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

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ABSTRACT

The effect of canbra oil on serum lipid patterns and hematology of whole blood was investigated with seven healthy male subjects. The 39-day study consisted of: 1) a 9-day preliminary period, when a mixed fat formulated to simulate the average Canadian fat intake was fed; 2) a 22-day experimental period, when canbra oil supplied the dietary fat; and 3) an 8-day post-experimental period, when the mixed fat again was fed. The diet, which contained 40 per cent of the total calories as fat, consisted of solid foods with textured soy protein substituted for meat. The mixed fat and the canbra oil (from B.campestris cv Span, 1.8 per cent erucic acid) supplied approximately 38 per cent of the total calories (i.e. 95 per cent of the total dietary fat). Fasting blood samples were taken on Days 1, 10, 18, 25, 32, and 39. Sera were analyzed for cholesterol, lipid phosphorus, and triglycerides. Electrophoresis was carried out on serum lipoproteins and the fatty acid patterns of the serum phospholipids were determined on Days 10, 32, and 39. Hemoglobin, hematocrit, reticulocyte count, total red cell count, and red cell fragility was determined in whole blood. All serum lipid levels decreased significantly ($P < 0.005$) in response to the canbra oil experimental diet. Mean serum cholesterol levels (mg/100 ml) on Days 1, 10, 18, 25, 32, and 39 were 203, 174, 159, 144, and 182, respectively. Lipid phosphorus followed a similar pattern to that of cholesterol but the magnitude of change tended to be greater. Changes in serum triglycerides, although small and of questionable biological significance, followed the same general pattern for all subjects. The ratio of beta-/pre-beta-lipoproteins decreased on the canbra oil diet, coincident

with similar decreases in serum cholesterol and then increased again on the mixed fat during the post-experimental period. However the beta-/pre-beta- ratio did not decrease during the preliminary mixed fat period in spite of a marked decrease in serum cholesterol. Changes in the fatty acid patterns of the phospholipids reflected changes in the fatty acid composition of the diet. The rapid change in phospholipid fatty acid patterns indicated a relatively rapid turnover of phospholipids in response to dietary manipulation. There was no evidence in the present study of a consistent effect of canbra oil on the blood hematology of young college men. Although changes occurred, the values for the various hematological parameters examined for each subject were within the range considered normal at all periods during the study. The appreciable effect of canbra oil on serum lipid patterns was consistent with the considerably lower levels of saturated fatty acids in the canbra oil diet. There was no evidence of any deleterious effect of canbra oil in the human; in fact, the changes observed in blood chemistry might even be considered desirable from a medical point of view.

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INTRODUCTION

Canadian rapeseed has become an important crop since its modest beginning in 1942 when a few western farmers co-operated in an experimental attempt at growing a few hundred pounds of seed. The finding that rape was adapted to certain areas of Western Canada, together with the wartime requirements for special marine lubricants, and encouragement from price supports for a few years, resulted in rape acreage increasing from 3,200 acres in 1943-44 to 80,000 acres in 1948-49. However, by 1950-51, the crop had almost disappeared with only 400 acres being sown that year. An amazing rebound in rape acreage occurred in the late '50s and early '60s as prospects brightened for exports and for increased domestic use of the oil (Bell, 1967). This trend, together with depressed wheat markets in the late '60s, pushed Canadian rapeseed acreage to approximately 4 million acres in 1970-71 (Weinberg, 1971).

As the consumer became more aware of the properties of rapeseed oil and as its use in the manufacture of food items continued to increase, domestic crush and consumption also expanded. However, a large quantity of rape in Canada is exported, with Japan being the leading customer. At present all producing and importing countries are primarily interested in rape as an edible oil source and so it seemed of interest to examine further its effects upon humans, since numerous reports suggest rapeseed oil produces adverse biochemical and physiological effects when fed to various species of experimental animals.

Since January 1973, low or zero erucic acid rapeseed oil, commonly known as canbra (contraction of Canadian Brassica) oil, has been the only type of rapeseed oil marketed in Canada. Canadian growers

switched their production to canbra oil which had been adapted to local growing conditions, as a result of experiments which indicated that diets containing more than 10 per cent rapeseed oil[1], with 33 to 50 per cent erucic acid, caused transient accumulation of fat in the hearts of rats, guinea pigs, and ducks (Abdellatif and Vles, 1970a,b,c; Beare-Rogers, 1970; Beare-Rogers et al., 1972a). Estimates indicated that the average Canadian consumption when expressed as a per cent of fat calories was much lower than that required to induce fatty infiltration in the hearts of experimental animals. Nevertheless, the switchover to canbra was considered prudent, although the likelihood of harm resulting from the use of rapeseed oil was considered remote.

No harmful effects have been attributed to the consumption of high levels of erucic acid by humans although until recently rapeseed oil products did not constitute a major source of fat in the Canadian diet. In fact, little work has been reported on the metabolism by the human of the oil from the original cultivars characteristically high in erucic acid (Δ13-cis-docosenoic acid) and essentially no work has been done with canbra oil. Work in our laboratory has shown that both canbra oil and rapeseed oil are very efficiently absorbed by the human when they accounted for approximately 58 per cent of the average daily fat intake (Vaisey et al., 1973).

The present study was designed to determine whether canbra oil is metabolized by the human in a manner similar to other dietary fats. In order to permit the testing of a single fat source, a test diet was formulated using meat analogues from soybean protein, egg albumin, and skim milk as the primary protein sources. Thus the mixed fat or canbra

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[1]The term 'rapeseed oil' used throughout the text refers to the oil from the original rape cultivars characteristically high in erucic acid.

oil used in the present study constituted approximately 95 per cent of the total daily fat intake. The subjects participating in the study were seven male college students. Lipid metabolism was assessed by whole blood hematology and determination of serum lipid constituents. Whole blood parameters measured included hemoglobin, hematocrit, reticulocyte count, total red cell count, and red cell fragility. Sera were analyzed for total cholesterol, lipid phosphorus, triglycerides, electrophoresis of lipoproteins, and determination of the fatty acid composition of the precipitated phospholipid fraction.

REVIEW OF LITERATURE

A. INTRODUCTION

Rapeseed oil is characterized by the presence of erucic acid, a 22-carbon monoenoic fatty acid (Δ13-cis-docosenoic acid), and small quantities of eicosenoic acid, a 20-carbon fatty acid (Δ11-cis-eicosenoic acid). The position of the double bond in these fatty acids (9 carbons from the methyl end) makes them members of the oleic acid series. In addition, rapeseed oil has a low level of saturated fatty acids, namely palmitic and stearic and so differs from most other vegetable oils.

Two species of rapeseed are grown in Canada, Brassica napus, commonly known as rape, colza, or Argentine-type rape, and Brassica campestris, commonly known as Polish-type or turnip rape. Internationally, the term 'rape' encompasses the seed of both species. The days to maturity for the B. campestris varieties are shorter (79-86 days vs. 100+ days for B. napus varieties) and so it is favored in Canada due to the length of the growing season on the Canadian prairies.

B. FATTY ACID COMPOSITION OF RAPESEED AND CANBRA OILS

The oils from B. campestris and B. napus are similar in the kinds of fatty acids present but different in the proportions of these fatty acids. B. campestris oil contains less erucic but more oleic and linoleic acids which would account for its higher iodine value (102-114 vs. 93-106). Noteworthy is the fact that European rapeseed oils differ from Canadian oils in that erucic acid makes up about 50 per cent by weight of the fatty acids.

In 1961, Stefansson *et al.* developed a new strain of *B. napus* rapeseed, having very little erucic acid. Subsequent work by plant breeders in the Department of Plant Science at the University of Manitoba and Canada Department of Agriculture, Saskatoon, has resulted in several varieties of low erucic acid rape in both the *B. napus* and *B. campestris* species. Comparison of the regular rapeseed oil with canbra oil shows the primary differences to be in the relative proportions of the monoene fatty acids (Table 1). The canbra oils have little or no erucic acid, very little eicosenoic acid, and appreciably high levels of oleic acid. The quantities of palmitic and stearic acids are slightly higher in canbra oil than rapeseed oil but both are low in these fatty acids when compared to other commercial vegetable oils such as soybean, olive, corn, and sunflower seed oil (Table 2). The linoleic acid content of canbra oil varies as it does in rapeseed oil with the level being slightly higher in the *B. campestris* than the *B. napus* varieties. The linolenic acid content of both canbra and rapeseed oil resembles that of soybean oil while the proportion of linoleic acid, though higher in the canbra oils, is still lower than that of other common vegetable oils. In addition, rapeseed and canbra oils contain small amounts of other fatty acids such as palmitoleic, C16:1, arachidic, C20:0, behenic, C22:0, and lignoceric, C24:0. The low level of saturation common to both rapeseed and canbra oils offers an advantage in the preparation of salad oils, as both give a higher yield than most vegetable oils.

With the changeover to canbra oil in Canada, considerable effort has been expended on the part of the plant breeder to develop new and improved varieties. Several generalizations have been made with regard to future genetic developments of canbra varieties (Weinberg, 1970).

Table 1

Fatty Acid Compositions of Rapeseed Oils[1]

	B. napus		B. campestris	
Fatty Acid, %	Nugget	Zero	Arlo	Zero
Palmitic, C16:0[2]	4	5	3	4
Stearic, C18:0	1	2	2	1
Oleic, C18:1	19	63	27	55
Linoleic, C18:2	14	20	18	31
Linolenic, C18:3	8	9	9	10
Eicosenoic, C20:1	14	1	12	-
Erucic, C22:1	40	-	31	-

[1]Data from Craig, 1970.

[2]Carbon number:number of double bonds.

Table 2

Comparison of Zero Erucic Rape (Canbra) Oils with Other Vegetable Oils[1]

Fatty Acid, %	Canbra Oil		Soya	Olive	Corn	Sunflower
	B.napus	B.campestris				
Palmitic, C16:0[2]	4.7	4.4	11.5	13.4	12.1	9.2
Stearic, C18:0	1.8	0.1	3.9	3.1	2.3	4.1
Oleic, C18:1	63.3	54.8	24.6	76.2	28.7	16.2
Linoleic, C18:2	20.0	31.1	52.0	5.5	56.2	72.5
Linolenic, C18:3	8.9	9.7	9.0	0.6	0.7	-
Eicosenoic, C20:1	1.3	-	-	-	-	-

[1]Data from Craig, 1970.

[2]Carbon number:number of double bonds.

These generalizations may be summarized as follows:

(i) canbra oils will contain only 4-6 per cent of saturated acids, i.e. palmitic and stearic combined;

(ii) canbra oil derived from B. campestris varieties can be expected to be about 10 per cent higher in linoleic acid than that derived from B. napus varieties;

and (iii) ideally, the eicosenoic and erucic acid content in the new canbra oils will be close to zero, although under practical conditions the content of erucic acid, C22:1, will probably be in the range of 5 per cent.

C. EFFECTS OF RAPESEED OIL AND CANBRA OIL ON ANIMAL GROWTH

Rapeseed oil became a crop of importance in Europe during the second World War when the interruption in shipping caused a shortage of edible oils. Once established, this highly unsaturated fat was found to be of great value for a balanced agriculture in northern Europe. Investigations with the oil, however, were hampered by lack of suitable analytical techniques for the study of unsaturated fatty acids. Nevertheless, comparative growth trials (Boer et al., 1947a,b) indicated inferior growth on rapeseed oil compared to other fat sources.

These experiments prompted Deuel et al. (1948a) to study the dietary effects of rapeseed oil. Their experiments confirmed earlier observations that weight gain was significantly lower in animals receiving rapeseed oil compared to butterfat or cottonseed oil. Furthermore, the efficiency of utilization of the rapeseed oil was poorer than that of the other two fats used in their study. The authors attributed the poor digestibility of rapeseed oil to the high content of erucic acid.

Carroll (1951; 1953) and Carroll and Noble (1952) confirmed the growth depressing effect of rapeseed oil and Barki et al. (1950) observed that poor growth was dependent on both the level and the kind of fat incorporated into the diet.

Thomasson (1955a) was the first to report, in feeding experiments of six weeks' duration, that average body weight gain decreased as the amount of rapeseed oil in the diet increased. At the highest level of rapeseed oil (73 calorie per cent) all animals died after being fed the diet an average of 17 days. Since the efficiency of food utilization for the rapeseed oil diets was comparable to the other diets containing one of the twenty fat sources tested, viz. lard, summer butterfat, olive oil, cottonseed oil, winter butterfat, beef fat, shea butter, maize oil, soyabean oil, groundnut oil, palm fat, sunflowerseed oil, poppyseed oil, coconut fat, sesame oil, owalanut oil, whale oil, herring oil, rapeseed oil, and kapokseed oil, growth retardation was assumed to be due to the effect of erucic acid on appetite. Thomasson concluded that fatty acids of 20 or more carbon atoms were causative and later showed that erucic acid was responsible for the decreased weight gains observed in rapeseed oil-fed rats (Thomasson and Boldingh, 1955b). Using a modification of Deuel's method for measuring the rate of intestinal absorption (Deuel et al., 1940), Thomasson found a significant correlation between the amount of rapeseed oil absorbed and the gain in weight of experimental animals. Of the eighteen oils tested, rapeseed oil was the most slowly absorbed at the end of 3 hours and among the slowest at the end of 6 hours. For 50 per cent of other common vegetable oils to be absorbed, only 6 hours was required while rapeseed oil required 8 to 9 hours for the same degree of absorption. Correspondingly, rapeseed oil produced

very poor growth; only kapokseed oil was inferior (Thomasson, 1956). Similarly, Carroll and Noble (1956) confirmed that rats grew and developed more slowly on diets containing erucic acid and that eicosenoic acid had a similar adverse effect on growth.

Beare et al. (1957) assessed the growth of rats fed Canadian-produced rapeseed oil. High levels of oil consistently retarded the weight gains of both sexes of Wistar strain rats. It was thought that appetite alone was responsible since there were significant differences in the amount of food consumed on the rapeseed oil diet compared with the corn oil diet. Thomasson (1955) also had suggested that the growth-retarding effect of rapeseed oil was associated with decreased food intake. However, when Beare et al. (1957) corrected body weight gain for the amount of food consumed by each rat, there was still a significant difference in weight gains after 3 weeks on the rapeseed oil diets, indicating differences in the utilization of these diets. However, rapeseed oil did not adversely affect corrected gains at 5, 7 and 9 weeks. Apparently, the animals were capable of adapting to rapeseed oil after 3 weeks, and were affected only through the appetite-depressing action of the rapeseed oil.

Murray et al. (1958a) studied the effect of pure fatty acid methyl esters on growth by feeding diets with methyl esters of oleic, eicosenoic, and erucic acid, thereby relating the effect of rapeseed oil to its major fatty acid constituents. However, no effect was found on the growth of males, although the growth of females was significantly depressed by rapeseed oil after 10 weeks on the diets.

Several authors have confirmed that when rapeseed oil was fed as more than 10 per cent by weight of the diet, growth was appreciably

reduced (Carroll, 1953; Beare et al., 1957; Roine and Uksila, 1959). Growth was equally as poor with 20 per cent erucic acid as with 20 per cent rapeseed oil in spite of an appreciably higher food intake with the 20 per cent erucic acid. The authors concluded that relatively small amounts of rapeseed oil in the diets of young rats had no deleterious effect on growth rate but as rapeseed oil or erucic acid was increased above the 10 per cent level in the diet, palatability decreased and growth was retarded.

Growth depression has been ascribed to the degree of saturation of the diet as well as the erucic acid content of the rapeseed oil (Hopkins et al., 1955; Murray et al., 1958b). In further work from the same laboratories, Beare et al. (1959a,b) concluded that both young and older animals utilized rapeseed oil and corn oil in the same manner. Subsequent work by Beare et al. (1963) substantiated their previous findings (Hopkins et al., 1955; Murray et al., 1958b) and they concluded that an unbalanced ratio of saturated to unsaturated fatty acids (expressed on a weight per cent basis), along with high amounts of erucic acid, was responsible for the adverse effect of rapeseed oil on animal growth. Acceptability of the diet was improved by increasing the saturation of the rapeseed oil by the addition of palm oil. The physiological mechanism underlying the food-intake-reducing action of rapeseed oil remains unknown but it has been suggested that hypothalmic control is involved since the decreased food consumption observed with rapeseed oil was temporary and occurred fairly rapidly (Beare and Beaton, 1967).

In general, these early experiments which found that rapeseed oil depressed growth and feed intake ascribed the effects to erucic acid and the degree of saturation of the diet. By contrast, high levels of canbra oil did not depress growth when studied in several animal species

(Cheneti et al., 1967; Craig and Beare, 1968; Rocquelin and Cluzan, 1968; Salmon, 1969a,b; Thoron, 1969). Furthermore, Craig and Beare (1968) found significantly higher food intakes and weight gains with canbra than with rapeseed oil. No further enhancement of weight gains was attained by the addition of saturated fatty acids to canbra oil nor by inter-esterification of the simple triglycerides. Thus canbra oil seemed to be nutritionally satisfactory when growth was the criterion of assessment.

D. DIGESTIBILITY OF RAPESEED AND CANBRA OILS AND THEIR CONSTITUENT FATTY ACIDS

In order to evaluate the nutritive value of dietary fats it is necessary to consider their digestibility, a term which describes the overall availability of a dietary fat (Deuel, 1955). That rapeseed oil could be digested and absorbed was shown as early as 1868 by Radziejewski (Aaes-Jørgensen, 1972) who found that erucic acid was deposited in the depot fat of rats fed rapeseed oil. Detailed experiments on the utilization of dietary rapeseed oil were first reported by Deuel et al. in 1940, although digestibility of rapeseed oil in man had been examined as early as 1918 by Holmes. Deuel et al. (1940) found rapeseed oil was more slowly absorbed in the rat than cottonseed oil, butterfat, or coconut oil. Additional digestibility studies on rapeseed oil by Deuel et al. (1948a,b) were prompted by the poor growth response of rats reported by Boer et al. (1947a,b). True digestibility was found to be 77 and 82 for crude and refined rapeseed oil, respectively, when fecal lipids were extracted by a method that also extracted the soaps. Deuel et al. (1948a) attributed the less efficient utilization of rapeseed oil by rats to its high content of erucic acid. When caloric intakes were corrected for the digestibility of the fats, the differences in efficiency of utilization

between the rapeseed oil diet and the butterfat or cottonseed oil diets disappeared.

The first report on the digestibility of rapeseed oil in humans was by Holmes (1918) who found a coefficient of digestibility of 98.8. Deuel and associates (1949) repeated these experiments in man since the possible presence of fecal soaps had not been taken into consideration by Holmes (1918). They similarly found coefficients of digestibility of 99.0 for rapeseed oil compared to 96.5 for cottonseed oil diets and concluded that a species difference exists between man and the rat with respect to the utilization of rapeseed oil. Recent observations by Vaisey et al. (1973) in humans fed diets containing either rapeseed oil or canbra oil confirm the earlier reports; digestibility coefficients were 98.7 for rapeseed oil and 99.9 for canbra oil.

Experiments with different species of animals including rats, guinea pigs, puppies, and pigs have indicated that strain as well as species affects the digestibility of rapeseed oil. Man and the puppy appear to be the only species that handle rapeseed oil well whereas digestibility is lower for the rat, guinea pig, and pig (Carroll, 1953; Carroll and Richards, 1958; Roine and Uksila, 1959; Crampton et al., 1960; Hamilton and McDonald, 1969; Rocquelin and Leclerc, 1969). Roine and Uksila (1959) and Crampton et al. (1960) found digestibility of rapeseed oil improved with age of the animal. However, no improvement was observed with age in the pig by Hamilton and McDonald (1969). On the other hand, Rocquelin and Leclerc (1969) found the digestibility of canbra oil to be high in the rat, namely 96.0 per cent. These findings suggest that there are species differences in the utilization of rapeseed oil but these differences vary depending on the erucic acid content of the oil. Of

importance to the present discussion, however, is the fact that both the original high erucic acid rapeseed oil and the new canbra oil are well digested and absorbed by the human (Holmes, 1918; Deuel et al., 1949; Vaisey et al., 1973).

Other dietary components have been found to affect the digestibility of rapeseed oil which may account for part of the apparent species differences. Removal of calcium and phosphorus markedly improved the digestibility of rapeseed oil in the adult female rat (Cheng et al., 1949) as did addition of 3 per cent lecithin to the diet (Augur et al., 1947). In fact, Carroll and Richards (1958) found that on a calcium-free diet the coefficient of digestibility of erucic acid was 92, whereas when calcium was present as 1 per cent of the diet and the Ca/P ratio was 4.25, the coefficient of digestibility for erucic acid was 21. High levels of dietary protein (65 per cent) also have been found to improve the digestibility of erucic acid (Carroll and Richards, 1958; Crampton et al., 1960).

Noteworthy is the fact that diets containing high amounts of rapeseed oil were well-accepted by human subjects (Deuel et al., 1949; Vaisey et al., 1973) and that no dietary upsets, such as diarrhoea occurred as has been noted by Trémolières and associates (1971) in France in humans given a single dose of liquid rapeseed oil. Vaisey et al. (1973) found no depression in appetite during an 8-day controlled feeding study when either rapeseed oil (35.6 per cent erucic acid) or canbra oil (3.3 per cent erucic acid) contributed over 50 per cent of the calories from fat.

