Hypercatabolism of Lipoprotein-Free Apolipoprotein A-I in HDL-Deficient Mutant Chickens

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Abstract The Wisconsin Hypoalpha Mutant (WHAM) chicken has a sex-linked mutation associated with a 90% reduction in high-density lipoprotein (HDL) cholesterol and apolipoprotein A-I (apoA-I). In the present studies, we did not detect a defect in apoA-I synthesis or secretion in liver or intestine. We tested the hypothesis that apoA-I is not binding properly to lipoprotein particles and is undergoing hypercatabolism. We therefore studied the in vivo turnover of lipid-free [125I]-apoA-I. Its turnover was fourfold faster in WHAM chickens than in normal chickens. The [125I]-apoA-I equilibrated more slowly with HDL in the WHAM chickens, and these animals had a much larger steady-state pool of lipid-free apoA-I than did control chickens. To determine the tissue sites of degradation of apoA-I, the tissue distribution of [125I]-tyramine cellobiose apoA-I was assessed. The liver and kidneys were the major sites of apoA-I degradation, but in the WHAM chickens, the kidney made a twofold larger contribution to apoA-I degradation than in normal chickens. Total plasma phospholipid levels are reduced by 44% to 78% in the WHAM chickens. A phospholipid deficit might explain the elevated lipid-free apoA-I pool and, secondarily, the HDL deficiency of the WHAM chickens. (Arterioscler Thromb. 1994;14:2053-2059.)

Key Words • HDL deficiency • apolipoprotein A-I • phospholipid deficiency

High-density lipoprotein (HDL) deficiency is considered a risk factor for coronary heart disease.1 Heritable HDL deficiencies are relatively common in the human population, with a frequency of approximately 3%.2 However, in most cases, the gene(s) responsible for the HDL deficit are unknown.

An obvious candidate gene for HDL deficiency is the one encoding the principal HDL protein, apolipoprotein A-I (apoA-I). Several apoA-I structural gene mutations have been shown to cause drastic reductions in HDL.1-3 Nevertheless, HDL deficiency is seldom attributable to mutations in the apoA-I structural gene. HDL deficiency may result from reduced HDL production, hypercatabolism,4 or a combination of these two factors.4-5 HDL production and turnover do not always reflect the synthesis rate or the physical properties of the apoA-I protein. Rather, the lipid components of HDL particles are intimately linked with the fate of the apoA-I protein. For example, apoA-I turns over much more rapidly when associated with triglyceride-enriched HDL particles than with cholesterol ester-enriched HDL particles. Through the action of lipoprotein lipase or hepatic lipase, triglyceride-enriched HDLs are reduced in size and rapidly cleared from the circulation.6

Because all of the components of HDL are exchangeable with other lipoprotein particles, it has been difficult to obtain a definitive model of HDL biogenesis and metabolism. ApoA-I is thought to associate with phospholipids and unesterified cholesterol before and/or after secretion. In the bloodstream and extravascular spaces, the phospholipid and cholesterol moieties are derived in part from the surface of triglyceride-rich lipoproteins, which is thought to "pinch off" on the lipase-catalyzed depletion of the triglyceride core.6 Nascent HDL particles are discoidal but rapidly acquire a core of nonpolar lipids to become spherical. Through the action of lecithin:cholesterol acyltransferase, the free cholesterol on the HDL particle is esterified to cholesterol ester and partitions into the core of the spherical particle.7 Cholesterol ester transfer protein (CETP) then catalyzes the exchange of HDL cholesterol esters with triglycerides from very-low-density lipoprotein (VLDL).7 The triglycerides can undergo lipolysis, resulting in smaller HDL particles, which are cleared more rapidly from the circulation than cholesterol ester–enriched HDLs.8 The CETP-mediated formation of small HDL particles helps to explain why CETP activity inversely correlates with HDL levels.9

Although lecithin:cholesterol acyltransferase, CETP, and triglyceride lipases influence HDL metabolism, variation in the genes encoding these enzymes accounts for only a small fraction of HDL abnormalities. It is therefore important to identify additional major genes that account for the large genetic contribution to HDL levels.

