SERUM FATTY ACID PATTERNS OF CLINICALLY HEALTHY WOMEN LIVING IN THE SOUTHEAST SECTION OF ARIZONA

by

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Mary Ann Kight

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I hereby recommend that this dissertation prepared under my direction by Mary Ann Kight entitled SERUM FATTY ACID PATTERNS OF CLINICALLY HEALTHY WOMEN LIVING IN THE SOUTHEAST SECTION OF ARIZONA be accepted as fulfilling the dissertation requirement of the degree of Doctor of Philosophy

B. L. Reid
Dissertation Director

July 7, 1966
Date

After inspection of the dissertation, the following members of the Final Examination Committee concur in its approval and recommend its acceptance:*

B. L. Reid	July 7, 1966
[illegible]	July 7, 1966
A. R. Kemmerer	July 8 1966
W. H. McCauley	July 8, 1966
W. Ferry	8 July 66
Peter E Pickens	8 July 1966

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SIGNED: Mary Anne Hight

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ABSTRACT

Seven professionally-active women dietitians twenty-eight to forty-seven years of age and living in Tucson, Arizona, were subjects from September 1, 1964, to November 10, 1964. The ten week mean daily temperature (^{o}F) was 73. Mean daily noon relative humidity (%) and percentage of possible sunshine for the ten weeks were 41 and 81, respectively. Total precipitation (In) was 2.77. The mean barometric pressure (In) was 27.34 at an average station elevation of 2,555 feet.

All subjects were within the usual findings for standard clinical blood and urine determinations. Mean height and weight for the group were 162 cm and 58 kg, respectively. Height-weight measurements when referred to the Society of Actuaries Per Cent Standard Weights gave a group mean of 97-101% of desirable weight. Blood pressures were normal and continuous residence in southeastern Arizona prior to the study ranged from 1½ to 29 years.

Thyrobinding Index (TBI), Basal Metabolic Rate (BMR), and Protein-Bound Iodine (PBI) mean values for the group were 1.04, -11%, and 5.4 mcg%, respectively.

Diets were self-selected and with few exceptions met the recommended daily dietary allowances set forth by the National Research Council. Average per cent of the recommended calorie allowance for the group was 101. Calories from fat, protein and carbohydrate consumed by

the subjects daily averaged 42, 14 and 44%, respectively. Daily linoleic acid intakes ranged from 4.23 to 11.84 gm or from 1 to 6% of total dietary calories. No significant correlations were observed between daily cholesterol intake (185 to 518 mg) or linoleate intakes and serum lipid levels, or between daily linoleic acid intake and TBI, BMR or PBI.

Weekly fasting serum total protein; five protein electrophoretic fractions (albumin, alpha -1 globulin, alpha -2 globulin, beta globulin, and gamma globulin); total lipids; five lipoprotein electrophoretic fractions (lipalbumin, alpha -1 globulin lipoprotein, alpha -2 globulin lipoprotein, beta globulin plus neutral fat lipoprotein, and gamma globulin lipoprotein); total fatty acids; total and free cholesterol values for the subjects showed no impressive clinical abnormalities.

The characteristic pattern of percentage distribution for the detected total lipid fatty acids gave mean values for lauric (12:0), myristic (14:0), palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), linoleic (18:2), and arachidonic (20:4) to be in the order of 0.47 $\pm$ 0.13; 1.21 $\pm$ 0.19; 20.68 $\pm$ 1.03; 3.92 $\pm$ 0.62; 3.94 $\pm$ 0.53; 26.37 $\pm$ 1.22; 39.39 $\pm$ 1.47 and 4.22 $\pm$ 1.03, respectively, with an oleic to linoleic acid (O:L) ratio of 0.66.

The subjects showed a significant negative correlation (-0.86) between total serum protein and serum linoleic plus arachidonic levels. A correlation of 0.87 was observed between serum cholesterol ester (mg) and either serum linoleic or arachidonic acid (mg). The correlation between serum linoleic and arachidonic acid was found to be 0.86. No significant correlations were observed between age and serum lipids.

CHAPTER I

INTRODUCTION

Evidence for the hypothesis that clinically healthy individuals, especially women, living in southeastern Arizona possess or acquire a metabolic behavior unique to this climate has been reported periodically for twenty-four years. The basic thread throughout the investigations has been the persistency of basal metabolisms falling within the negative acceptable range of widely used metabolism prediction standards. Tucson, Arizona, where the present and earlier studies were made, is located in southeastern Arizona at a latitude of 32° 15', longitude of 110° 57' and has a ground elevation of 2,430 feet. The area was first designated a "steppe" climate on the basis of a modification of the Koppen classification (1) but has more recently been listed by Green and Sellers (2) as one of six topographical sections in the state and as having a desert or semi-desert climate.

Willard (3) was the first to demonstrate that the basal metabolism of women born and reared in Arizona was lower than standards reported for similar women living in northern states. Campbell (4) determined whether a similar lower metabolism was present in men born and reared in Arizona. The average basal metabolism of the subjects in this study was significantly higher than those of the women in Willard's study. On an individual basis, however, forty-seven of the fifty-one men had negative values from the Mayo Foundation standard predictions of basal metabolism. Deviations from other prediction standards similarly showed a significant predominance of negative values. This might therefore suggest a lowering effect of climate in men with a "zone leveling" at a higher metabolic rate.

Ridgway (5) compared actual basal metabolisms of 103 girls, fourteen to nineteen years of age, in the Tucson public schools with similar standard prediction values. Data from this study were combined with those of Willard and reported by Thompson, Cox and Ridgway (6). At fourteen years of age a basal metabolic mean of 36.4 calories per square meter per hour was observed, which was lower than values for similar subjects living in the north and midwest. There was a progressive drop to a low level of 31.2 calories per square meter per hour at age eighteen at a mean annual temperature of 67.2^{o}F. This level was maintained through the twenty-third year of age although it was much below that predicted by widely used standards. This would seem to indicate the appearance of a "zone of minimal metabolism" in women as suggested by Hardy, Milhorat and DuBois (7). In the Hardy et al. study, women showed a marked drop in basal metabolism to an average of 30.9 calories per square meter per hour between 80.6 and 89.6^{o}F. Men used in the study showed no similar drop.

This early work led to the question of the effect of lowered energy requirements in females on growth and development during these and earlier ages. Since irrevocable damage may be caused by under-activity of the thyroid gland in growing children it became desirable to evaluate the observed metabolic function in terms of growth and development.

Subsequently Hurley (8) employed the Wetzel grid (9) as a measure of growth and development. The basal metabolism of fifty-three girls native to southern Arizona, twelve and thirteen years of age, was measured. Basal levels were 38.7 and 37.7 calories per square meter per hour, respectively, at mean annual temperatures similar to those in the Ridgway study. No diminishing effect of temperature on basal metabolism was observed for these groups at this age. However, early onset of puberty and advanced growth rates as compared with the grid predictions were observed, suggesting a favorable climatic effect on growth and development despite any later metabolic slowing effects.

Believing the "zone" to be masked by metabolic impetus resulting from the early onset of puberty, which could have been enhanced by greater outdoor activity and sunshine for these girls, Kight (10) continued with a study of Hurley's subjects together with additional subjects in the fourteen to eighteen age group. The anticipated decline in basal metabolism was observed up to the seventeenth year and was attributed to a physiological adjustment between diminishing growth impetus after the maximal developmental level was reached and the lowering effect of warm climate. Full climatic lowering effect was finally observed when growth as defined by the Wetzel grid ceased and only maintenance requirement remained. Yearly means of basal oxygen consumption when plotted on the basis of surface area confirmed Ridgway's findings for similar ages. Kight's subjects reached a mean low of 34.2 calories per square meter per hour at a mean annual temperature of 68.4°F. It appeared, therefore, that Hurley's subjects at a comparable age and Kight's additional subjects reaffirmed Ridgway's findings of a lowering effect on basal metabolism due to climate, and the possibility of Tucson, Arizona, representing a "zone of minimal metabolism".

In view of the above, interest was aroused with respect to other possible climatic-metabolic effects. Accordingly Thompson et al. (11) found the rate of depletion of total ascorbic acid in blood serum of women to be significantly higher in summer than in winter. Basal metabolismswere also observed in these seven subjects, and all were characteristically in the negative range of values at a mean minimum monthly temperature of 72.0°F., and a mean maximum monthly temperature of 99.0°F.

Efforts were then directed toward an investigation of the possibility that this lowered oxygen consumption was the result of lowered circulating thyroid hormone. At that time both protein-bound iodine (PBI) and cholesterol values were considered to be measures of thyroid activity. It had been found as early as 1933 (12) that the level of total iodine in the blood usually decreased in hypothyroidism, but correlations were questionable. Then in 1941 Salter, Bassett and Sappington (13) found that one could obtain a good evaluation of thyroid activity by determining the concentration of iodine bound to plasma proteins. In view of this and more recent findings, the level of PBI in the blood serum was used objectively as an index of the productive activity of the thyroid gland.

Thompson and Kight (14) related warm environmental temperatures to basal metabolism, PBI and total cholesterol levels in both men and women. Basal metabolisms for seven female student nurses were all in the negative range of the Mayo Foundation standards. Serum PBI concentrations were similarly in the lower one-half of the usual normal range, advancing the possibility of a lowered circulating thyroid activity in southeastern Arizona women which may be attributed to warm climate. Mean serum total cholesterol concentrations were 204 mg in summer and 209 mg per 100 ml in winter. Both values are well within the low normal range for human cholesterol levels.

Basal metabolism levels for the eleven postmen did not all fall within the low normal. Neither did their respective PBI concentrations fall within the lower one-half of the usual range throughout the study. These values were in the upper one-half of the usual limits during the months of January and February. It should be emphasized that January and February were the two coldest months during the investigation and that the postmen in handling their routes were exposed directly to these extremes. Total serum cholesterol concentrations in the men were 9 per cent higher in winter than in the summer. Values for the men were within the usual acceptable range but higher than values for the nurses. Observations in the men were probably influenced in some measure by age, but they do reflect the apparent inability of men to adapt by lowering the levels of basal energy exchange. The men and women in this particular study were not observed under similar conditions and therefore comparisons at this time should not be attempted. It is unfortunate that "male student nurses" and "postwomen" are not available for observation.

In a study with three women, Varnava (15) found basal metabolic deviations from both the Harris-Benedict and Mayo Foundation standards which were -4.15, -2.66, -16.06 and -5.32, -3.92, -14.79 per cent, respectively. This again demonstrates the typically negative values in women with warm weather.

Albagli (16) in a review of the effects of climate on basal metabolism expressed the belief that most differences ascribed to climate are due to differences in nutrition. It would therefore be of importance to observe selected aliment effects upon the basal metabolism of women living in this climate. We were given this opportunity in a study by Rodriguez (17). In defining the effects of natural and partially-hydrogenated safflower oil on serum lipid and protein constituents of women, she also found basal metabolisms to be negative for all three dietary treatments (natural oil, self-selected and hydrogenated oil). It is of particular interest that two of the three subjects demonstrated an increase in basal metabolism while consuming the hydrogenated safflower oil. This may or may not reflect a stress on metabolic function due to a difference in essential fatty acid (EFA) intake during the eight-week period on the hydrogenated oil. During the process of partial hydrogenation of the oil most of the linoleic acid was converted to elaidic, and the stearic was increased from 2.5 to

13.4 per cent. The dietary ratio of polyunsaturated to saturated fatty acids (P:S) was changed from 4.2 to 0.2, and polyunsaturated fatty acids as a result of the hydrogenation treatment dropped to an estimated 1 to 2 per cent of total calories. Metabolic rate has been shown to be higher in EFA deficient compared with normal control rats by Burr et al. (18, 19, 20). The possibility of a similar interrelationship between EFA and thyroid function in the human is obscure and in need of investigation.

Studies on EFA deficiency in man have been difficult, and for this reason literature references are meager in this area. Two gross criteria of fatty acid essentiality in experimental animals have been growth and dermal integrity (21). The deficiency syndrome has been described more recently to include scaliness of skin, loss of hair, emaciation, impairment of growth and reproduction capacity, increase in the intake of food and water, increased metabolic rate and early death (22).

As early as 1919 von Groer (23) reported a fat deficiency in two infants fed a skim-milk diet without vitamin supplementation. Growth impairment was the immediate manifestation. One infant grew normally for six months, after which growth ceased. The second infant grew normally for one hundred days before growth was impaired. It was only when the experiment was interrupted and a fat-containing diet was given that growth resumed.

Then in 1935 Holt et al. (24) reported another deficiency manifestation; namely, eczematous eruptions and spasmodic bronchitis in three infants maintained on a low fat diet for up to seven days.

In 1938 von Chwalibogowski (25) carried out an experiment similar to that of von Groer by keeping two infants on a skim-milk diet for up to fifteen months. One infant received, in addition to the skimmed milk diet, ultraviolet light treatments to protect against the development of rickets. After five months the milk diet was supplemented with fruit and carrots. Growth was normal and rickets developed only in the infant which did not receive ultraviolet light treatment. This author concluded that dietary fat was not essential for infants.

Hansen and Wiese in 1954 (26) observed four persons (three infants and one adult) maintained on an essentially fat-free diet for up to six months and concluded that man cannot synthesize EFA in adequate amounts. The respiratory quotient in the adult male subject was increased, and the iodine number of the total fatty acids of the serum was definitely lower for the low fat diet. The linoleic and arachidonic acid contents of the serum at the end of the low fat period were 3.2 and 1.9 per cent of the total fatty acids, respectively. Six months after resumption of the normal diet these values were 5.7 and 3.2 per cent, respectively.

James and Lovelock (27) in a 1958 review of the relationship between EFA and human disease were of the opinion that no human diseases are due to such a deficiency. However, studies in 1958 by Hansen et al. (28, 29) reported that most infants on a skim-milk diet, low in fat and EFA, developed frequent, large stools, perianal irritation, and alterations in the skin within a few weeks. The dermal symptoms appeared as dryness, thickening and desquamation with oozing in the intertriginous folds. Supplementation of the diet with linoleic acid restored the skin to normal within one to two weeks. Further indications of EFA deficiency were the extremely low values of dienoic and tetraenoic fatty acids and high trienoic fatty acid values of serum from the infants on the deficient diet. Thus the apparent need for dietary linoleic acid in the human was first demonstrated in Hansen's laboratory.

Additional work by Adam, Hansen and Wiese (30, 31) in 1958 which involved clinical observations and blood serum levels for dienoic, trienoic and tetraenoic fatty acids for infants fed milk mixtures containing varying amounts of linoleic acid became the basis for the first estimate of this fatty acid as a dietary essential. These workers found that the di-, tri-, and tetraenoic acids of blood serum varied as a function of the linoleate content of the diet. They believed the minimal dietary requirement of linoleic acid for the infant to be approximately 1 per cent of the total dietary calories. Minimal normal levels for polyenoic acids in the serum of the infant were found to be 10.5 ± 1.3, 2.7 ± 0.8, and 7.4 ± 2.4 per cent of the total fatty acids for di-, tri-, and tetranenoic acids, respectively. These values resulted when the dietary intake of linoleic acid constituted about 1 per cent of total dietary calories. Optimal levels for the di-, tri- and tetraenoic acids in the serum of the healthy infants appeared to be

23.7 ± 1.8, 0.6 ± 0.2, and 10.0 ± 1.1 per cent of the total fatty acids, respectively. These optimal values were attained in breast-fed infants in whom the linoleic acid intake was about 4 per cent of the total calories.

That EFA are definitely required by infants has more recently been indicated by Hansen et al. (32) who found that manifestations of the deficiency state disappeared promptly when linoleic acid was given as the ester or triglyceride or in a milk mixture providing 1 per cent or more of the calories as linoleic acid. Blood serum levels for dienoic acid of 5.6 ± 1.8 per cent of the total fatty acids were indicative of the deficiency state, whereas values of 12.9 ± 2.6 per cent of the total fatty acids represented the minimal normal values.

