:Monounsat.	1:1.1	1:10.6

[1]Carbon number: number of double bonds.

Table 13

Serum Cholesterol of Subjects During Experiment[1]

Subject	Day of Experiment					
	1	10[2]	18	25	32[2]	39
R.U.	181	161	155	138	145	176
M.Q.	204	170	158	168	216	193
T.T.	176	178	157	153	158	170
C.G.	213	175	179	174	197	204
S.K.	232	237	209	215	221	264
R.A.	205	184	158	151	155	162
R.D.	187	203	156	155	148	194
Group Mean	200	187	167	165	177	195

[1] mg cholesterol/100 ml of serum.

[2] Days on which dietary regimen was changed. Diets included:
1) mixed fat diet, Days 1-9 inclusive and Days 32-39 inclusive, and
2) rapeseed oil diet, Days 10-31 inclusive.

Table 14

Changes in Serum Cholesterol Level

Subject	Experimental Period					
	Mixed Fat	Rapeseed Oil Diet				Mixed Fat
	Day 1 vs 10	Day 10 vs 18	Day 18 vs 25	Day 25 vs 32	Overall Day 10 vs 32	Day 32 vs 39
R.U.	-20[1]	- 6	-17	+ 7	-16	+21
M.Q.	-34	-12	+10	+48	+46	-23
T.T.	+ 2	-21	- 4	+ 5	-20	+12
C.G.	-38	+ 4	- 5	+23	+22	+ 7
S.K.	+ 5	-28	+ 6	+ 6	-16	+43
R.A.	-21	-26	- 7	+ 4	-29	+ 7
R.D.	+16	-47	- 1	- 7	-55	+46
Group Mean	-13	-20	- 2	+12	-10	+18

[1] mg cholesterol/100 ml of serum.

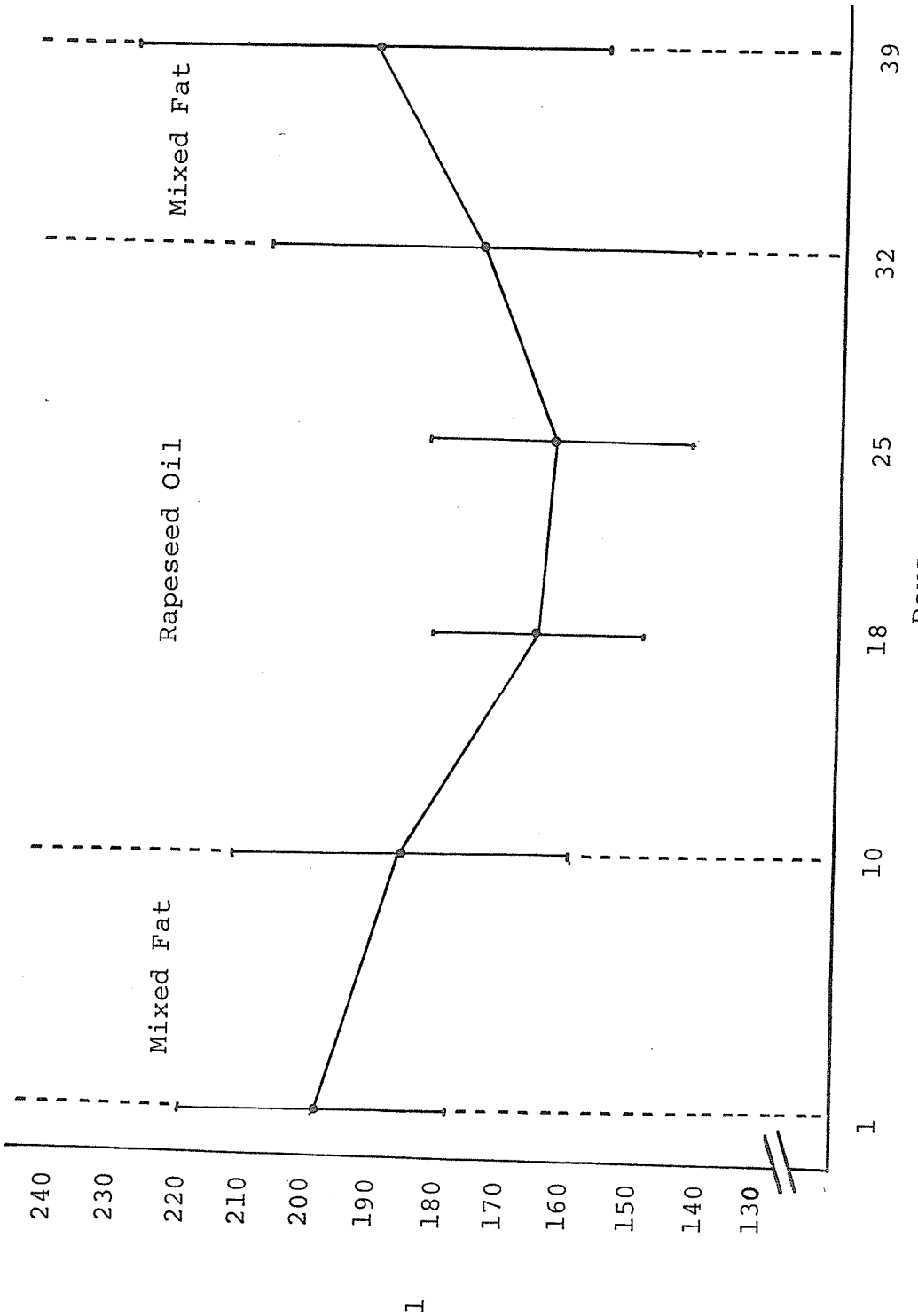

Figure 1. Mean serum cholesterol levels of subjects during experiment.

level in the first week of the rapeseed oil diet (Table 14; Appendix Table 5). Serum cholesterol values tended to plateau during the second week of the rapeseed oil diet (Day 18 vs 25, Table 14) and then rose 12 mg/100 ml of blood during the last week on the same diet (Day 25 vs 32, Table 14). The overall effect of the rapeseed oil diet during the 22-day period was a non-significant decrease in serum cholesterol of 10 mg/100 ml of blood. When the experimental data was fit to a response curve according to the method of Snedecor and Cochran (1972) it was found to have a significant quadratic component ($p = .05$). This is in contrast to observations of LeBlanc (1973) who found a significant ($p = .05$) linear decrease in serum cholesterol levels over the entire 22-day experimental period when canbra oil was fed. In LeBlanc's study the overall decrease was 30 mg/100 ml of blood. In both studies reversion to the mixed fat diet was accompanied by a significant increase in serum cholesterol to levels similar to those observed on Day 10. The changes in pattern of response during the experimental period may thus be attributed to the dietary fat.

The pattern of change of serum cholesterol on the rapeseed oil diet is analogous to observations of Malmros and Wigand (1957) who noted that cholesterol values dropped by 40 mg/100 ml of blood during the first week,plateaued during the second week, and rose by approximately 10 mg/100 ml of blood during the third week on a diet in which 40 percent of calories were supplied by rapeseed oil containing 50 percent erucic acid.

Keys et al. (1965) have suggested that changes in serum cholesterol are directly related to the percent of dietary calories contributed by the different groups of fatty acids. Their equation expressing the

expected change in serum cholesterol level in response to a change in dietary fat may be summarized as follows:

$$\Delta C = 1.2(2\Delta S' - \Delta P)$$

where ΔC is the change in cholesterol level in mg/100 ml of blood,

$\Delta S'$ is the change in total C_{12} to C_{16} saturated fatty acids,

ΔP is the change in total polyunsaturated fatty acids with $\Delta S'$ and ΔP expressed as a percent of total calories in the diet.

The decrease in cholesterol of 10 mg/100 ml of blood in the present study was considerably less than the 30 mg/100 ml of blood decrease observed by Malmros and Wigand (1957) and LeBlanc (1973) for subjects fed rapeseed and canbra oils respectively over a similar 3-week experimental period. It is also 6 mg/100 ml of blood less than the 16 mg/100 ml of blood change predicted by the equation of Keys et al. (1965) (Table 15). However Grande et al. (1962) found that rapeseed oil gave a mean serum cholesterol value 8 mg/100 ml of blood greater than that predicted by the same equation and suggested that the small difference indicated that the 20 and 22 carbon monoenoic fatty acids of rapeseed oil, like other monoenoic fatty acids, have little effect on serum cholesterol level.

C.2. Lipid Phosphorous

As with cholesterol, serum phosphorous values decreased on the mixed fat diet with a further significant decrease during the initial 2 weeks of the rapeseed oil diet, and then increased during the third week of the rapeseed oil diet. Lipid phosphorous also increased during the post-experimental mixed fat diet (Tables 16,17; Figure 2; Appendix Tables 5,8). The response of lipid phosphorous during the 39-day trial

Table 15

Change in Serum Cholesterol in Response to Change in Dietary Fatty Acids

Diet	% of Total Dietary Fatty Acids		% of Total Calories from fat	Change Expressed as % Total Calories in Diet	
	S'[1]	P[2]		S'[1]	P[2]
Mixed Fat	24.2	19.6	36.0	8.71	7.06
Rapeseed Oil	4.1	16.3	36.0	1.48	5.87

According to Keys et.al., 1965: $\Delta C = 1.2(2\,\Delta S' - \Delta P)$

$= 1.2(2(-7.23) - (-1.19)$

$= -15.92$

[1] S' : total saturated fatty acids minus stearic acid.

[2] P : total polyunsaturated fatty acids.

Table 16

Serum Lipid Phosphorous Levels of Subjects During Experiment[1]

Subject	Day of Experiment					
	1	10[2]	18	25	32[2]	39
	mg lipid phosphorous/100 ml serum					
R.U.	10.1	9.8	7.2	7.1	6.5	8.1
M.Q.	9.3	7.2	6.0	6.9	7.5	8.8
T.T.	9.5	6.8	6.8	6.5	6.5	7.1
C.G.	12.4	7.9	8.9	7.5	8.8	10.8
S.K.	10.6	8.4	7.6	7.8	8.0	12.0
R.A.	7.7	7.0	8.2	5.7	5.9	7.9
R.D.	9.7	7.6	6.8	4.8	6.5	7.5
Group Mean	9.9	7.8	7.4	6.6	7.1	8.9

[1]Mean of duplicate analyses.

[2]Days on which dietary regimen was changed. Diets included:
1) mixed fat diet, Days 1-9 inclusive and Days 32-39 inclusive, and
2) rapeseed oil diet, Days 10-31 inclusive.

Table 17

Changes in Serum Lipid Phosphorous Levels

Subject	Experimental Period					
	Mixed Fat	Rapeseed Oil Diet				Mixed Fat
	Day 1 vs 10	Day 10 vs 18	Day 18 vs 25	Day 25 vs 32	Overall Day 10 vs 32	Day 32 vs 39
	mg lipid phosphorous/100 ml serum					
R.U.	-0.3	-2.6	-0.1	-0.6	-3.3	+1.6
M.Q.	-2.1	-1.2	+0.9	+0.6	+0.3	+1.3
T.T.	-2.2	0.0	-0.3	0.0	-0.3	+0.6
C.G.	-4.5	+1.0	-1.4	+1.3	+0.9	+2.1
S.K.	-2.2	-0.8	+0.2	+0.2	-0.4	+4.0
R.A.	-0.7	+1.2	-2.5	+0.2	-1.1	+2.0
R.D.	-2.1	-0.8	-2.0	+1.7	-1.1	+1.0
Group Mean	-2.0	-0.5	-0.7	+0.5	-0.7	+1.8

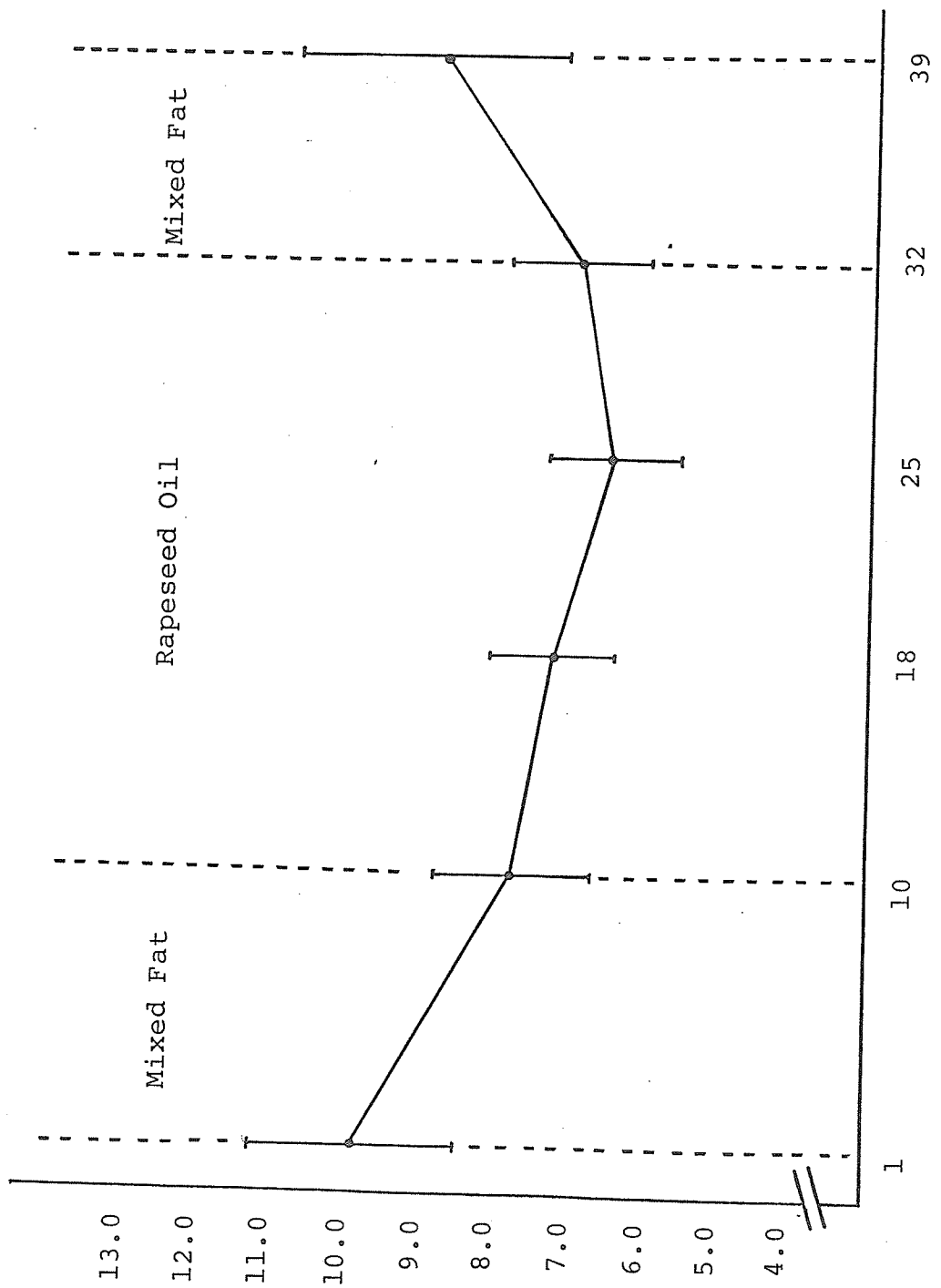

Figure 2. Mean serum lipid phosphorous levels of subjects during experiment.

