Diminished Macrophage Cholesterol Removal Rate by the Altered HDL Metabolism in the Nagase Analbuminemic Rat

Sergio Catanozi[a,*], Jussara C. Rocha[a], Marisa Passarelli[a], Carlos H. Mesquita[d], Vivian Y. Suguiama[a], Maria L. Guzzo[b], Antônio dos Santos Filho[c], Eder C.R. Quintão[a], and Edna R. Nakandakare[a]

[a]Lipids Laboratory (LIM 10), [b]Animal House, and [c]Department of Rheumatology (LIM 17) of Faculty of Medical Sciences, and [d]Institute of Nuclear Research (IPEN), University of São Paulo, São Paulo, Brazil

ABSTRACT: Dyslipoproteinemia of the Nagase analbuminemic rat (NAR) is characterized by elevated concentrations of VLDL and LDL attributed to increased rates of liver lipoprotein synthesis. Increased lysophosphatidylcholine (LPC) in NAR HDL has been attributed to high plasma LCAT activity. We show here that, as compared with Sprague-Dawley rats (SDR), NAR plasma triacylglycerol (TAG), total cholesterol (TC), HDL TAG, protein, total phospholipids (PL), LPC, and PS are increased. These alterations rendered the NAR HDL particle more susceptible to the activity of the enzyme hepatic lipoprotein lipase (HL), which otherwise was unaltered in our study. Fractional catabolic rates in blood of the autologous ^{125}I-apoHDL (median and lower quartile values), were, respectively, 0.231 and 1.645 (n = 10) in NAR as compared with 0.140 and 0.109 (n = 10) in SDR (P = 0.012), corresponding to synthesis rates of HDL protein of 89.8 ± 33.7 mg/d in NAR and 17.4 ± 6.5 mg/d in SDR (P = 0.0122). Furthermore, Swiss mouse macrophage free-cholesterol (FC) efflux rates, measured as the percent [^{14}C]-cholesterol efflux/6 h, were 8.2 ± 2.3 (n = 9) in NAR HDL and 11.2 ± 3.2 (n = 10) in SDR HDL (P = 0.03). Therefore, in NAR the modification of the HDL composition slows down the cell FC efflux rate, and together with the increased rate of plasma HDL metabolism influences the reverse cholesterol transport system.

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Hypoalbuminemia, proteinuria, hypercoagulability, edema, and dyslipoproteinemia that includes alterations of HDL composition and metabolism are common features of nephrotic syndrome (NS) (1). Understanding the mechanisms involved in this hyperlipidemia is critical to explain the increased cardiovascular risk in NS (2,3). Nephrotic syndrome animal models and the Nagase analbuminemic rat (NAR) share several alterations in the metabolism of lipoproteins (4).

From a stock of Sprague-Dawley rats (SDR), Nagase *et al.* (5) produced a strain of hyperlipidemic rats, NAR, completely deficient in serum albumin. The serum albumin deficiency is inherited as an autosomal recessive trait. Similar to NS, NAR hyperlipidemia has been characterized by increased total cholesterol (TC), TAG, free FA (FFA), and phospholipid (PL) plasma concentrations. Plasma apolipoproteins (apo) A-I, B, and E, as well as HDL lysophosphatidylcholine (LPC) are also significantly increased in NAR (6,7).

NAR total serum protein concentration is normal (5) but the plasma oncotic pressure is reduced (8). Although the relationship between low oncotic pressure and hyperlipidemia is not completely understood, previous studies have shown increased plasma TAG and VLDL synthesis rates in NAR that explain the pathogenesis of hyperlipidemia in NS (9,10).

In analbuminemia, substantial amounts of FFA and LPC (6,7) are bound to lipoprotein (LP) fractions, whereas in normal animals albumin is the main carrier of these components (11,12). Higher LPC concentration in NAR erythrocytes is normalized after albumin addition to blood (13), and monolayer cell permeability of human coronary artery endothelial cells is increased in a time- and dose dependent manner with LPC treatments (14). Furthermore, albumin protects endothelium-dependent relaxation against the inhibitory effect of LPC (15) and reduces LPC uptake by cultured endothelial cells (16). Thus, the role of albumin as a reservoir for LPC may be important to maintain the integrity of the monolayer endothelial cell. Injury or dysfunction of the monolayer endothelial cell is considered one of the critical events in the development of atherosclerosis (14).

Although rats are not suitable models for studies of atherosclerosis (17), NAR and human analbuminemia (5) are useful tools for understanding whether alterations in the metabolism of HDL play a role in the regulation of the body cholesterol homeostasis that may explain the mechanisms of premature atherosclerosis in humans. Thus, the current study aims at investigating whether the anti-atherogenic role of HDL is impaired by modifications of the HDL composition due to alterations of HDL metabolism in NAR.