Holman, Caster and Wiese (33) have in the last few years introduced estimation equations relating dietary linoleate to tissue polyunsaturated fatty acids. Such estimation equations permit the assessment of EFA nutritive status. The exponential equations relating di-, tri- and tetraenoic acids to dietary linoleate were derived by computer methods which yielded constants from which the minimal nutrient requirement could be calculated. The first such equation derived by Holman was for two-to-four-month old infants and was found to be:

$$\text{Log}_{10} \text{ dietary linoleate} = -1.087 + 0.0432 \text{ (di - tri + tetra)}$$

Holman, Caster and Wiese (34) in relating linoleate intake to polyunsaturated fatty acid composition of serum lipids of young men developed comparable equations for the estimation of dietary linoleate of adults. When the quantity (di - tri + tetra) for total serum fatty acids was plotted against the logarithm of the dietary linoleic acid as per cent of total calories for each subject and each diet period, a linear relationship became apparent. This equation for the least squares straight line was also derived by computer and was found to be:

$$\text{Log}_{10} \text{ dietary linoleate} = -0.296 + 0.0243 \text{ (di - tri + tetra)}$$

In this same study similar relationships were derived for serum triglyceride and cholesterol ester fatty acids. However, Holman concluded that comparable relationships for serum total lipid fatty acids and dietary linoleate gave such high correlation between these variables that fractionation of serum lipids seems unjustified.

The relationships offered by Holman serve as a means of estimating dietary linoleate from serum analyses and could therefore serve as a useful crosscheck of dietary record estimates of linoleic acid intake. This could afford a more accurate evaluation of EFA status than dietary records from which calculation of per cent of total calories are obtained. One must realize, of course, the importance of an ultimate evaluation which would include actual food fatty acid composition analyses.

In 1958 the Food and Nutrition Board in the United States (35) suggested 1 per cent of total dietary calories as EFA to be the minimal allowance for humans. More recently, 1964, (36) the Food and Nutrition Board indicated that linoleic acid in the range of 1 to 3 per cent of total calories would probably meet the infant's requirement and perhaps adult needs, which are thought to be less than those of infants.

It was decided, therefore, in view of 1) the accumulated evidence with respect to climatically lowered thyroid activity in women living in the southeast section of Arizona, 2) the trace evidence in the Rodriguez study (17) indicating a possible metabolic stress due to difference in level of EFA intake, and 3) the paucity of information regarding optimal EFA allowances in the human, that the major objective of this investigation would be to characterize the serum fatty acid patterns of clinically healthy women living in southeastern Arizona. This would primarily provide a means for assessment of EFA status and secondarily serve as a possible step toward relating climatically-lowered thyroid activity to human EFA requirements.

Certain factors became apparent for investigation and evaluation; namely, climatological conditions and nutritional and/or health status. In order to accomplish these the following selections were necessary; namely 1) a period of the year which would provide a relatively constant and overall climatic setting for the subjects, 2) women subjects who would reflect similar activities and accurate dietary nutrient records, and 3) indices of clinical health status, biochemical lipid nutriture, fatty acid distribution, and thyroid activity.

CHAPTER II

PROCEDURE AND METHODS

The experimental design for this investigation was divided into phases with respect to time, technical and financial assistance necessary to its realization. Figure 1 demonstrates the phases.

Climatological

Daily climatological data including temperature (^{o}F.), precipitation totals (inches), relative humidity (per cent) and percentage of possible sunshine were obtained from the Solar Energy Laboratory at the University of Arizona and the U. S. Weather Bureau at Tucson International Airport for the ten-week period beginning September 1, 1964, and ending November 10, 1964.

Clinical

Seven volunteer, professionally-active women dietitians with an average age of 37.5 years (Range: 28.8 to 47.0), who had lived in Tucson at least one and one-half years were subjects for the ten-week study. All were apparently in good health. Routine laboratory blood and urine examinations were made prior to the beginning of the study by the technical staff at Tucson Medical Laboratories, Tucson, Arizona, to estimate the clinical health status of each subject. Selected blood

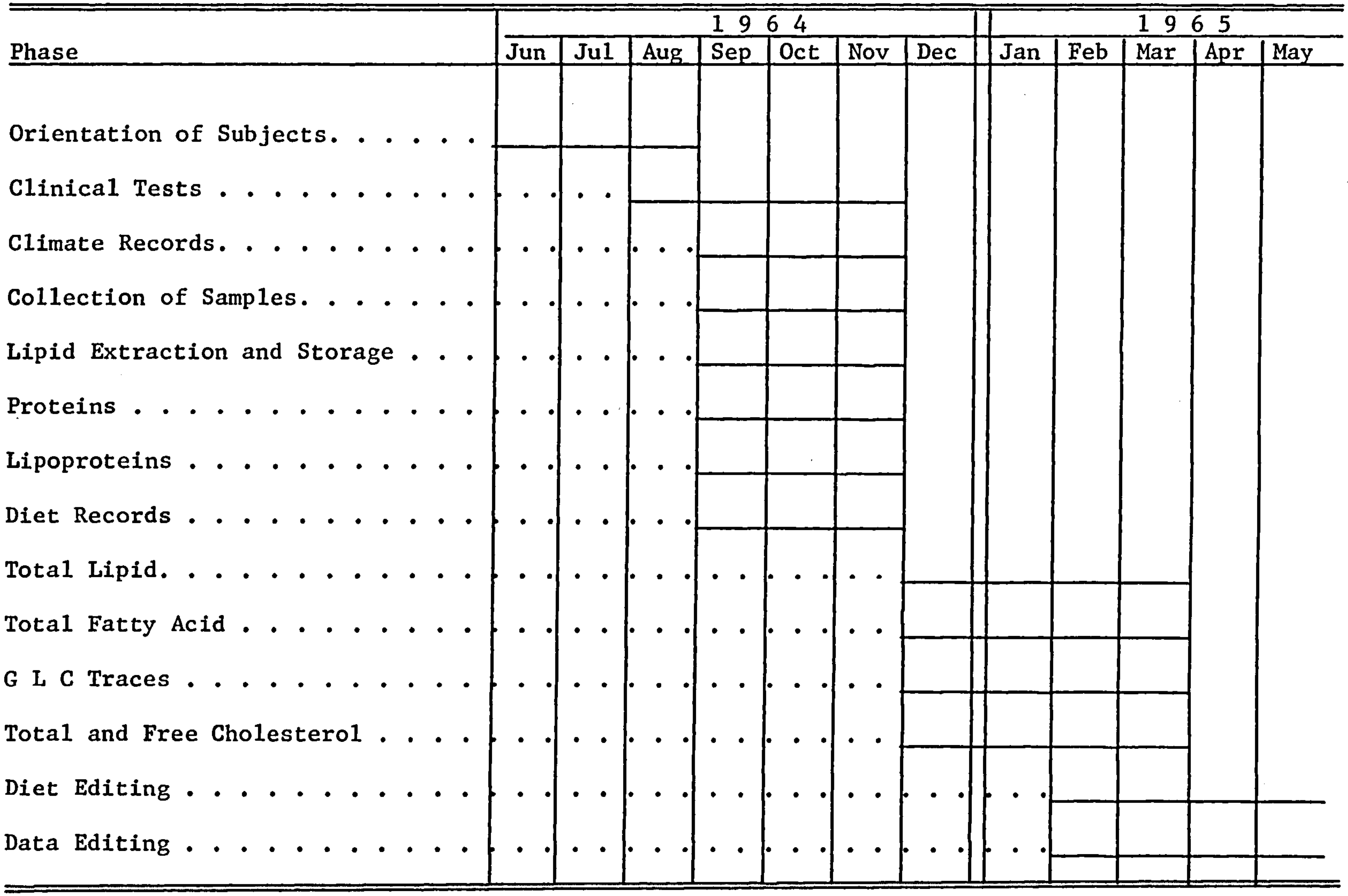

Figure 1. Scheme of Phases Involved in the Investigation

and urine analysis values were compared with normal values according to Davidsohn and Wells (37).

Following a preliminary orientation interview, additional clinical information was obtained from each subject in the human nutrition laboratory. Heights were taken without shoes and recorded to one-tenth of a centimeter as an average of two successive measurements which agreed within one-tenth of a centimeter. The standard stadiometer was used.

Weights were recorded weekly to one-tenth of a kilogram. Per cent standard weight for each subject was read directly from Table 7 in the Manual for Nutrition Surveys (38).

Blood pressures were measured using the standard blood pressure Baumanometer. The point of complete cessation of sound was used as the index of diastolic pressure. Readings were recorded as systolic/diastolic.

Since the study included measurements over a period of time equivalent to two or three menstrual cycles, the day of onset of menstruation was recorded as M day for each subject.

Continuous residence in Arizona prior to the study was recorded to the nearest one-half year. In addition, subjects were asked to maintain a personal logbook during the entire ten weeks of any factors which might affect the investigation.

Dietary

Two subjects, namely 5 and 7, consumed "controlled self-selected" diets consisting of three different daily self-selected dietaries which were estimated prior to the beginning of the study to maintain body weight ±five pounds. The three daily menus were rotated continuously throughout the ten weeks. The remaining five subjects consumed self-selected dietaries at daily levels estimated to maintain their individual weights ±five pounds.

All subjects recorded their individual dietaries during weeks three (September 15 to 21) and eight (October 20 to 26) of the experimental period. All diet records were evaluated on a seven-day basis for calories, protein, fat, carbohydrate, ash, calcium, phosphorus, iron, sodium, potassium, vitamin A, thiamine, riboflavin, niacin (equiv.), ascorbic acid, total saturated fatty acids, oleic, linoleic, and total cholesterol by means of the IBM 7072 computer and the 1963 revised Handbook No. 8, USDA (39).

The original program for evaluating dietary data (40) was revised for use on the 7072 computer. The revised program contained the compositions of approximately 2020 foods reported in Agriculture Handbook No. 8 on a 100-gram, edible-portion basis with corresponding values for selected fatty acids. The recommended daily dietary allowance information corresponded to values given in the Recommended Dietary Allowances, sixth edition revised, 1964 (36).

The new program provided for analysis of a diet for 1-99 days or up to 100 subjects and produced a list of average daily nutrient intake, recommended allowances for the corresponding age, height, weight, and sex, and excess or deficit from the recommended values.

The entire card deck (approximately 3,000 cards) included instructions for punching data and control cards, master food cards and daily allowance values, operating instructions, and program requiring Fortran II compiler, a computer of 10K words of storage with six tape drives which can be modified for other computers with very few changes.*

Biochemical

Blood specimens (20 to 30 ml) were obtained weekly by venipuncture at Tucson Medical Laboratories after an overnight fast. Serum was the preferred sample and was separated from the clot within two hours. Total lipid extraction and lipoprotein assays were initiated in the nutrition laboratory within five hours after collecting the sample. A time delay was necessary for protein fractionation, therefore appropriate aliquots were refrigerated at $3 \pm 1^{\circ}$ C. Any serum remaining after twenty-four hours was preserved at a maximum of 0° C. The latter samples were preserved for investigation of interests which might develop during the progress of the study.

Total Protein -- Concentration of each serum sample was measured weekly by refractometry with the American Optical** TS meter (a Goldberg refractometer) Model 10401. Duplicate 0.070 ml aliquots of serum were applied to the instrument and total protein concentration recorded in grams of protein per 100 ml of serum.

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* Numerical Analysis Laboratory, The University of Arizona, Tucson.

**American Optical Co., Instrument Division, Buffalo 15, New York.

Protein Components -- Relative concentration of five serum protein fractions were measured weekly with the Spinco Model R Paper Electrophoresis System (41). Duplicate 0.006 ml aliquots of serum were applied to filter paper strips in Spinco-Durrum Cells. Subjects' electrophoretic samples were always applied in the same order to correct for effects related to positioning of the strips in the cell. Cells were appropriately filled with barbital buffer, pH8.6 and ionic strength 0.075, totally sealed with plastic tape and run at room temperature for about eighteen hours at 2.5 ma. per cell. The electrophoretically-developed strips were dried for thirty minutes between 120 and 130° C., stained for thirty minutes in 0.1 per cent bromphenol blue in methanol, rinsed in methyl alcohol for ten minutes followed by rinses for six-minute periods in three separate 5 per cent aqueous acetic acid solutions, blotted, and dried at 120 to 130° C. again for fifteen minutes.

Strips were finally exposed in a sealed desiccator to ammonia vapor for fifteen minutes and scanned immediately with an Analytrol Model RB densitometer-integrator system using a 500 millimicron interference filter and B-5 balancing cam. Relative concentrations of each fraction were evaluated as percentage of the total area obtained from the densitometric tracings.

Total Lipids -- Quantitative determination of total serum lipids was accomplished by extracting the lipids from the serum according to the method of Lis (42), evaporating off the solvent from a 2-ml aliquot of the extract under nitrogen and weighing the lipid residue on a Mettler B-5 Analytical Balance. Total lipid concentrations were recorded in milligrams of lipid per 100 ml of serum.

Lipoprotein Components -- Relative concentration of five serum lipoprotein fractions were also resolved weekly using the Spinco Model R Paper Electrophoresis System. Duplicate 0.020 ml aliquots of serum were applied to filter paper strips in Spinco-Durrum Cells. Cells were filled with buffer under conditions identical to the protein fractionation procedure and run for about eighteen hours at 4.0 ma. per cell at $3 \pm 1^{\circ}$ C. The strips were dried at 100 to 110° C. for seven minutes and then stained using the procedure of Straus and Wurm (43). This method has consistently given precise correlation in this laboratory between the lipid fraction and its corresponding protein fraction. A saturated solution of the stain was prepared with 0.5 gm of fat red 7B (Ciba)* stain in absolute ethyl alcohol diluted to 1000 ml. This solution was allowed to stand forty-eight hours, filtered, and diluted with 667 ml redistilled water, capped and allowed to stand an additional twenty-four hours. Finally the stain solution was filtered twice before use. The strips were immersed in the stain solution for one hour at room temperature, then transferred to a decolorizing bath consisting of 0.1 per cent Clorox in 2 per cent aqueous acetic acid (the active ingredient in Clorox being reported on the label as sodium hypochlorite, 5.25 per cent) for one minute, fifteen seconds. Strips were then washed fifteen minutes each in four different 2 per cent

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*Ciba Pharmaceuticals, Fair Lawns, New Jersey.

aqueous acetic acid baths and were finally blotted and air dried. When completely dry, these strips were scanned with the Analytrol Model RB densitometer-integrator system. Relative fraction concentrations were evaluated as percentage of the total area obtained from the densitometric tracings.

Total and Free Cholesterol -- Serum was analyzed weekly for total and free cholesterol by the micro-determination of Galloway et al. (44). The procedure involved digitonide precipitation and the characterization of cholesterol by molar absorptivity with the Liebermann-Burchard reaction. Samples were read using a Beckman DU Spectrophotometer and cholesterol concentrations recorded in milligrams per 100 ml of serum.

Total Fatty Acids -- Determination of total serum fatty acids was accomplished gravimetrically following saponification of the 2 ml aliquot of total lipid extract, acid extraction and purification of the fatty acids as indicated below. These samples were also dried under nitrogen and weighed using the Mettler B-5 Analytical Balance. Total fatty acid concentrations were recorded in milligrams per 100 ml of serum. A single tared "control" flask was used for both total lipid and total fatty acid weighings.

Total Lipid Fatty Acids -- The method of Lis (42) as indicated previously was modified in this laboratory for extraction of total lipid. Aliquots of 3.0 ml of serum were pipetted a drop at a time into erlenmeyer flasks containing 30 ml of 95 per cent ethanol and 0.1 ml of 0.1 per cent hydroquinone in ethanol. These were incubated in a water bath shaker at 60° C. for one hour. The supernatant was then decanted through Whatman #40 filter paper into semi-micro Soxhlet flasks. A second extraction followed with 30 ml Bloor's reagent (95 per cent ethanol: ethyl ether, 3:1).

