Plasma decay of chylomicron remnants is not affected by heparin-stimulated plasma lipolytic activity in normal fasting man

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Abstract In an earlier study it was shown that retinyl palmitate appeared to be a satisfactory label for the core of chylomicrons and their remnants. When chylomicrons were endogenously labeled with retinyl palmitate and pulse-injected into healthy donors, retinyl palmitate was cleared from plasma by a first order process. Its fractional decay constant was very similar to the fractional catabolic rate of VLDL triglycerides, a lipoprotein lipase-dependent process, and 2-3 times slower than hepatic chylomicron remnant uptake in experimental animals. We, therefore, investigated whether plasma clearance of retinyl palmitate-labeled chylomicrons is accelerated by enhanced plasma triglyceride hydrolysis produced by heparin administration. Five healthy subjects took retinyl palmitate by mouth and 5-6 hr later two units of plasma were obtained by plasmapheresis. After storage for 42 hr, the units were pooled and separated into two equal volumes. The first half was injected into the donor and plasma retinyl palmitate and chylomicron triglyceride were measured for 3.5 hr (control study). Heparin was then given intravenously as a bolus followed by an infusion for 7 hr. A second retinyl palmitate clearance (postheparin study) was performed during the heparin infusion. Plasma lipolytic activity and retinyl palmitate and chylomicron triglyceride concentrations were measured serially. Total plasma lipolytic activity and hepatic triglyceride lipase activity were increased approximately 500-fold during postheparin studies, enhancing triglyceride decay 2.5- to 3-fold. Retinyl palmitate plasma decay, however, was unaffected. Retinyl palmitate plasma decay was a biexponential concentration-dependent function in eight of ten pre- and postheparin studies with the first, rapid exponential accounting for 90 ± 4% of total plasma retinyl palmitate decay. There were no significant differences in clearance rates of total plasma retinyl palmitate (100 ± 27 vs 108 ± 27 ml/min) or in the rapid or slow decay constants. The apparent volume of distribution of retinyl palmitate in relation to estimated plasma volume was comparable in control and postheparin studies (99 ± 11 vs 109 ± 22%). Although heparin accelerates chylomicron triglyceride hydrolysis and remnant formation, it does not affect plasma clearance of chylomicron remnants labeled with retinyl palmitate. We conclude that these findings are strong evidence that chylomicron remnant clearance is not limited by the rate of chylomicron remnant formation in normal fasting man. The plasma clearance rate of retinyl palmitate-labeled chylomicrons is an estimate of chylomicron remnant clearance. —Berr, F., R. Eckel, and F. Kern, Jr. Plasma decay of chylomicron remnants is not affected by heparin-stimulated plasma lipolytic activity in normal fasting man. J. Lipid Res. 1985. 26: 852-859.

Supplementary key words retinyl palmitate • postheparin lipoprotein lipase • heparin-enhanced triglyceride lipolysis

The plasma clearance of chylomicron remnants by the liver has not been quantitated in man. Labeling techniques for either core constituents or the remnant lipoproteins have not been satisfactory. Recently we evaluated a method for measuring chylomicron clearance using retinyl palmitate (RP) to label the core of chylomicrons and intestinal VLDL (3). Most RP remains in the lipid core of chylomicrons and their remnants until the remnants are cleared by the liver, and only 5 to 10% is transferred to other lipoproteins (3-6).

When RP-labeled chylomicrons are administered intravenously in RP-enriched autologous plasma, they distribute in the plasma volume and are cleared by a concentration-dependent process, probably reflecting hepatic chylomicron remnant uptake (3). The removal rate, however, is 2-3 times slower than reported for chylomicron remnants in animals (7-9) and similar to the plasma triglyceride turnover in man, a process mediated by lipoprotein lipase (10, 11).

To determine whether chylomicron triglyceride hydrolysis is the rate-limiting step in plasma clearance of RP-labeled chylomicron remnants, we measured plasma

Abbreviations: HDL, high density lipoproteins (d 1.063-1.21 g/ml); LDL, low density lipoproteins (d 1.019-1.063 g/ml); VLDL, very low density lipoproteins (d < 1.006 g/ml); HPLC, high performance liquid chromatography; V<sub>d</sub>, apparent volume of distribution; k<sub>e</sub>, apparent fraction elimination constant; RP, retinyl palmitate.

