Serum Lipids of Men Fed Diets Differing in Protein Quality and Linoleic Acid Content

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THERE is experimental evidence that dietary alterations in each of the three major food components, fat, carbohydrate and protein, can affect serum lipid levels in man.[1–3] Of the studies carried out with protein, variable results have been reported. Olson et al.[3] found decreased serum cholesterol in subjects when the daily protein content of their diet was reduced from 100 gm. of a mixture of animal and vegetable proteins to 25 gm. of vegetable proteins. Walker et al.[4] demonstrated significant decreases in serum cholesterol and phospholipids during some test periods when subjects received protein mainly from vegetable foods as compared with test periods when the chief source of protein was animal products. Albanese et al.[5] added a casein-containing supplement to the diet of older convalescent men and observed frequent increases in serum cholesterol. However, Keys and Anderson[6] increased the protein content of an experimental diet by an isocaloric substitution of skim milk powder for carbohydrate and reported no effect on the serum cholesterol level of subjects receiving this diet. Beveridge et al.,[7a] substituting calcium caseinate for carbohydrate in a formula-type diet, obtained similar results.

Beveridge[7a] has pointed out that since animal and vegetable foods have a variable lipid content, purified proteins must be used in experimental diets if changes observed in serum lipids are to be attributed solely to the protein parameter. To avoid the difficulties inherent in using food as a protein source and also to avoid changing the carbohydrate content of the diet, we attempted to alter the protein nutritive quality of the diet by substituting purified animal-type proteins for a purified vegetable protein. The possible effect of the kind of protein on serum lipids was tested by comparing a diet containing wheat gluten as the chief source of nitrogen with a diet containing an isonitrogenous amount of a mixture of casein and lactalbumin as a replacement for wheat gluten. The vegetable and animal proteins were each tested in diets with an assortment of fats having a 12 per cent linoleic acid content for one period of study and a 40 per cent linoleic acid content for another period.

SUBJECTS AND METHODS

Seven men ranging in age from fifty-three to seventy years and in weight from 59 to 84 kg. were selected to receive the diets. They resided in a metabolic balance ward during the study and their level of physical activity was relatively low. A medical examination showed each subject to be in a satisfactory state of health. Five men participated in all the experimental dietary periods and two men each in a different half of the study, giving a total of six subjects for each dietary treatment.

The entire study consisted of four experimental diet treatments, each of the two proteins being fed with each of the two fat mixtures. Subjects were maintained on a self-selected diet of ordinary foods for five days. For a succeeding fifty day period, subjects were fed the experimental diet, containing one of the fat assortments, supplemented with gluten

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TABLE I
Daily Menus for Experimental Dietary Periods

Breakfast*		Dinner*		Supper*	
Item	Amount (gm.)	Item	Amount (gm.)	Item	Amount (gm.)
Orange juice	100	Hash brown potatoes†	117	Bouillon	1
Biscuits†‡	86	Raw celery	25	Noodles with tomatoes†	120
"Blend B"§	15	Canned green beans	50	Lettuce	25
Canned applesauce	100	Biscuits†‡	86	Canned peaches	100
		"Blend B"§	5	White bread	20
		Canned pears	100	"Blend B"§	20
		Mints	0–25	Pudding†‡	175

* Each meal also included Sanka, sugar, jelly and 1.5 gm. of a mineral mixture prepared according to Rose.[13] One Unicap (Upjohn) multivitamin capsule was given per day.
† Containing "Blend A," the fats other than butter and margarine.
‡ Containing 1.6 gm. nitrogen. The weight for biscuits is weight unbaked.
§ The mixture of butter and margarine.

for the first twenty-five days and with a casein-lactalbumin mixture for the second twenty-five days. After another five-day period on ordinary foods, subjects were given the other fat assortment containing a different amount of linoleic acid for a fifty day period, twenty-five days with the gluten supplement and twenty-five days with the casein-lactalbumin mixtures. Because of limited space in the metabolic ward, only one or two subjects were studied at a time. Some subjects were given the fat assortment with the lower linoleic acid level first and some began with the higher level. The order in which the two proteins were given at each level of linoleic acid intake also was varied.

