Decreased production of low density lipoprotein by atorvastatin after apheresis in homozygous familial hypercholesterolemia

A. David Marais, Rossitza P. Naoumova,* Jean C. Firth, Candice Penny, Clare K. Y. Neuwirth,* and Gilbert R. Thompson[1,*]

Department of Internal Medicine, University of Cape Town Medical School, Cape Town, South Africa, and MRC Lipoprotein Team,* Clinical Sciences Centre, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London, W12 ONN, UK

Abstract Apheresis only partially controls raised low density lipoprotein cholesterol levels in patients with homozygous familial hypercholesterolemia, who usually respond poorly to lipid-lowering drugs. The efficacy and mechanism of action of a new 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, atorvastatin, was therefore investigated in seven homozygotes undergoing apheresis. One receptor-negative and six receptor-defective homozygotes undergoing plasma exchange or LDL apheresis every 2 weeks were studied during 2 months each on placebo and on atorvastatin 80 mg daily. Changes in plasma lipids and mevalonic acid, an index of cholesterol synthesis, were measured and the kinetics of the rebound of low density lipoprotein cholesterol and apolipoprotein B after apheresis were analyzed. All subjects had significant improvements on atorvastatin. Mean decreases in low density lipoprotein cholesterol were 31% greater both pre- and post-apheresis on atorvastatin compared with placebo, accompanied by a 63% decrease in mevalonic acid. Percentage changes in low density lipoprotein cholesterol and mevalonic acid were closely correlated ($r = 0.89$, $P = 0.007$). The mean production rates of low density lipoprotein cholesterol and apolipoprotein B were 21% and 25% lower, respectively, on atorvastatin than on placebo ($P < 0.005$ and <0.02) but changes in mean fractional clearance rates were not statistically significant. We conclude that atorvastatin enhances the efficacy of plasma exchange and low density lipoprotein apheresis in patients who lack low density lipoprotein receptors. This effect appears to be due to marked inhibition of cholesterol synthesis which results in a decreased rate of production of low density lipoprotein.—**Marais, A. D., R. P. Naoumova, J. C. Firth, C. Penny, C. K. Y. Neuwirth, and G. R. Thompson.** Decreased production of low density lipoprotein by atorvastatin after apheresis in homozygous familial hypercholesterolemia. *J. Lipid Res.* 1997. **38:** 2071–2078.

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Familial hypercholesterolemia (FH) is characterized by a dominantly inherited increase in the level of low density lipoprotein (LDL) in plasma, and a marked predisposition for the premature onset of atherosclerotic vascular disease. FH is due to mutations affecting one or both alleles encoding the LDL receptor, more than 150 of which have been described (1). Inheritance of two mutations gives rise to the clinical phenotype of homozygous FH, although in the majority of instances such individuals are genetically compound heterozygotes rather than true homozygotes (2). If left untreated, phenotypic homozygotes usually die in their second or third decade from severe atherosclerotic involvement of their coronary arteries, especially the ostia, and aortic root.

The highest frequency of FH occurs in South Africa where 1 in 100 white Afrikaners are affected, due to a founder gene effect in emigrants from Europe (3). The introduction of plasma exchange (4), and subsequently LDL apheresis (5), has improved the prognosis of homozygotes (6) but aortic stenosis remains a frequent and dangerous complication, often necessitating surgical intervention (7).

In an effort to reduce LDL levels still further, apheresis is frequently combined with treatment with a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor (statin). In homozygotes, lovastatin or simvastatin reduced LDL cholesterol by only an additional 11.5–16% below the level obtained with apheresis alone (8, 9). However, much greater reductions in LDL

Abbreviations: LDL, low density lipoprotein; FH, familial hypercholesterolemia; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; apoB, apolipoprotein B; MVA, mevalonic acid; HDL, high density lipoprotein; FCR, fractional catabolic rate; VLDL, very low density lipoprotein.

[1]To whom correspondence should be addressed.

are achievable in FH heterozygotes, whose better response to statins is attributable to their having only a partial deficiency of LDL receptors, the expression of which is markedly enhanced in vivo by pharmacological inhibition of endogenous cholesterol synthesis (10).