E. METABOLISM OF RAPESEED AND CANBRA OILS

Although studies have shown that erucic acid can be stored in animal tissue lipids, the ratio of erucic to other fatty acids has been found to be considerably lower than that of the diet. Several authors (Hopkins et al., 1957; Carroll, 1962; Craig et al., 1963; Craig and Beare, 1967) have suggested that the relatively low amount of erucic acid incorporated into tissue lipids is due to the in vivo metabolic conversion of erucic acid to oleic acid. Recently Carreau et al. (1968) checked the specific activity of liver fatty acids at specified times after injection of 14-^{14}C labelled erucic acid into rats. At two hours after injection, 20 to 28 per cent of the label present in liver fatty acids was in monoethylenic fatty acids other than erucic acid (Table 3). Lapous et al. (1970) have found that labelled erucic acid rapidly disappeared from liver, kidneys, brain, testicles, and seminal vesicles on withdrawal of rapeseed oil from the diet. Carroll (1966) had previously suggested that dietary erucic acid was deposited in the liver as free fatty acids, and was metabolized more slowly than oleic acid, whereas Bach et al. (1969) demonstrated that erucic oxidation proceeded at the same rate as oleic acid as evidenced by the specific activity of carbon dioxide expiration although the overall yield was lower. Walker (1972) found low amounts of eicosenoic, C20:1 and erucic acid, C22:1, deposited in liver, heart, plasma, and brain after feeding either rapeseed (B.campestris) or canbra oil as 30 calorie per cent to Wistar rats for 18 weeks (Table 4). Tissues, in general, with the exception of the heart, exhibited limited deposition of erucic acid. The heart requires more detailed consideration and will be discussed in Section F. Rocquelin et al. (1970) reported similar results for the liver and heart of rats fed diets containing 30 calorie per cent of

Table 3

Specific Activities of Rat Liver Fatty Acids, 2 Hours After Intravenous Injection of 14-^{14}C Erucic Acid in Rapeseed Oil Emulsions[1].

Methyl Esters[2]	DIETS		
	Fat-Free	Fat-Free + 1.6% Sunflower Oil	Fat-Free + 20% Rapeseed Oil
	Specific Activity: 10^{-2} x μC/mg.		
Saturated	0.13	0.06	0.06
Erucic	7.70	8.96	8.70
Mono-ethylenic[3]	2.30	3.30	3.70
Di-ethylenic	0.26	0.23	0.04
Tri-ethylenic	0.98	0.08	0.80
Poly-ethylenic	0.26	0.18	0.08

[1]Data from Carreau et al., 1968.

[2]Methyl esters of liver fatty acids were separated on TLC with silver nitrate impregnated silica gel.

[3]Erucic acid was not included; oleic acid was the main constituent of this fraction.

Table 4

Deposition of Fatty Acids in Liver, Heart, Plasma, and Brain of Rats Fed 30 Calorie Per Cent Rapeseed Oil or Canbra Oil for 18 Weeks[1]

	LIVER		HEART		PLASMA		BRAIN	
Fatty Acid	RSO[2]	ZRSO[3]	RSO	ZRSO	RSO	ZRSO	RSO	ZRSO
16:0[4]	17.9	18.5	10.5	11.4	16.1	15.0	16.2	18.7
16:1	3.0	2.4	1.3	1.6	2.8	2.0	0.1	0.5
18:0	12.5	12.6	15.2	15.4	9.1	7.8	19.6	21.5
18:1	28.6	30.5	21.8	29.4	27.8	37.0	25.0	26.3
18:2	9.6	11.6	16.5	15.7	13.3	15.5	0.8	1.2
18:3	1.3	1.3	1.2	1.2	1.6	3.0	0.2	0.1
20:1	2.4	0.6	3.6	0.6	3.3	0.8	3.0	3.3
20:4w6	14.4	12.5	17.7	12.2	18.4	12.7	10.2	9.9
22:1	1.3	0.2	3.1	0.4	2.9	0.9	0.7	0.2
22:4w6	0.5	0.2	0.6	0.6	0.3	Trace	3.4	3.0
22:5w6	0.5	Trace	0.4	Trace	Trace	Trace	0.5	Trace
22:5w3	0.9	1.1	1.0	1.6	0.3	0.6	2.0	0.1
22:6w3	4.6	5.6	4.9	6.2	1.6	2.4	15.4	12.8

[1]Data from Walker, 1972.

[2]RSO, Brassica campestris.

[3]ZRSO, Canbra oil.

[4]Carbon number:number of double bonds.

B. napus oil for 12 weeks. However, in meat-producing animals, namely the chicken, turkey, and lamb, substantial amounts of erucic (6 - 18 per cent of total fatty acids) and eicosenoic acid (7 - 12 per cent) were found in the adipose tissue when rapeseed oil was fed at levels of 10 per cent of the diet (Sell et al., 1968; Salmon, 1969a,b; Stokes and Walker, 1970). Similarly, the milk of female rats fed rapeseed oil at 20 per cent of the diet for 2 weeks contained erucic acid and eicosenoic acid at 15 and 9 per cent respectively (Beare, 1961a). In experiments of a longer duration, Beare et al. (1961b) observed somewhat lower amounts, 7 and 5 per cent respectively, which suggests that the female rat adapted to the intake of these fatty acids. Unlike milk fat, only small amounts (1 - 1.5 per cent) were found in the chicken egg yolk (Sell et al., 1968). On the other hand, Walker (1972) confirmed the observations of Rocquelin et al. (1970) that a greater proportion of eicosenoic acid than erucic acid was found in the brain.

Rocquelin et al. (1970) have suggested other characteristics of rapeseed oil, also common to canbra oil, namely the low level of palmitic acid, a high level of monounsaturated acids, and similar linolenic acid content, may play a part in the metabolism of tissue fatty acids. It also has been suggested (Mohrhauer and Holman, 1963; Mohrhauer et al., 1967 ; Mohrhauer and Holman, 1967) that this peculiar composition of rapeseed oil may affect the in vivo metabolism of linoleic acid in the liver of animals fed high levels of rapeseed oil. Several workers (Beare et al., 1963; Craig and Beare, 1968; Craig et al., 1963; Rocquelin et al., 1970) have observed that the conversion of linoleic to arachidonic acid is decreased in the liver in the presence of high erucic acid rapeseed oil in the diet. However, this also was found when canbra oil was present in the diet, suggesting that factors other than erucic acid may interfere with

fatty acid metabolism. More work is required in this area, particularly with regard to the comparison of canbra oil with rapeseed oil.

F. PHYSIOLOGICAL EFFECTS OF RAPESEED AND CANBRA OIL

One of the earliest reported physiological responses to rapeseed oil, other than depressed growth, was impaired reproduction. Carroll and Noble (1957) and Beare et al. (1959b) found a reduction in spermatogenesis in the absence of growth depression in both young and adult male rats. However, females were unaffected by treatment except for some interference with parturition. Recently, this effect of rapeseed oil on reproduction has been re-examined in the rat (Cheneti et al., 1967) and the pig (Thoron, 1969). These investigators have questioned whether rapeseed oil or erucic acid per se was the cause of the ill effects exhibited. In fact, they have attributed the deleterious effects seen by the early workers in this area to the feeding of hyperlipemic diets and inadequate supplies of vitamins such as E and A.

Of considerable concern today, particularly with regard to heart muscle, are the pathological changes accompanying the feeding of rapeseed oil. Although Roine and associates reported myocarditis in rats fed high amounts (50-70 calorie per cent) of rapeseed oil high in erucic acid as early as 1960, it was really the work of Rocquelin and associates (Rocquelin and Cluzan, 1967;1968; Rocquelin and Potteau, 1968; Rocquelin et al., 1970) that brought focus to this problem. Foci of histiocyte infiltration in the myocardium of rats fed rapeseed oil over a six-month period was first reported by Rocquelin and Cluzan (1967) and described in greater detail the following year by these workers (Rocquelin and Cluzan, 1968). The process of histiocyte cell infiltration was described further by Beare-Rogers (1970) and Abdellatif and Vles (1970a,c) who

subsequently demonstrated that lipid accumulation in cardiac and skeletal muscle began within a few hours after introducing rats to a dietary regimen containing rapeseed oil, reached peak infiltration after three to six days, as evidenced by a creamy heart, and then decreased to near normal levels within five to six weeks. The mechanism by which the heart disposes of the accumulated fat is equally as intriguing as is the cause of the fatty infiltration which occurs in the early stages of feeding rapeseed oil. These investigators (Abdellatif and Vles, 1970a,c; Beare-Rogers, 1970) found that the stage of histiocyte infiltration described by Roine (1960) and Rocquelin and Cluzan (1968) was present at the end of sixteen weeks, and after thirty-two and sixty-four weeks interstitial replacement fibrosis of the myocardium was observed.

Houtsmuller et al. (1970) have investigated the biochemical lesions that accompany the inclusion of rapeseed oil in the diet of rats. They observed a decrease in the capacity of isolated heart mitochondria to oxidize substrates concomitant with fatty accumulation, which mainly consisted of a rise in triglycerides (x 10) although there also was a small increase in free fatty acids (x 2.4). This has shown that erucic acid promoted the early deposition of cardiac fatty acids primarily in the form of triglycerides because upon feeding glyceryl trierucate, a linear relationship was observed between the amount of erucic acid ingested and the ATP production of the heart mitochondria.

The same lesions as described for the rats also have been observed in test pigs (Roine et al., 1960), ducklings (Abdellatif and Vles, 1970b; 1971; Abdellatif et al., 1972), rabbits (Vles and Abdellatif, 1970a), guinea pigs (Vles and Abdellatif, 1970b), miniature and regular baby pigs, gerbils and squirrel monkeys (Beare-Rogers and Nera, 1972). Although

the different species varied in the extent and pattern of response, all suffered some degree of myocardial change. In addition, ducklings (Abdellatif et al., 1972) and guinea pigs (Vles and Abdellatif, 1970b) both exhibited decreased hemoglobin, increased reticulocyte counts, and increased red cell fragility.

The prime causative agent in producing the ill effects in experimental animals therefore appeared to be erucic acid although other factors, as with growth, may participate in modifying the effect of dietary rapeseed oil. Increasing the saturation of the diet seemed to decrease the response to the erucic acid present in the regime (Beare et al., 1963; Beare-Rogers, 1970; Beare-Rogers et al., 1971; Abdellatif and Vles, 1971) but this was not always effective in reducing the pathogenicity (Beare-Rogers et al., 1972a). Not only erucic acid but other docosenoic acids such as those in marine oils also induced lipid accumulation in the heart of weanling rats (Beare-Rogers et al., 1971). These authors also have suggested that the position of the double bond may have some effect, but that the effect was basically minor compared to that of the chain length of the monoenoic acid (Beare-Rogers et al., 1972a).

Whether the occurrence and etiology of the later degenerative lesions in the heart are related to the early lipid infiltration still requires further investigation (Beare-Rogers et al., 1972b). However, Beare-Rogers et al. (1972a) have attributed the degenerative changes to erucic acid. Nevertheless, Rocquelin et al. (1970) and Rocquelin and Cluzan (1971) have found degenerative changes in heart muscle of rats fed canbra oil, though less pronounced than in rats fed rapeseed oil. This apparent conflict requires resolution because others have not observed lesions with canbra oil (Craig and Beare, 1968; Abdellatif and Vles, 1970c;

Beare-Rogers et al., 1971).

Whether the ingestion of high levels of rapeseed oil by the human will induce degenerative changes in cardiac muscle has been the subject of considerable debate over the past 2 to 3 years. The same aspects investigated in the rat cannot be looked at in the human. Research on the metabolism of rapeseed oil by the human, as with any studies on lipid metabolism, are primarily restricted to the investigation of changes in blood parameters accompanying the ingestion of rapeseed oil. A number of investigators (Carroll, 1951; Beare et al., 1959a;1960; Beare, 1961 ; Rozynkowa et al., 1962) have looked at changes in blood cholesterol in the rat upon feeding a regime containing rapeseed oil, although the rat is a poor experimental model for such research. In fact, Rozynkowa et al. (1962) even suggested that the pattern of change of cholesterol in the rat may be opposite to that in the human. Nevertheless, blood cholesterol was not affected by the ingestion of rapeseed oil or erucic acid in the diets fed to rats, (Carroll, 1951; Beare et al., 1959a;1960; Beare, 1961 ; Rozynkowa et al., 1962), mice, rabbits, and dogs (Carroll, 1957; Kritchevsky et al., 1972). Malmros and Wigand (1957) and Grande et al. (1962) seem to be the only investigators to have looked at the effect of rapeseed oil on serum lipids of humans. Small decreases in blood cholesterol analogous to that of other monoenoic fatty acids was found when rapeseed oil was present in the diet. However, no work has been reported on the effect of canbra oil upon serum lipid patterns. This is an area requiring further study.

Experimentally obtained myocarditis, including the influence of dietary fat source on lipid metabolism in the myocardium, as well as the relationship between the pathological changes in the myocardium and lipid

accumulation induced by particular fat sources, must be studied in different animal species fed rapeseed oil or canbra oil. Much of the present literature is not interpretable because comparison of results from various laboratories is possible only if the experimental conditions have been similar. Sources of variation in experimental conditions include the species of animal used as well as the strain. The age of the animal and the duration of the experiment determine the response to the oil, and the calorie per cent of the oil, as well as the erucic acid content, determines the magnitude of the response.

OBJECT OF RESEARCH

The objectives of the study herein described were:

1) to determine the effect of canbra oil as the major souce of dietary fat on a) serum lipid patterns and b) whole blood constituents of normal human subjects in so far as lipid metabolism is reflected by changes in blood lipid patterns;

2) to develop recipes which would facilitate the incorporation of either a mixture of fats or a single fat or oil as the major source of fat; and 3) as a result, to design a fat-controlled mixed fat or canbra oil diet resembling the diets normally consumed by Canadians.

EXPERIMENTAL METHODS

A. EXPERIMENTAL DESIGN

The 39-day metabolic trial was divided into 3 periods. The first nine days of the trial served as a stabilization period during which a mixed fat diet formulated to simulate the amount and composition of the fat in the average North American diet was fed. The purpose of this period was to provide time for the introduction of the subjects to the routine of the study, to determine individual caloric requirements, and to allow the blood lipid patterns to stabilize. The next twenty-two days of the trial served as the experimental period when the subjects received a canbra oil diet. The canbra oil diet was followed by eight days on the mixed fat diet.

The subjects maintained their normal activities and resided in their own homes. Any unusual activities were recorded for reference. Meals were served in the Home Economics Building, University of Manitoba, with the exception of Saturday dinner and Sunday lunch and dinner which were conveniently packaged for home preparation by the subjects. This protocol allowed for more normal family life and a more normal weekend routine. All meals were served at customary hours although attempts were made to accommodate individual lecture time-tables as all subjects were university graduate students. In addition to the three regular meals, the subjects received three snack items daily for between meals and post-dinner snacks. Standardized recipes (Appendix Tables 1-21) were followed in the preparation of all food items. Particular emphasis was placed on the fact that no other foods were to be consumed. General instructions given to the participants are present in Appendix Table 22.

Subjects were weighed daily before breakfast and individual caloric intake was periodically adjusted by alterations in the carbohydrate and fat intake to maintain constant body weight.

A two-day menu was used for both the mixed fat diet and the experimental regimen. Each daily menu was designed to include all food groups as well as provide texture and flavor. All recommended nutrient allowances specified by the Canadian Dietary Standard were met.

Fasting venous blood samples were obtained before breakfast on Days 1, 10, 18, 25, 32, and 39.

B. SUBJECTS

The subjects, seven healthy college males, aged 21-35 (average 24.6 years), were selected from a group who responded to personal contact and to posted notices. The subjects were chosen for the study on the basis of an interview with the project director, a physical examination and expressed co-operativeness. They were of average height and weight (Table 5) with no diagnosed metabolic disorders or recent history of poor health.

C. TEST FATS

The fat sources used for the mixed fat diet included butter[1],

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

Table 5

Physical Data of Subjects

Subject	Age (yr)	Height (cm)	Weight (Kg)	
			Initial	Over 39-Day Experimental Period
H.G.	23	175	74.2	73.5±0.4[1]
L.R.	24	182	71.2	71.0±0.2
C.W.	23	178	63.9	63.8±0.2
L.G.	24	179	64.2	64.1±0.3
D.R.	21	182	57.6	57.7±0.3
P.R.	35	175	72.0	73.2±0.4
H.R.	22	170	50.6	50.6±0.2

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

corn oil[2], beef tallow[3], margarine[4], lard[5], and vegetable shortening[6]. Canbra oil[7] and specially prepared canbra margarine[8] were utilized as the principle fats in the experimental diet. Fatty acid composition of the various fats was analyzed by gas-liquid chromatography.

D. STORAGE AND HANDLING OF DIETARY FATS AND OTHER FOOD STAPLES

Fats for both the mixed fat diet and experimental diet were purchased in single lots and stored in sealed containers. Butter, beef tallow, Parkay margarine, lard, Crisco shortening, and canbra margarine were stored at 7°C in a home-style electric refrigerator while the Mazola corn oil, and the canbra oil were kept at room temperature.

Other staples were similarly bought in single lots and stored at appropriate temperatures for the form of the product. Fresh skim milk was purchased bi-weekly, and a weekly supply of both bread (frozen until required) and produce were bought from a single local source. All the main dishes and snack items were prepared in advance and stored at -10°C until needed.

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

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

[7]Prepared from B. campestris cv. Span, Canada Packers Ltd., Winnipeg, Manitoba.

[8]Prepared from B. campestris cv. Span, Canada Packers Ltd., Research and Development Laboratories, 2211 St.Clair Ave.W., Toronto 9, Ontario.

E. DIET COMPOSITION

The mixed fat diet was formulated to simulate the amount and the composition of the fat in the average North American diet. Literature values on the trends in fat disappearance in North America indicated that the fatty acid pattern in the North American diet was in the ratio of 4.0:3.8:2.2 for saturated:oleic:linoleic (Table 6). Using the fats listed under Test Fats, Section C, a mixed fat diet was formulated to approximate these ratios. The proportion of each fat used in the diet is given in Table 7. When a mixture of these fats in the proportion used in the diet was analyzed, close agreement was found with the reported values, the ratios of saturated:oleic:linoleic being 4.0:3.9:2.2. Analysis by gas-liquid chromatography of the mixture made up of these fats is shown in Table 8. Butter was used primarily as the spread for the day, while the other fats were incorporated into a variety of menu items.

Textured vegetable protein (TVP)[9], fluid skim milk, and spray-dried egg albumin[10], were utilized as the primary protein sources. TVP, an extruded soybean product, was used to replace the meat normally found in the main entrées. TVP is essentially fat-free and is available in a variety of physical forms and simulated meat flavors. Composition of the three products used in the present study is shown in Table 9. TVP is supplemented with vitamins and minerals but is limiting in sulfur-containing amino acids. However, the skim milk and egg albumin added to the diet were sufficient to provide adequate amounts of the sulfur-containing amino acids. When TVP was hydrated with a mixture of water

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

[10]Cham Foods Ltd., Jarvis St., Winnipeg, Manitoba.

Table 6

Relative Proportion of Fatty Acids
in Mixed Fat Diet

	Sat.	Oleic	Linoleic
Literature values[1]	4.0	3.8	2.2
Calculated	4.0	3.8	2.2
Analyzed	4.0	3.9	2.2

[1]On the basis of, Call, D.R. and Sanchez, A.M. 1967. Trends in fat disappearance in the United States, 1909-65. J.Nutr. 93: Suppl.I, Part II.

Table 7

Composition of Fat Mixture

Ingredients	Per Cent
Butter	39.3
Corn oil	21.5
Tallow	7.1
Parkay Margarine	10.7
Lard	14.3
Crisco Shortening	7.1

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
cis-9 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.

Table 9

Typical TVP Analysis[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, %			
Phosphorus	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: Archer Daniels Midland Co., 733 Marquette Ave., Minneapolis, Minnesota 55440.

*Not determined at this time.

and the particular fats being studied, it closely simulated chunks of meat. In this form, it was readily incorporated into casserole type dishes, such as Sweet and Sour Pork or Beef Stew. The same product, in strips, was ground and formed into items such as Hamburgers and Meatballs. The four entrées, Hamburg Patties, Spaghetti with Meatballs and Tomato Sauce, Sweet and Sour Pork and Beef Stew, were prepared in advance in individual foil containers[11], frozen, and stored for up to three and a half months. No detectable changes were observed as a result of freezing and storage. Entrées for the various meals were taken directly from the freezer and heated and served in the original foil container.

A two-day menu rotation (Table 10) was developed with each menu designed to provide approximately 40 per cent of the total calories from fat. The experimental diet was similar to the mixed fat diet except that canbra oil and margarine were substituted for the fats contained in the mixed fat diet.

Breakfast was essentially the same for both Menu I and Menu II (Table 10). There was a choice of juice and subjects could select either rolled oats or cream of wheat. In addition, subjects could elect an omelette or French toast made from the egg albumin and bread instead of "scrambled" eggs[12] and toast. Lunch menus offered either Hamburg Patties on a Bun (Menu I) or Spaghetti with Meatballs and Tomato Sauce (Menu II). Variety in the two lunch menus was provided by the different salads, Coleslaw and Dressing (Menu I) or Tossed Salad with Piquant Salad Dressing (Menu II), and by random variation in the fruit served for

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

[12]"Scrambled" egg albumin - See recipe, Appendix Table 3.