The Wisconsin Hypoalpha Mutant (WHAM) chicken is the only known naturally occurring animal model with a spontaneous HDL deficiency due to a single-gene mutation.10-13 Because the mutation is sex-linked and all the known genes involved in lipid and lipoprotein metabolism are autosomal, this animal model provides a
unique opportunity to discover a new gene involved in the determination of HDL levels.

The WHAM chickens have a 90% reduction in HDL and apoA-I levels. In a prior study, we measured the in vivo turnover of HDL associated 125I-apoA-I in mutant animals; we found a twofold increase in the catabolic rate and estimated an 80% reduction in the HDL production rate. We therefore hypothesized that the major defect in the mutant chickens is defective apoA-I synthesis or secretion.

In the present work, experiments in cultured hepatocytes and intestinal loops did not detect an apoA-I synthetic defect. Rather, an in vivo assessment of the metabolism of lipid-free apoA-I revealed a profound impairment in the ability of mutant chickens to maintain apoA-I in HDL particles, resulting in rapid kidney-mediated apoA-I catabolism.

Methods

Animals

Chickens were obtained from a flock maintained by the University of Wisconsin-Madison Poultry Science Department. The chickens were fed ad libitum a standard University of Wisconsin poultry diet (0.01% cholesterol and 4.3% fat).

Southern Blots

Genomic DNA was isolated from the red blood cells of several chicken strains as described. Restriction fragments were obtained by digesting DNA with the restriction enzyme SacI (Promega). Digested DNA was electrophoresed through a 1.0% agarose gel and transferred to nitro membranes. The membranes were prehybridized for 1 hour using hybridization buffer (1.45% NaCl, 0.02 mol/L Na2HPO4, pH 7.0, 0.5% SDS, 25 mmol/L EDTA, 10% polyethylene glycol 68000, 0.5% dry milk, 200 μg/mL sheared salmon sperm DNA) and then hybridized overnight with 106 cpm 32P-labeled probes in hybridization solution at 65°C. The probes were produced by randomly priming a 650-bp EcoRI fragment of the chicken cDNA (kindly provided by A.J. Lusis) as described. Membranes were washed under high stringency conditions and exposed to film.

Hepatocytes

Cells were cultured using a method modified from Griendinger. Livers from 10- to 14-day-old chickens were perfused briefly with 50 to 100 mL of Hanks' balanced salts (Ca2+-free) buffer followed by 50 mL of Dulbecco's modified Eagle medium (DMEM) containing 5.0 mg collagenase (Promega). The liver was removed, minced, and shaken in a collagenase solution. The isolated cells were combined with 25 mL of chilled DMEM (GIBCO) plus 10% fetal bovine serum (FBS) and centrifuged at 50g for 5 minutes at 4°C. The cells were washed in DMEM-FBS and plated in 60-mm dishes. The plating medium consisted of DMEM, 10% FBS, 1 μg/mL insulin, and antibiotics. Two days after plating, the medium was removed and replaced with 2.0 mL of serum-free DMEM containing 100 μCi [35S]methionine (New England Nuclear) per dish.