The precipitate was then transferred quantitatively into filter paper and extracted eighteen hours with ethyl ether in a semi-micro Soxhlet apparatus. The final extract was evaporated to near dryness and the lipid recovered immediately in redistilled Skelly-solve F, transferred quantitatively to heavy-walled glass tubes and stored at 0° C.

For further analyses, extracts were removed from the freezer, centrifuged at 3000 rpm for 30 minutes and transferred to graduated glass-stoppered centrifuge tubes. The precipitate was washed three times with redistilled Skelly-solve F and the washes also transferred to the centrifuge tubes. The extract was diluted with redistilled Skelly-solve F to a final 10-ml volume, and two-ml aliquots were used for determination of total lipid, total fatty acids and total lipid fatty acids. Samples were saponified using the Lis (42) procedure. Three ml of redistilled absolute ethanol were added to the total lipid material followed by the addition of 0.3 ml of 33 per cent KOH (made fresh daily). The flasks were covered with glass marbles and placed in a 37° C. water bath shaker for thirty minutes, cooled and 10 ml redistilled Skelly-solve F and 3 ml water added. The flasks were capped and left overnight.

The alkaline aqueous phase of the saponification mixture was acidified with 0.3 ml of 10N sulfuric acid using methyl orange indicator. Ten ml of redistilled Skelly-solve F was added, the flasks capped and again left overnight. The acid phase was decanted together with 2 or 3 Skelly-solve F washes into 25 ml erlenmeyer flasks. The acid phase solvent was evaporated under nitrogen and fatty acid weights taken.

The fatty acids were then methylated using the method of Michaels (45) by immediately redissolving them in 10 ml of 1 per cent sulfuric acid-methanol, capping with a marble and allowing to reflux at 60^{o} C. for one hour in a water bath shaker. They were then evaporated to approximately 1 ml in a 45^{o} C. sand bath under nitrogen. Four ml of water and 10 ml of redistilled Skelly-solve F were added, flasks stoppered and left overnight.

The following day the ether phase was decanted into 15 ml glass vials together with 2 or 3 rinses of redistilled Skelly-solve F and evaporated to near dryness under nitrogen. The methyl ester residue was immediately dissolved in redistilled Skelly-solve F and the entire 35 to 40 microliters injected into a Wilkins Aerograph Model A-90-P gas chromatograph. Columns used for the separation of the fatty acid methyl esters were 20 per cent diethylene glycol succinate (DEGS) on 60/80 chromosorb W, hexamethyldisilazane (HMDS) treated. Fatty acids separated and detected by the A-90-P were recorded using the Speedomax H Leeds Northrup recorder. The method of choice for percentage composition calculations was triangulation or the multiplication of peak height by one-half the base width. Fatty acids were identified by comparison with known fatty acid* mixtures adjusted to proportions similar to those found in the unknown samples rather than mixtures containing equal proportions of all fatty acids. Relative concentrations of each of eight measurable fatty acid fractions were evaluated as percentage of the total area obtained from the recorded tracings.

Thyroid Activity -- PBI was determined on duplicate 1 ml serum samples with the dry ash method of Barker, Humphrey and Soley (46). The procedure involved essentially the precipitation of proteins along with the bound iodine, washing the precipitate free of inorganic iodide, ashing in a thermostatically controlled furnace, and quantitative analysis of iodide by evaluation of the catalytic effect of this ion on the reduction of colored ceric ion to colorless cerous ion, in the presence of arsenious acid. PBI values for Subjects 1 through 7 were evaluated as micrograms per 100 ml of serum.

PBI values for all subjects were confirmed by Tucson Medical Laboratories along with the routine clinical blood and urine tests at the end of the study. Thyrobinding Index (TBI) and Basal Metabolic Rates (BMR's) were obtained for five of the seven subjects as a further evaluation of thyroid activity.

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*Nutritional Biochemicals, Cleveland, Ohio.

CHAPTER III

RESULTS AND DISCUSSION

Climatological

Table 1 of the Appendix shows selected climatological data for the ten-week investigation. A period of the year was selected to represent as closely as possible the annual overall climatic conditions of the southeast section of Arizona; more specifically, Tucson. This approach hopefully would set the environmental stage for evaluation of the characteristic nutritional status, including serum fatty acid patterns, of individuals living on the Sonoran desert.

Cross, Shaw and Scheifele (1) in a 1960 comparison of representative cities throughout the United States with regard to climate report representative annual conditions of temperature, normal precipitation, 11:00 a.m. relative humidity and percentage of possible sunshine for Tucson to be 67.5, 10.36, 30 and 86, respectively. Upon examining the recordings more closely, September, October, and November were selected as appropriate for our studies with percentage of possible sunshine and relative humidity values at 90, 90, 90 and 29, 30, 29, respectively.

The actual 1964 temperature, noon relative humidity and percentage of possible sunshine values showed yearly means to be 66.8, 39 and 84, respectively. Normal precipitation totaled 12.42 inches for the year.

The mean temperature for the ten weeks of data collection was 73 with mean values of 78, 72 and 61° F. for September, October and the ten days in November.

Noon relative humidity for the ten weeks of the study averaged 41 per cent with mean values of 49, 35 and 32 for September, October and the ten days in November.

Average percentage of possible sunshine recorded during the study was 81 with values for September, October and November being 70, 89 and 88. Total precipitation for the ten weeks was 2.77 inches. The average barometric pressure (inches) for September was 27.289, for October 27.352, and November 27.382 at an average station elevation of 2,555 feet.

It becomes apparent that the ten-week period of sample collection beginning September 1, 1964, and ending November 10, 1964, did provide a relatively constant and characteristic overall climatic setting for the subjects being observed.

Clinical

Fasting blood and urine findings for each of the women subjects are given in Table 2 of the Appendix. The usual findings, used for comparison, are based primarily on data from the Department of Pathology, Mount Sinai Hospital, Chicago, Illinois (37). All standard blood and urine findings are given for the fasting state. In general, the subjects grouped within the usual limits and gave evidence of no gross pathology which might be reflected in abnormal fatty acid patterns. Any slight deviations such as those observed in the white blood cell differential count for Subjects 1, 3, and 4 might be explained by the fact that actual values may vary in different laboratories and with different techniques.

Additional clinical data for each subject are summarized in Table 3 of the Appendix. Age in years ranged from 28 to 47. Height-weight measurements when referred to the Society of Actuaries Standard weight groups (38) gave frequencies of three for the 90-94 per cent group, two for the 95-99 per cent group, one for the 105-109 per cent group, and a frequency of one for the 120-124 per cent group. The latter subject was beginning to enter obesity with a per cent standard weight of 120-124. However, this subject did maintain body weight at ± 5 pounds during the study. Two blood groups were represented by the subjects namely A and O with frequencies of three and four, respectively.

Table 3.1 presents the Lasser and Master (47) statistical analysis of blood pressure measurements in apparently healthy women which suggest a gradual rise in systolic pressure and a smaller rise in diastolic pressure with age. Blood pressures of the women dietitians as a group do not demonstrate such an increase with age (Table 3.1). However, the standard gradual increase with age with respect to difference between systolic and diastolic pressures was observed in the test subjects (correlation 0.96).

Table 3.1

MEAN BLOOD PRESSURE MEASUREMENTS IN APPARENTLY HEALTHY WOMEN, 25 TO 49 YEARS OF AGE

		Lasser Standard Females*		Actual Females		
Subject	Age Group	Systolic (mm)	Diastolic (mm)	Systolic (mm)	Diastolic (mm)	Difference (mm)
1	25 - 29	117 ± 11.4	74 ± 9.1	120	88	32
2	30 - 34	120 ± 14.0	75 ± 10.8	112	78	34
3	35 - 39	124 ± 13.9	78 ± 10.0	120	78	42
4	35 - 39	124 ± 13.9	78 ± 10.0	115	70	45
5	40 - 44	127 ± 17.1	80 ± 10.6	122	78	44
6	40 - 44	127 ± 17.1	80 ± 10.6	115	70	45
7	45 - 49	131 ± 19.5	82 ± 11.6	122	70	52

*Lasser and Master (47)

M days were in general regular in six of the subjects. Subject 2 was so extremely irregular that no attempt was made to record exact dates of onset of menstruation. Continuous time lived in Arizona prior to the study ranged from 1½ to 29 years.

Blood and urine findings within the usual acceptable limits were confirmed for each subject at the end of the study.

Dietary

Table 4 of the Appendix presents the final computer dietary evaluations for the third and eighth weeks of the study. The evaluations are given for each individual subject and represent a fourteen-day food item intake record. Morgan (48) concluded with respect to reliability of dietary records that "the one-day recall could be used to determine the characteristics of food use of groups but that a fourteen-day record was needed for a fair estimate of the intake of an individual."

All subjects maintained initial body weights ± 5 pounds during the ten week observation, indicating that total caloric intake was reasonably balanced with body expenditures. Total caloric intake, in per cent of the National Recommended Allowances (NRA), showed the following comparison with per cent standard weight (Table 4.1).

According to Goldsmith (49) the general nutritional status of the United States population shows a high incidence of caloric over-nutrition. Women are found to be either overweight or obese. Proudfit and Robinson (50) define a weight of 20 per cent or greater than

Table 4.1

PER CENT NRA CALORIES AND PER CENT STANDARD WEIGHT

Subject No.	Calories Per cent of NRA	Weight Per cent of Standard
1	133	95 - 99
2	102	120 - 124
3	101	90 - 94
4	94	90 - 94
5	99	105 - 109
6	84	95 - 99
7	99	90 - 94
Mean	101	97 - 101

desirable weight as indicating obesity. Overweight is the status between 10 and 20 per cent. In view of this, subject two was apparently consuming the necessary calories for maintenance. Subject 1, despite the 133 per cent of NRA intake, was apparently able to utilize the excess calories and thus maintain a desirable weight.

As a group, the mean values suggest desirable caloric intake levels and desirable weights. A balance of these two is preferred if we are to describe the subjects as clinically healthy. It is of interest that the subjects consuming slightly under 100 per cent NRA calories also fluctuated slightly under 100 per cent standard weight.

According to Stitt (51) in recent years there has been an increase in the proportion of food calories from fat (38 to 44 per cent), a slight increase in calories from protein (12 to 13 per cent) and a decrease in calories from carbohydrate foods (51 to 43 per cent). The subjects in this study conformed to this national trend (Table 4.2).

Table 4.2

CALORIES FROM FAT, PROTEIN AND CARBOHYDRATE

Subject No.	Per cent Fat Calories	Per cent Linoleic Calories	P:S* Ratio	Per cent Protein Calories	Per cent Carbohydrate Calories
1	45.33	1.36	0.19	15.80	36.69
2	41.14	2.82	0.35	13.42	44.90
3	45.01	4.51	0.62	13.96	41.29
4	39.59	2.50	0.35	13.73	48.77
5	40.17	3.76	0.97	12.76	48.03
6	40.26	2.87	0.30	17.93	43.19
7	41.79	5.64	1.17	12.26	48.05
Mean	41.89	3.35	0.56	14.26	44.41

*Polyunsaturated fatty acids:Saturated fatty acids

The proportion of total calories derived from fat in the diets of the test subjects was 39 to 45 per cent and linoleic acid accounted for 1 to 6 per cent of total calories. The calories from protein amounted to 12 to 17 per cent while per cent of calories from carbohydrate foods was 36 to 48 per cent. Therefore, the dietary pattern of the women subjects appeared to conform to the typical American diet with respect to total caloric intake distribution pattern.

For nutrients other than calories the Interdepartmental Committee on Nutrition for National Defense (ICNND) has published a "Suggested Guide to Interpretation of Nutrient Intake Data in 1957" (52). The second edition of the guide for reference man was published in 1963 (38) and is given in Table 4.3.

Table 4.3

SUGGESTED GUIDE TO INTERPRETATION OF NUTRIENT INTAKE DATA*

Nutrient	Low	Acceptable	High
Protein, gm/kg body weight	0.5-0.9	1.0-1.4	1.5+
Calcium, gm/day	0.30-0.39	0.4-0.7	0.8+
Iron, mg/day	6-8	9-11	12+
Vitamin A, IU/day	2000-3499	3500-4999	5000+
Ascorbic Acid, mg/day	10-29	30-49	50+
Thiamine, mg/1000 calories	0.20-0.29	0.3-0.4	0.5+
Riboflavin, mg/day	0.7-1.1	1.2-1.4	1.5+
Niacin, mg/day	5-9	10-14	15+

*ICNND (38)

Krehl and Hodges (53) point up that in the interpretation of nutrient intake data it is within reason to suggest that intakes less than the NRA may not be adequate for the continued maintenance of good health. Therefore the suggested ICNND guide terms of low, acceptable and high were used in combination with a per cent of NRA standard calculation to interpret the total daily nutrient intakes of our women subjects (Table 4.4). This approach has advantages over a single recommended intake or allowance value since it provides a means of evaluating the relative severity of nutrient problems which may be encountered on an individual basis. Also the use of percentage ranges of intake more accurately reflects our current knowledge and implies less finality than an absolute figure.

With regard to the word "high" this term is used in the sense of high for the prevention of recognizable clinical or well defined biochemical evidence of deficiency. The word "low" is also used in the sense of low for the prevention of recognizable clinical or biochemical evidence of deficiency. The entire interpretation is a provisional definition of an "adequate" diet. To attempt a more scientific appraisal of nutrient intake than has been accomplished here would necessitate actual food composite analyses which would include a sampling of the water consumed by each subject for calcium and iron.

In general, the self-selected diets were well balanced and, with few exceptions, met the recommended daily dietary allowances set forth by the National Research Council. The diet of subject 4 was somewhat low in thiamine by 0.15 mg per day. The diets of subjects 4 and 7 were low in calcium by 108 and 114 mg, respectively; and the diets of all, except for subject 1, were low in iron.

Table 4.4

SUGGESTED GUIDE* TO INTERPRETATION OF NUTRIENT INTAKE DATA USING PER CENT OF NRA VALUES**

	Subjects						
Nutrient	1	2	3	4	5	6	7
Protein	190	124	116	106	104	124	100
Calcium	130	94	110	86	106	100	86
Iron	112	77	73	52	58	73	67
Vitamin A	114	145	178	114	134	176	88
Ascorbic Acid	266	253	176	94	135	148	149
Thiamine	158	146	134	81	114	126	141
Riboflavin	158	116	150	112	130	140	113
Niacin	274	186	185	154	162	194	165
Calories	133	102	101	94	99	84	99

* Guide: less than 90% of NRA = low
90 to 124% of NRA = acceptable
125% or more of NRA = high

**Per cent of NRA Values:

$$\frac{\text{Mean Daily Nutritive Value (Table 4, Appendix)}}{\text{Mean Daily Recommended Allowance (Table 4, Appendix)}}$$

Rodriguez (17) reported two of three subjects in a pilot self-selected dietary study to be below the recommended allowances in iron, by 9 and 11 mg. In addition, Rodriguez reported a dietary calcium deficit in one subject to be 549 mg. Iron deficits for the six subjects in this study were 3, 4, 7, 6, 4, and 4 mg, respectively.
Goldsmith (49) in a review of the clinical nutritional problems in the United States indicated calcium and iron to be among the nutrients which are most often consumed in amounts less than those recommended.

The Food and Nutrition Board of the National Research Council (36) suggests that linoleic acid in the range of 1 to 3 per cent of total calories appears to meet the requirements of adults for this fatty acid. Therefore, the 1 to 6 per cent of total calories from linoleic acid observed in this study suggests no deficit in "dietary essential fatty acids" (54). Cholesterol intakes ranged from 185 to 518 milligrams daily (Appendix, Table 4).

