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PLASMA HDL LEVELS ARE REGULATED BY THE CATABOLIC RATE OF LARGE PARTICLES OF LIPOPROTEIN CONTAINING APO-A-I

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SUMMARY Lp-A-I was isolated by immunoaffinity chromatography and then separated into two fractions of large and small Lp-A-I particles by conventional ultracentrifugation with a cut-off density of 1.125 g/ml. The large and small particle-rich fractions were then radiolabeled with [^{125}I]-Na and [^{131}I]-Na, respectively. Both of the labeled lipoproteins were injected (20 μCi, i.v.) simultaneously into normolipidemic rabbits. The FCR of the large Lp-A-I particles was much less than that of the small Lp-A-I particles (0.801±0.026 /day vs. 2.227±0.067 /day, P<0.0001). These data indicate that the two particles have distinctly different metabolic pathways and that the lower FCR of larger Lp-A-I particles can effectively raise plasma HDL levels.

Lipoproteins containing apo-A-I (HDL) have recently been divided into two subspecies; those which contain apo-A-I but not apo-A-II (Lp-AI), and those which contain both apo-A-I and apo-A-II (Lp-A-I/A-II). Clinical studies have shown that low HDL levels are closely linked to low Lp-A-I levels, particularly in cases of coronary heart disease (1,2). Since plasma levels of Lp-A-I/A-II are fairly constant (1-3), in the plasma apo-A-I fractional catabolic rate (FCR) may reflect the FCR of Lp-A-I. Lp-A-I consists of two distinctly sized particles (large Lp-A-I; diameter 11.2 nm, and small Lp-A-I; 8.8 nm) (3), as determined by both gradient gel electrophoresis and electron-microscopy. We recently found that these particles differ functionally and that large Lp-A-I particles regulate the production of plasma cholesteryl ester (Ohta et al. unpublished data). In this study, we further analyzed the kinetic patterns of these particles in vivo using rabbits. To the best of our knowledge, this is the first report demonstrating the kinetics of Lp-AI separated by the particle sizes.

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MATERIALS and METHODS

Rabbits Male Japanese White normolipidemic rabbits (age approx. 10 months and weighing 2.8-3.0 kg, n=3) were obtained from Kyudo Co., Ltd., Fukuoka, Japan. All of the animals were housed individually with a 12 h light / 12 h dark cycle. The experimental project was assessed and approved by the Ethics Committee of Fukuoka University.

Lipoproteins Lp-A-I and Lp-A-I/A-II were isolated from pooled human plasma by a combination of anti-apo-A-I and anti-apo-A-II immunosorbent columns, as described previously (3-5). Briefly, pooled plasma was applied to an anti-apo-A-I immunosorbent column. After washing extensively with buffer A (0.01 M Tris-HCl, 0.5 M NaCl, 1 mM EDTA, pH 7.5), the column was eluted with 0.1 M acetic acid, and 1 mM EDTA (pH 3.0). Each effluent was immediately adjusted to pH 7.4 with 1.0 M Tris solution, and dialyzed against 0.15 M NaCl, and 1 mM EDTA (pH 7.4) (buffer B). Finally, the sample was concentrated in buffer B using an ultrafiltration cell (Amicon Corp., ME) equipped with a PM-10 membrane, and then applied to an anti-apo-A-II immunosorbent column. The column was washed with buffer A to obtain Lp-A-I. The Lp-A-I was then dialyzed and concentrated in buffer B. With this procedure, more than 90% of the lipids and apolipoproteins applied were recovered in the unbound and bound fractions. These lipoproteins were then dialyzed and concentrated. The purified Lp-A-I was further subfractionated into HDL_2 (d=1.063-1.125 g/ml) and HDL_3 (d=1.125 - 1.21 g/ml) fractions by ultracentrifugation at 15,000 g for 48 h at 4°C (6). Finally, these fractions were dialyzed against phosphate buffered saline (PBS). These lipoproteins were used immediately after isolation in the following kinetic experiment.

Iodination of large and small Lp-A-I particles Two hundred μg (approx. 0.5 ml) of the large and small particle-rich fractions of Lp-A-I were radiolabeled with [^{125}I]-Na and [^{131}I]-Na, respectively, according to McFarlane's method (7), as modified by Bilheimer et al. (8) and as previously described (9-11). After iodination, the protein solutions were passed through a Pharmacia PD-10 column equilibrated with saline-EDTA (0.1%), and the void volume (radiolabeled protein solution) was collected. The unbound iodide was removed by extensive dialysis against saline-EDTA.

