Metabolism of Plasma Lipoproteins in the Genetically Hypercholesterolemic Rat (RICO)

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Experiments were performed to determine the turnover processes of plasma cholesterol in genetically hypercholesterolemic rats (RICO). Specific activity of plasma cholesterol was monitored during 4 months following an intravenous injection of tritiated cholesterol. The results were subjected to two-pool model analysis. Cholesterol production in the RICO rat was significantly higher (28.9 ± 1.7 mg/d) than in the SW control (18.5 ± 0.7, $P < .01$). The study also revealed a 30% decrease in the rate constant for cholesterol movement from the plasma toward the majority of organs in the RICO rat versus the SW control. Very–low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) turnover were investigated following injection of labeled lipoproteins (on cholesteryl ester or apolipoproteins). Results from these experiments showed that the higher HDL cholesterol concentration in the RICO rat as compared with the control is due to the greater production rate of esterified cholesterol in these lipoproteins (1.3 ± 0.05 mg/h *v* 0.8 ± 0.03 in the control, $P < .001$). The fractional catabolic rate (FCR) or production rate for VLDL were not significantly different between the two groups (3.4 ± 0.01 and 3.6 ± 0.01 h^{-1} and 2.6 ± 0.4 and 3.3 ± 0.1 mg/h, respectively). However, radioactivity of VLDL recovered in LDL at death was considerably higher in RICO rats (14% ± 1% *v* 6% ± 1%, $P < .01$). The greater concentration of LDL cholesterol in RICO rats is due to a higher LDL production (0.40 ± 0.05 *v* 0.19 ± 0.03 mg/h, $P < .01$) together with a lower catabolism (FCR, 5.5 ± 0.6 *v* 7.9 ± 0.8%/h, $P < .05$). Cross-injection experiments showed that this lower catabolism of LDL is partly due to the nature of the lipoprotein particle. Taken together, these data are consistent with the hypothesis of a reduced uptake of apolipoprotein (apo)E–containing lipoproteins (VLDL and LDL), which results in a higher LDL cholesterol concentration in RICO rats.

AMONG ANIMAL MODELS of hypercholesterolemia, the RICO rat is particularly useful because its genetic hypercholesterolemia is not associated with hypertriglyceridemia.[1] This strain is characterized by a hypercholesterolemia that is essentially due to an increase in concentrations of low-density (LDL) and high-density (HDL) lipoproteins.[2] The aim of this study was to investigate the movement of cholesterol (particularly cholesteryl ester in LDL and HDL) from plasma to the various organs in RICO rats. To gain some insight into the nature of the hypercholesterolemia, we examined the flux of cholesterol first by a compartmental analysis of the plasma curve obtained following an intravenous injection of labeled cholesterol, and second, by a study of LDL and HDL catabolism. LDL was labeled on the protein moiety (essentially apolipoprotein [apo] B). Since cholesteryl ester can also leave the plasma HDL compartment independently of the protein uptake in rats,[3,4] HDL particles were labeled in both the protein moiety (essentially apo A-I) and the cholesteryl ester. Since very–low-density lipoproteins (VLDLs) are precursors of LDLs, catabolism of these lipoproteins was studied following in vivo labeling of the cholesteryl ester component. Labeled nondegradable analogs, ^{14}C-sucrose (as protein tracer) and ^{14}C-cholesteryl linoleyl ether (as a cholesteryl ester tracer), were used to follow tissue lipoprotein uptake.

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MATERIALS AND METHODS

Animals and Diet

Genetically hypercholesterolemic (RICO) and normocholesterolemic (SW) male rats were purchased from Ciba-Geigy (Basel, Switzerland). They were fed a semipurified diet of sucrose (53%), casein (23%), lard (9.2%), mineral mix (5%), skim milk (4%), yeast (2.3%), vitamin mix (2.5%), walnut oil (0.8%), and cystine (0.2%) as previously described.[5] Rats were fed the diet for 2 months before experimentation, at which time their body weight was 435 ± 7 g.

Lipoprotein Isolation

Rat blood was collected into tubes containing 4% EDTA and 4% monoiodoacetamide by intraaortic puncture under ether anesthesia. Plasma was separated from red blood cells by centrifugation (20 minutes, 4°C, 2,200 × *g*). Lipoproteins were isolated by KBr gradient from sequential ultracentrifugation.[6] After adjustment to the selected density, ultracentrifugation was performed at 4°C and 105,000 × *g* for 24 hours in a Ti 70 rotor (Beckman, Fullerton, CA).

Lipoprotein Labeling

In vivo VLDL labeling on cholesterol ester. VLDLs were labeled in vivo with ^{3}H-cholesterol. Fifty microcuries of ^{3}H-cholesterol (40 to 60 Ci/mmol; CEA, Saclay, France) were added to 0.2 mL Intralipid 20% (Kabi Pharmacia, Paris, France). After sonicating twice for 30 seconds each, the emulsion was injected intravenously into a donor rat. The rat was killed 6 hours following injection,[7] and VLDLs (1.006 < d) were separated. This rat was maintained in the fasting state to minimize contamination of VLDL by chylomicrons and intestinal VLDL. Under these conditions, 20% of VLDL labeling was recovered in cholesteryl ester.

Labeling of LDL and HDL apolipoproteins with ^{14}C-sucrose. LDL (1.020 < d < 1.050 g/mL) and HDL (1.09 < d < 1.21 g/mL) were obtained from 60 and 30 mL pooled plasma, respectively. The separation densities were chosen to minimize contamination of apo B LDL and apo A-IHDL fractions by apo E. The lipoprotein fractions were then dialyzed for 20 hours against a saline solution containing NaCl (0.15 mol/L), NaN_3 (0.02%), EDTA (1 mmol/L),

and phosphate (20 mmol/L) at pH 7.2, and were labeled on the protein moiety with ^{14}C-sucrose (540 μCi/μmol; CEA) according to Pittman et al.[8] Under these conditions, specific radioactivity was 4 μCi/mg protein and 1.5 μCi/mg protein for LDL and HDL, respectively. After the labeling procedure, the lipoproteins were dialyzed against 0.15 mol/L NaCl and 0.04% EDTA, pH 7.4, for 15 hours to remove any unbound tracers, and were then passed through a 0.45-μm Millipore filter before injection. The purity and stability of labeled LDL and HDL in the present study were checked throughout the experiments by gel filtration on Biogel (Biorad, Hercules, CA) columns equilibrated with buffer Tris 0.01 mol/L, NaCl 0.15 mol/L, and EDTA 0.01% at pH 7.[9] The flow rate was 4.8 mL/h, and the volume of each fraction collected was 0.6 mL.