ApoA-I Synthesis

Incubation with [35S]methionine (100 μCi per dish) was performed in serum-free DMEM without methionine (GIBCO). After the incubation period, the medium was removed and combined with 2x lysis buffer (0.04 mol/L Na2HPO4/H3PO4, 0.3 mol/L NaCl, 10 mmol/L EDTA, 2% Triton X-100, 200 μg/mL phenylmethylsulfonyl fluoride [PMSF], 4 mmol/L benzamidin, pH 7.2) to give a final concentration of 1x buffer. The cells were rinsed three times with cold PBS; then 2.0 mL of 1x lysis buffer was added. Cells were harvested, sonicated, and briefly centrifuged to remove cell debris. An aliquot of the cellular homogenate was precipitated with trichloroacetic acid (TCA) to determine total cellular protein synthesis. ApoA-I was immunoprecipitated from either the cells or media: 4 μL of rabbit anti-chicken apoA-I antibody was incubated with the sample overnight at 4°C. Protein A-Sepharose (1:1, 40 μL total volume, Sigma) in dilution buffer was added to each immunoprecipitation reaction, and samples were rotated 2 hours at room temperature. The beads were centrifuged, washed twice in wash buffer (0.1% Triton X-100, 0.02% SDS, 150 mmol/L NaCl, 5 mmol/L Tris pH 7.5, 5 mmol/L EDTA, 2 mol/L benzamidin), resuspended in electrophoresis sample buffer, and subjected to electrophoresis on a 12% denaturing polyacrylamide gel. The labeled apoA-I protein band was excised from the gel, and the polyacrylamide was dissolved in NH4OH:H2O2 (1:20) overnight. The radioactivity was measured in a Packard 1900CA liquid scintillation counter. The slopes of total protein synthesis (TCA-precipitable counts per minute per microgram of protein versus time) and apoA-I synthesis (apoA-I counts per minute per microgram of protein versus time) were measured. Both were linear over the time course. In Fig 2, the data are normalized to the metabolic activity of the different cell preparations as reflected in the rate of total protein synthesis.

Intestinal ApoA-I Secretion

Intestinal apoA-I secretion was quantified as described by Davidson and Glickman (in McGibbon). Chickens were anesthetized (0.25 mg/kg xylazine hydrochloride and 0.05 mg/kg ketamine hydrochloride), and 5 cm of jejunum was ligated. [35S]Methionine (100 μCi total volume) in 500 μL phosphate-buffered saline (PBS) was injected into the lumen, and the intestine was returned to the body cavity. After 9 minutes, the intestinal section was removed, sliced open, and rinsed with 20 to 30 mL of wash buffer (PBS plus 20 mmol/L methionine and 1.0 mmol/L DTT, pH 7.2). The mucosal cells were scraped into a tube containing fresh wash buffer. The cells were pelleted, resuspended in lysis buffer, homogenized using a motor-driven polytetrafluoroethylene (Teflon) pestle, and centrifuged briefly to remove cellular debris. Radioactivity incorporated into apoA-I was measured by immunoprecipitation and protein synthesis by TCA precipitation. On occasion, more than two rounds of immunoprecipitation were required to recover all of the apoA-I radioactivity.

125I-ApoA-I Turnover Studies

ApoA-I was purified from control chickens and radiolabeled as described previously. Specific radioactivity averaged approximately 200 cpm/μg apoA-I protein. ApoA-I (10 to 20 μCi) was injected into control and mutant hens, and plasma samples were collected. Radioactivity was measured using a Packard Multi-Prius 1 gamma counter. Fractional clearance rates were determined by graviometrically measuring the area under the radioactivity disappearance curves.

125I-Tyramine Cellobiose/ApoA-I Turnover Studies

Iodination of tyramine cellobiose and association with apoA-I was performed as previously described. The specific activity range was 200 to 450 cpm/μg. Essentially all of the radioactivity was insoluble in 10% TCA. Approximately 10 μCi apoA-I was injected into each chicken; plasma samples were collected at various times. An aliquot of each plasma sample was TCA-precipitated, and radioactivity in the pellet was determined. When approximately 20% to 30% of the injected dose remained (6 to 8 hours for the mutant and 24 hours for the controls), the animals were anesthetized and injected with 5000 U heparin (Sigma), and approximately 200 mL PBS and 1.0 mmol/L EDTA were perfused through the whole body through the left ventricle. Organs were removed, weighed, and a sample of tissue homogenized in PBS containing 1.0 mmol/L PMSF and 1.0 mmol/L benzamidin. Radio-
activity soluble in 10% TCA provided a quantitation of trapped $^{125}$I-labeled degradation products.$^{17}$

To determine the amount of labeled apoA-I that was associating with HDL, 400 μL of plasma was obtained approximately 10 minutes after $^{125}$I-tyramine cellobiose apoA-I injection and at the end of the time course. The density of the plasma sample was adjusted to 1.21 g/mL with NaBr, layered beneath 3.6 mL of 1.21 g/mL NaBr, and centrifuged for 9 hours at 208 000g using a TLA-100.3 rotor in a Beckman TL-100 centrifuge. The samples were fractionated into 10 aliquots, and the amount of radioactivity in each aliquot was determined.