Biochemical

Fatty acids are known to combine with certain protein and lipid components in blood as a means of transport. Therefore, in order to more adequately characterize the serum fatty acid patterns observed during this investigation, certain protein and lipid blood serum components were determined as additional defining parameters of the fatty acid status of the subjects.

Total Protein -- Weekly mean total protein concentrations were determined (Appendix, Table 5) and were found to remain constant within acceptable limits of 6.0 to 7.8 gm/100 ml of serum (37) for each subject during the entire study. Physiological variability for the subjects is presented in Table 5.1.

The lack of variability in the pooled control serum reflects the reproducibility usually observed in this laboratory for the American Optical protometer. Appropriate aliquots of this same lot of pooled human serum were carried through the subsequent biochemical analyses as the "Pooled Control Serum" to assess the accuracy and reproducibility (55) of other methods employed.

Variability in the subjects and the pooled control serum is expressed as Standard Deviation (S.D.), Standard Error (S.E.), and Coefficient of Variation (C.V.).

Table 5.1

VARIABILITY IN SERUM TOTAL PROTEIN

Subject	Weeks	N	Mean (gm)	S.D.	S.E.	C.V.
1	10	20	6.9	0.24	0.07	3
2	11	22	6.8	0.11	0.03	1
3	10	20	6.7	0.10	0.03	1
4	11	22	6.6	0.20	0.06	3
5	11	22	6.9	0.16	0.05	2
6	11	22	6.5	0.21	0.06	3
7	11	22	6.6	0.19	0.05	2
Pooled Control Serum	11	22	7.2	0.00	0.00	0

Protein Components -- The weekly means of five serum protein electrophoretic fractions; namely, albumin, alpha -1 globulin, alpha -2 globulin, beta globulin, and gamma globulin appear as percentage of total protein pattern in Table 6 of the Appendix.

Wall (56) reported the following average protein percentage pattern for normal adults: albumin 66 (60-70); alpha -1, 3 (2-5); alpha -2, 7 (5-10); beta, 9 (8-12); and gamma, 12 (10-15) per cent.

The serum protein fraction percentages for the seven subjects evaluated were fairly close to the above values. (Appendix, Table 6.1)

In all subjects, alpha -1 globulin, the smallest fraction, showed the greatest variability (27 to 33 per cent). The coefficients of variation of the subjects' sera were greater than that of the pooled control serum.

The albumin fraction was slightly low in all subjects with albumin: globulin (A:G) ratios for subjects 1 through 7 of 1.1, 1.0, 1.3, 1.0, 1.1, 1.3, and 1.0. The usual normal serum A:G ratio values are 1.1 to 1.9 (37). The lowest values of 1.0 for subjects 2, 4 and 7 are probably not critical in view of the variability encountered with the pooled control serum. The pooled control serum revealed a ratio of 1.4.

Summarized in terms of 1) the normal range of values, 2) expected variations in the configuration of patterns, 3) an awareness of pathophysiology involved in various disease states, and 4) a clinical history of the test subjects, the serum protein patterns observed during the study show no impressive clinical abnormalities.

Total Lipids -- Weekly mean total lipid values for the subjects are expressed as mg/100 ml serum in Table 7 of the Appendix. Variations for the subjects were large and are presented in Table 7.1.

Table 7.1

VARIABILITY IN SERUM TOTAL LIPID

Subject	Weeks	N	Mean (mg)	S.D.	S.E.	C.V.
1	9	18	501.8	156.76	52.25	31
2	10	20	428.3	120.98	38.25	28
3	10	20	553.5	172.40	54.51	31
4	11	22	474.2	200.88	60.57	42
5	11	22	550.0	144.37	43.53	26
6	11	22	662.0	144.61	43.60	21
7	11	22	542.4	167.68	50.56	30
Pooled Control Serum	11	22	703.0	17.96	5.41	2

The above mean values for all subjects were between 360 to 765 mg/100 ml serum and were considered to represent clinically normal serum total lipid values (37).

Wurm, Kotsitchek and Straus (57) report a mean total lipid value for 38 apparently healthy subjects to be as high as 946 $\pm$ 255. This same author reports a mean value for 35 abnormal (atherosclerotic) subjects to be 1066 $\pm$ 384. The group mean for the seven Arizona subjects was 530.

Lipoprotein Components -- About 95 per cent of the total serum lipids exist in the form of the several lipoproteins. Of particular concern is that in disease states such as coronary artery disease (58, 59) and in states of thyroid disfunction (60) total lipid and certain of the lipoprotein fractions may become specifically elevated. Therefore, it would seem justified to characterize the lipoprotein patterns although values within acceptable normal limits for the subjects would be expected.

The lipoprotein patterns obtained are given in Table 8 of the Appendix. Variations in lipoprotein estimations are summarized in Table 8.1 of the Appendix.

Table 8.2 presents for comparison lipoprotein normal control and abnormal group values of Wurm, Kotsitchek and Straus (57) for the five lipoprotein fractions visualized with fat red 7B stain in the test female subjects.

Table 8.2

LIPOPROTEIN VALUES IN SYMPTOMLESS CONTROLS AND ABNORMAL ATHEROSCLEROTIC PATIENTS*

Lipoprotein Fraction	Group: Controls Mean (%)	S.D.	Abnormals Mean (%)	S.D.
Lipalbumin	19.8	5.86	13.2	3.96
Alpha -1	4.67	2.07	3.80	1.43
Alpha -2	7.06	2.35	5.07	1.86
Beta	52.5	9.20	62.5	8.84
Gamma + neutral fat	15.5	4.56	15.4	6.78

*Wurm, Kotsitchek and Straus (57)

Of particular interest is the observation that in the abnormal patterns the beta-lipoprotein fraction increases at the expense of lipalbumin and the alpha-globulins. The beta-fraction values for the seven Arizona test subjects were 36.1, 39.3, 44.4, 40.6, 48.3, 47.1 and 46.4 per cent, respectively, in all cases below the standard group control mean reported by Wurm et al. (57).

The data dealing with serum lipoproteins, separated by electrophoresis, reveal visible normal patterns.

Total and Free Cholesterol -- For routine clinical use the preceding lipoprotein procedures are almost unavailable. More frequently cholesterol determinations are made to indicate a normal or abnormal status with respect to lipid metabolism. Because of the frequency of use the data for mean total serum cholesterol and free cholesterol are compiled in Tables 9 and 10 of the Appendix. Tables 9.1 and 10.1 summarize the variability in each subject for the cholesterol fractions.

The amounts of cholesterol observed in the sera from the seven subjects are similar to those frequently reported in the literature. Total cholesterol ranged from 147 to 233 mg/100 ml with the percentage in the free form ranging from 24 to 26 (Table 10.2). Mean total cholesterol concentration for the group was 172.

Hawthorne, Smith and Pescador (61) reported amounts of cholesterol in the sera of eight women 28 to 51 years of age to range from 152 to 244 mg/100 ml; the percentage in the free form varied from 25 to 32. Mean total cholesterol concentration was 195. These data are combined with the actual Arizona subjects data in Table 10.2. Subjects in both studies had a mean age of 37 years. Both laboratories used a micro-modification of the method of Sperry and Webb for cholesterol analysis.

Table 9.1

VARIABILITY IN SERUM TOTAL CHOLESTEROL

Subject	Weeks	N	Mean (mg)	S.D.	S.E.	C.V.
1	10	20	147.8	13.69	4.33	9
2	11	22	150.6	20.50	6.18	13
3	10	20	161.2	14.20	4.49	8
4	11	22	155.2	16.79	5.06	10
5	11	22	178.1	30.02	9.05	16
6	11	22	233.7	29.36	8.85	12
7	11	22	185.8	20.71	6.24	11
Pooled Control Serum	11	22	212.1	18.36	5.53	8

Table 10.1

VARIABILITY IN SERUM FREE CHOLESTEROL

Subject	Weeks	N	Mean (mg)	S.D.	S.E.	C.V.
1	10	20	37.4	6.89	2.17	18
2	11	22	38.0	5.24	1.58	13
3	10	20	40.2	6.43	2.03	15
4	11	22	41.4	8.75	2.64	21
5	11	22	44.2	7.53	2.27	17
6	11	22	58.3	7.49	2.25	12
7	11	22	47.5	5.53	1.66	11
Pooled Control Serum	11	22	52.4	8.67	2.61	16

Table 10.2

TOTAL AND FREE CHOLESTEROL IN APPARENTLY HEALTHY WOMEN 28 TO 51 YEARS OF AGE

	Serum Cholesterol		
Age (yrs)	Total (mg/100 ml)	Free (mg/100 ml)	Free:Total (%)
Hawthorne*			
28	210	51	25
32	200	55	27
33	152	48	32
33	209	62	30
40	244	69	28
40	158	48	30
44	169	48	28
51	218	54	25
Mean			
37	195	54	28
Actual			
28	147	37	25
31	150	38	25
36	161	40	24
38	155	41	26
40	178	44	24
43	233	58	24
47	185	47	25
Mean			
37	172	43	24

*Hawthorne, Smith and Pescador (61)

Davidsohn and Wells (37) give a range of 140 to 250 mg/100 ml serum for clinical normal total cholesterol values. The normal values for serum cholesterol esters are 65 to 75 per cent of the total cholesterol or normal free cholesterol values for serum of 25 to 35.

Expressed as per cent of total lipid, total and free cholesterol levels for the Arizona subjects exhibited a normal distribution (Table 10.3). These varied from 29 to 35 per cent for total cholesterol and from 7 to 8 per cent for the free cholesterol expressed as per cent of total serum lipids.

Bagchi, Halder and Chowdhury (62) report mean normal serum total cholesterol values for 45 "normal" subjects to be 156 mg/100 ml. Mean total lipid value for these subjects was 485 mg/100 ml of serum. This represents 32 per cent of total lipid as total cholesterol, confirmed by the Arizona results (Table 10.3).

Total Fatty Acids -- Lindgren et al. (63) approximate that two-thirds by weight of the total lipids of the blood are actually fatty acids. However, our results did not confirm this distribution. Values in all Arizona cases were lower and are given in Table 11.1. Weekly means for each subject are found in Table 11 of the Appendix.

It is recognized that definition of the total fatty acid values in terms of clinical norms is in need of clarification, especially in terms of methods and conditions used. The apparent low values observed in the immediate study might reflect a difference in procedures since Pernokis, Freeland and Kraus (64) have emphasized that values may be lower in some cases merely due to differences in lipid solvents used.

Table 10.3

DISTRIBUTION OF TOTAL LIPIDS IN THE TOTAL AND FREE CHOLESTEROL FRACTIONS

Subject	Total Lipid mg/100 ml	Total Cholesterol	Free Cholesterol
		Per cent of Total Lipid	
1	501	29	7
2	428	35	8
3	553	29	7
4	474	32	8
5	550	32	8
6	662	35	8
7	542	34	8
Mean	530	32	8
Pooled Control Serum	703	30	7

Table 11.1

DISTRIBUTION OF TOTAL LIPIDS IN THE TOTAL FATTY ACID FRACTION

Subject	Total Fatty Acids mg/100 ml	Total Fatty Acid
		Per cent of Total Lipid
1	137	27
2	111	25
3	171	30
4	138	29
5	163	29
6	227	34
7	154	28
Mean	157	29
Pooled Control Serum	302	42

Depending on the solvent, washing procedure, etc., nonlipid contaminants could contribute gravimetrically in varying degrees to the final lipid values.

Correlation between the total lipid values observed and the total fatty acids recovered from the total lipid extract in the present study was found to be 0.98.

White, Handler and Smith (65) give normal ranges for the total lipids and total fatty acids of the plasma of man to be 385 to 675 and 150 to 500 mg/100 ml, respectively. Thus the total fatty acid range as a percentage of total lipid is 38 to 74 per cent, with which our observed mean of 29 per cent (Range: 25 to 34) of total lipid value shows good agreement.

Bagchi, Halder and Chowdhury (62) report total fatty acid values of 329 $\pm$ 12.57 mg/100 ml for 45 normal subjects. This would confirm Lindgren's estimate with a 67 per cent of total lipid distribution. This work is of particular significance since lipid values were also reported for 61 subjects with the abnormal condition of phrynoderma, which the authors concluded is a manifestation of EFA deficiency in man.

Dorland's Illustrated Medical Dictionary (66) defines phrynoderma to mean a papular dry skin eruption, frequently accompanied by mild neuritic and eye symptoms, and seen in East Indian laborers fed a diet of maize meal. It is also called "toadskin."

The phrynoderma total lipid, total fatty acid and total cholesterol values reported were 422 $\pm$ 16.40, 282 $\pm$ 15.04, and 140 $\pm$ 6.52 mg/100 ml, respectively. All three means fall within usual limits and might, therefore, be of questionable significance.

The most significant difference between normal and phrynodermic subjects is found in the total dietary intake of essential fatty acid and the notable reduction in iodine number of the serum fatty acids of subjects with phrynoderma as compared with normal individuals. Daily intakes of linoleic acid for the normal subjects was 6.04 grams compared with 3.41 grams for those with phrynoderma. Our seven test subjects consumed linoleic acid in the amounts of 4.23, 6.75, 9.64, 5.01, 7.86, 5.11, and 11.84 grams daily (Mean: 7.20). This would suggest that at present the most meaningful criteria for EFA status would be a combination of dietary linoleic acid intake information and serum individual fatty acid content information. Variability in the subjects' serum total fatty acid levels was large and is presented in Table 11.2.

Total Lipid Fatty Acids -- Eight fatty acids were separated and detected by gas-liquid chromatography employing the thermoconductivity detector and were identified to be 12:0 (lauric), 14:0 (myristic), 16:0 palmitic), 16:1 (palmitoleic), 18:0 (stearic), 18:1 (oleic), 18:2 (linoleic), and 20:4 (arachidonic). Weekly means of the above fatty acids for each subject are expressed as percentage of total in Table 12 of the Appendix. Variability for the acids is shown in Table 12.1 (Appendix).

In six cases lauric, the smallest fraction, showed the greatest variability (50 to 61 per cent). Arachidonic was also detected in small

Table 11.2

VARIABILITY IN SERUM TOTAL FATTY ACIDS

Subject	Weeks	N	Mean (mg)	S.D.	S.E.	C.V.
1	9	18	137.2	73.08	24.36	53
2	10	20	111.8	79.79	25.23	71
3	10	20	171.7	76.09	24.06	44
4	11	22	138.0	67.11	20.23	48
5	11	22	163.6	79.58	23.99	48
6	11	22	227.2	70.09	21.13	30
7	11	22	154.5	71.61	21.59	46
Pooled Control Serum	11	22	302.9	43.81	13.21	14

amounts and showed large variation (25 to 51 per cent). The pooled control serum also showed a large variation for these two acids; namely 43 and 22 per cent, respectively. Palmitic, oleic, and linoleic were relatively constant in all cases, with linoleic, the largest fraction, being one of the least variable (7 to 10 per cent). The pooled control serum showed a C.V. of 4 for linoleic.

Fatty acid composition of serum for the Arizona group is presented in Table 12.2.

Tuna, Reckers and Frantz (67) reported mean per cent of total values for fatty acids in normal plasma to be: 14:0, 2.3; 16:0, 19.5; 16:1, 6.9; 18:0, 5.6; 18:1, 36.5; 18:2, 22.7; 18:3, trace; 20:0, 20:1 or 20:2, 0.3; and 20:4, 6.1. Other investigators report similar levels of fatty acids of human serum but have further separated the serum lipids into three major classes: the cholesterol esters, triglycerides

Table 12.2

FATTY ACID COMPOSITION OF SERUM IN SEVEN SOUTHEASTERN ARIZONA WOMEN

Acid	Mean (%)	Range (%)	S.D.	C.V.
12:0	0.47	(0.28 - 0.62)	0.13	27
14:0	1.21	(0.98 - 1.60)	0.19	15
16:0	20.68	(19.35 - 22.05)	1.03	4
16:1	3.92	(3.21 - 5.22)	0.62	15
18:0	3.94	(3.27 - 4.83)	0.53	13
18:1	26.37	(24.96 - 28.39)	1.22	4
18:2	39.39	(36.38 - 41.05)	1.47	3
20:4	4.22	(2.83 - 6.23)	1.03	24

and phospholipids. However, for the purposes of this work such fractionation was not considered as necessary, based on conclusions by Holman, Caster and Wiese (34, Chapter I). Lawrie et al. (68) reported the detection of 12:0 in the amount of 1.3 per cent by weight of the total fatty acids and determined this to occur in the triglyceride fraction.

