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Overfeeding increases very low density lipoprotein diameter and causes the appearance of a unique lipoprotein particle in association with failed yolk deposition[1]

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Abstract Structurally specific very low density lipoprotein (VLDL) particles are presumed to redirect VLDL uptake during yolk deposition. Egg production is an energy intensive process, but energy excess negatively affects egg production. This study sought to determine whether overfeeding changed hen lipoprotein profiles, and if so, whether changes were related to egg production. Overfeeding caused 33% of hens to stop yolk deposition and reabsorb unovulated follicles (involute). VLDL of control hens possessed characteristic small particle size (30 nm) and composition indicative of limited intravascular metabolism. Overfeeding generally increased VLDL diameter, the effect being slight (+6.6 nm) in overfed laying hens when compared to overfed-involuted hens (+51.2 nm). Changes in composition indicated that increased intravascular metabolism of VLDL parallelled increases in particle diameter. Overfed-involuted hens showed significant elevations of plasma low density lipoprotein, VLDL cholesteryl ester, and high density lipoprotein (HDL) triacylglycerol content. Overfeeding increased plasma HDL, and in association with involution, caused redistribution of HDL particle sizes, including the appearance of novel HDL ~20 nm in diameter, termed HDL_R, and a 97 kDa protein within the HDL fraction. The overfed hen model may provide new insight into the role of lipoprotein physical properties in determining the metabolism of triacylglycerol-rich lipoprotein, and the process of reverse lipid transport. ■ Overfeeding generally increases VLDL size and plasma HDL levels suggesting that increased peripheral metabolism, and perhaps mechanical exclusion of enlarged VLDL by the granulosa basal lamina, contribute to the obesity and impaired yolk deposition observed in hens with excessive energy intakes. Temporal definition of massive VLDL enlargement relative to follicular collapse remains to be established. Cessation of yolk deposition accompanied by reabsorption of partially developed follicles appears to be associated with specific redistributions of HDL subpopulations and appearance of HDL_R.—**Walzem, R. L., P. A. Davis, and R. J. Hansen.** Overfeeding increases very low density lipoprotein diameter and causes the appearance of a unique lipoprotein particle in association with failed yolk deposition. *J. Lipid Res.* 1994. **35:** 1354–1366.

Supplementary key words plasma lipoproteins • lipoprotein composition • lipoprotein sizes • reverse lipid transport

Strategies used by various species to control the metabolism of plasma triglyceride-rich lipoproteins comprise an area of intense scientific interest. Of particular interest are those factors and processes that control the conversion of very low density lipoprotein (VLDL) to intermediate density lipoprotein (IDL), and ultimately low density lipoprotein (LDL). The laying hen provides a unique model for studies investigating the role of lipoprotein physical properties in directing the metabolism and resultant tissue partitioning of triglyceride secreted by the liver. Dramatic alterations occur in the levels and physical properties of hepatic lipoproteins in plasma of hens with the onset of egg production (1–7). During this same period, the flow of triglyceride from liver to peripheral tissues such as muscle and adipose is largely redirected to yolk. Yolk deposition is a receptor-mediated event in which VLDL are transported intact from plasma into the developing oocyte (8). The VLDL deposited into yolk are rich in triglyceride (TG) in comparison to VLDL of non-laying chickens (4), and the physical modifications of this particle shield it from peripheral hydrolysis and ensure that sufficient TG-energy is deposited into yolk to support embryogenesis (9, 10).

Abbreviations: VLDL, very low density lipoprotein; HDL, high density lipoprotein; LDL, low density lipoprotein; IDL, intermediate density lipoprotein; apo, apolipoprotein; LPL, lipoprotein lipase; FLHS, fatty liver hemorrhagic syndrome; SCWL, single comb white leghorn; TG, triacylglycerol; UC, unesterified cholesterol; CE, cholesteryl ester; PL, phospholipid; PRO, protein; GGE, gradient gel electrophoresis; CBR-250, Coomassie Brilliant Blue R-250; SDS, sodium dodecyl sulfate.

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Food intake increases markedly to support the energy and protein requirements of egg production (11). Among "egg-type" hens, such as Single Comb White Leghorns (SCWL), energy intake is apparently driven by egg production, and the relationship between these two parameters can be described mathematically (12). Among chickens selected for rapid body growth, so called "meat-type" or broiler birds, tight linkage between food intake and egg production does not occur. Egg production among broiler hens is usually poor (13) and is improved by feed restriction (13, 14). Overfeeding of egg-type chickens is known to variably suppress egg production (15–18). Energy excess is also cited in the etiology of fatty liver hemorrhagic syndrome (FLHS), a spontaneous pathologic condition that can reduce egg production in commercial flocks (18–20).

It is unclear why excess energy intake should impair egg production, but untoward effects could be mediated by altered lipoprotein metabolism. Obesity is apparent in most poorly laying broiler breeder hens and hens with FLHS, suggesting that hepatic lipoprotein is ineffectively directed to yolk. To investigate these possibilities, lipoprotein profiles of SCWL hens with a genetic predisposition for high rates of egg production were examined during periods of both normal and excessive energy intake.

METHODS

Animal sources

Experiment 1. Twenty eight mature, W-36 Hyline, Single Comb White Leghorn (SCWL) laying hens, approximately 36 weeks old, were adapted to a purified diet (U.S. Biochemicals Corp., Cleveland, OH) consisting of 16.7% isolated soy protein, 2% spray dried egg white, 0.22% dl-methionine, 20% dextrin, 41% glucose, 3% cellulose, 5% corn oil with 0.2% ethoxyquin, 8.45% $CaCO_3$, 1.2% $CaHPO_4$, and 2.44% trace mineral and vitamin mix sufficient to meet the nutritional requirements for laying hens (12). Ten hens consumed the diet ad libitum (control), and the remaining 18 were intubated with this same diet to achieve 150% of their usual daily energy intake (overfed) (16). Prior to overfeeding, both groups of hens weighed an average of 1687 g and had egg production rates of 89% (16). Egg production was calculated for each hen as: (number of eggs laid/number of days observed) ×100%. A minimum of 7 days was used for calculation of egg production rates. Six overfed hens were necropsied each week for 3 weeks. Selection criteria, liver histology, and reproductive performance of these birds were reported elsewhere (16). Blood from six control hens fed ad libitum was taken at each weeks sampling. A total of 18 control hen blood samples and 18 overfed hen blood samples were used as sources of plasma lipoproteins. Three overfed hens which failed to lay eggs for several days were found to have maturing follicles, 1, or 2 ovulated ovum(s) in the oviduct at necropsy. Lipoprotein profiles of these hens were transitional in nature and possessed some characteristics of both laying and non-laying hens. The predominance of the characteristics changed in parallel with the degree to which yolk deposition was re-established. Data from these hens were not included in statistical analysis. One overfed hen died prior to blood sampling, and so the total number of overfed hens statistically analyzed was 14.

