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Received March 31, 1958. P.S.E.B.M., 1958, v98.

Effect on Serum Cholesterol in Man of Mono-Ene Fatty Acid (Oleic Acid) in the Diet.* (24052)

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From analysis of data from 41 sets of dietary comparisons in controlled experiments on groups of 12 to 27 men, we derived the equation: 1) Δ Chol. $= 2.74\ \Delta S - 1.31\ \Delta P$, for average change in serum cholesterol concentration when diet is changed in fats 2 to 4 weeks, ΔS being the difference between percentages of total calories provided in the 2 diets from glycerides of saturated fatty acids and ΔP being corresponding difference in glycerides of poly-unsaturated fats(1,2). This equation satisfactorily predicted group average responses of men in calorie equilibrium with constant amounts of protein and vitamins but with different amounts and kinds of fats in the diet. The original approach to this solution included provision for mono-enes (M) in the diet in the equation form: 2) Δ Chol. $= b\Delta S + c\Delta M + d\Delta P$. However, in all analyses the value of coefficient c proved not significantly different from zero, *i.e.* no influence of mono-ene could be proved, so final analysis was made in the form of Equation 1), above. After Equation 1) appeared, Ahrens *et al.*(3) published results from formula feeding experiments and more recently(4) they agree that their data satisfactorily conform to Equation 1). But they state that Equation 1), "could have been expressed just as accurately, and less mysteriously, in terms of iodine value of dietary fat" (ref. 4, p. 253). Further, they question the lack of effect of dietary mono-ene (oleic acid) on serum cholesterol level in man. *Iodine Value* vs. *2S-L.* Ahrens *et al.*(4) propose, in effect, that Equation 1) be simplified by approximation: 3) Δ Chol./1.35 $= 2\Delta S - \Delta L$, rounding the coefficients and using L to refer to linoleic acid, since almost all the poly-unsaturated fatty acid in most ordinary food fats is actually linoleic acid. Further, they

* This work was supported in part by National Dairy Council, Chicago, Minn. Heart Assn. and Winton Companies Fund, Minneapolis.

note that the iodine value of oleic acid (M) is 0.9 g. that of linoleic acid is 1.8/g, and the iodine value, $V = 0.9\,M' + 1.8L'$, where M' and L' are percentages of these fatty acids in total fat. It follows that V is a linear function of S'–L' where $S' = 100 - M' - L'$. But it does not follow that S'–L' is substantially the same as 2S'–L' and an even more egregious error is the assumption that S' and L' are equivalent to S and P in Equation 2). S and P are percentages of total calories in the diet furnished by glycerides of saturated and linoleic (or poly-unsaturated) fatty acids; S' and L' are percentages of FAT made up by these acids. Assuming that the glycerides contain 95.6% fatty acids, and taking for illustration the situation where calorie equilibrium is maintained at 3000 Cal. daily, we can re-write Equation 3) for this situation: 4) $2.36\,\Delta$ Chol. $= 2\Delta S'' - \Delta L''$, where S'' and L'' are grams of saturated and linoleic acids. In the situation where 50 g each of saturated and linoleic acids in the diet are replaced by 100 g of oleic acid, there is no change in iodine value but from Equation 4) we have 4a) $2.36\,\Delta$ Chol. $= 2(0-50) - (0-50) = -50$, and an average serum cholesterol fall of 21.2 mg 100 ml is predicted. In a 3000 Cal. diet as above, first 60 g each of saturated and linoleic acids and then only 10 g each of these could be fed replacing the 100 g of fatty acid with equivalent calories (941 Cal.) as carbohydrate. Again there is no change in iodine value, and hence no effect according to Ahrens *et al.*, but from Equation 4) we have: 4b) $2.36\,\Delta$ Chol. $= 2(10-60) - (10-60) = -50$, and again the prediction is an average fall of 21.2 mg of cholesterol/100 ml of serum. Elsewhere(2) we indicated how iodine values *plus* values for total grams or calories of fat might be used to make predictions in cases like the above. But we also showed that an equation in these terms is theoretically and actually inferior because oleic acid is not simply half of linoleic acid in cholesterol effect.

TABLE I. Composition of the Experimental Fats as g of the Glycerides/100 g of Each of the 3 Natural Fats and as g/32 g of Mixture of 52.8% Oleostearine and 47.2% of Safflower Oil.