In a more recent report by Tuna, Logothetis and Kammereck (69) 94 per cent of the detected serum total fatty acids in eleven normal subjects was found to be lauric, myristic, palmitic, palmitoleic, stearic, oleic, linoleic, and arachidonic. Their distribution is given in Table 12.3.

No serious attempt can be made at this time to compare values from different laboratories with regard to fatty acid values, since reports in the literature represent many different methods for separation, detection and quantification.

Table 12.3

FATTY ACID COMPOSITION OF SERUM IN ELEVEN NORMAL SUBJECTS*

Acid	Mean (%)	Range (%)	S.D.	C.V.
12:0	0.37	(0.20 - 0.91)	0.29	78
14:0	1.65	(1.03 - 2.88)	0.49	29
16:0	23.91	(22.0 -- 26.7)	1.42	5
16:1	5.04	(3.0 - 8.04)	2.0	39
18:0	6.83	(0.60 - 8.21)	0.71	10
18:1	25.82	(21.41 - 32.45)	3.51	13
18:2	24.39	(18.9 - 28.2)	4.9	20
20:4	6.04	(3.81 - 7.80)	1.43	23

*Tuna, Logothetis and Kammereck (69)

It is of interest, however, to compare the dissimilarities in relative size of the different fatty acid fractions reported by Tuna and found in our studies. The ratio of oleic to linoleic (O:L) is dissimilar in that Tuna found oleic as the major fraction with a mean O:L ratio of 1.05. We found linoleic as the major fraction with a mean O:L ratio of 0.66. This more closely resembles the ratio of 0.61 revealed by the cholesterol ester fraction in Lawrie's work (68).

The fatty acid percentages of the Arizona group appear to represent those for healthy human sera; therefore, it may be suggested that the observed patterns can serve as future clinical normal values.

Thyroid Activity

TBI and BMR's were obtained clinically for subjects 2, 4, 5, 6, and 7. Table 13 presents the combined PBI, TBI, and BMR data for the subjects.

Table 13

PBI, TBI AND BMR VALUES

Item	Usual Findings	Subjects 1	2	3	4	5	6	7
PBI*	4.0-8.0 mcg%	5.1	4.5	6.9	5.4	5.7	4.7	5.9
TBI*	0.86-1.20	--	1.08	--	1.01	1.06	1.00	1.05
BMR**	±10-15%	--	-2	--	-19	-17	-9	-9

* Davidsohn and Wells (37)
**Boothby, Berkson and Dunn (70)

All subjects appear to be within acceptable limits of thyroid activity for good health under desert or semi-desert conditions. The persistency of basal metabolisms in women within the negative area of prediction standards might be designated as the "subtle" type of hypothyroidism discussed by Danowski (71). These negative findings appear to be of no immediate concern with respect to the fatty acid status of our subjects. There is every likelihood that less than obvious degrees of hypothyroidism exist in other population groups, and that such a degree of lowering of metabolic activity would require innumerable years to produce an effect unfavorable to health.

It is noteworthy that Lawrie et al. (72) reported fatty acid patterns of the serum lipid fractions in diagnosed hypothyroidism to increase in saturation. The increase appeared with acids 16:0, 16:1 and 18:0. In addition there was a rise in serum cholesterol and a shift towards saturation of the fatty acids bound to it. The high correlation (0.87) between cholesterol ester and each of the two polyunsaturated acids detected in the present investigation, therefore, suggests no serious hypothyroid effect with respect to the fatty acid profile of our subjects.

Correlations were obtained for 1) dietary cholesterol, linoleate, P:S ratio, protein or carbohydrate and serum lipids, 2) age, per cent standard weight or PBI and serum lipids, 3) serum total protein or A:G ratio and serum lipids, 4) serum lipids, and 5) dietary linoleic acid levels of intake and PBI, TBI or BMR.

No significant* correlations were observed between daily cholesterol intake (185 to 518 milligrams), linoleate (absolute or per cent of total dietary calories or per cent of basal metabolism calories) or P:S ratio and serum levels for total cholesterol, cholesterol esters, free cholesterol, total lipid, total fatty acids, linoleic, arachidonic, linoleic plus arachidonic, P:S ratio or for beta lipoprotein levels.

Levels of linoleic acid dietary intake did show an increasing trend within the subjects in the order of 4.23, 5.01, 5.11, 6.75, 7.86, 9.64 and 11.84 grams per day which represented 1 to 6 per cent of total calories. Nevertheless serum linoleic acid did not respond with increased intake of this acid. This evidence may or may not concur with the hypothesis that homeostatic mechanisms maintain serum linoleic acid at maximal range levels in response to adequate dietary linoleic acid intakes. It would appear that all levels of linoleic acid intake were sufficient to maintain serum linoleic acid at high levels.

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*Significant at the .02 level of confidence or better.

Subject number one, who consumed the least amount of linoleic acid (4.23 grams or 1.36 per cent of total calories), maintained serum linoleic acid levels at 54.88 mg or 40.06 per cent of total fatty acids. Subject number seven consumed the greatest amount of linoleic (11.84 grams or 5.64 per cent of total calories) and maintained serum levels at 60.10 mg or 38.89 per cent of total serum fatty acids. The highest absolute serum linoleic acid level was obtained in subject number 6. Subject number 4 had the highest percentage of total fatty acids as linoleic; namely, 41.05 per cent. Subjects 4 and 6 consumed linoleic acid in the average daily amount of 5.01 and 5.11 grams, respectively.

The interrelationships between dietary fat and serum lipids have been studied extensively for a number of years and have resulted in no particular agreement. Mead (73) in a review of the present knowledge of dietary fat emphasizes that the optimal amount of fat in the diet cannot be stated with exactness, and that wide variations can be compatible with health. He indicates that the type of fat consumed may be the most important factor; since in many studies carried out with both human subjects and experimental animals, investigators have found that higher proportions of polyunsaturated fatty acids in the dietary fat are associated with, for example, lower serum cholesterol levels. It is suggested that a 15 per cent reduction in serum cholesterol can be achieved by an increase in the dietary P:S ratio from the usual 0.4 to at least 1.1. The Arizona subjects in general reflected usual P:S ratios (Table 4.2) with the exception of subject number 7 who demonstrated a ratio of 1.1. The absence of correlation between dietary

P:S ratios and serum lipids including cholesterol in the immediate study would indicate questionable meaning for the suggested effectiveness of dietary P:S ratios.

It is of course apparent that no extreme experimental dietary conditions existed in the immediate study and that this alone would contribute in some measure to the lack of significance in the correlations observed. It does appear that the range of dietary P:S ratios observed were effective in maintaining non-elevated serum cholesterol levels.

No significant correlations were expected or observed between dietary protein or carbohydrate and serum lipid levels. Friedman (74) in a study of diet and serum lipids states that the amount of protein in the diet, if adequate, does not influence serum cholesterol; but a fall in serum cholesterol, and beta lipoproteins does occur when the intake of protein is reduced to deficiency levels (i.e., 25 grams/day). Total lipids also tend to be low when protein deficiency levels are present. Protein intakes were adequate for all subjects in the present study.

Friedman further indicates that a high carbohydrate intake results frequently in hyperlipidemia, but that factors other than lipogenesis from carbohydrate are more apt to contribute to elevated serum lipid states. For instance, hypertriglyceridemia does not occur with diets of mixed ordinary foodstuffs at moderate intakes of carbohydrate, calories, total fats, and polyunsaturated fats, and a low intake of saturated fatty acid and cholesterol. These proportions in general describe the dietaries of our test subjects. Carbohydrates were in all cases consumed (Table 4.2) in moderate amounts. Total calories were adequate to maintain weight during the test period but moderate in intake.

No significant correlations were expected between age, per cent standard weight or PBI and serum lipids, since all subjects grouped within the usual clinical ranges acceptable in health and none were observed.

The group showed a negative correlation (-0.86) with respect to serum total protein and serum linoleic plus arachidonic acids (%). This may or may not imply that certain levels of serum protein are necessary to maintain metabolic relief of the serum linoleic plus arachidonic acid pool. Again, it may merely be an implication of subject individuality. No significant correlation was observed between serum A:G ratios and serum lipids.

Significant correlations were expected and observed between the level of total serum lipid and serum levels of total fatty acids (0.98), total cholesterol (0.89), free cholesterol (0.86), esterified cholesterol (0.90), and milligrams linoleic (0.97). Similar high correlations were observed between total fatty acids and total cholesterol, free cholesterol, esterified cholesterol, milligrams linoleic and arachidonic (0.89).

A correlation of (0.87) was observed between cholesterol ester and milligrams linoleic or arachidonic acid. It is well established that human serum cholesterol ester fractions are characterized by relatively high unsaturation with typical mean amounts of linoleic and arachidonic being 35.2 and 3.8 per cent of total fatty acids detected, respectively (68). The correlation between serum linoleic and arachidonic acid was found to be 0.86. This would suggest that arachidonic acid was formed from linoleate, as recently reported by Holman (75).

No significant correlations were observed between dietary linoleic acid levels of intake and PBI, TBI or BMR's.

CHAPTER IV

SUMMARY AND IMPLICATIONS

Self-selected nutrient intake and levels of serum proteins and lipids were determined for seven professionally-active women dietitians twenty-eight to forty-seven years of age and living in the southeast section of Arizona.

The ten-week period of investigation beginning September 1, 1964, and ending November 10, 1964, provided a relatively constant and characteristic overall climatic setting for the subjects.

Clinical, dietary and biochemical data: 1) characterized the subjects to be clinically healthy, 2) added to the twenty-four year accumulation of evidence with respect to climatically-lowered thyroid activity in women living in a semi-desert climate, 3) characterized the degree of lowering of thyroid activity observed in southeastern Arizona women to be "subtle" and of questionable meaning, 4) found apparent optimal levels of daily linoleic acid intake ranging from 4.23 to 11.84 grams or 1 to 6 per cent of adequate total calories, 5) contributed evidence that the level of linoleic acid in the diet had no effect upon the thyroid activity under the conditions of the study, and 6) suggested patterns of serum total lipid fatty acid distribution for future clinical normal values.

The characteristic pattern of distribution for the detected fatty acids gave mean values for lauric (12:0), myristic (14:0), palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), linoleic (18:2), and arachidonic (20:4) to be in the order of 0.47 $\pm$ 0.13; 1.21 $\pm$ 0.19; 20.68 $\pm$ 1.03; 3.92 $\pm$ 0.62; 3.94 $\pm$ 0.53; 26.37 $\pm$ 1.22; 39.39 $\pm$ 1.47; and 4.22 $\pm$ 1.03, respectively, with an oleic to linoleic acid (O:L) ratio of 0.66.

Data from this investigation supply useful parameters for better assessment of nutritional status and human health.

APPENDIX

Table 1

SELECTED CLIMATOLOGICAL DATA FOR TUCSON, ARIZONA (SEPT. 1 TO NOV. 10, 1964)

Date	Temperature (° F) Max	Min	Mean	Precipitation (Inches)	Relative Humidity (Per cent) Noon	5:00 p.m.	Percentage of Possible Sunshine
September							
1	100	69	84	0	46	35	98
2	101	65	83	0	39	44	100
3	103	64	83	0	33	31	73
4	103	72	87	0	34	33	50
5	100	76	88	0	40	47	56
6	96	69	82	0.23	29	76	38
7	97	66	81	0.15	74	76	84
8	92	68	80	0	60	62	14
9	87	66	76	0.25	67	65	2
10	78	68	73	0.67	87	87	2
11	94	69	81	Trace	59	72	38
12	96	73	84	0	56	47	60
13	94	70	82	Trace	54	73	67
14	93	68	80	0.15	56	83	54
15	90	65	77	0.08	42	42	98
16	92	56	74	0	48	44	100
17	94	57	75	0	42	38	99
18	92	63	77	0	38	47	83
19	87	64	75	0	58	55	63
20	89	66	77	0	51	59	92

Table 1 (continued)

Date	Temperature (° F) Max	Min	Mean	Precipitation (Inches)	Relative Humidity (Per cent) Noon	5:00 p.m.	Percentage of Possible Sunshine
September							
21	90	66	78	0	51	43	86
22	87	55	71	Trace	39	59	54
23	81	58	69	0.10	49	51	77
24	88	61	74	0.03	56	51	61
25	92	61	77	0.04	40	35	77
26	89	60	74	0	47	45	86
27	90	54	72	0	45	39	100
28	93	60	76	0	46	43	100
29	95	55	75	0	43	38	100
30	98	55	76	0	44	36	100
Sum	2781	1919	2341	1.70	1473	1556	2112
Mean	92.70	63.96	78.03	0.18	49.10	51.86	70.40

Table 1 (continued)

Date	Temperature (° F) Max	Min	Mean	Precipitation (Inches)	Relative Humidity (Per cent) Noon	5:00 p.m.	Percentage of Possible Sunshine
October							
1	97	56	76	0	38	38	100
2	97	55	76	0	35	38	100
3	96	55	75	0	35	37	83
4	98	56	77	0	42	37	98
5	94	69	81	0	43	41	100
6	99	68	88	0	34	26	100
7	99	57	78	0	30	25	89
8	99	58	78	0	35	26	94
9	99	55	77	0	28	22	99
10	98	54	76	0	27	15	100
11	97	54	75	0	26	26	100
12	98	53	74	0	24	23	100
13	93	55	74	0	29	28	95
14	91	56	73	0	28	31	85
15	88	54	71	Trace	42	67	40
16	78	62	70	0.45	68	90	19
17	78	56	67	0.23	55	84	27
18	83	56	69	--	--	46	79
19	80	51	65	0.32	39	39	100
20	82	59	70	0	36	37	100

Table 1 (continued)

Date	Temperature (° F) Max	Min	Mean	Precipitation (Inches)	Relative Humidity (Per cent) Noon	5:00 p.m.	Percentage of Possible Sunshine
October							
21	89	50	69	0	36	35	93
22	91	45	68	0	34	26	99
23	91	48	69	0	29	29	98
24	90	53	71	0	32	25	100
25	83	48	65	0	42	42	100
26	85	47	66	0	33	32	99
27	87	48	67	0	32	31	100
28	92	46	69	0	34	26	99
29	92	52	72	0	36	30	77
30	87	48	67	0	39	34	100
31	82	46	64	0	37	31	100
Sum	2813	1670	2237	1.00	1078	1117	2773
Mean	90.74	53.87	72.16	0.33	35.93	36.03	89.45

Table 1 (continued)

Date	Temperature (° F) Max	Min	Mean	Precipitation (Inches)	Relative Humidity (Per cent) Noon	5:00 p.m.	Percentage of Possible Sunshine
November							
1	83	43	63	0	34	39	86
2	78	44	61	0	42	40	100
3	79	39	59	0	30	28	100
4	78	37	57	0	32	26	100
5	80	43	61	0	27	25	100
6	86	40	63	0	25	21	100
7	87	41	64	0	22	26	100
8	86	43	64	0	30	33	93
9	75	44	59	0	38	46	15
10	75	47	61	0.07	45	55	93
Sum	807	421	612	0.07	325	339	887
Mean	80.70	42.10	61.20	0.07	32.50	33.90	88.70
10-wk Sum	6401	4010	5190	2.77	2876	3012	5772
10-wk Mean	90.15	56.47	73.09	0.21	41.08	42.42	81.29

