Apolipoprotein A-I kinetics in heterozygous familial hypercholesterolemia: a stable isotope study

R. Frénais,* K. Ouguerram,* C. Maugeais,* J. S. Marchini,† P. Benlian,§ J. M. Bard,*, T. Magot,* and M. Krempf[1],*,††**

Centre de Recherche en Nutrition Humaine,* Groupe Métabolisme, Hôtel Dieu, Nantes, France; Division of Clinical Nutrition,† School of Medicine of Ribeirão Preto, Brazil; Service de Biochimie,§ Biologie Cellulaire et Biologie Moléculaire, Hôpital Saint Antoine, Paris, France; Faculté de Pharmacie,** Laboratoire de Biochimie, Hôtel Dieu, Nantes, France; and Clinique d'Endocrinologie,†† Maladies Métaboliques et Nutrition, Hôtel Dieu, Nantes, France

Abstract Heterozygous familial hypercholesterolemia (FH) is associated with a moderate decrease of plasma apoA-I and HDL-cholesterol levels. The aim of the study was to test the hypothesis that these abnormalities were related to an increase of HDL-apoA-I fractional catabolic rate (FCR). We performed a 14-h infusion of [5,5,5-$^2H_3$]leucine in seven control subjects and seven heterozygous FH patients (plasma total cholesterol 422 ± 27 vs. 186 ± 42 mg/dL, $P <$ 0.001, respectively). Plasma apoA-I concentration was not changed in FH compared to controls (respectively 115 ± 18 vs. 122 ± 15 mg/dL, NS), and HDL-cholesterol level was decreased (37 ± 7 vs. 46 ± 19 mg/dL, NS). Kinetics of HDL metabolism were modeled as a single compartment as no differences were observed between HDL_2 and HDL_3 subclasses. Both mean apoA-I FCR and absolute production rate (APR) were increased in FH (respectively, 0.36 ± 0.14 vs. 0.22 ± 0.05 pool/d, $P <$ 0.05, and 18.0 ± 7.7 and 11.2 ± 2.3 mg/kg/d, $P <$ 0.05). Higher HDL-triglyceride and HDL-apoE levels were observed in patients with heterozygous FH. (Respectively 19 ± 8 vs. 8 ± 3 mg/dL, $P <$ 0.05, and 5.3 ± 0.8 vs. 3.7 ± 0.9 mg/dL, $P <$ 0.05). **We conclude that the catabolism of HDL-apoA-I is increased in heterozygous FH patients. However, plasma apoA-I concentration was maintained because of an increased HDL-apoA-I production rate.**—Frénais, R., K. Ouguerram, C. Maugeais, J. S. Marchini, P. Benlian, J. M. Bard, T. Magot, and M. Krempf. **Apolipoprotein A-I kinetics in heterozygous familial hypercholesterolemia: a stable isotope study.** ***J. Lipid Res.*** **1999.** 40**: 1506–1511.**

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Decreased plasma apolipoprotein (apo) A-I or high density lipoprotein (HDL)-cholesterol concentrations are reported as an independent risk factor for coronary heart disease (CHD) (1–3). The risk of CHD is very high in heterozygous familial hypercholesterolemia (FH) because of a large increase of plasma cholesterol and apoB-100 levels, related to a deficiency of half active low density lipoprotein (LDL) receptors (4). ApoB-100 pathway disturbances have been fully described in FH and result in an impaired LDL catabolism through receptor-mediated endocytosis and an overproduction of apoB-100-containing lipoproteins (5, 6). However, the effects of FH on apoA-I metabolism remain poorly documented. A recent in vitro study has shown that overexpression of scavenger receptor BI (SR-BI), an hepatic receptor for HDL, can mediate transport of sterols between LDL or HDL and endoplasmic reticulum of cells lacking functional LDL-receptors (7). This SR-BI overexpression in a transgenic mice model was associated with a decrease of HDL-cholesterol (8). In another in vivo study, it has been shown that the liver-specific overexpression of SR-BI led to a decrease of HDL, by enhancing HDL protein catabolism (9). This would suggest that in a situation of FH, removal of cholesteryl particles may be enhanced via HDL pathway. In one human homozygous FH, it was found that by using endogenous labeling with stable isotope tracers (10), low levels of HDL-cholesterol and apoA-I were related to a combined increased fractional catabolic rate (FCR) and decreased absolute production rate (APR) of HDL-apoA-I. However, kinetic data from hypercholesterolemic patients, obtained in two studies, failed to detect any significant change in both apoA-I catabolic and production rates compared to controls (11, 12). However, it must be pointed out that these studies were performed using exogenous labeling of HDL with radiotracers, which could change their physical features and alter their hepatic removal. Moreover, genetic features of the patients were not clearly indicated. In this