**Nondenaturing Polyacrylamide Gel Electrophoresis**

Plasma samples from control and mutant male chickens were electrophoresed through a 4% to 20% nondenaturing polyacrylamide gel. The protein was transferred onto nitrocellulose membranes and probed with an anti-chicken apoA-I antibody. The bands corresponding to apoA-I were detected using enhanced chemiluminescence according to the manufacturer’s procedure (Amersham).

**Plasma Phospholipid Determinations**

Lipids were extracted from plasma samples by the method of Bligh and Dyer.$^{18}$ The samples were corrected for extraction efficiency based on the recovery of $[^{14}]$C phosphatidylcholine. Lipid phosphate concentrations were measured by the Bartlett method.$^{19}$

**Results**

**Chromosomal Location of Chicken ApoA-I Gene**

The mutation in the WHAM chickens is linked to the Z (sex) chromosome.$^{12-14}$ To ascertain whether the apoA-I structural gene is a candidate for the WHAM mutation, we followed the segregation of apoA-I polymorphic markers. Southern blots of Sac I-digested genomic DNA from New Hampshire chickens that were probed for apoA-I displayed two bands corresponding to 1200 and 560 bp. By contrast, similarly digested White Leghorn chicken DNA produced bands at 1080 and 560 bp. The two strains were crossed, and genomic DNA from the progeny was analyzed. DNA from both the male and female progeny produced all three bands (Fig 1A). If the gene was sex-linked, the male offspring produced all three bands corresponding to apoA-I were detected using enhanced chemiluminescence (Amersham).

**ApoA-I mRNA Abundance and Translation**

Previous plasma turnover studies led us to hypothesize that defective HDL–apoA-I production makes a larger contribution to the HDL deficiency than does hypercatabolism.$^{11}$ We determined whether the HDL deficiency results from reduced apoA-I mRNA abundance. There was no reduction in apoA-I abundance in mutant liver or intestine mRNA samples; in fact, the apoA-I mRNA was twofold more abundant in the mutant liver samples (data not shown). In vitro translations were performed to determine if the apoA-I mRNA from the mutant is translationally competent. Liver and intestine mRNA were translated in vitro using the rabbit reticulocyte lysate system, and the apoA-I protein product was immunoprecipitated and detected by fluorography. The products resulting from normal and mutant tissues were of identical molecular weight and approximately equal abundance (data not shown). Therefore, there is no intrinsic defect in the apoA-I mRNA that would inhibit translation.

**Intracellular ApoA-I Synthesis**

Although the WHAM apoA-I mRNA is translationally competent, there could be trans-acting factors that inhibit apoA-I synthesis in vivo. To measure apoA-I synthesis in intact tissue, a short segment of jejunum was ligated, and $[^{35}]$S-methionine was introduced into the lumen. After 9 minutes, the segment was removed, the mucosal cells were scraped and homogenized, and $[^{35}]$S-labeled apoA-I was measured. After correction for total protein synthesis (determined by TCA precipitation), no differences in apoA-I synthesis were observed between animals (total protein synthesis: controls, 2.2±0.8% [n=3]; mutants, 2.3±0.3% [n=4]). Therefore, there is not an in vivo defect in apoA-I protein synthesis in WHAM chicken intestine.