thus coincides with reports of Erickson et al. (1964), Mc Gandy et al. (1970) and the observation of LeBlanc (1973) with canbra oil that the pattern of change of serum lipid phosphorous in response to a change in diet composition is similar to that of serum cholesterol. Although the overall pattern of response during the 39-day study was similar for cholesterol and lipid phosphorous there were several distinct differences between the two parameters. When fit to a response curve according to the method of Snedecor and Cochran (1972) lipid phosphorous data, in contrast to cholesterol data, did not have a significant quadratic component ($p = .05$). With serum cholesterol the major decrease occurred during the first week on the rapeseed oil diet whereas with lipid phosphorous the greater decrease occurred during the second week of the rapeseed oil diet (Figures 1 vs 2). As observed with serum cholesterol, levels of serum lipid phosphorous on Day 32 did not differ significantly from Day 10 (Appendix Table 5) which is in contrast to observations of LeBlanc (1973) with canbra oil where significant differences did occur in lipid phosphorous levels between the first and last day of the rapeseed oil diet. As with cholesterol, on both this and the canbra oil study reversion to the mixed fat diet resulted in a significant increase in serum phospholipid (Day 32 vs 39, Appendix Table 5). The changes in pattern of response during the experimental period may thus be attributed to the dietary fat.

C.3. Serum Triglycerides

The overall pattern of response of serum triglycerides to diet was similar to that observed for serum cholesterol and lipid phosphorous (Tables 18,19; Figure 3; Appendix Table 5). Mean serum

Table 18

Serum Triglyceride Levels of Subjects During Experiment[1]

Subject	Day of Experiment					
	1	10[2]	18	25	32[2]	39
	mg triglyceride/100 ml serum					
R.U.	140	91	65	59	71	66
M.Q.	123	52	76	98	99	74
T.T.	-[3]	99	69	75	120	83
C.G.	107	128	80	98	92	146
S.K.	108	237	122	120	101	129
R.A.	161	106	69	56	107	182
R.D.	186	93	112	67	101	66
Group Mean	138	115	85	82	99	107

[1]Mean of duplicate analyses.

[2]Days on which dietary regimen was changed, Diets included:
1) mixed fat diet, Days 1-9 inclusive and Days 32-39 inclusive, and
2) rapeseed oil diet, Days 10-31 inclusive.

[3]Blood sample lost. Group mean is for 6 subjects only.

Table 19

Changes in Serum Triglyceride Level

Subject	Experimental Period					
	Mixed Fat	Rapeseed Oil Diet				Mixed Fat
	Day 1 vs 10	Day 10 vs 18	Day 18 vs 25	Day 25 vs 32	Overall Day 10 vs 32	Day 32 vs 39
	mg triglyceride/100 ml of serum					
R.U.	- 49	- 26	- 6	+12	- 20	- 5
M.Q.	- 71	+ 24	+22	+ 1	+ 47	-25
T.T.	-[1]	- 30	+ 6	+45	+ 21	-37
C.G.	+ 21	- 48	+18	- 6	- 36	+54
S.K.	+129	-115	- 2	-19	-136	+28
R.A.	- 55	- 37	-13	+51	+ 1	+75
R.D.	- 93	+ 19	-45	+34	- 16	+ 8
Group Mean	-23	- 30	- 3	+17	-16	+ 8

[1]Blood sample lost. Group mean is for 6 subjects only.

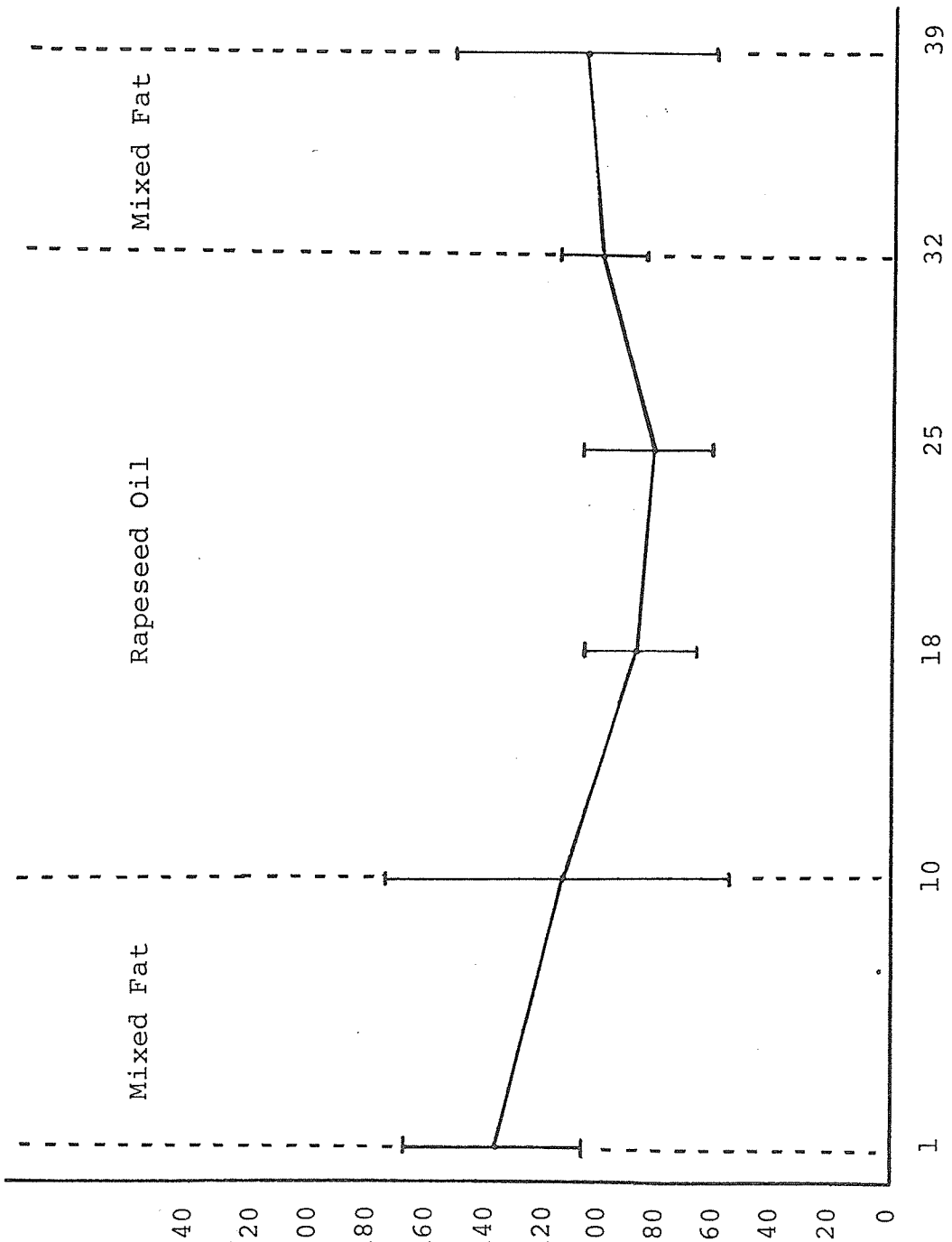

Figure 3. Mean serum triglyceride levels of subjects during experiment.

triglyceride levels dropped from Day 1 to Day 25 and increased from Day 25 to Day 39. However, the only significant change in serum triglyceride levels in response to dietary treatment was the decrease which occurred at Day 10 vs 25 (Appendix Table 5). As with serum cholesterol, serum triglyceride levels plateaued at this decreased level between Day 18 and Day 25 (Table 19; Figure 3).

Considerable variation was found among subjects and the variability was significant (Appendix Table 9). This is in accord with observations of Losier (1972) and LeBlanc (1973) who found variation in serum triglyceride levels among subjects to be significant when corn oil and canbra oil were fed.

Grande et al. (1972) have suggested that stearic acid will produce an elevation in serum triglycerides. Although the mixed fat diet in the present study contained 4 times as much stearic acid as the rapeseed oil diet (Table 12), there was a greater increase in serum triglycerides during the last week of the rapeseed oil diet than during the post-experimental period when the mixed fat diet was again fed (Table 19). Furthermore, the magnitudes of change in triglyceride levels in the present study were much greater than those observed by LeBlanc (1973) although the differences in dietary stearic acid levels between the mixed and experimental fats were similar in the two studies. It would have been interesting to see if serum cholesterol, lipid phosphorous and triglyceride levels continued to rise had the rapeseed oil diet been fed for a longer period of time.

C.4. Phospholipid Fatty Acid Patterns

The percent fatty acid composition of serum phospholipid fractions is shown in Table 20. A substantial increase in oleic acid was observed in response to the rapeseed oil diet. This change coincided with the high amount of monoenoic fatty acids in rapeseed oil. Although the long chain monoenoic fatty acids, eicosenoic and erucic, comprised approximately 52 percent of the dietary fatty acids (Table 10) only 3.9 percent of eicosenoic acid and 1.5 percent of erucic acid were present in the serum phospholipid fraction after 22 days on the rapeseed oil diet. Walker (1972) also noted low levels of erucic and eicosenoic acids in the serum phospholipids of rats fed 30 calories percent rapeseed oil. A decrease in palmitic and stearic acids in the present study in response to the rapeseed oil diet coincided with the lower levels of these acids in this diet than in the mixed fat diet. A similar increase in oleic acid and decrease in stearic and palmitic acids was noted on the canbra oil study (LeBlanc, 1973). All fatty acids returned to pre-rapeseed oil levels when the mixed fat diet was again fed.

D. WHOLE BLOOD PARAMETERS

Individuals vary considerably in their levels of different blood constituents. Normal hemoglobin values may vary from 13.5 to 18.0 g/100 ml of blood, hematocrit from 40 to 54 percent and red blood cell count from 4.5 to 6.5 million/cu mm of blood (Dacie and Lewis, 1963). Similarly there is a wide normal range for reticulocytes, 0.2 to 2 percent of the red blood cells; for leucocytes, 4 to 10 thousand/cu mm; and for platelets, 150 to 300 thousand/cu mm of blood (Dacie and Lewis, 1963). Thus for all of these parameters individuals may differ by more than 25

Table 20

Percent Fatty Acid Composition of Serum Phospholipids

Fatty Acid[1]	Day 10[2]	Day 32[2]	Day 39
Palmitic, C16:0	30.2	21.4	31.3
Palmitoleic, C16:1	1.5	1.4	-
Stearic, C18:0	14.9	12.0	16.1
Oleic, C18:1	14.3	23.7	14.2
Linoleic, C18:2	26.9	24.4	26.4
Linolenic, C18:3	-	1.0	tr.
Eicosenoic, C20:1	sl. tr.	3.5	1.8
Eicosatrienoic, C20:3	2.0	1.6	2.2
Arachidonic, C20:4	7.5	6.2	6.4
Erucic, C22:1	-	1.5	-

[1]Carbon number: number of double bonds.

[2]Days on which dietary regimen was changed. Diets included:
1) mixed fat diet, Days 1-9 inclusive and Days 32-39 inclusive, and
2) rapeseed oil diet, Days 10-31 inclusive.

percent and still be considered normal. Figures 4 to 8 show the mean levels of individual subjects for 5 blood components over the 39-day experiment. It is interesting to note that the level of each component for an individual, relative to the level of the same component for the other subjects was suprisingly consistent.