Table 2

FASTING BLOOD AND URINE FINDINGS FOR SOUTHEASTERN ARIZONA WOMEN

Item	Usual Findings	Subjects						
		1	2	3	4	5	6	7
Age		(28)	(31)	(36)	(38)	(40)	(43)	(47)
					Blood			
Hemoglobin gm/100 ml	12-16	14.1	12.3	14.1	13.2	13.4	12.3	12.3
Micro. Hct. vols.%	37-47	45	39	45	42	43	40	40
W.B.C./cu mm	5,000-10,000	5,900	8,650	7,200	6,450	7,350	9,950	7,250
Segs. %	50-70	41	59	48	48	56	58	56
Stabs. %	0-5	2	4	2	1	2	2	2
Lyms. %	20-40	45	33	46	45	38	35	38
Eos. %	1-5	9	2	3	6	3	4	1
Baso. %	0-1	2	0	0	0	0	1	1
Monos. %	1-6	1	2	1	0	1	0	2
Sed. Rate mm/hr.	0-20	9	28	15	--	13	2	17
Glucose mg/100 ml	65-100	80	86	80	89	80	77	89

Table 2 (continued)

Item	Usual Findings	Subjects						
		1	2	3	4	5	6	7
Age		(28)	(31)	(36)	(38)	(40)	(43)	(47)
					Urine			
Color	Yellow	Straw	Straw	Pale Yellow	Yellow	Clear Yellow	Clear Yellow	Turbid Yellow
Reaction (pH)	4.8 - 7.8	7.0	7.0	6.5	5.0	6.5	7.0	7.0
Sp. Gr.	1.002 - 1.030	1.003	1.001	1.004	--	1.014	1.014	1.014
Sugar	Negative	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Albumin	Negative	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
W.B.C.	Variable	Occ.	0-2	5-7	1-2	1-2	1-2	Rare
R.B.C.	Negative	None	None	None	None	None	None	None
Epith.	Variable	Num.	Few	Many	Many	Num.	Occ.	Few
Casts	Variable	None	None	None	None	None	None	None
Bacteria	Variable	Few	Occ.	Num.	Num.	Few	Scatt.	Few

Table 3

ADDITIONAL INFORMATION ABOUT THE SUBJECTS

Subject	Age (yrs.)	Height (cm)	Weight (Kg)	Standard Weight Group (per cent)	Blood Group	Blood Pressure (s/d*)	M** Day	Lived in Arizona (yrs.)
1	28	162	55	95- 99	O	120/88	21/24/25	3
2	31	169	75	120-124	O	112/78	Extremely irregular	6½
3	36	165	56	90- 94	O	120/78	7/9/8	1½
4	38	159	51	90- 94	A	115/70	21/21/	11
5	40	156	58	105-109	A	122/78	4/5/5	29
6	43	158	54	95- 99	A	115/70	11/21/	10
7	47	166	57	90- 94	O	122/70	24/28/20	6

* systolic/diastolic
** day of onset of menstruation -- September/October/November

Table 4

EVALUATION OF SELF-SELECTED DIETARY DATA

Nutrient	Mean Daily Recommended Allowance	Week 3 Mean Daily Nutritive Values	Difference	Week 8 Mean Daily Nutritive Values	Difference
			Subject Number 1		
Calories	2100.00	2879.94	+779.94	2714.46	+ 614.46
Protein gm	58.00	119.44	+ 61.44	101.55	+ 43.55
Fat gm	---	145.40	---	136.39	---
CHO Total gm	---	257.61	---	255.54	---
CHO Fiber gm	---	3.93	---	4.37	---
Ash gm	---	19.35	---	18.52	---
Calcium mg	800.00	1043.86	+243.86	1043.34	+ 243.34
Phosphorus mg	---	1568.34	---	1548.30	---
Iron mg	15.00	17.09	+ 2.09	16.40	+ 1.40
Sodium mg	---	2941.25	---	2282.88	---
Potassium mg	---	2541.66	---	3134.05	---
Vitamin A IU	5000.00	5125.76	+125.76	6319.71	+1319.71
Thiamine mg	0.80	1.09	+ 0.29	1.45	+ 0.65
Riboflavin mg	1.30	2.14	+ 0.84	1.97	+ 0.67
Niacin (equiv) mg	14.00	41.75	+ 27.75	34.87	+ 20.87
Ascorbic Acid mg	70.00	109.13	+ 39.13	263.13	+ 193.13
Total Saturated FA gm	---	22.67	---	22.75	---
Oleic gm	---	19.58	---	17.60	---
Linoleic gm	---	5.07	---	3.40	---
Cholesterol mg	---	453.00	---	518.00	---

Table 4 (continued)

Nutrient	Mean Daily Recommended Allowance	Week 3 Mean Daily Nutritive Values	Difference	Week 8 Mean Daily Nutritive Values	Difference
		Subject Number 2			
Calories	2100.00	2126.48	+ 26.48	2183.39	+ 83.39
Protein gm	58.00	77.12	+ 19.12	67.45	+ 9.45
Fat gm	---	98.30	---	98.72	---
CHO Total gm	---	226.56	---	257.22	---
CHO Fiber gm	---	3.63	---	5.17	---
Ash gm	---	14.13	---	33.32	---
Calcium mg	800.00	703.87	- 96.13	793.96	- 6.04
Phosphorus mg	---	1055.14	---	1094.40	---
Iron mg	15.00	11.33	- 3.67	11.91	- 3.09
Sodium mg	---	2300.61	---	8493.48	---
Potassium mg	---	2413.55	---	2859.61	---
Vitamin A IU	5000.00	4644.41	-355.59	9870.93	+4870.93
Thiamine mg	0.80	0.93	+ 0.13	1.41	+ 0.61
Riboflavin mg	1.30	1.40	+ 0.10	1.62	+ 0.32
Niacin (equiv) mg	14.00	27.22	+ 13.22	24.74	+ 10.74
Ascorbic Acid mg	70.00	150.40	+ 80.40	203.96	+ 133.96
Total Saturated FA gm	---	13.09	---	25.23	---
Oleic gm	---	11.75	---	27.78	---
Linoleic gm	---	6.02	---	7.49	---
Cholesterol mg	---	185.00	---	315.00	---

Table 4 (continued)

Nutrient	Mean Daily Recommended Allowance	Week 3 Mean Daily Nutritive Values	Difference	Week 8 Mean Daily Nutritive Values	Difference
		Subject Number 3			
Calories	1900.00	1999.22	+ 99.22	1849.03	- 50.97
Protein gm	58.00	70.52	+ 12.52	63.77	+ 5.77
Fat gm	---	103.64	---	88.82	---
CHO Total gm	---	200.60	---	196.66	---
CHO Fiber gm	---	3.48	---	3.67	---
Ash gm	---	13.90	---	12.59	---
Calcium mg	800.00	943.29	+ 143.29	822.94	+ 22.94
Phosphorus mg	---	1204.91	---	1108.21	---
Iron mg	15.00	11.27	- 3.73	10.71	- 4.29
Sodium mg	---	1604.13	---	1540.73	---
Potassium mg	---	2573.45	---	2216.03	---
Vitamin A IU	5000.00	9873.90	+4873.90	7967.81	+2967.81
Thiamine mg	0.80	0.97	+ 0.17	1.18	+ 0.38
Riboflavin mg	1.20	1.92	+ 0.72	1.70	+ 0.50
Niacin (equiv) mg	13.00	24.80	+ 11.80	23.36	+ 10.36
Ascorbic Acid mg	70.00	136.64	+ 66.64	109.15	+ 39.15
Total Saturated FA gm	---	14.73	---	16.10	---
Oleic gm	---	21.03	---	20.22	---
Linoleic gm	---	10.96	---	8.33	---
Cholesterol mg	---	369.00	---	348.00	---

Table 4 (continued)

Nutrient	Mean Daily Recommended Allowance	Week 3 Mean Daily Nutritive Values	Difference	Week 8 Mean Daily Nutritive Values	Difference
			Subject Number 4		
Calories	1900.00	1685.62	- 214.38	1910.89	+ 10.89
Protein gm	58.00	56.83	- 1.17	66.64	+ 8.64
Fat gm	---	76.22	---	81.99	---
CHO Total gm	---	201.39	---	237.14	---
CHO Fiber gm	---	2.28	---	4.31	---
Ash gm	---	10.22	---	12.70	---
Calcium mg	800.00	746.38	- 53.62	638.24	-161.76
Phosphorus mg	---	987.48	---	1009.49	---
Iron mg	15.00	6.90	- 8.10	8.87	- 6.13
Sodium mg	---	1375.49	---	1992.36	---
Potassium mg	---	1696.30	---	1792.31	---
Vitamin A IU	5000.00	6526.93	+1526.93	4891.36	-108.64
Thiamine mg	0.80	0.70	- 0.10	0.60	- 0.20
Riboflavin mg	1.20	1.39	+ 0.19	1.29	+ 0.09
Niacin (equiv) mg	13.00	19.33	+ 6.33	20.90	+ 7.90
Ascorbic Acid mg	70.00	72.27	+ 2.27	59.30	- 10.70
Total Saturated FA gm	---	12.78	---	15.91	---
Oleic gm	---	18.83	---	13.41	---
Linoleic gm	---	5.08	---	4.94	---
Cholesterol mg	---	292.00	---	287.00	---

Table 4 (continued)

Nutrient	Mean Daily Recommended Allowance	Week 3 Mean Daily Nutritive Values	Difference	Week 8 Mean Daily Nutritive Values	Difference
			Subject Number 5		
Calories	1900.00	1882.93	- 17.07	1880.25	- 19.75
Protein gm	58.00	59.96	+ 1.96	60.14	+ 2.14
Fat gm	---	84.21	---	83.74	---
CHO Total gm	---	225.93	---	225.96	---
CHO Fiber gm	---	2.89	---	2.99	---
Ash gm	---	13.43	---	13.81	---
Calcium mg	800.00	864.41	+ 64.41	846.82	+ 46.82
Phosphorus mg	---	1026.97	---	1036.82	---
Iron mg	15.00	8.56	- 6.44	8.93	- 6.07
Sodium mg	---	2286.03	---	2424.60	---
Potassium mg	---	2032.41	---	2070.99	---
Vitamin A IU	5000.00	7056.81	+2056.81	6434.46	+1434.46
Thiamine mg	0.80	0.90	+ 0.10	0.93	+ 0.13
Riboflavin mg	1.20	1.56	+ 0.36	1.55	+ 0.35
Niacin (equiv) mg	13.00	21.13	+ 8.13	20.89	+ 7.89
Ascorbic Acid mg	70.00	94.93	+ 24.93	94.49	+ 24.49
Total Saturated FA gm	---	8.21	---	8.04	---
Oleic gm	---	16.18	---	15.99	---
Linoleic gm	---	7.88	---	7.84	---
Cholesterol mg	---	221.00	---	188.00	---

Table 4 (continued)

Nutrient	Mean Daily Recommended Allowance	Week 3 Mean Daily Nutritive Values	Difference	Week 8 Mean Daily Nutritive Values	Difference
			Subject Number 6		
Calories	1900.00	1482.17	- 417.83	1716.91	- 183.09
Protein gm	58.00	64.96	+ 6.96	78.41	+ 20.41
Fat gm	---	67.36	---	75.73	---
CHO Total gm	---	161.01	---	184.38	---
CHO Fiber gm	---	4.11	---	4.18	---
Ash gm	---	14.40	---	13.98	---
Calcium mg	800.00	759.35	- 40.65	833.53	+ 33.53
Phosphorus mg	---	1045.56	---	1232.71	---
Iron mg	15.00	10.14	- 4.87	11.87	- 3.13
Sodium mg	---	2620.55	---	1916.69	---
Potassium mg	---	2382.50	---	2334.61	---
Vitamin A IU	5000.00	7789.99	+2789.99	9838.13	+4838.13
Thiamine mg	0.80	1.04	+ 0.24	0.97	+ 0.17
Riboflavin mg	1.20	1.52	+ 0.32	1.85	+ 0.65
Niacin (equiv) mg	13.00	22.05	+ 9.05	28.51	+ 15.51
Ascorbic Acid mg	70.00	112.54	+ 42.54	95.42	+ 25.42
Total Saturated FA gm	---	15.03	---	18.47	---
Oleic gm	---	15.54	---	14.24	---
Linoleic gm	---	4.61	---	5.61	---
Cholesterol mg	---	414.00	---	482.00	---

Table 4 (continued)

Nutrient	Mean Daily Recommended Allowance	Week 3 Mean Daily Nutritive Values	Difference	Week 8 Mean Daily Nutritive Values	Difference
			Subject Number 7		
Calories	1900.00	1892.60	- 7.40	1887.42	- 12.58
Protein gm	58.00	58.15	+ 0.15	57.45	- 0.55
Fat gm	---	87.80	---	87.73	---
CHO Total gm	---	227.70	---	226.38	---
CHO Fiber gm	---	4.44	---	4.51	---
Ash gm	---	11.80	---	11.47	---
Calcium mg	800.00	697.39	- 102.61	672.67	- 127.33
Phosphorus mg	---	968.99	---	939.04	---
Iron mg	15.00	10.03	- 4.97	10.08	- 4.92
Sodium mg	---	1672.17	---	1614.22	---
Potassium mg	---	2034.53	---	1978.74	---
Vitamin A IU	5000.00	4367.57	- 632.43	4431.31	- 568.69
Thiamine mg	0.80	1.10	+ 0.30	1.16	+ 0.36
Riboflavin mg	1.20	1.36	+ 0.16	1.36	+ 0.16
Niacin (equiv) mg	13.00	21.81	+ 8.81	21.20	+ 8.20
Ascorbic Acid mg	70.00	105.15	+ 35.15	103.50	+ 33.50
Total Saturated FA gm	---	10.21	---	9.98	---
Oleic gm	---	21.34	---	20.83	---
Linoleic gm	---	12.19	---	11.50	---
Cholesterol mg	---	202.00	---	240.00	---

Table 5

WEEKLY MEAN TOTAL PROTEIN CONCENTRATION -- GM PER 100 ML OF SERUM

Subject	Week 1	2	3	4	5	6	7	8	9	10	11
1	7.1	6.7	6.9	--	7.4	6.8	7.0	6.8	6.7	7.1	6.6
2	6.7	6.8	7.0	6.7	6.8	6.9	6.8	6.7	7.0	6.8	6.7
3	--	6.7	6.6	6.7	6.6	6.8	6.6	6.8	6.8	6.9	6.7
4	6.6	6.6	6.8	6.8	6.5	6.5	6.9	6.2	6.8	6.7	6.4
5	7.0	7.0	6.7	7.0	6.9	6.8	6.9	6.9	6.7	7.3	7.0
6	6.4	6.2	6.7	6.7	6.6	6.7	6.3	6.6	6.9	6.8	6.5
7	6.5	6.9	6.7	6.9	6.4	6.5	6.5	6.8	6.6	6.4	6.8
Pooled Control Serum	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2