**ApoA-I Synthesis and Secretion From Hepatocytes**

Because apoA-I mRNA is not deficient and is translationally competent in the mutant chickens, we measured the relative apoA-I protein synthesis and secretion rates in cultured control and mutant hepatocytes. The cells were labeled with $[^{35}]$S-methionine. ApoA-I was measured by immunoprecipitation from either the medium or cellular homogenate and normalized to total protein synthesis. The rates of apoA-I synthesis and secretion were indistinguishable in control and mutant chickens (Fig 2). Similar results were obtained when the hepatocytes were cultured in media containing 10% FBS, normal chicken serum, or WHAM chicken serum.
been shown to be preferentially catabolized by the liver. If the hypercatabolism of apoA-I in the chicken suggested that a lipid-poor pool of apoA-I was the kidney while HDL-associated apoA-I is catabolized in liver and the kidneys were the predominant and approximately coequal sites of apoA-I catabolism (Fig 4). In stark contrast, in the WHAM chicken the kidney made a disproportionate contribution to its catabolism. We measured the contributions of the various organs to apoA-I catabolism by quantifying the distribution of apoA-I labeled with 125I-tyramine cellobiose. Tyramine cellobiose is a nonbiodegradable tracer that becomes trapped on uptake into cells, thus providing an accurate estimate of the contribution of each organ to catabolism. The 125I-tyramine cellobiose apoA-I tracer was intravenously injected into control and mutant chickens. The plasma disappearance curves of 125I-tyramine cellobiose apoA-I were indistinguishable from those of directly iodinated apoA-I (Fig 4, inset). When approximately 20% to 30% of the initial plasma radioactivity remained (20 to 24 hours for the control, 5 to 7 hours for the mutant), the animals were killed, and organ uptake and degradation of the tracer were measured.

The tissue distribution of 125I radioactivity in the normal chickens was much like that previously reported after similar experiments were performed in rats; the liver and the kidneys were the predominant and approximately coequal sites of apoA-I catabolism (Fig 4). In stark contrast, in the WHAM chicken the kidney made a threefold higher contribution to apoA-I catabolism than did the liver (Fig 5).

**ApoA-I Association With Lipoproteins**

Enhanced kidney apoA-I catabolism is consistent with a larger pool of lipid-free apoA-I circulating in the plasma. Mutant female chickens without first reassociating the apolipoprotein with a lipoprotein particle. In control chickens, the apoA-I disappearance rate was similar to that of apoA-I associated with HDL. In striking contrast, the mutant chickens cleared the apoA-I at a rate approximately fourfold faster than HDL-associated apoA-I (Fig 3, Table 1).

**Sites of ApoA-I Degradation**

The rapid catabolism of 125I-apoA-I in the mutant chickens suggested that a lipid-poor pool of apoA-I was hypercatabolized. Lipid-free apoA-I has previously been shown to be preferentially catabolized by the kidney while HDL-associated apoA-I is catabolized in the liver. If the hypercatabolism of apoA-I in the liver was due to its inability to associate with HDL particles, the kidneys would be predicted to make a disproportionate contribution to its catabolism. We measured the contributions of the various organs to apoA-I catabolism by quantifying the distribution of apoA-I labeled with 125I-tyramine cellobiose. Tyramine cellobiose is a nonbiodegradable tracer that becomes trapped on uptake into cells, thus providing an accurate estimate of the contribution of each organ to catabolism. The 125I-tyramine cellobiose apoA-I tracer was intravenously injected into control and mutant chickens. The plasma disappearance curves of 125I-tyramine cellobiose apoA-I were indistinguishable from those of directly iodinated apoA-I (Fig 4, inset). When approximately 20% to 30% of the initial plasma radioactivity remained (20 to 24 hours for the control, 5 to 7 hours for the mutant), the animals were killed, and organ uptake and degradation of the tracer were measured.

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bloodstream of the mutant chickens and would predict a larger proportion of the $^{125}$I radioactivity that would be in the lipoprotein-free fraction (the $d>1.21$ g/mL fraction) of plasma after ultracentrifugation. When the ultracentrifugal separation was carried out immediately after injection, essentially all of the labeled apoA-I was in the $d<1.21$ g/mL fraction in control chicken plasma (Fig 6). In contrast, in mutant chicken plasma, only 50% was associated with lipoproteins (Fig 6). By the end of the experiment, essentially all of the tracer was lipoprotein-bound in both control and mutant plasma, reflecting the preferential clearance of lipid-free apoA-I in the mutant animals.