*To whom correspondence should be addressed at Lipids Lab—LIM 10, Faculty of Medical Sciences of University of São Paulo Av. Dr. Arnaldo 455 Room 3317 01246-000 São Paulo, Brazil. E-mail: catanozi@usp.br

Abbreviations: apo, Apolipoprotein; ^{14}C-PC, L-dipalmitoyl [choline-methyl-^{14}C] PC; DMEM, Dulbecco's modified Eagle`s medium; EDTA-PBS, ethylenediaminetetraacetic acid phosphate-buffered saline; FBS, fetal bovine serum; FC, free cholesterol; FCR, fractional catabolic rate; FAFA, fatty acid free albumin; FFA, free FA; HL, hepatic lipase; ^{125}I-apoHDL, radioactive HDL; LP, lipoprotein; LPC, lysophosphatidylcholine; NAR, Nagase analbuminemic rat; NS, nephrotic syndrome; PL, phospholipid; SDR, Sprague-Dawley rats; SM sphingomyelin; SR-BI, scavenger receptor B type I; RCT, reverse cholesterol transport system; TC, total cholesterol; TCA, trichloroacetic acid.

EXPERIMENTAL PROCEDURES

Animals. Breeding NAR and SDR colonies were kindly supplied by Professors Roberto Zatz and Clarice K Fujihara (Renal Division of University of São Paulo Medical School). Animals were raised and fed *ad libitum* a pelleted commercial chow (Nuvilab-Nuvital, São Paulo, Brazil) in conventional housing at 22 ± 2°C with a light-dark cycle of 12 h. Animal experiments were performed in accordance with protocols approved by the Institutional Animal Care and Research Advisory Committee (nos. 582/01 and 029/02). In order to avoid the interference of chylomicron TAG on plasma total measurements, all experiments were carried out in the morning after a 12-h overnight fasting period. Under ketamine (60 mg/kg body weight, i.p.) and xylazine (10 mg/kg body weight, i.p.) anesthesia, the right carotid and abdominal aorta arteries of 3-mon-old male NAR and SDR were cannulated with indwelling polyethylene catheters PE-50 and PE-100 (Intramedic, Clay Adams, Parsippany, NJ), respectively, previously rinsed with liquid silicon (Silicone Prontosil, Rio de Janeiro, Brazil). The right carotid artery isolation was carried out by a small incision in the ventral median line of the neck, and the abdominal aorta artery was cannulated after a median abdominal laparotomy. All experiments started after the animals had completely recovered from surgery. At the end of metabolic studies rats were sacrificed in a CO_2 chamber.

Isolation and labeling of HDL. Blood was drawn over heparin (20 IU, 5μL) by exsanguination from the rat abdominal aorta (NAR, $n = 12$; SDR, $n = 9$). HDL ($d = 1.063$–1.21 g/mL) was separated from plasma by sequential ultracentrifugation (L-80 ultracentrifuge, Beckman Instruments, Palo Alto, CA) in a 50Ti rotor after spinning at $d > 1.21$ g/mL, 200 000 × g, for 40 h at 4°C as previously described (18). HDL purification was performed by discontinuous gradient ultracentrifugation (19) utilizing an SW 41 rotor spinning at 200 000 × g for 24 h at 4°C. LP was dialyzed against EDTA-PBS. The protein content was determined according to Lowry *et al.* (20). HDL LPC, sphingomyelin (SM), PC, PS, and PE fractions were separated by TLC (21) and measured by the modified micro-procedure of Bartlett (22). TC and TAG were determined using commercially available kits (Roche Diagnostics, Mannheim, Germany). Fresh NAR ($n = 12$) or SDR ($n = 9$) HDL (500 μL) was incubated with 17 μl of L-dipalmitoyl [choline-methyl-^{14}C] PC (^{14}C-PC) (Dupont-New England Nuclear, Boston, MA) and their respective infranatants (3 mL; $d > 1.21$ g/mL) in a shaking water bath for 18 h at 37°C (23). The purity of the isotope was checked by silica gel TLC and shown to be higher than 98%. NAR and SDR ^{14}C-PC-HDL were then re-isolated by sequential ultracentrifugation as described above.

Hepatic post-heparin plasma lipolytic activity. Four minutes after intra-carotid heparin injection (100 IU/kg body weight), NAR ($n = 3$) and SDR ($n = 3$) post-heparin plasma lipases were obtained from the abdominal aorta. This time was selected based upon the time-course of appearance of lipolytic activities (24), and the hepatic lipase (HL) activity was determined after inhibition of the peripheral lipoprotein lipase activity as previously described (25). Nonetheless, the measurement of HL at a single time point does not reflect the HDL TAG hydrolysis rate over time. Briefly, NAR and SDR HDL were incubated with Tris-HCl (2 M NaCl, pH 8.0; Sigma Chemical Co., St Louis, MO) using autologous and heterologous post-heparin plasma in a shaking bath for 1 h at 37°C. Folch (3 mL chloroform/methanol, 2:1, vol/vol; E. Merck, Darmstadt, Germany) was added to the samples at the end of incubation. Following an overnight period at 4°C, the infranatant was then evaporated under N_2 flow. After separation by TLC, radioactivity counts in PC and LPC bands were determined in a beta scintillation counter (LS 6000-TA8, Beckman Instruments). HL activity was estimated as the percentage conversion of PC to LPC.

Iodination and kinetics of HDL. After dialysis, NAR HDL (3.69 mg/dL) and SDR HDL (1.61 mg/dL) protein pools were iodinated with iodine monochloride (Sigma Chemical Co.) and sodium iodide I-125 (Na-^{125}I) (MDS Nordion, Kanata, Ontario, Canada) (26). Radioactive HDL (^{125}I-apoHDL) pools were exhaustively dialyzed against PBS (pH 7.4), and an aliquot precipitated by TCA (E. Merck). More than 95% of ^{125}I-apoHDL was precipitated by TCA.

