IN VITRO CHARACTERIZATION OF TWO TYPES OF LDL APHERESIS MODULE AND EFFECT OF REPETITIVE LDL APHERESIS ON PLASMA CHOLESTEROL LEVELS AND AORTIC ATHEROSCLEROSIS IN HETEROZYGOUS WHHL RABBITS

Soo-Soo Kim, M.D., Yasunori Kutsumi, M.D., Tsuguhiko Nakai, M.D. and Susumu Miyabo, M.D.

In vitro filtration was used to characterize and compare the function of two types of LDL apheresis module: membrane filtration (module M: pore diameter, 0.04 μm; effective surface area, 0.1 m²) and LDL adsorption (module A: a column containing 20 ml of polyvinyl alcohol gels fixed with polyacrylic acid). Module A had better selectivity of LDL removal, while module M could rapidly remove a larger amount of LDL.

The effect of repetitive LDL apheresis with module A on the plasma cholesterol level and on the development of aortic atherosclerosis was examined in 6 heterozygous WHHL rabbits (5 to 10 months old; mean plasma total cholesterol level, 270 ± 39 mg/dl), treated with LDL apheresis at weekly intervals for 2 months. Plasma total and LDL cholesterol levels were lowered approximately 40% by a signal procedure. The LDL cholesterol level tended to decrease as treatment progressed, while the HDL cholesterol level was unchanged or rose above the baseline value in a week after LDL apheresis. The ratio of atherosclerotic lesion area to whole aortic area was relatively low in treated rabbits (6.5 ± 1.9%) in comparison with that in 5 untreated heterozygous WHHL rabbits (18.3 ± 7.7%). The mean cholesterol content in the thoracic aorta was 4.9 ± 1.3 mg/g wet tissue in treated rabbits vs 13.3 ± 6.1 mg/g wet tissue in untreated rabbits. These results suggest that repetitive LDL apheresis might be effective in maintaining a lower level of LDL cholesterol and retarding the atherosclerotic process in vivo.

Familial hypercholesterolemia (FH) is characterized by a massive increase in plasma total cholesterol (C) levels due to the abnormality in the metabolism of LDL, which is caused by a mutation in the gene encoding the LDL receptor. LDL-C is well known to have a strong correlation with coronary atherosclerosis, so severe and fatal ischemic heart disease usually occurs in these patients before the third or fourth decade of life. Thompson et al first tried plasma exchange in the management of FH patients and reported that this procedure brought symptomatic benefit with decreased anginal attacks in three out of four heterozygotes. Plasmapheresis by the double filtration

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Familial hypercholesterolemia
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Third Department of Internal Medicine, Fukui Medical School, Fukui, Japan
Mailing address: Soo-Soo Kim, M.D., Third Department of Internal Medicine, Fukui Medical School, 23 Shimoaizuki, Matsuoka-Cho, Fukui 910-11, Japan
method or the LDL adsorption method has been developed to overcome the disadvantages of this conventional plasmapheresis. Both methods are expected to remove LDL selectively. Quite recently, LDL apheresis using a specific sorbent for LDL has come into greater use since it was shown to remove LDL-C more selectively than double filtration plasmapheresis. Although various kinds of LDL adsorption columns have been used clinically, it is very difficult to come to a definite conclusion as to whether such LDL apheresis actually has a beneficial effect on atherosclerotic lesions in humans. For this purpose animal experiments offer an advantage over those in humans. It has been reported that the progress of atherosclerotic lesions in hypercholesterolemic rabbits was suppressed by plasmapheresis with a membrane filtration module.

In the present study, the functional characteristics of two types of modules, one with membrane filtration and the other with LDL adsorption, were first examined and compared by in vitro filtration tests. Second, we describe a new technique of LDL apheresis for rabbit experiments, based on continuous extracorporeal plasma separation and the removal of LDL by affinity binding to an LDL adsorption column containing polyvinyl alcohol gels fixed with polyacrylic acid as a ligand. Then, in order to investigate the effectiveness of the repeated use of this procedure on the plasma cholesterol level and the development of aortic atherosclerosis, we performed repetitive LDL apheresis in heterozygous WHHL rabbits, which are considered to serve as a model for FH in man. In this paper, the term plasmapheresis will be used to encompass both plasma exchange and LDL apheresis.