Table 6

WEEKLY MEANS OF FIVE SERUM PROTEIN COMPONENTS -- PERCENTAGE CONCENTRATION

	Weeks										
Component	1	2	3	4	5	6	7	8	9	10	11
					Subject Number 1						
Albumin	48.02	45.23	50.58	--	50.18	52.82	48.92	42.88	64.89	65.90	61.22
Alpha 1	6.10	4.94	6.98	--	5.54	5.38	6.37	7.36	4.02	3.35	3.06
Alpha 2	10.74	12.93	10.60	--	11.46	10.28	11.54	12.01	7.93	6.56	7.14
Beta	10.90	13.82	10.64	--	10.94	10.19	11.66	15.88	8.37	7.59	8.16
Gamma	24.26	23.09	21.20	--	21.87	21.34	21.52	21.88	14.79	16.60	20.41
					Subject Number 2						
Albumin	48.38	48.63	48.06	47.17	48.48	44.62	44.85	45.98	67.65	62.60	61.32
Alpha 1	8.18	6.69	6.77	9.06	7.26	7.92	6.74	7.80	3.24	4.54	4.50
Alpha 2	13.70	15.81	13.87	11.93	13.20	13.54	14.15	14.18	7.68	9.49	8.42
Beta	13.24	18.24	13.23	14.52	14.10	15.14	13.98	13.47	9.71	11.08	11.57
Gamma	16.50	10.64	18.06	17.32	16.96	18.78	20.28	18.56	11.72	12.28	14.20
					Subject Number 3						
Albumin	--	51.10	51.84	54.83	55.44	55.46	48.19	48.26	68.52	71.10	66.16
Alpha 1	--	8.37	6.91	6.31	6.48	6.19	8.86	7.91	4.72	3.30	4.10
Alpha 2	--	14.54	11.15	10.38	11.79	12.09	12.60	14.24	7.38	6.55	9.03
Beta	--	10.43	10.71	11.81	10.30	10.03	11.57	11.08	7.89	8.18	8.56
Gamma	--	15.56	19.39	16.67	16.00	16.22	18.79	18.51	11.42	10.87	12.16

Table 6 (continued)

Component	Weeks 1	2	3	4	5	6	7	8	9	10	11
					Subject Number 4						
Albumin	46.22	48.18	47.74	49.44	49.18	49.50	47.94	40.50	67.70	55.40	60.22
Alpha 1	6.58	6.59	7.60	5.16	5.13	5.22	6.61	9.19	2.78	4.64	3.23
Alpha 2	10.12	10.86	10.64	10.62	10.27	11.18	10.12	13.04	5.55	9.58	5.91
Beta	13.33	14.73	11.75	11.23	12.76	10.72	12.44	15.06	7.83	11.10	11.83
Gamma	23.76	19.65	22.27	23.56	22.66	23.38	22.89	22.22	16.15	19.28	18.82
					Subject Number 5						
Albumin	47.74	47.11	35.10	53.64	53.30	46.12	50.43	44.40	69.02	68.73	63.66
Alpha 1	7.30	6.38	7.14	5.74	4.84	6.99	5.38	7.14	2.76	2.64	3.78
Alpha 2	13.15	13.37	14.29	9.30	10.84	13.02	11.44	14.63	7.38	6.66	6.21
Beta	11.68	17.63	14.30	12.75	10.38	12.48	10.45	12.71	7.17	6.32	9.56
Gamma	20.12	15.50	29.17	18.58	20.64	21.39	22.29	21.12	13.67	15.66	16.80
					Subject Number 6						
Albumin	50.99	52.57	53.81	54.00	52.28	54.79	52.13	43.11	72.49	70.77	72.00
Alpha 1	6.48	7.10	6.06	5.79	7.66	5.62	6.37	7.57	3.44	2.74	2.56
Alpha 2	11.66	11.46	10.96	10.66	11.66	12.14	12.02	14.35	6.60	7.38	6.31
Beta	12.89	11.94	12.34	10.86	10.60	10.03	12.29	12.22	8.60	7.68	7.69
Gamma	17.97	16.94	16.82	18.70	17.79	17.41	17.19	16.38	8.88	11.44	11.43

Table 6 (continued)

Component	Weeks 1	2	3	4	5	6	7	8	9	10	11
					Subject Number 7						
Albumin	47.70	45.39	48.52	46.82	51.22	46.96	48.48	43.88	68.83	66.72	59.59
Alpha 1	6.67	8.01	6.24	8.07	5.45	8.04	7.40	8.61	4.24	3.26	4.28
Alpha 2	13.73	12.62	11.15	11.16	11.43	13.10	11.92	13.54	8.42	7.22	9.04
Beta	11.24	16.50	13.99	13.42	11.82	10.71	11.29	13.12	7.30	8.26	11.28
Gamma	20.66	17.48	20.10	20.54	20.08	21.18	20.92	20.86	11.21	14.53	15.80
					Pooled Control Serum						
Albumin	56.85	58.04	57.07	54.67	61.62	62.13	58.61	56.42	62.94	54.96	62.93
Alpha 1	4.22	3.62	5.88	5.46	4.04	4.04	4.46	4.48	3.52	5.44	5.62
Alpha 2	11.05	9.84	8.24	12.56	10.60	8.58	9.55	10.26	8.82	10.89	8.42
Beta	12.10	12.44	13.52	10.92	9.60	10.10	12.74	13.46	10.58	12.87	9.55
Gamma	15.78	16.06	15.29	16.39	14.14	15.15	14.64	15.38	14.12	15.84	13.48

Table 6.1

VARIABILITY IN SERUM PROTEIN COMPONENTS

Component	Weeks	N	Mean(%)	S.D.	S.E.	C.V.
			Subject Number 1			
Albumin	10	20	53.06	8.12	2.57	15
Alpha 1	10	20	5.31	1.47	0.47	27
Alpha 2	10	20	10.12	2.17	0.69	21
Beta	10	20	10.82	2.57	0.81	23
Gamma	10	20	20.70	2.87	0.91	13
			Subject Number 2			
Albumin	11	22	51.61	8.12	2.45	15
Alpha 1	11	22	6.61	1.79	0.54	27
Alpha 2	11	22	12.36	2.65	0.80	21
Beta	11	22	13.48	2.26	0.68	16
Gamma	11	22	15.94	3.22	0.97	20
			Subject Number 3			
Albumin	10	20	57.09	8.43	2.67	14
Alpha 1	10	20	6.32	1.83	0.58	29
Alpha 2	10	20	10.98	2.68	0.85	24
Beta	10	20	10.06	1.39	0.44	13
Gamma	10	20	15.56	3.10	0.98	19
			Subject Number 4			
Albumin	11	22	51.09	7.43	2.24	14
Alpha 1	11	22	5.70	1.87	0.56	32
Alpha 2	11	22	9.81	2.20	0.66	22
Beta	11	22	12.07	2.00	0.60	16
Gamma	11	22	21.33	2.47	0.75	11
			Subject Number 5			
Albumin	11	22	52.66	10.62	3.20	20
Alpha 1	11	22	5.46	1.75	0.53	32
Alpha 2	11	22	10.94	3.10	0.94	28
Beta	11	22	11.40	3.18	0.96	27
Gamma	11	22	19.54	4.27	1.29	21

Table 6.1 (continued)

Component	Weeks	N	Mean(%)	S.D.	S.E.	C.V.
			Subject Number 6			
Albumin	11	22	57.18	9.86	2.97	17
Alpha 1	11	22	5.58	1.85	0.56	33
Alpha 2	11	22	10.47	2.57	0.78	24
Beta	11	22	10.65	1.92	0.58	18
Gamma	11	22	15.64	3.31	1.00	21
			Subject Number 7			
Albumin	11	22	52.19	8.73	2.63	16
Alpha 1	11	22	6.39	1.84	0.56	28
Alpha 2	11	22	11.21	2.15	0.65	19
Beta	11	22	11.72	2.57	0.77	21
Gamma	11	22	18.49	3.31	1.00	17
			Pooled Control Serum			
Albumin	11	22	58.75	3.13	0.94	5
Alpha 1	11	22	4.62	0.84	0.25	18
Alpha 2	11	22	9.89	1.34	0.40	13
Beta	11	22	11.63	1.52	0.46	13
Gamma	11	22	15.12	0.92	0.28	6

Table 7

WEEKLY MEAN TOTAL LIPID CONCENTRATION -- MG PER 100 ML OF SERUM

	Week										
Subject	1	2	3	4	5	6	7	8	9	10	11
1	433	333	617	---	850	467	567	400	400	---	450
2	233	300	633	---	500	467	550	450	333	367	450
3	---	367	850	317	467	550	550	533	817	467	617
4	500	367	550	300	300	417	983	317	650	400	433
5	583	583	867	350	500	667	500	633	400	417	550
6	450	433	700	867	600	717	833	683	783	533	683
7	500	433	767	417	400	467	533	933	450	450	617
Pooled Control Serum	683	717	700	717	667	733	700	717	700	700	700

Table 8

WEEKLY MEANS OF FIVE SERUM LIPOPROTEIN COMPONENTS -- PERCENTAGE CONCENTRATION

Component	Week 1	2	3	4	5	6	7	8	9	10	11
					Subject Number 1						
Lipalbumin	26.80	28.33	29.19	--	29.30	28.99	25.96	28.28	30.88	32.13	30.15
Alpha 1	6.74	9.52	8.50	--	11.76	9.24	9.02	10.89	6.60	7.93	7.42
Alpha 2	13.54	15.49	16.32	--	13.76	15.55	13.64	15.90	8.84	8.46	9.28
Beta + Neutral Fat	37.48	33.10	33.34	--	34.11	33.61	36.36	34.60	41.90	36.80	39.84
Gamma	15.44	13.56	12.64	--	11.08	12.61	15.01	10.33	11.79	14.66	12.98
					Subject Number 2						
Lipalbumin	25.00	22.80	24.87	26.74	27.49	23.47	27.65	26.58	27.93	25.72	30.10
Alpha 1	6.82	7.76	7.95	8.50	7.54	8.92	8.20	7.07	5.16	8.56	6.12
Alpha 2	18.64	17.57	21.30	13.46	12.19	15.49	11.02	16.70	11.06	13.65	10.51
Beta + Neutral Fat	36.36	39.33	31.74	38.88	38.14	37.56	41.04	36.22	44.83	45.74	43.25
Gamma	13.18	12.54	14.14	12.42	14.64	14.55	12.09	13.42	11.02	6.33	10.02
					Subject Number 3						
Lipalbumin	--	22.46	26.62	27.48	27.23	23.93	27.96	25.23	25.91	24.28	27.34
Alpha 1	--	7.87	8.42	7.69	7.50	6.56	6.88	7.06	6.62	7.01	5.04
Alpha 2	--	19.90	15.70	8.15	11.94	10.82	8.12	15.31	7.47	7.01	9.48
Beta + Neutral Fat	--	38.20	38.34	45.52	42.22	46.23	44.78	39.30	46.75	53.44	49.65
Gamma	--	11.57	10.93	11.16	11.12	12.46	12.27	13.10	13.25	8.27	8.48

Table 8 (continued)

Component	Week 1	2	3	4	5	6	7	8	9	10	11
					Subject Number 4						
Lipalbumin	24.48	26.02	25.84	24.74	28.92	25.24	26.32	27.22	29.00	27.14	22.54
Alpha 1	9.50	8.15	6.48	7.11	7.66	7.84	7.26	8.42	5.94	8.08	4.77
Alpha 2	13.93	14.43	17.04	12.07	13.94	15.14	12.64	14.62	9.82	12.55	10.74
Beta + Neutral Fat	38.18	38.24	36.82	46.26	37.98	40.20	40.93	39.34	42.74	40.42	46.18
Gamma	13.92	13.16	13.82	9.82	11.49	11.58	12.86	10.40	12.49	11.82	15.78
					Subject Number 5						
Lipalbumin	19.41	21.20	21.74	26.56	27.20	21.55	23.27	22.10	22.02	22.29	23.15
Alpha 1	9.76	7.61	9.14	5.65	7.72	7.11	6.64	7.14	5.90	6.99	3.74
Alpha 2	11.98	13.32	13.74	9.62	9.96	13.02	9.56	13.70	6.70	10.74	7.46
Beta + Neutral Fat	43.79	45.50	46.02	47.19	44.23	45.78	48.83	44.47	56.08	52.43	57.46
Gamma	15.05	12.36	9.37	10.98	10.89	12.54	11.70	12.60	9.30	7.54	8.18
					Subject Number 6						
Lipalbumin	23.77	24.55	29.13	25.06	25.84	24.15	26.21	27.02	23.32	18.99	22.55
Alpha 1	5.68	6.72	6.74	6.92	7.58	9.08	7.13	6.10	5.81	6.82	6.13
Alpha 2	12.06	14.07	15.24	11.74	11.23	18.06	10.48	10.46	5.18	9.21	8.39
Beta + Neutral Fat	47.82	43.22	36.30	46.72	45.11	37.44	44.78	46.18	58.18	56.76	55.80
Gamma	10.68	11.43	12.60	9.54	10.24	11.26	11.40	10.24	7.52	8.23	7.12

Table 8 (continued)

Component	Week 1	2	3	4	5	6	7	8	9	10	11
					Subject Number 7						
Lipalbumin	18.64	21.90	20.44	26.18	24.88	21.08	23.42	24.47	21.26	21.55	23.74
Alpha 1	5.86	6.86	9.00	6.10	8.04	8.07	6.58	6.18	5.49	5.48	6.29
Alpha 2	16.88	13.26	13.04	8.11	11.71	15.70	11.00	11.74	9.40	9.76	7.86
Beta + Neutral Fat	43.34	43.82	45.50	46.72	43.90	40.36	45.04	43.74	52.74	50.60	54.70
Gamma	15.28	14.16	12.02	12.90	11.46	14.80	13.94	13.88	11.10	12.62	7.41
					Pooled Control Serum						
Lipalbumin	15.67	13.22	15.16	14.73	16.94	12.89	15.52	13.46	20.12	19.60	18.60
Alpha 1	4.48	4.96	4.54	3.88	5.08	5.30	5.42	4.20	7.54	6.29	5.81
Alpha 2	13.43	11.57	14.39	12.40	11.86	12.88	11.62	10.08	12.58	12.58	13.37
Beta + Neutral Fat	57.46	61.16	56.82	58.91	56.80	59.84	58.14	62.18	49.68	51.74	52.90
Gamma	8.96	9.09	9.09	10.08	9.32	9.09	9.30	10.08	10.08	9.79	9.32

Table 8.1

VARIABILITY IN SERUM LIPOPROTEIN COMPONENTS

Component	Weeks	N	Mean(%)	S.D.	S.E.	C.V.
			Subject Number 1			
Lipalbumin	10	20	29.00	1.82	0.58	6
Alpha 1	10	20	8.76	1.69	0.53	19
Alpha 2	10	20	13.08	3.08	0.97	23
Beta	10	20	36.11	2.96	0.94	8
Gamma	10	20	13.01	1.69	0.53	12
			Subject Number 2			
Lipalbumin	11	22	26.21	2.12	0.64	8
Alpha 1	11	22	7.51	1.14	0.34	15
Alpha 2	11	22	14.69	3.54	1.07	24
Beta	11	22	39.37	4.13	1.24	10
Gamma	11	22	12.21	2.42	0.73	19
			Subject Number 3			
Lipalbumin	10	20	25.84	1.82	0.58	7
Alpha 1	10	20	7.07	0.92	0.29	13
Alpha 2	10	20	11.39	4.30	1.36	37
Beta	10	20	44.44	5.01	1.58	11
Gamma	10	20	11.26	1.72	0.54	15
			Subject Number 4			
Lipalbumin	11	22	26.13	1.92	0.58	7
Alpha 1	11	22	7.38	1.29	0.39	17
Alpha 2	11	22	13.36	2.06	0.62	15
Beta	11	22	40.66	3.20	0.96	7
Gamma	11	22	12.47	1.70	0.51	13
			Subject Number 5			
Lipalbumin	11	22	22.77	2.28	0.69	10
Alpha 1	11	22	7.04	1.64	0.49	23
Alpha 2	11	22	10.89	2.47	0.75	22
Beta	11	22	48.34	4.84	1.46	10
Gamma	11	22	10.96	2.22	0.67	20

Table 8.1 (continued)