Four days before as well as during the ^{125}I-apoHDL kinetic study, animals had free access to drinking water containing potassium iodide and iodine (1.5 mg in each 500 mL) to avoid ^{125}I uptake by the thyroid. Before and after the experiment, blood samples (70 μL) were drawn for hematocrit, TAG, and TC measurements. Blood samples (100 μL) were sequentially drawn from the tail vein over a period of 48 h (at 3 min, 10 min, 6 h, 10 h, 24 h, 32 h, and 48 h) after an intra-carotid autologous infusion of NAR or of SDR ^{125}I-apoHDL. For this purpose rats were lightly restrained but not anesthetized. Radioactivity was measured in 40-μL plasma aliquots in a gamma counter (Cobra Model, Packard Instruments, Meriden, CT). Rats were normally fed 3 h after the ^{125}I-apoHDL infusion. Plasma ^{125}I-apoHDL clearance curves were analyzed as the fractional catabolic rate (FCR), according to a compartmental analysis of the plasma protein radioactivity vs. time. The system is based on a two-pool model that assumes the existence of an intravascular pool in dynamic equilibrium with an extravascular pool and that new input or exit of radiolabeled HDL occurs from the intravascular pool only (27). FCR values were derived directly from the kinetic model as the clearance rate from the intravascular pool. Biexponential ^{125}I-apoHDL decay curves for rats were individually modeled using a modified SAAM (simulation, analysis, and modeling) program, Compartmental Analysis Program (Institute of Nuclear Research IPEN, São Paulo, Brazil) (28) as summarized in Figure 1.

Cellular cholesterol efflux. Swiss mouse macrophages were harvested from peritoneal cavity in sterile PBS (0.8% NaCl, 0.006% Na_2HPO_4, 0.02% KCl, and 0.04% KH_2PO_4), with added penicillin (100 U/mL), streptomycin (100 U/mL), and fungizone (2.5 μg/mL) (Gibco BRL, Life Technologies, Rockville, MD). Pelleted cells after centrifugation at 90 × g for 2 min, at 4°C were suspended in the tissue culture medium (RPMI 1640; Gibco BRL, Life Technologies) including 10% FBS (Gibco BRL, Life Technologies), attaining final concentration of

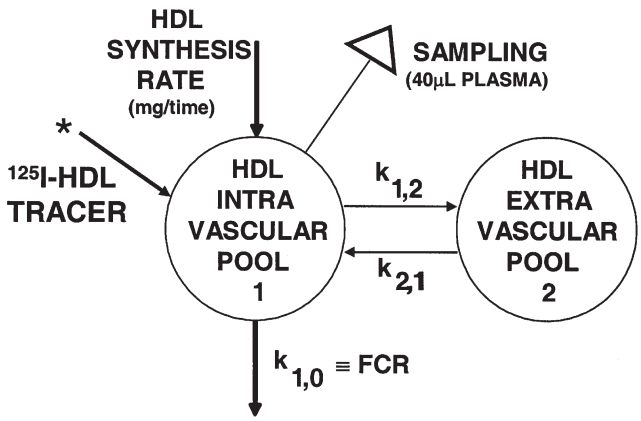

FIG. 1. Schematic model of the HDL kinetics. The model consists of two compartments, or pools, the intravascular and the extravascular compartments. It is assumed that the compartments are in dynamic equilibrium. The $k_{i,j}$ (min^{-1}) values represent the transfer rate of HDL among the compartments. The constants $k_{i,j}$ are associated with the half-life time (min) according to $t_{1/2} = 0.693/k_{i,j}$. In addition, the same quantity of synthesized HDL (mg/time) that is introduced in compartment 1 is removed from the system; thus, synthesis rate of HDL can be estimated using the following formula: HDL synthesis (mg/d) = plasma HDL (mg/mL) × plasma volume (mL) x FCR (h^{-1}) × 24 h/d. This model also assumes that ^{125}I-HDL is injected instantaneously in the intravascular compartment (arrow with asterisk). From compartment 1 a fraction $k_{1,2}$ of HDL is transferred to compartment 2 at each time unit. On the other hand, a fraction $k_{2,1}$ of HDL contained in the extravascular compartment returns to the intravascular compartment in each time unit. The tracer sampling was made in compartment 1 (line with a triangle).

1.5×10^6 cells/well. Cell solution were dispersed into 24-well plastic dishes and kept in a humidified incubator with a 5% CO_2 atmosphere at 37°C, for 24 h. Adherent cells were washed twice in PBS containing antibiotics and 0.1% FA-free albumin (FAFA; Sigma Chemical Co.). Macrophage cholesterol loading was carried out on 24-h incubation in Dulbecco's modified Eagle`s medium (DMEM; Gibco BRL, Life Technologies) containing 0.1% FAFA, 0.5 μCi/mL [4-^{14}C]-free cholesterol ([4-^{14}C]-FC; Dupont-New England Nuclear), and acetylated LDL (50 μg of protein/mL). The purity of the isotope was checked by silica gel TLC and shown to be higher than 98%. Cellular cholesterol pools were equilibrated for 24 h in DMEM/FAFA prior to the incubation with HDL. Cholesterol-enriched cells were next washed twice in PBS-FAFA, and thereafter incubated with NAR and SDR HDL (100 μg of HDL protein/mL) for 6 h. Control incubations were done with DMEM/FAFA only. The medium was drawn into tubes and centrifuged at $125 \times g$ for 10 min to remove cell debris, and the radioactivity was measured in a beta scintillation counter. Cells were rinsed twice with cold PBS/FAFA and twice with cold PBS and extracted with hexane/isopropanol (3:2, vol/vol) (E. Merck). Solvent was evaporated and radioactivity measured. The percentage of ^{14}C-FC efflux was calculated as (^{14}C-FC in the medium/^{14}C-FC in cells plus medium) × 100. The difference between the efflux elicited by HDL plus albumin and that by the albumin-enriched media expresses the HDL-mediated efflux. Protein measurement was done after cell lysis in 0.2 N NaOH (29,30).