Abbreviations: apo, apolipoprotein; APR, absolute production rate; CETP, cholesteryl ester transfer protein; CHD, coronary heart disease; FCR, fractional catabolic rate; FH, familial hypercholesterolemia; FPR, fractional production rate; LDL, low density lipoprotein; HDL, high density lipoprotein; SD, standard deviation; SRBI, scavenger receptor class B type I; VLDL, very low density lipoprotein.

[1] To whom correspondence should be addressed.

study, using endogenous apoA-I labeling with stable isotope tracers, we have tested the hypothesis that the FCR of HDL-apoA-I was increased in genetically defined heterozygous FH patients compared to controls.

SUBJECTS, MATERIALS, AND METHODS

Subjects

The kinetic study was performed in seven healthy, normolipidemic subjects and seven severely hypercholesterolemic patients, matched for mean age and body mass index. All control subjects were in good health and for at least 1 month prior to study none had been taking any medication that could affect carbohydrate or lipid metabolism. None of the study subjects had diabetes mellitus, proteinuria, or hypothyroidism. They were not regular cigarette smokers or alcohol consumers. Selected relevant clinical characteristics of the two groups are depicted in **Table 1**. Familial hypercholesterolemia was diagnosed according to the presence of a heterozygous mutation on the LDL-receptor gene (13), but also from characteristic clinical signs and an analysis of lipid parameters in family members (total plasma cholesterol >350 mg/dL, LDL-cholesterol >230 mg/dL, plasma triglycerides slightly increased and <250 mg/dL), showing a dominantly inherited hypercholesterolemia in the family from each proband (three or more affected relatives including at least one first degree relative). Hypolipidemic drugs (statins) were discontinued at least 2 weeks before the investigation. This washout phase allow us to recover the basal lipid profile of FH patients. None of them had ever been treated with probucol. Study subjects were instructed by a dietician to eat a weight-maintenance diet composed of 50% of the usual daily caloric intake as carbohydrate, 30% as fat, and 20% as protein, for at least 1 week prior to the study. The experimental protocol was approved by the Ethical Committee of Nantes University Hospital, and informed consent was obtained before the study was started.

Experimental protocol

The protocol was similar to that described in a previous study (14). Briefly, the endogenous labeling of apolipoprotein A-I was carried out by administration of l-[5,5,5-$^2H_3$]-leucine (99.8 Atom % $^2H_3$; Cambridge Isotope Laboratories, Andover, MA), dissolved in a 0.9% saline solution and tested for sterility and absence of pyrogens before the study. All subjects fasted overnight for 12 h prior to the study, and remained fasting during the entire protocol. Each subject received intravenously a prime of 10 $\mu mol \cdot kg^{-1}$ of tracer, immediately followed by a constant tracer infusion (10 $\mu mol \cdot kg^{-1} \cdot h^{-1}$) for 14 h. Venous blood samples were withdrawn in EDTA tubes (Venoject, Paris, France) at baseline, every 15 min during the first hour, every 30 min during the next 2 h, and then hourly until the end of the study. Plasma was immediately separated by centrifugation for 30 min at 4°C; sodium azide, an inhibitor of bacterial growth, and Pefabloc SC (Interchim, Montluçon, France), a protease inhibitor, were added to blood samples at a final concentration of 1.5 mmol/L and 0.5 mmol/L, respectively.