D.1. Hemoglobin, Hematocrit and Red Cell Count

Hemoglobin is an iron-containing pigment which occurs in the red blood cells and is commonly used to assess the physiological state of these cells. Hematocrit is the percent of blood volume comprised by the cells. One would thus expect hemoglobin, hematocrit and red cell count to follow similar trends over a period of time as was the case in the present study (Figures 9 to 11). There was a significant decrease for all three parameters between Day 10 and Day 18 and a significant increase between Day 18 and Day 25. Values decreased again for all three parameters between Day 25 and Day 32 but the decreases were statistically significant ($p = 0.05$) only for hematocrit and red cell count. There was a significant overall decrease for the three parameters between Day 10 and Day 32 and a non-significant increase between Day 32 and Day 39 (Tables 21 to 26 ; Appendix Tables 6,10,11,12). Diet alone does not appear to explain the changes observed. If the diet were the only factor affecting the changes one would have expected either a progressive increase or decrease during the experimental period. However, in the present study values for hemoglobin, hematocrit and red cell count decreased from Day 10 to 18, increased from Day 18 to 25 and then decreased again from Day 25 to 32. Other factors such as water retention, dehydration after muscular activity, muscular activity itself and different emotional states also

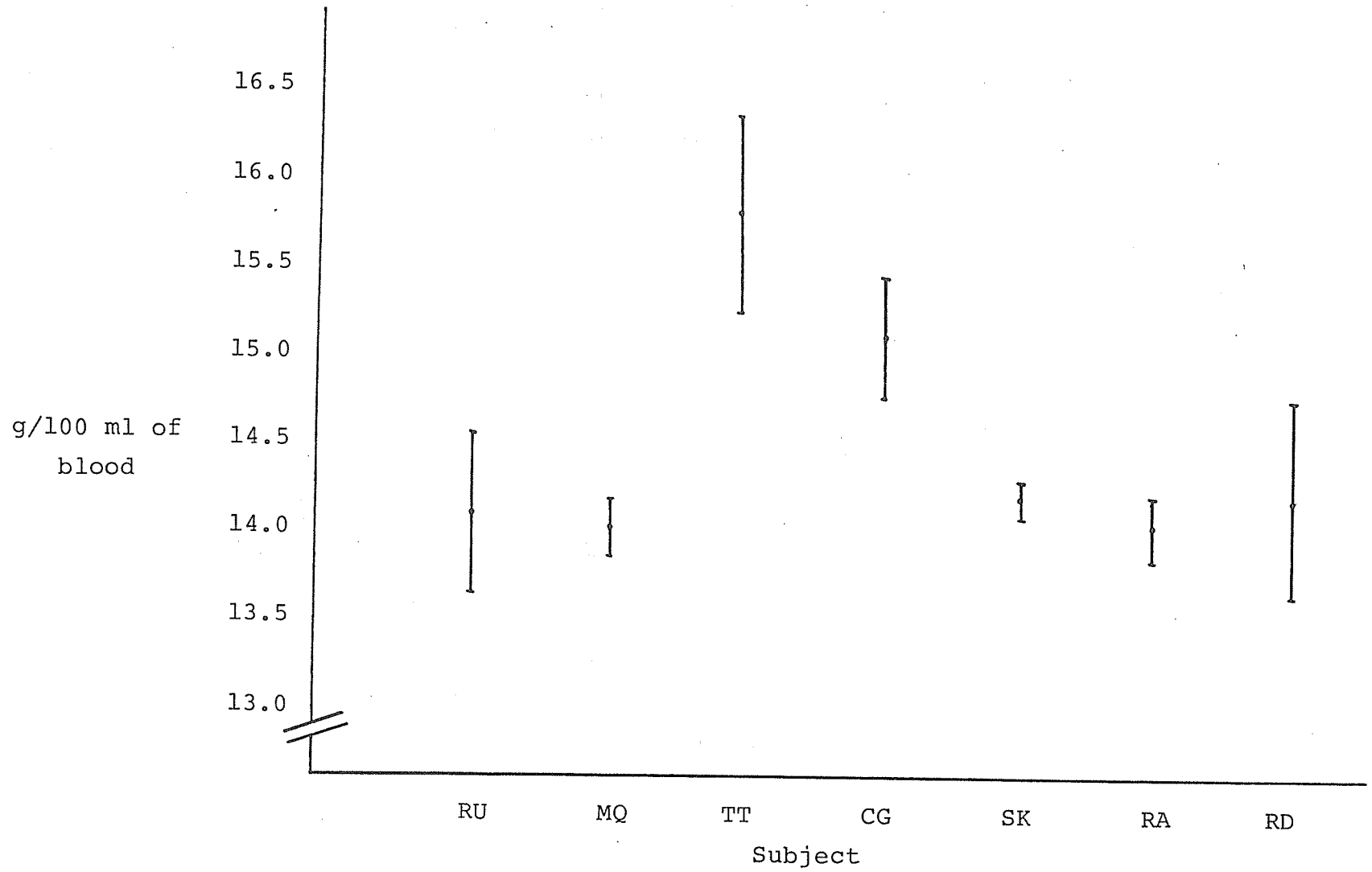

Figure 4. Mean hemoglobin levels of individual subjects over the 39-day experiment.

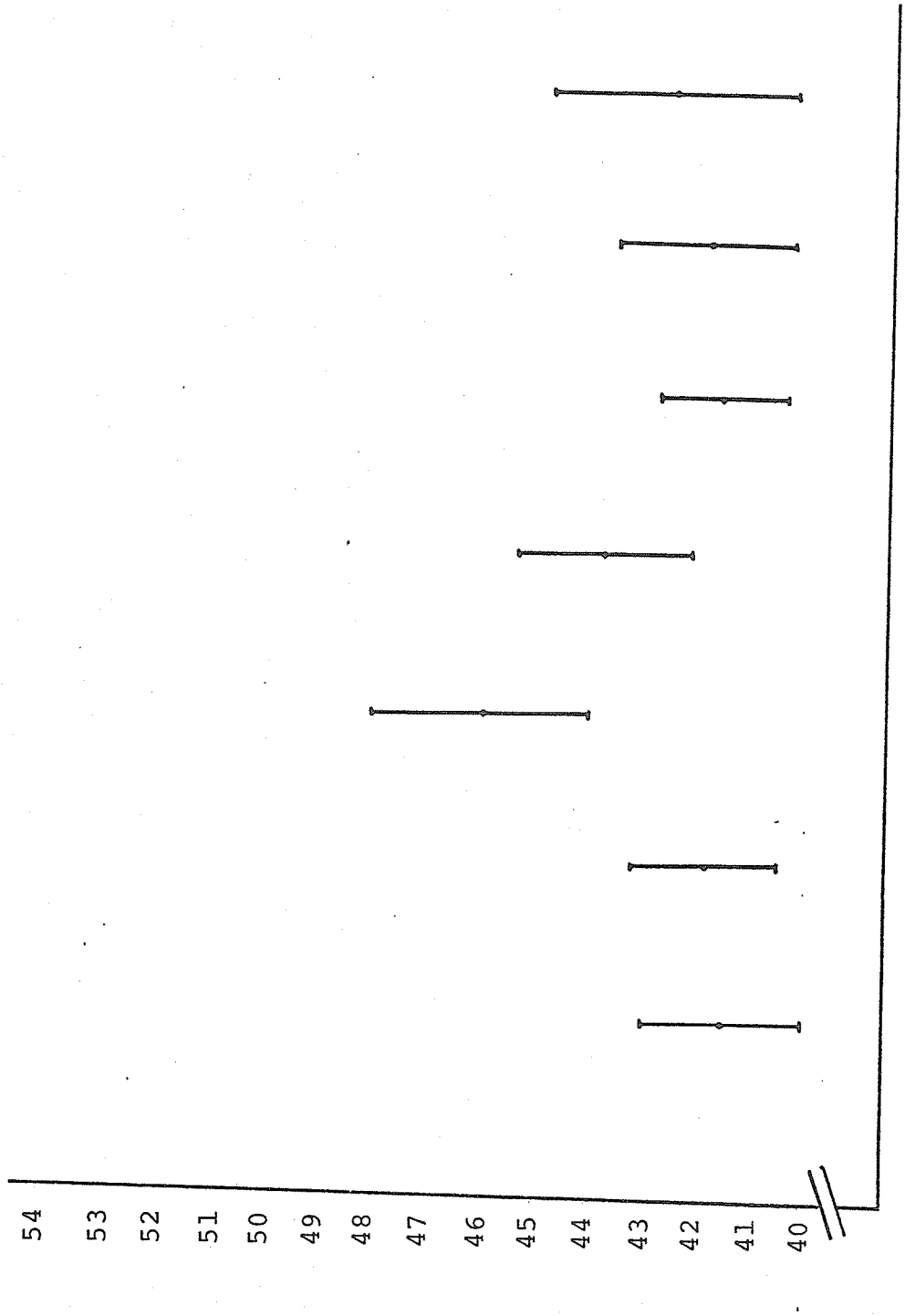

Figure 5. Mean hematocrit levels of individual subjects over the 39-day experiment.

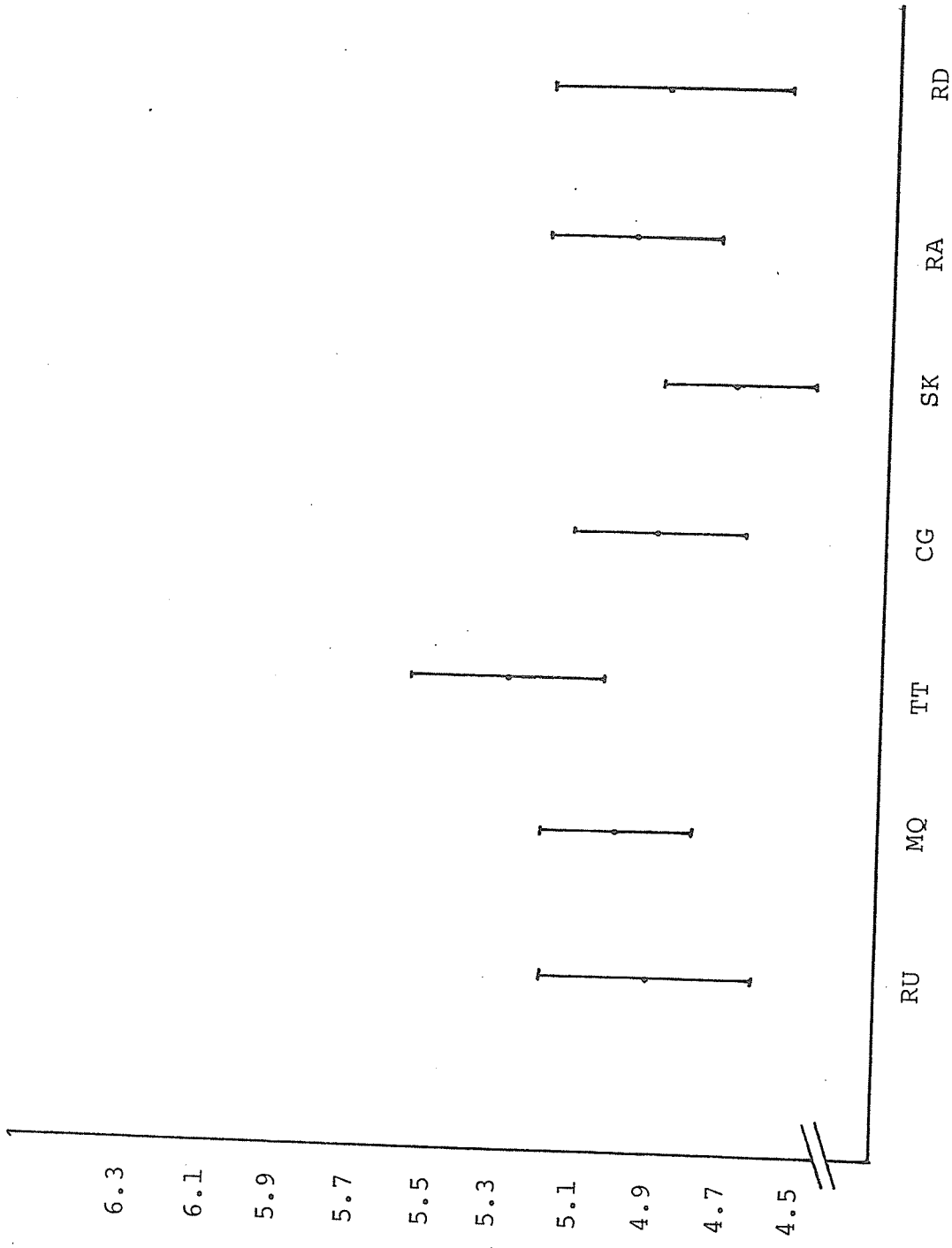

Figure 6. Mean red blood cell counts of individual subjects over the 39-day experiment.