Using these procedures, it has been previously demonstrated that these lipoproteins contain essentially apo B for LDL[10] and apo A-I for HDL.[11] Furthermore, LDL[12] and HDL[13] labeled by methods similar to the ones used here have been shown to have the same behavior as classic iodine-labeled lipoproteins, after injection.

HDL labeling on cholesteryl ester. For the in vivo methods, a rat received ^{3}H-cholesterol (500 μCi, 40 to 60 Ci/mmol; CEA) dissolved in walnut oil by intubation. Approximately 24 hours, later, the rat was killed by intraaortic puncture, blood was collected, and plasma was separated by centrifugation at 2,200 × *g* for 20 minutes. The plasma, essentially labeled on HDL,[14] was then injected in a second rat to eliminate labeled free cholesterol. One hour following injection, the rat was killed by intraaortic puncture, and HDL were isolated from plasma by ultracentrifugation ($1.063 < d < 1.21$). Under these conditions, 92% of the label was recovered in cholesteryl ester.

For the in vitro methods, ^{14}C-cholesteryl linoleyl ether (52 mCi/mmol; CEA) was incorporated into a HDL preparation as essentially described by Roberts et al.[15] Briefly, the fraction with a density higher than 1.21 g/mL obtained from rabbit plasma (which contains cholesteryl ester transfer protein in this species[16]) was used as the first acceptor for labeled cholesteryl ester, initially introduced in acetone. Rat HDLs, isolated by ultracentrifugation ($1.063 < d < 1.21$ g/mL), were then added after acetone evaporation. After mixing gently for 30 minutes at 4°C, HDLs were again separated by ultracentrifugation at density 1.063 to 1.21 g/mL. The yield of label incorporated in HDL was 7%. Using these techniques, it has been previously shown that labeled cholesteryl linoleyl ether and cholesteryl ester incorporated into HDL behave in a similar manner following injection in rats.[17]

Kinetic Studies

Whole-body cholesterol turnover. Rats received 0.5 mL Tween 20 containing ^{3}H-cholesterol (54 μCi, 40 to 60 Ci/mmol; CEA) intravenously. Blood samples (0.5 mL) were collected in the caudal vein at different time intervals during 4 months following the radioactive labeling. Specific radioactivity of total plasma cholesterol was measured. The curves were analyzed by compartmental analysis. The experimental curve was fitted to a pluriexponential equation on an IBM XT AT 286 microcomputer (Walnut, CA) using an iterative optimization program based on the least-squares error criterion.[18] Coefficient obtained were used to characterize the compartmental system to which the cholesterol system is approximated.[19] These values were also used to calculate the area under the curve of specific radioactivity ($\int_0^\infty a_1 \cdot dt$).

The total input flux in the system (R) was then calculated using the occupancy principle by the relation:

$$R = \frac{q_i}{\int_0^\infty a_1 \cdot dt}$$

where q_i is the injected radioactivity.[20]

Lipoprotein turnover. The rats received via the dorsal vein of the penis 0.5 mL ($\sim 10^6$ dpm) labeled lipoprotein preparation (LDL or HDL labeled with ^{14}C-sucrose, HDL labeled in vivo with ^{3}H-cholesteryl ester, HDL labeled in vitro with ^{14}C-cholesteryl linoleyl ether, or VLDL labeled in vivo with ^{3}H-cholesteryl ester). Blood samples (50 μL) were collected at different times from the caudal vein until 30 minutes after VLDL injection, until 8 hours after injection of in vivo–labeled HDL, and until 24 hours for the remaining two injections.

For injection of labeled analogs not degradable in the cells (^{14}C-sucrose and ^{14}C-cholesteryl linoleyl ether), the rats were killed by intraaortic puncture at the end of experiments (24 hours). The circulatory system was washed several times with physiological saline. The main organs were collected: liver, intestine, kidneys, adrenals, and testis. The intestine was fractionated into small intestine, cecum, and colon.

Experimental curves were adjusted to a pluriexponential equation with the program described earlier. The fractional catabolic rate (FCR) was calculated from the equation coefficients.[21,22] The production rate was determined by FCR × (plasma cholesteryl ester or protein mass) of the studied lipoprotein. After ^{14}C-sucrose and ^{14}C-cholesteryl linoleyl ether injection, the label was not released into the plasma after uptake by the cells.[4,8] Under these conditions, measuring the radioactivity in each organ collected at death, t_1, allowed the contribution (f) of each organ to total FCR to be determined by the equation,

$$f = \frac{q_0}{\int_0^{t_1} q_p \cdot dt},$$

where q_0 represents radioactivity of each organ at t_1 time of death, and q_p the decay of radioactivity in the plasma.[23,24] The ratio between this value (f) and that of FCR obtained from the complete curve gives the relative contribution of each organ to the total catabolism of the compound studied.

Results are expressed as the mean ± SEM, and statistical differences were determined by Student's *t* test.

Chemical and Radioisotopic Analyses

For plasma samples, the concentration of total apolipoproteins was determined by the Lowry method using bovine serum albumin as a standard. The concentration of cholesteryl ester was calculated by the difference between total and free cholesterol concentrations measured by enzymatic assay (CHOD-PAP; Boehringer Mannheim, Indianapolis, IN).

For VLDL experiments, lipids were first extracted with chloroform-methanol according to Folch et al.[25] Samples were then subjected to chromatography on a silicic acid microcolumn (Sep Pack; Waters, Millipore, Milford, MA) to separate esterified and unesterified cholesterol.[26]

Plasma and organ samples were digested by soluene 350 (50°C for 1 night). After cooling, the samples were neutralized with acetic acid. Radioactivity was measured in a scintillation counter (MR 300; Kontron, Milan, Italy) using lipoluma scintillation liquid. Correction for quenching was performed with an external standard.