Kinetic study of large and small Lp-A-I particles To prevent sequestration of radioiodide due to apolipoprotein catabolism, 3 mg of NaI was injected twice (12 h before and immediately prior to the injection of labeled protein) in a solution of approx. 1 ml via the marginal ear vein. Blood samples were taken at 4min, 3 h, 6 h, 8 h. 12 h and 24 h after injection of the labeled protein, [^{125}I]-large Lp-A-I and [^{131}I]-small Lp-A-I, and then once a day for 5 days. Blood was collected in tubes which contained EDTA (1 mg/dl) and plasma was obtained by low-speed centrifugation. Small aliquots of plasma taken at the various time points (4 min to 5 days) were again adjusted to a density of 1.063 g/ml, and then spun using a Hitachi RPL-42T rotor. Since labeled

apo-AI was immediately bound with HDL in rabbit plasma compartment, 99% of the total radioactivity was present in the d=1.063 g/ml bottom fraction. When the plasma was adjusted d=1.21 g/ml, 88% of the total radioactivity was in the 1.21 g/ml top fraction. More than 98% of the radioactivity was TCA-precipitable.

RESULTS and DISCUSSION

We used rabbits in this study since we previously injected rabbits with human HDL to observe the kinetics of human apo-AI in vivo. We observed the complete conversion of recombinant human Met-proapo-A-I to apo-A-I in rabbits in vivo (9). Therefore, we believe that rabbits offer a good model for the kinetics of human HDL, including the effects of drugs on HDL kinetics. In addition, rabbits offer a very safe method for examining HDL kinetics, considering that similar studies have not often been performed in humans.

Lp-A-I was isolated by immunoaffinity chromatography as reported previously (3), and then separated into two fractions of large and small Lp-A-I particles by conventional ultracentrifugation with a cut-off density of 1.125 g/ml. The large and small particle-rich fractions were then radiolabeled with [^{125}I]-Na and [^{131}I]-Na, respectively. Both of the labeled lipoproteins were injected (20 μCi, i.v.) simultaneously into normolipidemic rabbits as described previously (9-11). As shown in Figure 1, the FCR of the large Lp-A-I particles was much less than that of the small Lp-A-I particles (0.801±0.026 /day vs. 2.227±0.067 /day, P<0.0001), and no precursor-product relationship was found between the two fractions. These data indicate that the two particles have distinctly different metabolic pathways. Zech et al. developed a compartmental model for apo-A-I and apo-A-II using a human in vivo kinetic study. They proposed that apo-A-I was comprised of two plasma compartments that decayed at different rates (12). Rader et al. recently reported the in vivo kinetics of apo-A-I in both the Lp-A-I and Lp-A-I/A-II fractions (13). They found that the FCR of apo-A-I in Lp-A-I was much higher than that in Lp-A-I/A-II. Thus, the two apo-A-I plasma compartments described by Zech et al. may correspond to apo-A-I in the Lp-A-I and Lp-A-I/A-II fractions. However, our current results demonstrate the presence of apo-A-I compartments comprised of large and small Lp-A-I, which show different catabolic pathways and functionally different capacities for cholesyteryl esterification; large Lp-A-I has a potent effect in this regard. Further investigation will be needed to clarify the relationship between the catabolic pathways of apo-A-I in Lp-A-I, Lp-A-I/A-II and small, large Lp-A-I.

Our observations indicate that the lower FCR of larger Lp-A-I particles can effectively raise plasma HDL levels, since plasma levels of Lp-A-I/A-II are fairly constant in general populations. Several in vivo apo-A-I kinetic studies [10,11,14] have

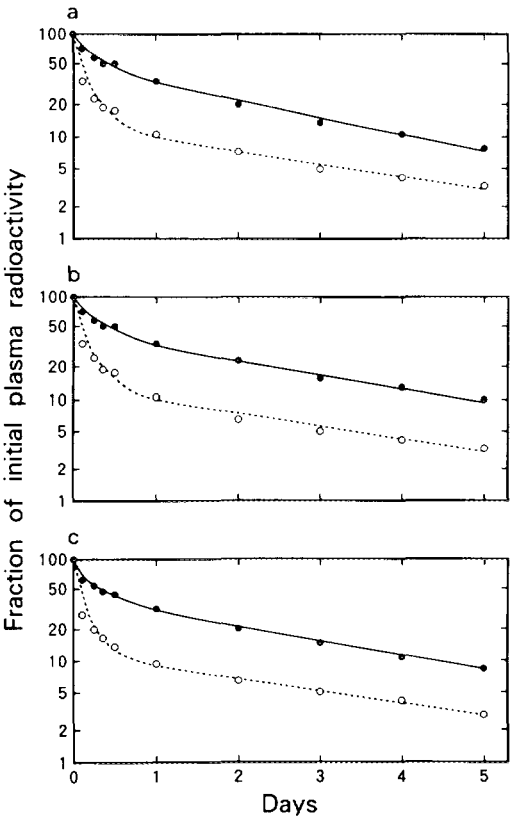

Figure 1. Fraction of initial plasma radioactivity in the [^{125}I]-Lp-A-I large particle-rich (—●—) and [^{131}I]-Lp-A-I small particle-rich (----○----) fractions in three (a, b, c) rabbits.

shown that the apo-A-I FCR is inversely correlated with plasma HDL levels in both diseased and control populations. Therefore, we conclude that the higher apo-A-I FCR of subjects with low HDL levels may result from a deficiency of large Lp-A-I particles, and that small Lp-A-I particles or Lp-A-I/A-II must be a major component of HDL in such patients.

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