Experiment 2. Twelve SCWL hens, approximately 60 weeks old and of a strain bred for high egg production (Line 9, Poultry Science Department, University of California-Davis), were adapted to the purified diet and overfed as in Experiment 1 for 2 weeks. Egg production was monitored prior to and during the intubation period. Blood was drawn prior to overfeeding, and again at necropsy after 2 weeks of intubation. Status of liver and reproductive tract were evaluated grossly at necropsy as described earlier (16). Plasma VLDL were centrifugally isolated for determination of particle size distributions by dynamic laser light scattering as described below.

Blood sampling and lipoprotein separation

Midmorning blood samples were drawn from either a brachial vein or directly from the heart of fed hens. Hens were anesthetized by intramuscular injection of sodium pentobarbital (45 mg/kg) prior to heart puncture. Whole blood was drawn into syringes or tubes, preloaded with 1 mg EDTA and 1 U each streptomycin sulfate and procaine penicillin G per ml whole blood, using a 20 G needle. Blood tubes were inverted 3 × and held on ice. Plasma was isolated within 2 h of blood sampling by centrifugation at 1470 *g*, 4°C, for 20 min. Plasma activity of lecithin:cholesterol acyltransferase (E.C. 2.3.1.43) was inhibited by addition of 15 μl dithionitrobenzoic acid/ml plasma. Lipoproteins were isolated from 2 ml plasma using sequential density gradient centrifugation (21). Density solutions were constructed by adding increasing amounts of NaBr to a basal NaCl (d 1.0063 g/ml) solution containing 0.01% EDTA and 50 kU/l each of streptomycin and penicillin. Very low density lipoprotein plus IDL (1) were isolated at d < 1.021 g/ml by aspiration with a narrow-bore pipet after centrifugation at 148,600 g_{avg} for 20 h at 14°C in a Sorvall 45.6 TFT rotor (Sorvall-DuPont, Wilmington, DE). Low density lipoprotein and HDL were isolated in a similar fashion at d 1.021–1.041 and d 1.041–1.21 g/ml, respectively, after centrifugation at 148,600 g_{avg} for 24 h at 14°C. Two milliliter samples of a salt solution with a density similar to plasma, d 1.0064 g/ml, were run in parallel with plasma samples to verify salt densities at each step of the density fractionation. Densities of all salt solutions were measured with a digital solution density meter (Mettler/Parr, DMA 46, Graz,

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Austria). Owing to the extreme lipemia of laying hen plasma, all centrifuge tubes and pipets were siliconized with Sigmacote® (Sigma Chemical Company, St. Louis, MO) prior to use. Recovery of plasma lipids (triglyceride, TG + unesterified cholesterol, UC + cholesteryl ester, CE) was calculated as quantity of lipid recovered in (VLDL + LDL + HDL)/plasma concentration. Lipid recoveries averaged 71% and were statistically similar among groups. Aliquots of freshly isolated density fractions were used to determine particle size distributions, while the remainder was dialyzed for 24 h at 4°C against 0.01% EDTA using Spectrapor dialysis tubing (Spectrum Medical Industries, Inc., Los Angeles, CA, exclusion limit ca. 3,500) in an M145.1 microdialysis chamber (Medical Research Apparatus Corp, Clearwater, FL).

The density intervals were designed to separate plasma into VLDL + IDL, LDL, and HDL density fractions (3). Others have reported that classical LDL is not present within laying hen plasma, and that laying hen HDL is unique, possessing characteristics typical of both LDL and HDL (1). Thus some overlap among functional classes of lipoproteins within a given density interval could occur. The VLDL, LDL, and HDL designations are retained to facilitate discussion, albeit with the caveat that the metabolic functions of the various density fractions of some groups may or may not correspond to the related human lipoproteins of the same density ranges.

Analysis of plasma and isolated lipoprotein composition

Plasma and dialyzed density fractions were used in composition studies. Protein (PRO) was measured by a modified Lowry method (22) using bovine serum albumin as the standard. Total phospholipid phosphorus was measured colorimetrically (23) after ashing (24). The weight of phospholipid (PL) was calculated as phosphorus weight × 24 (25). Quantities of choline-containing PL present in plasma were determined enzymatically using synthetic dipalmitoyl-phosphatidylcholine, dipalmitoyl, as the standard (26). Total cholesterol (TC) was measured using an enzymatic assay (27). Unesterified cholesterol (UC) was measured separately using the same techniques after deletion of cholesterol esterase (EC 3.1.1.13) from the reaction mixture. Weight of cholesteryl ester (CE) was calculated by multiplying the difference between TC and UC by 1.65. Triglycerides were measured enzymatically using a test kit, (Procedure No. 339, Sigma Chemical Company, St. Louis, MO). Commercially prepared lyophilized lipoprotein preparations (lipid control-N and lipid control-E, Sigma Diagnostics, St. Louis, MO) were reconstituted according to the manufacturers' directions and included in all triglyceride and cholesterol assays as a measure of assay accuracy.

Determination of VLDL particle size distributions

Diameters of VLDL were determined optically by dynamic light scattering using a Microtrak® Series 9200 Ultrafine Particle Analyzer (Leeds and Northrup, North Wales, PA) (28, 29). Centrifugally isolated VLDL were suspended as a 1:30 dilution in a NaCl solution (d 1.0063 g/ml) and placed into the sample well. System software, and 3 mWatt, $\lambda = 780$ nm laser beam were activated. Light scattering from lipoprotein particles was recorded for 4 min, adapted to the audio range, and deconvoluted by system software. Using this method, light from the laser diode is scattered from each particle and its frequency subsequently Doppler-shifted by Brownian motion of the particle. The Doppler effect is proportional to particle velocity. Velocity distribution is a known function of particle size, fluid temperature, and fluid viscosity. Both temperature and viscosity of the suspending fluid are known, thus with compensation, the velocity distribution becomes a unique function of particle size. Although considered a primary method (30), validity of size measurements were routinely assessed using monosized latex beads (Bangs Laboratory, Carmel, IN). Polydispersity of particle populations was calculated as the width, in nanometers, of the measured particle size distribution. Concentration of particles within the sample well is automatically calculated by summing all of the scattered light within the measurement region and dividing by the scattering efficiency of the differently sized particles. This value, corrected for dilution of sample, was designated as Concentration Index (C.I.) and its arbitrary units are proportional to particle concentration on a volume basis. VLDL triglyceride was found to correlate highly ($r^2 = 0.944$, n = 32) with C.I.

Determination of non-VLDL lipoprotein particle size distributions

Undialyzed aliquots of LDL and HDL were subjected to nondenaturing gradient gel electrophoresis (GGE) (31) using a Model GE-2/4 LS vertical electrophoresis apparatus (Pharmacia, Uppsala, Sweden). Gels with a gradient of 2-16% polyacrylamide (Pharmacia, Piscataway, NJ) were used to size LDL, and gels with a gradient of 4-30% polyacrylamide were used to size HDL. Protein staining with 0.05% Coomassie Blue R-250 (CBR-250) in methanol-acetic acid-water 45:10:45 for 24 h was used to visualize lipoproteins. Gels were destained in methanol-acetic acid-water 20:75:5 until backgrounds were clear. Migration of lipoproteins into the gel matrix was measured using a Hoefer GS 300 scanning densitometer and GS-365 software package (Hoefer Scientific Instruments, San Francisco, CA). Particle sizes were calibrated in the digitized scans by comparison with migration distances of standard proteins of known hydrated diameter (Pharma-

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cia, Piscataway, NJ) (32). Specific particle size ranges were established for each gel based on the migration distances of the duplicate standards run within each gel.

