Radioactive Fat Absorption Patterns in Obesity and Coronary Disease

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MANY attempts have been made to attribute the genesis of atherosclerosis in some manner to an error in fat metabolism. However, the results to date have been inconclusive and frequently conflicting. With the availability of newer technics utilizing radioactive substances it may be anticipated that more informative data will be forthcoming to help elucidate this basic problem.

During the past year we have been using radioactive triolein to study the absorption and utilization of fat in normal subjects and in patients with various diseases.[1-4] This report is concerned with the results obtained in patients with coronary artery disease and/or hypercholesteremia. In addition, because of the frequent association of coronary disease with obesity, the response of a group of obese subjects to a tagged fat meal has also been studied.

PROCEDURE

The procedure consists essentially of the administration of a test meal containing 25 μc. of I^{131} - triolein in a total volume of 1 ml. of peanut oil per kilogram of body weight. Samples of venous blood are taken at frequent intervals until a maximum radioactivity level has been reached, and then twenty-four hours later. A 2 ml. aliquot of unclotted blood is counted in a scintillation well counter (whole blood radioactivity). Another aliquot, treated with potassium iodide and trichloracetic acid to separate the lipoprotein-bound iodine, is also assayed for radioactivity. The total blood volume is assumed to be 7.2 per cent of the body weight. With the given activity in the 2 ml. portions and the calculated total blood volume, the total whole blood and lipid blood radioactivity can be determined and expressed as a percentage of the initially ingested fat.

The total urine output is collected for twenty-four hours after the meal, and also is assayed. In some cases, stools are collected over a seventy-two–hour period.

After the radioactive triolein is ingested, it enters the intestinal tract and is then absorbed. The radioactive iodine which circulates in the blood consists of two fractions. The first is contained in the fat which is in transport as lipoprotein. Normally, this portion does not exceed 50 per cent of the total radioactivity.

The second fraction is derived from the splitting of the iodine-fatty acid bond during utilization. The concentration of this inorganic fraction depends on the rate at which the fat is utilized and the speed of renal excretion. The influence of the thyroid on this fraction is negated by the use of blocking agents prior to the test meal.

The essential features of the blood radioactivity curves include (1) the time of maximum blood radioactivity; (2) the magnitude of this maximum value; (3) the twenty-four–hour activity level; and (4) the organic blood/whole blood radioactivity ratio. Although these values are only a partial reflection of the complete metabolic cycle which ensues after a

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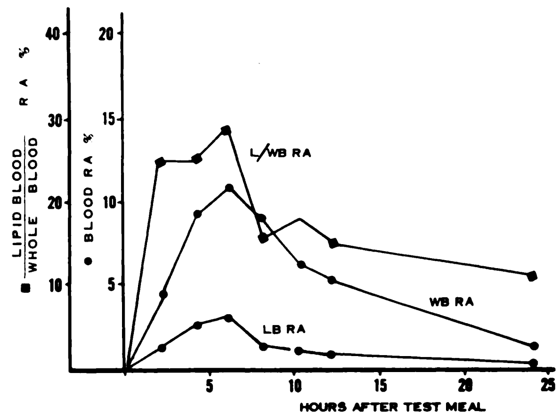

FIG. 1. Mean radioactive fat tolerance curves in a normal subject. In this and other illustrations, WB = whole blood, LB = lipid blood, RA = radioactivity. (From: LIKOFF, W., BERKOWITZ, D., WOLDOW, A., JACOBS, A. G. and SKLAROFF, D. M. *Circulation*, 18: 1118, 1958.[1])

labeled fat test meal, they appear to be the most informative.

RESULTS

In our normal control subject, after ingestion of a triolein test meal, the mean whole blood radioactivity usually increased to a peak value of 11.7 per cent (lipid blood value, 3.7 per cent) by the sixth hour and then gradually declined so that after twenty-four hours approximately 2 per cent (lipid blood value, 0.2 per cent) remained. The organic fraction represented less than 50 per cent of the total (Fig. 1). Twenty-four–hour urine collections contained between 20 and 40 per cent of the administered radioactive substance, and the average fecal radioactivity over this period is less than 2 per cent.

In our patients with coronary artery disease, the curves demonstrated marked differences from those in the normal subjects. Characteristic were unusually high blood levels of total and lipid blood radioactivity, delays in attaining these concentrations, and abnormal twenty-four–hour retention (Fig. 2). In addition, the lipid/whole blood activity ratio in many, showed that a fraction was present in the organic portion.

In a group of twenty-five obese female subjects (weighing 175 to 300 pounds) we again noted the elevated radioactivity values at the peak time and after twenty-four hours. This was reflected in both the whole blood and the lipid blood fractions (Fig. 3).