Table 1. Parameters of Cholesterol Transport and Distribution in the Whole Body Obtained by Two-Pool Model Analysis (n = 5)

Parameter	SW	RICO
Cholesterol concentration (mg/mL)		
Plasma	0.86 ± 0.05	1.73 ± 0.15*
VLDL	0.09 ± 0.01	0.14 ± 0.02
LDL	0.25 ± 0.02	0.63 ± 0.06*
HDL	0.35 ± 0.02	0.64 ± 0.03*
Parameters of the 2-pool model		
Q_1 (mg)	129 ± 10	210 ± 5*
Q_2 (mg)	367 ± 18	349 ± 22
k_{12} (d^{-1})	0.09 ± 0.02	0.11 ± 0.02
k_{21} (d^{-1})	0.26 ± 0.02	0.18 ± 0.02*
k_{01} (d^{-1})	0.15 ± 0.01	0.14 ± 0.01
R (mg · d^{-1})	18.5 ± 0.7	28.9 ± 1.7*

NOTE. See Fig 1 for model representation.

Abbreviations: k, rate constant in d^{-1}; Q, mass in mg; R, flux in mg · d^{-1}.

*$P < .01$.

RESULTS

Whole-Body Cholesterol Kinetics

The concentration of plasma total cholesterol at death was 0.86 ± 0.05 versus 1.73 ± 0.15 mg/mL in SW and RICO rats, respectively (Table 1). This doubled cholesterolemia is mostly due to an increase in both LDL and HDL cholesterol, 2.5 and 1.8 times greater in RICO versus SW rats, respectively. After an intravenous injection of tritiated cholesterol, the time course of specific activity in the semilogarithmic representation (Fig 1) shows that specific activity of plasma total cholesterol decreased rapidly during the first week and was linear thereafter for both RICO and SW rats. However, the decrease in specific radioactivity was more pronounced in RICO than in SW rats. For the same injected radioactivity, specific radioactivity at zero time was lower in RICO than in SW rats (by ~40%), reflecting higher mean plasma cholesterol concentration, and remained lower in RICO rats until the study concluded, with the decreases being 55% at 8 weeks and 70% at 16 weeks. The curves of the decrease in plasma cholesterol specific activity were fitted to a biexponential equation (Fig 1). Fitting to a triexponential equation provides no significant improvement and therefore was not taken into consideration, in agreement with other reports.[27-29]

A classic two-pool kinetic analysis[19] was performed involving a compartment in rapid exchange with the plasma (rapidly exchangeable pool) and a slower exchange pool (Fig 1). The first one corresponds principally to the liver, red blood cells, intestine, and adrenals. The second one corresponds principally to the nervous system, adipose tissue, and muscles.[19] Parameters of the model obtained for the two groups of rats are presented in Table 1. The value for total cholesterol input flux into the system is signifi-

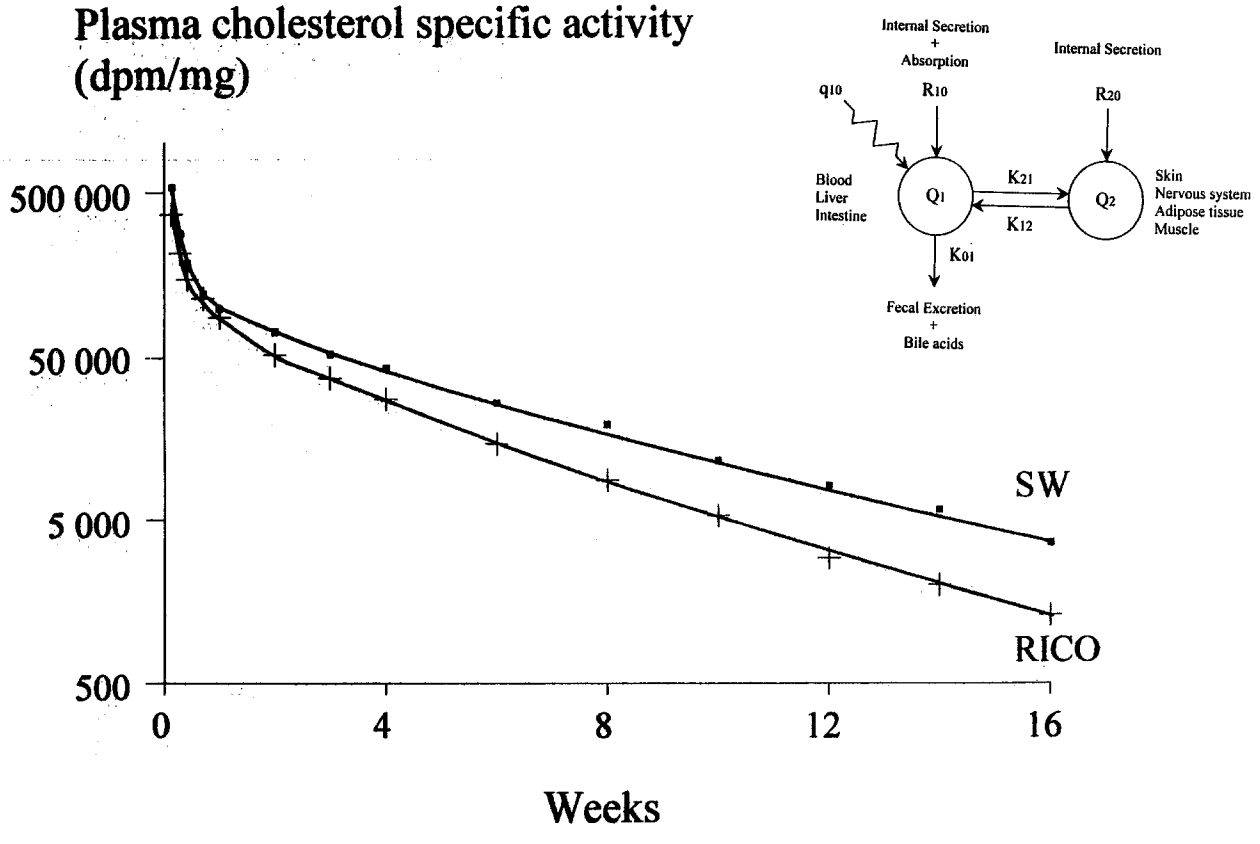

Fig 1. Time course of specific activity of plasma cholesterol (dpm · mg^{-1}) following an intravenous injection of ^{3}H-cholesterol in SW (●) and RICO (x) rats (n = 5). The lines represent the best fit generated by the model. Inset: Two-pool model of cholesterol turnover in the whole body. Compartment 1, rapidly exchangeable cholesterol pool; compartment 2, slowly exchangeable cholesterol pool.