Table 10

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 Albumin[3]	Scrambled Egg Albumin[3]
Slice Whole Wheat Toast	Slice Whole Wheat Toast
Skim Milk	Skim Milk
Strawberry Jam	Orange Marmalade
White Sugar	Brown Sugar
Butter or Margarine[4]	Butter or Margarine[4]
Lunch[1,2]	
Hamburg Patties[3]	Spaghetti/Meatballs/Tomato Sauce[3]
Bun	Slice Whole Wheat Bread
Coleslaw[3]	Tossed Salad: Lettuce and Tomato[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: Lettuce and Tomato[3]	Coleslaw[3]
Piquant Salad Dressing[3]	Coleslaw Dressing[3]
Slice Whole Wheat Bread	Slice Whole Wheat Bread
Canned Fruit with Juice[5]	Canned Fruit with Juice[5]
Skim Milk	Skim Milk
Snacks[1,2]	
Spicy Fruit Square, Iced[3]; Slice White Cake[3]; Raisin Oatmeal Cookie[3]	

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

[2]For quantities of each item, see Diet Calculations, Appendix Tables 23, 24, 25, and 26.

[3]See Recipes, Appendix Tables 1-21.

[4]20.0 gm Butter as Spread for day for Mixed Fat Diet; 20.0 gm canbra Margarine as Spread for day for Experimental Diet.

[5]Pears, apricots, pineapple, peaches, and plums (90.0 gm strained, with 10.0 gm juice added).

dessert. Bread was included at each meal to utilize the spread (butter or canbra margarine) and also to permit the subjects to wipe up any visible fat remaining on the serving dishes. Sweet and Sour Pork (Menu I) and Beef Stew (Menu II) were the main entrées at dinner. Variety again was provided by the different salads and variation in the fruit served. Pickles, sweet pickle relish, soy sauce, ketchup, and mustard were available at lunch and dinner. Subjects were provided specific amounts of each, for example - 1 tsp. of sweet pickle relish, 1 package of ketchup or mustard, and so forth.

The menus were designed for young adult male subjects and were calculated on an approximate 3000 calorie intake. Calorie and nutrient content of the diet is shown in Table 11, and the calculated nutrient composition of the menus is presented in Appendix Tables 23, 24, 25, and 26.

F. MEAL ANALYSIS

Composites were made of each of the daily menus of the mixed fat and experimental diets. The individual food items were thawed overnight in the refrigerator, and in the morning, weighed to the nearest gram on a Sartorius top-loading balance (Model 2254)[13]. Thawed composites of the meals were homogenized with 200 ml. of distilled water in a one-gal. Waring commercial blender (Model CB-5)[14]. A 70 gram portion of the homogenate was lyophilized in a Model 10-145MR-BA Virtis Freeze Drier[15]. The dried sample was ground with a mortar and pestle, and stored in

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[13]Sartorius-Werke AG, Gottingen, Germany.

[14]Waring Products Co., Winsted, Connecticutt.

[15]Virtis Company Inc., Gardiner, New York 12525.

Table 11

Calorie and 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	3077	3063	3060	3066	3100
Protein (g)	98.7	117.1	97.7	111.0	54.0
Fat (g)	140.3	136.4	138.8	137.6	-
Carbohydrate (g)	367.9	477.1	367.6	467.9	-
Calcium (mg)	1238	1317	1230	1313	500
Phosphorus (mg)	1224	1453	1219	1255	-
Iron (mg)	10.8	23.3	10.8	23.8	6.0
Vit.A (I.U.)	6036	10177	4650	8955	3700
Thiamin (mg)	2.6	1.6	1.6	1.6	0.9
Riboflavin (mg)	2.8	3.6	2.8	3.6	1.5
Niacin (mg)	8.0	12.5	8.0	12.5	9.0
Vit.C (mg)	144.2	115.6	144.2	88.0	30.0

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

[2]Based on Revised Dietary Standard for Canada (1964). Values given for males, 176 lb. (80 Kg), Activity Level A.

Whirl-Pak plastic bags (#B992, 510.30 gm)[16] at -10°C for later analysis.

Total lipid was extracted from the lyophilized food samples using the method of Bligh and Dyer (1959). Methyl esters of the fatty acids were prepared according to the method of Metcalfe et al. (1966) and were stored dissolved in petroleum ether in screw-top glass vials, flushed with nitrogen prior to storage, at -10°C until subjected to gas-liquid chromatographic (GLC) analysis.

The fatty acid methyl esters were separated using an Aerograph gas chromatograph (Model 1740-1)[17] equipped with dual columns, flame ionization detectors, a Varian Aerograph single pen recorder (Model 20)[17] and a Varian Aerograph digital integrator (Model 477)[17]. Helium[18] served as the carrier gas.

Samples were resolved on 2.7 m x 3.2 mm steel columns packed with 10 per cent EGSS-Y on 100/120 mesh GAS CHROM Q[19]. The flow rates were 30 ml./min. for helium[18], 25 ml./min. for hydrogen[18], and 250 ml./min. for air[18]. The columns were operated isothermally at a temperature of 195°C, with injector and detector temperatures maintained at 250°C and 230°C, respectively. The individual fatty acids were identified by comparing retention times with known fatty acid mixtures[20].

Bomb calorimetry on the diets, using a Parr Adiabatic Calorimeter (Model U30M) equipped with a #1241 automatic type calorimeter and a #1541

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[16]Canlab Laboratory Equipment, Winnipeg, Manitoba.

[17]Varian Aerograph, 6358 Viscount Rd., Malton, Ontario.

[18]Welder's Supplies, 25 McPhillips St., Winnipeg 3, Manitoba.

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

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

water heater[21], showed close agreement with calculated values (Table 12) when coefficient of digestibility was taken into consideration.

G. BLOOD INVESTIGATION

G.1. Sera and Blood Collection.

The subjects were instructed to have nothing to eat, specifically the post-dinner snack item, after 8:00 p.m. of the evening prior to taking the blood sample and to drink only water after midnight. Subjects were required to stand for at least 10 minutes prior to sampling.

Fasting blood samples were taken prior to breakfast on Days 1, 10, 18, 25, 32, and 39. Three 10 ml. BD Vacutainer tubes (No. 4710)[22] of blood were drawn from the antecubital vein of each person for use in serum analysis while a 3 ml. sample was obtained for whole blood analysis using a 3 ml. BD Vacutainer tube (No. 4854)[22]. The blood in the 10 ml. tubes was allowed to clot in a slanted position (approx. 45^{o} angle) for one hour at room temperature and then for 30 to 45 minutes in a refrigerator.

The clot was separated from the walls of the tube with the tip of a Pasteur pipet. The Vacutainer tubes were then centrifuged[23] at 1,400 x g for 10 minutes and the sera removed. The sera removed were re-centrifuged at 1,400 x g for 5 minutes and the clear sera pipetted into 1 dram glass screw-top vials[24]. The sera were aliquoted and stored

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[21]Parr Instrument Co., 211 Fifty-third St., Moline, Illinois 61625.

[22]Canlab Laboratory Equipment, Winnipeg, Manitoba.

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

[24]Kimble Co., Toledo, Ohio.

Table 12

Total Daily Caloric Intake

Diet	Calculated[1]	Analyzed[2]	% Difference[3]
MIXED FAT:			
Menu Day I	3077	3283.56	6.3
Menu Day II	3063	3242.88	5.5
EXPERIMENTAL:			
Menu Day I	3060	3310.62	7.6
Menu Day II	3066	3257.27	6.1

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

[2]Results obtained by bomb calorimetry expressed as Kilocalories.

[3]Per cent difference between analyzed and calculated values.

as follows: 0.5 ml., stored at 10°C for electrophoresis; 6.0 ml., stored at 10°C for ultracentrifugation; and the remainder distributed in approximately 2.0 ml. to a 1 dram vial and stored at -4°C for future analysis. Each vial was flushed with nitrogen before storage.

Prior to analysis, sera were thawed overnight at refrigerator temperature. Sera from the subjects were randomly analyzed in duplicate (two readings taken from each duplicate) for total cholesterol, lipid phosphorus, and triglyceride content. A standard (Moni-trol I, Lot No. Ltd. -112, B5103)[25] was run with all determinations. In addition, lipoprotein patterns were determined by electrophoresis and the serum phospholipids were precipitated from acetone and the fatty acid composition determined by gas-liquid chromatography. Red blood cell count, red blood cell fragility, reticulocytes, hemoglobin, and hematocrit were determined on the whole blood.

H. CHEMICAL ANALYSES

H.1. Serum.

H.1.a. Total Cholesterol. Total cholesterol was determined by the method of Pearson et al. (1952; 1953). This procedure involves a modified Leibermann-Burchard reaction, wherein serum was treated with acetic acid, p-toleunesulfonic acid, acetic anhydride, and sulfuric acid.

H.1.b. Lipid Phosphorus. Determination of lipid phosphorus was carried out using the method and standards of Fiske and Subbarow (1925) except that only 0.1 ml. of serum was used for the extraction of lipid. The ashing and the procedure for the analysis of phosphorus content were carried out using the method of Chen et al. (1956). All samples were

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[25]Dade Division, American Hospital Supply Corp., Miami, Florida 33152.

read against the blank using a Coleman Junior spectrophotometer (Model No. 6A-36715)[26].

H.1.c. Serum Triglycerides. Serum triglycerides were extracted according to the method of Ryan and Rasho (1967) except that only 0.1 ml. of serum was used for the determination. Each analysis was done in triplicate; two samples were saponified and one served as the unsaponified sample. After extraction, the samples were evaporated and then treated as in the procedure of Van Handel and Zilversmit (1957). Pure chromotropic acid was used as a blank against which the samples were read using a Unicam SP600, Series 2 spectrophotometer (Model No. 46511)[27].

H.1.d. Electrophoresis of Serum Lipoproteins. Electrophoresis of the sera was carried out according to the method of Beckering and Ellefson (1970). The cellulose acetate strips were scanned at a resolution of L on a Model 542 Densicord densitometer[28] fitted with filter No. N525. The areas of the peaks were determined with a planimeter and the ratio of the beta- to pre-beta-lipoproteins calculated.

H.1.e. Phospholipid Fatty Acid Patterns. Lipid was extracted from 2.0 ml. of serum by the procedure of Bligh and Dyer (1959). The chloroform extract was evaporated to dryness in 20 ml. polypropylene centrifuge tubes[29]. The phospholipids from the total lipid extracts were precipitated in acetone saturated with $MgCl_2$ according to the method of Beare-Rogers (1969). Methyl esters of the fatty acids in the

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[26]Coleman Instruments Inc., Maywood, Illinois.

[27]Pye Unicam Ltd., York St., Cambridge, CB12 PX, England.

[28]Photovolt Corp., New York, New York.

[29]Canlab Laboratory Equipment, Winnipeg, Manitoba.

phospholipids were prepared by the method of Barnes and Halloday (1972), except that only 0.50 ml. of 0.5N methanolic NaOH was used. The tightly stoppered vials were heated in a waterbath for five minutes at 80°C, cooled, 0.25 ml. BF_3-CH_3OH was added directly to each vial, and the contents reheated at 80°C for three minutes. After cooling, 1.5 ml. of saturated NaCl was added and the methyl esters extracted by shaking with 0.50 ml. n-hexane. The hexane layer was removed, concentrated under nitrogen and injected directly into the gas-liquid chromatograph.

The fatty acid methyl esters were resolved as described under MEAL ANALYSIS, Section F. Identification of the fatty acid methyl esters was made by the use of log plots and by comparison with known standards[30]. The fatty acid methyl esters also were resolved on 1.5 m x 3.2 mm stainless steel columns packed with 3 per cent SE-30 on 100/120 Varaport #30[31] as an additional aid in identification.

H.2. Whole Blood Parameters.

Blood parameters measured included hemoglobin, hematocrit, reticulocyte count, total red cell count and red cell fragility. All analyses on whole blood except red cell fragility were performed by the Winnipeg General Hospital, Hematological Laboratories.

H.2.a. Red Cell Fragility. The fragility of red cells was determined according to the method of Emerson et al. (1956). Two drops of each subject's blood were added from a Pasteur pipet to tubes containing 2.0 ml. of NaCl solution of the following concentrations (W/V): 0.25 per cent, 0.30 per cent, 0.35 per cent, 0.40 per cent, 0.45 per cent, 0.50 per cent, and 0.55 per cent. The tubes were inverted several times to mix and

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

[31]Applied Science Lab.Inc., P.O.Box 440, State College, Pennsylvania 16801.

allowed to stand for ten minutes. The tubes were then centrifuged[32] at 200 x g for 10 minutes and the colors of the supernatant solutions compared with standard tubes prepared as follows: 0.1 ml. of blood was dissolved in 2.0 ml. of distilled water (100 per cent hemolysis) and the latter diluted with appropriate amounts of distilled water to give standards corresponding to 10, 20, ...90 per cent hemolysis. Normally, hemolysis commenced at 0.45 per cent NaCl and was complete at 0.30 per cent.

I. STATISTICAL ANALYSES

All data were subjected to analysis of variance of a 2-way classification for a completely randomized block design according to Snedecor and Cochran (1967). The Treatments sum of squares were partitioned in order to make appropriate orthogonal comparisons to establish which of the treatments were significantly different from one another. Linear regressions were performed on the data for total serum cholesterol and lipid phosphorus to find the change that occurred per day for each of these parameters.

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

RESULTS AND DISCUSSION

A. SUBJECTS

The subjects remained in good health for the duration of the trial and all served successfully for the 39-day study. Body weight remained essentially constant during the entire experiment (Table 5). Thus, the changes observed in serum lipid patterns were attributed to dietary fat sources and not to sickness or changes in energy balance.

No dietary upsets were reported by the subjects during the course of the study even though they consumed approximately 134 g. of canbra oil daily on the experimental regimen. Trémolières et al. (1971) reported diarrhoea with humans given 30 ml. of liquid rapeseed oil in a single dose. The reason for the apparent difference between the present study and that of Trémolières et al. (1971) may be due to the composition of the oils fed, i.e. canbra oil vs. high erucic acid rapeseed oil, or to the method of administration, i.e. ingestion in a mixed diet vs. given orally as a single feeding of oil. Flatulence was a problem with the diet used in the present study but it was not associated with fat source because flatulence was equally as bothersome on the mixed fat as the canbra oil diet. This problem was attributed to relatively high levels of soybean protein (TVP) in the diet used in this study (28.5 - 37.5 grams per day).

B. DIETS

Both the mixed fat diet and the canbra oil diet were well accepted. The only problem encountered was with the Beef Stew where the TVP had been inadequately hydrated prior to the fat source being added.

The successful formulation of a solid test diet which permitted

incorporation of nearly all of the fat from a single source represented an improvement in experimental conditions for the study of the effect of dietary fat source on lipid metabolism of human subjects. The diet used in this study was similar to that used by Ravensdale (1972) except that a mixed fat diet, which was the same as the experimental diet except for fat source, was fed during the pre- and post-experimental periods. The diet used by Ravensdale (1972) during the pre-experimental period consisted of ordinary foods and contained a high level of butterfat.

Liquid formula test diets have been found to produce alterations in serum lipid levels, bile acid excretion, and plant sterol recovery which are independent of the fat contained in the formulae (Anderson et al., 1961). Nevertheless, several investigators (Lindstedt et al., 1965; Spritz et al., 1965; Wood et al., 1966; Connor et al., 1969; Grundy and Ahrens, 1970) have utilized liquid formula test diets for studies on the effect of dietary fat source on the composition of serum lipids in the human. Several investigators (Goldsmith et al., 1960; Eneroth et al., 1964; Moore et al., 1968) have used solid diets but the effect of dietary fat may have been confounded by the presence of fats other than those under investigation. In the diets used by Goldsmith et al. (1960), for example, 22 per cent of the fat calories were derived from sources other than the fat being tested. Similarly, the basal diet used by Moore et al. (1968) which consisted of three glasses of milk and two servings of meat, contributed up to 30 per cent of the daily fat calories.

Fatty acid composition of the diets is presented in Table 13. Fatty acid patterns were very similar for both menus with the mixed fat diet; myristic, palmitic, and stearic accounted for 34 per cent of the total, oleic another 35 - 36 per cent, and linoleic 21 per cent. There

Table 13

Per Cent Fatty Acid Composition of Diets

	Mixed Fat		Canbra Oil	
Fatty Acid	Menu I	Menu II	Menu I	Menu II
Myristic, C14:0[1]	3.0	3.5	-	-
Palmitic, C16:0	18.7	19.3	6.4	5.9
Palmitoleic, C16:1	1.8	1.9	tr	tr
Stearic, C18:0	12.5	12.1	3.0	4.5
Oleic, C18:1	36.5	35.4	54.5	57.8
Linoleic, C18:2	20.5	21.0	21.4	18.8
Linolenic, C18:3	1.7[2]	1.5[2]	9.5	7.5
Eicosenoic, C20:1	2.1	1.4	1.8	2.4
Erucic, C22:1	-	-	2.4	1.9

[1]Carbon number:number of double bonds.

[2]Linolenic, C18:3 and eicosanoic, C20:0 not resolved with columns used.

was also reasonable agreement in the fatty acid patterns for both menus with the canbra oil diet (Table 13). Saturated fatty acids provided only 10 per cent of the total while oleic acid accounted for 55 - 58 per cent of the total. Both the canbra oil and canbra oil margarine used in this study contained low levels of eicosenoic and erucic acid (Table 14). Thus the primary difference between the mixed fat and canbra oil diets was in the amounts of palmitic, stearic, oleic, and linolenic acid (Table 15). The canbra oil diet was much lower in palmitic and stearic acid than the mixed fat diet and higher in oleic and linolenic acid. The ratio of saturated to unsaturated fatty acids was 1.0:1.7 for the mixed fat diet compared to 1.0:8.9 for the canbra oil diet.

C. EFFECT OF DIET ON SERUM CHOLESTEROL

Changes in mean serum cholesterol at specific times throughout the study are presented in Tables 16 and 17 and Figure 1. There was a substantial decrease in serum cholesterol level for all subjects during the pre-experimental mixed fat period (Day 1 vs. Day 10), from an initial mean value of 203 to 174 mg/100 ml. (Table 16; Appendix Tables 27, 28). Notwithstanding this decrease during the preliminary period, serum cholesterol levels continued to decrease on the canbra oil diet. There was a mean decrease of 15 mg/100 ml. serum the first 8 days and 15 mg/100 ml. serum over the next 14 days (Table 17). Although the decrease during the first 8 days was as great as that during the last two weeks of the canbra oil diet, tests for linearity were significant over the entire 22-day period (Appendix Table 29). A decrease in serum cholesterol of 1.32 mg/100 ml. of serum per day was indicated by the slope of the linear regression line of the data covering the period on the canbra oil diet (Figure 2).

Table 14

Per Cent Fatty Acid Composition of Canbra Oil and Canbra Oil Margarine

Fatty Acid	Oil	Margarine
Palmitic, C16:0[1]	4.0	5.0
Palmitoleic, C16:1	tr	-
Stearic, C18:0	2.0	12.4
Oleic, C18:1	57.2	71.1
Linoleic, C18:2	21.2	5.6
Linolenic, C18:3	10.3	tr
Eicosenoic, C20:1	2.5	2.9
Erucic, C22:1	2.6	1.7

[1]Carbon number:number of double bonds.

Table 15

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

Fatty Acid	Per Cent Composition	
	Mixed Fat	Canbra Oil
Myristic, C14:0[1]	3.3	-
Palmitic, C16:0	19.3	6.2
Stearic, C18:0	12.3	3.8
Oleic, C18:1	35.9	56.2
Linolenic, C18:3	1.6	8.5
Ratio, Sat.:Unsat.	1.0:1.7	1.0:8.9

[1]Carbon number:number of double bonds.

Table 16

Total Serum Cholesterol of Subjects During Experiment[1]

Subject	Day of Experiment 1	10[2]	18	25	32[2]	39
	mg chol./100 ml. serum					
1 H.G.	213	190	177	161	159	189
2 L.R.	199	182	161	160	146	189
3 C.W.	196	155	147	141	137	167
4 L.G.	212	184	164	144	140	198
5 D.R.	197	165	147	143	139	172
6 P.R.	204	168	157	153	151	185
7 H.R.	203[3]	175	160	157	140	179
Group Mean	203	174	159	151	144	183

[1]Mean of duplicate analyses.

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

[3]Calculated value (serum lost) according to Snedecor, G.W. and Cochran, W.G. 1967. Statistical Methods, 6th.Edition. Iowa State University Press, Ames, Iowa. p.318.