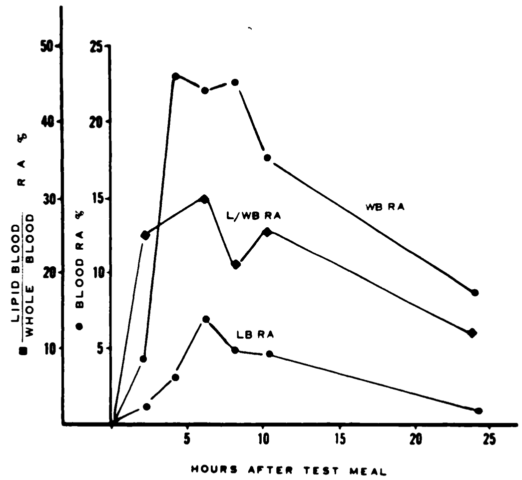

FIG. 2. Abnormal radioactive fat tolerance curves in patient with coronary artery disease.

COMMENTS

It would thus appear that patients with coronary artery disease, as well as obese subjects, demonstrate similar abnormalities after a radioactive fat tolerance test as compared to normal control subjects. Any attempt to explain these absorptive patterns presupposes a knowledge of various factors concerned therewith.

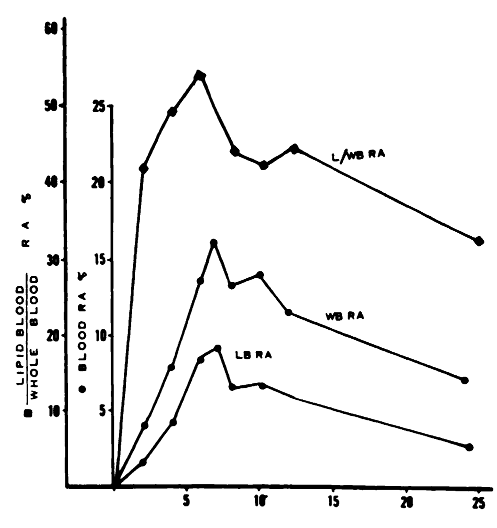

FIG. 3. Mean radioactive fat tolerance curves in obesity without coronary disease.

Under normal conditions, at least 97 per cent of the radioactive fat is absorbed within twenty-four hours. The amount present in the blood at any one precise moment, however, depends upon additional factors, notably the rate of utilization and the size of the fat pool. It is possible that an increased rate of absorption may be a factor in these higher blood curves. This could result from a decreased intestinal transit time allowing more time for the fat to be exposed to the absorbing surface, or else, a primary mucosal phenomenon may be responsible. Data showing that the intestinal mucosa is able to absorb abnormally high amounts of a basic foodstuff in certain conditions have already been advanced by Mayer.[5] He found that in certain mice with experimentally produced obesity, an increased rate of glucose absorption may be present—an apparent compensatory mechanism for the hyperphagia. On the other hand, abnormally high blood radioactivity levels may be the consequence of some derangement of fat transport. The delay in the disappearance of the isotope after twenty-four hours may have a similar explanation.

Thus far, the role of the pre-existing fat pool has not been taken into account. It may be that in those cases associated with hypercholesteremia, the abnormal curves are a result of the hyperlipemia and its attendant chemical dilution. Further studies correlating the basal triglyceride and total fat values are now in progress and may help to clarify this problem.[3]

The inability to define the precise mechanisms which are responsible for the abnormal radioactive fat tolerance curves we have found in these studies makes it difficult to offer any major conclusions. However, it would appear that the isotopic fat technic offers an excellent means for studying lipid metabolism. With this material it is possible to follow the fate of ingested fat in a much simpler and more precise manner than was heretofore available.

If further studies substantiate our preliminary data, it may be possible to utilize this procedure as a screening device to predict the presence of coronary disease in asymptomatic persons.

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DISCUSSION

Dr. Meyer Friedman (*San Francisco California*): When does the blood become completely free of radioactivity?

Dr. Berkowitz: In the normal subject, the blood radioactivity in twenty-four hours averaged about 2 per cent. In forty-eight hours, there was practically no radioactivity left.

Dr. Friedman: When did the blood radioactivity disappear completely in the most abnormal patient?

Dr. Berkowitz: Some maintained radioactivity for as long as seven days.

Dr. Friedman: Is the half-life about eight days?

Dr. Berkowitz: Yes.

Dr. W. Stanley Hartroft (*St. Louis, Missouri*): You said that obesity and coronary artery disease were associated and that more obese people have coronary artery disease than those who are not obese. There is evidence to question that, as I am sure you are aware. Actually, in autopsy data, where we have the full facts, we have not been able to obtain any correlation between weight and the incidence of coronary artery disease compared to control groups, either at time of death or as far back as we can go in the patient's history.

Have you been able to obtain any data of this type in your radioactive fat tests in diabetic and alcoholic patients or in those with cirrhosis?

It would also seem to me that there is evidence to suggest that the results obtained with this test would be affected by the state of caloric balance at the time. If the patients are gaining or losing weight during the time of this test, the results would be quite different. Were the patients who had had a coronary heart attack trying to lose weight, and do you have any data on the state of the fat balance? Also, have you discovered what are the results in this test when the quality of fat in the diet is varied, i.e., corn oil versus lard and so on?

DR. BERKOWITZ: We have not as yet had the opportunity to conduct such tests.

DR. J. POMERANZE (*New York, New York*): We have paralleled these tests a few years ago, but did not use radioactive substances. We used fat tolerance studies and our curves are exactly like those in obese patients with coronary artery disease.