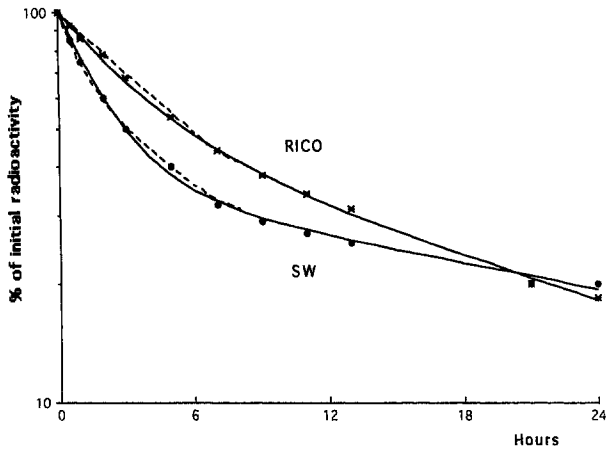

Fig 2. Radioactivity in plasma after injecting SW (●) and RICO (x) rats with autologous HDL labeled in vitro with ^{14}C-cholesteryl linoleyl ether (n = 5). Dashed lines represent the curves obtained after injection of HDL labeled in vivo with ^{3}H-cholesteryl ester.

cantly higher in the RICO rat (28.9 ± 1.7 mg/d) than in the SW rat (18.5 ± 0.7 mg/d). The mass (Q_1), or the rapidly exchangeable pool, is significantly higher in RICO rats than in SW rats: 210 ± 5 versus 129 ± 10 mg, respectively. It has been previously demonstrated[30] that cholesterol synthesis in the rat occurs exclusively in the organs included in the rapidly exchangeable pool. Therefore, cholesterol mass of the slowly exchangeable pool (compartment 2) was estimated by mass balance assuming that no cholesterol synthesis occurred in this compartment. Under these conditions, cholesterol mass in this compartment 2 is not significantly different in the two groups of rats (350 to 370 mg). Determination of the three parameters, k_{12}, k_{21}, and k_{01}, of the model shows that the values for k_{12} and k_{01} did not significantly differ between the two groups. Only the parameter (k_{21}) describing cholesterol movement from the plasma toward the slowly exchangeable pool was significantly lower in RICO rats as compared with SW rats: 0.18 ± 0.02 versus 0.26 ± 0.02 pools per day.

HDL Metabolism

Catabolism of HDL labeled on cholesteryl ester. Autologous HDLs labeled with ^{14}C-cholesteryl linoleyl ether were injected in RICO and SW rats. The curve of plasma radioactivity clearance was followed for 24 hours (Fig 2). At death, less than 20% of the initial radioactivity remained in the plasma of RICO and SW rats. At this time, 95% of plasma radioactivity was recovered in HDL cholesteryl ester. The FCR (Table 2) was similar in the two groups (8.9 ± 0.3 in RICO and 8.8 ± 0.5% · h^{-1} in SW rats). The production rate of HDL cholesteryl ester was higher in RICO than in SW rats: 1.3 ± 0.05 versus 0.8 ± 0.03 mg · h^{-1}, respectively. The curves obtained for the two groups of rats with HDL in vivo labeled on cholesteryl ester are shown in Fig 2.

Twenty-four hours after injection, uptake of HDL cholesteryl ether by the organs was compared for the two groups of rats (Table 2). In RICO and SW rats, the part of the collected organs represented 73% to 79% of total FCR. The liver was by far the major site of uptake and was 30 times greater than the most efficient organs (adrenals and intestine). Adrenals and testis showed a significant decrease in the uptake in RICO rats: 92% and 50%, respectively.

Catabolism of HDL labeled on apolipoproteins. Four and 24 hours after HDL injection, virtually all the radioactivity was recovered in HDL (Fig 3A). Figure 4 shows the mean time course of HDL labeled on the protein moiety in RICO and SW rats. At 24 hours, 24% to 26% of the initial radioactivity remained in plasma for both groups. Despite a lower mean value for FCR in RICO rats (5.8 ± 0.3 *v* 6.6 ± 0.3% · h^{-1}), this difference was not statistically significant. The production rate was not statistically significantly higher in RICO rats (1.61 ± 0.06 *v* 1.42 ± 0.06 mg HDL protein per hour) as compared with SW rats.

Twenty-four hours after injection, uptake of HDL protein by the organs was compared for the two groups of rats (Table 2). No difference was observed in HDL protein uptake by the various organs between RICO and SW rats.

Table 2. Parameters of HDL and LDL Cholesteryl Ester and Apolipoprotein Metabolism in SW and RICO Rats

	HDL Cholesteryl Ester		HDL Apolipoproteins		LDL Apolipoproteins	
Parameter	SW (n = 5)	RICO (n = 5)	SW (n = 5)	RICO (n = 5)	SW (n = 5)	RICO (n = 5)
Production rate (mg · h^{-1})	0.80 ± 0.03	1.30 ± 0.05*	1.42 ± 0.06	1.61 ± 0.06	0.19 ± 0.03	0.40 ± 0.05†
FCR (% · h^{-1})	8.8 ± 0.5	8.9 ± 0.3	6.6 ± 0.3	5.8 ± 0.3	7.9 ± 0.5	5.5 ± 0.5†
Participation in FCR (%)						
Liver	73.0 ± 3.3	70.0 ± 2.9	34.5 ± 0.4	38.0 ± 2.3	68.6 ± 3.1	61.9 ± 4.6
Adrenals	2.6 ± 0.2	0.2 ± 0.01*	0.10 ± 0.01	0.13 ± 0.01	0.06 ± 0.01	0.06 ± 0.02
Kidney	0.32 ± 0.01	0.42 ± 0.1	3.7 ± 0.1	3.5 ± 0.8	2.3 ± 0.2	3.1 ± 0.3
Intestine	2.4 ± 0.3	1.8 ± 0.1	2.1 ± 0.2	2.9 ± 0.4	2.8 ± 0.3	3.9 ± 0.4
Colon	0.16 ± 0.02	0.22 ± 0.02	0.25 ± 0.01	0.32 ± 0.09	0.16 ± 0.02	0.21 ± 0.02
Cecum	0.16 ± 0.01	0.22 ± 0.03	0.45 ± 0.02	0.57 ± 0.11	0.10 ± 0.03	0.19 ± 0.03
Testis	0.45 ± 0.01	0.20 ± 0.01*	1.25 ± 0.1	1.46 ± 0.17	0.12 ± 0.02	0.18 ± 0.03
Unsampled organs	20.9 ± 2.5	26.5 ± 1.9	57.6 ± 0.55	53.1 ± 2.3	29.9 ± 2.4	30.5 ± 3.5