Recently we reported the ability of atorvastatin, a new HMG-CoA reductase inhibitor, to decrease cholesterol synthesis and lower LDL cholesterol in heterozygous FH (11). Here we describe the efficacy of atorvastatin as an adjunct to plasma exchange or LDL apheresis in the treatment of homozygous FH, including a receptor-negative subject, and provide evidence that the reduction in LDL is achieved mainly via a decreased rate of production.

SUBJECTS AND METHODS

Seven post-pubertal patients with phenotypically homozygous FH were studied, five in South Africa and two in Britain. Their clinical features and the nature of the associated LDL receptor gene mutations have been described previously (9, 12, 13). Patients were designated as receptor negative or receptor defective, depending upon whether LDL binding by their cultured fibroblasts or Epstein-Barr virus transformed lymphoblasts was $<$ or $>$ 2% of normal (14).

All patients had been undergoing apheresis at 2-week intervals for several years and continued to do so during the study. The South African patients were treated with plasma exchange (9), the British patients underwent LDL apheresis, using disposable dextran sulphate/cellulose columns (15). The study design was an open label comparison of placebo and atorvastatin, 80 mg daily, each for a period of 8 weeks during which apheresis was maintained as usual. Exclusion criteria were previous portacaval shunt, serum triglyceride $>$4.5 mmol/L, hepatic dysfunction, and taking of drugs influencing lipid metabolism or known to interact with HMG-CoA reductase inhibitors.

Serum transaminase and creatinine phosphokinase measurements were performed every 2 weeks, prior to apheresis. Blood samples for serum lipid and lipoprotein assays were taken between 8–9 AM after an overnight fast immediately before and after apheresis on three consecutive occasions during the second half of each treatment period; mean values were used for statistical analysis. The rebound of LDL was studied in each patient for 14 days after apheresis first on placebo and then on atorvastatin, each on three occasions in Cape Town and once in London. Total and high density lipoprotein (HDL) cholesterol, triglycerides, and apolipoprotein(apo)B were assayed by standard methods in the local laboratory and LDL cholesterol was calculated (16). Plasma mevalonic acid, an index of cholesterol synthesis, was analyzed in frozen samples in London, as previously described (11). Fasting levels and diurnal changes in plasma mevalonic acid (MVA) in humans have been shown to correlate with the results of several other methods of estimating cholesterol synthesis, including sterol balance (17), deuterium uptake into newly synthesized cholesterol (18), and assay of hepatic HMG-CoA reductase activity (19). In addition, we recently showed a good correlation between changes in plasma MVA and lathosterol, a later precursor of cholesterol, in FH subjects undergoing LDL apheresis (20). Plasma volume in liters was calculated as 4.5% of body mass in kilograms.

The production and fractional clearance rates of LDL cholesterol and apoB during the 2-week interval between consecutive apheresis were calculated from the equation:

LDL cholesterol (mmol/L) or

$$\text{apoB (mg/dl) at time t (days)} = (u/k)(1 - e^{-k \cdot t}) + m_0 e^{-k \cdot t},$$

Although independently derived, this equation can be re-arranged to that published by Apstein et al. (21) for use in the non-steady state (see Appendix) and assumes that LDL mass (m) is contained in a single intravascular compartment that has a post-apheresis mass (m_0), a zero order production rate (u), and a first order clearance rate (k) and that these parameters do not vary during the 2 weeks rebound period. The parameters u and k were calculated by non-linear regression using Prism (Graphpad Software). The validity of the equation was tested by calculating LDL apoB production rates in two homozygotes (not in this study) in whom non-steady state turnover of ^{125}I-labeled LDL had been determined in the past (22). By summing the absolute catabolic rate of LDL apoB and the daily increment in plasma LDL apoB after apheresis, production rates in the two subjects undergoing non-steady state isotopic turnover studies were estimated as 26.8 and 23.3 mg/kg per day versus 31.5 and 19.4 mg/kg per day by kinetic analysis of the post-apheresis rebound of LDL-apoB using non-linear regression.