Equal chromogenicity of protein was assumed in area calculations. The manual integration procedure within the system software was used following baseline adjustment to determine individual peak areas. Areas of individual peaks within a single sample were then added together to give the total area. The percentage of particles within a given size range was calculated by dividing the peak areas falling within migration limits defined by the protein standards by the total area.

Apoprotein separation and estimation of molecular weight

Aliquots of the d 1.041–1.210 g/ml HDL fraction were boiled for 5 min in an equal volume of application buffer consisting of 10% glycerol, 5% mercaptoethanol, 3% sodium dodecyl sulfate (SDS), and 0.0625 M Tris, pH 6.8, prior to protein separation by PAGE (33). Apolipoproteins were applied to 180 × 200 × 0.7 mm gels consisting of a 4.5% acrylamide stacking gel, and a 16.7% acrylamide separation gel (37:1 ratio of acrylamide/bis-acrylamide, Sigma Chemical Company, St. Louis, MO). Samples were run into the gel matrix using a 60 V, 2-h initial electrophoresis step, and then separated at 120 V. Separation of protein bands required 4–6 h as judged by movement of tracking dye. Gels were first stained for >3 h in isopropanol–glacial acetic acid–water 25:10:65 containing .05% CBR-250, followed by >1 h in isopropanol–glacial acetic acid–water 10:10:80 containing 0.005% CBR-250, and finally >1 h in glacial acetic acid–water 10:90 containing 0.0005% CBR-250. Gels were completely destained in methanol–glacial acetic acid–water 20:5:75. Separate lanes containing protein standards of known molecular weight (Rainbow™ protein molecular weight markers, Amersham, Arlington Heights, IL) were included in each gel. Calculation of total protein peak areas within samples and the fractional distribution of the total protein among individual proteins were as described for native lipoprotein particles.

Statistics

Data are expressed as the arithmetic mean ± standard error of the mean (SEM). Values are reported as grams per liter plasma rather than millimoles per liter as others have shown that overfeeding results in changes in acyl chain length and saturation (19), and individual fatty acid species were not determined. Preliminary statistical analysis of data from Experiment 1 showed that the degree of liver hemorrhage experienced by individual hens due to overfeeding was not associated with any alterations in plasma lipoprotein characteristics. All overfed hens had a similar degree of hepatic steatosis. Based on these preliminary analysis three groups of hens were identified: *1)* control hens, which consumed food ad libitum; *2)* overfed-layers, overfed hens which continued to lay eggs; and *3)* overfed-involuted, overfed hens which stopped laying eggs and whose reproductive tracts regressed (involuted). Data were then analyzed by both one-way (group) and two-way (feeding level × lay status) analysis of variance (AOV) using a general linear model and Type III sums of squares (34). Statistical analysis of apolipoprotein data was restricted to those proteins that appeared in all samples. Differences in mean values were tested by the least-squares means procedure. Differences between means that were considered significant differed at a minimum of $P \leq 0.05$.

RESULTS

Density distribution, concentration, and chemical composition of plasma lipids

In experiment 1, total plasma lipids were similar and very high (~22.6 g/l) in all three groups of hens (**Table 1**). Plasma UC was significantly ($P < 0.05$) elevated in both groups of overfed hens when compared to control hens (Table 1). The 1.6-fold higher concentration of plasma CE present in overfed hens was not statistically different from control values.

Total quantities of plasma lipid recovered as centrifugally isolated lipoprotein were similar among groups; however, the density distribution of recovered lipoprotein was affected by both feeding level and egg production status (Table 1). Lipoprotein recovered in the d < 1.021 g/ml VLDL + IDL fraction of overfed-laying hens was about 30% higher, and that of overfed-involuted hens about 10% lower than that of control hens fed ad libitum. Lipoprotein within the d < 1.021 g/ml fraction accounted for 82% of recovered lipoprotein in control hens, and a statistically equivalent 77% of overfed laying hens (Table 1). In contrast, VLDL accounted for a significantly ($P < 0.01$) lower 64% of recovered lipoprotein among overfed involuted hens. A 3-fold increase in the amount of lipoprotein recovered from the d 1.021–1.041 g/ml LDL fraction ($P < 0.05$), and a 1.7-fold increase in the amount of lipoprotein recovered from the d 1.041–1.210 g/ml HDL ($P < 0.05$) fraction accounted for the density redistribution of lipoprotein among overfed-involuted hens (Table 1). The total mass of HDL lipoprotein was also significantly ($P < 0.001$) increased among overfed laying hens in comparison to controls, but was not accompanied by changes in the either the amount or fractional contribution of LDL lipoprotein (Table 1). Overfeeding consistently increased plasma fractional HDL content from 13% in control hens to 15% and 20% in overfed-layers and overfed-involuted hens, respectively. Also apparent by examination of staining intensity of volume-equivalent aliquots of HDL separated by GEE, this increase failed to

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TABLE 1. Plasma total lipid concentration and density distribution of plasma lipoprotein isolated from laying hens with differing rates of feed intake and egg production

Parameter	Hens Consuming Diet ad libitum: Laying Eggs (n = 18)	Hens Overfed to 150% Usual Daily Energy Intake: Laying Eggs (n = 8)	Hens Overfed to 150% Usual Daily Energy Intake: Involuted (n = 6)
Concentration of plasma total lipid and lipid classes, g/l plasma			
TG	14.08 ± 2.51	13.67 ± 2.36	18.19 ± 11.37
UC	0.72 ± 0.06[a]	1.31 ± 0.21[b]	1.25 ± 0.38[b]
CE	0.77 ± 0.20	1.23 ± 0.42	1.17 ± 0.17
PL[c]	5.82 ± 0.41 (12)	6.26 ± 0.90 (6)	5.58 ± 0.54 (4)
Total	21.4	22.5	26.2
Amount of lipoprotein recovered within specific density intervals, g/l plasma			
d < 1.021 g/ml, VLDL + IDL	11.67 ± 1.34	15.12 ± 2.37	10.81 ± 3.05
d 1.021-1.041 g/ml, LDL	0.87 ± 0.06[a]	1.23 ± 0.24[a]	3.28 ± 1.63[b]
d 1.041-1.210 g/ml, HDL	1.47 ± 0.8[a]	2.41 ± 0.21[b]	2.48 ± 0.23[b]
Distribution of lipoprotein among density intervals, % total recovered lipoprotein			
VLDL + IDL	82 ± 2[a]	77 ± 6[a]	64 ± 2[b]
LDL	7 ± 1[a]	8 ± 2[a]	16 ± 3[b]
HDL	11 ± 1	15 ± 4	20 ± 4

Values are given as means ± SEM. Statistical analysis used a complete weighted least-square means analysis. Hens consumed a purified diet for 1-3 weeks prior to blood sampling. At weekly intervals, six control hens and six overfed hens were selected for analysis. Overfed hens were intubated with 150% of their usual energy intake as calculated from food consumption during 10 days preceding intubation. Egg production of control hens = 82% (eggs per hen per 100 days), overfed laying hens = 79%, and overfed-involuted hens = 37%. Abbreviations: n, number of hens; TG, triacylglycerol; UC, unesterified cholesterol; CE, cholesteryl ester; PL, phospholipid; VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein.