NOTE. Values concerning cholesteryl ester metabolism were obtained by injection of lipoproteins labeled with ^{14}C-cholesteryl linoleyl ether, and those concerning apolipoprotein metabolism, by injection of lipoproteins labeled with ^{14}C-sucrose.

*$P < .001$.

†$P < .01$.

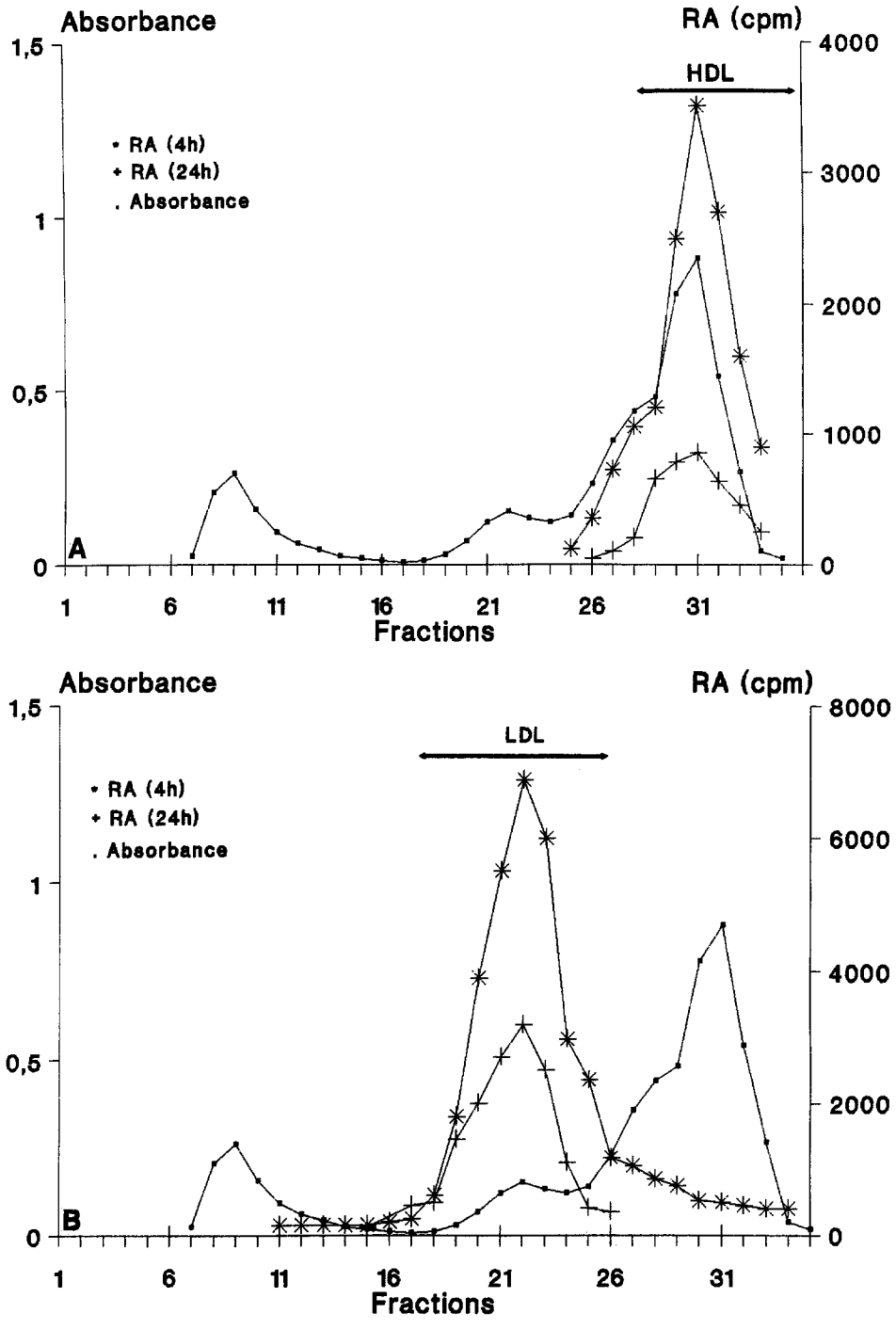

Fig 3. Elution profile on Biogel column chromatography of 100-μL rat plasma samples 4 and 24 hours after injection of ^{14}C-sucrose–labeled lipoproteins. (A) Injection of ^{14}C-sucrose–labeled HDL; (B) injection of ^{14}C-sucrose–labeled LDL.

In RICO and SW rats, 42% to 47% of the total FCR was accounted for by the collected organs. Among these organs, the liver was the major site for uptake, but represented only 35% to 38% of total uptake. The kidney and small intestine were the next most important tissues in the overall uptake.

VLDL Metabolism

Autologous VLDLs labeled with ^{3}H-cholesterol were injected into RICO and SW rats. The curves of plasma cholesteryl ester radioactivity were followed for 30 minutes (Fig 5). At death, 24% and 25% of the radioactivity injected as cholesteryl ester were detected in plasma from SW and RICO rats, respectively. Cholesteryl ester radioactivity was recovered in VLDL (85% ± 2% *v* 80% ± 2%), in LDL (6% ± 1% *v* 14% ± 1%, $P < .001$), and in HDL (9% ± 2% *v* 6% ± 1%) for SW plus RICO rats, respectively. FCR was not significantly changed in RICO versus SW rats (3.4 ± 0.01 *v* 3.6 ± 0.01 h^{-1}). The production rate of cholesteryl esters into VLDL was also unchanged (2.6 ± 0.4 and 3.3 ± 0.1 mg/h, respectively, in SW and RICO rats).