Data are presented as means ± standard error of the mean (SEM). Statistical significance of differences during treatment was evaluated by paired *t*-test and by one-way analysis of variance for repeated measurements (ANOVA) for normally distributed data. The Mann-Whitney test was used to compare data with a skewed distribution, i.e., serum triglycerides. The relationship between the changes in LDL cholesterol and plasma

TABLE 1. Clinical characteristics of seven homozygous FH patients

No.	Age at Time of Study	Sex	Plasma Lipids[a] TC	TG	HDL-C	LDL-C	LDL-Receptor Mutation
			mmol/l				
1	13	M	15.1	1.2	0.60	13.9	Exon 7/8 deletion[b] and exon 7/8 deletion[c]
2	13	F	28.7	1.7	0.65	27.3	Pro664Leu and null[d]
3	19	F	18.1	0.9	0.50	17.2	Asp154Asn[e] and Unknown[d]
4	19	M	18.1	2.0	0.60	16.6	Asp206Glu[f] and Unknown[d]
5	24	F	19.5	0.4	0.80	18.5	Asp206Glu[f] and Asp206Glu[d]
6	26	M	23.5	1.0	0.80	22.2	Glu387Lys and Glu387Lys[d]
7	32	M	15.4	1.3	—	—	Asp206Glu[f] and Val408Met[g,d]
Mean ± SE	20.8 ± 2.6		19.8 ± 1.8	1.22 ± 0.2	0.65 ± 0.05	19.2 ± 1.9	

[a]Values were recorded before commencing apheresis.
[b]Cape Town 2.
[c]Receptor negative.
[d]Receptor defective.
[e]Afrikaner 3.
[f]Afrikaner 1.
[g]Afrikaner 2.

MVA was examined by linear regression analysis. A value of $P < 0.05$ was considered significant.

These studies were approved by the Research Ethics Committees of the University of Cape Town and the Hammersmith Hospitals Trust. All patients gave informed consent and continue to receive atorvastatin on a compassionate use basis.

RESULTS

The ages at time of the study and pre-treatment serum lipids of the four male and three female patients are shown in **Table 1.** Three were true homozygotes, one of whom was receptor-negative. The other two and the remaining four compound heterozygotes were receptor-defective.

Changes in serum lipids and lipoproteins

Mean LDL cholesterol levels on placebo and on atorvastatin are shown in **Fig. 1.** Pre- and post-apheresis values were both 31% lower while on atorvastatin (8.17 ± 0.30 and 2.04 ± 0.11 versus 11.90 ± 0.42 and 2.97 ± 0.33 mmol/L, $P = 0.0004$ and 0.026).

Decreases in LDL cholesterol on atorvastatin compared with placebo (**Table 2**) were mirrored by decreases in total cholesterol (9.21 ± 0.33 vs. 13.08 ± 0.51 mmol/L or −29%, $P = 0.0004$). Decreases in apoB (183 ± 10 vs. 238 ± 20 mg/dl or 23%, $P = 0.001$) were less marked (**Table 3**). Corresponding changes in serum triglyceride were 0.58 ± 0.05 vs. 0.77 ± 0.06 mmol/L (−23%, $P = 0.04$). HDL cholesterol values were similar on atorvastatin and placebo (0.89 ± 0.14 vs. 0.81 ± 0.07 mmol/L, respectively).

Kinetic analysis of LDL cholesterol and apoB

The mean post-apheresis rebounds in LDL cholesterol are shown during the placebo and treatment phase for the group in **Fig. 2.** The best fit values for production and clearance in each individual are indicated in Table 2. Analysis of these results showed that the slower rate of rebound of LDL cholesterol on atorvastatin was due more to decreased production than to increased clearance. This trend was especially marked in the only receptor negative homozygote (Patient 1), in whom production rate decreased by 21% but the computed fractional catabolic rate (FCR) did not change. Patient 2 behaved anomalously in that she showed not only a greater decrease in production rate than other patients on atorvastatin but also a marked decrease in FCR. The reasons are unclear but it is note-

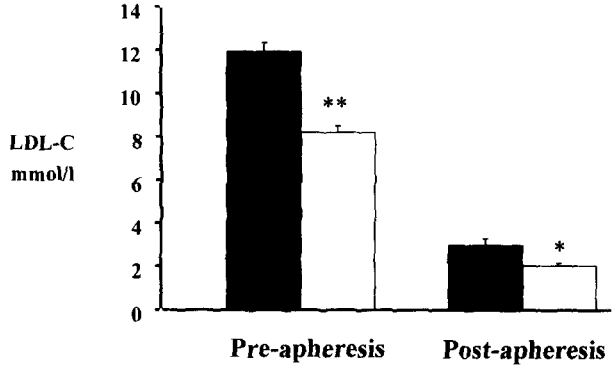

Fig. 1. Plasma LDL cholesterol (C) in seven FH homozygotes undergoing apheresis. Values represent the mean of three consecutive estimations at bi-weekly intervals during the second month on placebo (■) and atorvastatin 80 mg daily (□). $^{**}P = 0.0004$ placebo vs. atorvastatin (pre-apheresis); $^{*}P = 0.026$ placebo vs. atorvastatin (post-apheresis).