1Preliminary reports have appeared in abstract form (1, 2).
2To whom reprint requests should be addressed at: GI-Division B-158, University of Colorado School of Medicine, 4200 East Ninth Avenue, Denver, CO 80262.
decay of RP before and during triglyceride lipolysis enhanced by heparin in five healthy subjects. We used continuous intravenous heparin infusion, which accelerates chylomicron triglyceride removal 2- to 3-fold by release of lipoprotein lipase (12).

METHODS

Study design

This study was approved by the Human Subjects Committee of the University of Colorado School of Medicine. All subjects gave written informed consent, were paid volunteers, and were hospitalized for study in the Clinical Research Center.

Three women and two men ages 21 to 35 years were studied. They were in good health and were not taking any drugs. Their plasma lipid levels were in the normal range. Pregnancy was excluded by history, and by the rapid-HCG-test.

After an overnight fast, each subject took 110 mg of retinyl palmitate, equivalent to 60 mg of retinol/mL of body surface area in a gelatin capsule and 100 mL/m² heavy cream (39% fat) by mouth and 5 to 6 hr later two units of plasma were obtained by plasmapheresis. The plasma was stored in ACD buffer, pH 7.0, light-shielded at room temperature for 42 hr, well mixed, and then separated into two equal volumes. RP concentration and its distribution in individual lipoprotein fractions were measured in the pooled plasma (3).

Two days later, at 8:00 AM, half of the stored plasma was pulse-injected intravenously into the fasting donor within 2 min and blood samples for retinyl palmitate assay were obtained from the contralateral forearm prior to the injection, every 4 min for 28 min, at 35, 45, 60, 75, and 90 min and then every 30 min until 11:30 AM. Heparin was then given intravenously as a bolus (2280 IU/m² body surface area) followed by an infusion (1985 IU/m² per hr) for 7 hr (13). At 3:30 PM the other half of the plasma was pulse-injected and a second retinyl palmitate clearance was performed.

The subjects remained fasting for the entire study period. Except for transient (3-5 min) symptoms of hypocalcemia (hot flushes) due to the citrate injected with the plasma, no adverse reactions to the procedure occurred.

Plasma triglyceride and cholesterol levels, total plasma lipolytic activity, and activity of hepatic triglyceride lipase were determined at the beginning and at the end of each clearance period, 15 min after heparin bolus injection, and 90 min after beginning the second RP clearance. To assess decay of chylomicron triglyceride, 9-ml blood samples were drawn immediately prior to plasma injection and at 5, 15, 25, and 35 min of each clearance period. These blood samples contained 1.4 mg/ml Na EDTA and 2.7 mg/10 mL diethyl-p-nitrophenyl phosphate (ICN Pharmaceutical, Plainview, NY), an inhibitor of plasma lipolytic activity (14). They were immediately cooled on ice and the plasma was separated by centrifugation (5 min, 1000 g) within 45 min. At 90 or 120 min of each clearance period, a blood sample was obtained and analyzed for RP distribution in plasma lipoprotein fractions separated by ultracentrifugation (15).

Analyses

RP levels in total plasma (0.5 ml aliquot) or individual lipoprotein fractions were measured by HPLC (16) as previously described (3) using 100 pmol of retinyl undecanoate as internal standard and a chloroform-methanol lipid extraction procedure (17).

In a SW 40 rotor of a Beckman L 5-75 ultracentrifuge, chylomicrons (d < 0.90 g/ml), VLDL fractions A (approx. d 0.933 g/ml), B (approx. d 0.957 g/ml), and C (approx. d 0.967-0.984 g/ml) were prepared from 4 ml of plasma by sequential flotation on a discontinuous salt gradient, which was finally fractionated into an intermediate density fraction, a visible LDL band, and a d 1.10 g/ml infranatant according to Redgrave and Carlson (15) as previously described (3).

