# Serum Lipid Levels in Rats Fed Vegetable Oils with and without Cholesterol.* (24724)

COLEMAN R. SESKIND, MARJORIE T. SCHROEDER, RICHARD A. RASMUSSEN AND ROBERT W. WISSLER

*Dept. of Pathology, University of Chicago School of Medicine, Chicago, Ill.*

Recent work has pointed to the importance of degree of saturation and fatty acid distribution of dietary fat in affecting serum lipid levels(1-7). The present study was designed to determine the effects of a known atherogenic diet on serum lipid levels in rats with source of fat and presence or absence of cholesterol as the only variables. Two experiments were performed each with a similar plan but each with a different group of dietary lipids to obtain varying levels of saturation and fatty acid content.

*Materials and methods. Animals.* In 2 experiments 36, 9-month-old albino male Sprague-Dawley rats were marked, weighed, and bled for serum lipid determinations. Animals in first experiment had average weight of 540 g (486-625 g) and had been maintained on Purina laboratory chow. They were divided into 6 groups comparable with respect to distribution of initial weight and serum lipid concentrations. All rats were kept in thermostatically controlled room (76°F), in large wire-bottomed cages with 6 rats/cage. In second experiment rats were 4½ months old and average weight was 424 g (378-456 g).

*Diet.* A liquid ration, described by Moskowitz(8) of 50% fat, 28% carbohydrate, 5% protein, and 1% choline (on dry weight basis) and containing adequate minerals, vitamins, and roughage, was used. In the first experiment, 6 diets were prepared, each pair contained one of 3 vegetable fats, all presumably free of cholesterol: soybean oil with iodine number (I#) of 129, olive oil (I# 80) and 50% olive oil - 50% palmitic acid mixture (I# 40).† Each fat was used to prepare 2 rations one with and one without 1% cholesterol on dry weight basis. Diets were prepared biweekly and fed 3 times daily by stomach tube(9,10) thus eliminating variation in dietary intake. During first week, amount of diet (half of which was water)/feeding was increased gradually from 10 to 15 ml. Each rat then received an estimated 135 calories/day. Animals were allowed tap water *ad lib.* but no additional diet. In second experiment the plan was similar except that a different approach was used to achieve various levels of dietary lipid saturation. Cottonseed oil, commonly used in commercial vegetable shortening, was hydrogenated to levels of saturation similar to those utilized in Exp. 1.† Six diets with the same basic composition as Exp. 1 were prepared. The 3 pairs of diets, one with and one without 1% cholesterol, utilized fresh oil (I# 107), hydrogenated oil (I# 78), and laboratory hardened oil (I# 43).

*Serum lipids.* Twelve-hour fasting blood samples were obtained from lateral tail vein at biweekly intervals. One ml blood was taken from each rat, pooled with that removed from others of the group, and allowed to clot. The serum was then removed after centrifugation and total cholesterol and lipid phosphorus determined by standard methods(11,12). The baseline, 6 week, and in first experiment, 10 week values were from averaged individual determinations. Animals were all killed at

* This investigation was supported by grant from Nat. Inst. Health, U.S.P.H.S.

† Palmitic acid was furnished through courtesy of Dr. Robert J. Vander Wal, Food Research Division, Armour and Co.

These fats were furnished and analyzed by Research Labs. of Swift and Co. *Soybean oil* I# 128.6 Linoleic 41.1, Linolenic 6.4, Arachidonic .00, Oleic 39.8, Saturated Acids 7.4; *Olive oil* I# 81.7 Linoleic 8.6, Linolenic .58, Arachidonic .01, Oleic 71.8, Saturated acids 14.6; *Olive and Palmitic* I# 40 Linoleic 3.39, Linolenic .28, Arachidonic .00, Oleic 38.03, Saturated acids 53.9. *Fresh Cottonseed* I# 109.5, Linoleic 40.1, Linolenic .08, Arachidonic .00, Oleic 41., Saturated acids 18.8, Trans isomers, 1., M.P. −2 to +2°C; *Hydrogenated Cottonseed* I# 77.3 Linoleic 9.27, Linolenic 0.04, Arachidonic .00, Oleic 67.3, Saturated Acids 23.4, Trans isomers 30.9, M.P. 35°C; *Lab Hardened Cottonseed* I# 42.5 Linoleic .0, Linolenic, .0 Arachidonic .0, Oleic 50 (calc.) Saturated Acids 45.6, Trans isomers 28.4, M.P. 50°C.