TABLE 2. Derived production and clearance rates of LDL-cholesterol in homozygous FH patients undergoing bi-weekly apheresis while on placebo and on atorvastatin

No.	Production Rate		Fractional Clearance Rate		Serum LDL Cholesterol[a]	
	Placebo	Atorvastatin	Placebo	Atorvastatin	Placebo	Atorvastatin
	mmol/day		*pools/day*		*mmol/l*	
1	2.52	1.99	0.126	0.126	10.7	8.7
2	3.98	2.02	0.194	0.117	11.9	8.0
3	5.52	4.23	0.116	0.131	11.4	8.1
4	3.67	2.60	0.111	0.123	10.5	7.2
5	4.72	4.07	0.086	0.152	12.6	7.3
6	3.91	3.68	0.099	0.156	14.0	8.2
7	6.51	5.65	0.141	0.161	12.1	9.7
Mean	4.40	3.46[b]	0.125	0.138[c]	11.90	8.17
SE	0.45	0.47	0.01	0.007	0.42	0.30

[a]Pre-apheresis values.
[b]$P = 0.0047$, atorvastatin vs. placebo.
[c]NS, atorvastatin vs. placebo.

worthy that she was one of the two youngest patients in the series and was undergoing a post-pubertal growth spurt (Table 1).

Analysis of the apoB rebound curves gave similar results with a significant decrease in production rate on atorvastatin but no change in mean FCR (Table 3). Individual values of the latter decreased in two patients (nos. 2 and 4), increased in two (nos. 5 and 6), and were virtually unchanged in the remainder. In contrast, production rates decreased to a greater or lesser extent in all seven patients. Mean decreases in LDL cholesterol and apoB production rates on atorvastatin were of similar magnitude (21% and 25%, respectively).

Changes in cholesterol synthesis

The acute effects of apheresis on MVA levels were examined in each patient, once while on placebo and once while on atorvastatin. As shown in **Fig. 3,** a significant rise in mean MVA levels was observed on the third day after apheresis while on placebo but not while on atorvastatin.

Longer term effects of atorvastatin on cholesterol synthesis were examined by comparing mean pre-apheresis levels of plasma MVA during the second month of each treatment period. As shown in **Fig. 4,** mean values were 63% lower while on atorvastatin than on placebo. As shown in **Figs. 5A and B,** percentage decreases in plasma MVA correlated more closely with percentage change in LDL cholesterol ($r = 0.89$, $P = 0.007$) than with percentage change in apoB ($r = 0.61$, $P = 0.06$).

DISCUSSION

Homozygous FH has hitherto been regarded as refractory to lipid-lowering drugs, necessitating the use of radical forms of therapy such as apheresis, portacaval

TABLE 3. Derived production and clearance rates of apolipoprotein B in homozygous FH patients undergoing bi-weekly apheresis while on placebo and on atorvastatin

No.	Production Rate		Fractional Clearance Rate		Serum apoB[a]	
	Placebo	Atorvastatin	Placebo	Atorvastatin	Placebo	Atorvastatin
	mg/day		*pools/day*		*mg/dl*	
1	624.1	526.1	0.187	0.196	197	159
2	1644.8	786.8	0.344	0.217	268	193
3	1739.6	1320.8	0.229	0.214	200	172
4	944.2	538.3	0.175	0.121	190	157
5	1416.6	1094.6	0.169	0.191	233	170
6	1247.6	1213.6	0.136	0.200	340	218
7	1821.5	1641.7	0.223	0.219	237	215
Mean	1348.3	1017.4[b]	0.209	0.197[c]	237.9	183.4
SE	166.7	158.1	0.026	0.012	20.0	9.6

[a]Pre-apheresis values.
[b]$P = 0.019$, atorvastatin vs. placebo.
[c]NS, atorvastatin vs. placebo.