Using this density gradient centrifugation technique, chylomicron fractions were prepared in a single spin (4.5 × 10⁶ g-min), and the top 1.5 ml of each gradient was harvested with a Beckman tube slicer. Total lipids were extracted three times using two parts of diethylether and one part of water phase. Samples were dried under nitrogen and analyzed for triglycerides using a fluorometric microassay for glycerol after alkaline hydrolysis (18).

For assay of lipolytic activities, blood specimens were immediately cooled on ice and the plasma was separated by centrifugation. From 1 to 2 ml of fresh plasma lipolytic activity was adsorbed to 0.5 ml columns of heparin-Sepharose 6B (Pharmacia) and the columns were washed with 0.5 ml of 0.2 M Na barbital buffer. The lipolytic enzyme activity was eluted with 1.5 ml of Na barbital buffer containing 6 mg/ml Na⁺-heparin (Fischer) and analyzed by an enzymatic assay using a radiolabeled triolein substrate as described (19). Hepatic triglyceride lipase activity was assayed in the absence of serum activator and in the presence of 1.2 M NaCl, conditions that completely inhibit peripheral lipoprotein lipase (19). Total serum triglyceride and cholesterol levels were measured by standard techniques (20).

Calculations

RP clearance was calculated by dividing the dose of RP administered by the area under the total (RP) time curve, as determined by the trapezoidal method. In addition, RP

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3Vitamin A palmitate 1,000,000 IU/g in corn oil was a generous gift from Dr. B. I. Wolff, Roche Chemical Division, Nutley, NJ.

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plasma disappearance data were tested for fit to a mono-, bi-, or triexponential decay function by an exponential stripping program; final fit of the data to the resulting biexponential function was performed with a nonlinear least squares program as previously described (3). The apparent volume of distribution of retinyl palmitate was calculated by dividing the retinyl palmitate dose injected by the y-intercepts of the biexponential decay curve. Clearance rates were calculated by dividing the dose of RP administered by the area under the plasma RP concentration time curve (dose/AUC).

Significance of the differences between the clearance rates before and during heparin administration was calculated by the Student's t-test for paired observations after testing the equality of variances by an F-test (21, 22). P < 0.05 was considered statistically significant. All results, unless otherwise stated, are expressed as mean ± 1 standard deviation.

**RESULTS**

**Heparin effects on plasma lipolytic activity**

Heparin administration caused total plasma lipolytic activity and hepatic triglyceride lipase activity to increase 500-fold (Fig. 1). Plasma lipolytic activities remained at maximum levels throughout the period of heparin infusion.

**Heparin effects on plasma triglyceride lipolysis**

Plasma total triglyceride levels were in a steady state during both clearance periods. Mean total plasma triglyceride levels were higher during the control study (65 ± 27 mg/dl) than during the heparin study (50 ± 34 mg/dl), whereas total plasma cholesterol levels were unaltered during the entire study period.

The plasma decay of chylomicron triglyceride after the injection of retinyl palmitate-rich plasma could be ana-

![Fig. 1. Total plasma lipolytic activity (n = 5) (●) and hepatic triglyceride lipase activity (n = 4) (○). Data points are mean ± SEM. At the open arrow RP-labeled plasma was injected and its clearance was measured. At the closed arrow a bolus of heparin was given intravenously and heparin was infused continuously for 6.5 hr as shown by the horizontal line.](image_url)
lyzed in only three studies (Table 1). The half-lives for chylomicron triglyceride decay ranged from 5.7 min to 10 min in the control state and was shortened 2- to 3-fold during heparin administration. One subject (No. 5) had slight chylomicronemia prior to the control study. His serum triglyceride levels decreased from 66 to 27 mg/dl during the control study and was, therefore, not useful for further analysis. Another set of samples (No. 2) was accidentally destroyed during work-up.