MATERIALS AND METHODS

Plasma: Plasma used in the in vitro study was obtained from the outlet of the second membrane during double filtration plasmapheresis in a patient with FH. One unit of heparin per milliliter of plasma was added to this pooled plasma, and then the total volume of plasma was adjusted to 200 ml with saline. Modules: A membrane filtration module (module M: pore diameter, 0.04 μm; effective surface area, 0.1 m²) and an LDL adsorption module (module A) with polyacrylic acid linked to polyvinyl alcohol were examined by in vitro filtration. Module A was a column filled with 20 ml of wet-packed adsorbent which had a binding affinity to apolipoprotein (apo) B-containing lipoproteins. Both modules were manufactured by Asahi Medical Co., Ltd., Tokyo, Japan. Module A was used for in vivo experiments in WHHL rabbits.

In vitro study: The in vitro circuit used in the present study is shown in Fig. 1. The temperature of the plasma pool was kept at 36-37°C with a heat controller. Then the plasma was circulated through each module at a flow rate of 2 ml/min by a peristaltic pump. Samples for biochemical analysis were drawn from the module inlet (plasma pool) first, and from the outlet every 5 or 10 min after the heparinized saline used for priming the circuit had been washed out. While module M was almost completely occluded in approximately 20 min, the experiment could be continued for 60 min in module A. The total flow volume was designed to be almost the same (70-80 ml) in both modules.

Animals: Heterozygous WHHL rabbits were kindly provided by Dr. Y. Watanabe (Kobe University School of Medicine). Heterozygous WHHL rabbits were raised by mating pairs of male homozygous WHHL rabbits and female New Zealand White rabbits. All rabbits were fed standard laboratory chow (Oriental Co., Ltd., Tokyo, Japan) containing 0.1% cholesterol. Rabbits with plasma total-C levels of 100 to 400 mg/dl were considered to be heterozygotes. Six heterozygous WHHL rabbits (rabbit A-F), five males and one female, were chosen to be treated with LDL apheresis. Each rabbit, except for rabbit D, was compared individually with its own sibling as an untreated control, which showed similar plasma total-and LDL-C levels at the beginning of the study. All of them were housed individually in metal cages with a mesh floor, kept in an air-conditioned (23°C) room on a 12 hr light/12 hr dark cycle. Food and water were provided ad libitum. Food intake and body weight were monitored at weekly intervals.

Procedure and study design of LDL apheresis in rabbits: The extracorporeal circuit for LDL apheresis employed in this rab-
bit experiment is illustrated in Fig. 2. During LDL apheresis, rabbits were anesthe-
tized with an intraperitoneal nembutal injection of 30 mg/kg body weight (Pentobarbital sodium solution, Abbott Lab., Illinois, USA). At first the whole circuit was filled with physiologic saline containing heparin. The plasma separator consisted of cellulose diacetate hollow fibers with an average pore diameter of 0.2 μm and an effective surface area of 0.1 m² (Asahi Medical Co.). Blood was withdrawn from the central ear or femoral artery, through a 22-gauge Surflo intravenous catheter (Terumo Co., Tokyo, Japan) at a rate of 4—8 ml/min, and plasma was pumped from the separator at a rate of 1.5—3 ml/min with a peristaltic pump. Before passing through the plasma separator, blood was mixed continuously with heparin (5—10 U/ml). Plasma separated from blood cells through the hollow fibers was then perfused through the LDL adsorption.

Fig. 3. Changes in total inflow amounts (dotted line) and total removal amounts (solid line) of total and lipoprotein cholesterols, and protein at every sampling time in the experiment using a membrane filtration module (module M). VLDL = very low density lipoprotein; IDL = intermediate density lipoprotein; LDL = low density lipoprotein; HDL = high density lipoprotein; C = cholesterol.

column (module A). Finally, processed plasma and blood cells were recombined and returned to the rabbit by apheresis. The total extracorporeal circulation volume was less than 50 ml. LDL apheresis was performed once a week for 2 months in each rabbit. Processed plasma volume was over 100 ml per apheresic procedure on the basis that plasma volume (ml) in a rabbit was estimated to be 3.5–4.0% of body weight. Blood samples for biochemical analysis were taken immediately before and after every LDL apheresis. On the same day, blood samples were also obtained from corresponding untreated rabbits given a similar dose of intraperitoneal nembutal. Rabbits were sacrificed by intravenous nembutal injection after eight apheresic treatments, each with a control rabbit. The whole aorta, from the aortic root to the iliac bifurcation, was removed rapidly and reserved for determination of the atherosclerotic lesion area and cholesterol content.

