Disposal of Human Chylomicrons Administered Intravenously in Ischemic Heart Disease and Essential Hyperlipemia


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After a fatty meal, plasma lipemia, as measured by increase in optical density in a colorimeter, is significantly greater throughout the period of absorption in patients with ischemic heart disease than in control subjects.1,2 The extent of the lipemia depends on contemporary processes of absorption and fat clearance. The rate of absorption depends on such factors as gastric emptying and the form and quantity of the ingested fat; the total amount of fat absorbed is not likely to be greater in subjects with ischemic heart disease, since normal subjects absorb at least 95 per cent of ingested fat.

The variable effect of absorption can be excluded by intravenous infusions of fat. The mean rates of removal of artificially emulsified cottonseed oil (Lipomul), when administered intravenously, were found to be similar in patients with ischemic heart disease and in matched controls.3,4 However, such emulsions are initially removed by the reticuloendothelial system, and in this way their metabolism differs significantly from that of chylomicrons.

A similar study of the removal of intravenously administered human chylomicrons would be preferable and will be presented in this paper. However, several factors which may influence the rate of removal of chylomicrons from plasma must be considered.

First, there may be an abnormality in the rate of removal of chylomicrons, in which form nearly all long chain fatty acids are transported. This may be due to deficient activity of lipoprotein lipase, an enzyme involved in the uptake of triglyceride by certain tissues.5,6 In patients with ischemic heart disease, a defect in the postheparin clearing activity of plasma has been reported.7

Secondly, the rate of removal may be different because of an abnormality in the physical or chemical state of chylomicrons. The studies of Carlson and Olhagen,8 and of Havel and Gordon,9 when taken together, suggest that in idiopathic hyperlipemia fat particles of different chemical composition are hydrolyzed at different rates.

Thirdly, the rate of removal may be delayed if chylomicrons have to equilibrate with an abnormally large pool of plasma triglyceride; there is evidence that, in patients with ischemic heart disease, the concentration of fasting plasma triglyceride is abnormally high.10

In the experiments to be presented, the first two possibilities have been examined. In the first, a comparison has been made between the rates of removal of standard suspensions of human lymph chylomicrons administered intravenously to a group of patients with ischemic heart disease and to a group of control subjects. In the second, chylomicron suspensions were prepared from the plasmas of patients and control subjects and comparisons made between the rates of removal of paired suspensions when infused into common recipients.

In these experiments, the influence of in-
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creased plasma triglycerides was probably excluded by studying groups of subjects whose mean plasma total esterified fatty acid levels did not differ significantly. The importance of this factor was, however, examined in an experiment in which chylomicrons were infused into two subjects with hyperlipemia.

Methods

EXPERIMENT 1

A standard infusion of chylomicrons was prepared as follows: The thoracic duct of a patient with metastatic carcinoma of the lung was cannulated at the time of a lymph node biopsy. The patient was given a meal which contained 60 Gm. of cream fat with which 600 μC. of glyceryl tri-C-14 had been emulsified. Chyle was collected over the next 12 hours, and the chylomicrons were separated by spinning the chyle under 0.85 per cent saline for one hour at 20,000 r.p.m. at 4 C. in a model L Spinco ultracentrifuge. The creamy supernatant layer was washed in saline, recentrifuged, and resuspended in saline. This procedure was carried out under aseptic conditions. The chylomicrons were stored at 4 C. and appeared to remain unaltered microscopically for at least two weeks.

Aliquots of this preparation were infused into eight male patients with ischemic heart disease and into eight male control subjects. The eight patients had all recovered from a transmural myocardial infarction at least three months previously and were not receiving anticoagulant therapy or a special diet. Their mean age was 56. The eight control patients were selected, each of whom had all recovered from a transmural myocardial infarct at least three months previously, his plasma was lipemic, but this had largely cleared on a diet which was low in animal fat and high in unsaturated fat. At the time of the investigation, the plasma esterified fatty acids were 540 mg. per 100 ml., and the plasma chol-

The optical density of the plasma samples was then measured in a Unicam spectrophotometer at a wave length of 650 μm. The plasma samples were then layered under 0.85 per cent saline and spun at 20,000 r.p.m. for one hour. The supernatant chylomicrons were extracted with a 20-fold dilution of 3:1 alcohol-ether. The slight precipitate of protein was spun off and the solvent evaporated. The fat was redissolved in 10 ml. of a 0.3 per cent solution of diphenyloxazole in toluene and the radioactivity counted in a Packard Tri-Carb liquid scintillation counter.

