Effect of a Therapeutic Regime on Hyperchylomicronemia and Hypercholesterolemia

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THE PRIMARY object of this investigation was to study some of the effects of the so-called lipotropic substances on fat metabolism in general and on disease conditions allegedly associated with derangement of fat metabolism.

There are two possible approaches to the study of serum lipids. The lipoid substances are distributed over the wide spectrum of the colloidal suspension; these lipoprotein macromolecules range from the most minute ones first fractionized by Pederson[1] to the largest chylomicrons well within the visible spectrum first studied in greater detail by Frazer and associates.[2,3] Thus, through the extraction methods, one may ascertain the total amount of a given lipid material contained in various proportions in the macromolecules of varying size and density; or, using as a yardstick some of the physical properties, one may attempt to determine the level of a given class of suspended lipoprotein molecules. In this study, both approaches have been applied, using the size of the particles and the overall levels of total and esterified cholesterol as indices.

A distinction has been made in this report between atherosclerotic patients (cases of proved myocardial infarction) and presumably "normal" subjects. This clinically arbitrary division was dictated by two facts: (1) elevated serum cholesterol levels have been reported to correlate with an increased tendency toward atherosclerosis[4,5]; (2) the increased tendency to myocardial infarction appears to correlate in a statistically significant manner with elevated fasting chylomicron levels.[6-9]

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MATERIALS AND METHODS

Sixteen patients with electrocardiographically proved myocardial infarction in the past were initially put on a lipotropic regime. All but two of them were males and all but one Caucasian. Their mean age was 48.8 years. Of this group, 11 remained on the lipotropic regime for nine weeks or longer, and the data on these 11 are considered for evaluation.

Because we have observed a significant increase of the chylomicron levels in presumably non-atherosclerotic females 65 years of age and older, it was deemed advisable to administer the substances to 26 white females with a mean age of 77 years, all of whom are permanent residents of the Old Ladies Home. Of this group, 25 remained on the regime for four weeks, 25 for nine weeks, and 12 for twelve weeks or longer. Up to the time of this report, five control subjects, non-psychotic in-patients at a State Hospital, have been receiving a placebo for four to six weeks. We expect to follow a larger group for a longer period.

Determinations of total serum cholesterol, esterified cholesterol, and chylomicron concentration form the basis of this report. The cholesterol determinations were made according to modified Bloor's technique. Chylomicron concentration refers to the percentage of lipoprotein particles, 0.3 micron in diameter and larger, microscopically visible under proper dark field setup. Our technique was a modification[7] of the technique cited by Zinn and Griffith[6] and was uniformly carried out by the same personnel.

At least two laboratory workups were performed on all the subjects prior to administra-

tion of lipotropes. All tests were performed on fasting (18 to 20 hours) serum. If any marked deviation occurred, the sample was discarded and the test repeated. No changes of diet, daily habits, etc., were instituted. On our advice several subjects interrupted administration and resumed it again, repeating this procedure several times. Any such change was immediately preceded by a laboratory study.

As this represents the first phase of our experiment, it was considered advisable to administer mixed lipotropic material*, as this approach appeared to have some experimental justification, particularly in the realm of hepatic therapy[10-13]. The arbitrary average daily dose (6–8 capsules per day) provided about 2 Gm. of choline dihydrogen citrate, 800 mg. of methionine, and over 600 mg. of inositol, together with vitamin B_{12} and liver extract. (A study is also being conducted on the effect of individual lipotropic substances administered alone.) Only the subjects who followed the regime strictly were considered.

Results

The problem was studied and the results interpreted by appropriate statistical methods. Changes were evaluated carefully, considering both the normal variability of serum cholesterol levels and chylomicron concentrations in fasting serum and the range of error in the techniques employed. Intermittent periods of therapy served as controls in the same individual.

Usually two independent groups of measurements are compared for significance by using their means and standard deviations to determine whether the ratio of difference between the means to the standard error of the difference of the means is larger or smaller than 2.5 (P = 1 per cent). Since the two groups of values in these tests are not independent, but are on the same individuals tested at separate times, a different method of analysis was used. The method takes into account individual variations rather than group variations, in that the difference between the values before the initiation of the regime and the levels of each person after administration is compared with the mean difference of the "before" and "after" levels for the group. Their standard deviation measurement ("S") was computed by dividing the sum of the squares of the deviations of each individual difference from the overall mean difference by the number of persons measured, less one, and then extracting the square root of the number:

$$S = \sqrt{\frac{\text{sum of squares of deviations}}{n - 1}}$$

Significance was determined by using the mean difference, the "S" value, and Snedecor's "Z" chart,* which estimates the number of pairs of observations necessary for significance. Tables I and II list the values obtained and their respective significance.