Component	Weeks	N	Mean(%)	S.D.	S.E.	C.V.
			Subject Number 6			
Lipalbumin	11	22	24.60	2.63	0.79	10
Alpha 1	11	22	6.79	0.95	0.29	14
Alpha 2	11	22	11.47	3.48	1.05	30
Beta	11	22	47.12	7.26	2.19	15
Gamma	11	22	10.02	1.75	0.53	17
			Subject Number 7			
Lipalbumin	11	22	22.51	2.22	0.67	9
Alpha 1	11	22	6.72	1.16	0.35	17
Alpha 2	11	22	11.68	2.90	0.88	24
Beta	11	22	46.41	4.42	1.33	9
Gamma	11	22	12.69	2.21	0.67	17
			Pooled Control Serum			
Lipalbumin	11	22	15.99	2.53	0.76	15
Alpha 1	11	22	5.23	1.04	0.31	19
Alpha 2	11	22	12.43	1.15	0.35	9
Beta	11	22	56.88	3.94	1.19	6
Gamma	11	22	9.47	0.45	0.13	4

Table 9

WEEKLY MEAN TOTAL CHOLESTEROL CONCENTRATION -- MG PER 100 ML OF SERUM

	Week										
Subject	1	2	3	4	5	6	7	8	9	10	11
1	155.8	118.9	159.5	---	158.9	140.1	141.4	140.0	161.7	159.7	142.1
2	132.5	152.8	181.4	127.7	164.5	171.5	146.0	153.2	173.9	133.2	120.7
3	---	141.5	182.4	167.3	142.4	147.4	158.8	164.5	180.4	162.9	164.4
4	161.4	147.6	191.2	165.5	144.1	130.0	152.4	140.0	152.3	172.4	150.7
5	196.8	171.0	222.0	201.4	170.1	177.9	189.8	102.0	178.6	167.0	183.2
6	204.3	186.6	267.7	226.6	222.2	218.1	214.0	257.0	280.1	263.2	231.2
7	173.5	197.9	220.1	182.6	167.1	165.1	149.6	203.9	203.9	187.0	193.5
Pooled Control Serum	189.4	203.1	236.0	208.6	193.1	194.3	218.1	215.7	250.0	208.9	216.6

Table 10

WEEKLY MEAN FREE CHOLESTEROL CONCENTRATION -- MG PER 100 ML OF SERUM

	Week										
Subject	1	2	3	4	5	6	7	8	9	10	11
1	48.5	26.0	40.1	--	41.7	28.3	35.8	40.2	42.3	39.2	32.1
2	42.0	34.7	39.9	35.1	50.8	36.0	35.1	36.4	41.7	33.3	33.4
3	--	32.1	47.3	45.9	44.3	31.9	31.5	43.7	47.0	38.1	40.7
4	43.4	28.2	52.0	46.3	58.4	31.5	43.6	39.9	39.5	38.3	34.7
5	55.5	45.1	30.1	53.1	46.0	42.0	45.6	34.1	48.9	39.7	46.7
6	58.3	47.7	63.9	62.9	58.6	51.1	49.3	65.6	72.4	56.1	55.7
7	48.5	44.3	50.8	55.8	53.0	38.1	39.2	49.8	51.1	44.5	48.4
Pooled Control Serum	54.6	33.9	52.7	56.7	64.9	44.3	52.2	56.4	62.5	45.6	53.3

Table 11

WEEKLY MEAN TOTAL FATTY ACID CONCENTRATION -- MG PER 100 ML OF SERUM

Subject	Week										
	1	2	3	4	5	6	7	8	9	10	11
1	33	67	167	---	267	183	167	167	117	---	67
2	---	17	217	17	83	183	167	217	117	67	33
3	---	67	233	83	67	250	217	233	250	167	150
4	117	83	200	50	67	250	217	117	200	117	100
5	67	133	267	83	83	267	200	283	133	117	167
6	233	117	217	250	133	367	250	267	283	183	200
7	67	133	267	100	50	200	183	267	167	133	133
Pooled Control Serum	333	283	333	333	383	317	283	300	283	267	217

Table 12

WEEKLY MEANS OF EIGHT MEASURABLE SERUM FATTY ACID COMPONENTS -- PERCENTAGE CONCENTRATION

Acid	Week 1	2	3	4	5	6	7	8	9	10	11
					Subject Number 1						
12:0	0.10	0.96	0.18	--	0.64	0.36	0.36	0.30	0.75	0.74	0.36
14:0	0.78	1.37	1.20	--	1.05	1.19	1.51	1.30	0.99	0.86	0.90
16:0	22.06	21.58	24.38	--	25.82	19.68	20.99	18.68	17.49	17.97	17.38
16:1	3.70	3.68	3.94	--	3.42	3.90	3.19	3.87	3.57	4.28	5.91
18:0	6.80	3.29	6.65	--	5.22	4.51	5.07	3.40	4.21	5.64	3.49
18:1	25.81	29.08	23.76	--	23.16	19.49	24.81	26.16	26.49	30.34	29.03
18:2	37.60	37.68	36.18	--	36.20	44.40	44.07	42.72	42.12	40.17	39.43
20:4	3.15	2.36	3.71	--	4.49	6.47	--	3.57	4.38	--	3.50
					Subject Number 2						
12:0	0.46	0.70	0.68	--	1.25	0.22	0.56	0.62	0.28	0.38	0.88
14:0	1.38	1.84	1.02	1.92	1.95	1.29	1.70	1.70	1.27	1.59	1.97
16:0	24.26	20.56	23.04	19.82	22.88	22.82	21.04	21.78	21.32	22.16	22.88
16:1	4.43	5.08	4.43	6.58	5.41	4.86	4.76	6.02	5.85	6.65	3.40
18:0	3.46	2.24	5.95	2.86	5.57	4.16	2.94	3.96	2.30	3.40	2.12
18:1	30.96	27.32	25.13	27.83	26.04	21.98	23.50	28.82	26.63	25.16	28.98
18:2	33.62	42.26	34.65	37.72	32.58	38.31	39.74	32.66	38.02	35.74	34.87
20:4	1.43	--	5.10	3.27	4.32	6.36	5.76	4.44	4.33	4.92	4.90

Table 12 (continued)

Acid	Week 1	2	3	4	5	6	7	8	9	10	11
						Subject Number 3					
12:0	--	0.66	0.36	0.34	0.50	0.24	0.64	0.54	0.92	1.06	0.14
14:0	--	0.98	1.00	0.86	0.93	1.08	1.59	1.30	1.46	1.47	1.00
16:0	--	19.10	22.11	20.98	21.92	20.62	20.57	21.36	23.59	20.90	21.20
16:1	--	3.25	2.54	4.69	4.40	2.80	4.46	3.28	3.60	4.28	3.14
18:0	--	2.28	6.74	2.41	4.72	3.99	3.80	4.37	4.99	3.48	4.00
18:1	--	23.02	19.34	25.14	23.90	24.14	28.94	31.18	26.93	27.64	24.30
18:2	--	46.41	43.28	41.60	41.58	42.16	36.26	33.85	33.61	37.42	40.03
20:4	--	4.30	4.63	3.98	2.05	4.97	3.74	4.12	4.90	3.75	6.19
						Subject Number 4					
12:0	0.64	0.62	0.32	0.46	1.20	0.61	1.13	1.13	0.26	0.10	0.36
14:0	2.34	1.48	0.72	1.14	1.60	0.99	1.12	1.20	1.20	0.99	0.74
16:0	24.60	16.84	22.04	20.16	17.54	19.24	21.23	18.14	18.28	17.08	18.51
16:1	3.38	4.60	3.64	4.38	3.06	4.28	3.28	3.14	3.63	4.56	4.35
18:0	6.77	2.62	3.98	4.16	1.34	5.39	5.26	3.86	5.54	3.86	3.07
18:1	23.34	26.86	25.40	26.74	25.36	24.26	26.80	26.50	23.41	28.46	27.82
18:2	36.70	45.26	39.68	41.19	47.70	38.66	37.20	42.88	42.38	39.30	40.62
20:4	2.23	1.72	4.22	1.77	2.20	6.57	3.98	3.15	5.30	5.65	4.53

Table 12 (continued)

Acid	Week 1	2	3	4	5	6	7	8	9	10	11
					Subject Number 5						
12:0	0.24	0.16	0.36	0.68	0.25	0.30	0.69	0.53	--	0.36	0.25
14:0	0.66	0.75	0.72	1.13	0.86	0.74	1.92	0.78	0.86	1.44	0.95
16:0	21.82	23.14	21.09	20.41	19.95	23.36	19.50	18.71	18.88	18.40	20.44
16:1	3.16	3.44	2.64	4.13	3.54	2.92	4.16	3.74	4.36	4.70	4.93
18:0	5.00	4.96	5.18	2.54	2.50	4.30	2.62	2.32	3.42	2.00	4.14
18:1	27.77	26.76	23.86	31.88	24.06	29.14	29.81	30.38	29.83	28.00	30.76
18:2	39.13	37.66	40.18	37.41	44.92	35.61	40.72	40.76	42.65	43.06	36.36
20:4	2.22	3.13	5.97	1.82	3.92	3.63	0.58	2.78	--	2.04	2.17
					Subject Number 6						
12:0	0.64	0.46	0.39	--	0.44	0.16	0.69	0.26	0.69	0.14	0.20
14:0	1.52	1.28	1.03	1.00	1.72	1.32	1.56	0.92	1.12	1.16	1.00
16:0	20.02	17.02	20.14	23.36	20.08	20.71	18.84	19.39	18.84	16.44	17.98
16:1	3.74	3.72	3.38	3.32	3.48	4.02	4.66	3.58	3.31	4.10	4.25
18:0	3.67	1.92	5.62	6.58	3.94	4.70	2.18	4.55	5.68	3.53	3.68
18:1	25.08	26.74	23.25	22.72	26.10	25.00	26.30	28.16	22.84	23.78	24.63
18:2	40.76	45.51	40.50	34.86	37.86	35.20	40.93	36.87	39.56	43.64	43.06
20:4	4.57	3.35	5.69	8.16	6.38	8.89	4.84	6.27	7.96	7.21	5.20

Table 12 (continued)

Acid	Week 1	2	3	4	5	6	7	8	9	10	11
					Subject Number 7						
12:0	0.48	0.40	0.07	0.25	0.08	0.10	0.48	0.48	0.34	0.22	0.22
14:0	2.38	1.08	0.85	1.02	1.64	0.72	1.07	1.26	0.89	0.98	0.67
16:0	27.37	20.06	24.32	19.96	20.50	22.44	20.24	20.90	21.38	19.58	20.59
16:1	3.78	2.24	2.23	4.28	4.06	2.40	3.50	3.56	2.98	3.61	2.70
18:0	5.28	2.60	4.44	3.08	2.70	3.99	3.46	2.68	2.70	2.16	2.88
18:1	27.98	29.02	26.22	30.33	27.06	27.32	25.52	30.31	25.66	27.46	25.95
18:2	31.43	41.88	37.17	38.19	40.82	36.30	39.34	36.61	40.88	42.09	43.13
20:4	1.30	2.72	4.70	2.89	3.12	6.73	6.39	4.00	5.17	3.90	3.86
					Pooled Control Serum						
12:0	0.84	0.43	0.44	0.28	0.31	0.40	0.27	0.32	0.29	0.28	0.31
14:0	1.65	1.63	1.39	1.18	1.55	1.37	1.30	1.28	1.16	1.72	1.43
16:0	22.65	21.76	23.12	24.53	22.56	25.50	22.38	21.31	23.19	23.96	23.09
16:1	4.74	4.05	4.33	4.22	3.99	4.48	4.47	4.32	4.95	4.26	4.30
18:0	4.59	4.55	4.17	4.56	4.50	3.77	4.55	4.22	4.59	4.41	4.78
18:1	29.49	28.19	28.16	30.75	31.09	29.11	27.49	31.77	28.87	29.81	30.47
18:2	31.19	31.44	33.13	27.71	31.72	31.22	32.38	31.77	31.12	31.35	30.16
20:4	4.85	7.95	5.26	6.77	4.28	4.15	7.16	5.01	5.83	4.21	5.46

Table 12.1

VARIABILITY IN SERUM FATTY ACID COMPONENTS

Acid	Weeks	N	Mean(%)	S.D.	S.E.	C.V.
			Subject Number 1			
12:0	10	20	0.48	0.28	0.09	58
14:0	10	20	1.12	0.24	0.08	21
16:0	10	20	20.60	2.91	0.92	14
16:1	10	20	3.95	0.75	0.24	19
18:0	10	20	4.83	1.28	0.40	26
18:1	10	20	25.81	3.24	1.02	12
18:2	10	20	40.06	3.13	0.99	7
20:4	8	16	3.95	1.22	0.43	30
			Subject Number 2			
12:0	10	20	0.60	0.30	0.10	50
14:0	11	22	1.60	0.32	0.10	20
16:0	11	22	22.05	1.29	0.39	5
16:1	11	22	5.22	0.99	0.30	19
18:0	11	22	3.54	1.29	0.39	36
18:1	11	22	26.58	2.59	0.78	9
18:2	11	22	36.38	3.09	0.93	8
20:4	10	20	4.48	1.36	0.43	30
			Subject Number 3			
12:0	10	20	0.54	0.29	0.09	53
14:0	10	20	1.17	0.26	0.08	22
16:0	10	20	21.24	1.17	0.37	5
16:1	10	20	3.64	0.76	0.24	20
18:0	10	20	4.08	1.29	0.41	31
18:1	10	20	25.45	3.35	1.06	13
18:2	10	20	39.62	4.21	1.33	10
20:4	10	20	4.26	1.07	0.34	25
			Subject Number 4			
12:0	11	22	0.62	0.38	0.11	61
14:0	11	22	1.23	0.45	0.14	37
16:0	11	22	19.42	2.39	0.72	12
16:1	11	22	3.85	0.60	0.18	15
18:0	11	22	4.17	1.52	0.46	36
18:1	11	22	25.90	1.70	0.51	6
18:2	11	22	41.05	3.34	1.01	8
20:4	11	22	3.76	1.67	0.50	44

Table 12.1 (continued)

Acid	Weeks	N	Mean(%)	S.D.	S.E.	C.V.
			Subject Number 5			
12:0	10	20	0.38	0.19	0.06	49
14:0	11	22	0.98	0.38	0.12	38
16:0	11	22	20.52	1.70	0.51	8
16:1	11	22	3.79	0.73	0.22	19
18:0	11	22	3.54	1.21	0.36	34
18:1	11	22	28.39	2.62	0.79	9
18:2	11	22	39.86	2.95	0.89	7
20:4	10	20	2.83	1.46	0.46	51
			Subject Number 6			
12:0	10	20	0.41	0.21	0.07	52
14:0	11	22	1.24	0.26	0.08	21
16:0	11	22	19.35	1.89	0.57	9
16:1	11	22	3.78	0.43	0.13	11
18:0	11	22	4.19	1.43	0.43	34
18:1	11	22	24.96	1.75	0.53	6
18:2	11	22	39.89	3.44	1.04	8
20:4	11	22	6.23	1.71	0.51	27
			Subject Number 7			
12:0	11	22	0.28	0.16	0.05	57
14:0	11	22	1.14	0.49	0.15	42
16:0	11	22	21.58	2.53	0.71	10
16:1	11	22	3.21	0.74	0.22	22
18:0	11	22	3.27	0.94	0.28	28
18:1	11	22	27.55	1.77	0.53	6
18:2	11	22	38.89	3.41	1.02	8
20:4	11	22	4.07	1.61	0.49	39
			Pooled Control Serum			
12:0	11	22	0.38	0.16	0.05	43
14:0	11	22	1.42	0.19	0.06	13
16:0	11	22	23.10	1.21	0.36	5
16:1	11	22	4.37	0.28	0.08	6
18:0	11	22	4.43	0.28	0.08	6
18:1	11	22	29.56	1.35	0.41	4
18:2	11	22	31.20	1.38	0.42	4
20:4	11	22	5.54	1.27	0.38	22

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