Analytical procedures

Measurement, isolation and preparation of apolipoproteins. VLDL ($d < 1.006$ g/mL), were isolated from 3 mL of plasma by a sequential ultracentrifugation using an angle rotor at 40000 $rev \cdot min^{-1}$ for 24 h at 10°C (Himac CP70, Hitachi). HDL_2 ($1.063 < d < 1.125$ g/mL) and HDL_3 ($1.125 < d < 1.210$ g/mL) were then isolated by a density gradient ultracentrifugation modified method (15) using a swinging bucket rotor at 40000 $rev \cdot min^{-1}$ for 24 h at 10°C (Centrikon T 2060, Kontron Instruments). Plasma and HDL cholesterol and triglycerides levels were measured using commercially available enzymatic kits (Boehringer Mannheim GmbH, Germany). ApoA-I concentration was measured in plasma and HDL fractions by immunonephelometry (Behring, Rueil Malmaison, France). HDL-apoE level was evaluated by electroimmunodiffusion assay (Sebia, Issy-les-Moulineaux, France). The apoA-I pool size ($mg \cdot kg^{-1}$) was calculated by multiplying the mean plasma apoA-I concentration by 0.038–0.049 ($l \cdot kg^{-1}$), assuming a plasma volume of 3.8 to 4.9% of body weight according to age, gender, and body weight of each study subject (16). The plasma apoA-I concentration was taken to be the HDL-apoA-I concentration, with the assumption that >90% of plasma apoA-I resides in HDL fraction (17).

HDL-apoA-I and VLDL-apoB-100 were concentrated and isolated from other apolipoproteins by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) using a 4–5–10% discontinuous gradient. Apolipoproteins were identified by com-

TABLE 1. Selected clinical and biological characteristics of study subjects

Subject	Sex	Age	BMI	LDL-CH	LDL-Receptor Mutation[a]	ApoE Isoform	Clinical Signs
		yr	*kg/m²*	*mg/dl*			
FH 1	F	64	19.2	312	Phe105Ser, exon 4	E3/E3	GX, TX
FH 2	M	55	24.9	338	frameshift, exon 4	E3/E3	CAD, GX, TX
FH 3	F	50	31.2	327	Trp66Gly, exon 3	E3/E3	CAD, GX, TX
FH 4	M	28	27.9	237	ND[b]	E3/E3	AG, GX, MI, TX
FH 5	F	39	32.9	326	—[c]	E3/E3	AG, CAD, TX
FH 6	M	57	24.9	415	Ala370Thr, exon 8 Glu702STOP, exon 15[d]	E3/E3	CABG, GX, PA, TX
FH 7	M	41	23.2	333	frameshift, exon 4	E3/E3	CAD, TX
FH, mean	4/3	47.7	26.6	326.9			
(SD)	M/F	(12.4)	(4.6)	(52.0)			
Controls, mean	6/1	39.0	27.0	90.7	ND	ND	none
(SD)	M/F	(11.7)	(5.2)	(33.2)			

AG, angioplasty (PCTA, percutaneous coronary angioplasty); CABG, coronary artery bypass grafting; CAD, coronary artery disease; GX, gerontoxon (corneal arcus); MI, myocardial infarction; PA, peripheral atherosclerosis; TX, tendon xanthoma; ND, not determined. FH patients (FH 4 not determined) were negative for the Arg3500Gln mutation of apoB-100.

[a] All mutations were found heterozygous on the LDL-receptor gene.

[b] FH 4 was dead.

[c] FH 5 was negative for LDL receptor mutation (candidate for a *third* gene mutation).