LDL Metabolism

Four and 24 hours after LDL injection, virtually all the radioactivity was recovered in LDL (Fig 3B). Figure 6

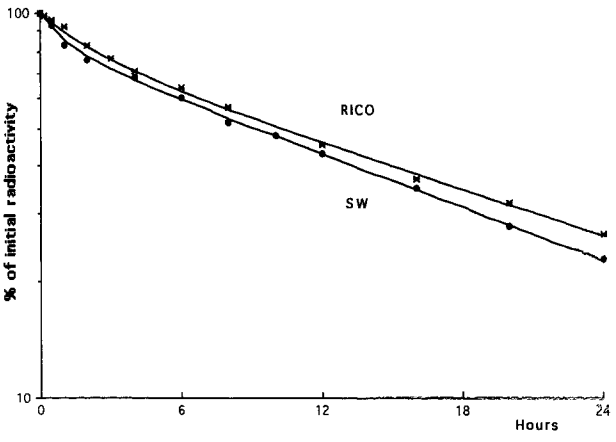

Fig 4. Radioactivity in plasma after injecting SW (●) and RICO (x) rats with autologous HDL labeled in vitro with ^{14}C-sucrose (n = 5).

shows the mean time course of autologous LDL in RICO and SW rats. LDL disappeared faster in SW than in RICO rats: 18% and 28% of the initial radioactivity was detected after 24 hours in SW and RICO rat plasma, respectively. The FCR was significantly lower ($-30\%, P < .01$) in RICO ($5.5 \pm 0.5\% \cdot h^{-1}$) than in SW ($7.9 \pm 0.5\% \cdot h^{-1}$) rats. The production rate of LDL protein in RICO rats was twice that in SW rats (0.40 ± 0.05 *v* 0.19 ± 0.03 mg $\cdot h^{-1}$).

Twenty-four hours after injection, uptake of LDL protein by the organs was measured in the two groups of rats (Table 2). In RICO and SW rats, 60% to 70% of the total FCR was accounted for by the collected organs. Among the various organs studied, the liver, small intestine, and kidney constituted, in order of importance, the majority of LDL uptake in SW and RICO rats. Comparison of LDL uptake by organ in the two groups of rats (Table 2) shows no significant difference between the two groups.

To determine whether the decrease in LDL catabolism is linked to the LDL particle itself and/or to the catabolic system, plasma disappearance curves of heterologous LDL (LDL from SW to RICO rats and LDL from RICO to SW rats) labeled with ^{14}C-sucrose were compared. The time course of heterologous LDL radioactivity is shown in Fig 6. At death (24 hours), 29% to 31% of the injected radioactivity was detected in plasma in the two groups of rats. This radioactivity was totally recovered in LDL. The FCR for LDL was similar in the two groups: 5.4 ± 0.5 versus $5.5 \pm 0.2\%$/h for SW and RICO recipient rats, respectively. The FCR of RICO rats receiving autologous LDL was also similar to that of the heterologous group ($5.5 \pm 0.6\% \cdot h^{-1}$), but differed significantly for SW rats receiving autologous LDL ($7.9 \pm 0.8\% \cdot h^{-1}, P < .01$).

DISCUSSION

Whole-body kinetics show an important increase in the cholesterol production flux of the RICO rat versus the SW rat. This increase can be linked to the increased intestinal and hepatic cholesterogenesis already demonstrated in RICO rats[31] and to the increased cholesterol lymph flow rate.[2] The total mass of the rapidly exchangeable cholesterol pool undergoes a marked increase (× 1.6) similar to that of hypercholesterolemia (× 2) without an increase in the slowly exchangeable pool. Thus, the higher cholesterol mass of the RICO rat would only involve the rapidly exchangeable pool. For the parameters of whole-body cholesterol turnover, only the one concerning cholesterol movement from the rapid pool (containing plasma) toward the slow pool (containing most of the organs) is altered. These studies suggest the existence of a disturbance in cholesterol catabolism of LDL and/or HDL, the lipoproteins that distribute cholesterol to the tissues in the rat. This observation justifies the present study of LDL and HDL metabolism.

Since the various chemical components of the HDL particle may follow distinct catabolic pathways,[3,4] HDLs were dual-labeled. After HDLs were labeled in their protein moiety and injected in rats, radioactivity remained in the HDL during the period of study. Therefore, the decrease in plasma radioactivity essentially reflects that of HDL. After injecting HDL, labeled in vivo on esterified cholesterol, the curve is perfectly superimposed on the one obtained after injecting in vitro labeled HDL. This observation validates the in vitro labeling used.

HDL catabolic rates measured in SW rats are similar to those measured in other normocholesterolemic strains. This is true for the protein moiety (6.6 *v* 5.6 to 7.6% $\cdot h^{-1}$)[32,33] and for esterified cholesterol (8.8 *v* 8.6 to 12% $\cdot h^{-1}$).[4,32,33]

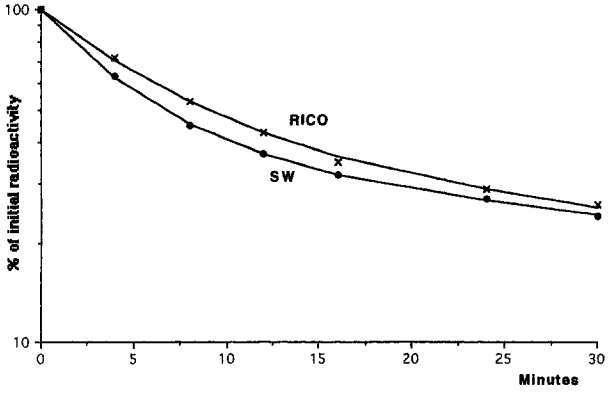

Fig 5. Radioactivity in plasma cholesteryl ester after injecting SW (●) and RICO (x) rats with autologous VLDL labeled in vivo with ^{3}H-cholesterol (n = 5).

