Spontaneous high density lipoprotein deficiency syndrome associated with a *Z*-linked mutation in chickens

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Abstract A mutant strain of chicken previously identified by a "recessive white skin" phenotype was found to have a profound deficiency in high density lipoprotein (HDL) and apolipoprotein A-I (apoA-I). ApoA-I levels in the mutant chickens were reduced by >90%. Since HDL is the predominant cholesterol transporter in chickens, the HDL deficiency was associated with a >80% decrease in total plasma cholesterol. The mutation segregates with markers linked to the *Z*-chromosome. The structure of the apoA-I produced by the mutant chickens appeared normal as judged by two-dimensional gel electrophoresis. The genetic and biochemical evidence, therefore, suggests that the mutation is not in the apoA-I structural gene. Turnover studies were performed on labeled HDL or on labeled apoA-I preincubated with HDL prior to intravenous injection. Both types of experiments showed that both defective apoA-I production and hypercatabolism contributed to the HDL deficiency, although defective production made a much larger contribution. —**Poernama, F., S. A. Schreyer, J. J. Bitgood, M. E. Cook, and A. D. Attie.** Spontaneous high density lipoprotein deficiency syndrome associated with a *Z*-linked mutation in chickens. *J. Lipid Res.* 1990. **31:** 955–963.

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High density lipoprotein (HDL) deficiency syndromes in humans are strongly correlated with an increased risk of coronary heart disease (1, 2). Little is known about the mechanisms that regulate plasma HDL levels, and few systems exist in which to study this phenomenon. The understanding of the processes that regulate plasma HDL has been aided by studies of animal models and certain human lipoprotein abnormalities, which indicate that several factors may influence plasma HDL concentrations. Some of these factors involve synthesis of the major protein component of HDL, apolipoprotein A-I (apoA-I). Sorci-Thomas et al. (3) have correlated large differences in plasma HDL between cynomolgus and African Green monkeys with differences in the level of hepatic and intestinal apoA-I mRNA. In addition, increased HDL levels in response to feeding African Green monkeys saturated fat and cholesterol correlates with increased levels of apoA-I mRNA (4). A higher apoA-I synthetic rate has been implicated in the observed human gender differences in plasma apoA-I concentration (5). However, Brinton et al. (6) have concluded from their studies that higher HDL levels in women are attributable to a lower apoA-I clearance rate.

ApoA-I structure is another factor that has been shown to affect HDL concentration. Weisgraber et al. (7) have described an individual in which a $cys_{173} \rightarrow arg$ substitution in apoA-I resulted in hypercatabolism of HDL, thereby lowering the patient's HDL level fivefold. Similarly, a $pro_{165} \rightarrow arg$ substitution has been associated with lower HDL levels in three unrelated families (8).

In the majority of individuals with genetic HDL deficiency syndromes, however, apoA-I has a normal structure (1, 2). Therefore, other genes are likely to be responsible for the low plasma apoA-I levels (and concomitant low HDL levels) characteristic of such heritable disorders as Tangier disease and several types of familial hypoalphalipoproteinemias (2, 9, 10).

Tangier disease is a rare disorder associated with hypocholesterolemia, mild hypertriglyceridemia, and a profound deficiency of HDL cholesterol, apoA-I, and apoA-II

Abbreviations: HDL, high density lipoprotein; VLDL, very low density lipoprotein; LDL, low density lipoprotein; IDL, intermediate density lipoprotein; WHAM, Wisconsin Hypo-Alpha Mutant; apoA-I, apolipoprotein A-I; FCR, fractional clearance rate; SDS, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetic acid.

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(11). While metabolic studies in Tangier disease patients revealed normal rates of apoA-I production, an increase greater than 30-fold in the FCR of apoA-I was detected (9). The causative mutation in Tangier disease does not appear to be in the structural gene for apoA-I (12), thus a separate metabolic defect may be responsible for the observed HDL hypercatabolism characteristic of this syndrome.

In this report we describe a strain of chickens which is the first known animal model for a spontaneous HDL deficiency syndrome. These animals were first identified by McGibbon in 1981 and were distinguished by a *Z*-linked[2] white shank, white beak phenotype, called "recessive white skin" (13). The chickens were from a flock that had been closed since 1948, hence the mutation apparently arose spontaneously after that time. Since 1981, the mutant allele (*y*) was preserved against a random genetic background by outcrossing and backcrossing in succeeding generations, thereby selecting animals with the white shank phenotype. In 1988, Bitgood (14) carried out two three-point test crosses and concluded that the mutation is in a linear order with two other *Z*-linked loci. We demonstrate that this mutation is also associated with a severe decrease in plasma HDL, a decrease that does not appear to involve defective apoA-I structure. We have named the mutant chickens Wisconsin Hypo-Alpha Mutant (WHAM) chickens.