Statistical analyses. Data were expressed as mean ± SD. The student's t-test was used for statistical comparison of the data, except in the study of ^{125}I-apoHDL kinetics, where the Mann-Whitney rank sum test was used and data expressed as median and lower quartile. Data evaluation was calculated using GraphPad Prism version 2.01 (GraphPad Software Inc., San Diego, CA). Results of the statistical tests were considered significant at the 95% confidence level ($P < 0.05$).

RESULTS

Similarity of body weight was attained in both experimental groups; however, plasma TAG and TC (Table 1) as well as HDL TAG, TC, PL, and protein concentrations were higher in NAR than in SDR. HDL LPC and PS concentrations were also higher in NAR, but HDL SM, PC, and PE concentrations did not differ between the two groups (Table 2).

We have investigated whether the differences in the HDL composition could be ascribed to differences in post-heparin lipase activity. SDR and NAR HDL particles labeled with L-dipalmitoyl [choline-methyl-^{14}C] were incubated with plasma containing HL according to several combinations possible. In autologous as well as in heterologous incubations HDL-PL hydrolysis *in vitro* was significantly higher when NAR HDL was used as substrate (Table 3). The higher PL hydrolysis of NAR HDL was solely attributed to alteration of the HDL composition and not to differences in intrinsic HL activities between NAR and SDR.

Autologous ^{125}I-apoHDL was infused intra-arterially to further explore whether altered NAR HDL composition brings on modifications in the kinetics of HDL. Total plasma HDL from pools of donor NAR and SDR were labeled with ^{125}I and pulse-infused into the carotid of NAR and SDR rats. Thereafter, radioactivity was measured over the course of 48 h and expressed per mL of plasma. Data derived from the FCR curves expressed as median and lower quartile were, respectively, 0.231 and 1.645 ($n = 10$) in NAR, as compared with 0.140 and 0.109 ($n = 10$) in SDR ($P = 0.012$), indicating a faster rate of HDL metabolism in NAR that can only be explained by a higher HDL synthesis rate in this animal (Fig. 2).

As HDL is the main LP fraction involved in the reverse cholesterol transport system, that is, in the FC removal mechanism from extra-hepatic tissues and its transport to the liver, it was then investigated whether the HDL-mediated cell cholesterol efflux of [^{14}C]-cholesterol enriched macrophages from donor mice was altered in the presence of HDL drawn from NAR. Indeed,

TABLE 1
Characteristics of NAR and SDR Used in This Study

	NAR ($n = 40$)	SDR ($n = 30$)
Body weight (g)	377 ± 26	386 ± 33
Plasma TAG (mg/dL)	116 ± 32[a]	56 ± 18[a]
Plasma TC (mg/dL)	141 ± 30[a]	73 ± 14[a]

Data are expressed as mean ± SD.
[a]$P < 0.0001$, NAR vs. SDR, statistical comparison by student's *t* test.

TABLE 2
HDL Composition in TAG, TC, Protein, Total PL, and Phospholipid Fractions in NAR and in SDR

	NAR (n = 8)	SDR (n = 7)
	mg/dL	
HDL-TAG	3.94 ± 0.78	2.30 ± 0.41[a]
HDL-TC	99 ± 28	55 ± 7[a]
HDL-protein (10)	119 ± 30	37 ± 13[a]
	µmol/mL	
HDL-PL	1.56 ± 0.17	0.78 ± 0.17[a]
HDL-LPC	0.064 ± 0.012	0.023 ± 0.013[a]
HDL-SM	0.035 ± 0.023	0.034 ± 0.028
HDL-PC	0.102 ± 0.047	0.104 ± 0.044
HDL-PS	0.102 ± 0.048	0.044 ± 0.038[b]
HDL-PE	0.029 ± 0.034	0.026 ± 0.033

Data are expressed as mean ± SD. [a]$P < 0.001$, NAR vs. SDR; [b]$P < 0.05$, NAR vs. SDR, statistical comparison by student's t test.