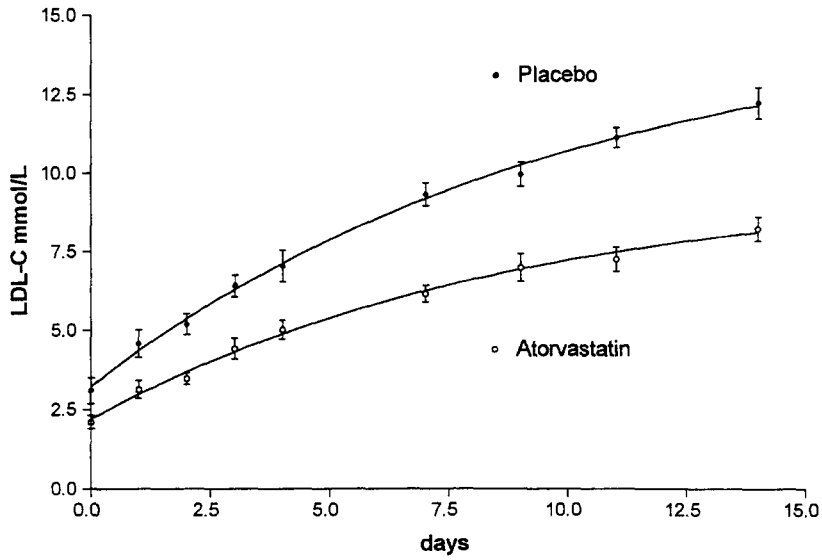

Fig. 2. Rate of rebound (mean ± SE) of LDL cholesterol (C) after apheresis in seven homozygotes while on placebo or atorvastatin 80 mg daily. Goodness of fit (r^2) for placebo and atorvastatin rebounds were 0.91 and 0.88, respectively.

shunt, and liver transplantation. Although more hazardous, the latter procedure can sometimes result in normalization of serum lipids, by substituting a liver with a normal complement of LDL receptors for one that is deficient or defective in that respect (23). The greater severity of hypercholesterolemia in receptor-negative homozygotes and their reported lack of response to HMG-CoA reductase inhibitors (24) led to the concept that the raised LDL is largely attributable to hypocatabolism. However, there is also evidence of increased production of LDL via a very low density lipoprotein (VLDL)-independent pathway in homozygous FH (25), presumably reflecting hepatic cholesterol overload secondary to increased influx of LDL via receptor-independent pathways (26). Thus there are grounds for believing that not only hypocatabolism but also increased production of LDL contribute to elevated LDL levels in homozygotes. If so, this might account for the LDL-lowering effect of portacaval shunting, which reduces both cholesterol synthesis and LDL secretion (27, 28), and also for the response to simvastatin seen in an FH homozygote with a mutation that precluded the formation of LDL receptors (29).

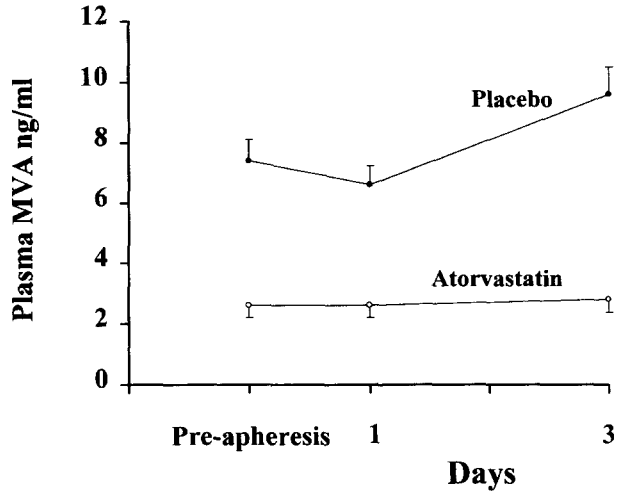

Fig. 3. Plasma mevalonic acid (MVA) immediately before and on days 1 and 3 after apheresis in seven homozygotes while on placebo ($P < 0.05$) and atorvastatin ($P = 0.80$).