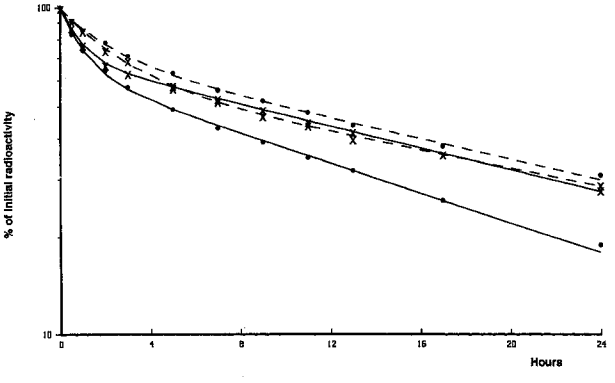

Fig 6. Radioactivity in plasma after injecting SW (●) and RICO (x) rats with autologous (—) and heterologous (----) LDL labeled with ^{14}C-sucrose (n = 5).

Turnover parameters of the protein moiety (FCR and production rate) were unchanged in RICO versus SW rats. It was similar for the catabolic process of cholesterol esters, in contrast to the production rate of cholesteryl ester, which was 1.30 ± 0.05 and 0.80 ± 0.03 mg/h, respectively. Therefore, in RICO rats, the increase in HDL esterified cholesterol is only due to an increase in HDL esterified cholesterol production without a major disturbance in their catabolism. This increase in production of HDL could be linked to the higher release of intestinally derived triglyceride-rich lipoproteins, as previously reported for the RICO rat.[2] Indeed, conversion of these lipoproteins is one of the sources of HDL cholesterol.[34]

Our results obtained in the SW rat confirm the data from previous studies[3,4] that the liver is responsible for greater than two thirds of the uptake of HDL esterified cholesterol. In RICO rats compared with SW rats, only the adrenals and the testis showed a smaller uptake of HDL esterified cholesterol. Since hepatic lipase appears to be involved in uptake of cholesteryl ester by steroidogenic organs,[35] our results could suggest a defect with hepatic lipase in both organs of the RICO rat. The weak contribution of the adrenals and testis to FCR (~3%; Table 2) explains why this disturbance has no effect on the FCR. In the SW and the RICO rat, the main organs involved in HDL apolipoprotein uptake are the liver (~33%) and the kidneys and intestine (~6%).[13]

After injection of labeled VLDL, radioactivity of plasma cholesteryl ester at the time of death was almost exclusively carried by VLDL (80% to 85%). Clearance of the radioactivity of plasma cholesteryl ester therefore reflects clearance of the cholesteryl esters carried by VLDL. The value obtained for the SW rat (3.6 ± 0.01 h^{-1}) was similar to that obtained in other normocholesterolemic rats (3 to 4 h^{-1}).[36,37] This clearance was not significantly decreased in RICO rats. This was the same for the production rate. The only disturbance was that two to three times more VLDLs were transformed into LDLs in RICO rats, therefore suggesting that direct removal of VLDL by the liver was then reduced in this strain of rats.

After labeled-LDL injection, the label remained in the LDL fraction during the experiment. This shows that the plasma decrease essentially reflects that of LDL. The FCR of LDL in SW rats was similar to the one observed in other normocholesterolemic strains (7.9 *v* 7.9 to 13% · h^{-1}).[10,12,38] The most active organs in the uptake process are the liver (>66%), the kidneys, and the intestine, as previously reported.[10]

The results of autologous LDL injections show that the higher LDL cholesterol concentration in the RICO rat is due to an increase in LDL production together with a defective catabolism. The 30% decrease in the catabolic rate observed in RICO rats concerns both the collected organs (included in the rapidly exchangeable pool with the plasma) and the uncollected ones (included in the slowly exchangeable pool with the plasma), as shown by the absence of a significant disturbance in their contribution to the total uptake. Such a decrease concerning the uncollected organs explains the similar decrease (30%) in the parameter of cholesterol movement from plasma to the slowly exchangeable pool, observed above for whole-body kinetics.

Two possible explanations could account for the decreased FCR of LDL in the RICO rat: (1) a reduced capacity to eliminate LDL, or (2) a defect in the LDL particle itself. The results of heterologous LDL injection experiments indicate that low catabolism in RICO rats is due to both an abnormality in LDL composition and an abnormality in the catabolic system (Fig 6).

The high LDL concentration in the RICO rat is consistent with a defect in LDL uptake and LDL overproduction. Increased production could be explained by an increased conversion of VLDL to LDL without a change in the secretion of VLDL. It has been previously demonstrated in humans that modulation of LDL receptor activity can influence plasma LDL concentration by modulating both the uptake of LDL and its precursors.[39] We hypothesize that the increased plasma LDL cholesterol in the RICO rat is due to the existence of an apo E that is poorly recognized by the receptors. Indeed, these apolipoproteins are found in all lipoprotein classes in rats, including RICO rats,[31] and are recognized by LDL receptors.[40] This hypothesis is also supported by alterations in chylomicron catabolism associated with apo E in RICO rats[2] and is the subject of further study in our laboratory.

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REFERENCES

1. Muller KR, Li JR, Dinh DM, et al: The characteristics and metabolism in the genetically hypercholesterolemic rat. Biochim Biophys Acta 574:334-343, 1979

2. Ouguerram K, Magot T, Lutton C: Metabolism of intestinal triglyceride rich lipoprotein in the genetically hypercholesterolemic rat (RICO). Atherosclerosis 93:201-208, 1992

3. Glass CK, Pittman RC, Winstein DB, et al: Dissociation of tissue uptake of cholesterol ester from that of apoprotein A-1 of rat plasma high density lipoprotein: Selective delivery of cholesterol ester to liver, adrenal and gonad. Proc Natl Acad Sci 80:5435-5439, 1983

4. Stein Y, Dabach Y, Hollander G, et al: Metabolism of HDL cholesterol ester in the rat, studied with a nonhydrolysable analog, cholesteryl linoleyl ether. Biochim Biophys Acta 752:98-105, 1983

5. Mathé D, Lutton C, Rautureau J, et al: Effects of dietary fibers and salt mixtures or cholesterol metabolism in rats. J Nutr 197:466-474, 1977

6. Havel RJ, Eder HA, Bragdon JH: Composition of ultracentrifugally separated lipoproteins in human serum. J Clin Invest 34:1345-1353, 1955