[d] Both mutations supposedly on the same chromosome.

paring migration distances with known molecular weight standards (cross-linked phosphorylase b markers, Sigma, St. Louis, MO, and electrophoresis calibration kit, Pharmacia LKB, Biotechnology Inc., Piscataway, NJ). Apolipoprotein bands were excised from polyacrylamide gels and dried in vacuum for 1 to 2 h (RC 10-10 Jouan, Saint Herblain, France). The desiccated gel slices were hydrolyzed with 1 mL of 4 mol/L HCl (Sigma, St. Quentin Fallavier, France) at 110°C for 24 h. Hydrolysates were then evaporated to dryness and the amino acids were purified by cation exchange chromatography using a Temex 50W-X8 resin (Bio-Rad, Richmond, CA). Amino acids and plasma leucine were esterified with propanol/acetyl chloride, and further derivatized using heptafluorobutyric anhydride (Fluka Chemie AG, Buchs, Switzerland) prior to analysis.

Determination of tracer-to-tracee ratios. Chromatographic separations were carried out on a 30 m × 0.25 mm i.d. DB-5 capillary column (J & W Scientific, Rancho Cordova, CA). The column temperature program was as follows: initial temperature was held at 80°C, then increased at 10°C·min^{-1} to a final temperature of 180°C. Electron-impact gas chromatography–mass spectrometry was performed on a 5890 gas chromatograph connected with a 5971A quadrupole mass spectrometer (Hewlett-Packard, Palo Alto, CA). The isotopic ratio was determined by selected ion-monitoring at m/z 282 and 285. Calculations of apoA-I kinetic parameters were based on the tracer-to-tracee mass ratio (18).

Modeling

For HDL modeling, we used a one-compartment model, as previously described (14). Kinetic analysis of tracer-to-tracee ratios was achieved by a computer software for simulation, analysis, and modeling (SAAM II v 1.0.1, Resource Facility for Kinetic Analysis, Dept. of Bioengineering, SAAM Institute, Seattle, WA). VLDL-apoB-100 and HDL-apoA-I data were kinetically analyzed using a monoexponential function (18): $A(t) = Ap[1 - e^{x}(-k(t - d))]$, where A(t) is the tracer-to-tracee ratio at time t, Ap, the tracer-to-tracee ratio at the plateau of the VLDL-apoB-100 curve, d, the delay between the beginning of the experiment and the appearance of tracer in the apolipoprotein, and k the fractional production rate (FPR) of the apolipoprotein. For the estimation of apoA-I synthesis, we used the plateau of VLDL-apoB-100 tracer-to-tracee ratio as precursor pool enrichment. It was assumed that this plateau value, obtained using a monoexponential function, corresponded to the tracer-to-tracee ratio of the leucine precursor pool. This estimation is made upon the assumption that apoB-100 and the majority of apoA-I are synthesized by the liver (19). We estimated the FPR, i.e., the proportion of apolipoprotein A-I entering the pool per unit time (d^{-1}), and the absolute production rate (APR), i.e., the amount of apolipoprotein AI entering the pool per unit time (mg·kg^{-1}·d^{-1}). APR was the product of FPR multiplied by apolipoprotein A-I mass in HDL. ApoA-I pool was considered to be constant, as no significant variation was observed between measurements made at three different sampling times (data not shown). Under these steady state conditions, FPR equals fractional catabolic rate (FCR).

Statistical analysis

Data were reported as mean ± standard deviation (SD) unless otherwise specified. Statistical analysis was performed using Instat Software package (GraphPad, San Diego, CA). The non parametric Mann-Whitney U-test was used to compare clinical and kinetic data between heterozygous FH patients and controls. A two-tailed probability level of 0.05 was accepted as statistically significant.

RESULTS

Apolipoprotein and lipid concentrations

Individual data for plasma and HDL composition are presented in **Table 2**. FH patients showed higher fasting plasma cholesterol concentrations ($P < 0.001$). Although in a normal range, triglycerides were also higher in FH ($P < 0.05$). Furthermore, compared to controls, higher HDL-triglycerides and HDL-apoE concentrations were observed in FH patients ($P < 0.05$). Plasma apoA-I concentration and pool size, as well as HDL-cholesterol level, showed a nonsignificant trend toward decrease in hypercholesterolemic patients.