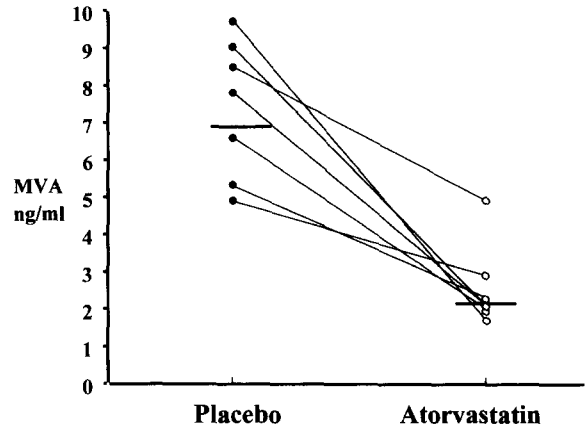

Fig. 4. Effect of atorvastatin on plasma mevalonic acid (MVA) in seven FH homozygotes undergoing apheresis. Each value represents the mean of three consecutive estimations at bi-weekly intervals during the second month on placebo (●) and on atorvastatin 80 mg daily (○). $P = 0.001$ placebo vs. atorvastatin.

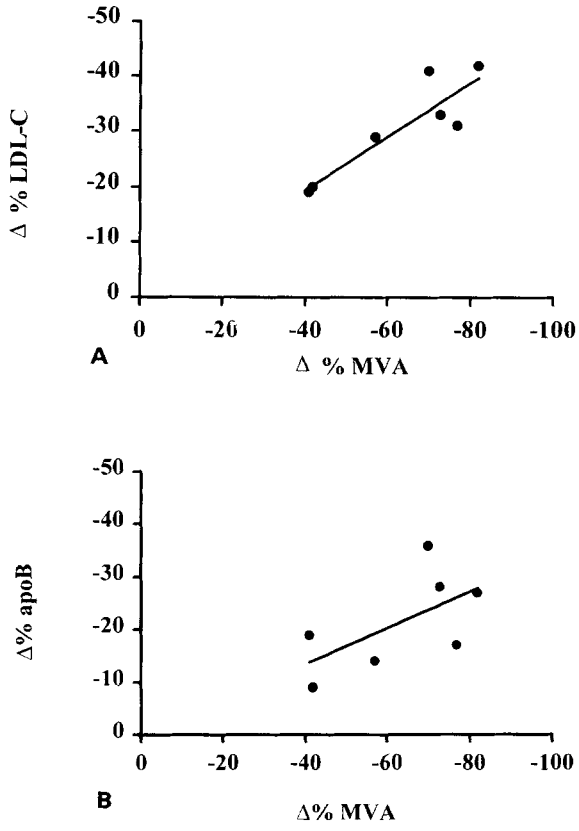

Fig. 5. A: Correlation between percentage reductions in LDL cholesterol and plasma MVA on atorvastatin ($r = 0.89$, $P = 0.007$). B: Correlation between percentage reductions in LDL apoB and plasma MVA on atorvastatin ($r = 0.61$, $P = 0.06$).

Atorvastatin is a synthetic and highly effective HMG-CoA reductase inhibitor (30) which has recently been licensed in Britain and the USA. In FH heterozygotes, atorvastatin (80 mg daily) has been shown to lower LDL cholesterol and plasma mevalonic acid levels by 54% and 59%, respectively (11). During the present study, reductions in LDL cholesterol additional to those achieved by either plasma exchange or LDL apheresis in homozygotes averaged 31%. Measurement of plasma MVA levels during the 24 h after single, equal doses of atorvastatin and simvastatin in two homozygotes (31) and in 20 heterozygotes (32) showed that suppression of cholesterol synthesis was more prolonged after atorvastatin. Animal studies indicate that atorvastatin is retained in the liver longer than lovastatin (33) and an analogous pharmacokinetic differential may exist in humans with respect to atorvastatin and simvastatin.