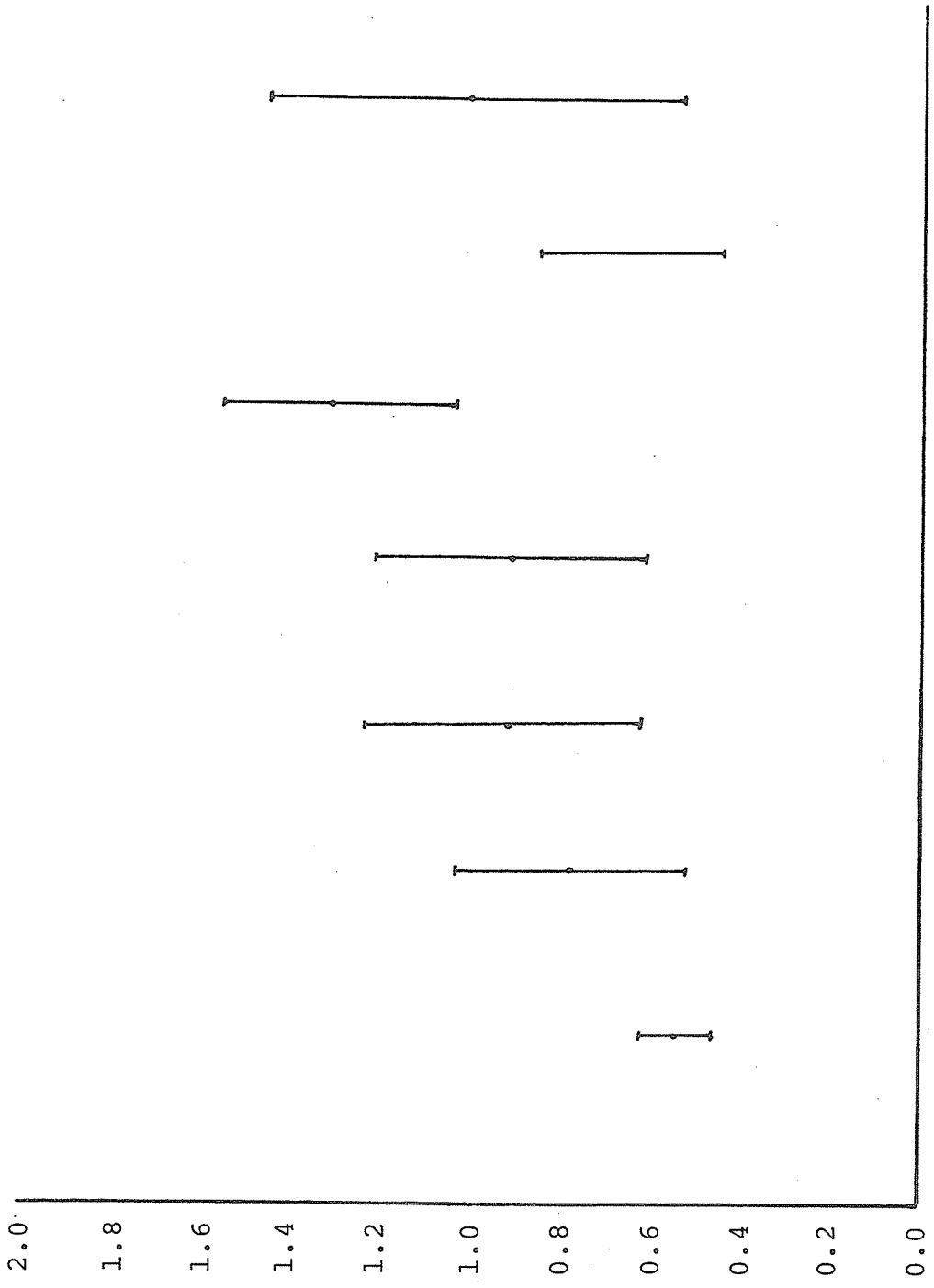

Figure 7. Mean reticulocyte counts of individual subjects over the 39-day experiment.

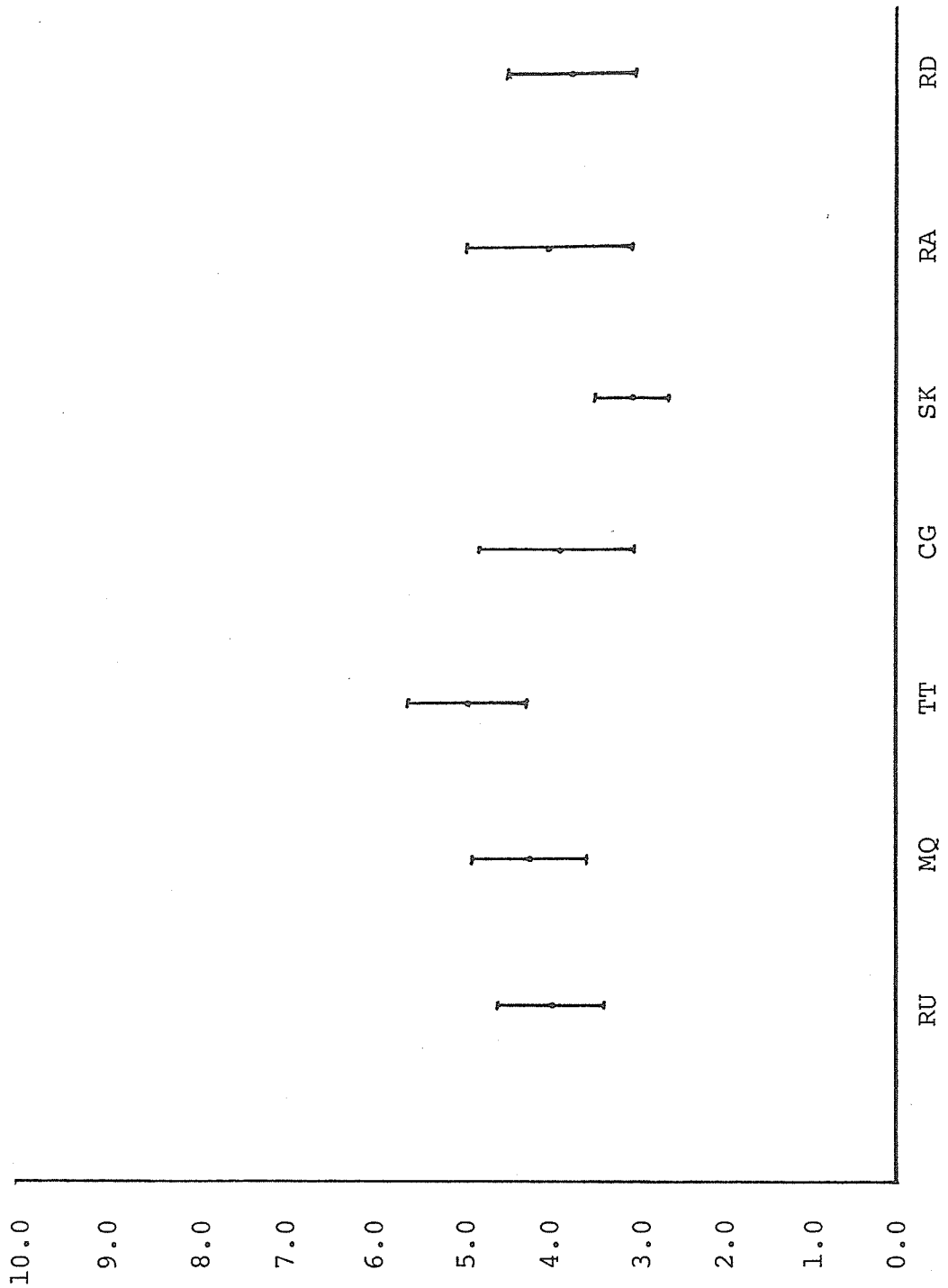

Figure 8. Mean leucocyte counts of individual subjects over the 39-day experiment.

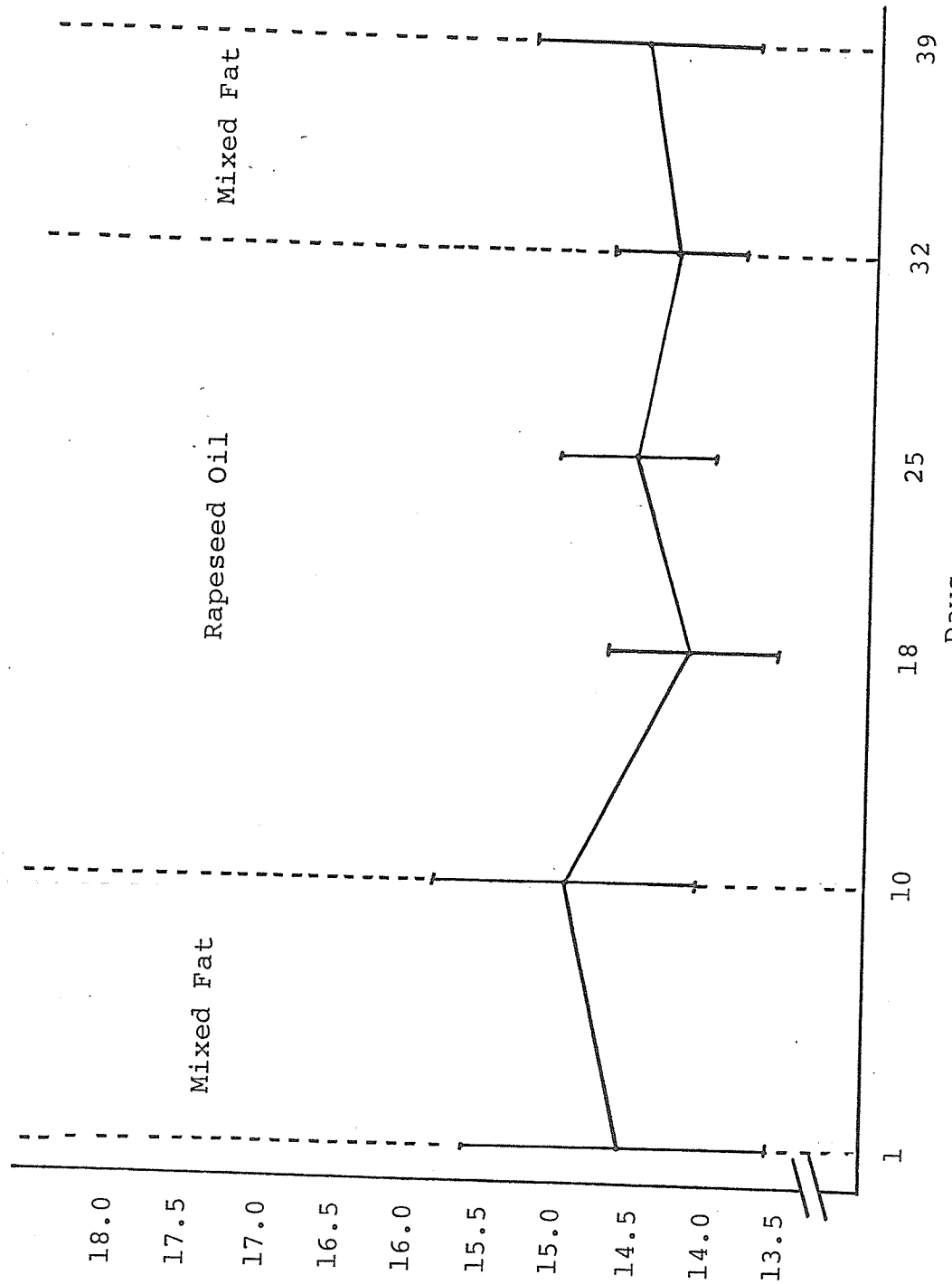

Figure 9. Mean hemoglobin levels of subjects during experiment.

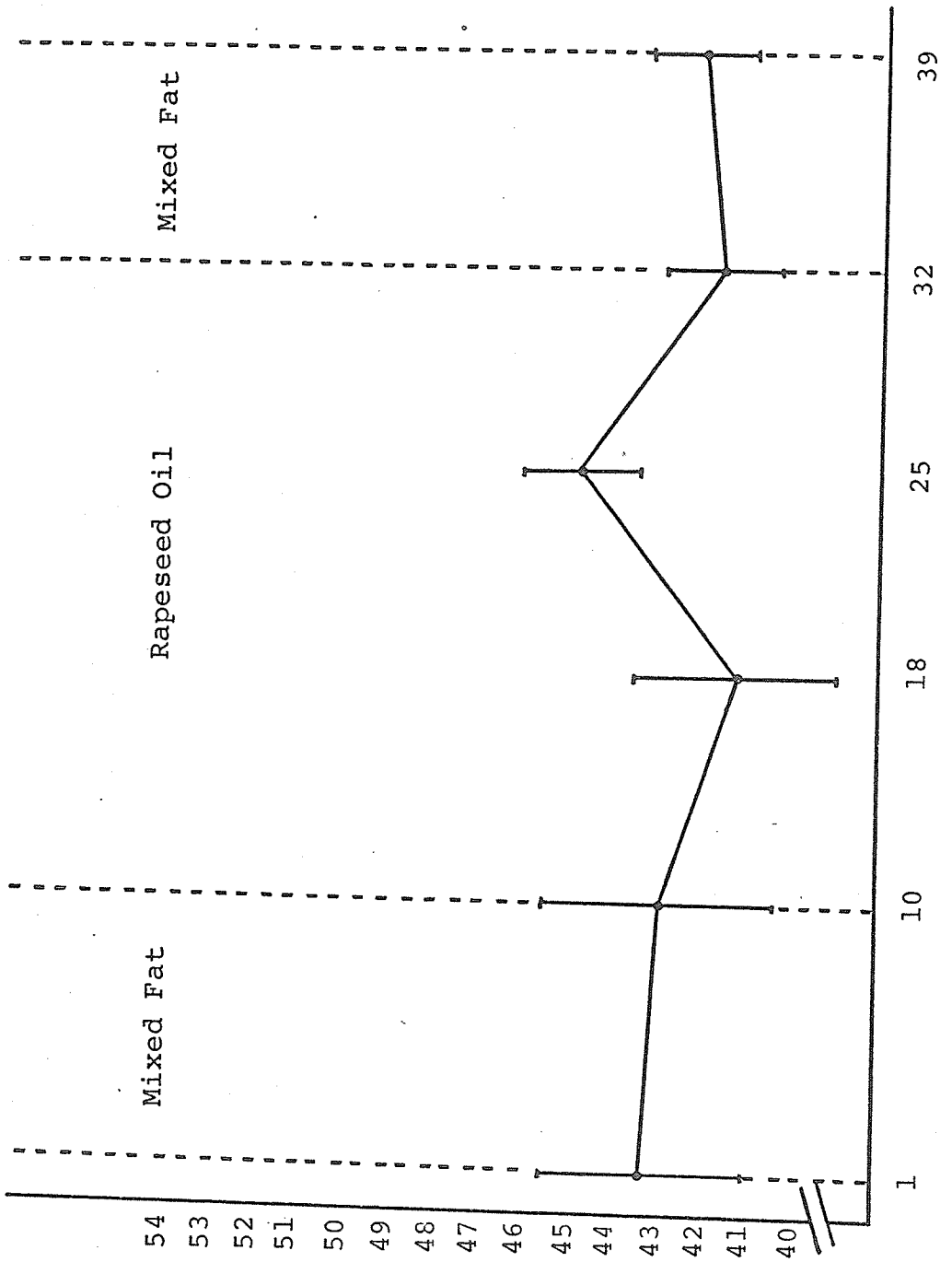

Figure 10. Mean hematocrit levels of subjects during experiment.