Kinetic data

Enrichment in plasma free leucine reached a plateau value after 30 min and remained stable through to the end of the study (data not shown). The tracer-to-tracee ratio curves in VLDL and HDL are shown in **Fig. 1**. A plateau of tracer-to-tracee ratio was observed for VLDL-apoB-100 but not for HDL-apoA-I, reflecting a slow rate of synthesis for this apolipoprotein. Experimental data for HDL_2 and HDL_3 were similar (**Fig. 2**) in both control and FH population. Then both lipoprotein subfractions were pooled for the following results. Kinetic parameters of

TABLE 2. Plasma apoA-I, cholesterol, and triglyceride concentrations (mg/dL) and HDL composition (mg/dL) in study subjects

Subject	Plasma ApoA-I	Plasma CH	Plasma TG	HDL-CH	HDL-TG	HDL-apoE
FH 1	117	388	159	50	21	5.3
FH 2	101	448	132	31	7	6.1
FH 3	93	405	163	35	23	4.4
FH 4	110	407	189	31	32	4.7
FH 5	132	408	245	42	14	6.2
FH 6	144	461	179	34	21	5.9
FH 7	111	440	164	39	17	4.5
FH, mean	115	422	176	37	19	5.3
(SD)	(18)	(27)	(35)	(7)	(8)	(0.8)
Controls, mean	122	186	106	46	8	3.7
(SD)	(15)	(42)	(44)	(19)	(3)	(0.9)
P^a	NS	<0.001	<0.05	NS	<0.05	<0.05

ApoA-I, apolipoprotein A-I; CH, cholesterol; TG, triglycerides; HDL, high density lipoprotein.
[a] Statistics FH vs. Controls.

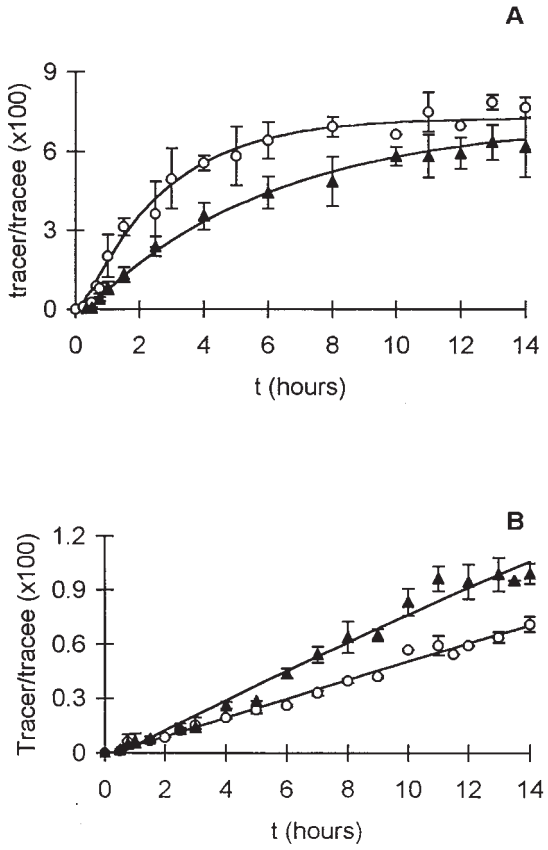

Fig. 1. Mean experimental values (symbols) of the tracer-to-tracee ratio for VLDL-apoB-100 (panel A) and for HDL-apoA-I (panel B) in control subjects (open circles) and heterozygous FH patients (triangles). Fits (lines) were calculated using monocompartmental analysis during a primed constant infusion of [$^2H_3$]leucine. Data are shown as mean ± SEM.