[a,b]Values across rows not sharing a common superscript differ at $P \leq 0.05$.

[c]Values determined by enzymatic assay with choline oxidase (E.C. 1.1.3.17). Approximately 70% plasma total phospholipid detected (26). Numbers of samples analyzed in parentheses.

reach statistical significance ($P = 0.08$ for control vs. overfed-involuted).

Concentration and fractional composition of chemical constituents of density-fractionated lipoproteins

Both the total amounts and fractional contributions of individual lipoprotein constituents varied among groups within each density class. Overfed layers had the highest levels, overfed-involuted the next highest, and control hens the lowest levels of both VLDL-UC and VLDL-CE (**Table 2**). Differences between overfed layers and control hens were significant ($P < 0.05$). Quantities of VLDL-PL were clearly, and significantly elevated ($P < 0.05$) in overfed layers in comparison to either control hens or overfed-involuted hens. However, the amounts of VLDL-TG and VLDL-PRO were similar among the three groups. Control hen VLDL contained 55.4% TG, 5.1% UC, 2.0% CE, 22.5% PL, and 15.1% PRO (Table 2). Also shown in Table 2, the fractional contributions of UC and PRO to VLDL were similar among groups. VLDL isolated from either overfed group were relatively richer in PL and poorer in TG than that of control hens. Fractional VLDL-TG loss was due to an increased fractional VLDL-CE content in both overfed groups. The increase in fractional VLDL-CE was significant ($P < 0.05$) for overfed-involuted hens.

In hens, overfeeding alone appeared to increase the amounts of LDL-TG and LDL-PL, but overfeeding in conjunction with involution produced a significant ($P < 0.05$) 5.7-fold increase in LDL-TG, and a 1.9-fold increase ($P < 0.01$) in LDL-PL when compared to control hens (**Table 3**). Overfed-involuted hens also had significant 3-fold elevations in the amounts of LDL-UC ($P < 0.01$) and 4.8-fold increases in LDL-PRO ($P < 0.01$) in comparison to either overfed layers or control hens. Calculation of the fractional composition showed that LDL of control hens contained 41.2% TG, 7.2% UC, 17.4% CE, 18.9% PL, and 15.4% PRO (Table 3). Low density lipoproteins from overfed-involuted hens contained 22% PRO, a significant ($P < 0.01$) enrichment in comparison to either control or overfed laying hens (Table 3).

Overfeeding had significant ($P < 0.05$) main effects to increase all components except UC within the HDL fraction (**Table 4**). Involution produced additional significant increases in HDL-PRO ($P < 0.05$), and HDL-TG content ($P < 0.001$) in overfed hen plasma. Control hen HDL contained 5.9% TG, 3.3% UC, 19.9% CE, 44.1% PL, and 26.9% PRO. Overfeeding significantly reduced the fractional HDL-PL to an average of 34% ($P < 0.0001$) while increasing the fractional protein content. The increase in the fractional HDL-PRO content was significant for overfed-involuted hens ($P < 0.01$). Fractional HDL-

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TABLE 2. Concentration and fractional chemical composition of lipoproteins recovered from the d ≤ 1.021 g/ml density fraction of ultracentrifugally separated plasma taken from laying hens differing in energy intake and egg production

Parameter	Hens Consuming Diet ad libitum	Hens Overfed to 150% Usual Daily Energy Intake	
	Laying Eggs (n = 18)	Laying Eggs (n = 8)	Involuted (n = 6)
Concentration, g/l plasma			
TG	6.72 ± 0.89	8.18 ± 1.92	5.29 ± 1.91
UC	0.53 ± 0.05[a]	0.95 ± 0.2[b]	0.86 ± 0.28[a,b]
CE	0.21 ± 0.03[a]	0.52 ± 0.17[b]	0.47 ± 0.13[a,b]
PL[c]	2.47 ± 0.26[a]	3.50 ± 0.37[b]	2.74 ± 0.43[a]
PRO	1.75 ± 0.21	1.97 ± 0.30	1.45 ± 0.56
Fractional composition, %			
TG	55.4 ± 1.8	46.2 ± 9.5	43.9 ± 4.8
UC	5.1 ± 0.6	6.6 ± 1.2	7.6 ± 1.0
CE	2.0 ± 0.4[a]	3.9 ± 1.7[a]	5.4 ± 1.9[b]
PL	22.5 ± 1.4	27.4 ± 4.7	30.7 ± 5.1
PRO	15.1 ± 0.6	15.8 ± 3.5	12.3 ± 1.3

Values are given as means ± SEM. Statistical analysis used a complete weighted least square means analysis. Hens consumed a purified diet for 1-3 weeks prior to blood sampling. At weekly intervals, six control hens and six overfed hens were selected for analysis. Overfed hens were intubated with 150% of their usual energy intake as calculated from food consumption during 10 days preceding intubation. Egg production and abbreviations given in legend of Table 1.

[a,b]Values across rows, not sharing a common superscript, differ at $P \leq 0.05$.

[c]Values determined by measurement of total lipoprotein phosphorus.

TG content of both egg laying groups averaged 6%, but more than doubled ($P < 0.05$) to 13% in overfed-involuted hens.

Size distributions of density-fractionated lipoproteins

In Experiment 1, attempts to determine size of VLDL by nondenaturing GGE were unsuccessful as particles larger than 37 nm are excluded from the gel matrix (32). The inadequacy of this technique proved informative, however, as the lack of stainable material in lanes containing aliquots of VLDL from overfed-involuted hens, which were known to contain significant amounts of lipid and protein, suggested that VLDL size was increased.

In Experiment 2, a different technique based on Doppler-shifted laser light, as described in Methods, was used to measure VLDL diameters. Control hen VLDL was found to have a modal diameter of 27.1 nm, polydispersity of 10 nm, and a concentration index (C.I.) of 4.97 (**Table 5, Fig. 1A**). Overfeeding significantly ($P < 0.05$) increased VLDL diameter (Table 5), but the

TABLE 3. Concentration and fractional chemical composition of lipoproteins recovered from the d 1.021-1.041 g/ml density fraction of ultracentrifugally separated plasma taken from laying hens differing in energy intake and egg production