(Analyses made by Research Division of Procter and Gamble Co., Miami Valley Labs.)

Class of fatty acid	Olive oil	Oleo-stearine	Saf-flower	Mixture
Saturated	13	67	12	13
Mono-ene	75	31	10	7
Poly-ene	12	2	78	12
Total	100	100	100	32

TABLE II. Composition of Fat Extracted from 4 Menus of Basic L.F. Diet as Served in Standard Portions to Provide 3200 Calories Daily.

	Menu 1	Menu 2	Menu 3	Menu 4	Mean
Iodine value	58.8	51.1	61.4	61.4	58.1
% of fatty acids					
Saturated	45.9	54.4	42.1	42.6	46.0
Mono-ene	44.6	36.4	49.5	47.9	44.6
Poly-ene	10.5	9.2	8.4	9.5	9.4

Methods. A critical test of the effect or lack of effect of oleic acid glyceride is to feed a diet in which it is isocalorically substituted for carbohydrate. Equation 1) predicts that there would be no significant change in serum cholesterol. One experimental fat diet (O.O.) was high in oleic acid provided by substituting, daily, 100 g olive oil for 900 Cal. of simple carbohydrates in the L.F. (low-fat) diet. The other experimental fat diet (S.O.) was made by substituting for 288 Cal. of carbohydrate in the L.F. diet, 32 g of a mixture of 47.2% safflower oil and 52.8% of oleostearine so as to match the O.O. diet in respect to saturated and poly-ene fats, but to be very much lower in oleic acid glyceride. Table I shows the composition of the several fats used. The calorie difference between O.O. and S.O. diets was balanced by carbohydrates. Gelatine was added to the O.O. diet to correspond with the very small amount of protein in carbohydrate foods in the S.O. diet but not in O.O. diet. Otherwise S.O. and O.O. diets were identical. 22 men aged 42 to 62 years were selected and controlled at Hastings State Hospital as previously described(2,5). After 2 weeks on L.F. diet they were divided into groups A and B, matched in regard to age, relative body weight, psychiatric diagnosis and habitual physical activity. Group A was transferred to the S.O. diet, Group B to the O.O. diet. After 2 weeks the diets of the groups were reversed, for a final 2 weeks.

TABLE III. Means of Actual Intakes on O.O. and S.O. Diets and Estimates for Low Fat Diet Based on Measured Servings on Low Fat Diet and Individual Adjustments as Made on O.O. and S.O. Diets. Values in g/day and as % of total calories. Also mean total daily calories for each period and mean body weights at ends of these periods.

Item	L.F. diet g	L.F. diet % cal.	O.O. diet g	O.O. diet % cal.	S.O. diet g	S.O. diet % cal.
Protein	115.0	14.1	112.5	13.5	110.4	13.8
Fat	34.0	9.5	134.3	36.3	65.1	18.3
Saturated	15.2	4.2	30.6	8.3	29.7	8.4
Mono-ene	15.0	4.2	89.9	24.3	22.1	6.2
Poly-ene	3.8	1.1	13.8	3.7	13.4	3.7
Total calories	3260		3330		3190	

	Period I	Period II	Period III
Total calories per day	3260	3280	3240
Body wt (kg)			
Group A	67.8	67.5	67.8
" B	65.9	65.7	66.1

Blood samples were drawn from each man twice on 2 different days at end of each of 3 dietary periods. Serum total cholesterol was determined in duplicate, as described previously(6). In addition, serum phospholipid was estimated in duplicate on each sample. The serum sample (0.2 ml) was extracted with hot Bloor's mixture, filtered, the filtrate evaporated to dryness. The dry residue was extracted with hot chloroform and the analysis proceeded as in the method of Fiske and Subbarow(7). Table II shows the composition of the L.F. diet as served in 4 basic menus. In each case the complete menu was ground and homogenized, adding water as needed. Total lipid was measured in one set of aliquots, another being used for estimation of fatty acids. For the latter the slurry was lyophilized and the dry powder was saponified with alcoholic KOH, acidified and extracted at room temperature with ethyl ether. The ether was evaporated from the filtrate and the residue re-extracted with petroleum ether, the final analysis being made with official methods of the American Oil Chemists' Society. All of these manipulations were carried out at low temperature in an atmosphere of nitrogen. Table III shows average daily nutrients actually consumed on O.O. and S.O. diets and an estimate for nutrient intake on L.F. diet based on measured servings on L.F. diet and individual adjustments in Periods II and III. Master menus, from which basic servings were taken, were fixed at an average of 3200 Cal. in each of the 3 diets. The "adjustments" were small amounts of bread or jelly, or both, added to or removed from individual rations as needed to maintain weight constancy, and allowances for occasional plate waste. Small discrepancies between averages for O.O. and S.O. in Table II and the planned level of 3200 Cal. represent these adjustments which were recorded in Periods II and III but not in Period I (L.F.). In view of the high degree of weight constancy in all periods, it seemed safe to apply the average of these adjustments to Period I, but the effect is trivial in any case. It will be observed that amounts of saturated and poly-unsaturated fats eaten on O.O. and S.O. diets were substantially identical but the O.O. diet contained 67.8 g of oleic acid glycerides/day more than in the S.O. diet. Further, O.O. and S.O. diets provided about 3 times as much linoleic acid as the L.F. diet.