In both the infarction and control groups there was a statistically significant depression of chylomicron levels (Table I). This effect seems to become demonstrable three to four weeks after initiation of the regime and continues during the period of administration and for a short time following discontinuation. This "after effect" varies from individual to individual but usually lasts for several weeks. It should be pointed out that the depression appears to be greater in the infarction group than in the non-infarction group as the regime continues. This may be significant, inasmuch as the initial mean chylomicron level for the non-infarction group was 44.56, and for the infarction group 45.15. The similar values of total cholesterol were 316.02 and 307.41, and for cholesterol esters 211.42 and 217.75—all mg. per 100 ml.

In the myocardial infarction group, prolonged administration of the drug (nine weeks and longer) resulted in borderline statistically significant lowering of total serum cholesterol levels (Table II). There was little or no ap-

* Methischol, supplied through the courtesy of U. S. Vitamin Corporation.

* The detailed description of this statistical technique may be found on page 37 of *Statistical Methods* by George W. Snedecor, published in 1940 by Ohio State College Press.

TABLE I

Effect of Administration of Lipotropic Substances on Fasting Chylomicron Levels

Period of administration, weeks	4	9	12
Non-infarction group			
Number of persons	25	24	11
Mean difference	−5.56	−10.36	−8.19
"S" value	4.73	6.34	3.22
Statistical significance	Yes	Yes	Yes
Period of administration, weeks	**Up to 9**		**9 or More**
Infarction group			
Number of persons	11		11
Mean difference	−7.94		−15.72
"S" value	6.16		7.57
Statistical significance	Yes		Yes

− signifies decrease.

TABLE II

Effect of Administration of Lipotropic Substances on Total Serum Cholesterol and Cholesterol Esters

Period of administration, weeks	4	9	12
Non infarction group			
Cholesterol			
Number of persons	25	24	12
Mean difference	+11.16	−0.02	+0.29
"S" value	20.80	20.42	26.50
Statistical significance	Borderline	No	No
Cholesterol esters			
Number of persons	25	24	12
Mean difference	−6.22	+0.79	+4.38
"S" value	20.16	25.27	43.23
Statistical significance	No	No	No
Period of administration, weeks	**Up to 9**		**9 or More**
Infarction group			
Cholesterol			
Number of persons	12		11
Mean difference	−13.46		−25.44
"S" value	30.42		37.50
Statistical significance	No		Borderline
Cholesterol esters			
Number of persons	12		11
Mean difference	+2.25		−14.80
"S" value	28.37		46.50
Statistical significance	No		No

− signifies decrease; + signifies increase.

parent effect on the cholesterol levels when the regime was continued for a period of less than nine weeks. During the first four weeks of administration of the lipotropic compound to the non-atherosclerotic group, there was an increase in total cholesterol levels of borderline statistical significance; subsequent periods of the regime were characterized by minimal variations in the cholesterol levels.

As was mentioned before, the non-infarction subjects were purposely chosen from among females over 65 years of age, since we found that these individuals exhibit chylomicron levels comparable to the values of atherosclerotic subjects in much younger age groups. Thus the starting point in the two groups under consideration in this study was strikingly similar.

The differences obtained, if considered due to the regime instituted, should be viewed against the known mean variability in the chylomicron concentration. This mean for 31 individuals tested on repeated occasions during the first year of our long-range project was 2.3 per cent; for 16 individuals repeatedly tested by the same technical personnel during the second year of the study, it was 1.7 per cent, giving the overall mean of 2.0 per cent. The split samples of the *same test* performed blindly rarely differ by more than 2.0. In our hands, a comparable mean value for cholesterol variation was 17.9 mg. per 100 ml., and 12.9 mg. per 100 ml. for the esterified fraction of cholesterol.

The third test of significance was carried out on four subjects from among the infarction group. The results are presented in Figure 1. It is apparent that the degree to which the lowering of pre-regime chylomicron levels can be obtained varies considerably from subject to subject, but the trend is similar in all of them. This fact is particularly brought out by intermittent discontinuation of the regime.

The figures in Table III show the results in five patients currently under placebo administration.

Discussion

The visible lipoprotein macromolecules and aggregates are only part of a long spectrum of which chylomicrons are the "high end." The