MATERIALS AND METHODS

Animals

Chickens were all obtained from a flock maintained by the University of Wisconsin-Madison Poultry Science Department. The chickens were fed ad libitum a diet containing 0.01% cholesterol and 4.3% fat (University of Wisconsin Poultry Diet). For turnover studies, the females were studied at 6 months of age, prior to the normal onset of hypertriglyceridemia that accompanies ovulation. Males were studied at 7–11 months of age.

Lipoproteins

For the analysis of the distribution of cholesterol, triglyceride, and protein among lipoprotein fractions, VLDL (d < 1.006 g/ml), IDL (d 1.006–1.019 g/ml), and LDL (d 1.019–1.046 g/ml) were isolated by sequential ultracentrifugation in a Beckman Ti 50.2 rotor for 24 h at 40,000 rpm, 15°C. WHAM and control HDLs (d 1.046–1.21 g/ml) were isolated by ultracentrifugation for 48 h under the same conditions previously described (15). For compositional analyses and turnover studies, HDL was subjected to the following purification. To remove contaminating LDL, the densities of the HDL fractions were adjusted to 1.09 g/ml with solid NaBr. One volume of this fraction was layered beneath three volumes of a NaBr solution (d 1.08 g/ml) and centrifuged (Beckman Ti 50.2 rotor, 48 h, 40,000 rpm, 15°C). HDL was collected from the bottom of the tube. The density of the HDL was adjusted to 1.215 g/ml with solid NaBr, and one volume of HDL was layered beneath three volumes of a NaBr solution (d 1.21 g/ml) and centrifuged as described above. Purified HDL was collected from the top of the tube. The average recovery of cholesterol (lipoprotein cholesterol/plasma cholesterol) was 99% for wild type and 96% for mutant chickens. The HDL was then dialyzed against phosphate-buffered saline/EDTA (PBS/EDTA: 0.9% sodium chloride; 8 mM sodium and potassium phosphate, pH 7.4; 1 mM EDTA). Finally, the HDL was passed through a 0.2-μm filter (Gelman Acrodisc) and used within 3 weeks. Total triglyceride and cholesterol were determined by enzymatic assays (Sigma kit #336 and #352, respectively). Quantitation of phospholipids (16), protein (17), and free and esterified cholesterol (18) were also performed. Student's *t*-test (19) was used to compare the plasma lipoprotein levels of control and mutant chickens. When repeated analyses were performed on one dataset, the probability threshold was adjusted using Bonferroni's method (19).

Gel electrophoresis

Lipoproteins were delipidated as described by Utermann et al. (20). One-dimensional discontinuous gel electrophoresis was performed by the method of Laemmli (21) with a 10% polyacrylamide resolving gel. Two-dimensional gel electrophoresis was performed by The Kendrick Laboratory as described (22). The gels were stained with Coomassie blue R-250 (one-dimensional gels) or silver (two-dimensional gels).

ApoA-I purification

The HDL was delipidated 3 times with diethyl ether–ethanol 3:1 (v/v) at 4°C was washed 3 times with cold (4°C) ether (23). The precipitate was dried under nitrogen and dissolved overnight by gentle end-over-end mixing in 5.4 M urea, 0.1 M Tris, 0.01% EDTA, pH 8.0. The dissolved HDL protein was applied to a Sephacryl S-300 column (0.7 × 50 cm) and eluted with the aforementioned Tris/urea buffer. The purity of apoA-I was checked by polyacrylamide gel electrophoresis. The apoA-I-containing fractions were dialyzed overnight against PBS/EDTA and stored at −20°C.