Table 17

Changes in Serum Cholesterol Level

Subject	Experimental Period					
	Mixed Fat	Canbra 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 chol./100 ml. serum					
1 H.G.	-23	-13	-16	- 2	-31	+30
2 L.R.	-17	-21	- 1	-14	-36	+43
3 C.W.	-41	- 8	- 6	- 4	-18	+30
4 L.G.	-28	-20	-20	- 4	-44	+58
5 D.R.	-32	-18	- 4	- 4	-26	+33
6 P.R.	-36	-11	- 4	- 2	-17	+34
7 H.R.	-28	-15	- 3	-17	-35	+39
Group Mean	-29	-15	- 8	- 7	-30	+39

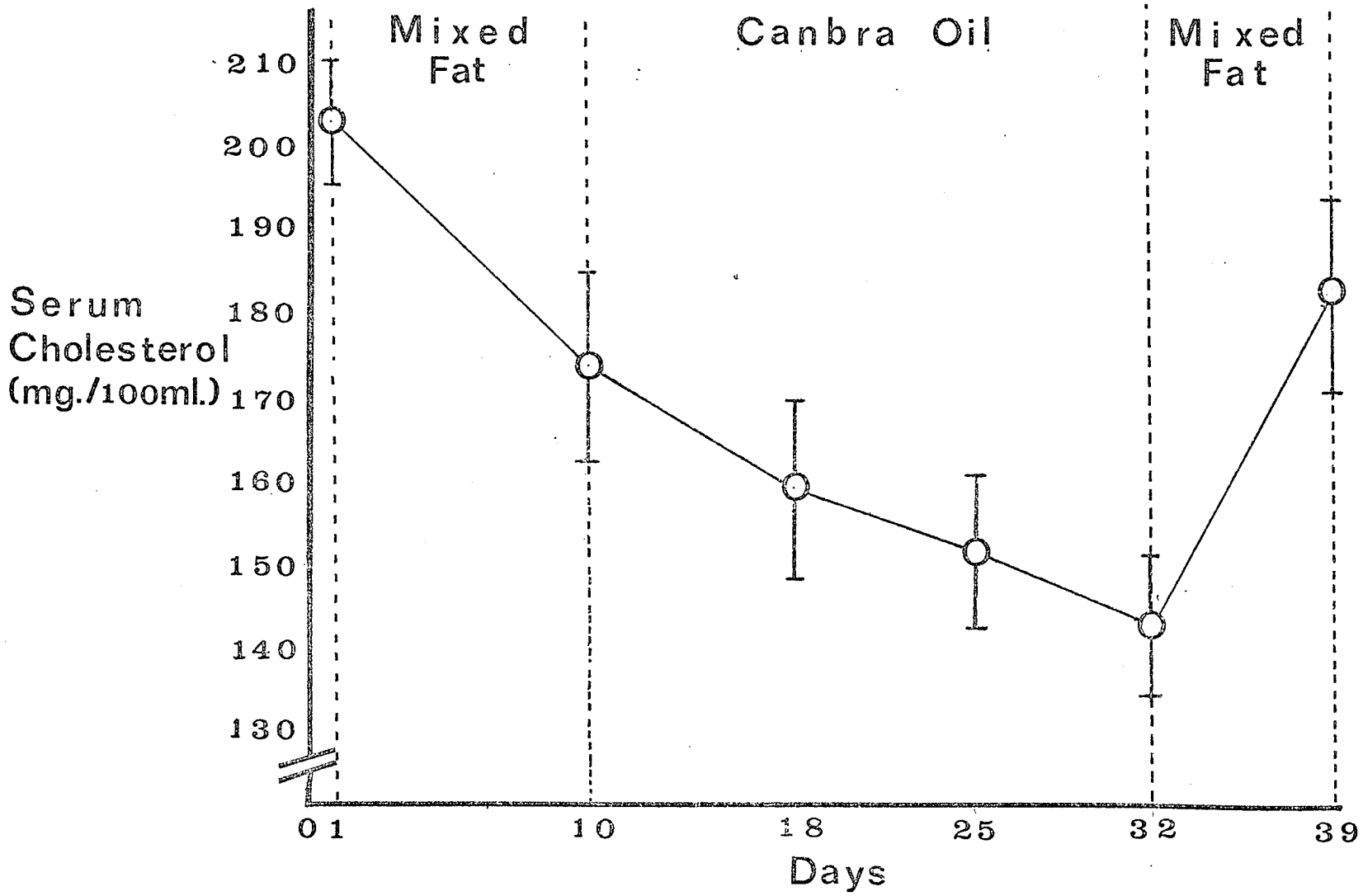

Figure 1. Mean serum cholesterol levels of subjects during experiment.

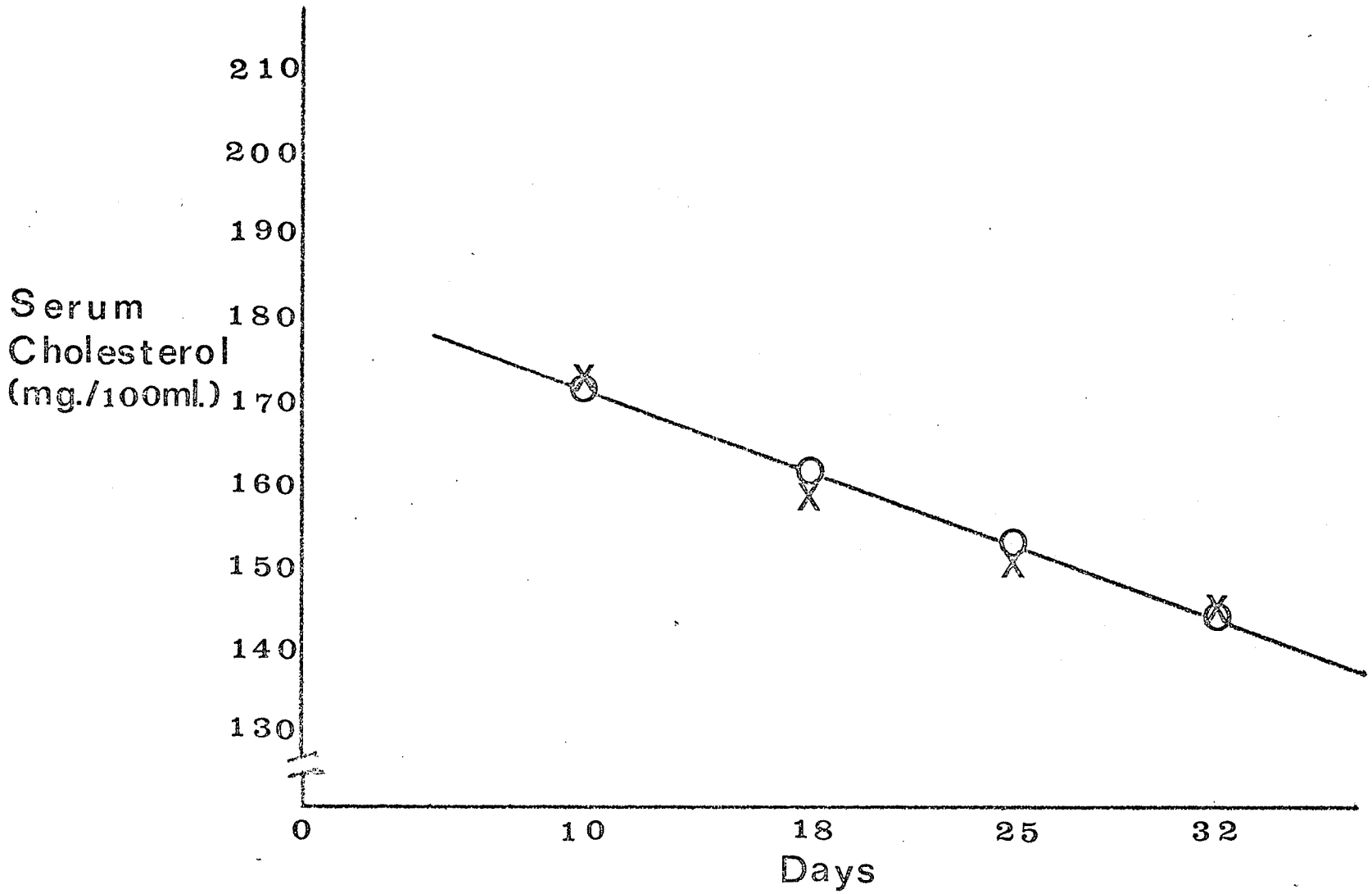

Figure 2. Linear regression of serum cholesterol on days of experiment for the canbra oil period. Slope of the regression line was -1.32.
Symbol: X - observed value; 0 - calculated value.

Serum cholesterol values did not appear to reach a stable plateau during the period the canbra oil diet was fed. Keys et al. (1957) stated that the major change in serum cholesterol following a change in dietary fat occurs during the first week with a further small decrease during the second week after which no further significant changes should be expected over the next one to two months. Although the major change on the canbra oil diet occurred during the first week, as had been observed by Keys et al. (1957) and Losier (1972) following a change in dietary regimen, mean serum cholesterol values did not plateau on the canbra diet due primarily to the marked decrease in serum cholesterol for two subjects (#2 L.R. and #7 H.R., Table 17) during the third week on this diet (Day 25 vs. Day 32). It would have been interesting to continue the experimental diet for a longer period of time in order to see whether a plateau would have occurred.

The data from the present study with canbra oil coincide with the observations of Malmros and Wigand (1957) and Grande et al. (1962) who found a similar cholesterol-lowering effect (approximately 40 mg/100 ml. decrease in serum cholesterol over a similar period of time) for subjects fed diets containing high erucic acid rapeseed oil. However, the decrease in serum cholesterol level which occurred during the experimental period in the present study was greater than might be expected on the basis of the changes in fatty acid composition of the diet, if one assumes that the equation derived by Keys et al. (1965) accurately predicts the change in cholesterol in response to a change in fatty acid composition of the diet. Keys et al. (1957) suggested that changes in serum cholesterol level were directly related to changes in fatty acid composition of the diet when expressed in terms of calories. Their original prediction

equation derived by multiple regression (Keys et al., 1957) showed changes in serum cholesterol to be primarily associated with saturated and polyunsaturated fatty acids while monounsaturated fatty acids had little or no effect on serum cholesterol levels (Keys et al., 1958). These relationships were subsequently confirmed by Hegsted et al. (1965). Further work by Keys et al. (1965) led to a simplified form of the original equation wherein stearic acid, C18:0 was omitted from the calculation since it was found not to affect serum cholesterol. The latter equation is written as follows:

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

where ΔC is the change in cholesterol level in mg/100 ml.,
$\Delta S'$ is the change in the total C12 to C16 saturated fatty acids,
ΔP is the change in the total polyunsaturated fatty acids with
$\Delta S'$ and ΔP expressed in terms of total calories in the diet.

The change in serum cholesterol level is dependent only on the changes in the dietary levels of C12 to C16 saturated fatty acids, relative to the changes in the polyunsaturated fatty acids both expressed as a per cent of total caloric intake.

The observed change in serum cholesterol in response to the canbra oil diet was found to be 30 mg/100 ml. serum (Table 17) whereas the predicted change in serum cholesterol in response to change in dietary fatty acids on the basis of the equation by Keys et al. (1965) would be 19 mg/100 ml. serum (Table 18). Grande et al. (1958) and Losier (1972) also found that observed values were slightly higher than predicted values when corn oil diets were fed. Higher values than those predicted by the regression equation also were observed by Grande et al. (1962) when subjects were fed high erucic acid rapeseed oil diets.

Table 18

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

DIET	% Total Daily Calories from Fatty Acids		% of Total Calories from fat	Change expressed as % of Total Caloric Fat Intake	
	S'[1]	P[2]		S'[1]	P[2]
Mixed Fat	22.6	22.6	40.6	9.18	9.18
Canbra Oil	6.2	28.6	40.6	2.52	11.62

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

$= 1.2\ [2(-6.66) - (2.44)]$

$= -18.91$

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

[2] P : total polyunsaturated fatty acids.

The substantial decrease in serum cholesterol levels noted on the mixed fat diet during the initial 9 days of the experiment could be due to the fact that literature values (Call and Sanchez, 1967) giving the composition of fat ingestion by the average North American are in error or that the subjects in this study customarily consumed a much higher level of saturated fat than the average North American. However, the possibility also exists whether type of protein has any effect on serum lipid patterns. Hamilton and Carroll (1971) have reported lower levels of serum cholesterol in the rabbit in response to diets containing plant proteins, particularly soybean protein, compared to those containing animal protein. Olson et al. (1964, 1970) reported that glutamate, a major component of most vegetable proteins, produced lower serum cholesterol levels. However, Olson's lab (Bazzano and Olson, 1969) later questioned the effect of glutamate on serum cholesterol levels and Anderson et al. (1971) found that source of dietary protein did not significantly alter serum cholesterol levels in the human. Whether type of protein has any effect on serum lipid patterns is a topic that requires further elucidation. Nevertheless, the response during the experimental diet can be attributed to canbra oil since the only difference between the mixed fat diet and the experimental regimen was the source of fat. In addition, the reversion to the mixed fat diet was accompanied by a concomitant increase in serum cholesterol. The pattern of change was consistent for all subjects during the 39-day trial in spite of the differences among subjects.

D. EFFECT OF DIET ON LIPID PHOSPHORUS

Lipid phosphorus response followed a pattern similar to that of serum cholesterol. This coincides with reports in the literature that

the response of serum lipid phosphorus to changes in diet composition is similar to that of serum cholesterol (Connor, 1969; Erickson et al., 1964; McGandy et al., 1970).

Changes in lipid phosphorus during the study are presented in Tables 19 and 20. There was a decrease in lipid phosphorus during the preliminary mixed fat period (Day 1 vs. Day 10), a further decrease during the canbra oil experimental diet (Day 10 vs. Day 32), followed by a sharp increase upon return to the mixed fat diet (Day 32 vs. Day 39). This pattern was very similar to that of serum cholesterol (Figure 3 vs. Figure 1) including the fact that reversion to the mixed fat regimen for the last eight days (Day 32 vs. Day 39) resulted in an increase in serum lipid phosphorus to a level slightly above that on Day 10 when the canbra oil diet was introduced. Although the overall pattern of response for lipid phosphorus was similar to serum cholesterol, there were distinct differences between lipid phosphorus and serum cholesterol with regard to the magnitude of change during the different dietary periods. The per cent change in lipid phosphorus was greater than the per cent change in serum cholesterol during the preliminary mixed fat period. In addition, the pattern of response for these two constituents differed somewhat on the canbra diet; with serum cholesterol the major change occurred during the first week whereas with lipid phosphorus the greater change took place during the last two weeks of the canbra oil diet. Substitution of the mixed fat diet for the 8 days following the canbra oil diet (Day 32 vs. Day 39) resulted in a greater increase in phospholipid[1] (i.e. mg lipid P x 25) than that which

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[1]Phospholipid calculated by multiplying mg lipid phosphorus/100 ml. serum/day by 25.

Table 19

Serum Lipid Phosphorus Levels of Subjects During Experiment[1]

Subject	Day of Experiment					
	1	10[2]	18	25	32[2]	39
	mg lipid phosphorus/100 ml. serum					
1 H.G.	11.3	9.2	8.2	7.4	6.4	10.0
2 L.R.	11.9	10.8	10.0	8.7	7.1	11.0
3 C.W.	11.8	9.8	8.9	7.4	6.4	9.8
4 L.G.	10.4	9.2	8.0	7.0	5.6	9.8
5 D.R.	12.8	10.8	10.1	8.8	7.5	11.3
6 P.R.	11.5	10.0	9.4	7.8	6.8	10.5
7 H.R.	12.2[3]	11.0	9.9	8.3	7.1	11.2
Group Mean	11.7	10.1	9.2	7.9	6.7	10.5

[1]Mean of two duplicate analyses.

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

[3]Calculated value (serum lost) according to Snedecor, G.W. and Cochran, W.G. 1967. Statistical Methods, 6th.Edition. Iowa State University Press, Ames, Iowa. p.318.

Table 20

Changes in Serum Lipid Phosphorus Level

Subject	Experimental Period					
	Mixed Fat	Canbra 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 phosphorus/100 ml. serum					
1 H.G.	-2.1	-1.0	-0.8	-1.0	-2.8	+3.6
2 L.R.	-1.1	-0.8	-1.3	-1.6	-3.7	+3.9
3 C.W.	-2.0	-0.9	-1.5	-1.0	-3.4	+3.4
4 L.G.	-1.2	-1.2	-1.0	-1.4	-3.6	+4.2
5 D.R.	-2.0	-0.7	-1.3	-1.3	-3.3	+3.8
6 P.R.	-1.5	-0.6	-1.6	-1.0	-3.2	+3.7
7 H.R.	-1.2	-1.1	-1.6	-1.2	-3.9	+4.1
Group Mean	-1.6	-0.9	-1.3	-1.2	-3.4	+3.8

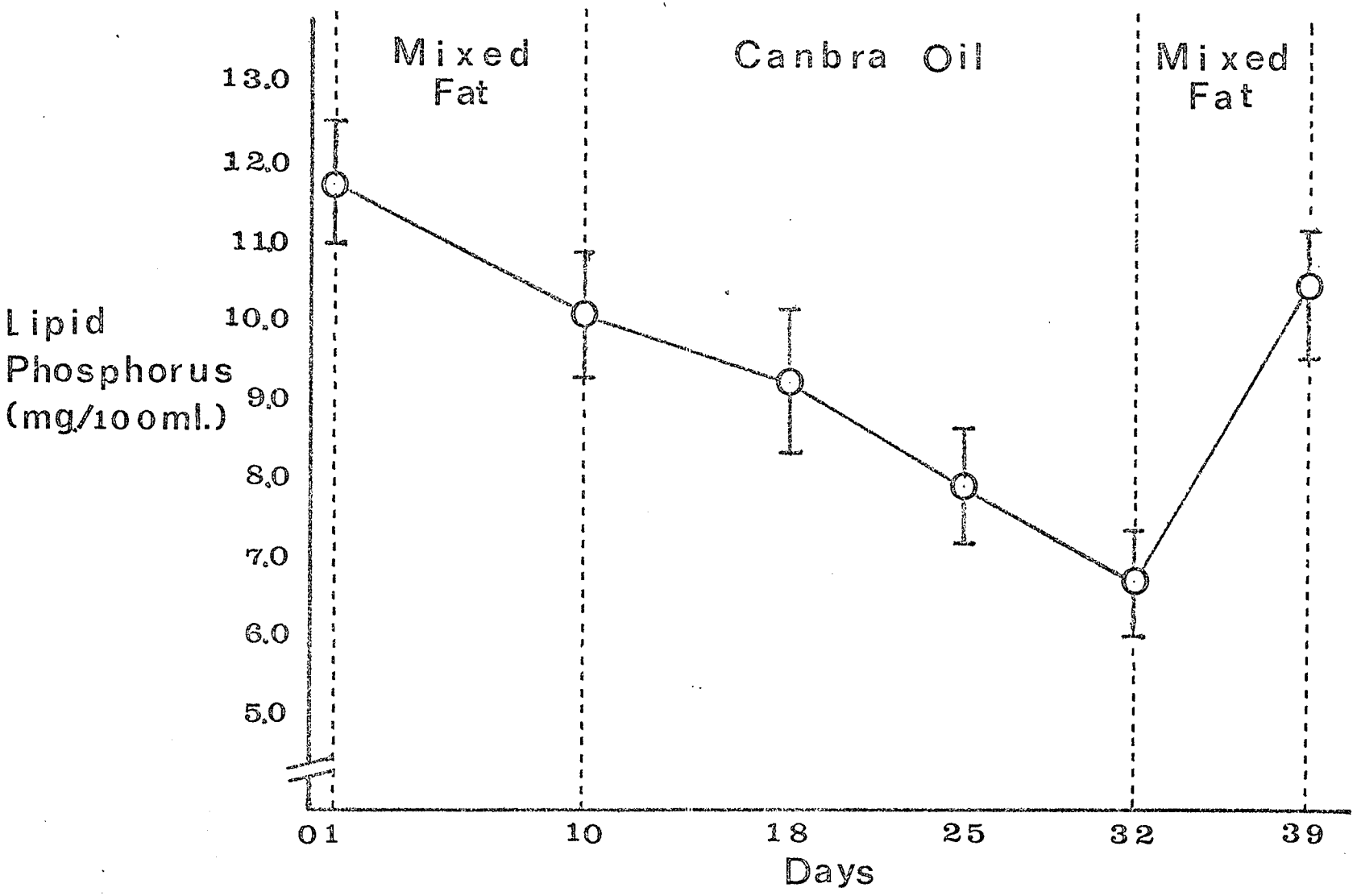

Figure 3. Mean serum lipid phosphorus levels of subjects during experiment.

occurred for serum cholesterol. However, in neither case was the level at Day 32 significantly greater than the level at Day 10 ($P > 0.005$, Day 10 vs. Day 32, Appendix Tables 30, 31). The decrease in lipid phosphorus, as in the case of serum cholesterol, was linear with time during the canbra oil diet (Appendix Table 32). The decrease in lipid phosphorus during the canbra oil diet was approximately 0.15 mg/100 ml. serum/day or 3.75 mg phospholipid/100 ml. serum/day (Figure 4).

Thus the present study found that lipid phosphorus responded to changes in dietary fat source and that the overall pattern of change for lipid phosphorus was similar to that of serum cholesterol. However, the magnitude of decrease in lipid phosphorus or phospholipid tended to be slightly greater for lipid phosphorus than for serum cholesterol.

E. EFFECT OF DIET ON SERUM TRIGLYCERIDES

Considerably more variation was found among subjects for serum triglycerides (Tables 21, 22; Appendix Table 33) than for either serum cholesterol or lipid phosphorus. Hegsted et al. (1965) and Turpeinen et al. (1968) also noted that serum triglyceride levels were characterized by a greater coefficient of variation than that observed for serum cholesterol levels.

The changes in mean serum triglycerides associated with diet manipulation, although small, reached significance ($P < 0.005$) due to the fact that all subjects followed the same general pattern. There was a significant decrease in serum triglycerides during the preliminary mixed fat diet, a further significant decrease on the canbra oil diet with most of the change occurring during the third week, followed by a significant increase upon return to the mixed fat diet (Figure 5 and Appendix Table 34).