Because ultracentrifugation in high salt concentrations can cause the dissociation of apolipoproteins from lipoproteins, the steady-state distribution of plasma apoA-I was assessed by nondenaturing gel electrophoresis. When probed on an immunoblot, a fraction of the apoA-I in the mutant chicken plasma migrated as lipid-free apoA-I, while essentially all of the apoA-I in the control chicken plasma migrated with HDL particles (Fig 7).

**Plasma Phospholipid Concentrations**

The stabilization of apoA-I in the bloodstream involves apoA-I binding to phospholipids at the surface of lipoprotein particles. Thus, one possible explanation for an increased pool of lipoprotein-free apoA-I in the WHAM chickens is a phospholipid deficit. The total plasma phospholipid concentrations were measured in male and female chickens, including females before and after sexual maturity, when hens produce large quantities of VLDL. In all three groups, there was a large phospholipid deficit. The phospholipid reductions ranged from 44% to 78% (Table 2).

**Discussion**

In this study, we have shown that the heritable HDL deficiency of the WHAM chickens is not caused by a mutation in the apoA-I structural gene; the HDL deficiency mutation is sex-linked but markers within the apoA-I gene segregate like autosomal genes. The WHAM mutation therefore is a model for the vast majority of human HDL deficiency syndromes in not being caused by an apoA-I mutation. Because the mutation is sex-linked, elucidation of the mutant gene.

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**Fig 4** Graph shows organ contribution to apolipoprotein A-I (apoA-I) catabolism in control hens. The data represent the means and SDs of three control hens injected with $^{125}$I-tyramine cellobiose (TC)-apoA-I. The animals were killed when 20% to 30% of the initial dose remained in the bloodstream. The inset shows a representative plasma disappearance curve carried out to the time of death.

**Fig 5** Graph shows organ contribution to apolipoprotein A-I (apoA-I) catabolism in mutant hens. The data represent the means and SDs of three mutant hens injected with $^{125}$I-tyramine cellobiose (TC)-apoA-I. The animals were killed when 20% to 30% of the initial dose remained in the bloodstream. The inset shows a representative plasma disappearance curve carried out to the time of death.

**Fig 6** Graph shows ultracentrifugal fractionation of plasma after injection with $^{125}$I-tyramine cellobiose-apoA-I. Plasma samples were isolated immediately after injection of the tracer and at the end of the experiment, when approximately 20% to 30% of initial plasma radioactivity remained. The densities of the samples were adjusted to 1.21 g/mL, and the samples were centrifuged. After centrifugation, each sample was fractionated into 10 tubes, and radioactivity in each tube was determined. Left, density distribution of the tracer immediately after injection. Right, density distribution of the tracer at the end of the time course.

**Fig 7** Immunoblot of nondenaturing gradient gel of control and mutant plasma. Plasma containing approximately equal amounts of apolipoprotein A-I (apoA-I) was subjected to electrophoresis through a 4% to 20% nondenaturing polyacrylamide gel, transferred to nitrocellulose, probed with an anti-apoA-I antibody, and detected using enhanced chemiluminescence. The figure shows the mobilities of samples from four control and three mutant chickens. HDL indicates high-density lipoprotein.
may shed light on gender differences in human HDL metabolism.

The genetic defect of the WHAM chickens does not cause abnormalities in apoA-I synthesis or secretion. Rather, the defect affects the stability of the apoA-I in the circulation; apoA-I disappeared from the bloodstream in WHAM chickens more than four times faster than in normal chickens.

The hypercatabolism of apoA-I detected in this study with a lipid-free 125I-apoA-I tracer contrasts with earlier turnover studies, which used 125I-HDL or 125I-apoA-I that was reassocaited with HDL before intravenous injection. These experiments detected much less hypercatabolism. The results underscore the methodological difficulties in HDL tracer experiments. Because the apoA-I pool is not metabolically homogeneous, different tracers selectively trace different apoA-I pools. The use of intact 125I-HDL or preassociating 125I-apoA-I with HDL before injection selectively traces the less exchangeable apoA-I pools. If the association of apoA-I with HDL particles is a critical step affecting the stability of apoA-I in the circulation, then this step cannot be properly traced with 125I-HDL. This helps to explain why in our previous experiments we apparently underestimated HDL catabolism.