Fasting plasma esterified fatty acids were measured by the method of Stern and Shapiro.11

EXPERIMENT 2

In this experiment, plasma chylomicrons were obtained from six patients with ischemic heart disease and from six control subjects after a meal containing labeled fat. The mean ages of the two groups were 62 and 60, respectively. The majority of the controls were suffering from apparently localized cancers (three had carcinoma of the lung). They had no evidence of cardiovascular disease and had not lost any weight. After an overnight fast, each subject was given a meal of 100 Gm. fat with 100 μC. of C-14 tripalmitin. Three and a half hours later, at the probable peak of the lipemia, 500 ml. blood was removed, and chylomicrons were prepared for infusion as before.

A preliminary calculation showed that the regression coefficients which related the radioactivity of the infusions to their esterified fatty acid content and to their optical density, respectively, were both significant (P < 0.01). Each infusion from a patient with a myocardial infarct was paired with one from a control subject according to the amount of radioactivity present and to the optical density. The average dose given contained 0.5 Gm. fat and approximately 0.5 μC. of C-14. Six normal fasting recipients were selected, each of whom received two infusions, one each from a patient and a control. The infusions were administered consecutively, the second about two hours after the first, when very little plasma radioactivity remained from the first infusion. The first infusion given was, on three occasions, from a patient with ischemic heart disease, and on the other three, from a control subject. Venous samples were taken at two-minute intervals for 12 minutes, and the radioactivity was measured in the plasma chylomicrons, as before.

EXPERIMENT 3

To observe the effect of a large pool of triglyceride in plasma on the rate of removal of infused chylomicrons, standard infusions of lymph chylomicrons were given, as in the first experiment, to two patients with hyperlipemia. Patient A was a 45-year-old male with tendinous xanthomata of several years' duration. When first seen 12 months previously, his plasma was lipemic, but this had largely cleared on a diet which was low in animal fat and high in unsaturated fat. At the time of the investigation, the plasma esterified fatty acids were 540 mg. per 100 ml., and the plasma chol-
terol was 390 mg per 100 ml. Patient B was a 33-year-old man with a 13-year history of abdominal pain, hepatosplenomegaly, and grossly lipemic plasma. At the time of the investigation, his plasma was creamy with an esterified fatty acid content of 2,485 mg per 100 ml and a serum cholesterol of 600 mg per 100 ml.

Results

EXPERIMENT 1

The mean levels of esterified fatty acids were 232 mg per 100 ml in the control group and 248 mg per 100 ml in the ischemic heart disease group. These did not differ significantly (0.1 > P > 0.05), and it is, therefore, probable that the sizes of the plasma triglyceride pools which equilibrated with the infused fat were similar in the two groups.

The mean rates of removal of the standard chylomicron infusions by the two groups are shown in figure 1. The rates of removal were identical in the two groups, and when plotted semilogarithmically were linear, that is, exponential, at least over the first 10 minutes. The half times for both groups were seven minutes.

Figure 2 shows the mean rates of fall of optical density of the plasmas after the chylomicron infusions. The curves for the two groups were very similar with half times of six to seven minutes.

EXPERIMENT 2

Figure 3 shows the mean rates of removal by the six recipients of the six paired chylomicron infusions from the patients and from the controls. The rates of removal were exponential over the first six to eight minutes. The mean half times were six and seven minutes for the control and ischemic heart disease infusions, respectively, and did not differ significantly. Regression coefficients were calculated for the rates of removal of all infusions and the significance of the difference between the two groups sought by a paired t-test analysis of the coefficients. The difference was not significant.

EXPERIMENT 3

Figure 4 compares the rates of removal of standard chylomicron infusions by the control group and by the two patients with hyperlipemia. The removal of chylomicron radioactivity was much slower than normal in the two patients. The rate of removal was slowest in the patient with the highest lipid levels. The half times in the two patients were 21 and more than 32 minutes, respectively.