Results. Cholesterol and phospholipid data from this experiment are given in Table IV for each of 22 men at the end of each of 3 dietary periods. There was a general rise in serum cholesterol level when diet was changed from L.F. to either O.O. or S.O. Apparently this effect was incomplete at end of 2 weeks and progressed further during succeeding 2 weeks but, because of switchback design of the experiment, it is possible to compare the 2 experimental fats without interference by this trend. Mean cholesterol was 171.15 on S.O., 171.20 on O.O. diet. The mean for both groups on L.F. diet was 158.7 $\pm$ 4.9, while that on O.O. and S.O. diets was 171.2 $\pm$ 4.1. The difference between these means is 12.5 $\pm$ 6.3, and $p = 0.04$.

A more sensitive test of significance is to analyze results in terms of differences for each man on the several diets. The time trend from Period II to Period III complicates application of this method but correction can be made because it was not significantly different (+ 10.1 for Group A and + 10.0 for Group B). This method of individual differences also fails to show any difference in cho-

TABLE IV. Total Cholesterol and Phospholipid Concentrations, mg per 100 ml of Serum, at the Ends of the 3 Dietary Periods. Type of diet is indicated in parentheses at heads of columns.

	Cholesterol			Phospholipid		
Group	Nov. 12-14 (L.F.)	Nov. 26-27 (S.O.)	Dec. 10-12 (O.O.)	Nov. 12-14 (L.F.)	Nov. 26-27 (S.O.)	Dec. 10-12 (O.O.)
A	130	154	181	140	134	130
	166	170	168	107	124	125
	127	132	140	90	100	112
	182	180	190	120	145	126
	169	165	194	126	141	156
	136	142	150	106	110	120
	196	224	226	130	147	148
	187	166	199	130	114	131
	211	215	207	145	144	144
	156	140	143	111	110	102
	165	183	184	132	131	130
Mean	166.4	170.1	180.2	121.5	127.3	129.5
S.E.	± 8.0	± 8.9	± 2.2	± 5.0	± 5.0	± 4.7
	(L.F.)	(O.O.)	(S.O.)	(L.F.)	(O.O.)	(S.O.)
B	170	186	186	131	128	144
	148	163	185	128	136	152
	139	140	131	122	107	104
	152	140	174	115	110	140
	149	148	156	108	85	103
	133	150	148	101	114	114
	156	188	208	100	136	144
	144	174	170	118	124	132
	128	142	138	108	109	102
	152	180	186	99	122	132
	190	173	212	120	117	154
Mean	151.0	162.2	172.2	113.6	117.0	129.1
S.E.	± 5.2	± 5.7	± 8.1	± 3.4	± 4.4	± 6.0
A + B (N = 22)						
Mean	158.7	166.1	176.4	117.6	122.2	129.3
S.E.	± 4.9	± 5.2	± 4.2	± 3.1	± 3.5	± 3.7

lesterol values on O.O. and S.O. diets; the difference is zero and standard error is ± 3.1. Applying this method to comparison of L.F. with both O.O. and S.O. diets, shows that the mean: (O.O + S.O. diets) − (L.F. diet) = 12.5 ± 2.9, with $p < 0.001$.