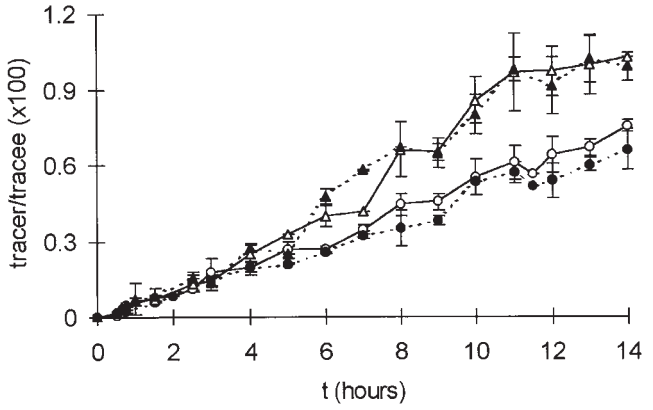

Fig. 2. Experimental values of the tracer-to-tracee ratio for HDL_2-apoA-I (open symbols) and HDL_3-apoA-I (solid symbols) in control subjects (circles) and heterozygous FH patients (triangles), during a primed constant infusion of [$^2H_3$]leucine. Data are shown as mean ± SEM.

HDL-apoA-I are shown in **Table 3**. There was an increase in the FCR of HDL-apoA-I ($P < 0.05$) in hypercholesterolemic patients compared to controls (63%). The APR was also significantly increased (61%) in hypercholesterolemic subjects compared to controls ($P < 0.05$).

DISCUSSION

This study was designed to test the hypothesis that HDL clearance is enhanced in heterozygous familial hypercholesterolemia. We actually found a 63% increase of FCR in FH patients compared to healthy subjects, using an endogenous labeling of apoA-I. Furthermore, both plasma apoA-I and HDL-cholesterol concentrations were not significantly decreased in heterozygous FH patients, as previously reported (11, 12, 20, 21), because of a concomitant increase in the HDL-apoA-I production rate.

Endogenous labeling of apolipoproteins by infusion of an amino acid labeled with a stable isotope is now widely used for physiological studies and exploration of dyslipoproteinemia (22–24). One advantage of this approach is that changes in lipoprotein kinetics are not related to potential alterations by the exogenous labeling (25). As we already observed (14), the tracer-to-tracee ratios in HDL_2 and HDL_3 were similar, indicating a fast rate of interconversion between these subclasses of lipoproteins. Then, within our study conditions, HDL_2 and HDL_3 were assimilated to a single HDL compartment (26). Our experimental enrichment data could not be adjusted on a two-pool model, as previously used (12), because our study design with a constant infusion of tracer and our period of sampling did not allow the characterization of tracer exchanges with a second pool. This is a limitation of our experimental conditions, but the one-pool model, widely used in other studies on apoA-I kinetics (27–29), allows us to test our hypothesis on overall HDL catabolism. We have taken VLDL-apoB-100 enrichment at the plateau as an estimate of apoA-I leucine precursor pool enrichment, assuming that apoA-I was mainly synthesized by the liver (19), which is likely to occur in the fasting state. The use of the plateau value of VLDL apoB-100 tracer-to-tracee ratio as a basis for calculation of HDL-apoA-I kinetics (27, 28) also could introduce a source of error if the enrichments of

TABLE 3. Kinetic parameters of apoA-I-HDL in study subjects

Subject	ApoA-I FCR	ApoA-I Pool Size	ApoA-I APR
	pool·d^{-1}	*mg·kg*$^{-1}$	*mg·kg*$^{-1}$*·d*$^{-1}$
FH 1	0.32	52.7	17.0
FH 2	0.34	43.4	14.9
FH 3	0.24	39.1	9.5
FH 4	0.59	51.7	30.6
FH 5	0.34	54.8	18.9
FH 6	0.16	61.2	10.1
FH 7	0.49	50.5	24.9
FH, mean	0.36	50.5	18.0
(SD)	(0.14)	(7.3)	(7.7)
Controls, mean	0.22	53.5	11.2
(SD)	(0.05)	(10.9)	(2.3)
P^a	<0.05	NS	<0.05

FCR, fractional catabolic rate; APR, absolute production rate.
[a] Statistics FH vs. Controls.

the precursor pool of apoB-100 in the hepatocyte were different between patients and controls. This is unlikely as the hepatic enrichment of the precursor pool was not different between the two study groups (Fig. 1A), despite significantly different cholesterol contents in hepatocytes.