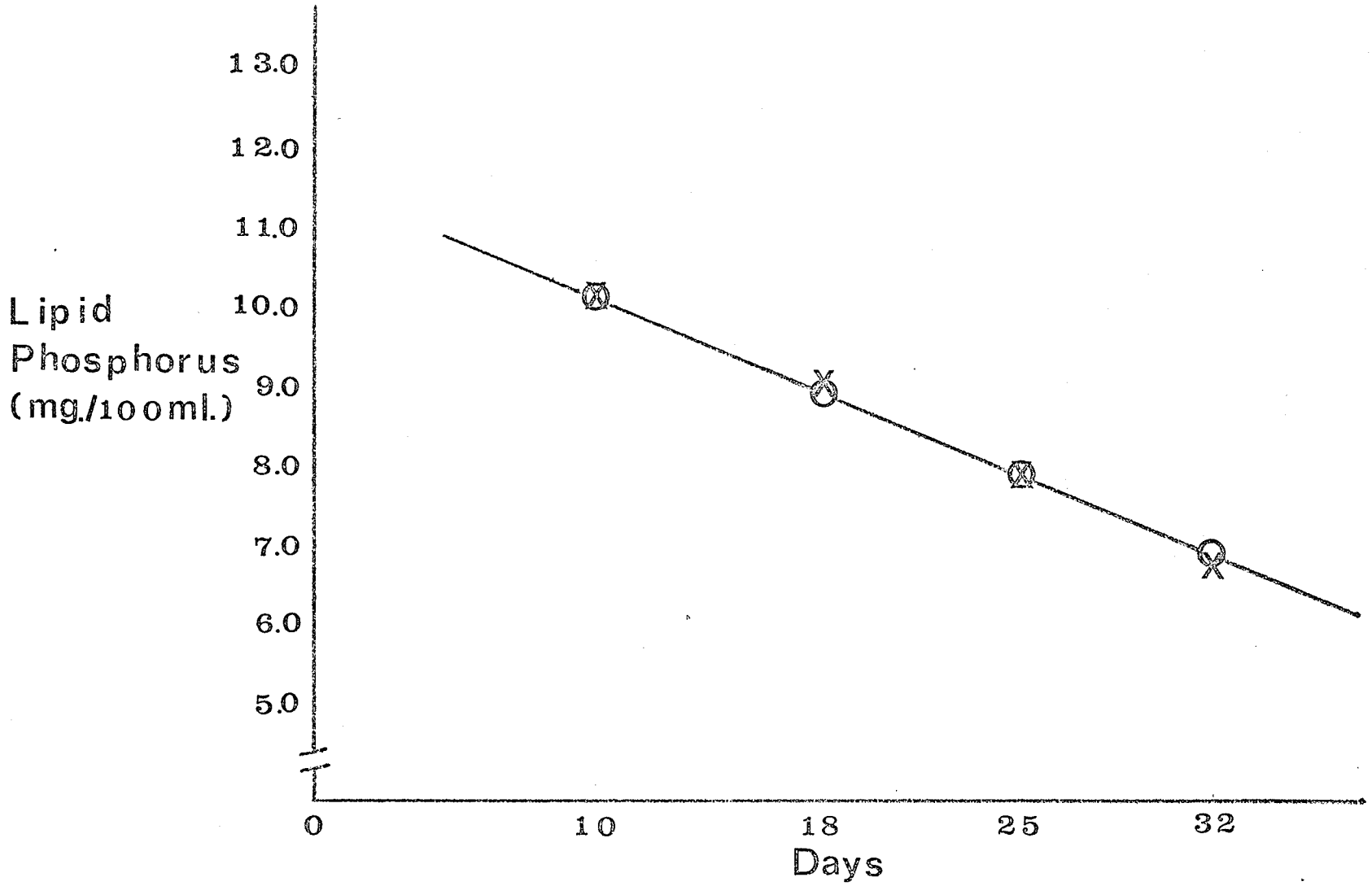

Figure 4. Linear regression of lipid phosphorus on days of experiment for the canbra oil period. Slope of the regression line was -0.15. Symbol: X - observed value; 0 - calculated value.

Table 21

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					
1 H.G.	85	84	83	82	80	84
2 L.R.	77	70	70	70	69	71
3 C.W.	74	68	68	68	65	68
4 L.G.	78	74	74	74	72	75
5 D.R.	83	80	80	80	78	80
6 P.R.	111	106	106	106	104	107
7 H.R.	86[3]	82	82	82	80	83
Group Mean	84	80	80	80	78	81

[1]Mean of two duplicate analyses.

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

[3]Calculated value (serum lost) according to Snedecor, G.W. and Cochran, W.G. 1967. Statistical Methods, 6th.Edition. Iowa State University Press, Ames, Iowa. p.318.

Table 22

Changes in Serum Triglyceride Level

Subject	Experimental Period					
	Mixed Fat	Canbra 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. serum					
1 H.G.	-1	-1	-1	-2	-4	+4
2 L.R.	-7	0	0	-1	-1	+2
3 C.W.	-6	0	0	-3	-3	+3
4 L.G.	-4	0	0	-2	-2	+3
5 D.R.	-3	0	0	-2	-2	+2
6 P.R.	-5	0	0	-2	-2	+3
7 H.R.	-4	0	0	-2	-2	+3
Group Mean	-4	-0.1	-0.1	-2	-2	+3

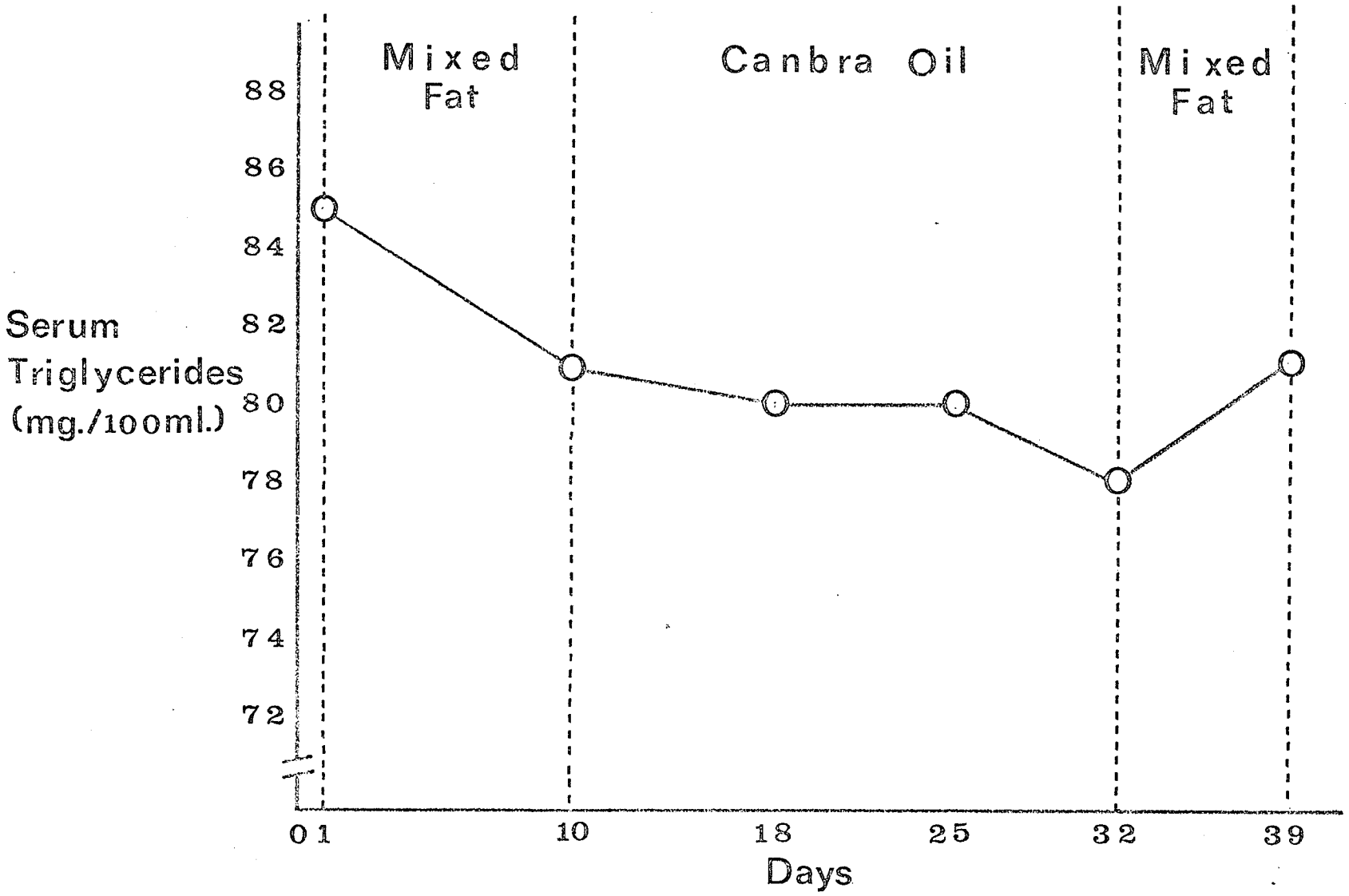

Figure 5. Mean serum triglyceride levels of subjects during experiment.

Serum triglyceride levels were slightly higher at Day 39 than at Day 10 when the canbra oil diet was introduced, a pattern similar to that of serum cholesterol and lipid phosphorus.

The primary difference in composition between the mixed fat diet and the canbra oil diet was in the amounts of saturated and unsaturated fatty acids. The canbra oil diet contained no myristic and less palmitic and stearic acids but higher amounts of oleic and linolenic acids. The relatively minute change observed in serum triglyceride levels on the experimental diet may be related to the fact that substitution of the mixed fat diet by the canbra oil diet resulted in only a small change in the quantity of stearic acid in the diet. Grande et al. (1972) have suggested that saturated fatty acids with fewer than 12 carbon atoms and stearic acid produce elevation of serum triglycerides, although they do not affect serum cholesterol levels. The substantial increase in serum triglycerides on the return to the mixed fat diet tended to substantiate Grande's hypothesis that an increase in stearic acid concomitantly results in an increase in serum triglycerides.

The results of the present investigation indicate that the substitution of canbra oil for a mixed fat diet, similar to the average North American diet, was accompanied by a substantial decrease in serum cholesterol and lipid phosphorus and a slight decrease in serum triglycerides. This effect appeared to be related to the considerably lower level of saturated fatty acids in the canbra oil diet.

F. ELECTROPHORESIS OF SERUM LIPOPROTEINS

Lipoprotein patterns have been widely used as a diagnostic tool in hyperlipidemias, particularly the genetically mediated type, because lipo-

proteins are associated with the transport of different fat moieties in the blood (Levy et al., 1966). Furthermore, it has been found that these inherited hyperlipidemic defects respond to diet manipulation. Although there is limited information on the precise effects of diet composition on the serum lipid patterns in the hyperlipidemic, there is even less available information on the effect of diet manipulation upon lipid parameters in the normal individual. The beta-lipoproteins are the primary cholesterol-bearing fraction while the pre-beta-lipoproteins serve as the transport vehicle for the endogenously synthesized triglycerides. Thus changes in the lipoprotein patterns should coincide with observed changes in serum lipids.

In the present study only the beta-/pre-beta-lipoprotein ratios were reported because there is no satisfactory method for quantitation of the electrophoretograms. Densitometric quantitation of lipoproteins separated by electrophoresis have been criticized because of the non-linear relationship between the concentration and density of bound dye, due mainly to the variable dye-binding capacity of different lipids (Lopez-S. et al., 1971). Furthermore no satisfactory lipoprotein standards are available against which electrophoretograms of unknown samples can be compared for the concentration of the various lipoprotein fractions. Therefore the results obtained must be interpreted with some reservation.

The observed changes in beta-/pre-beta-lipoprotein ratios for each of the subjects throughout the 39-day trial are presented in Tables 23 and 24. A set of representative cellulose acetate strips and the densitometric scans of the electrophoretograms are presented in Figure 6. There was a decrease in the beta-/pre-beta-lipoprotein ratio on the canbra oil diet, the only statistically significant change associated with dietary change in fat source (Appendix Tables 35, 36). It is interesting

Table 23

Beta-/Pre-Beta-Lipoprotein Ratios of Subjects During Experiment[1]

Subject	Day of Experiment					
	1	10[2]	18	25	32[2]	39
1 H.G.	2.43	1.74	1.86	1.59	1.32	1.54
2 L.R.	1.83	2.92	2.63	2.32	1.59	3.00
3 C.W.	2.50	1.60	1.69	1.55	1.75	1.43
4 L.G.	2.28	2.38	2.13	1.70	1.78	2.83
5 D.R.	1.55	2.83	1.47	1.22	1.50	1.40
6 P.R.	1.42	1.90	2.70	1.50	1.17	2.13
7 H.R.	3.00	4.75	1.89	1.83	2.00	2.25
Group Mean	2.14	2.59	2.05	1.67	1.59	2.08

[1]Ratio of areas for beta-/pre-beta-lipoproteins determined from densitometer scans of electrophoretic strips.

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

Table 24

Changes in Beta-/Pre-Beta-Lipoprotein Ratios

Subject	Experimental Period					
	Mixed Fat	Canbra 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
1 H.G.	-0.69	+0.12	-0.27	-0.27	-0.42	+0.22
2 L.R.	+1.09	-0.29	-0.31	-0.73	-1.33	+1.41
3 C.W.	-0.90	+0.09	-0.14	+0.20	+0.15	-0.32
4 L.G.	+0.10	-0.25	-0.43	+0.08	-0.60	+1.05
5 D.R.	+1.28	-1.36	-0.25	+0.28	-1.33	-0.10
6 P.R.	+0.48	+0.80	-1.20	-0.33	-0.73	+0.96
7 H.R.	+1.75	-2.86	-0.05	+0.17	-2.75	+0.25
Group Mean	+0.45	-0.54	-0.38	-0.09	-1.00	+0.50

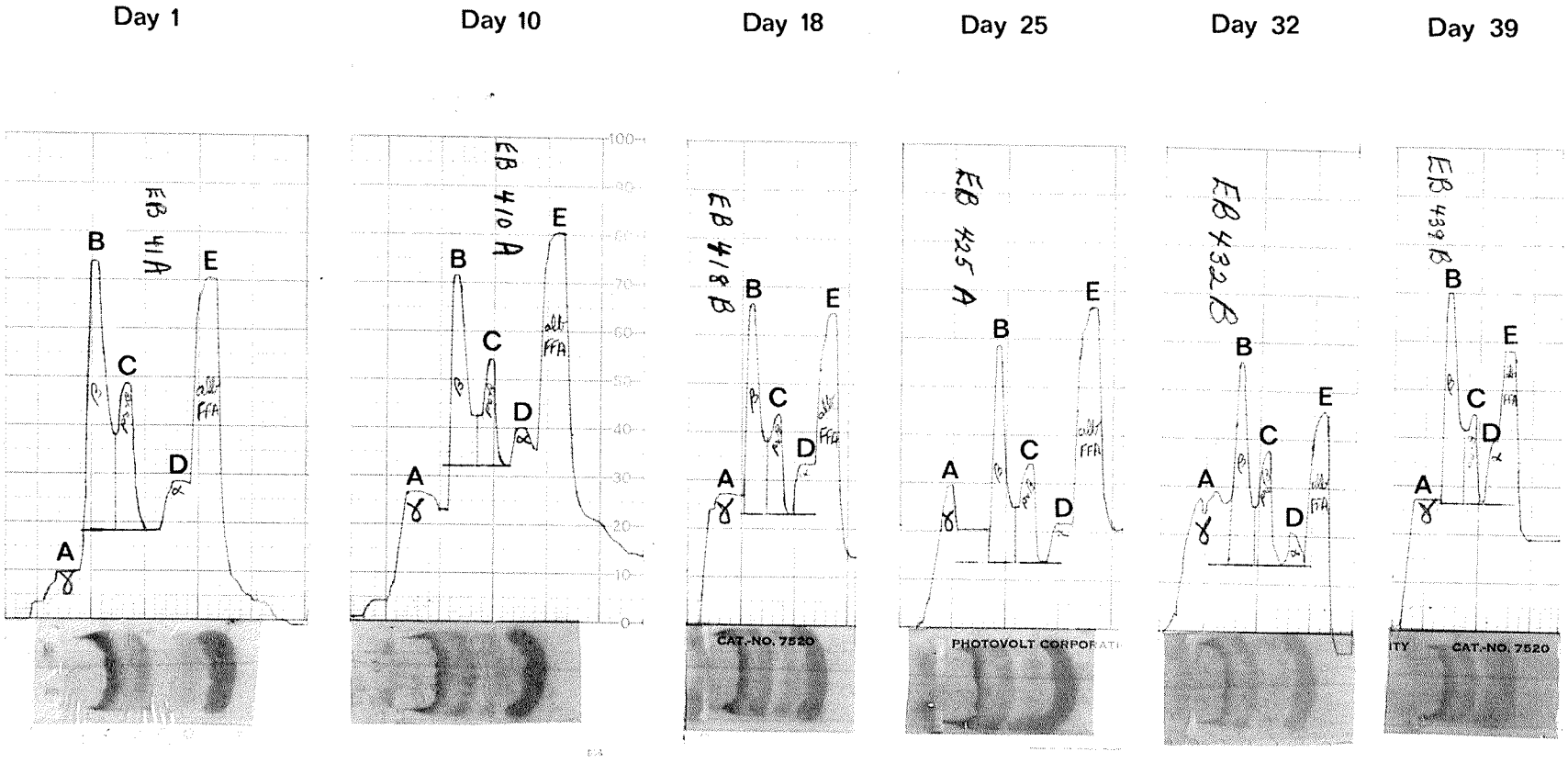

Figure 6. Representative electrophoretograms and densitometric scans of serum lipoproteins for subject No. 4 (L.G.) at specific times throughout the study. The symbol A denotes the origin and gamma-lipoprotein fraction, B the beta-lipoprotein fraction, C the pre-beta-lipoprotein fraction, D the alpha-lipoprotein fraction, and E the albumin bound free fatty acid fraction.

to note that the change observed on the canbra diet was what would have been expected in spite of the limitations of the method. Only a small decrease occurred in serum triglycerides during this period whereas an appreciable change occurred in serum cholesterol. In addition, there was a concomitant increase in the beta-/pre-beta-lipoprotein ratio as for serum cholesterol and triglycerides during the post-experimental mixed fat diet. However, the expected pattern did not prevail during the preliminary mixed fat period where there was a slight increase in the beta-/pre-beta-lipoprotein ratio. There was considerable variation among subjects in the beta-/pre-beta-lipoprotein ratios (Table 24). This subject variability might be attributable to the method employed.

G. EFFECT OF DIET ON PHOSPHOLIPID FATTY ACID PATTERNS

Fatty acid patterns of the phospholipid fraction precipitated from acetone lipid extracts are shown in Table 25. A decrease in palmitic and stearic acids was exhibited in response to the canbra oil diet. These changes coincided with the substantially lower levels of these fatty acids in the canbra oil diet than in the mixed fat diet. Linoleic and eicosenoic acids also decreased slightly on the canbra oil diet. These changes were compensated for by a substantial increase in oleic acid and a slight increase in arachidonic acid. With the exception of linoleic acid, all fatty acids returned to pre-canbra oil levels upon the return to the mixed fat diet for the final 8 days of the study.

Failure to detect erucic acid in the phospholipids after 22 days on the canbra oil diet was confirmed by employing two different liquid phases for the gas chromatographic analysis, EGSS-Y and SE-30. The stationary phase of the SE-30 columns separate fatty acids primarily on the basis of

Table 25

Per Cent Fatty Acid Composition of Serum Phospholipids

Fatty Acid	DAY 10	32	39
Palmitic, C16:0[1]	24.6	19.6	22.4
Palmitoleic, C16:1	2.0	2.0	2.2
Stearic, C18:0	14.2	12.9	14.8
Oleic, C18:1	11.8	19.2	11.2
Linoleic, C18:2	24.0	20.9	20.0
Eicosenoic, C20:1	2.6	1.6	2.0
Eicosatrienoic, C20:3	2.1	2.4	2.8
Arachidonic, C20:4	7.2	8.8	7.5
Unknown[2]	2.9	2.7	3.1

[1]Carbon number:number of double bonds.

[2]Resolved between C20:4 and C20:5.

carbon number. Comparisons with known standards and log plots of carbon number vs. retention time for fatty acids resolved on the EGSS-Y columns further substantiated the results that no erucic acid was incorporated into the phospholipids. The changes exhibited in the phospholipid fatty acid patterns confirm the observations that dietary fat has an appreciable influence on serum lipid patterns.

H. HEMATOLOGY OF WHOLE BLOOD

One of the major problems in studying metabolism in the human, particularly the subclinical effects of antimetabolites, is the limitation associated with the fact that assays, as far as experimental purposes are concerned, are restricted to blood and urine. A variety of factors have been found to affect hematological parameters in both humans and experimental animals. Most studies on factors affecting hematological parameters have dealt with nutrients known to be directly involved in the synthesis of blood constituents, especially nutrients such as iron, copper, folic acid, and vitamin B_{12}. Only a limited number of observations have been reported on animals fed rapeseed oil and although rapeseed oil has been shown to have an adverse effect on metabolism in experimental animals (Abdellatif and Vles, 1970a,b,c; Beare-Rogers, 1970; Houtsmuller *et al.*, 1970; Rocquelin and Cluzan, 1967; 1968; 1971), very few studies have reported on hematological parameters. Decreased hemoglobin and increased reticulocytes were observed in both ducklings (Abdellatif *et al.*, 1972) and guinea pigs (Vles and Abdellatif, 1970b) fed high levels of rapeseed oil. These workers also reported increased red cell fragility in the duckling and guinea pig. Determination of whole blood hematology reflects the physiological state of the total system since it indicates the oxygen carrying capacity of the

blood and the functioning of the hematopoietic system. Some of the most frequently used hematological measurements are total red cell count, hematocrit, hemoglobin, and reticulocyte count. In addition, red cell fragility is used as an indication of membrane integrity.