Radioimmunoassay of apoA-I

Rabbit anti-chicken apoA-I antiserum was obtained from rabbits immunized with subcutaneous injections of

[2]The *Z*-chromosome is a sex chromosome in avian species, analogous to the *X*-chromosome in mammals. In avians, unlike mammals, the female is the heterogametic gender. Thus females are *WZ* and males are *ZZ*.

purified chicken apoA-I (1 mg) mixed in Freund's complete adjuvant. After immunization, the rabbit was boosted with 300 μg apoA-I in incomplete Freund's adjuvant. The antiserum reacted only with apoA-I on an immunoblot of total chicken plasma protein. The antiserum was diluted so as to bind 40–60% of radioiodinated apoA-I (~15,000 cpm). All dilutions were made in sodium borate (0.1 M), bovine serum albumin (100 mg/ml), and sodium azide (1 mM), pH 8.5 (dilution buffer). The assay was carried out by mixing 100 μl diluted plasma or 100 μl apoA-I standard with 100 μl of ^{125}I-labeled apoA-I. The volume was brought to 500 μl with Tween-20 (final concentration, 400 mg/ml) (24). The mixture was incubated overnight at 4°C and the bound apo-AI was precipitated by adding 100 μl of a suspension containing protein A-Sepharose-CL-4B (Sigma). The pellet was collected by centrifugation and washed 3 times in dilution buffer containing Tween-20. The radioactivity in the pellet was determined in a Packard Multiprias 3801 gamma counter.

Turnover studies of radiolabeled HDL

Control and WHAM HDLs were iodinated with either ^{131}I or ^{125}I to a specific activity of 250–600 cpm/ng (25). More than 98% of the radioactivity was precipitable by 10% trichloroacetic acid; less than 6% of the radioactivity was lipid-bound.

Female chickens (aged 6 months) were fed ad libitum the standard diet and had free access to water. Approximately 150 μCi each of nonautologous ^{131}I-labeled control and ^{125}I-labeled WHAM HDL were simultaneously injected through the wing vein. Blood (1.0 ml) was collected by venipuncture or heart puncture into tubes containing solid EDTA (final concentration 8 mg/ml). Thirteen samples were collected over 50 h. Plasma samples were precipitated in cold trichloroacetic acid (final concentration: 10% w/v). ^{125}I and ^{131}I radioactivities in the pellets were determined. In studies where apoA-I radioactivity was determined, only ^{125}I-labeled control or WHAM HDL was injected. Blood samples were collected and the radioactivity was measured as described above. An aliquot of plasma was subjected to SDS-PAGE (10% resolving gel); the apoA-I band was excised and radioactivity was determined. Fractional clearance rates were determined by gravimetric measurement of the areas under the plasma disappearance curves (FCR = (area under the plasma disappearance curve)$^{-1}$). Upon completion of the experiment, plasma HDL protein levels were determined. ApoA-I from each HDL sample was quantitated by subjecting aliquots of HDL to SDS gel electrophoresis along with purified apoA-I standards. The gels were scanned on a two-dimensional gel scanner and protein mass was estimated by interpolation of the standard curve. ApoA-I synthetic rates were calculated by multiplying the HDL apoA-I pool size by the HDL apoA-I fractional clearance rate.

Turnover studies of radiolabeled apoA-I

ApoA-I from control and WHAM male chickens was radioiodinated with ^{125}I to a specific activity of 490–930 cpm/ng. More than 95% of the radioactivity was precipitable with trichloroacetic acid (10% w/v). Radiolabeled apoA-I was incubated with HDL for 2–4 h at 37°C as modified from the method of Shepherd et al. (26). Free ^{125}I-labeled apoA-I was separated from HDL-bound ^{125}I-labeled apoA-I by gel permeation chromatography on Bio-Gel A-1.5m (Bio-Rad). Male chickens (33 weeks old) were injected with 5 μCi ^{125}I-labeled apoA-I-labeled HDL (150–400 μg HDL protein). Ten to 13 blood samples were collected over 72 h into tubes containing dry EDTA. Plasma samples were precipitated with trichloroacetic acid (10% w/v) and the radioactivity in the pellets was quantitated. In these experiments, the FCRs were determined by measuring the areas under the plasma disappearance curves using the DEXP program, supplied by W. W. Cleland (27). The program uses the Gauss-Newton curve-fitting procedure. HDL was isolated from a plasma sample from each animal for HDL compositional analysis. Plasma apoA-I pool size was determined by radioimmunoassay, and apoA-I production rates were calculated as described above.