A recent in vitro study on a Chinese hamster ovary cell line lacking functional LDL-receptors has shown that overexpression of scavenger receptor BI (SR-BI) can mediate transport of sterols between LDL or HDL and endoplasmic reticulum of cells (7). In addition, in a transgenic mice model of LDL-receptor knock-outs and liver-specific overexpression of SR-BI, a decrease of HDL-cholesterol was observed (8). It was furthermore demonstrated that the liver-specific overexpression of SR-BI resulted in a marked decrease in HDL-cholesterol and apoA-I levels, related to an enhanced clearance of HDL protein in vivo (9). Our hypothesis of increased HDL catabolism in FH, extrapolated from these findings on animal models, is validated by our kinetic observations. Schaefer et al. (10) previously investigated the kinetics of apoA-I in a single homozygous FH patient and two normal control subjects by endogenous labeling using a stable isotope. The FCR of apoA-I in their homozygous FH subject was 48% increased compared to controls. This is in good agreement with the 63% increase observed in the current study, suggesting that HDL-apoA-I hypercatabolism may not be related to the degree of magnitude of hypercholesterolemia. Our control FCR values are close to those reported in studies in healthy subjects (27–29). However, our results in heterozygous FH are in sharp contrast with data previously related in two studies performed in hypercholesterolemic patients (11, 12). It must be emphasized that the exogenous labeling of HDL with radiotracers performed in these two studies could change their physical features, and hence alter their hepatic removal (25). Furthermore, the genetic defect on LDL-receptor in the hypercholesterolemic patients was not clearly depicted.

Besides the role of SR-BI, other alterations can be drawn to explain the increase in HDL-apoA-I catabolic rate. Factors that alter HDL composition could modify HDL-apoA-I kinetics. As previously suggested, CETP-mediated exchange of cholesteryl ester from HDL to LDL and reciprocal transfer of triglycerides affected HDL core composition (30), and thus contributed to an enhanced apoA-I catabolism (31, 32). An increased CETP activity is usually reported in FH patients (21, 30, 33). As apoA-I has a lower affinity for triglyceride-enriched particles, the pool of this easily dissociable apolipoprotein is greater (32) and this may partly account for the increased FCR of HDL-apoA-I in heterozygous FH. Another potential explanation has been suggested by Schaefer et al. (10), related to the expanded pool size of apoE in FH. ApoE-enriched HDL could be catabolized either via a specific receptor-dependent pathway, mediated by apoE, or via the hepatic apoB/E receptor, present in almost half the normal amount in heterozygous FH. As we reported, higher HDL-apoE level in heterozygous FH, apoB/E, or apoE receptor pathways could lead to an increased clearance of HDL-apoA-I in this group.

Contrasting with some previous studies (33–36), both plasma apoA-I and HDL-cholesterol levels were nonsignificantly decreased in heterozygous FH, because of the increase of HDL-apoA-I production rate. This is in discrepancy with studies performed in the homozygous FH animal model (37) or in humans (10, 38, 39). Nevertheless, the increased HDL-apoA-I production in the heterozygous FH group we observed is consistent with results using the human hepatocellular carcinoma model (40). Monge et al. (40) observed that LDL uptake by HepG2 cells led to increased levels of apoA-I mRNA in these cells. They concluded that LDL may play an important role in apoA-I gene expression and regulation. Unlike the situation in homozygous FH, LDL could help produce HDL-apoA-I in patients with heterozygous FH. In homozygous FH, defective apoA-I gene regulation could be due to an absolute LDL receptor dysfunction (37). Although the LDL-apoB-100 fractional catabolic rate is low in heterozygous FH patients, absolute uptake of LDL is overall increased, because of a larger LDL pool size. Thus, apoA-I synthesis could be enhanced by LDL, leading to the increased HDL-apoA-I production rate. This concurs with the hypothesis of Schaefer et al. (10), suggesting a feedback pathway involving the LDL receptor that regulates the hepatic expression of both apoA-I and apoB-100.

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