Fractionation of plasma lipoprotein density classes and analytic procedures of biochemical factors: Both plasma and blood samples were placed in glass tubes containing EDTA-2Na (Terumo Co.). Plasma was separated by centrifugation at 2500rpm for 15 min at 8°C soon after blood was taken from the rabbit. Lipoprotein fractions were isolated by sequential ultracentrifugation at densities of 1.006, 1.019, 1.063 and 1.210 g/ml in a type 50.3Ti-664 rotor (Beckman Instruments, Inc., Palo Alto, CA) in an ultracentrifuge (Hitachi 70P-72-A; Hitachi Koki Co., Ltd., Ibaragi, Japan).

Cholesterol levels in original plasma, VLDL (d<1.006 g/ml), LDL (1.006 to 1.019 g/ml), HDL (1.019 to 1.063 g/ml) and HDL (HDL₂, 1.063 to 1.125 g/ml; HDL₃, 1.125 to 1.210 g/ml) were determined enzymatically with a Determiner TC 555 (Kyowa Medex Co., Ltd., Tokyo, Japan). This method was also used in the determina-
Fig. 4. Changes in total inflow amounts (dotted line) and total removal amounts (solid line) of total and lipoprotein cholesterols, and protein at every sampling time in the experiment using an LDL adsorption module (module A). Abbreviations as in Fig. 3.

tion of aortic cholesterol content. The plasma triglyceride level was also measured enzymatically with a Determiner TG-S 555 (Kyowa Medex Co.). The plasma protein level was measured by the method of Lowry et al.

**Determination of atherosclerotic lesion area and cholesterol content in aorta:** At the end of the study, after the rabbit had been killed, the aorta was dissected and removed. The aorta was opened longitudinally along its anterior margin, flattened on a transparent bag, and photocopied on the glass plate of a photocopier (NP-3525; Canon Inc., Tokyo, Japan). The degree of atherosclerotic surface involvement was evaluated by the xerographic method of Hata et al. Whole aortic and lesion areas were calculated by planimetry (X-PLAN 360; Ushikata Mfg. Co., Ltd., Tokyo, Japan). Aortic samples were stored at −80°C soon after the lesion evaluation until the time of determination of cholesterol content. After the surrounding connective tissues had been removed, the tissue sample of the thoracic aorta was minced and homogenized in preparation for lipid isolation. Then it was extracted with chloroform/methanol, 2:1 (vol/vol) and the chloroform phase was separated, evaporated, and dissolved in isopropyl alcohol before determination of the cholesterol level.

**Statistical analysis:** Data analysis was performed by Student’s paired-t or unpaired-t test as appropriate. Values were expressed as mean ± standard error of the mean (SEM). A p value of <0.05 was considered to be significant.

**RESULTS**

**In vitro study**

Four pools of plasma were prepared for in vitro study so that each module could be tested twice. Data were given as the average of two trials for each experiment. Levels of cholesterol and protein in plasma pools used

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TABLE 1  INITIAL CLINICAL AND LABORATORY DATA IN FIVE CONTROL (PARENTHESSES) AND SIX TREATED HETERozygous WHHL RABBITS