RESULTS

Plasma lipoprotein levels in control and WHAM chickens

When the total plasma cholesterol levels of control and WHAM chickens were compared, WHAM females were found to have a sevenfold reduction relative to control females (**Table 1**). Similarly, homozygous male WHAM

TABLE 1. Lipoprotein cholesterol concentration in control and WHAM female chickens

Fraction	Control (Y/−)	WHAM (y/−)
	mg/dl	
Plasma	100.1 ± 10.8	14.0 ± 3.7[a]
VLDL	0.2 ± 0.1	0.1 ± 0.1
IDL	0.5 ± 0.3	0.3 ± 0.4
LDL	11.4 ± 3.7	4.7 ± 3.6[b]
HDL	82.5 ± 10.6	6.3 ± 2.0[a]

Values are mean ± standard deviation of six animals; animals were 6 weeks old.

[a] $P < 0.00001$.

[b] $P < 0.05$; one-tailed t-test; P values were calculated with correction for Bonferroni's inequality.

TABLE 2. Lipoprotein cholesterol concentration in control and WHAM male chickens

Fraction	Control (*Y/Y*)[a]	WHAM (*Y/y*)[b] Heterozygotes	WHAM (*y/y*)[b] Homozygotes
		mg/dl	
Plasma	73.1 ± 4.9	56.9 ± 2.9[c]	14.2 ± 2.8[d]
VLDL	0.32 ± 0.11	0.17 ± 0.13	0.09 ± 0.06
IDL	0.58 ± 0.15	0.39 ± 0.06	0.27 ± 0.12
LDL	23.9 ± 8.7	9.7 ± 1.3[e]	8.8 ± 3.53
HDL	49.5 ± 13.8	45.9 ± 3.0	4.6 ± 0.66[f]

Values are mean ± standard deviation of three animals.
[a]Animals were 42 weeks old.
[b]Animals were 33 weeks old.
[c]$P < 0.01$ by one-tailed *t*-test for the comparison with controls.
[d]$P < 0.005$ by one-tailed *t*-test for the comparison with heterozygotes; $P < 0.005$ for the comparison with controls.
[e]$P < 0.05$ by one-tailed *t*-test for the comparison with controls.
[f]$P < 0.005$ by one-tailed *t*-test for the comparison with heterozygotes; $P < 0.05$ for the comparison with controls.

TABLE 4. Plasma triglyceride concentration in control and WHAM male chickens

Fraction	Control (*Y/Y*)[a]	WHAM (*Y/y*)[b] Heterozygotes	WHAM (*y/y*)[b] Homozygotes
		mg/dl	
Plasma	27.50 ± 5.62	28.18 ± 3.60	17.60 ± 3.20[c]
VLDL	2.34 ± 0.07	3.14 ± 0.24	1.95 ± 0.11
IDL	2.63 ± 0.66	1.96 ± 0.12	2.85 ± 1.66
LDL	8.30 ± 2.76	5.84 ± 0.27	5.90 ± 2.06
HDL	11.62 ± 3.26	10.99 ± 1.95	4.75 ± 1.11[d]

Values are mean ± standard deviation of three animals.
[a]Animals were 42 weeks old.
[b]Animals were 33 weeks old.
[c]Not significantly different from controls; $P = 0.078$.
[d]Not significantly different from controls; $P = 0.055$.

chickens exhibited a fivefold decrease in total plasma cholesterol compared to control males (**Table 2**). In both genders, the cholesterol reduction was most pronounced in the HDL fraction, where WHAM chickens had <10% of the cholesterol of normal chickens. Additionally, LDL cholesterol was found to be reduced in both female and male mutant chickens (Tables 1 and 2). No significant reductions were found in the other lipoprotein fractions studied. **Table 3** and **Table 4** show, respectively, plasma and lipoprotein triglyceride concentrations in female and male control and WHAM chickens.

Plasma cholesterol was measured in chickens from 3 days prior to hatching to 8 weeks of age. Control, heterozygous, and homozygous male chickens experienced the characteristic cholesterol rise at hatching (28), although this increase was attenuated in the latter two groups, indicating that the expression of the phenotype was not subject to developmental modulation (**Fig. 1**).

Plasma apoA-I was quantitated by radioimmunoassay. The homozygous male WHAM chickens (*y/y*) and mutant female chickens (*y/–*) had apoA-I levels <5% of control male (**Fig. 2**). Heterozygotes (*Y/y*) and hemizygotes (female control; *Y/–*) had apoA-I levels 57 and 69% of control male, respectively, consistent with a gene dosage-dependence of apoA-I concentration.