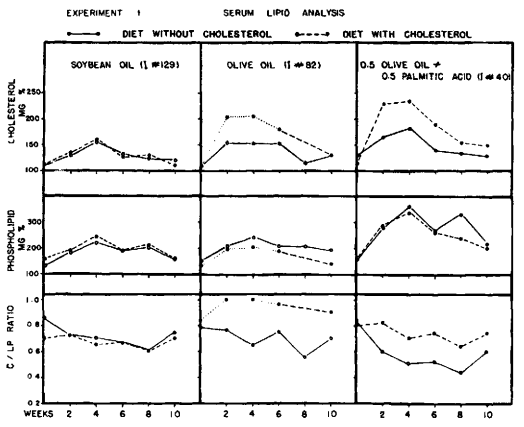


FIG. 1. Serum lipid analysis of Exp. 1.

10 weeks in Exp. 1 and 6 weeks in Exp. 2.

*Results of First Experiment. Body Weights.* All groups had initial average weight of about 540 g. Both soybean and olive oil groups regardless of whether cholesterol was added, showed similar total weight gains of 200-250 g at end of 10 weeks. On the other hand, rats receiving the most saturated fat consistently showed less weight increase, and at end of experiment this was only one-half that of other groups or about 125 g. Fecal fat excretion study during seventh week with animals in Groups 1 and 5 showed an average 0.3 g more lipid material was excreted/day by rats fed palmitic acid-olive oil ration. Part, if not all, of lesser weight gain may be accounted for by this fact.

*Serum Lipid Determinations.* Fig. 1 shows results of serum lipid determinations. In every group serum cholesterol rose to a peak at 2-4 weeks, then declined during remainder of experiment even though diets were constant during entire 10 weeks. Groups fed soybean oil (I# 129) showed a slow rise reaching a peak of 160 mg% followed by decline to intial levels whether cholesterol was fed or not. In groups fed olive oil (I# 82), there was a sharper rise to about the same peak level as in preceding groups. When dietary cholesterol was added, there was a definite elevating effect compared to that observed with comparable cholesterol-free ration. Elevations of these serum cholesterol values were maintained longer but at 10 weeks they had declined almost to initial levels. The same phenomena were noted for the 50:50 mixture as for olive oil except that peaks for this ration were the highest attained. Moreover, although a subsequent decrease was noted, serum cholesterols of the group receiving 50:50 fat mixture with added cholesterol did not return to initial levels of blood cholesterol. Serum phospholipid curves resembled cholesterol curves with initial rise and subsequent decline for all diet groups. Soybean and olive oil groups were quite similar. With 50:50 mixture however, phospholipid levels were definitely elevated above levels of other groups. In no instance, however, did addition of dietary cholesterol raise the phospholipid level above its cholesterol-free counterpart as it did with serum cholesterol levels. Phospholipid levels were not elevated until the greatest degree of saturation (or lowest levels of essential fatty acids) had been reached.

With soybean oil diet, serum cholesterol-phospholipid ratio did not change appreciably and addition of cholesterol had no apparent influence. Olive oil alone also had no influence on the ratio, but when cholesterol was added, there was a definite elevating effect. In groups fed the most saturated 50:50 mixture only, the ratio was depressed. Addition of dietary cholesterol again resulted in higher ratio, this time near normal levels. The only apparent correlation between dietary variables and behavior of ratio was observed in relation to oleic acid content of rations.

For cholesterol-free diet groups, increasing saturation of dietary lipid caused increasing liver weights and progressively greater liver percentage of body weights. Moreover, for a given dietary fat, addition of cholesterol resulted in increase of liver weights above those of corresponding cholesterol-free ration.

*Results of Second Experiment. Weight Changes.* Groups fed the most unsaturated and the moderately saturated cottonseed oil gained weight similarly. Groups fed highly saturated fat declined after first week and showed little net gain at end of experiment. Fecal excretion study for fat at 5½ weeks, on animals in Groups 2 and 6 showed that over 1 g more lipid material was excreted/day by rats receiving laboratory hardened fat. This correlates with their lower body weight.