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>Age (month)</th>
<th>Sex</th>
<th>Weight (kg)</th>
<th>Total-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>5 (5)</td>
<td>male (male)</td>
<td>3.3 (3.0)</td>
<td>266 (250)</td>
<td>205 (189)</td>
<td>14 (30)</td>
<td>54 (113)</td>
</tr>
<tr>
<td>B</td>
<td>10 (10)</td>
<td>male (male)</td>
<td>3.6 (4.0)</td>
<td>327 (278)</td>
<td>206 (181)</td>
<td>23 (20)</td>
<td>46 (53)</td>
</tr>
<tr>
<td>C</td>
<td>10 (10)</td>
<td>male (male)</td>
<td>3.4 (3.4)</td>
<td>211 (207)</td>
<td>159 (129)</td>
<td>17 (22)</td>
<td>8 (15)</td>
</tr>
<tr>
<td>D</td>
<td>5</td>
<td>male</td>
<td>3.9</td>
<td>387</td>
<td>287</td>
<td>29</td>
<td>32</td>
</tr>
<tr>
<td>E</td>
<td>7 (7)</td>
<td>female (male)</td>
<td>3.4 (3.1)</td>
<td>311 (249)</td>
<td>196 (196)</td>
<td>30 (18)</td>
<td>29 (76)</td>
</tr>
<tr>
<td>F</td>
<td>7 (7)</td>
<td>male (female)</td>
<td>3.1 (3.8)</td>
<td>115 (172)</td>
<td>62 (74)</td>
<td>18 (65)</td>
<td>25 (28)</td>
</tr>
<tr>
<td>Mean</td>
<td>7.3 (7.8)</td>
<td></td>
<td>3.5 (3.5)</td>
<td>270 (231)</td>
<td>186 (154)</td>
<td>22 (31)</td>
<td>32 (57)</td>
</tr>
<tr>
<td>S.E.M.</td>
<td>0.9 (1.0)</td>
<td></td>
<td>0.1 (0.2)</td>
<td>39 (19)</td>
<td>30 (23)</td>
<td>3 (9)</td>
<td>7 (17)</td>
</tr>
</tbody>
</table>

Abbreviations as in Fig. 3. There are no significant differences in any of the parameters between the two groups.

in the experiment of module M were as follows: total-C, 785 and 445 mg/dl (VLDL-C, 84 and 26 mg/dl; IDL-C, 32 and 17 mg/dl; LDL-C, 585 and 361 mg/dl; and HDL-C, 84 and 41 mg/dl), and protein, 6.0 and 4.1 g/dl, respectively. Fig. 3 shows changes in total inflow amounts (TIA) and total removal amounts (TRA) of total and lipoprotein cholesterol, and protein calculated from the data in the experiment of module M. TIA is equal to the cumulative amount of each factor entering the module. The TRA at every sampling time was similarly calculated on the assumption that the rate of removal was constant during the sampling interval. The ratios of TRA to TIA in VLDL-C, IDL-C and LDL-C were kept constant at 1.0 throughout the experiment. This means that perfect removal of these lipoprotein cholesterol could be achieved by module M. However, the same ratio in HDL-C was as high as 0.90 or more throughout the study. The final ratios of TRA/TIA at the end of the study were 0.99, 1.0, 1.0, 0.997, 0.95 and 0.81 for total-C, VLDL-C, IDL-C, LDL-C, HDL-C and protein, respectively.

The data in the study using module A are shown in Fig. 4. The levels of cholesterol and protein in the two pools of plasma employed in the experiment using module A were as follows: total-C, 520 and 560 mg/dl (VLDL-C, 56 and 35 mg/dl; IDL-C, 31 and 14 mg/dl; LDL-C, 397 and 466 mg/dl; and HDL-C, 36 and 45 mg/dl), and protein, 3.9 and 5.2 g/dl, respectively. In VLDL-C, TRA began to fall below TIA immediately after 5 min. The TRA curve of IDL-C reached a plateau at 10 mg. Although TRA of LDL-C became lower than TIA after 20 min, it showed a linear and sharp increment even in the later period of the experiment. TRAs of HDL-C and protein were much lower than TIAs from the very beginning of the experiment. The final ratios of TRA/TIA were 0.78, 0.64, 0.57, 0.82, 0.58 and 0.26 for total-C, VLDL-C IDL-C, LDL-C, HDL-C and protein, respectively.

There was no significant difference in mean total-(615±170 vs 540±20 mg/dl) and LDL-C(473±112 vs 432±35 mg/dl) levels between plasma pools used in the experiments of modules M and A.

LDL apheresis in WHHL rabbits

The clinical and laboratory data of the rabbits at the start of the study are summarized in Table I. They were 5 to 10 months old, weighing 3.1 to 3.9 kg (treated group) and 3.0 to 4.0 kg (untreated group). The values of plasma total-C in each treated rab-
### TABLE II

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>Mean removal rate (%)</th>
<th>Total-C</th>
<th>LDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>8</td>
<td>40.4±3</td>
<td>42.9±4.2</td>
</tr>
<tr>
<td>B</td>
<td>8</td>
<td>32.8±2.9</td>
<td>35.7±3.0</td>
</tr>
<tr>
<td>C</td>
<td>8</td>
<td>41.9±4.2</td>
<td>43.9±4.3</td>
</tr>
<tr>
<td>D</td>
<td>8</td>
<td>46.4±5.3</td>
<td>46.5±8.2</td>
</tr>
<tr>
<td>E</td>
<td>8</td>
<td>40.1±1.6</td>
<td>41.0±1.3</td>
</tr>
<tr>
<td>F</td>
<td>8</td>
<td>38.1±2.1</td>
<td>29.7±4.8</td>
</tr>
</tbody>
</table>

### TABLE III

Mean removal rates of plasma total-and LDL-C by LDL apheresis in heterozygous WHHL rabbits.