TABLE 3. Plasma triglyceride concentration in control and WHAM female chickens

Fraction	Control (*Y/–*)	WHAM (*y/–*)
	mg/dl	
Plasma	28.9 ± 3.3	23.0 ± 6.5
VLDL	5.2 ± 0.8	4.0 ± 1.1
IDL	5.6 ± 0.9	4.8 ± 1.4
LDL	8.0 ± 0.9	7.1 ± 3.7
HDL	14.2 ± 1.2	5.6 ± 1.2[a]

Values are mean ± standard deviation of six animals; animals were 6 weeks old.
[a]$P < 0.0001$.

Lipoprotein composition

In WHAM HDL particles there was a greater than threefold enrichment in triglyceride (**Table 5**). The triglyceride enrichment was at the expense of cholesteryl ester. It should be emphasized that, although HDL was enriched in triglyceride, the concentration of plasma HDL triglyceride was reduced as a consequence of a reduction in HDL particle number (Tables 3 and 4).

The apolipoprotein compositions of VLDL, LDL, and HDL were similar in WHAM and control chickens (**Fig. 3**) and were qualitatively similar to that described by Hermier, Forgez, and Chapman (15).

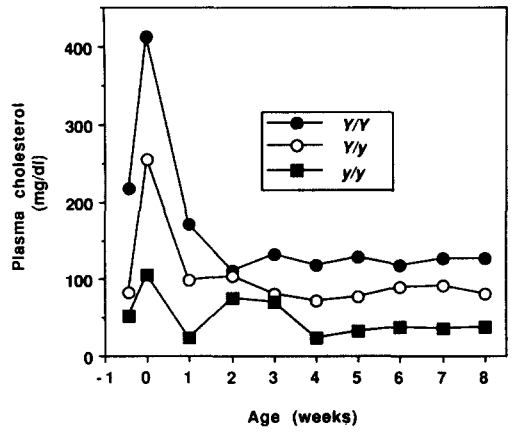

Fig. 1. Plasma cholesterol concentration in male chickens from 3 days before hatching to 8 weeks of age; *Y/Y*, controls; *Y/y*, heterozygotes; *y/y*, homozygous mutants.

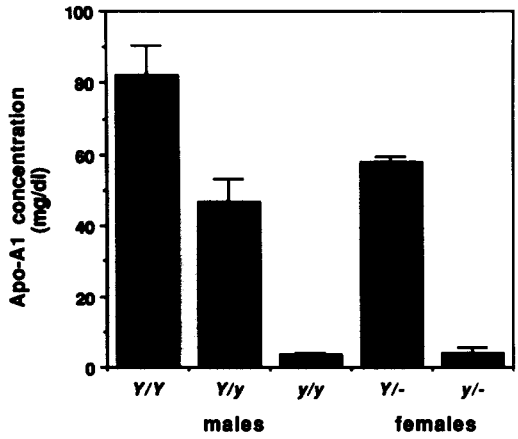

Fig. 2. Plasma apoA-I concentrations in 5-week-old chickens; *Y/Y*, control males; *Y/y*, heterozygous males; *y/y*, homozygous mutant males; *Y/–*, hemizygous control females; *y/–*, hemizygous mutant females. ApoA-I was quantitated by radioimmunoassay.

Mass and charge of apoA-I

Since several apoA-I variants have been discovered in HDL from humans with HDL deficiency syndromes (1, 29), HDLs from WHAM and control chickens were screened for variant forms of apoA-I using two-dimensional gel electrophoresis. ApoA-I from both chicken strains displayed identical apoA-I isoform distributions (Fig. 3), suggesting that apoA-I is not altered in the WHAM chicken.

Plasma turnover studies

To quantitate the potential contribution of defective production and/or hypercatabolism to the HDL deficiency in the WHAM chickens, HDL plasma turnover studies were performed. There are two methods for studying HDL turnover (30). HDL particles can be directly radiolabeled or apoA-I can be radiolabeled and then allowed to equilibrate with HDL particles. Studies with labeled whole HDL invariably suffer from a small portion of the radiolabel being associated with minor protein constituents in the HDL particles. On the other hand, incorporation of apoA-I into HDL particles might selectively trace a more rapidly exchanging pool of HDL apoA-I. In our experiments, both techniques were used. The first set of experiments used female chickens and the HDL turnover studies were performed with radiolabeled HDL particles. A second set of experiments was performed with male chickens injected with HDL that had been pre-incubated with radioiodinated apoA-I.