TABLE 3
Post-Heparin Hepatic Lipase Activity on HDL Particles

HDL NAR + HL NAR vs.	HDL SDR + HL SDR	P[a]
92 ± 7 (14)	4.6 ± 2.1(14)	0.0001
HDL NAR + HL SDR vs.	HDL SDR + HL SDR	
89 ± 10 (12)	4.6 ± 2.1 (14)	0.0001
HDL NAR + HL SDR vs.	HDL SDR + HL NAR	
89 ± 10 (12)	3.7 ± 1.4 (9)	0.0001
HDL NAR + HL SDR vs.	HDL NAR + HL NAR	
89 ± 10 (12)	92 ± 7 (14)	0.37
HDL SDR + HL NAR vs.	HDL SDR + HL SDR	
3.7 ± 1.4 (9)	4.6 ± 2.1 (14)	0.25

L-Dipalmitoyl [choline-methyl-^{14}C] HDL particles were incubated with hepatic lipase (HL). HDL particles and HL were drawn from SDR and from NAR, and experiments were designed in a crossover fashion.
Data are expressed as mean ± SD. Values express percent hydrolysis of the radioactive phospholipids; figures in parentheses represent n for the measurement.
[a]Statistical comparison by student's t test

the percent [^{14}C]-cholesterol macrophage efflux was 8.2 ± 2.3 (n = 9) utilizing NAR HDL and 11.2 ± 3.2 (n = 10) utilizing SDR HDL, a difference significant at $P = 0.03$.

DISCUSSION

Albumin plays an important role on the transport of FFA, bilirubin, ions, heavy metals, drugs, hormones, enzymes, and bile acids (31–33). NAR are characterized by analbuminemia, hypercholesterolemia, hypertriacylglycerolemia, and high plasma PL levels (5). These are also characteristics of human analbuminemia (1,2). In addition, several of these features are present in the hypoalbuminemia of NS. The absence of albumin presents potential problems regarding plasma FFA and LPC transport. For instance, LPC and FFA are mainly bound to LP and not to albumin in NAR plasma (7).

The two- to threefold elevated LPC concentration in NAR HDL can be explained by synergistic processes. A faster rate of HDL catabolism in NAR reflects an increased production of HDL, and, consequently, a greater HDL particle number exposure to the action of the HL, which otherwise is normal in these animals. Although Kaysen *et al.* (34) had reported a reduced

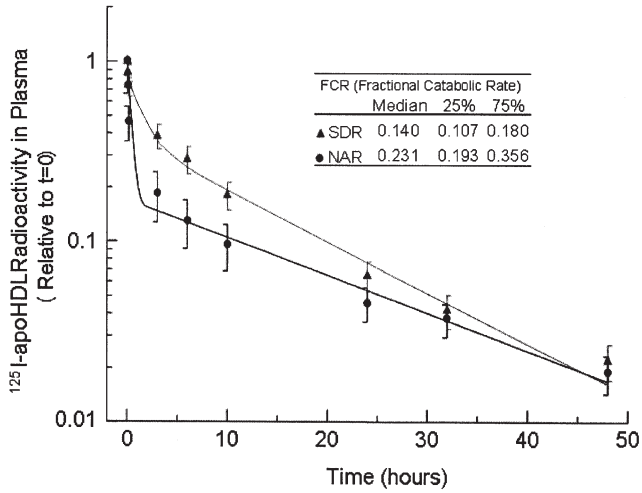

FIG. 2. Plasma radioactivity curves (in 40 µL aliquots) from tail vein blood sequentially drawn over a period of 48 h (3 min, 10 min, 6 h, 10 h, 24 h, 32 h, and 48 h) after an intracarotid autologous infusion of NAR (circles) or of SDR ^{125}I-apoHDL (triangles). Each experimental point represents the mean ± SEM (n = 10) relative to t = 0. Median FCR values (h^{-1}) were derived directly from the kinetic model as the clearance rate from the intravascular pool. NAR FCR 0.231 *vs* SDR FCR 0.140, difference significant at $P = 0.012$. FCR and the HDL-protein concentration in plasma allowed for the calculation of the absolute HDL-protein synthesis rates as mg/d ± SEM, which were 89.8 ± 33.7 mg/d in NAR and 17.4 ± 6.5 mg/d in SDR (difference significant at $P = 0.0122$).

apo-AI fractional catabolic rate in NAR, their apo-AI kinetics had been carried out utilizing apo-AI HDL drawn from normal SDR, and not from NAR. The enhanced conversion of PC to LPC (35) should be accounted for by the HL-induced modification of the LP. In this regard, the HL activity provides the major (~55%) route for PC removal from plasma, which is consistent with the elevated plasma PC concentration observed in humans with HL deficiency (36). The faster plasma clearance of ^{125}I-apoHDL in NAR compared with SDR reflects an increased synthesis rate and plasma HDL concentration simultaneous with an elevated concentration of NAR HDL TAG, PL, and apo-AI. The latter is likely the outcome of the metabolism of TAG-rich particles; a previous study from our laboratory has shown faster plasma TAG and VLDL synthesis rates in NAR than in SDR (10). Our data are compatible with an increased rate of hepatic cholesterol synthesis in NAR as reported by others (37,38).

Hepatic scavenger receptor B type I (SR-BI) protein is not altered in NAR (38). SR-BI internalizes HDL cholesteryl ester but little HDL protein. Thus, the faster turnover rate of HDL protein means that specific receptors, such as kidney cubilin and megalin, must be involved in HDL protein metabolism in NAR (39).