Parameter	Hens Consuming Diet ad libitum	Hens Overfed to 150% Usual Daily Energy Intake	
	Laying Eggs (n = 18)	Laying Eggs (n = 8)	Involuted (n = 6)
Concentration, g/l plasma			
TG	0.34 ± 0.04[a]	0.55 ± 0.18[a,b]	1.93 ± 1.31[b]
UC	0.06 ± 0.01[a]	0.08 ± 0.02[a]	0.22 ± 0.09[b]
CE	0.20 ± 0.06	0.26 ± 0.11	0.25 ± 0.09
PL[c]	0.14 ± 0.01[a]	0.21 ± 0.03[a,b]	0.27 ± 0.07[b]
PRO	0.12 ± 0.01[a]	0.14 ± 0.04[a]	0.62 ± 0.24[b]
Fractional composition, %			
TG	41.2 ± 3.4	42.6 ± 5.8	42.3 ± 6.9
UC	7.2 ± 1.0	5.9 ± 1.3	8.0 ± 2.4
CE	17.4 ± 5.2	19.8 ± 8.0	14.6 ± 4.5
PL	18.9 ± 1.7	20.7 ± 4.6	12.7 ± 3.2
PRO	15.4 ± 1.2[a]	10.9 ± 1.6[a]	22.4 ± 3.7[b]

Values are given as means ± SEM. Statistical analysis used a complete weighted least square means analysis. Hens consumed a purified diet for 1-3 weeks prior to blood sampling. At weekly intervals, six control hens and six overfed hens were selected for analysis. Overfed hens were intubated with 150% of their usual energy intake as calculated from food consumption during 10 days preceeding intubation. Egg production and abbreviations given in legend of Table 1.

[a,b]Values across rows, not sharing a common superscript, differ at $P \leq 0.05$.

[c]Values determined by measurement of total lipoprotein phosphorus.

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TABLE 4. Concentration and fractional chemical composition of lipoproteins recovered from the d 1.041-1.210 g/ml density fraction of ultracentrifugally separated plasma taken from laying hens differing in energy intake and egg production

Parameter	Hens Consuming Diet ad libitum	Hens Overfed to 150% Usual Daily Energy Intake	
	Laying Eggs (n = 18)	Laying Eggs (n = 8)	Involuted (n = 6)
Concentration, g/l plasma			
TG	0.09 ± 0.01[a]	0.16 ± 0.02[b]	0.32 ± 0.06[c]
UC	0.05 ± 0.01	0.09 ± 0.03	0.09 ± 0.01
CE	0.31 ± 0.02[a]	0.63 ± 0.19[b]	0.47 ± 0.07[a,b]
PL[d]	0.70 ± 0.03[a]	0.85 ± 0.07[b]	0.77 ± 0.09[a,b]
PRO	0.43 ± 0.03[a]	0.68 ± 0.04[b]	0.82 ± 0.08[c]
Fractional composition, %			
TG	5.9 ± 0.4[a]	7.0 ± 0.9[a]	13.3 ± 2.7[b]
UC	3.3 ± 0.5	3.1 ± 0.8	3.7 ± 0.6
CE	19.9 ± 0.9	24.0 ± 4.3	18.7 ± 1.9
PL	44.1 ± 1.1[b]	36.3 ± 3.0[a]	31.0 ± 1.8[a]
PRO	26.9 ± 0.6[a]	29.5 ± 2.1[a,b]	33.4 ± 1.9[b]

Values are given as means ± SEM. Statistical analysis used a complete weighted least square means analysis. Hens consumed a purified diet for 1-3 weeks prior to blood sampling. At weekly intervals, six control hens and six overfed hens were selected for analysis. Overfed hens were intubated with 150% of their usual energy intake as calculated from food consumption during 10 days preceeding intubation. Egg production and abbreviations given in legend of Table 1.

[a,b,c]Values across rows not sharing a common superscript differ at $P \leq 0.05$.

[d]Values determined by measurement of total lipoprotein phosphorus.

degree of increase was dependent upon rate of egg production (Fig. 1B). Overfed hens that continued to lay eggs produced VLDL similar to those of control hens, and possessed a monodisperse population polydispersity of 8.8 nm and a C.I. of 7.53 (Table 5). Overfed hens with failed egg production produced VLDL that were both significantly ($P < 0.01$) larger and more polydisperse than either egg laying group (Table 5, Fig. 1). The C.I. of the overfed involuted hens was significantly ($P < 0.05$) lower than that of the overfed layers, and only 41% of control hen values; this latter difference was not found to be statistically significant (Table 5). In both egg laying groups, the percentage of VLDL with diameters in excess of 46 nm was less than 20%. However, in overfed-involuted hens, 91% of the VLDL were larger than 46 nm in diameter.

Low density lipoprotein is well resolved by nondenaturing GGE. In general agreement with literature values (1, 35), control hen LDL segregated into two or three bands with the major band having a diameter of either 24 nm or 25 nm (**Table 6**). All control hens contained an additional minor band of 21 nm in diameter. Nearly 70% of control hen LDL was found in particles with diameters in excess of 22 nm. The profile of overfed layers was nearly identical to that of control hens. In contrast, LDL from overfed-involuted hens had average diameters of only 24 nm, 23 nm, and 21 nm. Among these hens, 70% of stainable LDL was 18-22 nm in diameter.

Scanning densitometry of high density lipoprotein from control hens showed three major bands with diameters of 10 nm, 12 nm, and 13 nm that blended into a nearly continuous size distribution (**Table 7, Fig. 2**).

TABLE 5. Size characteristics of lipoproteins recovered from the d ≤ 1.019 g/ml density fraction of ultracentrifugally separated plasma taken from laying hens differing in energy intake and egg production

Parameter	Hens Consuming Diet ad libitum	Hens Overfed to 150% Usual Daily Energy Intake	
	Laying Eggs (n = 10)	Laying Eggs (n = 6)	Involuted (n = 4)
VLDL modal diameter, nm	27.1 ± 0.7[a]	33.7 ± 0.9[b]	78.3 ± 4.1[c]
Polydispersity, nm	10.0 ± 0.8[a]	8.8 ± 1.1[a]	35.9 ± 2.5[b]
Percentage of particles >46 nm	12.2 ± 1.8[a]	16.6 ± 4.4[a]	91.0 ± 5.0[b]
Concentration Index	4.97 ± 1.25[a,b]	7.52 ± 2.22[b]	2.05 ± 0.84[a]

Values are given as means ± SEM. Hens were fed a purified diet for 2 weeks prior to blood sampling. Overfed hens were intubated with 150% of their usual energy intake as calculated from food consumption during 10 days preceeding intubation.

[a,b,c]Values across rows not sharing a common superscript differ at $P \leq 0.05$.