The experimental proteins* were obtained at the beginning of the study and the casein and lactalbumin were mixed in a large mixer. Throughout the study, the level of dietary nitrogen was maintained at 6.5 gm per day, of which the experimental protein provided 4.8 gm.

Fat provided 40 per cent of the total calories throughout the experiment. Two fat assortments differing in linoleic acid content were prepared at the beginning of the study. Assortment I was planned to approximate the fat composition of the average diet in the United States and contained in per cent: butter, 25; margarine, 7; rendered beef fat, 17; hydrogenated vegetable oil, 12; lard, 31; cottonseed oil, 9. For preparing assortment II, 32 gm. of safflower oil was added to 68 gm. of assortment I. In the interests of palatability, butter and margarine were mixed separately and used as a spread. At the beginning of the study the assortments were prepared in an institutional type mixer, packaged in one and a half gallon tubs and stored at approximately −20°c. The linoleic acid concentration as determined by gas liquid chromatography was 12 per cent in assortment I and 40 per cent in assortment II.

Menus for the study were unchanged from day to day and are shown in Table I. Biscuits served as carrier for the experimental protein at the morning and noon meals, and a starch-thickened pudding contained the protein at the evening meal. Daily caloric intakes varied slightly, ranging from 2,250 to 2,360 kcal. When caloric adjustment was necessary to maintain weight, it was achieved by changing the amount of experimental fat to the level providing 40 per cent of the calories and by varying the amount of wheat starch in the biscuits. All subjects received the same number of calories from sugars.

Nitrogen balance studies were carried out by the Kjeldahl method.[7b] Dietary nitrogen was determined on one-day food composites prepared every five days. Urinary nitrogen was determined daily and fecal nitrogen for each five-day period.

Fat in food and feces was analyzed by the Van de Kamer method.[8] Dietary fat was determined for one-day food composites prepared in alternating five-day periods and fecal fat for each five-day collection.

Fasting blood samples were drawn at least once during the five-day foreperiod and at the conclusion

* Gluten: Vicrum, Hercules Powder Co. Casein-lactalbumin: 80 per cent high-nitrogen casein, Sheffield Chemical Company. 20 per cent lactalbumin, Nutritional Biochemicals Co.

TABLE II
Serum Lipid Values* for the Various Experimental Diets

Serum Component	Self-Selected (mg./100 ml.)	12% Linoleic Acid (mg./100 ml.)		40% Linoleic Acid (mg./100 ml.)	
		Gluten	Casein-Lactalbumin	Gluten	Casein-Lactalbumin
Total lipid	815 ± 135	756 ± 210	685 ± 173	785 ± 293	667 ± 211
Cholesterol	258 ± 28	232 ± 33	250 ± 32	218 ± 26	209 ± 37
Sterol ester	217 ± 23	195 ± 36	200 ± 33	192 ± 45	169 ± 26
Glyceride	207 ± 51	204 ± 71	169 ± 62	268 ± 131	210 ± 87
Phospholipid	299 ± 62	272 ± 95	238 ± 72	237 ± 94	208 ± 78
Unesterified fatty acids† (in μ Eq/-liter serum)[2]	762 ± 192†	795 ± 202†	788 ± 166†	804 ± 114†	818 ± 143†

* Each value is the mean value for six men ± standard deviation.
† Based on palmitic acid. Values given in μEq. per L. serum.

of each twenty-five day period. The serum was frozen at −20°c. in tubes with screw caps. When a serum sample was thawed, portions were used immediately for preparation of filtrate for cholesterol determination and for extraction of total lipids. Cholesterol was measured by the procedure of Sperry and Webb[9] and lipids were extracted and fractionated by the procedure described by Fillerup and Mead.[10] The remainder of the serum (under nitrogen) was refrozen immediately and held in the freezer until a convenient number of samples was on hand for determination of unesterified fatty acids by Dole's method.[11]

Fatty acid methyl esters of the glyceride, sterol ester and phospholipid fractions were prepared by the method of Stoffel et al.[12] and subjected to gas liquid chromatographic analysis. A Barber-Colman chromatograph, Model 10, with a packed column* and an argon ionization detector with a radium source was used.