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>n</th>
<th>Mean removal rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>8</td>
<td>40.4±3</td>
</tr>
<tr>
<td>B</td>
<td>8</td>
<td>32.8±2.9</td>
</tr>
<tr>
<td>C</td>
<td>8</td>
<td>41.9±4.2</td>
</tr>
<tr>
<td>D</td>
<td>8</td>
<td>46.4±5.3</td>
</tr>
<tr>
<td>E</td>
<td>8</td>
<td>40.1±1.6</td>
</tr>
<tr>
<td>F</td>
<td>8</td>
<td>38.1±2.1</td>
</tr>
</tbody>
</table>

All values are given as the mean±SEM. n=number of treatments. Other abbreviations as in Fig. 3.

The effects of repetitive LDL apheresis on plasma total and lipoprotein cholesterol levels in each rabbit are summarized in Table II. Plasma total-and LDL-C levels were decreased significantly by this treatment (p<0.05), while both HDL2- and HDL3-C levels changed only slightly. The amount of IDL-C removed was considerable, and its removal rate often became higher than that of LDL-C. There was no significant difference between the mean pretreatment level of plasma total-C in each treated rabbit and the mean total-C level in its control. The mean pretreatment levels of LDL-C in rabbits B and C were significantly higher than the mean levels of LDL-C in the corresponding controls. In rabbits B and C, the mean pretreatment levels of HDL2- and HDL3-C in rabbit F were constantly lower throughout the study, while the mean pretreatment level of LDL-C (134±15 mg/dl) tended to be
higher than the mean level of LDL-C (94±18 mg/dl) in the control.

Mean removal rates (r) of plasma total- and LDL-C were calculated from the data before and after eight apheresic treatments (Table III). The equation used is \( r \) (\%) = \( \frac{(Lb-La) \times 100}{Lb} \), where Lb and La represent the cholesterol levels before and after LDL apheresis, respectively. Mean removal rates of plasma total- and LDL-C were very similar in all rabbits except rabbit F, reaching approximately 40%. The mean \( r \) of LDL-C was as low as 29.7±4.8% (8.8 to 49.5%) in rabbit F.

Figure 5 shows the weekly changes in mean plasma total and lipoprotein cholesterol levels before and after each of eight apheresic treatments in treated rabbits, and those in untreated rabbits during the study. The mean pretreatment levels of all parameters in the treated group showed no significa-

significant difference from the mean levels in the control group throughout the study. In the treated group, the changes in LDL-C levels were quite similar to those in total-C levels. In addition, plasma total-, IDL-and LDL-C levels tended to decrease along with the treatment course. On the other hand, HDL\(_2\)-and HDL\(_3\)-C levels tended to return at least to the baseline value, and sometimes even greater. There was no definite trend in any of the parameters in the control group.

As illustrated in Fig. 6, atherosclerotic lesions were much more severe in the thoracic aorta, especially in the arch, than in the abdominal aorta, even though they appeared in various patterns. The degree of atherosclerotic surface involvement was expressed as a ratio of the lesion area to the whole aortic area. This value was lower in every treated rabbit than in the corresponding untreated rabbit. Rabbit D had no control rabbit with

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which to compare, but its lesion ratio was extremely low (0.6%) though its initial plasma total-C level was the highest among all the rabbits including the controls. Inhibition of atherosclerosis was obviously great in rabbit D, even if compared with rabbit A and its control which were the same age as rabbit D. Evaluation of the cholesterol content in the thoracic aorta, where atherosclerotic lesions were most densely located, revealed that the mean value in the treated group (4.9±1.3 mg/g wet tissue) was much lower than in the control group (13.3±6.1 mg/g wet tissue). However, the cholesterol content was a little higher in rabbits B and F than in their controls.