Erythrocyte count is occasionally the most valuable indicator of disturbed erythropoiesis. However, in general, erythrocyte count is not of much worth since the oxygen carrying capacity of the blood is reflected in the hematocrit and hemoglobin. In normal man the average number of red blood cells per cubic millimeter of blood is 5,400,000 (±600,000) with the normal range being regarded as 4.5 - 6.5 million/cu.mm. (Dacie and Lewis, 1963). Two of the most frequently used parameters in assessing the physiological state of red blood cells are hematocrit and hemoglobin. Hematocrit is the percentage of red cells in the blood and is sometimes referred to as "packed cell volume" as determined from the level of packed cells in "hematocrit tubes" upon centrifugation. Hematocrit values can vary tremendously, depending not only on whether the person has anemia but on a variety of factors such as level of activity and altitude at which the person resides. Nevertheless hematocrit is probably the best single test for the diagnoses of the presence and degree of anemia, polycythemia, or hemoconcentration because it can be accurately determined (±1 per cent). Normal values for the human range from 40 - 54 per cent with a mean of 42 (Dacie and Lewis, 1963). Hemoglobin levels are the balance between synthesis, which depends on precise control of the appropriate substrates, and degradation. Whole blood of man contains a mean of 14.8 grams of hemoglobin/100 ml. of blood with the range accepted as normal for adult man in North America being 13.5 - 18.0 (Dacie and Lewis, 1963).

The number of reticulocytes, immature erythrocytes, is a reflection

of the normal functioning of the hematopoietic system. In general, reticulocytes account for 0.5 per cent of the total number of red blood cells but the range of values considered normal for adults is 0.2 - 2 per cent (Dacie and Lewis, 1963).

Hematological measurements over the 39-day test period are summarized in Tables 26 - 33. Values for all of the parameters measured were within the normal range throughout the trial. Although changes were observed in some of the parameters, none were considered attributable to the canbra oil test fat.

The decrease in total red cell count, hematocrit, hemoglobin, and reticulocyte count during the preliminary mixed fat period was significant ($P < 0.005$; Appendix Tables 37-44). Only the hematocrit and reticulocyte count changed during the subsequent 22-day experimental period on the canbra oil diet and the 8-day mixed fat period at the end of the experiment. The hematocrit increased gradually over this 30-day period as evidenced by the fact that there was a significant ($P < 0.005$) overall improvement in hematocrit (Day 10 vs. Day 32 vs. Day 39, Appendix Table 40). However, hematocrit values at Day 32 did not differ from those at Day 10 or Day 39 (Tables 28,29; Appendix Tables 39,40). A similar pattern of response was noted for the reticulocyte count except that recovery occurred exclusively during the canbra oil period. There was a significant ($P < 0.005$) increase in reticulocyte count from Day 10 to Day 32 but Day 32 did not differ ($P > 0.005$) from Day 39 (Tables 32, 33; Appendix Tables 43, 44). In spite of these changes, the various hematological values for each subject were within the range considered normal at all periods.

There were no changes observed in red cell fragility during the study. Red cell hemolysis for all subjects occurred between 0.30 per

Table 26

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					
1 H.G.	5.5	4.9	5.2	5.3	5.0	5.6
2 L.R.	5.1	4.9	5.1	5.1	4.9	5.0
3 C.W.	4.9	4.5	4.5	4.8	4.9	4.8
4 L.G.	4.9	5.1	5.0	5.2	5.1	5.3
5 D.R.	4.9	4.6	4.8	4.9	4.7	4.8
6 P.R.	4.7	4.7	4.6	4.8	4.7	4.7
7 H.R.	5.3	4.9	5.0	5.0	4.8	4.9
Group Mean	5.0	4.8	4.9	5.0	4.9	5.0

[1]As determined by Hematological Laboratories, Winnipeg General Hospital.

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

Table 27

Changes in Total Red Cell Count

Subject	Experimental Period: Mixed Fat	Canbra 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					
1 H.G.	-0.6	+0.3	+0.1	-0.3	+0.1	+0.6
2 L.R.	-0.2	+0.2	0.0	-0.2	0.0	+0.1
3 C.W.	-0.4	0.0	+0.3	+0.1	+0.4	-0.1
4 L.G.	+0.2	-0.1	+0.2	-0.1	0.0	+0.2
5 D.R.	-0.3	+0.2	+0.1	-0.2	+0.1	-0.1
6 P.R.	0.0	-0.1	+0.2	-0.1	0.0	0.0
7 H.R.	-0.4	+0.1	0.0	-0.2	+0.1	+0.1
Group Mean	-0.2	+0.1	+0.1	-0.1	+0.1	+0.1

Table 28

Hematocrit of Subjects During Experiment[1]

Subject	Day of Experiment					
	1	10[2]	18	25	32[2]	39
	percentage of blood that is cells					
1 H.G.	46.5	42.5	45.0	45.0	43.5	48.5
2 L.R.	46.0	44.0	45.0	44.5	45.0	46.5
3 C.W.	43.5	40.0	40.0	42.5	45.0	43.5
4 L.G.	42.5	44.0	42.5	44.0	45.0	46.0
5 D.R.	45.5	42.5	44.0	45.0	44.0	45.0
6 P.R.	43.0	43.0	41.5	43.5	44.0	43.5
7 H.R.	45.5	41.5	42.5	42.0	42.5	43.0
Group Mean	44.6	42.5	42.9	43.8	44.1	45.1

[1]As determined by Hematological Laboratories, Winnipeg General Hospital.

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

Table 29

Changes in Hematocrit

Subject	Experimental Period					
	Mixed Fat	Canbra 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
	percentage of blood that is cells					
1 H.G.	-4.0	+2.5	0.0	-1.5	+1.0	+5.0
2 L.R.	-2.0	+1.0	-0.5	+0.5	+1.0	+1.5
3 C.W.	-3.5	0.0	+2.5	+2.5	+5.0	-1.5
4 L.G.	+1.5	-1.5	+1.5	+1.0	+1.0	+1.0
5 D.R.	-3.0	+1.5	+1.0	-1.0	+1.5	+1.0
6 P.R.	0.0	-1.5	+2.0	+0.5	+1.0	+0.5
7 H.R.	-4.0	+1.0	-0.5	+0.5	+1.0	+0.5
Group Mean	-2.1	+0.4	+1.0	+0.4	+1.6	+1.1

Table 30

Hemoglobin of Subjects During Experiment[1]

Subject	Day of Experiment					
	1	10[2]	18	25	32[2]	39
	grams/100 ml. of blood					
1 H.G.	16.2	14.5	15.4	15.2	14.1	15.5
2 L.R.	15.2	15.3	15.9	15.1	15.0	14.9
3 C.W.	15.0	14.0	14.2	14.7	14.5	14.1
4 L.G.	15.1	14.8	14.8	14.7	14.6	15.1
5 D.R.	15.4	14.5	15.0	15.0	14.4	14.5
6 P.R.	14.4	14.7	14.2	14.5	14.1	14.0
7 H.R.	15.8	13.9	14.4	14.0	13.7	13.8
Group Mean	15.3	14.5	14.8	14.7	14.4	14.6

[1]As determined by Hematological Laboratories, Winnipeg General Hospital.

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

Table 31

Changes in Hemoglobin

Subject	Experimental Period					
	Mixed Fat	Canbra 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
				grams/100 ml. of blood		
1 H.G.	-1.7	+0.9	-0.2	-1.1	-0.4	+1.4
2 L.R.	+0.1	+0.6	-0.8	-0.1	-0.3	-0.1
3 C.W.	-1.0	+0.2	+0.5	-0.2	+0.5	-0.4
4 L.G.	-0.3	0.0	-0.1	-0.1	-0.2	+0.5
5 D.R.	-0.9	+0.6	0.0	-0.6	-0.1	+0.1
6 P.R.	+0.3	-0.5	+0.3	-0.4	-0.6	-0.1
7 H.R.	-1.9	+0.5	-0.4	-0.3	-0.2	+0.1
Group Mean	-0.8	+0.3	-0.1	-0.4	-0.2	+0.2

Table 32

Reticulocyte Count of Subjects During Experiment[1]

Subject	Day of Experiment					
	1	10[2]	18	25	32[2]	39
	per cent of reticulocytes					
1 H.G.	1.1	0.3	1.0	1.2	1.2	1.3
2 L.R.	0.9	0.6	0.8	1.7	1.1	1.4
3 C.W.	1.3	0.3	1.0	1.0	0.7	1.7
4 L.G.	1.5	1.0	1.0	1.3	1.5	1.7
5 D.R.	1.6	0.3	0.7	0.8	0.9	0.8
6 P.R.	1.6	0.2	1.2	0.8	1.7	0.8
7 H.R.	0.6	0.0	0.5	0.8	1.1	0.6
Group Mean	1.2	0.4	0.8	1.1	1.2	1.2

[1]As determined by Hematological Laboratories, Winnipeg General Hospital.

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

Table 33

Changes in Reticulocyte Count

Subject	Experimental Period					
	Mixed Fat	Canbra 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
	per cent of reticulocytes					
1 H.G.	-0.8	+0.7	+0.2	0.0	+0.9	+0.1
2 L.R.	-0.3	+0.2	+0.9	-0.6	+0.5	+0.3
3 C.W.	-1.0	+0.7	0.0	-0.3	+0.4	+1.0
4 L.G.	+0.5	0.0	+0.3	+0.2	+0.5	+0.2
5 D.R.	-1.3	+0.4	+0.1	+0.1	+0.6	-0.1
6 P.R.	-1.4	+1.0	-0.4	+0.9	+1.5	-0.9
7 H.R.	-0.6	+0.5	+0.3	+0.3	+1.1	-0.5
Group Mean	-0.7	+0.4	+0.2	+0.1	+0.8	+0.01

cent NaCl and 0.45 per cent NaCl. Hemolysis was first observed at 0.45 per cent NaCl and was complete at 0.30 per cent NaCl.

The present results indicate that ingestion of canbra oil by young adult men at levels providing approximately 38 per cent of the total calories (i.e. 95 per cent of the total fat) had no deleterious effect upon hematology. The changes which occurred did not result in values outside the range considered normal for the human. Abdellatif and Vles (1972) and Vles and Abdellatif (1970b) have reported adverse effects of high erucic acid rapeseed oil on the hematology of ducklings and guinea pigs. Whether this apparent failure to find similar changes in the human is related to species or composition of the oil fed awaits further elucidation. Nevertheless there is no evidence to suggest that canbra oil had a deleterious effect on the hematology of the young men participating in this study.

SUMMARY AND CONCLUSIONS

The present study investigated the effect of canbra oil on serum lipid patterns and whole blood hematology of healthy college men fed a diet in which the added fat supplied approximately 38 per cent of the total calories (i.e. 95 per cent of the total dietary fat). The diet was composed of solid foods in which meat was replaced by soy protein (TVP). The 39-day study was divided into 3 periods: 1) a 9-day stabilization period during which a mixed fat diet, formulated to simulate the composition of fat in the average North American diet, was fed; 2) a 22-day period when canbra oil supplied the fat; and 3) an 8-day period when the mixed fat diet was again fed. The primary difference between the mixed fat and canbra oil diets was in the amounts of palmitic, oleic, and linolenic acid. The canbra oil diet was much lower in palmitic and stearic acid than the mixed fat diet and higher in oleic and linolenic acid. The ratio of saturated to unsaturated fatty acids was 1.0:1.7 for the mixed fat diet compared to 1.0:8.9 for the canbra oil diet.

Blood samples were taken for the determination of serum lipid patterns and whole blood hematology on Days 1, 10, 18, 25, 32, and 39. Serum cholesterol decreased for all subjects during the preliminary mixed fat period (Day 1 vs. Day 10), from an initial mean value of 203 to 174 mg/100 ml. serum. Serum cholesterol levels continued to decrease on the canbra oil diet with a mean decrease of 15 mg per 100 ml. the first 8 days and another 15 mg per 100 ml. over the next 14 days.

Reversion to the mixed fat diet for the last 8 days of the study resulted in an increase in serum cholesterol to a mean level slightly above that at the commencement of the canbra regimen (183 vs. 174 mg/100 ml.). Lipid phosphorus followed a pattern similar to that of serum cholesterol. There was a decrease of 40 mg phospholipid (mg lipid P x 25) during the preliminary mixed fat period (Day 1 vs. Day 10), a further decrease of 85 mg phospholipid during the canbra diet (Day 10 vs. Day 32), followed by a sharp increase (95 mg phospholipid) upon return to the mixed fat diet. Although the patterns followed by serum cholesterol and lipid phosphorus were similar, the per cent change in lipid phosphorus was greater than the per cent change in serum cholesterol during the preliminary mixed fat period. In addition, the major changes in lipid phosphorus on the canbra oil diet took place during the last two weeks whereas with serum cholesterol the greater change occurred during the first week of the canbra oil diet.

Statistically significant changes in mean serum triglycerides accompanied the change from the mixed fat to the canbra oil diet and vice versa. A decrease in triglycerides of 4 mg/100 ml. serum occurred during the preliminary mixed fat period. There was a further decrease of 2 mg/100 ml. serum on the canbra regimen (Day 10 vs. Day 32) with most of the change taking place during the third week. The return to the mixed fat diet for the last 8 days resulted in an increase of 3 mg/100 ml. serum. The changes in triglycerides were small and thus probably of minor physiological importance.

The results of the present investigation indicated that substitution of canbra oil for a more saturated mixed fat was accompanied by a

substantial decrease in serum cholesterol and lipid phosphorus and a slight decrease in serum triglycerides. These changes are what one might expect on the basis of the composition of the fats fed during the different dietary periods, although the change in serum cholesterol on the canbra oil regimen was somewhat greater (30 mg/100 ml. serum) than might have been expected on the basis of the prediction equation derived by Keys and associates at the University of Minnesota wherein the calculated expected change in serum cholesterol was 19 mg/100 ml. serum. The results with canbra oil, however, were analogous to those found with corn oil and high erucic acid rapeseed oil where the observed values also were higher than those predicted by the regression equation.

Electrophoretic patterns of the lipoproteins basically followed what might be expected on the basis of serum lipid patterns. There was a decrease of 1.00 in the ratio of beta-/pre-beta-lipoprotein on the canbra oil diet followed by an increase of 0.50 in the ratio upon return to the mixed fat diet for the last 8 days of the study. This finding is consistent with the fact that there was a substantial decrease in serum cholesterol and only a slight decrease in serum triglycerides on the canbra diet. However, the beta-/pre-beta-lipoprotein ratio did not follow the expected pattern during the preliminary mixed fat period since an increase rather than a decrease was observed.

Because phospholipids are an integral part of the vehicle of lipid transport, the lipoproteins, it was considered of interest to examine the changes in the fatty acid patterns of the phospholipids as a result of diet manipulation. The changes exhibited reflected the changes brought about in fatty acid composition of the diet as a result of

changing from a mixed fat to canbra oil and vice versa. The fact that the observed changes occurred abruptly with a change in diet suggests a rapid turnover of phospholipids in the serum.

No consistent effect of diet on blood hematology was observed. Although there were changes in hematology, some of which were statistically significant ($P < 0.005$), the major change in total red cell count, hematocrit, hemoglobin, and reticulocyte count occurred during the first 9 days of the study when the mixed fat was fed. These changes were followed by a gradual recovery during the following 30-day period, with only hematocrit and reticulocyte count exhibiting statistically significant changes during this period. The various hematological values for each subject were within the range considered normal for the adult male at all periods during the study. No changes were found in red cell fragility.

The results of the present investigation indicate no consistent effect of canbra oil on the blood hematology of young college men in spite of reports of decreased hemoglobin, increased reticulocyte count, and increased red cell fragility for ducklings and guinea pigs fed high erucic acid rapeseed oil. However, inclusion of canbra oil in a solid diet, at a level providing 38 per cent (i.e. 95 per cent of the total fat), had an appreciable effect on serum lipid patterns. These results are consistent with the lower levels of saturated fat intake associated with the canbra diet. Thus, inclusion of a high level of canbra oil in the diet of young college men had no deleterious effect upon metabolism in so far as metabolism is reflected by changes in serum lipid patterns and blood hematology. In fact, the changes observed in blood chemistry in response to canbra oil might be considered desirable from a cardiological point of view.

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APPENDIX

Appendix Table 1

ROLLED OATS (1 serving) (Mixed Fat)

200 ml. boiling water
40 g. (½ c.) rolled oats
1/8 tsp. salt
10.0 g. Corn oil (Mazola)

Method:

Bring water, salt, and oil to a rapid boil. Slowly add rolled oats, while stirring. Cook until thickened.

N.B. Do not overcook as fat separates out.

ROLLED OATS (1 serving) (CANBRA)

200 ml. boiling water
40 g. (½ c.) rolled oats
1/8 tsp. salt
10.0 g. Canbra oil

Method:

Bring water, salt, and oil to a rapid boil. Slowly add rolled oats, while stirring. Cook until thickened.

N.B. Do not overcook as fat separates out.

Appendix Table 2

CREAM OF WHEAT (1 serving) (Mixed Fat)

180 ml. boiling water
21 g. cream of wheat
1/8 tsp. salt
10.0 g. Corn oil (Mazola)

Method:

Bring water, salt, and oil to a rapid boil. Slowly add cream of wheat while stirring. Cook until thickened.

N.B. Do not overcook as fat separates out.

CREAM OF WHEAT (1 serving) (CANBRA)

180 ml. boiling water
21 g. cream of wheat
1/8 tsp. salt
10.0 g. Canbra oil

Method:

Bring water, salt, and oil to a rapid boil. Slowly add cream of wheat while stirring. Cook until thickened.

N.B. Do not overcook as fat separates out.

Appendix Table 3

SCRAMBLED EGG (1 serving) (Mixed Fat)

100 g. egg albumin, reconstituted 6:1
1 tsp. skim milk powder
¼ drop yellow food colouring
8.0 g. butter

Method:

Combine albumin, colouring, and milk powder. Melt butter in individual fry pans; add the egg mixture to the pans, stirring frequently while cooking. Sprinkle with a dash of salt and pepper.

Variations: Whip ingredients into a meringue using a mixer; place in pan with fat - yields one fluffy omelet.

SCRAMBLED EGG (1 serving) (CANBRA)

100 g. egg albumin, reconstituted 6:1
1 tsp. skim milk powder
¼ drop yellow food colouring
5.0 g. Canbra margarine

Method:

Combine albumin, colouring, and milk powder. Melt margarine in individual fry pans; add the egg mixture to the pans, stirring frequently while cooking. Sprinkle with a dash of salt and pepper.

Variations: Whip ingredients into a meringue using a mixer; place in pan with fat - yields one fluffy omelet.

Appendix Table 4

HAMBURG PATTIES (16 - 115.80 g. servings) (Mixed Fat)

Combine in a large covered pot:

540 g. TVP beef strips
550 ml. water
197.82 g. butter
92.70 g. tallow

Heat and simmer until the water and fat are absorbed. Grind. Cool well.

Meanwhile, melt in pot over low heat:

193.32 g. lard
185.40 g. Crisco shortening

Combine in a blender with a removable bottom:

252.00 g. dry cottage cheese
108.84 g. reconstituted egg albumin
5.09 g. Kitchen Bouquet
26.46 g. tomato paste

Method:

Blend the above until creamy. Add the melted fat to the blended ingredients gradually, blending well after each addition. Add the mixture from the blender to the ground TVP; combine until thoroughly mixed.

Add: 2 tbsp. dehydrated minced onion. Mix well. Cool well.

Weigh out in amounts of 115.80 g. onto foil. Shape into 2 patties of approximately equal size. Wrap and label. Freeze.

Appendix Table 5

HAMBURG PATTIES (16 - 115.80 g. servings) (CANBRA)

540.0 g. TVP beef strips
550.0 ml. water
253.80 g. Canbra oil

Heat and simmer for 10 minutes until water is absorbed. Grind.

Meanwhile, combine in a blender with a removable bottom:

252.0 g. dry cottage cheese
108.84 g. reconstituted egg albumin
5.09 g. Kitchen Bouquet
26.46 g. tomato paste
307.80 g. Canbra oil

Method:

Blend the first four ingredients until creamy. Add the Canbra oil to the blended ingredients in the blender. Blend until well mixed. Add the mixture from the blender to the ground TVP mixture. Combine until thoroughly mixed. Add 2 tbsp. rehydrated minced onion. Mix well. Weigh out in amounts of 115.80 g. onto foil. Shape into 2 patties of approximately equal size. Wrap and label. Freeze.

Appendix Table 6

SPAGHETTI (1 serving) (Mixed Fat)

Cook together:

1/3 tsp. salt
0.765 g. Crisco (approximately ¼ tsp.)
41.0 g. dry wt. spaghetti
410 g. water

Yield: 1 serving of approximately 102.0 g.

Place into large foil container.

SPAGHETTI (1 serving) (CANBRA)

Cook together:

1/3 tsp. salt
0.765 g. Canbra oil
41.0 g. dry wt. spaghetti
410.0 g. water

Yield: 1 serving of approximately 102.0 g.