*Serum Lipid Analysis.* Fig. 2 shows results

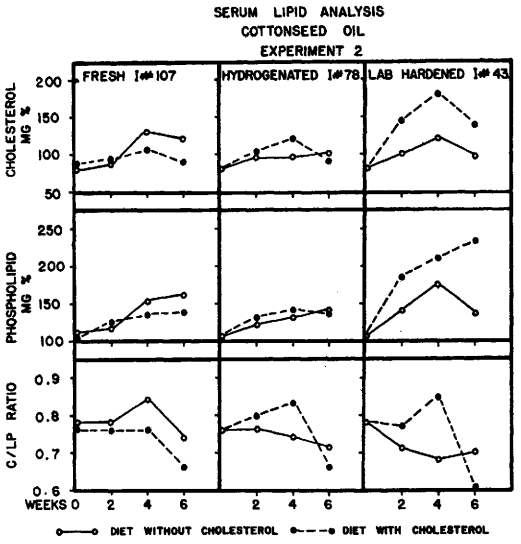


FIG. 2. Mean serum lipid analyses of Exp. 2.

of serum lipid analyses. As in Exp. 1, the general pattern included a peak at 4 weeks and subsequent decline for serum cholesterol values in all groups. In this experiment neither increased lipid saturation nor added dietary cholesterol influenced serum cholesterol level, until dietary fat was very saturated. Then these factors appeared additive. This suggests that a critical degree of saturation (or a threshold level of essential fatty acid deficiency) is necessary to produce elevation of serum cholesterol and to result in further elevation by dietary cholesterol. In almost every case phospholipid values were parallel to serum cholesterol values. The one exception was continued rise with feeding of very saturated fat with added dietary cholesterol. This phenomenon was similar to that observed in the first experiment. Kinsell(7) has reported a similar result in man.

Serum cholesterol-phospholipid ratios paralleled serum cholesterol when either natural or hydrogenated oils were fed. This was also true until 4 weeks, when "lab. hardened" plus cholesterol ration was fed, but at 6 weeks there was a precipitous drop due to continued phospholipid elevation. As in the first experiment the ratio of the group fed the most saturated fat alone was depressed.

*Discussion.* When one varies saturation of dietary lipids under experimental conditions, other dietary constituents may be changed also. For example such factors as dietary estrogenic substances, sitosterol, and cholesterol content of experimental rations may be inadvertently altered. Furthermore, caloric intake and intestinal absorption may be significant but hidden variables in an otherwise well controlled study. In our experiments care was taken to establish conditions which would allow a valid comparison of effects of saturation of fatty acids with and without added cholesterol on blood lipid levels of the rat. By employing stomach tube feeding technic, and by selected fecal fat analyses, variables of caloric intake and gastrointestinal fat absorption were either well controlled or evaluated. Use of vegetable oils avoided cholesterol contamination of those rations to which it was not added. Employing a biological assay for dietary estrogens(13) ancillary studies in conjunction with the first experiment, indicated that soybean oil had estrogenic activity. Therefore in the second experiment a single fat source, free of estrogenic effect, was employed and degree of saturation was varied by hydrogenation. The sitosterol content of various oils is a variable that was not measured.‡

Insofar as our results can be evaluated, it appears that no matter whether saturation of dietary fat is increased by additional palmitic acid or by hydrogenation of cottonseed oil, there is no great tendency for serum cholesterol to rise unless dietary cholesterol is added to the diet. Perhaps of more importance is the observation that dietary cholesterol has little elevating effect on serum cholesterol unless it is combined with highly saturated fat-containing diet. In both experiments the cholesterol-phospholipid ratios were relatively higher when dietary cholesterol was added to the more highly saturated fats.

These results aid in interpretation of several recent reports on relationship between dietary lipids and blood lipids. For example under

‡ Since this manuscript was submitted we have completed sitosterol analyses on the lipids used in this experiment. These are (mg%): Soybean oil .59, Olive oil .38, Cottonseed oil .44. Hydrogenation of cottonseed oil did not alter the sitosterol content. These results indicate that dietary sitosterol was probably not a significant variable in this study.

some dietary conditions there is evidence that dietary cholesterol has little if any effect on blood cholesterol in rats(17). This is said to be the case in man also(4,7,16), but it may be true only when individuals already have an elevated blood cholesterol level or when they receive diets containing considerable unsaturated fat. Although most recent evidence supports the relationship between saturation of dietary fatty acids and elevation of blood cholesterol(3,7), there are also interesting exceptions to this general correlation(14,15). Such variables as amount of fat consumed, initial level of serum cholesterol, length of diet period, and presence or absence of dietary cholate must be evaluated in interpreting these experiments. Furthermore, it is evident from our data that homeostatic factors probably play a major role in influencing ultimate serum cholesterol levels in dietary experiments. In both experiments here summarized, at 2 to 4 weeks, blood lipids of groups receiving the less saturated oils showed differences between groups fed diets with and without dietary cholesterol. These either disappeared or changed at 6 or 10 weeks. It appears, however, that this homeostatic mechanism is least effective when cholesterol is fed with highly saturated lipid.