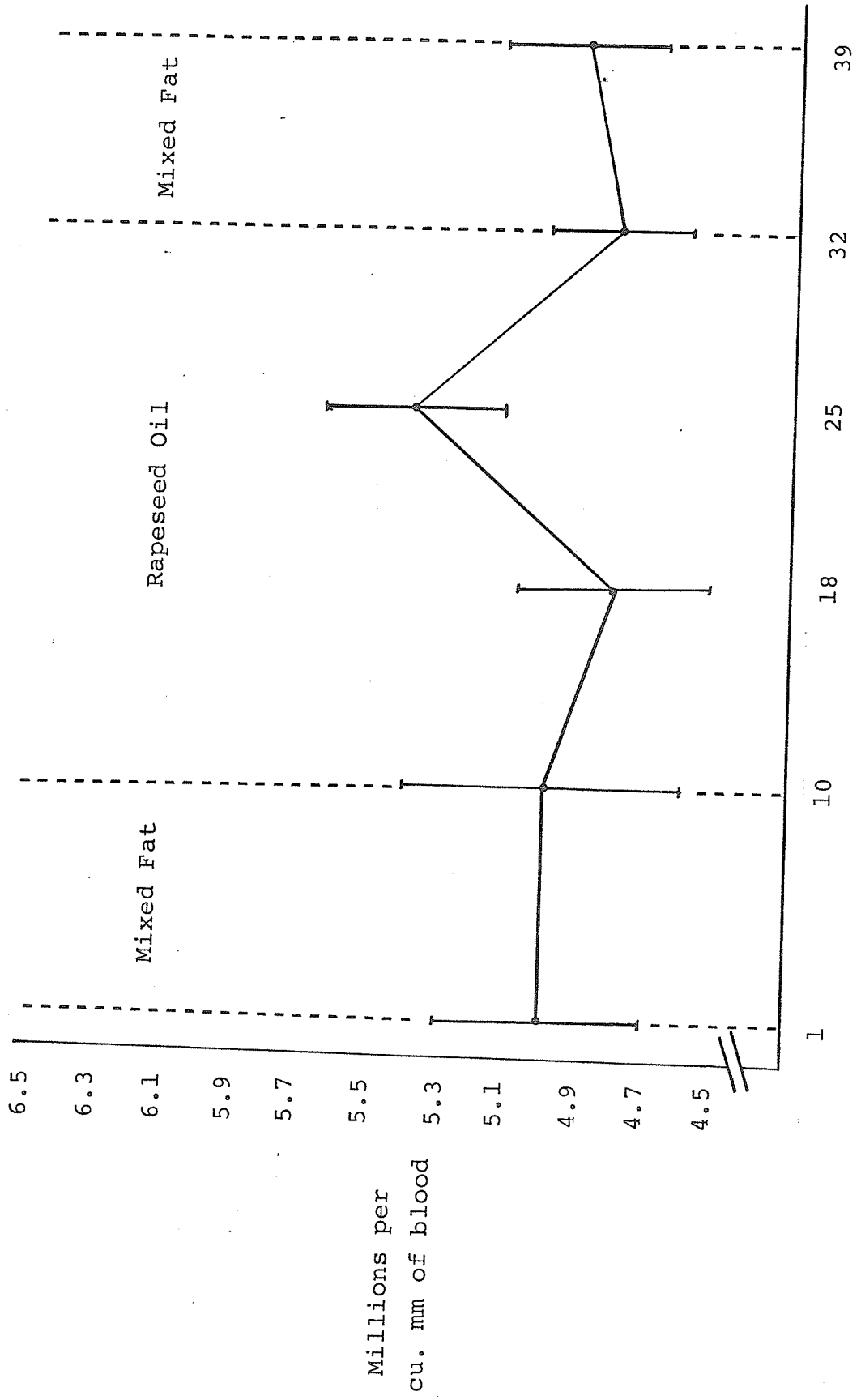

Figure 11. Mean red blood cell count of subjects during experiment.

Table 21

Hemoglobin of Subjects During Experiment[1]

Subject	Days of Experiment					
	1	10[2]	18	25	32[2]	39
	g hemoglobin/100 ml of blood					
R.U.	13.5	14.4	13.6	14.9	14.3	13.9
M.Q.	14.1	14.1	13.7	14.1	13.9	14.2
T.T.	16.3	16.9	15.9	15.4	15.3	15.5
C.G.	15.1	15.0	14.6	15.2	15.2	15.6
S.K.	-[3]	14.4	14.1	14.2	14.4	14.3
R.A.	13.9	14.0	13.7	14.0	14.1	14.3
R.D.	-[3]	15.2	13.6	14.4	13.9	14.0
Group Mean	14.6	14.9	14.2	14.6	14.4	14.5

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

[2]Days on which dietary regimen was changed. Diets included:
1) mixed fat diet Days 1-9 inclusive and Days 32-39 inclusive, and
2) rapeseed oil diet, Days 10-31 inclusive.

[3] Blood sample lost. Group mean is for 5 subjects only.

Table 22

Changes in Hemoglobin

Subject	Experimental Period					
	Mixed Fat	Rapeseed Oil Diet				Mixed Fat
	Day 1 vs 10	Day 10 vs 18	Day 18 vs 25	Day 25 vs 32	Overall Day 10 vs 32	Day 32 vs 39
	g hemoglobin/100 ml of blood					
R.U.	+0.9	-0.8	+1.3	-0.6	-0.1	-0.4
M.Q.	0.0	-0.4	+0.4	-0.2	-0.2	+0.3
T.T.	+0.6	-1.0	-0.5	-0.1	-1.6	+0.2
C.G.	-0.1	-0.4	+0.6	0.0	+0.2	+0.4
S.K.	-[1]	-0.3	+0.1	+0.2	0.0	-0.1
R.A.	+0.1	-0.3	+0.3	+0.1	+0.1	+0.2
R.D.	-[1]	-1.6	+0.8	-0.5	-1.3	+0.1
Group Mean	+0.3	-0.7	+0.4	-0.2	-0.4	+0.1

[1]Blood sample lost. Group mean is for 5 subjects only.

Table 23

Hematocrit of Subjects During Experiment[1]

Subject	Day of Experiment					
	1	10[2]	18	25	32[2]	39
	percentage of blood that is cells					
R.U.	41.0	42.0	40.0	44.5	41.0	41.0
M.Q.	42.5	41.5	40.0	44.5	41.5	41.5
T.T.	47.0	48.5	46.0	47.0	44.5	43.0
C.G.	43.5	42.5	42.0	46.5	43.0	45.0
S.K.	-[3]	41.5	40.5	43.5	40.5	42.0
R.A.	42.5	41.0	40.5	44.5	40.5	42.5
R.D.	-[3]	44.5	40.0	45.5	41.5	41.5
Group Mean	43.3	43.1	41.3	45.1	41.8	42.4

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

[2]Days on which dietary regimen was changed. Diets included:
1) mixed fat diet, Days 1-9 inclusive and Days 32-39 inclusive, and
2) rapeseed oil diet, Days 10-31 inclusive.

[3]Blood sample lost. Group mean is for 5 subjects only.

Table 24

Changes in Hematocrit

Subject	Experimental Period					
	Mixed Fat	Rapeseed Oil Diet				Mixed Fat
	Day 1 vs 10	Day 10 vs 18	Day 18 vs 25	Day 25 vs 32	Overall Day 10 vs 32	Day 32 vs 39
	percertage of blood that is cells					
R.U.	+1.0	-2.0	+4.5	-3.5	-1.0	0.0
M.Q.	-1.0	-1.5	+4.5	-3.0	0.0	0.0
T.T.	+1.5	-2.5	+1.0	-2.5	-4.0	-1.5
C.G.	-1.0	-0.5	+4.5	-3.5	+0.5	+2.0
S.K.	-[1]	-1.0	+3.0	-3.0	-1.0	+1.5
R.A.	-1.5	-0.5	+4.0	-4.0	-0.5	+2.0
R.D.	-[1]	-4.5	+5.5	-4.0	-3.0	0.0
Group Mean	-0.2	-1.8	+3.9	-3.4	-1.3	+0.6

[1]Blood sample lost. Group mean is for 5 subjects only.

Table 25

Total Red Cell Count of Subjects During Experiment[1]

Subject	Day of Experiment					
	1	10[2]	18	25	32[2]	39
	millions/cu mm of blood					
R.U.	4.7	5.0	4.7	5.4	4.8	4.9
M.Q.	5.1	5.0	4.8	5.4	4.9	5.0
T.T.	5.4	5.7	5.4	5.7	5.2	5.1
C.G.	4.8	4.7	4.7	5.3	4.8	5.0
S.K.	-[3]	4.7	4.6	5.1	4.6	4.7
R.A.	5.0	4.8	4.8	5.4	4.9	5.0
R.D.	-[3]	5.2	4.6	5.4	4.7	4.8
Group Mean	5.0	5.0	4.8	5.4	4.8	4.9

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

[2]Days on which dietary regimen was changed. Diets included:
1) mixed fat diet, Days 1-9 inclusive and Days 32-39 inclusive, and
2) rapeseed diet, Days 10-31 inclusive.

[3]Blood sample lost. Group mean is for 5 subjects only.

Table 26

Changes in Total Red Cell Count

Subject	Experimental Period					
	Mixed Fat	Rapeseed Oil Diet				Mixed Fat
	Day 1 vs 10	Day 10 vs 18	Day 18 vs 25	Day 25 vs 32	Overall Day 10 vs 32	Day 32 vs 39
	millions/cu mm of Blood					
R.U.	+0.3	-0.3	+0.7	-0.6	-0.2	+0.1
M.Q.	-0.1	-0.2	+0.6	-0.5	-0.1	+0.1
T.T.	+0.3	-0.3	+0.3	-0.5	-0.5	-0.1
C.G.	-0.1	0.0	+0.6	-0.5	+0.1	+0.2
S.K.	-[1]	-0.1	+0.5	-0.5	-0.1	+0.1
R.A.	-0.2	0.0	+0.6	-0.5	+0.1	+0.1
R.D.	-[1]	-0.6	+0.8	-0.7	-0.5	+0.1
Group Mean	0.0	-0.2	+0.6	-0.5	-0.2	+0.1

[1]Blood sample lost. Group mean is for 5 subjects only.

are known to affect these parameters (Wintrobe, 1956; Dacie and Lewis, 1963). In light of the number of factors affecting blood parameters it is surprising that the pattern of change for the three parameters was so consistent for all subjects in the present study (Tables 22,24,26). Perhaps it is suffice to observe that all values for hemoglobin, hematocrit and red cell count were well within the normal range.

D.2. Reticulocyte Count

Reticulocytes are immature red blood cells which normally constitute 0.2 to 2.0 percent of the total number of red blood cells (Dacie and Lewis, 1963). There was considerable variability among subjects in the initial reticulocyte count although all values were within the normal range (Table 27; Figure 12). In contrast to Abdellatif et al. (1972) who found increased reticulocyte counts in the blood of ducklings fed rapeseed oil, values remained essentially constant for each individual during feeding of the rapeseed oil diet and no significant changes occurred in this period (Tables 27,28; Figures 7,12; Appendix Tables 6, 13). Although the mean reticulocyte count was significantly higher at Day 39 than at Day 25 and 32 (Appendix Table 6) the higher mean value at Day 39 was primarily due to the relatively high values for subjects SK and RD.

D.3. Leucocyte Count

Leucocyte count describes the number of white blood cells/cu mm of blood. Results of leucocyte counts taken during the study are shown in Tables 29 and 30 and Figure 13 Statistical analyses of the counts are summarized in Appendix Tables 6 and 14. Leucocyte counts decreased

Table 27

Reticulocyte Count of Subjects During Experiment

Subject	Day of Experiment					
	1	10[2]	18	25	32[2]	39
	percent of reticulocytes					
R.U.	0.4	0.6	0.6	0.5	0.5	0.6
M.Q.	0.4	0.6	0.8	1.1	0.8	0.9
T.T.	1.0	1.1	0.6	0.5	1.0	1.3
C.G.	0.5	1.2	1.3	0.6	0.9	0.9
S.K.	-[3]	1.0	1.5	0.8	1.2	2.2
R.A.	0.4	0.8	0.5	0.5	0.9	0.7
R.D.	-[3]	0.9	1.2	0.6	0.6	1.7
Group Mean	0.5	0.9	0.9	0.7	0.8	1.2

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

[2]Days on which dietary regimen was changed. Diets included:
1) mixed fat diet, Days 1-9 inclusive and Days 32-39 inclusive, and
2) rapeseed oil diet, Days 10-31 inclusive.

[3]Blood sample lost. Group mean is for 5 subjects only.