Heparin effects on plasma decay of RP-labeled chylomicrons

The plasma injected for control clearances and clearances during heparin infusion contained equal amounts of RP, triglyceride (Table 2), and cholesterol (data not shown). After storage, 85 ± 4% of the RP was associated with the chylomicron and VLDL fraction, whereas the remaining 15 ± 4% floated in lipoprotein fractions of d > 1.006 g/ml, primarily in LDL. The plasma volumes injected were 314 ± 17 ml and 323 ± 11 ml and caused the hematocrit to drop 4.3 ± 2.4% and 4.1 ± 1.1% in control clearances and clearances during heparin administration, respectively.

Heparin-enhanced chylomicron triglyceride lipolysis did not affect plasma clearance of RP-labeled intestinal lipoproteins (Fig. 2, Table 3). The mean clearance rate calculated as RP dose divided by the area under the RP plasma decay curve was 104 ± 21 ml/min in the control period and 113 ± 20 ml/min during heparin administration.

RP plasma decay obeyed a biexponential function in four of five subjects. The rapid kinetic process cleared 90 ± 5% of the injected RP in studies before and during heparin-induced lipolysis. Neither the fast nor the slow decay constant was affected by heparin (Table 2). In the fifth subject (No. 3), a possible second kinetic component was not adequately described. Since it is calculated from an interval of 0.3 and 0.6 times its half-life, in control and heparin studies, respectively, it is not physiologically significant.

Average half-life of the rapid RP plasma decay (n = 5) was 11.6 ± 4.0 min during the control studies and 11.9 ± 5.3 min during heparin infusion. It was 81 ± 39 min and 99 ± 28 min for the slow decay function (n = 4). During the slow RP decay more than 90% of the RP was associated with lipoproteins of d > 1.006 g/ml (Table 4). The apparent volume of distribution was comparable in control studies and during heparin-induced lipolysis (3032 ± 307 vs 3318 ± 648, respectively). The slight mean increase in volume of distribution (286 ml) observed during heparin administration corresponded to the additional plasma volume injected (323 ± 11 ml) during the heparin study.

DISCUSSION

The purpose of this study was to enhance chylomicron remnant formation by heparin-induced chylomicron triglyceride hydrolysis and to determine whether chylomicron remnant removal was also enhanced. The results show that chylomicron remnant removal was unchanged, suggesting that, under the conditions of the study, chylomicron triglyceride hydrolysis is not rate-limiting for chylomicron remnant clearance.

The rate-limiting step in chylomicron and VLDL remnant formation is hydrolysis of the core triglyceride by lipoprotein lipase (23, 24). In studies in the isolated perfused rat heart, the rate of chylomicron and VLDL triglyceride hydrolysis parallels the rate of their remnant formation (23). Intravenous administration of heparin stimulates remnant formation by releasing lipoprotein lipase and hepatic triglyceride lipase from their endothelial binding sites into the circulation (12, 25, 26). Heparin accelerates hydrolysis of infused lymph chylomicrons in dogs 2- to 3-fold (12), similar to the 2- to 3-fold

<table>
<thead>
<tr>
<th>TABLE 1. Concentrations and plasma half-lives of chylomicron triglyceride (TG)*</th>
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<tbody>
<tr>
<td>Subject</td>
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<tr>
<td>---------</td>
</tr>
<tr>
<td>TG Concentration</td>
</tr>
<tr>
<td>Control Studies</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
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</table>

*During control studies and in studies during release of lipolytic activity by heparin, chylomicrons were prepared from 4 ml of fresh plasma at 0, 5, 15, 25, and 35 min after pulse injection of plasma rich in retinyl palmitate-labeled chylomicrons and analyzed for triglycerides (18). Decay of chylomicron triglyceride obeyed a monoexponential first-order function in all three subjects and was 2- to 3-fold enhanced by heparin administration.

*This sample was obtained at 36 min; all others were obtained 25 min after the injection of plasma.
enhancement of chylomicron triglyceride decay during heparin administration in our study. Heparin administration also enhances the concomitant release of C apolipoproteins from chylomicrons and VLDL (27), a critical step in remnant formation. In more physiological systems, such as the isolated perfused rat heart (28) or hepatectomized or intact rats, heparin produces similar changes (29, 30).