Although there is evidence suggesting that degree of saturation of dietary fat may influence esterification of serum lipids, especially serum cholesterol(18), further study is necessary to ascertain the relation of these observations to elevation of serum lipids and deposition of lipids in arteries. It appears that among the many factors that influence the level of serum lipids, highly saturated fat and dietary cholesterol combined have a definite additive effect.

*Summary.* 1) In adult male albino rats tube feeding diets containing large quantities of vegetable fats of differing degrees of saturation and essential fatty acid content, suggests that serum cholesterol is elevated by increasing saturation and that dietary cholesterol augments this effect for the more saturated oils. Elevation appears transient for the less saturated oils but is more sustained for highly saturated fats. 2) Serum phospholipid values in general are parallel to serum cholesterol values. Only with feeding of fats with lowest I# was there an elevation of phospholipid levels to especially high values as compared with serum cholesterol. This effect is particularly striking for "lab. hardened" cottonseed oil fed with cholesterol. 3) Cholesterol/phospholipid ratio shows no difference between groups fed high I# oils with or without dietary cholesterol. The more saturated fats showed elevation of ratios in groups with added cholesterol above groups fed no cholesterol. Highly saturated fat fed alone had a lowering effect on the C/LP ratio.

1. Ahrens, E. H., Jr., *Am. J. Med.,* 1957, v23, 933.
2. Kinsell, L. W., Sinclair, H. M., *Lancet,* 1957, v1, 883.
3. Bronte-Stewart, B., Antonis, A., Eales, L., Brock, J. F., *ibid.,* 1956, v1, 521.
4. Gordon, H., Lewis, B., Eales, L., Brock, J. F., *ibid.,* 1957, v2, 1299.
5. Beveridge, J. M. R., Connell, W. F., Mayer, G. A., *Canad. J. Biochem. and Physiol.,* 1956, v34, 441.
6. Malmros, H., Wigand, G., *Lancet,* 1957, v2, 1.
7. Kinsell, L. W., Michaels, G. D., *Fed. Proc.,* 1955, v14, 661.
8. Moskowitz, M. S., Moskowitz, A. A., Bradford, W. L., Wissler, R. W., *Arch. Path.,* 1956, v61, 245.
9. Shay, M., Gruenstein, M., *J. Lab. and Clin. Med.,* 1946, v31, 1384.
10. Talalay, P., Takano, G. M. V., *ibid.,* 1952, v40, 486.
11. Abell, L. L., Levy, B. B., Brodie, B. B., Kendall, F. E., *J. Biol. Chem.,* 1952, v195, 357.
12. Fister, H. J., Lipid Phosphorous, Method 10-37.1, *Manual of Standardized Procedures for Spectrophotometric Chemistry,* N. Y. Standard Scientific Supply Corp., 1950.
13. Huggins, C., Jensen, E. V., Cleveland, A. S., *J. Exp. Med.,* 1954, v100, 228.
14. Swell, L., Flick, D. F., Field, H., Jr., Treadwell, C. R., *Am. J. Physiol.,* 1955, v180, 124-128.
15. Kritchevsky, D., Moyer, A. W., Tesar, W. C., McCandles, R. F. J., Logan, J. B., Brown, R. A., Englert, M. E., *ibid.,* 1956, v185, 279-280.
16. Keys, A., Anderson, J. T., Michelsen, O., Adelson, S. F., Fidenza, F., *J. Nutr.,* 1956, v59, 39.
17. Jones, R. J., Wissler, R. W., Huffman, S., *Arch. Path.,* 1957, v63, 593.
18. Ahrens, E. H., Jr., Hirsch, Jules, Insull, W., Jr., Peterson, M. L., p245, *Chemistry of Lipids as Related to Atherosclerosis.* Edited by Page, Irvine H., Charles C. Thomas, 1958.

Received December 15, 1958. P.S.E.B.M., 1959, v100.