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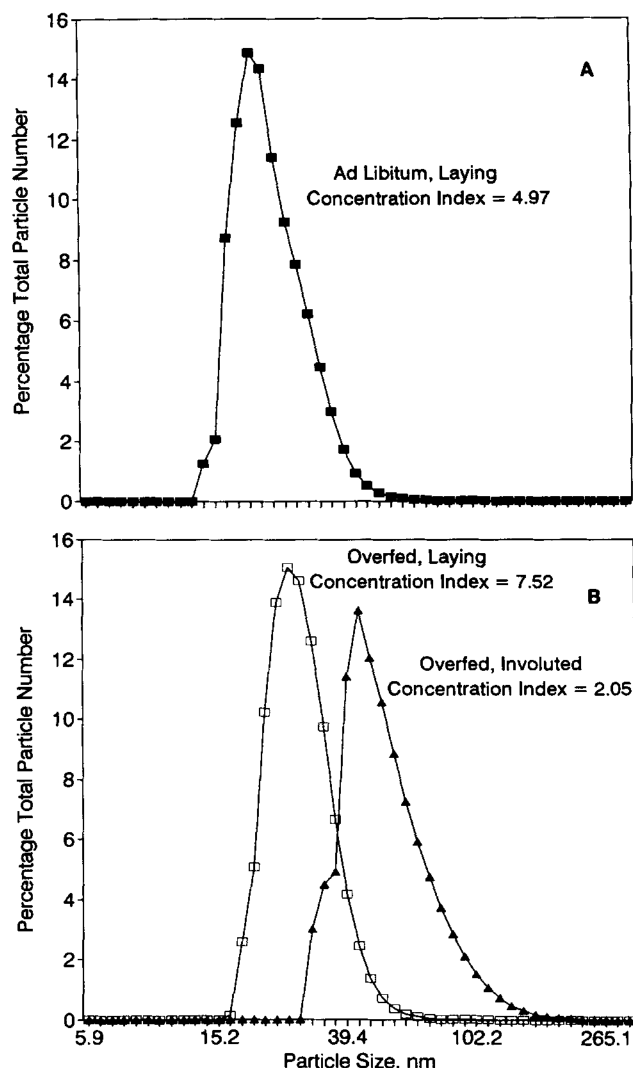

Fig. 1. Dynamic light scattering-determined diameter distributions of very low density lipoprotein (VLDL), d < 1.021 g/ml, isolated from hens before (panel A), and after (panel B) overfeeding for 2 weeks. Differences in egg production after overfeeding reflect individual responses of hens to overfeeding. Freshly isolated VLDL were diluted 30-fold in 0.9 N NaCl prior to sample analysis. Concentration index is a measure of optical turbidity and is proportional to particle concentration on a volume basis.

Control hens contained virtually no HDL with diameters in excess of 17 nm, and a relatively even distribution of particles between the diameter ranges of 8–12 nm and 12–17 nm (Table 7). HDL profiles of overfed layers resembled those of control hens although mean particle diameters were smaller, and a band appeared at 10.6 nm. Size distributions of HDL from overfed layers were shifted towards the smaller classes of particles, with 69% of protein found in the 8–12 nm range. HDL profiles of overfed-involuted hens were in marked contrast to the other two groups (Table 7, Fig. 2). Particles with diameters in the 12–17 nm range were absent and the vast majority of protein (81%) was contained in the 8–12 nm range. Major bands within this size range were located at 8.8 nm, 9.2 nm, and 10.5 nm. Fifteen percent of HDL particles from overfed-involuted hens were located in bands with diameters between 20 and 21 nm. One hen, which involuted rapidly following initiation of overfeeding and exhibited a total plasma TG concentration of 75 g/l, had three separate HDL bands in the >17 nm range. As shown in Fig. 2, appearance of the 20 nm HDL band was most closely associated with involution as indicated by reduction in follicular weight at necropsy. The HDL profile shown in lane H has a transitional pattern midway between the profiles of the overfed layer in lane I and the overfed-involuted hen in lane F. Data from the hen shown in lane H were excluded from statistical analysis because egg production (yolk deposition) stopped for 5 days and then restarted, as judged by the presence of developing yolk follicles at necropsy.

Molecular weight estimation and fractional distribution of protein moieties in the d 1.041–1.210 g/ml fraction

Five primary protein bands were detected in the HDL fraction. As shown in **Table 8** and **Fig. 3**, a 28 kDa band, corresponding to apolipoprotein A-I (apoA-I), was the most abundant band in all groups. Control laying hens contained additional notable bands with apparent molecular masses of 16, 13, and 8 kDa. The 8 kDa band cor-

TABLE 6. Size characteristics of lipoprotein recovered from the d 1.021–1.041 g/ml density fraction of plasma taken from hens with differing rates of energy intake and egg production

	Hens Consuming Diet ad libitum	Hens Overfed to 150% Usual Daily Energy Intake	
Parameter	Laying Eggs (n = 18)	Laying Eggs (n = 8)	Involuted (n = 6)
Percentage of stainable protein with diameters			
>22 nm	69 ± 3	71 ± 3	33 ± 9
18–22 nm	31 ± 3	30 ± 3	68 ± 9
Lipoprotein diameters, nm			
Band 1	24.6 ± 0.2 (8)	24.6 ± 0.1 (4)	24.2 ± 0.2 (5)
Band 2	23.6 ± 0.1 (11)	23.4 ± 0.2 (4)	22.6 ± 0.1 (3)
Band 3	20.9 ± 0.1 (18)	21.0 ± 0.1 (8)	20.8 ± 0.1 (6)

Values are given as means ± SEM. Number of hens exhibiting protein band is shown in parentheses. Hens consumed a purified diet for 1–3 weeks prior to blood sampling. At weekly intervals, six control hens and six overfed hens were selected for analysis. Overfed hens were intubated with 150% of their usual energy intake as calculated from food consumption during 10 days preceeding intubation. Egg production in legend of Table 1. Total protein in the d 1.021–1.041 g/ml density fraction is shown in Table 3.

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TABLE 7. Size characteristics of lipoprotein recovered from the d 1.041–1.210 g/ml density fraction of plasma taken from hens with differing rates of energy intake and egg production

	Hens Consuming Diet ad libitum	Hens Overfed to 150% Usual Daily Energy Intake	
Parameter	Laying Eggs (n = 18)	Laying Eggs (n = 8)	Involuted (n = 6)
Percentage of stainable protein with diameters			
>17 nm	1 ± 1	1 ± 0.4	15 ± 4
12–17 nm	43 ± 5	30 ± 1.3	5 ± 1
8–12 nm	56 ± 5	69 ± 0.4	80 ± 3
Lipoprotein diameters, nm			
Band 1	ND	ND	20.3 ± 0.3 (6)
Band 2	13.1 ± 0.1 (16)	12.7 ± 0.1 (5)	ND
Band 3	11.7 ± 0.1 (16)	11.4 ± 0.1 (6)	ND
Band 4	ND	10.6 ± 0.2 (4)	10.5 ± 0.10 (6)
Band 5	9.9 ± 0.1 (18)	9.7 ± 0.1 (7)	9.2 ± 0.10 (6)
Band 6	ND	ND	8.8 ± 0.03 (3)

Values are given as means ± SEM. Number of hens exhibiting protein band is shown in parentheses. ND, not detected. Hens consumed a purified diet for 1–3 weeks prior to blood sampling. At weekly intervals, six control hens and six overfed hens were selected for analysis. Overfed hens were intubated with 150% of their usual energy intake as calculated from food consumption during 10 days preceeding intubation. Egg production in legend of Table 1. Total protein in the d 1.041–1.210 g/ml density fraction is shown in Table 4.

responds to monomeric apolipoprotein VLDL-II (2, 35). Approximately half of all hens had a 13 kDa protein. Additional trace bands were detected in some samples that had apparent molecular masses of 200, 105–125, 11 kDa, and 97 kDa. Overfeeding increased apoA-I, with the increase becoming statistically significant ($P < 0.05$) in overfed-involuted hens (Table 8). Involution of overfed hens resulted in a doubling of both the amount and fractional protein contribution of the 97 kDa protein. In contrast, involution decreased the amount of both the 16 kDa and 8 kDa protein bands to 25% and 35% of control values. The fractional contributions of these two proteins were also markedly reduced by involution (Table 8). Lane D of Fig. 3 shows that the LDL fraction of laying hen plasma contained considerable quantities of the 16 kDa protein as well as the 8 kDa protein, and a ladder of larger protein bands which probably are apoB-derived (2).