Despite potential artifacts with postheparin plasma (14), in vitro incubation of such plasma with plasma chylomicrons and VLDL yields remnants that are normal in composition and function (28-31). In summary, there is strong evidence that the function of functionally intact chylomicron remnants is enhanced during postheparin lipolysis under conditions similar to those of our study. Thus, in spite of enhanced chylomicron remnant formation, plasma removal of RP was not accelerated.

RP appears to be a stable label for the core of chylomicrons and their remnants (3). In our earlier study, only 5-7% of the RP was transferred from chylomicrons and VLDL to LDL during postprandial lipemia or during in vitro incubation (3). RP is transported in the core of chylomicrons and their remnants in plasma and removed with the remnant particle (32). Therefore, plasma removal of RP, at least its major kinetic component, reflects plasma removal of chylomicron remnants.

In our previous study, RP decay was monoexponential in seven of eight subjects, but in this study it was clearly biexponential in eight of ten clearances (i.e. four of five subjects excluding subject No. 3).

The explanation for the difference between the two studies is not obvious. The only systematic difference in procedures is that smaller plasma volumes (323 ± 11 ml vs 516 ± 132 ml) containing smaller doses of RP were injected in this study. In this study the injected plasma contained more RP in the d > 1.006 g/ml (15 ± 4% compared to 5 to 7%), primarily in the LDL fraction. During the second, slow kinetic component of RP decay, almost all of the RP was recovered in the d > 1.006 g/ml lipoprotein fractions, primarily LDL. This second kinetic component, therefore, probably represents clearance of RP transferred to higher density lipoproteins. Clearance rates of RP-labeled chylomicrons, calculated by non-compartmental analysis, during the control state and during heparin-induced triglyceride hydrolysis were not significantly different, indicating that remnant formation was not rate-limiting for the removal of RP-labeled chylomicron remnants from the plasma. Other possibilities for the failure to observe enhanced chylomicron remnant removal in this study will be considered below.

To assure valid results, clearances during control and post-heparin triglyceride hydrolysis states were measured in the same individuals, 7 hr apart. Since triglyceride removal from chylomicrons obeys non-linear, dose-dependent pharmacokinetics (33, 34), the same doses of RP-labeled particles and of chylomicron and VLDL triglycerides were used in each pair of studies.

There are no data suggesting a circadian variation in hepatic chylomicron remnant uptake, but it is unlikely that a circadian rhythm would affect the clearance measurements. Obviously, the sequence of control and post-heparin studies could not be reversed. Furthermore, after conclusion of the control clearance study, heparin was administered for 3 hr until a new steady state of plasma triglyceride was achieved (14). The long interval between studies (7.5 hr) assures the availability of hepatic receptor sites for chylomicron remnant during the second clearance (34). The study design, therefore, provides for two RP clearances per subject, differing only by one variable: heparin-induced lipolysis.

TABLE 2. Kinetic analysis of plasma RP disappearance after intravenous injection of RP-labeled chylomicrons: lack of effect of heparin-enhanced lipolysis

<table>
<thead>
<tr>
<th>Subject</th>
<th>Dose of RP Injected</th>
<th>Dose of Triglyceride Injected</th>
<th>Apparent $V_d$</th>
<th>$k_1$ of the Rapid Decay</th>
<th>$k_2$ of the Slow Decay</th>
<th>Fraction of RP Dose Cleared by the Rapid Process</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>H</td>
<td>mg</td>
<td>C</td>
<td>H</td>
<td>min$^{-1}$</td>
</tr>
<tr>
<td>Mean</td>
<td>1.394 ± 0.0758</td>
<td>1.435 ± 0.0765</td>
<td>239 ± 70</td>
<td>3032 ± 307</td>
<td>3318 ± 648</td>
<td>0.065 ± 0.020</td>
</tr>
<tr>
<td>SD</td>
<td>NS</td>
<td>NS</td>
<td>NS'</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

$^a$C, control; H, heparin.

$^bP < 0.05$ was considered statistically significant.