We also tried to change the picture and succeeded in doing so by reducing the patients weight or eliminating fat from the diet at that time. Unfortunately, the technics for measuring fatty acids in the blood are difficult and we had to discard the test. However, this radioactive test achieves the same results in a simpler manner, and I believe it should be used in an attempt to treat these people by various means—use of a special diet and oils and lipotropics to see whether or not it has any effect on the absorptive curve.

DR. S. RODBARD (*Buffalo, New York*): Is it justifiable to draw the conclusion that because patterns of patients who have had coronary diseases are similar to those who are obese, one may say that the obese are "precoronaries" or "non-clinical coronaries?" Are these not separate conditions with similar patterns and not necessarily related to each other?

DR. POMERANZE: Obesity in itself is not a precursor of coronary artery disease, but I would guess that in a person who is predisposed to coronary artery disease, obesity will be a triggering mechanism. This is analogous to the obese patient with a predisposition to diabetes, in whom it will develop sooner if obesity is maintained. I would emphasize that the relation is possibly the same with coronary artery disease.

DR. CAMILLO ARTOM (*Winston-Salem, North Carolina*): This technic with iodized fat is certainly an interesting one. However, there may be some limitations, especially the possible liberation of iodine from fatty acid molecules.

When we used non-radioactive iodized fat, the amounts of iodine used had to be much higher to detect it. Large amounts of iodine were liberated, but it became evident that the whole molecule of fatty acid was not necessarily demolished. The possibility exists that the liberation of iodine occurred much before the fatty acid was demolished. For instance, when iodized fatty acids were administered, there was considerable fatty infiltration of the liver which did correspond to the amount of lipid iodine. Moreover, many of the fatty acids which were deposited in the liver had lost their iodine and were not detectable by the iodine technic. They were probably not natural and were changing their form. I don't know exactly what they were. They were not metabolized to the same extent as the natural fatty acids. That does not take away interest from this technic but implies an additional reservation about the interpretation of the results.

DR. BERKOWITZ: I think Dr. Rodbard's point is well taken. It may be that we have demonstrated similar metabolic alterations which have no more in common than that.

As to Dr. Hartroft's question, in the majority of diabetic patients we have studied, there has been an abnormal curve.

We were particularly interested in patients with cirrhosis. For a long time many have maintained that high fat diets are injurious to people with liver disease. We studied this and found normal patterns.

Relative to the state of nutrition of the patients in our studies, those with coronary disease were all of normal weight. None were attempting to lose weight. Our obese patients had been in that condition for a long time and this test was made soon after we saw them for the first time in a special obesity clinic.

DR. COLIN C. LUCAS (*Toronto, Canada*): My reaction may be colored by some experiences I had many years ago while working with Dr. Banting in a different field. We were interested in compounds analogous to the sulfa drugs. Most of you probably know that a compound almost indistinguishable chemically from sulfanilamide has no effect whatsoever on bacteria. If you move the 2-acid group from the para position to the meta position it is completely inactive. If you put a chlorine, a bromine or an iodine in either the para or the meta position of the OH group, the activity disappears completely; and if you put in a methyl or methoxy it would disappear completely.

That has always made me dubious about incorporating foreign additives. We often say that the amount being added is small compared to the total, but it is that small bit being measured, and whether or not it is any measure of the body's handling of the normal thing, I don't know. I wouldn't say, "Do not do it," but on the other hand I would be hesitant about drawing conclusions. You must have tools to work with and you must do the best you can with the tools. I am warning about the interpretation of the results.

DR. BACON F. CHOW (*Baltimore, Maryland*): I support the viewpoint of Dr. Lucas in that we want to make similar studies with radioactive vitamin B_{12}. We have studied tolerance curves produced by the intravenous injection of different amounts of cobalt^{60} labeled vitamin B_{12}. You can obtain different types of curves because part of the cyano group would be replaced by the hydroxy group. This type of study, while interesting clinically, should be interpreted carefully in terms of physiologic effects.

DR. BERKOWITZ: Dr. Spitzer, our biochemist, might like to comment on this.

DR. J. J. SPITZER (*Philadelphia, Pennsylvania*): It has been demonstrated that as far as absorption and transport are concerned—and I emphasize this as opposed to metabolism on the cellular level—there is good correlation between neutral fats and iodine-labeled neutral fats.

This does not hold true for metabolism on the cellular level, that iodine and the fatty acids part company; but absorption and transport through the body are the same.

DR. ARTOM: The difference has been pointed out at least in the rate of metabolism, and possibly also in the type of products first formed when the iodine is taken away from the fatty acid molecule. In other words, there is probably a dehydration—by what mechanism I do not know, but it happens with brominated fatty acids. We have used brominated fatty acids and the bromine goes out even faster than the iodine, but this de-brominated fatty acid is still accumulating in the liver at a rate which is much higher than the normal fatty acid with hydrogen instead of iodine.

DR. B. A. SACHS (*New York, New York*): How are the total lipids extracted before measuring the radioactivity?

DR. BERKOWITZ: With trichloroacetic acid.