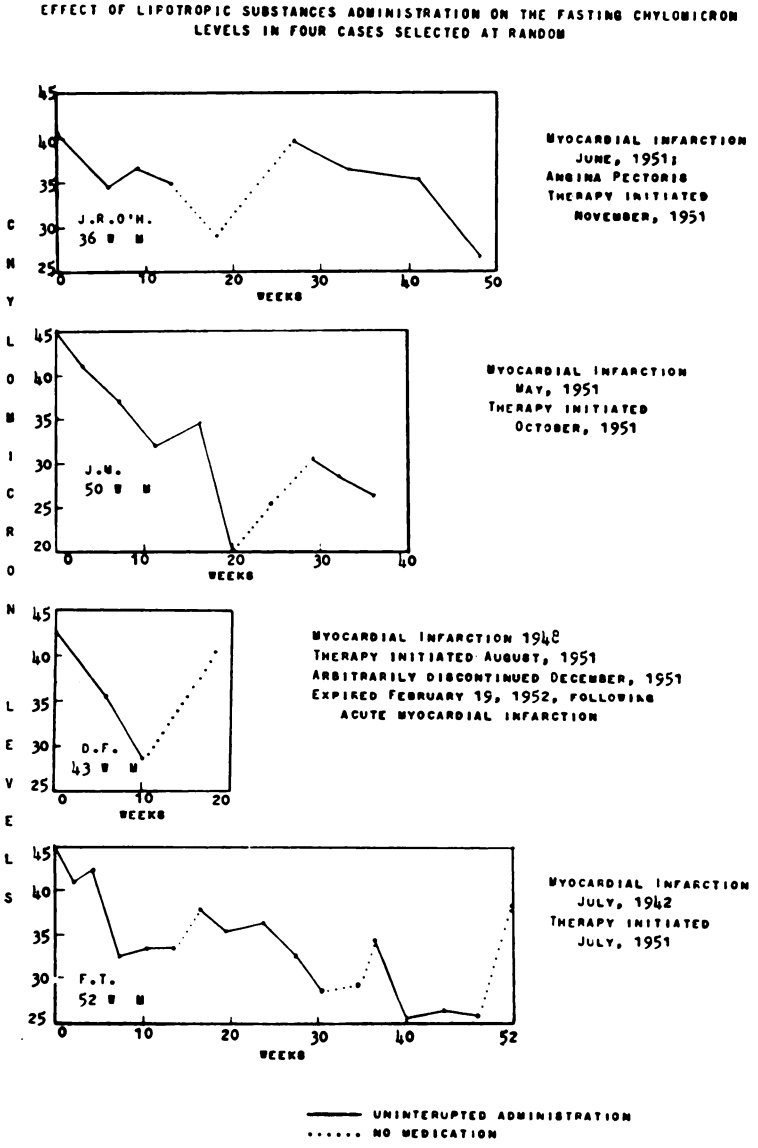

Figure 1

TABLE III
Results of Placebo Administration to Five Patients

Patient	Initial chylomicron level (average)	Length of administration	Level after administration
M. L. W.	40.2	4 weeks	43.8
J. A. G.	46.2	6 weeks	44.8
E. L. S.	29.7	7 weeks	34.6
R. C. McB.	39.2	9 weeks	40.4
R. M.	32.4	1 week	33.3
		5 weeks	33.6

reproducibility of results on the fasting serum (as contrasted with *varying* chylomicron levels in the post-prandial period) reflects the base line which an individual exhibits. The presence of fatty substances is explained by the fact that the systemic lipids do not comprise an inert mass but are part and parcel of a dynamic system. It appears from the classic experiments of Schoenheimer and associates that before fatty acids of dietary origin can be oxidized they are first incorporated into the body fat depots; there is a simultaneous removal of fatty acids from those depots, and this change is both constant and dynamic. This was demonstrated by tagging fatty acids with deuterium. Fats are transported in the form of myriads of macromolecules; the most minute ones studied with the aid of an ultracentrifuge (they may be termed ultramicroscopic) contain considerable amounts of cholesterol per macromolecule, in addition to small amounts of tri-glycerides and proteins (globulin fraction or fractions) and moderate amounts of phospholipids. The greater the size of the particle, the smaller the content of cholesterol, phospholipid, and proteins, with an increase in the neutral fat fraction. A statistically and experimentally significant change in the level of a given fraction may represent a shift caused by the increase or decrease of the total amount of substances such as cholesterol and phospholipids. The reflection of that purely chemical change, often difficult to demonstrate by present chemo-analytical methods, is found in the change of respective levels of various fractions created arbitrarily by dividing the lipoprotein molecular spectrum into segments within the "ultramicroscopic" part (studied ultracentrifugally) and the "microscopic" one (studied in the dark field). The lowered phospholipid-cholesterol ratio of Ahrens and Kunkel might suggest either too much cholesterol (considerable amounts of which are contained in lipoprotein macromolecules on the low end of the spectrum) or too little phospholipids. If the latter be the case, let us remember that a higher level of chylomicrons reflects large amounts of triglycerides per particle, with much smaller concentration of phospholipid. That again would tally with the observation of Zinn and Griffith[6] and Labecki[7,8,9] on the correlation between elevated chylomicron levels and the incidence of myocardial infarction.