The serum phospholipid values in general were parallel with the cholesterol values and there were no significant changes in cholesterol/phospholipid ratio, the averages being 1.4, 1.3, and 1.4 on the L.F., O.O., and S.O. diets, respectively. The mean phospholipid values were 117.6 ± 3.1, 123.3 ± 3.4 and 128.2 ± 3.8, on the L.F., O.O. and S.O. diets, respectively. The difference, S.O. − O.O., is 4.9 ± 5.1. The phospholipid rise in going from L.F. to the O.O. and S.O. diets is highly significant, the difference being 8.2 ± 3.0, with $p = 0.01$.

Discussion. The olive oil used is typical in fatty acid composition of the majority of olive oil samples considered by Hilditch(8) who notes, however, the report of Cattaneo *et al.* of a variation, according to maturity of the fruit, from 14.6% saturated and 6.6 linoleic, to 15% saturated and 22.5% linoleic. According to Equation 1), the former oil would have a small cholesterol-promoting effect when substituted for carbohydrate in the diet, whereas the latter would have very little effect. At a substitution level of 100 g/day and in balance at 3000 Calories, Equation 1) would predict an average rise in 2 to 4 weeks of around 10 mg of cholesterol/100 ml of serum from the former oil and only about 4 mg from the latter. It is understandable, therefore, that Bronte-Stewart *et al.*(9) could show no effect on serum cholesterol when olive oil was introduced into a low-fat diet for a few days in the diets of 2 Bantu men.

From a study of 2 persons on formula diets, Kinsell *et al.*(10) concluded that triolein and

ethyl oleate are not equivalent in effect on cholesterol of half of the corresponding linoleic acid compounds. They cite us as arguing that "sufficient oleate would be quite as effective as linoleate." A major point in our refs. 1) and 2) was precisely the reverse. Malmros and Wigand(11) observed in 11 subjects a fall in serum cholesterol when olive oil was substituted for hydrogenated coconut oil in the diet and a further fall when corn oil was substituted for olive oil. These results are all predicted, at least semi-quantitatively, by Equation 1). Ahrens *et al.*(4) insist that effect of dietary fats on serum cholesterol is related to iodine value as closely as, or better than, our expression 2S-L, and they showed that for fats they used, there is a rough inverse correlation between iodine value and 2S-L *when total fat is constant.* The iodine values of fats in our diets were: L.F.—58, O.O.—75, S.O.—65 and the mean cholesterol values on these diets were L.F.—158.7, O.O. —171.2, S.O.—171.2. Obviously, in this set of diets the serum cholesterol levels bear no relationship to the iodine value.

Finally, it is interesting to apply Equation 1) in the prediction of cholesterol results in this experiment. First, of course, is the prediction of no difference between the O.O. and the S.O. diets, and this is confirmed. Second, Equation 1) predicts a rise in changing from L.F. to either O.O. or S.O. and this is also confirmed. The mean cholesterol rise from the L.F. diet to O.O. and S.O. diets predicted from Equation 1) is 7.4. The standard error of estimate of the prediction equation was S.E.E. $= \pm 5.0$ when tested with the 41 sets of data used in deriving Equation 1). Hence we may write: Δ Observed $= 12.5 \pm 2.9$, Δ Predicted $= 7.4 \pm 5.0$.

Summary. 1) A controlled dietary experiment was performed on 22 men who subsisted first on a low-fat diet and then, in a switch-back arrangement of 2 matched groups of 11 men each, for periods of 2 weeks, on each of 2 diets differing by 67.8 g daily of mono-ene, the difference being matched by simple carbohydrate calories. The 2 diets were identical in all other respects and calorie equilibrium was maintained throughout all 3 dietary periods. 2) Results confirmed a prediction equation in showing no cholesterol difference on the 2 experimental fat diets, in spite of differences in iodine value and oleic acid content. Serum cholesterol rose significantly when the diet was changed from low fat to either of other diets and this change also conformed to that predicted by the equation. Serum phospholipid in general responded in parallel to cholesterol responses.

We are grateful to A. Giurlani and Bro., San Francisco, for generously providing olive oil and to Drs. L. W. Beck, F. H. Mattson and P. W. Ifland of Procter and Gamble Co., Miami Valley Labs., for analyses of oils used. At Hastings State Hospital, we are indebted to volunteers from the Brethren Service Com. for constant aid in management of patients and their diets.

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Received April 2, 1958. P.S.E.B.M., 1958, v98.