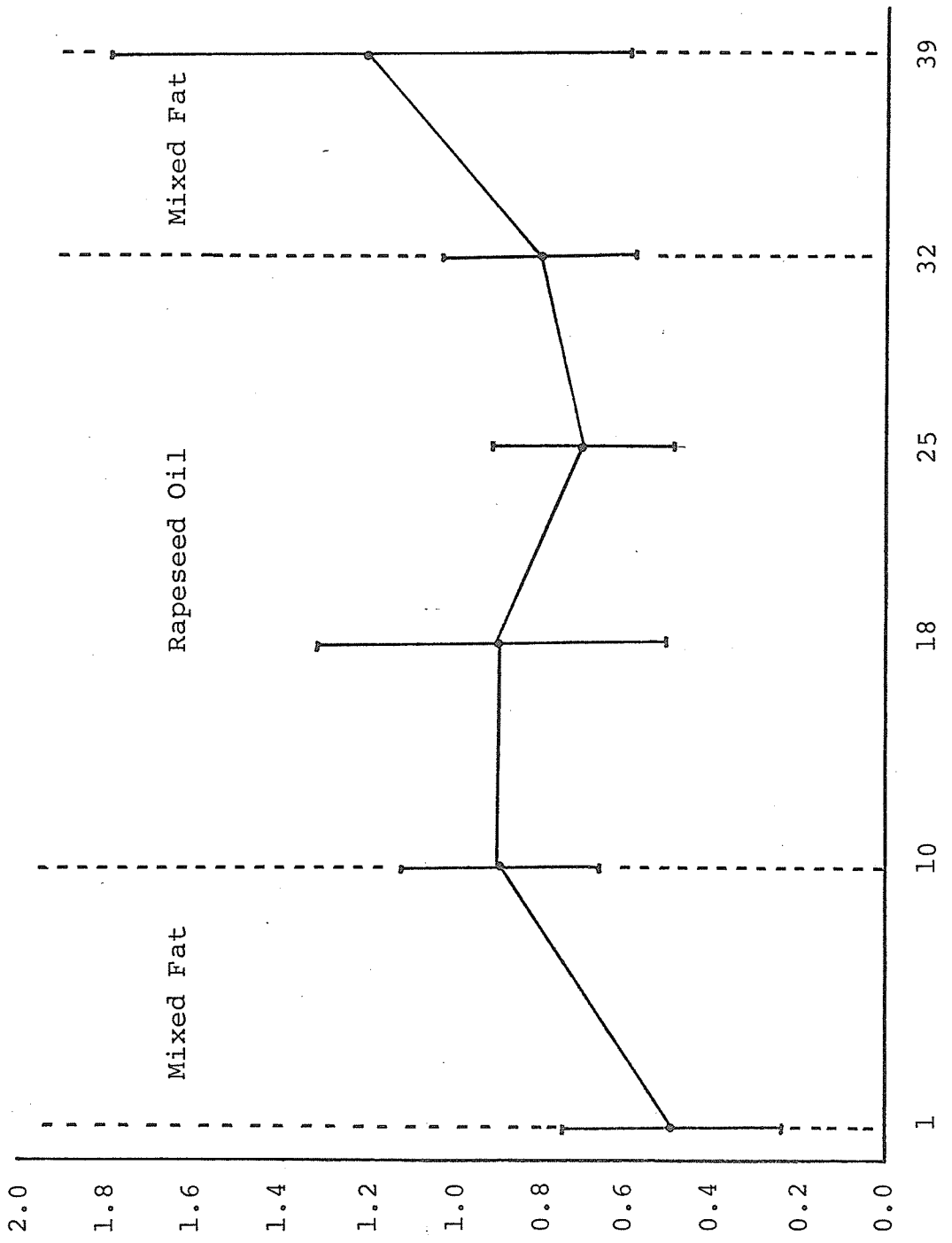

Figure 12. Mean reticulocyte counts of subjects during experiment.

Table 28

Changes in Reticulocyte Count

Subject	Experimental Period					
	Mixed Fat	Rapeseed Oil Diet				Mixed Fat
	Day 1 vs 10	Day 10 vs 18	Day 18 vs 25	Day 25 vs 32	Overall Day 10 vs 32	Day 32 vs 39
	percent of reticulocytes					
R.U.	+0.2	0.0	-0.1	0.0	-0.1	+0.1
M.Q.	+0.2	+0.2	+0.3	-0.3	+0.2	+0.1
T.T.	+0.1	-0.5	-0.1	+0.5	-0.1	+0.3
C.G.	+0.7	+0.1	-0.7	+0.3	-0.3	0.0
S.K.	-[1]	+0.5	-0.7	+0.4	+0.2	+1.0
R.A.	+0.4	-0.3	0.0	+0.4	+0.1	-0.2
R.D.	-[1]	+0.3	-0.6	0.0	-0.3	+1.1
Group Mean	+0.3	+0.0	-0.3	+0.2	-0.0	+0.3

[1]Blood sample lost. Group mean is for 5 subjects only.

Table 29

Leucocyte Count of Subjects During Experiment[1]

Subject	Day of Experiment					
	1	10[2]	18	25	32[2]	39
	thousands/cu mm of blood					
R.U.	4.4	3.5	4.0	3.1	4.2	5.0
M.Q.	4.9	3.7	4.2	3.7	4.1	5.3
T.T.	5.5	5.0	5.8	4.3	4.0	5.2
C.G.	4.2	4.5	4.4	3.4	2.5	4.5
S.K.	-[3]	3.5	3.3	2.5	3.0	3.7
R.A.	5.4	4.2	4.1	3.9	2.7	4.7
R.D.	-[3]	4.0	4.4	3.2	2.8	4.4
Group Mean	4.9	4.1	4.3	3.4	3.3	4.7

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

[2]Days on which dietary regimen was changed. Diets included:
1) mixed fat diet, Days 1-9 inclusive and Days 32-39 inclusive, and
2) rapeseed oil diet, Days 10-31 inclusive.

[3]Blood sample lost. Group mean is for 5 subjects only.

Table 30

Changes in Leucocyte Count

Subject	Experimental Period					
	Mixed Fat	Rapeseed Oil Diet				Mixed Fat
	Day 1 vs 10	Day 10 vs 18	Day 18 vs 25	Day 25 vs 32	Overall Day 10 vs 32	Day 32 vs 39
	thousands/cu mm of blood					
R.U.	-0.9	+0.5	-0.9	+1.1	+0.7	+0.8
M.Q.	-1.2	+0.5	-0.5	+0.4	+0.4	+1.2
T.T.	-0.5	+0.8	-1.5	-0.3	-1.0	+1.2
C.G.	+0.3	-0.1	-1.0	-0.9	-2.0	+2.0
S.K.	-[1]	-0.2	-0.8	+0.5	-0.5	+0.7
R.A.	-1.2	-0.1	-0.2	-1.2	-1.5	+2.0
R.D.	-[1]	+0.4	-1.2	-0.4	-1.2	+1.6
Group Mean	-0.8	+0.3	-0.9	-0.1	-0.7	+1.4

[1]Blood sample lost. Group mean is for 5 subjects only.

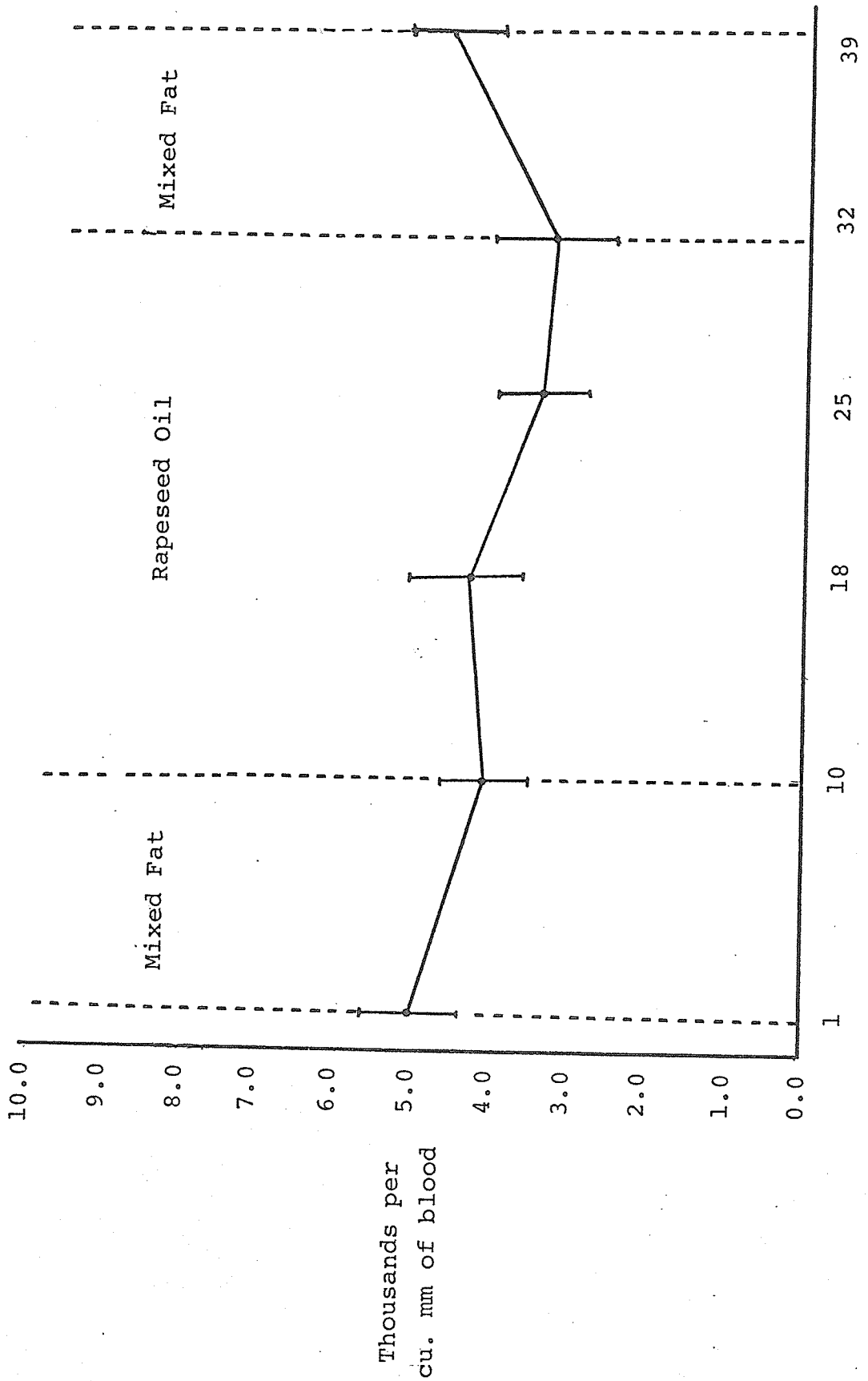

Figure 13. Mean leucocyte counts of subjects during experiment.

significantly during the second week of the rapeseed period and then remained constant during the third week on this diet. The fact that leucocyte counts increased appreciably for all subjects when the mixed fat diet was again fed suggests that the decrease in leucocyte count noted during the rapeseed oil period was related to dietary fat. The normal range for leucocyte count is between 4 and 10 thousand/cu mm of blood (Dacie and Lewis, 1963). As shown in Table 29 values throughout the study ranged from 2.5 to 5.8 thousand/cu mm of blood. Wintrobe (1956) has suggested that leucocyte values below 5 thousand per cu mm of blood indicate leukopenia, an abnormally low level of leucocytes in the blood. As all the subjects were active and healthy at all times during the experiment the meaning of the observations in the present study is not clear.

D.4. Platelet Count

The normal concentration of platelets in the blood is between 150 and 300 thousand/ cu mm of blood (Guyton, 1971). Platelet counts above 150,000/cu mm of blood were considered normal in the present study. Counts between 120 and 150 thousand were classed low-normal and counts below 120 thousand were considered low. Table 31 summarizes platelet counts of subjects during the 39-day trial. There was a definite and apparently progressive drop in platelet counts between the first day of the rapeseed oil period, when all 7 subjects had normal platelet counts, and the last day of the rapeseed oil period, when 5 of the subjects were classed as low and the other 2 subjects as low-normal.During the latter part of the rapeseed oil period subject TT experienced several nosebleeds, while petechial hemmorhages on the back were reported by subject RU.

Table 31

Platelet Count[1] of Subjects During Experiment[2]

Subject	Day of Experiment					
	1	10[3]	18	25	32[3]	39
R.U.	N	N	LN	L	L	N
M.Q.	N	N	LN	LN	LN	N
T.T.	N	N	LN	LN	L	LN
C.G.	N	N	LN	LN	L	N
S.K.	-[4]	N	LN	LN	LN	N
R.A.	N	N	L	LN	L	N
R.D.	-[4]	N	LN	LN	L	LN
Group Summary	5N	7N	6LN; 1L	5LN; 2L	2LN; 5L	5N; 2LN

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

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

[3]Days on which dietary regimen was changed. Diets included:
1) mixed fat diet, Days 1-9 inclusive and Days 32-39 inclusive, and
2) rapeseed oil diet, Days 10-31 inclusive.

[4]Blood sample lost. Summary is for 5 subjects only.

Both conditions had been experienced by the subjects in the year prior to the study but had not been noticed for several months. In addition, a large hematoma developed around the site where a fat biopsy was taken from subject CG on Day 32. Platelets play a major role in the blood clotting process (Guyton, 1971). The decrease in platelet counts observed for all subjects on the rapeseed oil diet may thus account for the nosebleeds and hemmorhages observed in this study. Platelet counts rose for all subjects when the mixed fat diet was again fed (Table 31) so it is probable that decreases in platelet count were related to dietary fat source. The nosebleeds and petechial hemmorhages disappeared during the post-experimental mixed fat period.