*The increase in $V_d$ of 286 ml corresponded to the plasma volume of 323 ± 11 ml injected during the second, heparin, clearance study.
Fig. 2. RP decay after intravenous pulse-injection of RP-labeled chylomicrons into the same subjects, first in the control state (O—O) and subsequently during enhanced triglyceride hydrolysis (■—■) due to continuous intravenous heparin administration. In four subjects, RP decay obeyed a biexponential function. In four subjects, heparin-enhanced lipolysis did not affect RP plasma decay. In one subject (No. 3) plasma RP decay was slower during the first control study than during heparin administration. This subject, however, had endogenous chylomicronemia at the start of the study due to a failure to fast, which could have decreased the decay rate of RP-labeled chylomicrons.

Heparin binds to apoproteins B and E in LDL and VLDL at physiological ionic strength and pH (35). Goldstein et al. (36) demonstrated impaired LDL binding to fibroblast LDL-receptors in the presence of heparin, presumably due to competition of heparin at the receptor binding site of these apolipoproteins. The lowest heparin concentrations required, however, exceeded the calculated heparin concentration in our studies by approximately 100-fold. Heparin preferentially binds to LDL rather than VLDL (37) and equally to apolipoprotein E in HDL (38). Therefore, it is not likely that competition of heparin for apolipoprotein E binding sites would substantially decrease chylomicron remnant clearance in these studies.

In conclusion, these studies show that heparin administration, which enhances chylomicron triglyceride hydrolysis and the formation of chylomicron remnants, does not affect the rate of plasma decay of RP-labelled chylomicrons. This supports the usefulness of RP as a marker.

TABLE 3. Plasma clearance of retinyl palmitate prior to (control) and during heparin-enhanced lipolysis

<table>
<thead>
<tr>
<th>Subject</th>
<th>Control</th>
<th>Heparin</th>
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<tr>
<td></td>
<td>ml/min</td>
<td></td>
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<tr>
<td>1</td>
<td>107</td>
<td>116</td>
</tr>
<tr>
<td>2</td>
<td>91</td>
<td>88</td>
</tr>
<tr>
<td>3</td>
<td>68</td>
<td>71</td>
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<tr>
<td>4</td>
<td>140</td>
<td>128</td>
</tr>
<tr>
<td>5</td>
<td>92</td>
<td>136</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>100 ± 27</td>
<td>108 ± 27</td>
</tr>
</tbody>
</table>

*Study design, RP doses administered, and kinetic data for RP decay are given in Table 2. Clearance was calculated by Dose/AUC.
This work was supported by grants AM 31765 and AM 26356 from the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, and by the Clinical Research Center, University of Colorado School of Medicine, Grant RR-00051 from the General Clinical Research Centers Program of the Division of Research Resources, National Institutes of Health. Dr. Berr was supported by a Research Training Grant of the Deutsche Forschungsgemeinschaft. The authors are grateful to Dr. R. Chapman, Director of the Belle Bonfils Memorial Blood Center, Denver, for help with the plasmaphereses performed on the study volunteers. We thank Mrs. Carol McKinley, R. N., for assistance during the clinical studies and Mrs. Radene Showalter, Trudy Yost, and Judith Prasad for expert technical assistance.

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REFERENCES


TABLE 4. Distribution of plasma RP in lipoprotein classes during slow exponential decay of plasma RP*

<table>
<thead>
<tr>
<th>lipoprotein classes</th>
<th>Control</th>
<th>Heparin-Enhanced Lipolysis</th>
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<tbody>
<tr>
<td>Chylomicrons and VLDL A</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>VLDL B + C</td>
<td>7 ± 8</td>
<td>3 ± 6</td>
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<tr>
<td>d &gt; 1.006 g/ml</td>
<td>93 ± 7</td>
<td>97 ± 6</td>
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*Recovery from lipoprotein classes was 106 ± 36% (high variation was due to low levels of RP). The RP concentrations in the plasma samples obtained at the end of the study were 3.6 ± 3.3 and 5.7 ± 3.3% of the peak concentrations in the control and heparin studies, respectively.