The higher rate of LCAT activity in NAR (7) prevents the concentration of HDL PC from further rising. In other words, an elevated concentration of PC must have occurred early on due to the increased HDL production rate. On the other hand, the higher HDL PS concentration in NAR may have rendered the HDL particle more susceptible to the action of HL, as previously reported

(40). Furthermore, greater plasma apoE and apo-AI concentrations in NAR, attributed mostly to the apoE-loaded HDL (7), may also activate the HDL PC hydrolysis by HL (7,35,41–45). Finally, LPC is known to modulate the metabolism of lipoproteins upon modifying their clearance *in vivo* and *in vitro* (46,47).

Considering that HDL is a critical protecting factor against premature atherosclerosis (48,49), we investigated the role of this particle on the removal of unesterified cholesterol from mouse peritoneal macrophages. This is the first step in the reverse cholesterol transport system (RCT) that ultimately delivers peripheral cholesterol to the liver (43,50). As compared with SDR the NAR HDL capacity for FC efflux from the lipid-loaded macrophages is significantly impaired. In this regard, it is known that the PL content and composition are important factors determining the HDL capacity for FC efflux from peripheral cell plasma membranes (51–53). For instance, HDL PC hydrolysis by phospholipase A2 slows down the rate of the SR-BI-mediated cholesterol efflux (53). Thus, the increased LPC content in NAR HDL may have contributed simultaneously to the impaired cell FC efflux rate and to the faster hepatic HDL uptake. Increased formation of HDL LPC and decreased HDL-dependent FC efflux from lipid-loaded mouse macrophages have also been reported in the human HDL pretreated with human secretory phospholipase (54). However, an impaired adenosine triphosphate-binding cassette transporter A1 receptor expression in NAR could not be ruled out and needs further investigation.

In summary, this study indicates that in NAR, plasma concentrations of TAG, PL, and TC are elevated largely due to the HDL fraction reflecting higher rate of HDL protein synthesis. The increased HDL lipid content renders the particle more susceptible to the otherwise unaltered intrinsic HL activity. Moreover, the efficiency of the NAR HDL to remove cell cholesterol is impaired. Therefore, alterations in the HDL metabolic profile described in NAR help explain disturbances of the RCT.

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REFERENCES

1. Kaysen, G.A., and Velden, M.G.M.S. (1999) New Insights into Lipid Metabolism in the Nephrotic Syndrome, *Kidney Int. 55 (Suppl. 71)*, S18–S21.
2. Ordoñez, J.D., Hiatt, R.A., Killebrew, E.J., and Fireman, B.H. (1993) The Increased Risk of Coronary Heart Disease Associated with Nephrotic Syndrome, *Kidney Int. 44*, 638–642.
3. Watts, G.F., Herrmann, S., Dogra, G.K., Playford, D.A., Best, J.D., Thomas, M.A.B., and Irish, A. (2001) Vascular Function of the Peripheral Circulation in Patients with Nephrosis, *Kidney Int. 60*, 182–189.
4. Vaziri, N.D. (2003) Molecular Mechanisms of Lipid Disorders in Nephrotic Syndrome, *Kidney Int. 63*, 1964–1976.
5. Nagase, S., and Shimamune, K. (1979) Albumin-Deficient Rat Mutant, *Science 205*, 590–591.
6. Joles, J.A., Koolschijn, N.W., Scheek, L.M., Koomans, H.A., Rabelink, T.J., and Van Tol, A. (1994) Lipoprotein Phospholipid Composition and LCAT Activity in Nephrotic and Analbuminemic Rats, *Kidney Int. 46*, 97–104.
7. Van Tol, A., Jansen, E.H.J.M., Koomans, H.A., and Joles, J.A. (1991) Hyperlipoproteinemia in Nagase Analbuminemic Rats: Effects of Pravastatin on Plasma (apo) Lipoproteins and Lecithin: Cholesterol Acyltransferase Activity, *J. Lipid Res. 32*, 1719–1728.
8. Joles, J.A., Koolschijn, N.W., Braam, B., Kortlandt, W., Koomans, H.A., and Mees, E.J.D. (1989) Colloid Osmotic Pressure in Young Analbuminemic Rats, *Am. J. Physiol. 257,* F23–F28.
9. Joles, J.A., Bijleveld, C., Van Tol, A., Geelen, M.J.H., and Koomans, H.A. (1995) Plasma Triglyceride Levels Are Higher in Nephrotic Than in Analbuminemic Rats Despite a Similar Increase in Hepatic Triglyceride Secretion, *Kidney Int. 47*, 566–572.
10. Catanozi, S., Rocha, J.C., Nakandakare, E.R., Oliveira, H.C.F., and Quintão, E.C.R. (1994) Nagase Analbuminemic Rats Have Faster Plasma Triacylglycerol and VLDL Synthesis Rates, *Biochim. Biophys. Acta 1212*, 103–108.
11. Spector, A.A. (1975) Fatty Acid Binding to Plasma Albumin, *J. Lipid Res. 16*, 165–179.
12. Switzer, S., and Eder, H.A. (1965) Transport of Lysolecithin by Albumin in Human and Rat Plasma, *J. Lipid Res. 6*, 506–511.
13. Stoll, L.L., Oskarsson, H.J., and Spector A.A. (1992) Interaction of Lysophosphatidylcholine with Aortic Endotelial Cells, *Am. J. Physiol. 262*, H1853–H1860.
14. Yan, S., Chai, H., Wang, H., Yang, H., Nan, B., Yao, Q., and Chen, C. (2005) Effects of Lysophosphatidylcholine on Monolayer Cell Permeability of Human Coronary Artery Endothelial Cells, *Surgery 138*, 464–473.
15. Vuong, T.D., Kimpe, S., Roos, R., Rabelink, T.J., Koomans, H.A., and Joles, J.A. (2001) Albumin Restores Lysophosphatidylcholine-Induced Inhibition of Vasodilation in Rat Aorta, *Kidney Int. 60*, 1088–1096.
16. Joles, J.A., Willekes-Koolschijn, N., and Koomans, H.A. (1997) Hypoalbuminemia Causes High Blood Viscosity by Increasing Red Cell Lysophosphatidylcholine, *Kidney Int. 52*, 761–770.
17. Thie, G.M.D., Schneijderberg, V.L.M., and Van Tol, A. (1986) Identification and Characterization of Rat Serum Lipoprotein Subclasses. Isolation by Chromatography on Agarose Columns and Sequential Immunoprecipitation, *J. Lipid Res. 27*, 1035–1043.
18. Havel, R.J., Eder, H.A., and Bragdon, J.H. (1955) The Distribution and Chemical Composition of Ultracentrifugally Separated Lipoproteins in Human Serum, *J. Clin. Invest. 34,* 1345–1353.
19. Redgrave, T.G., Roberts, D.C.K., and West, C.E. (1975) Separation of Plasma Lipoproteins by Density-Gradient Ultracentrifugation, *Anal. Biochem. 65*, 42–49.
20. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951) Protein Measurement with the Folin Phenol Reagent, *J. Biol. Chem. 193*, 265–275.
21. Marinetti, G.V. (1962) Chromatographic Separation, Identification, and Analysis of Phosphatides, *J. Lipid Res. 3*, 1–20.
22. Bartlett, G.R. (1959) Phosphorus Assay in Column Chromatography, *J. Biol. Chem. 234,* 466–468.
23. Albers, J.J., and Segrest J.P. (1986) *Methods in Enzymology*: *Plasma Lipoproteins,* in *Isolation, Characterization, and Assay of Plasma Lipid Transfer Proteins,* Tollefson, J.H., and Albers, J.J., eds., Vol. 129, pp. 797–816, Academic Press, New York.
24. Nilsson, A., Chen, Q., and Dahlman, E. (1994) Metabolism of