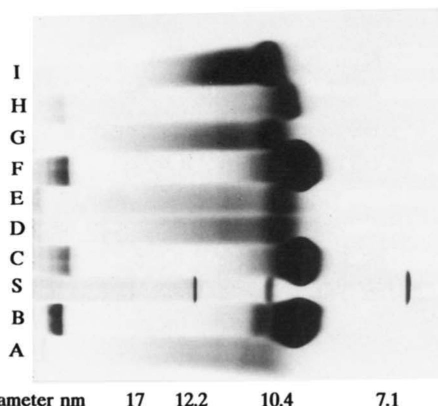

Fig. 2. Nondenaturing gradient gel electrophoresis of native high density lipoprotein (HDL) isolated at d 1.041–1.210 g/ml by ultracentrifugation. Diameters of HDL particles were determined by comparison of the migration of 20-μl aliquots isolated from 2 ml plasma, relative to co-migrating protein standards of known hydrated diameters as indicated at the bottom. Lane A: control hen, egg production = 80% (eggs per 100 hen days), follicle weight = 52.2 g; lane B: overfed-involuted hen, egg production = 0%, follicle weight = 6.5 g; lane S: protein standards; lane C: overfed-involuted hen, egg production = 60%, follicle weight = 15.7 g; lane D: control hen, egg production = 100%, follicle weight = 43.8 g; lane E: control hen, egg production = 90%, follicle weight = 52.9 g; lane F: overfed-involuted hen, egg production = 60%, follicle weight = 20 g; lane G: control hen, egg production = 80%, follicle weight = 50.4 g; lane H: overfed laying hen, egg production = 40%, follicle weight = 55.1 g; lane I: overfed laying hen, egg production = 90%, follicle weight = 50.3.

DISCUSSION

Yolk formation requires both mobilization and subsequent deposition of significant amounts of lipid within specific cells of selected tissues. Avians appear to achieve yolk-specific VLDL deposition by directly altering the physical properties of this class of lipoprotein (10, 36). In the present study, the essentiality of small diameter, LPL-resistant VLDL for continued yolk deposition was confirmed. Overfeeding generally increased VLDL diameter, but the response was individual in nature, as was the reproductive failure previously reported for these same hens (16). Hens that persisted in egg production despite overfeeding retained an average modal VLDL diameter of 34 nm, well within the putative size exclusion limits of the granulosa basal lamina (37) through which VLDL must pass in order to access the oocyte's receptor (38). In contrast, hens that involuted as a result of overfeeding exhibited an average modal VLDL diameter of 78 nm, much larger than the 27 nm mode of control hens. The smallest VLDL observed in overfed-involuted hens was 30.4 nm, showing that the present results were not due to

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TABLE 8. Concentration and fractional distribution of protein moieties in high density lipoproteins isolated from hens with differing rates of energy intake and egg production

		Quantities of Electrophoretically Separated Apoprotein, AU		
		Hens Consuming Diet ad libitum	Hens Overfed to 150% Usual Daily Energy Intake	
Band	M_R	Laying Eggs (n = 18)	Laying Eggs (n = 8)	Involuted (n = 6)
1	97.0 ± 0.42 (19)	137 ± 38 (7)	200 ± 42 (6)	529 ± 79 (6)
2	28.3 ± 0.27 (32)	5509 ± 792[a] (18)	7022 ± 1313[a,b] (8)	9981 ± 1864[b] (6)
3	15.8 ± 0.09 (28)	857 ± 143[a,b] (15)	1239 ± 515[b] (8)	198 ± 75[a] (5)
4	13.4 ± 0.16 (18)	387 ± 108 (10)	706 ± 172 (3)	456 ± 238 (3)
5	7.8 ± 0.07 (31)	722 ± 113[b] (18)	1083 ± 293[b] (8)	251 ± 36[a] (6)

Values are given as means ± SEM. Number of hens exhibiting the protein band is given in parentheses. Differences in the quantities of the 28.3, 15.8, and 7.8 kDa proteins were statistically analyzed by complete weighted least-square means analysis. Values for the 97 and 13.4 kDa proteins were not statistically analyzed due to the numbers of missing values. Hen information is provided in the legend for Table 7 and egg production data in the legend for Table 1. M_R, the apparent molecular mass (kDa), was calculated from mobility relative to protein standards shown in Fig. 3. Protein quantity is expressed as AU or arbitrary densitometer units of band densities visualized from 10 μl of isolated HDL stained with Coomassie Blue. Total protein content of HDL fraction is shown in Table 4.

[a,b]Values across rows not sharing a common superscript differ at $P \leq 0.05$.

simple weighting of the distribution by a few large particles. Further, the marked increase in VLDL size among overfed-involuted hens was not due to portomicron contamination as equally overfed hens that continued to lay eggs exhibited a monodisperse population of VLDL centered at 34 nm. Mechanical exclusion of enlarged VLDL may contribute to reproductive dysfunction in overfed hens. The granulosa basal lamina has been shown to exclude portomicrons (39). It is not known whether involution precedes or follows gross VLDL enlargement, but altered VLDL and HDL metabolism clearly precede, and probably contribute to, involution.

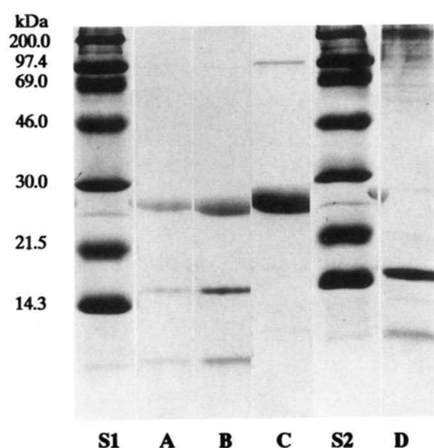

Fig. 3. SDS-polyacrylamide gel electrophoresis, under reducing conditions, of protein moieties recovered within an ultracentrifugally isolated d 1.041–1.210 g/ml high density lipoprotein (HDL) fraction. A 10-μl aliquot of ultracentrifugally isolated HDL, containing ~6 μg of protein was applied to each lane. In this gel system 0.1 μg protein was detectable as a single band, 2 μg protein within a single band overloads gel. Lanes S1 and S2: protein standards of known molecular mass (kDa) as indicated in margin; lane A: control laying hen, compare to S1; lane B: overfed laying hen, compare to S1; lane C: overfed-involuted hen, compare to S2; lane D: protein moieties of d 1.021–1.041 g/ml low density lipoprotein (LDL) fraction of laying hen, compare to S2. Lane D shown to indicate potential reservoir of the 14 kDa protein band found in lanes A and B.