D.5. Red Cell Fragility

Red cell fragility is frequently taken as an indication of membrane integrity. Although increased permeability of red blood cells has been reported for ducklings and guinea-pigs fed high levels of rapeseed oil (Abdellatif and Vles, 1970), red cell fragility did not change during the present study. Hemolysis was first observed at 0.45 percent NaCl and was complete at 0.30 percent NaCl.

Feeding of rapeseed oil at levels providing 36 percent of the total calories had no deleterious effects on hematology except for a decrease in platelet counts. After feeding canbra oil at the same level and for the same period of time, LeBlanc (1973) concluded that canbra oil also had no deleterious effect on blood hematology. Although not discussed by LeBlanc, platelet counts were normal throughout the canbra oil study.

E. FAT BIOPSIES

Abdominal fat biopsies were taken from two volunteer subjects on the first and last day of the rapeseed oil diet with the object of seeing if erucic acid was deposited in depot fats. Erucic acid was not present in the initial sample of either subject. After 22 days on the rapeseed oil diet erucic acid made up 0.4 percent of the total fatty acids of one subject and 2.05 percent of the total fatty acids of the other subject (Table 32). By contrast, erucic acid was found to make up 7 to 12 percent of total carcass fatty acids of rats fed a diet containing 20 percent rapeseed oil (37 percent erucic acid) for 3 months (Craig et al., 1963) and of turkeys and chickens fed rapeseed oil at levels of 10 percent of the diet for 6 weeks (Salmon, 1969ab). It is interesting to note that the ratios of eicosenoic acid to erucic acid were similar in both animals and man even though much less of each fatty acid was deposited in the human. In the present study none of the fatty acids changed by more than 2 percent during the experimental period. Subject weight was controlled during the experiment so the amount of fat laid down would be minimal. Variance also may be due to differences in length of time on the diet or to the stages of physiological development of each of the different species involved. It is possible however that the fatty acids of rapeseed oil are deposited at different levels in the body fat of humans and the carcass fat of other species.

Table 32

Percent Fatty Acid Composition of Fat Biopsy Samples

Fatty Acid	Subject CG		Subject SK	
	Day 10[1]	Day 32[2]	Day 10[1]	Day 32[2]
Lauric, C12:0[3]	0.54	0.84	0.60	0.86
Myristic, C14:0	3.05	3.15	2.80	2.91
Palmitic, C16:0	21.63	22.60	20.80	19.00
Palmitoleic, C16:1	4.00	3.77	4.50	4.33
Stearic, C18:0	6.73	6.09	6.60	6.16
Oleic, C18:1	44.24	45.36	42.60	40.87
Linoleic, C18:2	15.42	14.82	15.90	16.59
Linolenic, C18:3	0.57	1.09	0.90	1.66
Eicosenoic, C20:1	1.32	1.02	1.80	2.67
Erucic, C22:1	-	0.40	-	2.05

[1]Day 10 : first day of rapeseed oil diet.

[2]Day 32 : last day of rapeseed oil diet.

[3]Carbon number: number of double bonds.

SUMMARY AND CONCLUSIONS

The present study investigated the effect of rapeseed oil on serum lipid patterns and whole blood constituents of normal male subjects when rapeseed oil provided about 36 percent of the total calories in a mixed diet. The study consisted of a 39-day metabolic trial and included an initial 9-day stabilization period throughout which a mixed fat diet was fed, a 22-day experimental period when the subjects received a rapeseed oil diet, and an 8-day follow-up period when subjects were again fed the mixed fat diet.

There was a mean decrease in serum cholesterol level of 13 mg/100 ml of blood during the pre-experimental mixed fat period and a further significant decrease in cholesterol of 20 mg/100 ml during the first week of the rapeseed oil diet. Serum cholesterol values tended to plateau during the second week of the rapeseed oil diet and then increased by 12 mg/100 ml during the last week of the same diet. The pattern of change of serum cholesterol on the rapeseed oil diet is analogous to that observed by Malmros and Wigand (1957) who noted a decrease of 40 mg/100 ml in serum cholesterol during the first week, no appreciable change during the second week and an increase of approximately 10 mg/100 ml during the third week on a diet in which 40 percent of calories were supplied by 50 percent erucic acid rapeseed oil, but it is in contrast to observations of LeBlanc (1973) who found a significant ($p = .05$) linear decrease in serum cholesterol levels over the entire 22-day experimental period when canbra oil was fed. The change in serum cholesterol observed during the rapeseed oil period in the present study and that predicted

by the equation of Keys et al. (1965) suggests that the 20 and 22-carbon monoenoic fatty acids of rapeseed oil, like other monoenoic fatty acids, have little effect on serum cholesterol. Reversion to the mixed fat diet was accompanied by a significant increase in serum cholesterol.

Although there were differences among changes in serum cholesterol, lipid phosphorous and triglyceride levels, the overall pattern of response of the three parameters were similar throughout the study. Serum levels of all three decreased during the first week on the rapeseed oil diet, tended to plateau during the second week and then increased slightly during the last week on this diet. As a result the overall change during the 22-day experimental period was not significant for any of these patterns. Reversion to the mixed fat diet was accompanied by an increase in triglycerides and a significant increase in serum lipid phosphorous level. The response of lipid phosphorous during the study thus coincides with reports of Erickson et al. (1964), McGandy et al. (1970) and Le Blanc (1973) that the pattern of change of serum lipid phosphorous in response to a change in diet composition is similar to that of serum cholesterol. The overall pattern of response for serum lipids during the study was attributed to dietary fat source.

Eicosenoic and erucic acids made up very little of the serum phospholipid fraction (5.4 percent) after 22 days on the rapeseed oil diet even though these fatty acids comprised 52 percent of the dietary fatty acids. The decrease in saturated fatty acids and the increase in oleic acid observed in the phospholipids reflect the low level of saturated and high level of monounsaturated fatty acids in the rapeseed oil diet. A rapid turnover of phospholipids in the serum is suggested as the changes

which occurred on the rapeseed oil diet returned to pre-rapeseed oil levels after only one week on the mixed fat diet. Erucic acid was found in fat biopsies of 2 subjects after 22 days on the rapeseed oil diet but the levels were low; erucic acid made up 0.4 percent of total fatty acids of one subject and 2.05 percent of total fatty acids of the other subject. As weight of the subjects was controlled during the experiment, little fat would be expected to be laid down and only minimal changes would be expected in the fatty acid composition of adipose tissue. The fact that body weight was controlled in the present experiment may partially explain the difference in level of erucic acid found in the depot fat of man and other animals. In general, the depot fat of animals fed rapeseed oil has been found to contain 7 to 12 percent erucic acid. In this study none of the fatty acids changed by more than 2 percent during the experimental period.

Statistically significant changes in hematology occurred during the experiment, but all values for hemoglobin, hematocrit, red cell count and reticulocyte count were within the ranges considered normal for the adult male. Although increased permeability of red blood cells has been reported for ducklings and guinea-pigs fed high levels of rapeseed oil (Abdellatif and Vles, 1970), there were no changes in red cell fragility during the present study. Hemolysis was first observed at 0.45 percent NaCl and was complete at 0.30 percent NaCl. Leucocyte counts decreased significantly during the second week of the rapeseed oil period and then remained constant during the third week on this same diet. The fact that leucocyte counts increased appreciably for all subjects when the mixed fat diet was again fed suggests that the decrease in leucocyte count noted during the rapeseed oil period was related to dietary fat.

Leucocyte counts were below normal at most periods throughout the entire 39-day trial period. The meaning of these observations is not clear. Platelet counts were normal for all 7 subjects on the first day of the rapeseed oil period but counts progressively decreased during the 22-day experimental period to where counts for 5 subjects were low and 2 subjects were low-normal. Platelet counts above 150,000/cu mm were considered normal in the present study. Counts between 120 and 150 thousand were classed low-normal and counts below 120 thousand were considered low. Nosebleeds, petechial hemmorhages and a hematoma at the site where a fat biopsy was taken were reported during the last week of the rapeseed oil period. These conditions disappeared during the post-experimental mixed fat diet. The fact that platelet counts returned to normal in 5 of the subjects when the mixed fat diet was again fed strongly suggests that the decrease in platelet count during the experimental period was related to the rapeseed oil.

Rapeseed oil provided 36 percent of the dietary calories of 7 healthy male subjects for 22 days. Except for the drop in platelet count, no marked effects in metabolism were observed insofar as metabolism is reflected by changes in serum lipid patterns and whole blood hematology.

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APPENDIX

Appendix Table 1

Calculated Nutrient Composition of Mixed Fat Diet, Menu 1[1]

Item	Grams	Cal.	Pro. (g)	Fat (g)	CHO (g)	Ca (mg)	P (mg)	Fe (mg)	Vitamins: A (I.U.)	Thiamin (mg)	Ribo-flavin (mg)	Niacin (mg)	C (mg)
Breakfast:													
Orange Juice 1437[2]	120	54	0.8	0.1	12.8	10	19	0.12	240	0.11	0.01	0.36	54
Rolled oats 1390[2]	20	78	2.8	1.5	13.6	11	81	0.90	0	0.12	0.03	0.20	0
Scrambled egg albumen 981[2]	14	53	11.5	0	0.8	9	16	0.14	0	0.01	0.29	0.10	0
Brown sugar 2229[2]	14	53	0	0	13.5	12	3	0.48	0	0	0	0.03	0
Strawberry jam 1148[2]	19	52	0.1	0	13.3	4	2	0.19	2	0	0.01	0.04	3
Bread 459[2]	30	81	2.6	1.0	15.1	21	26	0.72	0	0.08	0.05	0.69	0
Skim milk 1322[2]	240	86	8.6	0.2	12.2	290	228	0	0	0.10	0.43	0.24	2
Lunch:													
Hamburger patties[3]	125	489	23.1	39.8	11.8	18	32	0.13	46	0.08	0.21	0.89	1
Hamburger bun 1902[2]	45	134	3.7	2.5	23.6	33	38	0.86	0	0.13	0.08	0.99	0
Coleslaw[3]	65	17	0.8	0.1	3.9	29	19	0.31	1186	0.04	0.03	0.24	31
Coleslaw dressing[3]	26	84	0	5.0	10.0	0	0	0.01	0	0	0	0	0
Fruit[4]	100	96	0.4	0.1	25.0	8	10	0.40	672	0.03	0.02	0.30	3
Skim milk 1322[2]	240	86	8.6	0.2	12.2	290	228	0	0	0.10	0.43	0.24	2
Dinner:													
Sweet'n'Sour[3]	308	595	13.7	37.1	53.7	36	19	0.96	702	0.18	0.36	0.89	24
Rice 1877[2]	30	109	2.0	0.2	24.1	7	28	0.24	0	0.02	0.01	0.48	0
Tossed salad[3]	100	18	1.0	0.2	3.8	17	25	0.50	615	0.06	0.05	0.50	15
Salad dressing[3]	10	54	0.1	5.9	0.1	0	0	0	0	0	0	0	0
Bread 459[2]	30	81	2.6	1.0	15.1	21	26	0.72	0	0.08	0.05	0.69	0
Fruit[4]	100	96	0.4	0.1	25.0	8	10	0.40	672	0.03	0.02	0.30	3
Skim milk 1322[2]	240	86	8.6	0.2	12.2	290	228	0	0	0.10	0.43	0.24	2
Plus:													
Cookies (2)[3]	55	206	3.3	6.5	35.1	14	56	1.06	233	0.11	0.06	0.51	0
Spicy fruit square[3]	30	114	1.4	3.3	20.3	14	11	0.68	133	0.03	0.04	0.23	0
White cake[3]	53	162	2.1	5.8	25.9	22	25	0.08	231	0.01	0.05	0.10	0
Butter 505[2]	11	79	0.1	8.9	0.1	2	2	0	363	-	-	-	0
TOTALS	1925	2963	98.3	119.6	383.2	1164	1132	8.90	4495	1.38	2.66	8.26	140

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

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

[3]Recipe after LeBlanc (1973).

[4]Calculated average assuming that equal quantities of pears, apricots, pineapple, peaches and plum were served.