**DISCUSSION**

Two types of module are available for the LDL adsorption procedure, one by affinity adsorption with heparin17 or dextran sulfate18 and the other by immunoadsorption with anti-LDL serum19. One of the disadvantages of the adsorption method resides in its limitation of binding capacity. Additionally, there is the risk of sensitizing patients to animal gamma globulin by the immunoadsorption method. In the present study, we developed and employed a module filled with polyvinyl alcohol gels20 as a new LDL adsorption column. Polyvinyl alcohol is a water-soluble synthetic polymer made by alcoholysis of polyvinyl acetate, having both good chemical stability and water solubility, and easily forms gels with low toxicity. One possible mechanism of the LDL adsorption21 is electrostatic interaction between apo B and the ligands such as polycrylic acid or dextran sulfate, both of which have a negative charge. Apo B is known to have a positive charge and to account for 95% of LDL apolipoproteins. Apolipoproteins of VLDL and IDL consist of apo A-I, B(40%), C and E. Since VLDL and IDL are larger than LDL in diameter and volume, LDL is likely to diffuse more easily than VLDL and IDL into the beads with ligands.

The results of in vitro study demonstrated that both modules M and A were able to remove an appreciable amount of LDL. Module M had a greater capacity and a higher speed for removal of LDL than did module A. In contrast, module A showed relatively better selectivity of LDL removal and allowed more HDL and protein to pass through the column than did module M. The capacity of module A to bind apo B-
containing lipoproteins appeared to be rather limited. Module A will be a more reliable and useful device for LDL apheresis if its binding capacity can be adequately increased.

The amount of HDL-C removed by module M was unexpectedly large. This is certainly because too many particles larger than HDL in the plasma pool led to rapid occlusion of the membrane filter.

The plasma employed originated from human plasma trapped by the second membrane filter during double filtration plasmapheresis in a FH patient. So, in every plasma pool degeneration of lipoprotein particles could occur to a certain degree in addition to the problem that LDL-C level was different among the plasma pools. These problems might make it difficult to evaluate quantitatively the removal capacity of each module. However, the plasma pools were obtained from the same patient and were processed by the same procedure. Therefore, it should be reasonable to discuss a relative difference in the selectivity of LDL removal between the two modules.

We have already reported an efficient plasmapheresis system that makes it possible to study the long-term effect of plasmapheresis on plasma cholesterol metabolism in rabbits. In the present study of LDL apheresis in rabbits, a modified system was devised, which was well tolerated by rabbits. Methodological improvements to enable repetitive LDL apheresis in small animals include minimizing the priming volume of the extracorporeal circuit and securing good blood access. The priming volume should be 50 ml or less in the rabbit experiment. It must be of particular interest to get good blood access. We used the rabbit's own vessels for blood access, but a few artificial devices have been proposed for repeated use.

It is difficult to breed heterozygous WHHL rabbits which are of the same age and have similar plasma cholesterol levels since the heterogeneity of their characteristics is so remarkable. Nevertheless, we dared to use heterozygous WHHL rabbits in this study for the following reasons: 1) In humans the incidence of heterozygous FH is so high that the management of heterozygotes is clinically important. 2) It is uncertain that homozygous WHHL rabbits can tolerate repeated LDL apheresis. 3) 20 ml of gel in module A is considered to adsorb approximately 400 mg of cholesterol, but this capacity may be insufficient for homozygous WHHL rabbits.

The decrease in plasma total-and LDL-C averaged approximately 40% after a single LDL apheresis procedure and none of the treated rabbits suffered physical damage, at least from this degree of reduction in cholesterol. The influence of a longer period of treatment on rabbits is unknown because it is impossible to perform the procedure more than 10 times at the present. The effect of LDL apheresis on VLDL-C is equivocal. Since the absolute value of IDL-C was low, similar to that of HDL_{2}- and HDL_{3}-C, its reduction was due partly to the fact that 50 ml or less of plasma was replaced by saline for priming of the adsorption column. A slight reduction in HDL-C immediately after LDL apheresis might also be caused by the adsorption of apo E-containing HDL. Further study will be needed to clarify this possibility.