The validity of our analysis of LDL kinetics is dependent upon certain assumptions, one of which is that all LDL is contained in a single intravascular compartment. However, it has long been recognized that approximately 25% of LDL apoB is located in an extravascular compartment which is in close equilibrium with the plasma pool (34). After apheresis, there is an influx of LDL from the extravascular compartment which appears to reach equilibrium with the intravascular LDL pool within 4 days, based upon a study in a homozygote in whom the distribution of ^{131}I-labeled LDL before and after apheresis was determined using a whole body counter (unpublished data). Influx of extravascular LDL during the first 4 days after apheresis might lead to overestimation of production by the one-pool model but to a similar extent on placebo and atorvastatin, as pharmacological reduction of LDL does not alter its intravascular/extravascular distribution (35). In contrast, the assumption that the production rate remains constant during the rebound period would probably underestimate the initial production rates, but once again both the placebo and atorvastatin phases would be affected to a similar extent in relative terms. As stated under Methods, non-steady state analysis of ^{125}I-labeled LDL apoB turnover in two homozygotes studied previously gave values for production rates that were in reasonable agreement with those obtained in the same subjects by the method used in this study (17.5% lower and 16.7% higher). With regard to the estimation of FCR, analysis of post-apheresis rebound curves in hypercholesterolemic subjects has been shown to give values similar to those obtained in steady-state studies, as was discussed by Apstein et al. (21). Despite its theoretical limitations, the one-pool model originally described by those authors, which we have adapted by the addition of non-linear regression analysis, appears to provide estimates of production rate and FCR that approximate quite closely to those obtained by other methods.

Based on the analysis of post-apheresis rebound curves and on the reduction of LDL cholesterol and apoB in a receptor-negative subject, our data suggest that the LDL-lowering effect of atorvastatin in FH homozygotes was exerted more via a decrease in LDL production than by an increase in LDL catabolism. Reductions in LDL cholesterol and plasma MVA correlated closely, implying that measures that decrease cholesterol synthesis lead to an accompanying decrease in the production of LDL or its precursors. It has been previously shown that limitation of the cholesterol supply by inhibition of HMG-CoA reductase reduced lipoprotein secretion in the perfused rat liver model (36) and we recently reviewed work in cultured cells, animal models, and humans which led us to conclude that the supply of cholesterol to the liver is a determinant of lipoprotein secretion (37); the data from the present study are in keeping with this hypothesis.

Further support has come recently from the observation that atorvastatin exerts marked triglyceride-lowering properties (38) which, it could be speculated, reflects reduced VLDL secretion. An alternative mecha-

nism, that atorvastatin promotes clearance of remnant particles by stimulating residual LDL receptor activity and thus reducing LDL production, would not explain its effects in the receptor-negative subject. Recently presented data in hypercholesterolemic minipigs showed that atorvastatin decreased LDL apoB production by reducing both the secretion of VLDL and its conversion to LDL but without influencing the FCR of either lipoprotein (39). However, it should be noted that in the present study five of the six receptor-defective homozygotes exhibited an increase in the FCR of LDL cholesterol on atorvastatin, consistent with stimulation by the latter of whatever residual LDL receptors there were in the liver.

Atorvastatin was well tolerated in these relatively short-term studies and no significant clinical or biochemical adverse events were observed. Assuming that the decreases in LDL cholesterol which occurred are maintained in the long-term, the combination of apheresis with atorvastatin would enable significantly better control of hypercholesterolemia in FH homozygotes than has been achievable hitherto other than by liver transplantation (40). If so, this should delay the onset of aortic stenosis, which has been shown to correlate with the product of age and cholesterol concentration throughout life (41), as well as help postpone death from coronary heart disease.

APPENDIX

The equation for the rebound of lipoprotein was derived for a simple compartmental model. This model assumes that the mass (m) of LDL cholesterol or apoB is contained in a single intravascular compartment that has a post-apheresis mass (m_0), a zero order production rate (u), and a first order clearance rate (k) and these parameters do not vary during the rebound. The equation describing the change in mass with time ($dm/dt = u - k \cdot m$) was integrated for the boundary conditions where $t = 0$, $m = m_0$ to yield $m_{(t)} = (u/k) - [(u/k) - m_0]e^{-kt}$ or $m_{(t)} = (u/k)(1 - e^{-kt}) + m_0e^{-kt}$.

Apstein et al. (21) made the same assumptions when they described the cholesterol concentration (C) at time t, in terms of a minimum (i.e., post-apheresis) cholesterol concentration (C_M), the steady state cholesterol concentration before apheresis (C_0), and production rate (S) as well as a clearance constant (k). Equation 3 thus became $C/C_M = (C_0/C_M) - [(C_0/C_M) - 1]e^{-kt}$. Noting that the steady state concentration of cholesterol gives the value for (S/k), their equation can be shown to be identical with ours by substitution and rearrangement.

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