Appendix Table 2

Calculated Nutrient Composition of Mixed Fat Diet, Menu 2[1]

Item	Grams	Cal.	Pro. (g)	Fat (g)	CHO (g)	Ca (mg)	P (mg)	Fe (mg)	Vitamins: A (I.U.)	Thiamin (mg)	Ribo-flavin (mg)	Niacin (mg)	C (mg)
Breakfast:													
Apple juice 27[2]	120	56	0.1	0	14.3	7	11	0.72	0	0.01	0.02	0.12	42
Cream of wheat 993[2]	20	11	2.1	0.2	14.9	107	113	8.40	0	0	0	0	0
Scrambled egg albumen 981[2]	14	53	11.5	0	0.8	9	16	0.14	0	0.01	0.29	0.10	0
White sugar 22302	14	54	0	0	13.9	0	0	0	0	0	0	0	0
Bread 459[2]	30	81	2.6	0	15.1	21	26	0.73	0	0.08	0.05	0.69	0
Orange marmalade 1318[2]	19	49	0.1	0	14.0	7	2	0.11	0	0	0	0.02	1
Lunch:													
Spaghetti/sauce/meatballs[3]	367	527	23.5	26.0	50.6	31	119	3.09	2383	0.54	0.41	5.02	42
Tossed salad[3]	100	18	1.0	0.2	3.8	17	25	0.51	210	0.06	0.05	0.51	15
Salad dressing[3]	15	80	0.2	8.8	14.0	0	0	0	0	0	0	0	0
Bread 459[2]	30	81	2.6	1.0	15.1	21	26	0.72	0	0.08	0.05	0.69	0
Fruit[4]	100	96	0.4	0.1	25.0	8	10	0.40	672	0.03	0.02	0.30	2
Skim milk 1322[2]	240	86	8.6	0.2	12.2	290	228	0	0	0.10	0.43	0.24	2
Dinner:													
Beef stew[3]	395	654	29.6	43.3	38.2	38	89	2.44	6961	0.42	0.76	3.26	26
Mashed potato 1797[2]	30	109	2.2	0.2	25.2	11	52	0.51	0	0.07	0.02	1.62	10
Coleslaw[3]	65	17	0.8	0.1	3.9	29	19	0.31	1186	0.04	0.03	0.24	28
Coleslaw dressing[3]	26	84	0	5.0	10.0	0	0	0.01	0	0	0	0	0
Bread 459[2]	30	81	2.6	1.0	15.1	21	26	0.72	0	0.08	0.05	0.69	0
Fruit[4]	100	96	0.4	0.1	24.9	8	10	0.40	672	0	0.02	0.30	3
Skim milk 1322[2]	240	86	8.6	0.2	12.2	290	228	0	0	0.01	0.43	0.24	2
Plus:													
Cookies (2)[3]	56	206	3.3	6.5	35.1	14	56	1.06	233	0.11	0.06	0.51	0
Spicy fruit square[3]	30	114	1.4	3.3	20.3	14	11	0.68	133	0.03	0.04	0.23	0
White cake[3]	53	162	2.1	5.8	25.9	22	25	0.08	231	0.01	0.05	0.10	0
Butter 505[2]	20	143	0.1	16.2	0.1	4	3	0	660	-	-	-	0
TOTALS	2114	2944	103.2	117.9	404.6	969	1095	21.00	13341	1.77	2.78	14.88	177

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

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

[3]Recipe after LeBlanc (1973).

[4]Calculated average assuming that equal quantities of pears, apricots, pineapple, peaches and plum were served.

Appendix Table 3

Calculated Nutrient Composition of Rapeseed Diet, Menu 1[1]

Item	Grams	Cal.	Pro. (g)	Fat (g)	CHO (g)	Ca (mg)	P (mg)	Fe (mg)	Vitamins: A (I.U.)	Thiamin (mg)	Ribo-flavin (mg)	Niacin (mg)	C (mg)
Breakfast:													
Orange juice 1437[2]	120	54	0.8	0.1	12.8	11	19	0.12	240	0.11	0.01	0.36	54
Rolled oats 1390[2]	30	117	4.3	2.2	20.5	16	122	1.35	0	0.18	0.04	0.30	0
Scrambled egg albumen 981[2]	14	53	11.5	0	0.8	9	16	0.14	0	0.01	0.29	0.10	0
Brown sugar 2229[2]	14	52	0	0	13.5	12	3	0.48	0	0	0	0.03	0
Strawberry jam 1148[2]	19	51	0.1	0	13.3	4	2	0.19	2	0	0.01	0.04	3
Bread 459[2]	30	80	2.6	1.0	15.1	21	26	0.72	0	0.08	0.05	0.70	0
Skim milk 1322[2]	240	86	8.6	0.2	12.2	290	228	0	0	0.10	0.43	0.24	2
Lunch:													
Hamburger patties[3]	125	400	18.7	31.5	10.4	14	32	0.12	50	0.07	0.18	0.79	1
Hamburger bun 1902[2]	45	134	3.7	2.5	23.9	33	38	0.86	0	0.13	0.08	0.99	0
Coleslaw[3]	65	17	0.8	0.1	3.9	29	19	0.31	1186	0.04	0.03	0.24	28
Coleslaw dressing[3]	26	83	0	5.0	10.0	0	0	0.01	0	0	0	0	0
Fruit[4]	100	96	0.4	0.1	25.0	8	10	0.40	672	0.03	0.02	0.30	3
Skim milk 1322[2]	240	86	8.6	0.2	12.2	290	228	0	0	0.10	0.43	0.24	2
Dinner:													
Sweet'n'Sour[3]	308	616	13.6	39.4	53.6	33	16	0.96	108	0.18	0.36	0.89	26
Rice 1877[2]	30	108	2.0	0.1	24.1	7	28	0.24	0	0.02	0.01	0.48	0
Tossed salad[3]	100	17	1.0	0.2	3.8	17	25	0.51	210	0.06	0.05	0.51	15
Salad dressing[3]	15	80	0.2	8.8	0.1	0	0	0	0	0	0	0	0
Bread 459[2]	30	80	2.6	1.0	15.1	21	26	0.72	0	0.08	0.05	0.69	0
Fruit[4]	100	96	0.4	0.1	25.0	8	10	0.40	672	0.03	0.02	0.30	3
Skim milk 1322[2]	240	86	8.6	0.2	12.2	290	228	0	0	0.10	0.43	0.24	2
Plus:													
Cookies (2)[3]	55	201	3.3	5.8	35.1	14	56	1.06	207	0.11	0.06	0.51	0
Spicy fruit square[3]	30	120	1.4	4.1	20.3	14	11	0.68	1	0.03	0.04	0.23	0
White cake[3]	53	163	2.1	5.8	25.9	22	25	0.08	231	0.01	0.05	0.10	0
Margarine 1317[2]	14	100	0.1	11.3	0.1	3	2	0	462	-	-	-	0
TOTALS	2043	2977	95.4	119.5	389.9	1166	1170	9.35	4041	1.47	2.64	8.28	139

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

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

[3]Recipe after LeBlanc (1973).

[4]Calculated average assuming that equal quantities of pears, apricots, pineapple, peaches and plum were served.

Appendix Table 4

Calcualted Nutrient Composition of Rapeseed Diet, Menu 2[1]

Item	Grams	Cal.	Pro. (g)	Fat (g)	CHO (g)	Ca (mg)	P (mg)	Fe (mg)	Vitamins: A (I.U.)	Thiamin (mg)	Ribo-flavin (mg)	Niacin (mg)	C (mg)
Breakfast:													
Apple juice 27[2]	120	56	0.1	0	14.3	7	11	0.72	0	0.01	0.02	0.10	42
Cream of wheat 993[2]	20	71	2.1	0.2	14.9	107	113	8.40	0	0	0	0	0
Scrambled egg albumen 981[2]	14	53	11.5	0	0.8	9	16	0.14	0	0.01	0.29	0.10	0
White sugar 2230[2]	14	54	0	0	13.9	0	0	0.01	0	0	0	0	0
Bread 459[2]	30	81	2.6	1.0	13.7	21	26	0.72	0	0.08	0.05	0.69	0
Orange marmalade 1318[2]	19	59	0.1	0	14.0	7	2	0.11	0	0	0	0.02	1
Lunch:													
Spaghetti/sauce/meatballs[3]	367	538	23.4	27.1	50.6	30	118	3.09	2215	0.54	0.41	5.02	42
Tossed salad[3]	100	18	1.0	0.2	3.8	17	25	0.51	210	0.06	0.05	0.51	15
Salad dressing[3]	15	80	0.2	8.8	0.1	0	0	0	0	0	0	0	0
Bread 459[2]	30	81	2.6	1.0	15.1	21	26	0.72	0	0.08	0.05	0.69	0
Fruit[4]	100	96	0.4	0	25	8	10	0.40	672	0.03	0.02	0.30	3
Skim milk 1322[2]	240	86	8.6	0.2	12.1	290	228	0	0	0.10	0.43	0.24	2
Dinner:													
Beef stew[3]	395	651	29.5	42.8	38.1	35	86	2.44	6350	0.42	0.76	3.26	26
Mashed potato 1797[2]	30	109	2.2	0.2	25.2	11	52	0.51	0	0.07	0.02	1.62	10
Coleslaw[3]	65	17	0.8	0.1	3.9	29	19	0.31	1186	0.04	0.03	0.24	28
Coleslaw dressing[3]	26	84	0	5.0	10.0	0	0	0.01	0	0	0	0	0
Bread 459[2]	30	81	2.6	1.0	15.1	21	26	0.72	0	0.08	0.05	0.69	0
Fruit[4]	100	96	0.4	0	25.0	8	10	0.40	672	0.03	0.02	0.30	3
Skim milk 1322[2]	240	86	8.6	0.2	12.2	290	228	0	0	0.10	0.43	0.24	2
Plus:													
Cookies (2)[3]	56	201	3.3	5.8	35.1	14	56	1.06	207	0.11	0.06	0.51	0
Spicy fruit square[3]	30	121	1.4	4.1	20.3	14	11	0.68	1	0.03	0.04	0.23	0
White cake[3]	53	162	2.1	5.8	25.9	22	25	0.83	231	0.01	0.05	0.10	0
Margarine 1317[2]	20	144	0.1	16.2	0.1	4	3	0	660	-	-	-	0
TOTALS	2114	3025	103.1	118.9	389.3	965	1091	21.72	12404	1.80	2.78	14.88	174

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

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

[3]Recipe after LeBlanc (1973).

[4]Calculated average assuming that equal quantities of pears, apricots, pineapple, peaches and plum were served.

Appendix Table 5

t-test[1]: Serum Parameters

Day	Cholesterol	Triglyceride	Lipid Phosphorous
10 vs 18	2.64	ns	ns
10 vs 25	2.90	1.96	2.45
10 vs 32	ns	ns	ns
10 vs 39	ns	ns	2.24
18 vs 25	ns	ns	ns
18 vs 32	ns	ns	ns
18 vs 39	3.69	ns	3.06
25 vs 32	ns	ns	ns
25 vs 39	3.96	ns	4.69
32 vs 39	2.37	ns	3.67

[1]Probability of chance occurrence = 0.05.

Appendix Table 6

t-test[1]: Whole Blood Parameters

Day	Hemo-globin	Hemato-crit	Red cell count	Reticulo-cyte count	Leuco-cyte count
10 vs 18	3.54	2.17	2.86	ns	ns
10 vs 25	ns	3.33	5.71	ns	2.92
10 vs 32	2.53	2.17	2.86	ns	3.33
10 vs 39	2.02	ns	ns	ns	2.50
18 vs 25	2.02	6.33	8.56	ns	3.75
18 vs 32	ns	ns	ns	ns	4.17
18 vs 39	ns	ns	ns	ns	ns
25 vs 32	ns	5.50	8.57	ns	ns
25 vs 39	ns	4.50	7.14	3.13	5.42
32 vs 39	ns	ns	ns	2.50	5.83

[1]Probability of chance occurrence = 0.05.

Appendix Table 7

Analysis of Variance: Total Serum Cholesterol[1]

Source of Variation	df	SS	MS	F-value	P[2]
Replications (days)	4	4499.89	1124.97	5.48	0.01
Subjects	6	18706.40	3117.73	15.18	0.01
Residuals (error)	24	4929.31	205.39		
Total	34	28135.60			

[1]Days 10,18,25,32 and 39.

[2]P = probability of chance occurrence.

Appendix Table 8

Analysis of Variance: Lipid Phosphorous[1]

Source of Variation	df	SS	MS	F-value	P[2]
Replication (days)	4	21.05	5.26	6.14	0.01
Subjects	6	24.96	4.16	4.85	0.01
Residuals (error)	24	20.58	0.86		
Totals	34	66.59			

[1]Days 10,18,25,32 and 39.

[2]P = probability of chance occurrence.

Appendix Table 9

Analysis of Variance: Serum Triglycerides[1]

Source of Variation	df	SS	MS	F-value	P[2]
Replication (days)	4	5622.11	1405.53	1.39	ns
Subjects	6	16715.20	2785.87	2.76	.05
Residuals (error)	24	24255.09	1010.63		
Total	34	46592.40			

[1]Days 10,18,25,32 and 39.

[2]P = probability of chance occurrence.

Appendix Table 10

Analysis of Variance: Hemoglobin[1]

Source of Variation	df	SS	MS	F-value	P[2]
Replication (days)	4	1.81	0.45	3.22	.05
Subjects	6	13.78	2.30	16.36	.01
Residuals (error)	24	3.37	0.14		
Total	34	18.96			

[1]Days 10,18,25,32 and 39.

[2]P = probability of chance occurrence.

Appendix Table 11

Analysis of Variance: Hematocrit[1]

Source of Variation	df	SS	MS	F-value	P[2]
Replication (days)	4	63.39	15.85	12.26	0.01
Subjects	6	73.27	12.21	9.45	0.01
Residuals (error)	24	31.01	1.29		
Total	34	167.67			

[1]Days 10,18,25,32 and 39.

[2]P = probability of chance occurrence.

Appendix Table 12

Analysis of Variance: Total Red Cell Count[1]

Source of Variation	df	SS	MS	F-value	P[2]
Replication (days)	4	1.53	0.38	21.37	0.01
Subjects	6	1.30	0.22	12.09	0.01
Residuals (error)	24	0.43	0.02		
Total	34	3.26			

[1]Days 10,18,25,32 and 39.

[2]P = probability of chance occurrence.

Appendix Table 13

Analysis of Variance: Reticulocyte Count[1]

Source of Variation	df	SS	MS	F-value	P[2]
Replication (days)	4	1.01	0.25	2.93	.05
Subjects	6	1.89	0.31	3.64	.05
Residuals (error)	24	2.08	0.09		
Total	34	4.98			

[1]Days 10,18,25,32 and 39.

[2]P = probability of chance occurrence.

Appendix Table 14

Analysis of Variance: Leucocyte Count[1]

Source of Variation	df	SS	MS	F-value	P[2]
Replication (days)	4	9.29	2.32	11.24	.01
Subjects	6	7.48	1.25	6.03	.01
Residuals (error)	24	4.96	0.21		
Total	34	21.74			

[1]Days 10,18,25,32 and 39.

[2]P = probability of chance occurrence.