Place into large foil container.

Appendix Table 7

MEATBALLS (Mixed Fat)

In quantity, for 16 servings, combine in covered saucepan:

400 g. TVP beef strips
550 ml. hot water
108.40 g. Crisco
34.08 g. lard
81.76 g. Parkay margarine

Method:

Heat to boiling, reduce heat and simmer, still covered, until all the water and fat are absorbed. Run the rehydrated TVP through a meat grinder. Cool well.

Add:

400.0 g. reconstituted egg albumin
4 tsp. dehydrated minced onion
pepper
garlic powder

Cool the mixture well in the freezer. Shape into meatballs of 36 g. each.

MEATBALLS (CANBRA)

In quantity, for 16 servings, combine in covered saucepan:

400 g. TVP beef strips
550 ml. hot water
240.00 g. Canbra oil

Method:

Heat to boiling, reduce heat and simmer, still covered, for 5 minutes. Run the rehydrated TVP through a meat grinder. Cool well.

Add:

400.0 g. reconstituted egg albumin
8 tsp. hydrated instant minced onion (4 tsp. dehydrated)
pepper
garlic powder

Cool the mixture well in the freezer. Shape into meatballs of 36 g. each.

Appendix Table 8

TOMATO SAUCE (1 serving) (Mixed Fat)

.008 g. dehydrated onion (¼ tsp.)
1.25 g. green pepper
119.0 g. canned tomatoes
2.25 g. salt (¼ tsp.)
6.00 g. spices (approximately ¼ tsp.each of black pepper, oregano, celery salt; 1/8 tsp. each of curry powder, garlic powder).
8.16 g. corn oil
2.84 g. lard
62.00 g. tomato juice
15.60 g. tomato paste
8.00 g. tomato sauce

Method:

Combine in individual pots. Cover, bring to a boil, reduce heat, let simmer for at least 15 minutes, stirring occasionally.

TOMATO SAUCE (1 serving) (CANBRA)

.008 g. dehydrated onion (¼ tsp.)
1.25 g. green pepper
119.0 g. canned tomatoes
2.25 g. salt (¼ tsp.)
6.00 g. spices (approximately ¼ tsp. each of black pepper, oregano, celery salt; 1/8 tsp. each of curry powder, garlic powder).
11.00 g. Canbra oil
62.00 g. tomato juice
15.60 g. tomato paste
8.00 g. tomato sauce

Method:

Combine in individual pots. Bring to a boil, reduce heat, cover with lid, let simmer for 15 minutes, stirring occasionally.

Appendix Table 9

COLESLAW AND DRESSING (1 serving) (Mixed Fat)

50 g. cabbage, shredded
5 g. green pepper, diced
½ tsp. dehydrated onion
10 g. carrot, shredded

Dressing:

6.0 g. water
10.0 g. sugar
5.0 g. vinegar
5.0 g. corn oil

Method:

Mix sugar and corn oil; add water and vinegar. Bring to a boil in a small pot. Pour over the vegetables while hot; let stand overnight.

COLESLAW AND DRESSING (1 serving) (CANBRA)

50 g. cabbage, shredded
5 g. green pepper, diced
½ tsp. dehydrated onion
10 g. carrot, shredded

Dressing:

6.0 g. water
10.0 g. sugar
5.0 g. vinegar
5.0 g. Canbra oil

Method:

Mix sugar and Canbra oil; add water and vinegar. Bring to a boil in a small pot. Pour over the vegetables while hot; let stand overnight.

Appendix Table 10

TOSSED SALAD

50.0 g. lettuce
50.0 g. tomato

PIQUANT SALAD DRESSING (16 - 15.0 g. servings) (Mixed Fat)

51.5 g. vinegar
2 tbsp. water
1 tsp. salt
½ tsp. dry mustard
1 tsp. onion juice
½ tsp. sugar
½ tsp. paprika
¼ tsp. pepper
25 g. reconstituted egg albumin
146.00 g. corn oil

Method:

Measure all ingredients into a blender which has a close-fitting top. Cover and "blend" vigorously.

PIQUANT SALAD DRESSING (16 - 15.0 g. servings) (CANBRA)

51.5 g. vinegar
2 tbsp. water
1 tsp. salt
½ tsp. dry mustard
1 tsp. onion juice
½ tsp. sugar
½ tsp. paprika
25 g. reconstituted egg albumin
146.00 g. Canbra oil

Method:

Measure all ingredients into a blender which has a close-fitting top. Cover and "blend" vigorously.

Appendix Table 11

SWEET AND SOUR PORK (1 serving) (Mixed Fat)

Combine in a saucepan:

25 g. TVP pork chunks
128 g. pineapple juice (drained from can)
½ tsp. minced dehydrated onion
2 tbsp. water
9 g. diced green pepper
7.05 g. corn oil
5.15 g. beef tallow
9.99 g. lard
15.51 g. Parkay margarine
2.50 g. butter

Cover and bring to a boil. Reduce heat and simmer covered for 15 minutes.

Combine and add to the first mixture:

1 tbsp. brown sugar
1½ tsp. cornstarch
1 tsp. vinegar
1 tsp. soy sauce
1/8 tsp. salt

Add:

50 g. pineapple tidbits.

Simmer until thickened only. Place in foil container, cover and label, freeze.

Appendix Table 12

SWEET AND SOUR PORK (1 serving) (CANBRA)

Combine in a saucepan:

25 g. TVP pork chunks
128 g. pineapple juice (drained from can)
½ tsp. minced dehydrated onion
2 tbsp. water
9 g. diced green pepper
39.00 g. Canbra oil

Cover and bring to a boil. Reduce heat and simmer, covered, for 15 minutes.

Combine and add to the first mixture:

1 tsp. vinegar
1 tsp. soy sauce
1 tbsp. brown sugar
1/8 tsp. salt
1½ tsp. cornstarch

Add:

50 g. pineapple tidbits.

Simmer only until thickened, stirring occasionally. Place into foil containers. Cover and label, freeze.

Appendix Table 13

BEEF STEW (1 serving) (Mixed Fat)

Cook frozen carrots and frozen peas sufficient for the number of stew servings to be prepared.

Combine in a saucepan:

50 g. TVP beef chunks
100 ml. water
10.66 g. tallow
1 tbsp. dehydrated minced onion
7.53 g. butter
16.51 g. lard
10.96 g. Parkay margarine

Bring to a boil and simmer covered for 5-10 minutes (or until all the liquid is taken up). Set aside, still covered.

Weigh into foil container:

20 g. blanched frozen peas
20 g. partially cooked frozen carrots (approx. 8 min./lb.)

Refrigerate until needed.

Combine in a custard cup to make a paste:

50.0 ml. water
15.0 g. flour

Stir well.

When TVP is hydrated, add:

150.0 ml. water
1½ tsp. Bovril (1 small envelope)

Bring to a boil.

Pour contents of foil container into saucepan; add flour/water paste; stir only until thickened. Pour into foil containers. Seal with labeled covers; freeze.

Appendix Table 14

BEEF STEW (1 serving) (CANBRA)

Cook frozen peas and carrots sufficient for the number of stew servings to be prepared.

Combine in a saucepan:

50 g. TVP beef chunks
70 ml. water
42.00 g. Canbra oil
1 tbsp. dehydrated minced onion

Bring to a boil and simmer covered for 5-10 minutes (or until all the water is taken up).

Weigh into foil container:

20 g. blanched frozen peas
20 g. partially cooked frozen carrots (approx.8 min./lb.)

Refrigerate until needed.

Combine in a custard cup to make a paste:

50 ml. water
15 g. flour

Stir well.

When TVP is hydrated, add:

170 ml. water
1½ tsp. Bovril (1 small envelope)

Bring to a boil.

Pour contents of foil container into saucepan; add flour/water paste; stir only until thickened. Pour into foil container; seal labelled covers; freeze.

Appendix Table 15

RICE (100.0 g. = 1 serving)

43.0 g. rice (Delta)
100 ml. water
1/8 tsp. salt

Method:

Add salt to water and bring to boil. Add rice and cook covered for approximately 15 minutes over low heat.

INSTANT MASHED POTATO (100 g. = 1 serving) (Mixed Fat)

16.0 g. mashed potato flakes
63.25 g. water
15.0 g. skim milk
1/8 tsp. salt
8.0 g. butter

Method:

Place water and salt in small casserole dish. Add skim milk, flakes, then butter. Cover with lid and keep warm in oven (250^{o}F).

INSTANT MASHED POTATO (100 g. = 1 serving) (CANBRA)

16.0 g. mashed potato flakes
63.25 g. water
15.0 g. skim milk
1/8 tsp. salt
5.0 g. Canbra margarine

Method:

Place boiling water and salt in small casserole dish. Add skim milk, flakes, then margarine. Cover with lid and keep warm in oven (250^{o}F).

Appendix Table 16

SPICY FRUIT SQUARES (Mixed Fat)

Temp. 350°F

200 g. lightly packed brown sugar
100 g. reconstituted egg albumin
1½ tsp. vanilla
85 g. pre-sifted A.P.flour
1 tsp. baking powder
¼ tsp. salt
½ tsp. cinnamon
40 g. raisins
40 g. chopped cherries
64.00 g. butter

Method:

Preheat oven to 350°F. Toss raisins and cherries in small amount of the allowed flour. Blend or sift together flour, baking powder, salt, cinnamon; set aside. Cream the fat and brown sugar. Add the egg albumin. Gradually add the dry ingredients blending well after each addition. Stir in the fruit. Bake at 350°F for 25 minutes. Squares should be approximately 29 g. each.

Yield: 16 squares

ICING (Mixed Fat)

Sufficient for 18 squares:

128 g. sifted icing sugar
15 g. skim milk
1 tsp. vanilla
pinch salt
18.00 g. butter

Total = 162.5 g. Add 9 g. icing to each square.

Appendix Table 17

SPICY FRUIT SQUARES (CANBRA)

Temp. 350°F.

64.00 g. Canbra oil
200 g. lightly packed brown sugar
100 g. reconstituted egg albumin
1½ tsp. vanilla
85 g. pre-sifted A.P.flour
1 tsp. baking powder
¼ tsp. salt
½ tsp. cinnamon
40 g. raisins
40 g. chopped cherries

Method:

Preheat oven to 350°F. Toss raisins and cherries in small amount of the allowed flour. Blend or sift together flour, baking powder, salt, cinnamon; set aside. Cream the canbra oil and brown sugar. Add the egg albumin. Gradually add the dry ingredients blending well after each addition. Stir in the fruit. Bake at 350°F for 25 minutes. Squares should be approximately 29 g. each.

Yield: 16 squares

ICING (CANBRA)

Sufficient for 18 squares:

15.75 g. Canbra oil
128 g. sifted icing sugar
15 g. skim milk
1 tsp. vanilla
pinch salt

Total = 162.5 g. Add 9 g. icing to each square.

Appendix Table 18

WHITE CAKE (Mixed Fat)

Temp. 350°F.

200 g. sugar
1 tsp. vanilla
½ tsp. almond extract
192 g. sifted cake flour
3 tsp. baking powder
½ tsp. salt
80 g. reconstituted egg white
50 g. sugar
244 g. fluid skim milk
112.00 g. butter

Method:

Cream the fat, add sugar slowly, creaming until fluffy; add flavourings. Mix and sift dry ingredients. Beat the egg whites until stiff, but not dry; beat in the sugar. Add the milk and flour alternately to the sugar-fat mixture; fold in the egg whites using a wire whisk. Bake in glass 10" tube for about 40 minutes at 350°F.

Yield: 16 - 42.0 g. slices/cake. (Baked wt.)

Appendix Table 19

WHITE CAKE (CANBRA)

Temp. 350°F.

112.00 g. Canbra margarine
200 g. sugar
1 tsp. vanilla
½ tsp. almond extract
192 g. sifted cake flour
½ tsp. salt
3 tsp. baking powder
80 g. reconstituted egg white
50 g. sugar
244 g. fluid skim milk.

Method:

Cream the fat; add the sugar slowly, creaming until fluffy; add flavourings. Mix and sift dry ingredients. Beat the egg whites until stiff, but not dry; beat in the sugar. Add the milk and flour alternately to the sugar-fat mixture; fold in the egg whites using a wire whisk. Bake in glass 10" tube pan for about 40 minutes at 350°F.

Yield: 16 - 42.0 g. slices/cake. (Baked wt.)

Appendix Table 20

RAISIN OATMEAL COOKIES (Mixed Fat)

Temp. 400°F.

Mix thoroughly together:

250 g. sugar
100 g. reconstituted egg albumin
126.00 g. butter

Sift together and stir in:

192 g. sifted A.P.flour
1 tsp. soda
1 tsp. salt
2 tsp. cinnamon

Stir in:

160 g. rolled oats
164 g. seedless raisins

Drop from spoon to make balls of 27.5 g. . Place on lightly-greased baking sheet about 2 inches apart. Bake until lightly browned, about 8-10 minutes.

Yield: 36 - 25 g. cookies.

Appendix Table 21

RAISIN OATMEAL COOKIES (CANBRA)

Temp. 400°F.

Mix thoroughly together:

112.00 g. Canbra margarine
250 g. sugar
100 g. reconstituted egg albumin

Sift together and stir in:

192 g. sifted A.P.flour
1 tsp. soda
1 tsp. salt
2 tsp. cinnamon

Stir in:

160 g. rolled oats
164 g. seedless raisins

Roll into balls of 25.0 g. Place on lightly-greased baking sheet about 2 inches apart. Bake until lightly browned, about 8-10 minutes.

Yield: 36 - 25 g. cookies.

Appendix Table 22

General Instructions for Subjects
Participating in the Metabolic Study

1. This is a reversal metabolic study looking at the metabolism of canbra oil by the human. It will be 39 days in length, consisting of 9 days preliminary mixed fat diet, 22 days experimental diet, followed by another 8 days on the mixed fat diet.

2. Meals will be served in Room 402, Home Economics Building, 7 days a week at the following times:

Breakfast	:	8:00 a.m.
Lunch	:	12:00 p.m.
Dinner	:	5:00 p.m.

However, arrangements will be made to accommodate your class schedules and we are considering the possibility of sending the Sunday meals home on Saturday.

Promptness for meals will be appreciated and if you expect to be early or late, we expect to be informed.

3. All of the food must be eaten, since it has been carefully weighed and measured; calories will be adjusted so that the subjects maintain normal weight throughout the study.

4. Only food or drink served or specified by the project director will be permitted. Nothing else is to be consumed during the period of the study.

5. Body weight will be recorded daily before breakfast. A scale and form for recording weight will be available in Room 402.

6. Since the Home Economics Building is locked on the weekend, keys will be available from the project director at the beginning of the study and returned at the conclusion.

N.B. The success of the study depends definitely on YOU.....on your cooperation in eating only the food provided and in observing the instructions for the collection of biological samples. We expect your complete cooperation if selected for this study. If you have any questions, we will be glad to answer them at any time. If you have any serious doubts concerning this study you should withdraw before the trial begins.

Collection of Biological Samples

Blood

1. Six fasting blood samples will be taken during the study: day 1, 10, 18, 25, 32, and 39. All samples will be taken by a qualified technician.

2. No foods are to be consumed for at least 12 hours before the blood sample is to be taken (clear coffee, tea, or water are permitted). Coffee, tea and smoking are prohibited during the period 1 hour before the time the blood sample is to be taken.

Stools

1. Total feces will be collected for three 5-day periods: days 6-10, 16-20, and 35-39, inclusive of this study.

2. Feces will be collected in containers that can be easily transported to the laboratory. Separate containers will be available for each voiding.

Appendix Table 23

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

Item	Grams	Cal.	Pro.	Fat	CHO	Ca	P	Fe	Vitamins: A	Thiamin	Ribo-flavin	Niacin	C
Breakfast:													
Orange juice 1437[2]	113.4	51.0	0.794	0.113	12.10	10.20	18.10	0.113	226.80	0.102	0.013	0.340	51.00
Rolled oats from recipe[3]	250.8	244.4	5.680	13.000	27.30	23.10	162.00	1.880	0	0.240	0.056	0.400	0
Scrambled egg from recipe[3]	110.4	128.0	14.300	6.530	2.24	41.41	42.20	0.181	264.71	0.015	0.764	0.139	0.168
Skim milk 1322[2]	226.8	81.7	8.170	0.227	11.60	274.40	215.50	trace	trace	0.091	0.408	0.227	2.270
Brown sugar 2229[2]	14.0	52.2	0	0	13.50	11.90	2.66	0.476	0	0.001	0.004	0.028	0
Strawberry jam 1148[2]	19.0	51.7	0.114	0.019	13.30	3.81	1.71	0.190	1.90	0.002	0.006	0.038	3.000
Toast 462[2]	28.4	89.2	2.870	1.050	16.70	27.82	32.10	0.824	trace	0.065	0.068	0.795	trace
Lunch:													
Hamburg patties from recipe[3]	121.7	424.7	19.200	34.590	10.50	16.01	27.60	0.119	419.80	0.070	0.187	0.674	0.720
Bun 1902[2]	47.0	140.1	3.850	2.630	24.90	34.83	40.00	0.890	-	0.130	0.080	1.030	-
Coleslaw from recipe[3]	65.0	17.3	0.766	0.130	3.91	28.74	19.20	0.305	1186.00	0.035	0.034	0.235	30.700
Dressing from recipe[3]	38.0	84.0	0	5.000	10.50	0	0	0.010	0	0	0	0	0
Fruit as calculated[4]	100.0	61.4	0.400	0.120	15.90	8.01	10.00	0.400	700.00	0.028	0.022	0.034	3.400
Skim milk 1322[2]	226.8	81.7	8.170	0.227	11.60	274.40	215.50	trace	trace	0.091	0.408	0.227	2.270
Dinner:													
Sweet 'n' sour from recipe[3]	308.2	577.1	14.400	36.700	51.30	49.61	26.70	1.520	749.20	0.272	0.076	0.809	29.300
Rice 1872[2]	100.0	109.0	2.000	0.100	24.20	10.00	28.00	0.900	0	1.100	-	1.000	0
Tossed salad from recipe[3]	100.0	17.5	1.000	0.150	3.80	16.51	24.50	0.500	615.00	0.060	0.050	0.500	14.500
Salad dressing from recipe[3]	14.7	83.2	0.210	9.130	0.44	1.12	0.30	0.003	0	0	0.005	0.002	0
Fruit as calculated[4]	100.0	61.4	0.400	0.120	15.90	8.01	10.00	0.400	700.00	0.028	0.022	0.034	3.400
Bread 461[2]	28.4	76.7	2.500	0.909	14.30	23.91	27.60	0.710	trace	0.071	0.060	0.682	trace
Skim milk 1322[2]	226.8	81.7	8.170	0.227	11.60	274.40	215.50	trace	trace	0.091	0.408	0.227	2.270
Plus:													
Butter 505[2]	20.0	143.2	0.120	16.200	0.08	4.01	3.20	0	660.00	-	-	-	0
Spicy fruit sq. from recipe[3]	33.5	114.5	1.440	3.300	20.40	43.72	19.10	0.686	132.50	0.028	0.038	0.231	0.025
White cake from recipe[3]	55.9	163.5	2.240	5.780	26.20	41.24	53.30	0.126	231.00	0.012	0.131	0.134	0.153
Cookie from recipe[3]	27.9	103.4	1.700	3.230	17.60	7.50	28.30	0.525	116.40	0.055	0.033	0.257	0.046
Icing-square from recipe[3]	9.0	38.3	0.040	0.811	8.01	1.32	1.05	0.008	33.00	0	0.002	0.001	0.938
TOTALS:	2385.7	3076.9	98.734	140.393	367.88	1237.67	1224.12	107.66	6036.31	2.587	2.875	8.021	144.260

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

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

[3]Calculated totals from recipe given in appropriate Appendix Table.

[4]Calculated average assuming that equal quantities of pears, apricots, pineapple, peaches, and plums were served (90.0 gm. strained fruit with 10.0 gm. juice added).