Chylomicron Phosphatidylinositol in the Rat: Fate *in vivo* and Hydrolysis with Lipoprotein Lipase and Hepatic Lipase *in vitro*, *J. Lipid Res. 35*, 2151–2160.
25. Albers, J.J., and Segrest, J.P. (1986) Methods in Enzymology: Plasma Lipoprotein, in *Preparation, Characterization, and Measurement of Hepatic Lipase,* Ehnholm, C., and Kuusi, T., eds., Vol. 129, pp. 717–738, Academic Press, New York.
26. McFarlane, A.S. (1958) Efficient Trace Labelling of Proteins with Iodine, *Nature 182*, 53.
27. Lewis, G.F., Lamarche, B., Uffelman, K.D., Heatherington, A.C., Honig, M.A., Szeto, L.W., and Barrett, P.H.R. (1997) Clearance of Postprandial and Lipolytically Modified Human HDL in Rabbits and Rats, *J. Lipid Res. 38*, 1771–1781.
28. Marchese, S.R.M., Mesquita, C.H., and Cunha, I.I.L. (1998) Compartmental Analysis (AnaComp) Program: Application to Calculate ^{137}Cs Transfer Rates in Marine Organisms and Dose on the Man, *J. Radioanal. Nucl. Ch. 232*, 233–236.
29. Passarelli, M., Shimabukuro, A.F.M., Catanozi, S., Nakandakare, E.R., Rocha, J.C., Carrilho, A.J.F., and Quintão, E.C.R. (2000) Diminished Rate of Mouse Peritoneal Macrophage Cholesterol Efflux Is Not Related to the Degree of HDL Glycation in Diabetes Mellitus, *Clin. Chim. Acta 301*, 119–134.
30. Harada, L.M., Carvalho, M.D.T., Passarelli, M., and Quintão, E.C.R. (1998) Lipoprotein Desialylation Simultaneously Enhances the Cell Cholesterol Uptake and Impairs the Reverse Cholesterol Transport System: *in vitro* Evidences Utilizing Neuraminidase-Treated Lipoproteins and Mouse Peritoneal Macrophages, *Atherosclerosis 139*, 65–75.
31. Roda, A., Cappelleri, G., Aldini, R., Roda, E., and Barbara, L. (1982) Quantitative Aspects of the Interaction of Bile Acids with Human Serum Albumin, *J. Lipid Res. 23*, 490–495.
32. Rothschild, M.A., Oratz, M., and Schreiber, S.S. (1972) Albumin Synthesis, *New Engl. J. Med. 286*, 748–757.
33. Rudman, D., and Kendall, F.E. (1957) Bile Acid Content of Human Serum: The Binding of Cholanic Acids by Human Plasma Proteins, *J. Clin. Invest. 36*, 538–542.
34. Kaysen, G.A., Hoye, E., Jones, H.Jr., Van Tol, A., and Joles, J.A. (1995) Effect of Oncotic Pressure on Apolipoprotein A-I Metabolism in the Rat, *Am. J. Kidney Dis. 26*, 178–186.
35. Thuren, T., Weisgraber, K.H., Sisson, P., and Waite, M. (1992) Role of Apolipoprotein E in Hepatic Lipase Catalyzed Hydrolysis of Phospholipid in High-Density Lipoproteins, *Biochemistry 31*, 2332–2338.
36. Breckenridge, W.C., Little, J.A., Alaupovic, P., Wang, C.S., Kuksis, A., Kakis, G., Lindgren, F., and Gardiner, G. (1982) Lipoprotein Abnormalities Associated with a Familial Deficiency of Hepatic Lipase, *Atherosclerosis 45*, 161–179.
37. Shin, Y., Vaziri, N.D., Willekes N., Kim C.H., and Joles J.A. (2005) Effects of Gender on Hepatic HMG-CoA Redutase, Cholesterol 7α-Hydroxylase, and LDL Receptor in Hereditary Analbuminemia, *Am. J. Physiol. 289,* E993–E998.
38. Liang, K., and Vaziri, N.D. (2003) HMG-CoA Redutase, Cholesterol 7 Alpha- Hydroxylase, LCAT, ACAT, LDL Receptor, and SRB-1 in Hereditary Analbuminemia. *Kidney Int. 64,* 192–198.