RESULTS

The substitution of wheat gluten for the casein-lactalbumin mixture appeared to have an effect on nitrogen balance that was not influenced by the type of fat in the diet. For the five subjects who completed the entire study, the mean nitrogen balance values during the last fifteen days of both wheat gluten-containing dietary periods were −0.22, −0.93, +0.49, +0.42 and −0.12 gm. nitrogen per day. In the same order, the mean nitrogen balances for similar time intervals with the casein-lactalbumin supplemented diet were +0.28, +0.07, +0.85, +1.26 and −0.15 gm. nitrogen per day. The first four of the five subjects as listed showed a significant decrease in nitrogen retention ($P < 0.001$ by t test) during the administration of the wheat gluten, an indication that (based on the criterion of nitrogen balance) this diet was less adequate than the diet containing the casein-lactalbumin mixture in meeting protein needs.

The mean fecal fat excretion was 3.8 gm. per day with the lower level of linoleic acid and 3.2 gm. per day with the higher linoleate concentration. The values were not significantly different and are well within the normal limits of fat excretion.

The values obtained for the serum lipid fractions from blood serum drawn at the conclusion of the various experimental diet periods are given in Table II. There were no significant differences in total lipids, sterol esters, glycerides, phospholipids or unesterified fatty acids associated with any of the dietary treatments. In the case of serum cholesterol, the values observed with the casein-lactalbumin diet did not differ significantly from those observed with the wheat gluten diet, regardless of the linoleic acid content. However, since the change in dietary fat had a greater effect when the diet contains casein-lactalbumin (from a mean of 250 to 209 mg. per 100 ml.) than wheat gluten (from a mean of 232 to 218 mg. per 100

* 20 per cent ethylene glycol succinate polyester on silanized Chromosorb, 80–100 mesh.

ml.), the data were also tested for an interaction between the kind of dietary protein and the composition of dietary fat by the method of linear contrasts. No significant interaction was found. When the cholesterol values obtained during all periods in which the diets contained the higher level of linoleic acid were compared with those obtained during periods of self-selected diet, there was a significant difference ($P < 0.05$ by t test). This confirms the results of other workers that use of a highly unsaturated dietary fat is associated with a decrease in serum cholesterol.[1,14-16]

In conclusion, the ingestion of two diets differing in kind of protein and in nutritive quality of protein as judged by nitrogen balance criteria could not be shown to have any effect on the levels of various serum lipid fractions with the number of subjects and the experimental conditions used in this study.

Serum fatty acid patterns were also determined in sterol ester, glyceride, and phospholipid fractions at the two concentrations of dietary linoleic acid and with both dietary proteins. The type of dietary protein had no effect on the distribution of fatty acids in any of the fractions and hence the results are not tabulated. However, again in confirmation of other workers,[15,17] in all three fractions the percentage of linoleic acid increased with the increase in dietary linoleic acid from 62 to 73 per cent in the sterol esters, from 13 to 26 per cent in the glycerides, and from 23 to 28 per cent in the phospholipids. The increase in linoleate was accompanied by a decrease in the per cent of oleate in all three fractions and by a decrease in palmitate in the phospholipid and sterol ester fractions.

SUMMARY

When wheat gluten was substituted for a casein-lactalbumin mixture as the chief source of nitrogen in an experimental diet, less nitrogen was retained in four of five subjects tested. With six subjects studied and under the experimental conditions employed, this treatment had no significant effect on the serum content of total lipids, sterol esters, glycerides, phospholipids or unesterified fatty acids. This situation pertained whether the diet contained 12 per cent of linoleic acid or 40 per cent of linoleate as fat. However, serum cholesterol levels were lower and the per cent of linoleate in the sterol ester, glyceride and phospholipid serum fractions was increased when the subjects were fed diets containing the larger amount of the polyunsaturated fatty acid with either type of protein.

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