In the first set of experiments, ^{125}I-labeled WHAM HDL and ^{131}I-labeled control HDL were simultaneously injected into the blood streams of female control and WHAM chickens. The fractional clearance rates (FCRs) of the two types of HDL differed significantly. Regardless of the HDL injected, the WHAM chickens cleared HDL about 40% faster than did control chickens (**Fig. 4; Table 6**). When plasma clearance of control and WHAM HDL particles was compared, the disappearance rate of WHAM HDL was approximately 20% faster than that of control HDL (Fig. 4; Table 6). Although a faster HDL clearance was observed in the WHAM chickens, this degree of hypercatabolism could not account for the large HDL deficit. Rather, defective HDL production in the WHAM chicken was the major contributor to the HDL deficiency. When the HDL apoA-I production rates for the two female strains were calculated, there was a ninefold difference between control (7.54 mg $dl^{-1}h^{-1}$) and WHAM (0.83 mg $dl^{-1}h^{-1}$) chickens.

In a second set of experiments, male control and homozygous WHAM chickens were injected with autologous HDL that had been incubated with ^{125}I-labeled apoA-I. As was observed with ^{125}I-labeled HDL particles, ^{125}I-labeled apoA-I was cleared about twofold more quickly from the bloodstream of WHAM chickens (**Fig. 5**). However, the calculated production rates (**Table 7**) show a fivefold difference between the two chicken variants. Together, all of the turnover data suggest that the abnormality primarily responsible for the HDL deficiency syn-

TABLE 5. HDL lipid composition in control and WHAM chickens

Animals	Protein	Phospholipid	Cholesterol	Cholesteryl Ester	Triglyceride
			%		
Males[a]					
Control	41.3 ± 0.8	33.7 ± 1.2	4.6 ± 0.2	14.0 ± 1.0	6.3 ± 1.4
WHAM	38.7 ± 2.2	30.6 ± 3.0	7.1 ± 0.8	2.8 ± 1.8	20.7 ± 1.4
Females[b]					
Control	46.4	18.6	5.2	23.4	6.4
WHAM	37.6	23.6	2.6	12.8	23.4

[a]Each value represents the mean ± standard deviation of three different HDL preparations, each from a different animal.

[b]Each value represents the mean value from two HDL preparations. Each HDL preparation was isolated from the pooled plasma of three or more animals.

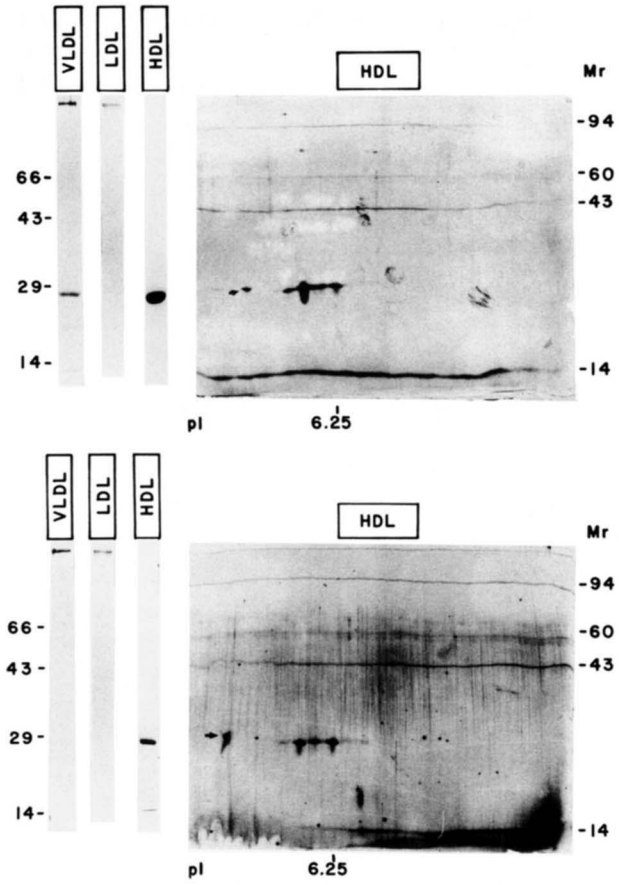

Fig. 3. One-dimensional and two-dimensional SDS-polyacrylamide gel electrophoresis of VLDL, LDL, and HDL apolipoproteins from control (top) and WHAM (bottom) chickens. Isoelectric points of the four major isoforms are 5.85, 5.96, 6.08, 6.25. The arrow identifies the position of the internal standard, vitamin D-dependent calcium binding protein (M_r = 27,000; pI = 5.2).