39. Moestrup, S.K., and Nielsen L.B. (2005) The Role of the Kidney in Lipid Metabolism, *Curr. Opin. Lipidol. 16,* 301–306.
40. Thuren, T., Sisson, P., and Waite, M. (1990) Hydrolysis of Lipid Mixtures by Rat Hepatic Lipase, *Biochim. Biophys. Acta 1046*, 178–184.
41. Bensadoun, A., and Berryman D.E. (1996) Genetics and Molecular Biology of Hepatic Lipase, *Curr. Opin. Lipidol. 7*, 77–81.
42. Hime, N.J., Barter, P.J., and Rye, K.A. (2001) Evidence That Apolipoprotein A-I Facilitates Hepatic Lipase-Mediated Phospholipid Hydrolysis in Reconstituted HDL Containing Apolipoprotein A-II, *Biochemistry 40,* 5496–5505.
43. Tall, A.R. (1990) Plasma High-Density Lipoproteins. Metabolism and Relationship to Atherogenesis, *J. Clin. Invest. 86*, 379–384.
44. Thuren, T., Sisson, P., and Waite, M. (1991) Activation of Hepatic Lipase Catalyzed Phosphatidylcholine Hydrolysis by Apolipoprotein E, *Biochim. Biophys. Acta 1083*, 217–220.
45. Thuren, T. (2000) Hepatic Lipase and HDL Metabolism, *Curr. Opin. Lipidol. 11*, 277–283.
46. Portman, O.W., and Alexander, M. (1976) Influence of Lysophosphatidylcholine on the Metabolism of Plasma Lipoproteins, *Biochim. Biophys. Acta 450*, 322–334.
47. Windler, E.E.T., Preyer, S., and Greten, H. (1986) Influence of Lysophosphatidylcholine on the C-Apolipoprotein Content of Rat and Human Triglyceride-Rich Lipoproteins During Triglyceride Hydrolysis, *J. Clin. Invest. 78*, 658–665.
48. Von Eckardstein, A., Nofer, J.R., and Assmann, G. (2001) High-Density Lipoproteins and Arteriosclerosis. Role of Cholesterol Efflux and Reverse Cholesterol Transport, *Arterioscler. Thromb. Vasc. Biol. 21*, 13–27.
49. Wang, M., and Briggs, M.R. (2004) HDL: the Metabolism, Function, and Therapeutic Importance, *Chem. Rev. 104,* 119–137.
50. Eisenberg, S. (1984) High-Density Lipoprotein Metabolism, *J. Lipid. Res. 25*, 1017–1058.
51. Fournier, N., Moya, M.L., Burkey, B.F., Swaney, J.B., Paterniti, J., Jr., Moatti, N., Atger, V., and Rothblat, G.H. (1996) Role of HDL Phospholipid in Efflux of Cell Cholesterol to Whole Serum: Studies with Human apoA-I Transgenic Rats, *J. Lipid Res. 37,* 1704–1711.
52. Fournier, N., Paul, J.L., Atger, V., Cogny, A., Soni, T., Moya, M.L., Rothblat, G., and Moatti, N. (1997) HDL Phospholipid Content and Composition as a Major Factor Determining Cholesterol Efflux Capacity from FU5AH Cells to Human Serum, *Arterioscler. Thromb. Vasc. Biol. 17,* 2685–2691.
53. Yancey, P.G., Moya, M.L., Swarnakar, S., Monzo, P., Klein, S.M., Connelly, M.A., Johnson, W.J., and Williams, D.L. (2000) High Density Lipoprotein Phospholipid Composition Is a Major Determinant of the Bi-directional Flux and Net Movement of Cellular Free Cholesterol Mediated by Scavenger Receptor BI, *J. Biol. Chem. 275*, 36596–36604.
54. Ishimoto, Y., Yamada, K., Yamamoto, S., Ono, T., Notoya, M., and Hanasaki, K. (2003) Group V and X Secretory Phospholipase A_2s-Induced Modification of High-Density Lipoprotein Linked to the Reduction of Its Antiatherogenic Functions, *Biochim. Biophys. Acta 1642,* 129–138.

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