7. Faergeman O, Havel R: Metabolism of cholesteryl esters of rat very low density lipoproteins. J Clin Invest 55:1210-1218, 1975

8. Pittman RC, Green SR, Attie AD, et al: Radiolabeled sucrose covalenty linked to protein. A device for quantifying degradation of plasma proteins catabolized by lysosomal mechanisms. J Biol Chem 254:6876-6879, 1979

9. Rudel LL, Lee JA, Morris MD, et al: Characterization of plasma lipoproteins separated and purified by agarose column chromatography. Biochem J 139:89-95, 1974

10. Battacharya S, Balasubramaniam S, Simons LA: Role of the liver in LDL catabolism in the rat. Biochem J 220:333-336, 1986

11. Van Tol A, Dallinga-Thie GM, Van Gent T, et al: Specific saturable binding of rat HDL to rat kidney membranes. Biochim Biophys Acta 876:340-351, 1986

12. Pittman RC, Attie AD, Carew TE, et al: Tissue sites of catabolism of rat and human LDL in rats. Biochim Biophys Acta 710:7-14, 1982

13. Glass CK, Pittman RC, Keller GA, et al: Tissue sites of degradation of apolipoprotein A-1 in the rat. J Biol Chem 258:7161-7167, 1983

14. Magot T, Verneau C, Lutton C, et al: Origin and fate of cholesterol metabolism in rat plasma lipoproteins in vivo. I. Qualitative analysis. Annu Nutr Metab 29:147-159, 1985

15. Roberts DCK, Miller NE, Grook D, et al: An alternative procedure for incorporating radiolabelled cholesteryl ester into plasma lipoproteins in vitro. Biochem J 226:319-322, 1985

16. Ha YC, Barter PJ: Difference in plasma cholesteryl ester transfer activity in sixteen vertebrate species. Comp Biochem Physiol 71:265-269, 1982

17. Groener JEM, Van Gent T, Van Tol A: Effect of lipid transfer protein on plasma lipids, apolipoproteins and metabolism of HDL cholesteryl ester in the rat. Biochim Biophys Acta 1002:93-100, 1989

18. Yamaoka K, Tanigawara Y, Nakagawa T, et al: A pharmacokinetic analysis program (Multi) for microcomputer. J Pharm Dym 4:879-885, 1981

19. Goodman DS, Noble RP: Turnover of plasma cholesterol in man. J Clin Invest 47:231-241, 1968

20. Perl W, Samuel P: Input-output analysis for total input rate and total traced mass of body cholesterol in man. Circulation Res 25:191-199, 1969

21. Matthews CME: The theory of tracer experiment with ^{131}I-labelled plasma proteins. Phys Med Biol 2:36-53, 1957

22. Langer T, Strober W, Levy RI: The metabolism of low density lipoproteins in familial type II. J Clin Invest 51:1528-1536, 1972

23. Day AJ, Wahlquist ML, Campbell DJ: Differential uptake of cholesterol and of different cholesterol esters by atherosclerotic intima in vivo and in vitro. Atherosclerosis 11:301-320, 1970

24. Magot T: La modélisation des métabolismes. Application au metabolisme du cholestérol et des lipoprotéines plasmatiques. Paris, France, Masson, 1990

25. Folch L, Lees M, Stahley GH: A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497-509, 1957

26. Hirsh J, Ahrens ED: The separation of complex lipid mixture by use of silicic acid chromatography. J Biol Chem 233:311-320, 1958

27. Cohn JS, Kimpton WG, Nestel PJ: The effects of dietary casein and soy protein on cholesterol and very low density lipoprotein metabolism in the rat. Atherosclerosis 52:219-231, 1984

28. Oh SY, Dupont J, Clow DJ: Kinetic analysis of cholesterol turnover in rat tissues. Steroids 27:637-655, 1976

29. Robins S, Russo A: Regulation of body cholesterol pools. Influence of cholesterol input and excretion in an animal model. Biochim Biophys Acta 573:343-353, 1979

30. Magot T, Chevallier F: Measurement of the rate of cholesterol synthesis in various organs of the rat in vivo. Ann Biol Anim Biochim Biophys 19:1757-1770, 1979

31. Cardona-Sanclemente LE, Verneau C, Mathe D, et al: Cholesterol metabolism in the genetically hypercholesterolemic rat (RICO). I. Measurement of turnover processes. Biochim Biophys Acta 919:205-212, 1987

32. Carr TP, Lei KY: HDL cholesteryl ester and protein catabolism in hypercholesterolemic rats induced by copper deficiency. Metabolism 39:518-524, 1990

33. Gross G, Sykes M, Arenallo R, et al: HDL clearance and receptor mediated catabolism of LDL are reduced in hypothyroid rats. Atherosclerosis 66:269-275, 1987

34. Eisenberg S: HDL metabolism. J Lipid Res 25:1017-1058, 1984

35. Jansen H, Hulsmen WC: Heparin releasable lipases may play a role in the uptake of cholesterol by steroid-secreting tissues. Trends Biochem Sci 5:265-268, 1980

36. Cooper AD, Shrewsbury MA, Erickson SK: Comparison of binding and removal of remnant of triglyceride-rich lipoproteins of intestinal and hepatic origin by rat liver in vitro. Am J Physiol 243:389-395, 1982

37. Magot T, Champarnaud G, Anfreville R, et al: Origin and fate of cholesterol in rat plasma lipoproteins in vivo. II. Modelling of cholesterol absorption and its release into plasma lipoproteins. Ann Nutr Metab 29:160-174, 1985

38. Stein Y, Halperin G, Stein O: The fate of cholesterol linoleyl ether and cholesteryl linoleate in the intact rat after injection of biologically labeled human LDL. Biochim Biophys Acta 486:569-574, 1981

39. Magot T, Malmendier CL, Ouguerram K, et al: In vivo effect of simvastatin on lipoprotein cholesteryl ester metabolism in normocholesterolemic volunteers. Clin Chim Acta 196:59-68, 1991

40. Nagata Y, Chen J, Cooper AD: Role of low density receptor-dependent and -independent sites in binding and uptake of chylomicrons remnants in rat liver. J Biol Chem 263:15151-15158, 1988