It would be premature at present to imply that lowering of chylomicron levels (percentage of particles 0.3 micron in diameter and larger, in relation to all *visible* lipoprotein macromolecules) has a preventive or curative effect. However, our past and current studies indicate the constancy of the chylomicron level under normal circumstances (no special dietary or drug regime) and a very good statistical correlation between elevated chylomicron levels and the occurrence of myocardial infarction. The effect of lipotropic agents in lowering the chylomicron levels appears to be surprisingly consistent. Whether or not the action of choline and methyl group donors (methionine, betaine) and inositol is to provide components of phospholipids and thus increase the phospholipid serum levels can only be speculated. Such an increase might be reflected from the physical standpoint by the increase in the smaller (0.1 micron to 0.2 micron))visible fatty particles—and perhaps others in the ultramicroscopic segment—thus causing a relative decrease in the chylomicron concentration. The action of certain substances may create a shift in the levels of lipoprotein macromolecules of various sizes. This might be the explanation of the suggested cholesterol-stabilizing effect of the phospholipids. It would be of particular interest to study the effect on that spectrum of lipoproteins which represent a gap between the lipoprotein macromolecules of high flotation rates

(about S_f 400 and higher) and the smallest (about 0.1 micron) microscopically visible particles. Unfortunately there are considerable technical difficulties connected with the study of that intermediary segment of the lipoproteins.

The preliminary observations reported here not only should receive long-term clinical evaluation, but should also be extended by further studies utilizing all known biochemical and biophysical approaches to supplement each bit of additional knowledge by a broad, unbiased, and detached approach to this perplexing problem.

Summary

Lipotropic substances—choline, methionine, and inositol in a balanced formula (Methischol)—were administered to 11 patients with proved past myocardial infarction for not less than nine weeks and to 24 elderly women for nine weeks, and to 12 for twelve weeks, the latter group having no history of myocardial infarction.

After nine or more weeks of administration of the lipotropic mixture to the myocardial infarction group, there was a decrease of borderline statistical significance in the total serum cholesterol levels. No such variation was observed in the control group even after prolonged therapy; neither was there any appreciable variation following the administration of a lactose placebo in capsules of identical appearance.

There was a statistically highly significant depression of chylomicron levels (per cent of microscopically visible lipoprotein particles 0.3 micron in diameter and larger) in all subjects on the above regime. Intermittent discontinuation and repeated administration of these substances in four atherosclerotic subjects, followed from five to twelve months, resulted alternately in elevation and depression of chylomicron levels, respectively. Placebo administration did not result in a change in chylomicron levels beyond the statistically expected and experimentally confirmed variation.

Addendum

Since our original data were tabulated and statistically analyzed we have maintained 18 more patients with clinically demonstrable coronary atherosclerosis on lipotropic therapy. The mean chylomicron levels for that group of 18 patients, on the basis of repeated tests prior to any medication, was 44.96 per cent; all patients without exception showed the same trend manifested by the subjects in the original study. The mean chylomicron levels at the end of lipotropic therapy was 31.29 per cent. The shortest period of administration was 15 weeks; the longest period, 54 weeks; the average was 29 weeks. It is of interest that discontinuation of therapy for a certain period of time or decrease in the daily dose resulted in the increase of the fasting chylomicron levels; resumption of therapy or increase in the dose resulted in the lowering of the fasting chylomicron levels. Our observation on the effect of lipotropic therapy recently received confirmation by another group.[14]

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Avoiding Osteoporosis

"In the ballet, age is usually depicted with the tremor and festinant gait of Parkinsonism, the ineptitude and labile mood of an early senile dementia, and the painful, bent back of senile osteoporosis. And the artist is right, in stressing the trouble that ageing bone can cause. . . . If we could bring our patients into old age with strong bones we would save them a vast amount of suffering and the country a great deal of money. . . .

On the one hand, not enough osteoid tissue may be formed: on the other, calcium may be deposited in it in inadequate amounts or removed from it too fast. The first pathological change is called osteoporosis, and the second osteomalacia.

"Nature has not ordained that patients should pay close attention to this classification, and many of them, particularly the elderly, have a combination of conditions.

"We have. . .as some of the known and suspected causes why many old people have brittle bones: (1) An imbalance of hormones. (2) A diet deficient in calcium, protein, vitamin D, and, perhaps, other essential factors. (3) Deficient absorption of calcium. (4) Inactivity, or even immobility.

"At least three of these causes are preventable: all are treatable. . . . We do not know how to keep elderly people 'in endocrine balance.' We can only attempt to diagnose the condition and treat it early in its course. . . . On the other hand, we can ensure an adequate intake of calcium.

"Our greatest endeavours must. . .be directed toward. . .prevention, or, failing that, . . . early detection and treatment. As people grow old, they should keep as active as possible, and take a diet containing adequate amounts of calcium, vitamin D, and protein."

—J. R. Bolton. *The Medical Press* 232: 381–385, 1954.