Appendix Table 24

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

Item	Grams	Cal.	Pro.	Fat	CHO	Ca	P	Fe	Vitamins: A	Thiamin	Ribo-flavin	Niacin	C
Breakfast:													
Apple juice 27[2]	85.1	40.00	0.085	trace	10.10	5.11	7.66	0.511	-	0.009	0.018	0.085	0.851
Cream of wheat from recipe[3]	211.8	162.30	2.500	10.300	15.40	107.80	118.50	8.970	0	0	0	0	0
Scrambled egg from recipe[3]	110.4	128.00	14.310	6.530	2.24	41.40	42.20	0.181	264.72	0.015	0.764	0.139	0.168
Skim milk 1322[2]	226.8	81.70	8.170	0.227	11.60	274.40	215.50	trace	trace	0.091	0.408	0.227	2.270
White sugar 2230[2]	10.0	38.50	0	0	9.95	0	trace	0	0.01	-	-	-	-
Orange marmalade 1318[2]	19.0	48.80	0.095	0.019	133.20	6.65	1.71	0.114	-	0.004	0.004	0.019	1.140
Toast 461[2]	28.4	89.20	2.870	1.050	16.70	27.80	32.10	0.824	trace	0.065	0.068	0.795	trace
Lunch:													
Skim milk 1322[2]	226.8	81.70	8.170	0.227	11.60	274.40	215.50	trace	trace	0.091	0.408	0.227	2.270
Spaghetti from recipe[3]	41.0	158.10	5.130	1.260	30.80	15.20	66.40	1.190	0	0.360	0.150	2.500	0
Tomato sauce from recipe[3]	224.0	140.98	2.430	10.280	11.50	22.60	47.70	1.850	2214.27	0.130	0.079	1.931	42.030
Meatballs from recipe[3]	101.8	243.55	16.420	15.700	9.68	4.32	6.62	0.051	161.70	0.003	0.189	0.638	0.105
Tossed salad from recipe[3]	100.0	17.50	1.000	0.150	3.80	13.50	24.50	0.500	615.00	0.060	0.050	0.500	14.500
Dressing from recipe[3]	14.7	83.20	0.210	9.130	0.44	1.12	0.30	0.003	0	0	0.005	0.002	0
Fruit as calculated[4]	100.0	61.40	0.400	0.120	15.90	8.00	10.00	0.400	700.00	0.028	0.022	0.034	3.400
Bread 461[2]	28.4	76.70	2.500	0.909	14.30	23.90	27.60	0.710	trace	0.071	0.060	0.682	trace
Dinner:													
Beef stew from recipe[3]	394.9	606.05	33.690	38.240	37.20	25.92	216.61	4.931	2898.48	0.316	0.693	2.066	6.080
Coleslaw from recipe[3]	65.0	17.30	0.766	0.130	3.91	23.70	19.20	0.305	1186.00	0.035	0.034	0.235	30.700
Coleslaw dressing from recipe[3]	38.0	84.00	0	5.000	10.50	0	0	0.010	0	0	0	0	0
Skim milk 1322[2]	226.8	81.70	8.170	0.227	11.60	274.40	215.50	trace	trace	0.091	0.408	0.227	2.270
Fruit as calculated[4]	100.0	61.40	0.400	0.120	15.90	8.00	10.00	0.400	700.00	0.028	0.022	0.034	3.400
Bread 461[2]	28.4	76.70	2.500	0.909	14.30	23.90	27.60	0.710	trace	0.071	0.060	0.682	trace
Mashed potato from recipe[3]	103.1	120.90	1.740	6.590	14.20	27.30	43.30	0.274	264.00	0.043	0.037	0.875	5.270
Plus:													
Butter 505[2]	20.0	143.20	0.120	16.200	0.08	5.00	3.20	0	660.00	-	-	-	0
Spicy fruit sq.from recipe[3]	33.5	114.50	1.440	3.300	24.40	43.70	19.10	0.686	132.50	0.028	0.038	0.231	0.025
White cake from recipe[3]	55.9	163.50	2.240	5.780	26.20	41.20	53.30	0.126	231.00	0.012	0.131	0.134	0.153
Cookie from recipe[3]	27.9	103.43	1.700	3.230	17.60	7.46	28.30	0.525	116.40	0.055	0.033	0.257	0.046
Icing-square from recipe[3]	9.0	38.30	0.040	0.811	8.01	1.34	1.05	0.008	33.00	0	0.022	0.001	0.938
TOTALS:	2630.7	3062.61	117.106	136.439	477.11	1317.12	1453.45	23.279	10177.08	1.606	3.683	12.521	115.616

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

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

[3]Calculated totals from recipe given in appropriate Appendix Table.

[4]Calculated average assuming that equal quantities of pears, apricots, pineapple, peaches, and plums were served (90.0 gm. strained fruit with 10.0 gm. juice added).

Calculated Nutrient Composition of Canbra Diet[1], Menu I

Item	Grams	Cal.	Pro.	Fat	CHO	Ca	P	Fe	Vitamins: A	Thiamin	Ribo-flavin	Niacin	C
Breakfast:													
Orange juice 1437[2]	113.4	51.00	0.794	0.113	12.10	10.20	18.10	0.113	226.80	0.102	0.013	0.340	51.000
Rolled oats from recipe[3]	250.8	244.40	5.680	13.000	27.30	23.10	162.00	1.880	0	0.240	0.056	0.400	0
Scrambled egg from recipe[3]	107.4	114.90	14.300	5.050	2.21	39.80	40.90	0.181	0.72	0.015	0.764	0.139	0.168
Skim milk 1322[2]	226.8	81.70	8.170	0.227	11.60	274.40	215.50	trace	trace	0.091	0.408	0.227	2.270
Brown sugar 2229[2]	14.0	52.20	0	0	13.50	11.90	2.66	0.476	0	0.001	0.004	0.028	0
Strawberry jam 1148[2]	19.0	51.70	0.114	0.019	13.30	3.80	1.71	0.190	1.90	0.002	0.006	0.038	3.000
Toast 462[2]	28.4	89.20	2.870	1.050	16.70	27.80	32.10	0.824	trace	0.065	0.068	0.795	trace
Lunch:													
Hamburg patties from recipe[3]	115.8	396.30	18.800	31.500	10.50	13.70	25.83	0.119	50.90	0.070	0.187	0.674	0.720
Bun 1902[2]	47.0	140.10	3.850	2.630	24.90	34.80	40.00	0.890	trace	0.130	0.080	1.030	trace
Coleslaw from recipe[3]	65.0	17.30	0.766	0.130	3.91	28.70	19.20	0.305	1186.00	0.035	0.034	0.235	30.700
Coleslaw dressing from recipe[3]	38.0	84.00	0	5.000	10.50	0	0	0.010	0	0	0	0	0
Fruit as calculated[4]	100.0	61.40	0.400	0.120	15.90	8.00	10.00	0.400	700.00	0.028	0.022	0.034	3.400
Skim milk 1322[2]	226.8	81.70	8.170	0.227	11.60	274.40	215.50	trace	trace	0.091	0.408	0.227	2.270
Dinner:													
Sweet 'n' Sour from recipe[3]	306.4	597.50	14.000	39.500	51.20	45.90	23.80	1.520	144.60	0.272	0.076	0.809	29.300
Rice 1872[2]	100.0	109.00	2.000	0.100	24.20	10.00	28.00	0.900	0	0.110	-	1.000	0
Tossed salad from recipe[3]	100.0	17.50	1.000	0.150	3.80	16.50	24.50	0.500	615.00	0.060	0.050	0.500	14.500
Piquant salad dressing from recipe[3]	14.7	83.20	0.210	9.130	0.48	1.12	0.30	0.003	0	0	0.005	0.002	0
Fruit as calculated[4]	100.0	61.40	0.400	0.120	15.90	8.00	10.00	0.400	700.00	0.028	0.022	0.034	3.400
Bread 461[2]	28.4	76.70	2.500	0.909	14.30	23.90	27.60	0.710	trace	0.071	0.060	0.682	trace
Skim milk 1322[2]	226.8	81.70	8.170	0.227	11.60	274.40	215.50	trace	trace	0.091	0.408	0.227	2.270
Plus:													
Canbra margarine 1317[2]	20.0	144.00	0.120	16.200	0.08	4.00	3.20	0	660.00	-	-	-	0
Spicy fruit sq.from recipe[3]	35.9	121.20	1.410	4.060	20.30	42.60	18.50	0.698	0.50	0.028	0.038	0.231	0.025
White cake from recipe[3]	55.9	163.50	2.240	5.780	26.20	41.20	53.50	0.126	231.00	0.012	0.131	0.134	0.153
Raisin Oatmeal Cookie from recipe[3]	25.0	100.80	1.700	2.910	17.60	7.38	28.20	0.525	103.60	0.055	0.033	0.257	0.046
Icing-Square from recipe[3]	9.0	37.40	0.039	0.710	8.01	1.32	1.03	0.008	28.90	0	0.002	0.001	0.938
TOTALS:	2374.5	3060.00	97.703	138.862	367.69	1226.92	1217.43	10.778	4649.92	1.597	2.875	8.044	144.160

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

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

[3]Calculated totals from recipe given in appropriate Appendix Table.

[4]Calculated average assuming that equal quantities of pears, apricots, pineapple, peaches, and plums were served (90.0 gm. strained fruit with 10.0 gm. juice added).

Calculated Nutrient Composition of Canbra Diet[1], Menu II

Item	Grams	Cal.	Pro.	Fat	CHO	Ca	P	Fe	Vitamins: A	Thiamin	Ribo-flavin	Niacin	C
Breakfast:													
Apple juice 27[2]	85.1	40.00	0.085	trace	10.10	5.11	7.66	0.511	-	0.009	0.018	0.085	0.851
Cream of wheat from recipe[3]	211.8	162.30	2.500	10.300	15.40	108.80	118.50	8.970	0	0	0	0	0
Scrambled egg from recipe[3]	107.4	114.90	14.300	5.050	2.21	39.80	40.90	0.681	0.72	0.015	0.764	0.139	0.168
Skim milk 1322[2]	226.8	81.70	8.170	0.227	11.60	274.40	215.50	trace	trace	0.091	0.408	0.227	2.270
White sugar 2230[2]	10.0	38.50	0	0	9.95	0	0	0.010	0	-	-	-	-
Orange marmalade 1318[2]	19.0	48.80	0.095	0.019	133.20	6.65	1.71	0.114	-	0.004	0.004	0.019	1.140
Toast 461[2]	28.4	89.20	2.870	1.050	16.70	27.80	32.10	0.824	trace	0.065	0.068	0.795	trace
Lunch:													
Skim milk 1322[2]	226.8	81.70	8.170	0.227	11.60	274.40	215.50	trace	trace	0.091	0.408	0.227	2.270
Spaghetti from recipe[3]	41.0	158.05	5.125	1.260	30.83	16.13	66.42	1.190	-	0.360	0.150	2.500	0
Tomato sauce from recipe[3]	225.1	150.20	2.430	11.400	11.50	22.60	47.70	1.850	2214.10	0.130	0.079	1.930	42.000
Meatballs from recipe[3]	100.3	238.70	16.400	15.200	9.61	3.35	5.84	0.051	0.60	0.053	0.189	0.638	0.105
Tossed salad from recipe[3]	100.0	17.50	1.000	0.150	3.80	16.50	24.50	0.500	615.00	0.060	0.050	0.500	14.500
Piquant dressing from recipe[3]	14.7	83.20	0.210	9.130	0.48	1.12	0.30	0.003	-	-	0.005	0.002	-
Fruit as calculated[4]	100.0	61.40	0.400	0.120	15.90	8.00	10.00	0.400	700.00	0.028	0.022	0.034	3.400
Bread 461[2]	28.4	76.70	2.500	0.909	14.30	23.90	27.60	0.710	trace	0.071	0.060	0.682	trace
Dinner:													
Beef stew from recipe[3]	393.6	635.20	27.700	42.400	37.10	24.00	214.00	4.930	2350.00	0.316	0.693	2.070	6.080
Coleslaw from recipe[3]	65.0	17.30	0.766	0.130	3.91	28.70	19.20	0.305	1186.00	0.035	0.034	0.235	3.070
Coleslaw dressing from recipe[3]	38.0	84.00	0	5.000	10.50	-	-	0.010	-	-	-	-	-
Skim milk 1322[2]	226.8	81.70	8.170	0.227	11.60	274.40	21.55	trace	trace	0.091	0.408	0.227	2.270
Fruit as calculated[4]	100.0	61.40	0.400	0.120	15.90	8.00	10.00	0.400	700.00	0.028	0.022	0.034	3.400
Bread 461[2]	28.4	76.70	2.500	0.909	14.30	23.90	27.60	0.710	trace	0.071	0.060	0.682	trace
Mashed potato from recipe[3]	100.0	99.64	1.720	4.160	14.23	26.65	42.73	0.274	165.00	0.043	0.037	0.880	5.270
Plus:													
Canbra margarine 1317[2]	20.0	144.00	0.120	16.200	0.08	4.00	3.20	-	660.00	-	-	-	-
Spicy fruit sq.from recipe[3]	35.9	121.20	1.410	4.060	20.30	42.60	18.50	0.698	0.50	0.028	0.038	0.231	0.025
White cake from recipe[3]	55.9	163.80	2.240	5.780	26.20	41.20	53.30	0.126	231.00	0.012	0.131	0.134	0.153
Raisin oatmeal cookie from recipe[3]	25.0	100.80	1.700	2.910	17.60	7.38	28.20	0.520	103.60	0.055	0.033	0.257	0.046
Icing-square from recipe[3]	9.0	37.40	0.039	0.710	8.01	1.32	1.03	0.008	28.90	-	0.002	0.001	0.938
TOTALS:	2622.4	3065.89	111.020	137.658	476.91	1310.71	1253.54	23.795	8955.42	1.656	3.683	12.529	87.956

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

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

[3]Calculated totals from recipe given in appropriate Appendix Table.

[4]Calculated average assuming that equal quantities of pears, apricots, pineapple, peaches, and plums were served (90.0 gm. strained fruit with 10.0 gm. juice added).

Appendix Table 27

Analysis of Variance: Total Serum Cholesterol

Source of Variation	df	SS	MS	F-value	P[1]
Replications (days)	5	16871.32	3374.26	111.98	0.005
Subjects	6	2431.14	405.19	13.44	0.005
Residuals (error)	30	904.01	30.13		
Total	41	20206.47			

[1]P = probability of chance occurrence.

Appendix Table 28

Orthogonal Comparisons: Total Serum Cholesterol

No.	Comparison	df	SS	MS	F-value	P[1]
1	Day 10 vs Day 39	1	257.14	257.14	8.53	ns
2	Day 32 vs Day 39	1	5092.07	5092.07	168.98	0.005
3	Day 10 vs Day 32	1	3060.64	3060.64	101.57	0.005
4	Day 10 vs Day 32 vs Day 39	1	514.50	514.50	17.07	0.005
5	Day 1 vs Day 10	1	3001.78	3001.78	99.62	0.005
	Residuals (error)	30	904.01	30.13		

[1]P = probability of chance occurrence.

Appendix Table 29

Analysis of Variance, Linear Regression:
Total Serum Cholesterol of Subjects During Canbra Oil Diet

Source of Variation	df	SS		MS	F-value	P[1]
Replications (days)	3	3393.00				
linear regression	1	3295.82		3295.82	116.71	0.005
experimental error	2	97.18		48.59	1.72	ns
Subjects	6	1852.00	SSE = 2457.43			
Residuals (error)	18	508.25		28.24		
Total	27	5753.25				

[1]P = probability of chance occurrence.

Appendix Table 30

Analysis of Variance: Lipid Phosphorus

Source of Variation	df	SS	MS	F-value	P[1]
Replications (days)	5	115.95	23.19	414.09	0.005
Subjects	6	17.62	2.94	52.43	0.005
Residuals (error)	30	1.70	0.06		
Total	41	135.27			

[1]P = probability of chance occurrence.

Appendix Table 31

Orthogonal Comparisons: Lipid Phosphorus

No.	Comparison	df	SS	MS	F-value	P[1]
1	Day 10 vs Day 39	1	0.56	0.56	9.33	ns
2	Day 32 vs Day 39	1	50.92	50.92	909.28	0.005
3	Day 10 vs Day 32	1	40.80	40.80	728.57	0.005
4	Day 10 vs Day 32 vs Day 39	1	10.60	10.60	189.28	0.005
5	Day 1 vs Day 10	1	8.80	8.80	157.14	0.005
	Residuals (error)	30	1.70	0.06		

[1]P = probability of chance occurrence.

Appendix Table 32

Analysis of Variance, Linear Regression:
Lipid Phosphorus of Subjects During Canbra Oil Diet

Source of Variation		df		SS		MS	F-value	P[1]
Replications (days)		3		46.89				
linear regression	1		46.35			46.35	1158.75	0.005
experimental error	2		0.54			0.27	6.75	ns
Subjects		6		12.50	SSE = 13.85			
Residuals (error)		18		0.81		0.04		
Total		27		60.20				

[1]P = probability of chance occurrence.

Appendix Table 33

Analysis of Variance: Serum Triglycerides

Source of Variation	df	SS	MS	F-value	P[1]
Replications (days)	5	157.62	31.52	46.52	0.005
Subjects	6	5745.91	957.65	1410.38	0.005
Residuals (error)	30	20.38	0.68		
Total	41	5923.91			

[1]P = probability of chance occurrence.

Appendix Table 34

Orthogonal Comparisons: Serum Triglycerides

No.	Comparison	df	SS	MS	F-value	P[1]
1	Day 10 vs Day 39	1	1.14	1.14	1.68	ns
2	Day 32 vs Day 39	1	25.78	25.78	37.97	0.005
3	Day 10 vs Day 32	1	14.07	14.07	23.67	0.005
4	Day 10 vs Day 32 vs Day 39	1	2.88	2.88	4.24	ns
5	Day 1 vs Day 10	1	64.29	64.29	94.68	0.005
	Residuals (error)	30	20.38	0.68		

[1]P = probability of chance occurrence.

Appendix Table 35

Analysis of Variance: Beta-/Pre-Beta-Lipoprotein Ratios

Source of Variation	df	SS	MS	F-value	P[1]
Replications (days)	5	4.81	0.96	2.66	ns
Subjects	6	5.86	0.98	2.70	ns
Residuals (error)	30	10.85	0.36		
Total	41	21.52			

[1]P = probability of chance occurrence.

Appendix Table 36

Orthogonal Comparisons: Beta-/Pre-Beta-Lipoprotein Ratios

No.	Comparison	df	SS	MS	F-value	P[1]
1	Day 10 vs Day 39	1	0.46	0.46	1.28	ns
2	Day 32 vs Day 39	1	1.42	1.42	3.94	ns
3	Day 10 vs Day 32	1	3.51	3.51	9.75	0.005
4	Day 10 vs Day 32 vs Day 39	1	2.17	2.17	6.02	ns
5	Day 1 vs Day 10	1	0.69	0.69	1.91	ns
	Residuals (error)	30	10.85	0.36		

[1]P = probability of chance occurrence.

Appendix Table 37

Analysis of Variance: Total Red Cell Count

Source of Variation	df	SS	MS	F-value	P[1]
Replications (days)	5	0.341	0.068	3.400	ns
Subjects	6	1.525	0.254	12.700	0.005
Residuals (error)	30	0.593	0.020		
Total	41	2.459			

[1]P = probability of chance occurrence.

Appendix Table 38

Orthogonal Comparisons: Total Red Cell Count

No.	Comparison	df	SS	MS	F-value	P[1]
1	Day 10 vs Day 39	1	0.161	0.161	8.050	ns
2	Day 32 vs Day 39	1	0.071	0.071	3.550	ns
3	Day 10 vs Day 32	1	0.178	0.178	8.900	ns
4	Day 10 vs Day 32 vs Day 39	1	0.095	0.095	4.750	ns
5	Day 1 vs Day 10	1	0.206	0.206	10.300	0.005
	Residuals (error)	30	0.721	0.020		

[1]P = probability of chance occurrence.

Appendix Table 39

Analysis of Variance: Hematocrit

Source of Variation	df	SS	MS	F-value	P[1]
Replications (days)	5	35.430	7.086	5.146	0.005
Subjects	6	44.400	7.400	5.374	0.005
Residuals (error)	30	41.320	1.377		
Total	41	121.150			

[1]P = probability of chance occurrence.

Appendix Table 40

Orthogonal Comparisons: Hematocrit

No.	Comparison	df	SS	MS	F-value	P[1]
1	Day 10 vs Day 39	1	24.446	24.446	17.753	0.005
2	Day 32 vs Day 39	1	3.500	3.500	2.542	ns
3	Day 10 vs Day 32	1	9.446	9.446	6.860	ns
4	Day 10 vs Day 32 vs Day 39	1	21.428	21.428	15.561	0.005
5	Day 1 vs Day 10	1	16.071	16.071	11.671	0.005
	Residuals (error)	30	41.320	1.377		

[1]P = probability of chance occurrence.

Appendix Table 41

Analysis of Variance: Hemoglobin

Source of Variation	df	SS	MS	F-value	P[1]
Replications (days)	5	3.902	0.780	4.936	0.005
Subjects	6	5.591	0.932	5.898	0.005
Residuals (error)	30	4.752	0.158		
Total	41	14.245			

[1]P = probability of chance occurrence.

Appendix Table 42

Orthogonal Comparisons: Hemoglobin

No.	Comparison	df	SS	MS	F-value	P[1]
1	Day 10 vs Day 39	1	0.002	0.002	0.018	ns
2	Day 32 vs Day 39	1	0.161	0.161	1.014	ns
3	Day 10 vs Day 32	1	0.121	0.121	0.762	ns
4	Day 10 vs Day 32 vs Day 39	1	0.028	0.028	0.182	ns
5	Day 1 vs Day 10	1	2.082	2.082	13.149	0.005
	Residuals (error)	30	4.752	0.158		

[1]P = probability of chance occurrence.

Appendix Table 43

Analysis of Variance: Reticulocyte Count

Source of Variation	df	SS	MS	F-value	P[1]
Replications (days)	5	3.593	0.718	8.159	0.005
Subjects	6	1.816	0.302	3.432	ns
Residuals (error)	30	2.627	0.088		
Total	41	8.036			

[1]P = probability of chance occurrence.

Appendix Table 44

Orthogonal Comparisons: Reticulocyte Count

No.	Comparisons	df	SS	MS	F-value	P[1]
1	Day 10 vs Day 39	1	2.239	2.239	25.443	0.005
2	Day 32 vs Day 39	1	0.001	0.001	0.011	ns
3	Day 10 vs Day 32	1	2.161	2.161	24.556	0.005
4	Day 10 vs Day 32 vs Day 39	1	2.934	2.934	33.341	0.005
5	Day 1 vs Day 10	1	2.486	2.486	28.250	0.005
	Residuals (error)	30	2.627	0.088		

[1]P = probability of chance occurrence.