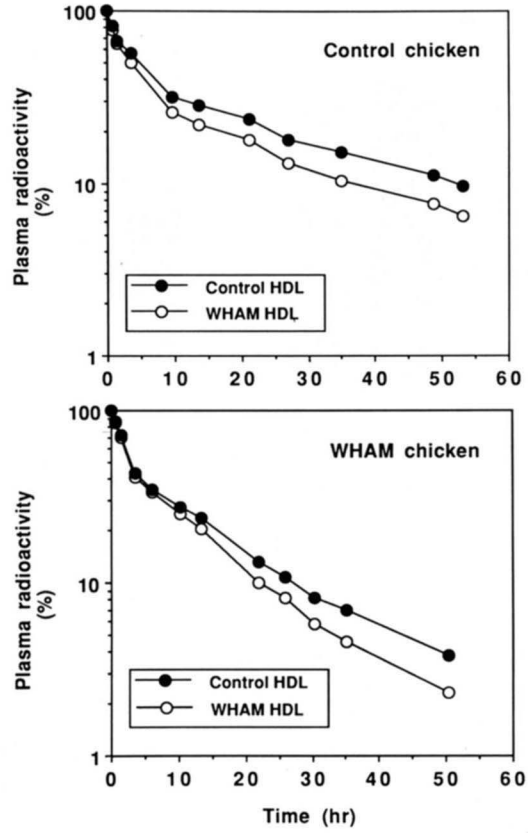

Fig. 4. Plasma disappearance of control and WHAM HDLs. HDLs were simultaneously injected into control (top) and WHAM (bottom) female chickens.

drome of the WHAM chickens is diminished production of apoA-I.

DISCUSSION

The phenotype that originally distinguished the WHAM chickens from wild-type Single Comb White Leghorn chickens was white, rather than the normal yellow, shanks and beaks. All white-shanked chickens studied (>100 animals) had the pronounced cholesterol deficiency, and all the wild-type chickens studied (>100 animals) had normal cholesterol levels. The lower cholesterol levels in the WHAM chickens were primarily due to a reduction in plasma HDL. Since xanthophylls are transported on lipoproteins, the white skin color could very well be a consequence of the HDL deficiency.

It is interesting to note that the wild-type male chickens have a >41% higher plasma apoA-I concentration than wild-type females, and that heterozygous WHAM males have plasma apoA-I levels within the same range as wild-type females (46.8 vs 57.8 mg/dl). Since the WHAM mutation is linked to the *Z*-chromosome, and in chickens the heterogametic gender is the female, the similarity in apoA-I concentration between the heterozygous male and the hemizygous female (Fig. 2) is consistent with the defect in the WHAM chickens being a loss-of-function mutation.

TABLE 6. Fractional clearance rates of ^{125}I-labeled HDL in control and WHAM female chickens

Animals	Control HDL	WHAM HDL
	pools/h	
Control		
1	0.074	0.091
2	0.066	0.093
3	0.072	0.094
4	0.091	ND
Mean ± SD	0.076 ± 0.011	0.093 ± 0.002
WHAM		
1	0.105	0.127
2	0.106	0.133
3	0.108	0.120
4	0.103	0.128
Mean ± SD	0.106 ± 0.002	0.127 ± 0.005

Samples were collected for approximately 50 h; ND, not determined.

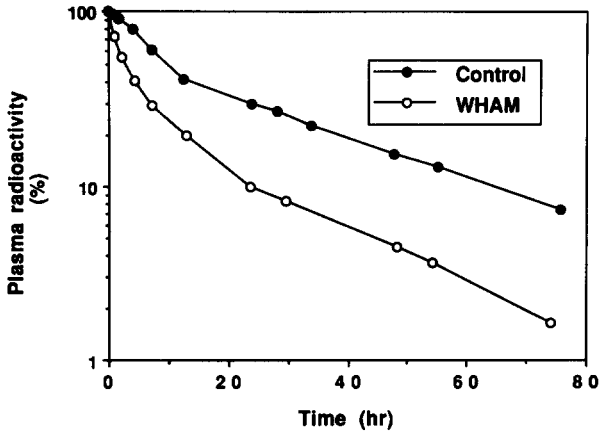

Fig. 5. Plasma disappearance of autologous control and WHAM HDL in control and WHAM male chickens.