Our turnover studies with lipid-free 125I-apoA-I as a tracer clearly demonstrated that the ability of apoA-I to associate with HDL particles is critical to its stability in the bloodstream. The detection of a larger pool of lipid-free apoA-I is consistent with an impairment in the ability of mutant chickens to carry apoA-I in HDL particles. Since virtually all of the increase in apoA-I catabolism occurred in the kidneys, the data also support the conclusion that the affinity of apoA-I for HDL particles largely determines its catabolic rate and its tissue site of degradation. In a similar fashion, Horowitz et al found that HDL from HDL-deficient patients had apoA-I that was more loosely associated with lipoprotein particles. Perfusion of HDL from these patients resulted in greater apoA-I clearance by rabbit kidneys.

HDL represents a "snapshot" of lipids and apolipoproteins that continually transfer between lipoprotein particles. Within the HDL particle, there appears to be both a rapidly exchangeable and a much more slowly exchangeable pool of apoA-I.21 When Glass et al traced the protein and cholesterol ester moieties of HDL, they detected more protein catabolism in the kidney and more cholesterol ester uptake in steroidogenic tissues. Because Glass et al found 36-fold more apoA-I than cholesterol ester uptake into the kidney, it seemed plausible that a lipid-free apoA-I pool was selectively targeted to the kidney. This paradigm is now strongly supported by the demonstration that a nonexchangeable apoA-I analogue is not targeted to the kidneys.24

Variability in the affinity of apoA-I for HDL is principally a consequence of HDL lipid composition. Even a reduction in the size of HDL particles, such as occurs after the action of hepatic lipase22,23 or lipoprotein lipase,8 leads to increased apoA-I catabolism. The increased HDL accompanying CETP-deficiency states demonstrates how preservation of the cholesterol ester core stabilizes HDL particles.9

At present, it is not known what accounts for the impaired association of apoA-I with lipids in the WHAM chickens. Other studies have ruled out the possibility of defects in several enzymes affecting HDL. Mutations affecting CETP activity or hepatic lipase have been shown to result in changes in HDL levels or pool sizes of lipid-free apoA-I. However, we found no differences in CETP activity (F. Poernama, unpublished observations, 1991) or hepatic lipase activity (H. Wong, unpublished observations, 1991) between control and mutant chickens. In addition, deficiencies in lipoprotein lipase have been shown to result in hypertriglyceridemia, triglyceride-enriched HDL, and reduced plasma HDL levels due to hypercatabolism.26-30 Although the mutant chickens do have triglyceride-enriched HDL,13 they are not hypertriglyceridemic, suggesting that lipoprotein lipase is not defective in these animals.

Apart from mutations in specific enzymes affecting HDL, the WHAM chickens may have a defect in a lipid component of HDL. Phospholipids are known to play a role in determining the stability of apoA-I on HDL particles. Reduced levels of phospholipids or alterations in phospholipid structure may be expected to affect HDL production and stability. The acyl chain composition of phosphatidylcholine affects the apoA-I binding capacity of the phospholipid. For example, enrichment of the sn-2 position with ω-3 fatty acids results in reduced apoA-I binding affinity.31

The WHAM chickens were originally identified because they had an abnormally severe response to a choline-deficient diet (Poernama and Cook, unpublished observations, 1988). Plasma phospholipid is reduced by 40% to 80% in the mutant chickens. The phospholipid deficit could be a consequence of abnormal choline transport or metabolism or of a defect in phospholipid synthesis or intracellular phospholipid trafficking. Experiments to test these hypotheses in the WHAM chickens are currently being pursued.

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References

4. Mosiander TA, Long MD, Forte TM, Nichols AV, Gong EL, Blanche PJ, Krauss RM. Dissociation of high density lipoprotein precursors from apolipoprotein B-containing lipoproteins in the
Hypercatabolism of lipoprotein-free apolipoprotein A-I in HDL-deficient mutant chickens.
S A Schreyer, L K Hart and A D Attie

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