Changes in total-and LDL-C levels during the study resembled each other in every treated rabbit (Table II and Fig. 5). This result confirms the hypothesis that the absolute amount of LDL-C removed substantially influences in vivo cholesterol metabolism. Interestingly enough, the pretreatment levels of atherogenic cholesterol such as IDL-C and LDL-C tended to decrease with treatment, while HDL_{2}- and HDL_{3}-C levels were unchanged or rose above the baseline values in one week after each LDL apheresis. Many reports on a similar phenomenon in humans are available. Homma et al investigated the recovery process of plasma lipoprotein cholesterol after double filtration plasmapheresis in five heterozygous FH patients and reported that HDL_{2}- and HDL_{3}-C reached their preplasmapheresis levels in one week. The mechanism whereby LDL apheresis improves HDL metabolism is explained as follows: 1) Periodic removal of a small amount of total HDL apolipoprotein (vascular + extravascular) may stimulate HDL synthesis. 2) The LDL-to-HDL ratios may be restored to more normal values. 3) Depletion of cellular cholesterol may be expected to reduce receptor-mediated binding of HDL in tissues, which depends direct-
ly upon cellular cholesterol content, and thus to promote the movement of extravascular HDL back into plasma. The present study not only provides support for these findings but also suggests that repetitive LDL apheresis with an adsorption column may be effective in maintaining lower levels of atherogenic lipoprotein cholesterol.

Comparison of aortic atherosclerosis between untreated and treated heterozygous WHHL rabbits clearly demonstrated that repetitive LDL apheresis reduced atheromatous plaque formation in the aorta. For instance, the ratio of the lesion area in rabbit C was 1/5 that in the control, whereas mean pretreatment levels of HDL$_2$-and HDL$_3$-C were significantly lower in rabbit C than in the control, and the reverse was true with regard to LDL-C. Furthermore, plasma total-and LDL-C levels did not necessarily tend to decrease during the course of repetitive LDL apheresis in this rabbit. An extremely low ratio (0.6%) of lesion area in rabbit D deserves special mention in consideration of its relatively high initial plasma total-C level (387 mg/dl). The mean removal rates of plasma total-and LDL-C were also the highest in this rabbit. These results suggest that a very rapid decrease in atherogenic lipoprotein cholesterol is definitely responsible for the retardation of the atherosclerotic process. On the other hand, in rabbit F and its control the ratio of the lesion area was the same, as was the cholesterol content in the thoracic aorta. LDL apheresis was not always carried out effectively in rabbit F partly because this rabbit was often irritable during treatment in spite of having the same anesthetic procedure as the others. The cholesterol content in the thoracic aorta in the treated group tended to be small, but was not necessarily correlated with the ratio of the lesion area. This was probably because histobiochemical factors other than the cholesterol content also influenced the lesion area evaluated by the xerographic method.

The progress of atherosclerotic lesions was greatly suppressed in rabbits C, D and E. In the treatment of these rabbits, mean removal rates of total-and LDL-C were over 40% and an absolute amount of LDL-C removed was 150 mg or more by a single LDL apheresic procedure. From this result it is speculated that 3 g or more of LDL-C should be removed by a single procedure to prevent the progress of atherosclerosis in humans.

Coronary arteries were not examined in this study because few atheromas would be expected to be found out in heterozygous WHHL rabbits, which had not been selectively bred. The heterozygous WHHL rabbits used in this study were all under 10 months of age at the beginning of the study, and their plasma total-C levels were not so high as those of homozygotes reported previously. However, since the ratio of the content of atherogenic lipoprotein cholesterol to plasma total-C was considered to be high enough to promote an atherosclerotic process, we speculated that even in these young rabbits some degree of atherosclerosis was already present and in progress just before LDL apheresis was started. Thus, they were good candidates for testing the beneficial effects of LDL apheresis. The results of this study convince us that repetitive LDL apheresis, even if carried out for only 2 months, is a reliable and effective treatment to retard the progression of atherosclerotic lesions in heritable hypercholesterolemic rabbits lacking LDL receptors.

Published reports on plasmapheresis have encouragingly documented regression of human atherosclerosis on the basis of clinical observations or sequential angiographic evaluations. Further research should be directed toward producing regression of atherosclerosis in much larger numbers and with a longer period of LDL apheresis alone or in combination with lipid-lowering agents. Abbreviations WHHL: Watanabe Heritable Hyperlipidemic VLDL: very low density lipoprotein LDL: intermediate density lipoprotein HDL: high density lipoprotein

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