The small particle size typical of laying hen VLDL is thought to enhance resistance to lipolysis by LPL. In accordance with this, the larger VLDL of either group of overfed hens appeared to be more susceptible to peripheral LPL-lipolysis as indicated by elevated plasma HDL content and reduced fractional plasma VLDL content (40, 41). Differences in density distributions were most obvious among comparisons between control laying hens and overfed-involuted hens, the two groups that also showed the greatest difference in modal VLDL diameter (27.1 vs. 78.3 nm). Compared to control hens, the percentage of VLDL present in plasma decreased from 82% to 64% in overfed-involuted hens, while both LDL (7 vs. 16%) and HDL (11 vs. 20%) doubled in their fractional contributions to plasma total lipoprotein. VLDL from both laying and non-laying overfed hens possessed increased amounts of FC and PL surface lipids, and a substitution of cholesteryl ester for triglyceride within core lipids. Such changes are indicative of enhanced intravascular metabolism (4, 41), and point to a loss among overfed hens of the normal control systems that create VLDL resistant to peripheral use and intravascular metabolism.

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Both reduction of VLDL diameter and its resistance to LPL hydrolysis are thought to be mediated by the estrogen-dependent synthesis of apolipoprotein VLDL-II (apoVLDL-II) (4, 7). It is unclear whether overfeeding has a direct progressive effect, or an abrupt secondary effect on VLDL properties and metabolic fate. Thus, despite VLDL size and compositional changes, the total concentration of LDL recovered from overfed laying hens was low and of similar size and composition to that of control hens. As noted earlier, "LDL" from egg laying groups may not be stereotypical (1), and in fact the recovered lipoprotein had size distributions typical of small VLDL or IDL. In contrast, greatly increased quantities of LDL, containing significantly more protein, and of a characteristic LDL size, were recovered from the plasma of overfed-involuted hens with large VLDL (3, 35). Thus, temporal definition of gross changes in VLDL susceptibility to hydrolysis as either pre- or post-involution remain to be established. A straightforward explanation of the present results is that overfeeding somehow suppresses plasma estrogen, halts apoVLDL-II synthesis, enlarging VLDL diameter, and returning lipoprotein metabolism to an immature or non-female state. Among involuted hens, loss of the small follicles that produce estrogen (42), and absence of an apoVLDL-II protein band in overfed non-laying hen HDL makes this an attractive possibility. However, in another study (43), plasma estrogen levels of overfed non-laying hens were elevated relative to controls. Also, overfeeding in the absence of estrogen does not produce the liver pathology (44) documented for these hens (16). Moreover, chronic estrogen treatment in roosters was shown to enhance liver apoA-I content (45) as was observed among all overfed hens. Recent work related to apoVLDL-II gene expression finds that estrogen removal may not be required for silencing of gene expression (46). Follicular reabsorption occurs rapidly, and thus reduction of plasma estrogen and decompensated VLDL enlargement may well be a terminal event in a much more dynamic process.

Overfeeding and involution had distinct effects on HDL metabolism within the laying hen. As previously noted, laying hens typically have reduced circulating levels of HDL that possess characteristics of both typical LDL and HDL (1). Resistance of laying hen VLDL to LPL hydrolysis probably contributes to the lower circulating levels of HDL since, presumably, relatively little redundant surface material is generated during their residency in plasma (41). Overfeeding generally increased plasma total HDL, enriching protein, but reducing phospholipid content of the particles. Involution was associated with the appearance of novel HDL particles ~20 nm in diameter. Designated as HDL_R to denote the association with yolk reabsorption (47), its appearance in plasma produced 4-fold increases in HDL-TG content, loss of lipoprotein within the 12-17 nm size range, and reduction in the diameter of the remaining HDL particles. This particle may be analogous to HDL_I or HDL_c found in humans (48). Triglyceride is presumed to reside principally within the HDL_R particle, and the fractional TG content of the isolated particle is expected to be greater than the 13% average of the complete HDL fraction. An analogous yolk absorption process occurs in the newly hatched chick, which is also reported to exhibit two similarly sized lipoprotein populations based on either apoB or apoA-I (49). Apolipoprotein profiles of overfed-involuted hens support the view that similarly sized LDL and HDL exist simultaneously as only minor traces of apoA-I were found in LDL and equally negligible amounts of large protein fragments, presumably derived from apoB (2), were seen in HDL apolipoprotein profiles.

Apolipoprotein content of HDL from controls and overfed layers were similar, and contained the expected bands of apoA-I and apoVLDL-II at 28.3 and 7.8 kDa, respectively. A protein band was typically observed at 15.8 kDa which could correspond to dimeric apoVLDL-II, but analytic conditions make this unlikely (2). Additional protein bands, similar in size to those reported earlier (1, 2), were variably observed and require additional studies with increased numbers of hens to assess their physiologic relevancy. The role of the 97 kDa protein in avian HDL metabolism is unknown at this time. Studies are currently underway to isolate and characterize the 97 kDa protein which is not Lp[a] (Dr. Jane Hoover-Plow, personal communication). Specific postendocytotic proteolysis of apoB accompanies VLDL deposition into yolk, but does not generate a 97 kDa fragment (50). Similarly, postendocytotic proteolysis of vitellogenin (51) may contribute to the variably observed protein bands with M_Rs in excess of 100 kDa. Reassociation of lipovitellin, a vitellogenin subunit, with plasma HDL has been shown for trout (52).

The redistribution of HDL particle sizes within overfed-involuted hens may well be mediated by elevations in levels of free fatty acids (53, 54) generated by enhanced VLDL hydrolysis generally, or at the surface of the reabsorbing yolk follicle specifically. This scenario is consistent with the association of stress with the onset of fatty liver hemorrhagic syndrome (17, 19), and is supported by the observation of cortisone-induced follicular collapse in laying hens (55). These findings suggest that apoVLDL-II gene expression may be transiently influenced by fluctuations in a variety of hormones or factors either directly or indirectly. Alternatively, gene expression may not be altered but the quantity of gene product may become inadequate to sustain alterations in lipoprotein metabolism necessary for yolk deposition. These latter possibilities are consistent with persistent but erratic lay patterns present in overfed (16) and spontaneously hyperphagic avian populations (56).

Overfeeding laying hens creates conditions favoring al-

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teration of VLDL biophysical properties and metabolic fate. Some hens are able to resist these conditions and continue to produce VLDL with requisite structural features for continued yolk deposition. The basis for this resistance is currently unknown, but may relate to an individual hen's ability to continue producing apoVLDL-II at a level sufficient to maintain the unique biophysical properties of yolk-targeted VLDL. Failed egg production (yolk deposition) in association with involution of the reproductive tract and reabsorption of the lipid material in the follicles produces specific alterations within the HDL including the formation of HDL_R. Investigation of the mechanisms underlying the responses of plasma lipoproteins in overfed hens may provide new insight into the role of lipoprotein physical properties in determining the metabolism of triacylglycerol-rich lipoprotein, and the process of reverse lipid (e.g., cholesterol) transport.

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