It is unlikely that the mutation responsible for the HDL deficiency in WHAM chickens is in the apoA-I structural gene. On two-dimensional polyacrylamide gels, the WHAM apoA-I protein showed the same mass and isoform distribution as control apoA-I. The apoA-I gene has not been mapped in the chicken but is autosomal in the mouse (31) and in the human (32). Preliminary data suggest that apoA-I is also autosomal in chickens (S. Schreyer and A. D. Attie, unpublished observations). Hence, the association of the WHAM phenotype with a sex-linked mutation (13, 14) suggests that a locus distinct from the apoA-I structural gene is involved.

The principal metabolic defect in the WHAM chickens was a major reduction in the apoA-I production rate. In females, there was a ninefold decrease in HDL production, while in males there was a fivefold lower apoA-I production rate, compared with their respective controls.

TABLE 7. Turnover of HDL labeled with ^{125}I-labeled apoA-I in male chickens

Animals	Fractional Clearance Rate	ApoA-I Concentration	Production Rate
	pools/h	*mg/dl*	*mg/dl/h*
Control			
1	0.040	127.1	5.02
2	0.042	163.8	6.86
3	0.043	134.6	5.77
4	0.042	122.0	5.12
Mean ± SD	0.042 ± 0.001	136.9 ± 18.7	5.70 ± 0.85
WHAM			
1	0.117	10.6	1.24
2	0.100	7.6	0.76
3	0.114	11.6	1.33
4	0.106	10.5	1.12
5	0.086	12.6	1.09
Mean ± SD	0.105 ± 0.012	10.6 ± 1.9	1.11 ± 0.22

However, a faster FCR also contributed to the reduction in plasma apoA-I levels. WHAM HDL, injected into either control or WHAM chickens, was catabolized more rapidly than control HDL.

In contrast to the majority of humans with low plasma HDL, the mutant chickens did not have hypertriglyceridemia (Tables 3 and 4). There was a reduction in total plasma triglyceride, reflecting the dearth of HDL particles. However, the HDL particles were themselves triglyceride-rich; this triglyceride enrichment of WHAM HDL may account for its hypercatabolism. Like the WHAM chickens, hypertriglyceridemic patients have triglyceride-rich HDL (33) and a higher FCR for HDL (30, 34). Additionally, Goldberg et al. (35) observed hypercatabolism of triglyceride-enriched HDL in cynomolgus monkeys made hypertriglyceridemic by injection of anti-lipoprotein lipase antibody. Although the WHAM chickens do not have hypertriglyceridemia, the triglyceride enrichment in HDL particles may be a consequence of the approximately eightfold increased VLDL-to-HDL ratio caused by the HDL deficiency. Such an altered lipoprotein profile would favor augmented triglyceride transfer from VLDL to HDL, facilitated by the plasma cholesteryl ester transfer protein (36). Within this schema, the triglyceride enrichment of WHAM HDL could very well explain the HDL hypercatabolism in the WHAM chickens. The speculation that HDL hypercatabolism is a consequence of altered HDL particles rather than an intrinsic difference in the animals is supported by the experiments showing more rapid catabolism of WHAM HDL particles in normal chickens (Fig. 4, Table 6).

Another syndrome affecting HDL levels has been identified in C57BL/6 mice (37). In these animals, a high fat diet brings about a 50% reduction in HDL cholesterol. The HDL reduction in C57BL/6 mice and in the WHAM chickens probably occurs by a different mechanism. Genetically, they appear to be unrelated because the WHAM chicken phenotype is associated with a sex-linked mutation while the mouse phenotype is associated with a gene on chromosome 1 (assuming synteny between chicken and mouse). Moreover, the apoA-I synthetic rate is not reduced in fat-fed C57BL/6 mice, suggesting that the HDL reduction in these animals is due to enhanced HDL catabolism (38).

The WHAM chicken may prove to be a valuable animal model for the study of HDL deficiency syndromes. An examination of tissues from animals up to 13 months of age showed no evidence of spontaneous atherosclerosis; however, they may be more susceptible to diet-induced atherosclerosis than normal chickens. Current studies in our laboratory are aimed at answering this question as well as discovering the molecular basis of the defect in WHAM chicken apoA-I production; these studies should advance our understanding of HDL metabolism in humans.

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