No febrile responses or other undesirable
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side effects were observed during the infusions and over the following six months.

Discussion

The results show that, under the conditions of our experiments, the rate of removal of radioactivity from chylomicrons administered intravenously was similar in patients with ischemic heart disease and in control subjects. The radioactivity of chylomicrons obtained from such patients was as readily removed from the circulation of recipients as was that of chylomicrons from control subjects.

However, these conditions may not represent the way in which chylomicrons are removed under all circumstances. The dose of fat given was deliberately small and was designed to produce a blood level of fat which might be found after a moderate fat meal. It is possible that a difference between the two groups might emerge if a larger amount of fat was given, or if instead of a rapid infusion, a steady more prolonged infusion was used. In animals, the rate of removal is slowed by increasing the load of chylomicrons.12

The fasting patient may also be unsuitable, since it has been shown that in fasting animals chylomicrons are removed largely by the liver.13 If a difference exists between the two groups of subjects, it may be in the ability of their adipose tissue to remove fat, and this may not have been tested.

A most important point to consider is the size of the plasma triglyceride pool with which the chylomicrons equilibrated. The similar mean esterified fatty acid concentration in the two groups suggests that the pool sizes were similar. This was possibly due to the selection of an older population, since abnormally high lipid concentrations are more usually found in younger patients with ischemic heart disease.14 It is possible that in younger patients differences in the removal of chylomicrons might be found.

Although Carlson and Olhagen,9 and Havel and Gordon9 have infused lipemic plasmas in man, this appears to be the first reported experiment in which separated human chylomicrons have been infused into man. Numerous similar experiments have been performed in animals, and our observations made in man
closely parallel those made in the dog\textsuperscript{15,16} and in the rat\textsuperscript{12,13} in that the half times were similar and the rates of removal were exponential over the initial rapid phase, although the amounts of lipid infused in this experiment were considerably less.

Chemical analysis of the injected lipid was not performed, and therefore it is uncertain whether significant contamination with very low density lipoproteins had occurred. The relatively slow and short centrifugation used in these experiments would have favored the exclusion of such lipoproteins from the chylomicron preparations\textsuperscript{17} and the similar rates of disposal of infused chylomicrons obtained from lymph and plasma, respectively, is against significant contamination of the plasma preparations. It is also not known to what extent free fatty acids and phospholipids had become labeled.

The good correlation between plasma turbidity and chylomicron radioactivity agrees with previous studies which have shown a close relationship between serum optical density and triglyceride concentration after fatty meals\textsuperscript{18}. However, changes in optical density are less accurate and can be determined only when much fat in the form of large particles is present.

In the two patients with hyperlipemia, the removal of radioactivity from plasma chylomicrons was very much delayed. In patient B, who had the higher level of esterified fatty acids (2,845 mg. per 100 ml.), the rate of removal was slower than in patient A (esterified fatty acids, 540 mg. per 100 ml.). This suggests that the rate of removal of chylomicrons may be related to the size of the plasma triglyceride pool.

These findings do not, in any way, refute observations made in the past that patients with ischemic heart disease have abnormal alimentary lipemias, although it should be remembered that a definitive study of this problem, in which parameters other than turbidity are measured, has yet to be made adequately. From the data obtained in the present experiments and with the previously stated qualifications, it is possible that if plasmas of patients with ischemic heart disease do remain unduly lipemic after a fatty meal, it is in part due to the higher level of fasting plasma triglyceride.

**Summary**

Human lymph chylomicrons labeled in vivo with C-14 palmitate were infused into eight patients with ischemic heart disease and into eight control subjects. The mean rates of removal of radioactivity from the chylomicron fraction of the plasmas of these subjects were found to be the same in the two groups. Labeled chylomicrons were also prepared from the blood of six patients with ischemic heart disease and of six controls, and infused into six recipients as paired infusions. Radioactivity from chylomicrons obtained from patients with ischemic heart disease was as readily removed from the circulations of recipients as was radioactivity from chylomicrons obtained from control subjects. When lymph chylomicrons were infused into two patients with hyperlipemia, the rate of removal of chylomicron radioactivity was considerably less than normal and appeared to be related to the levels of endogenous esterified fatty acids.

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**References**


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