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Assessment of Egg Nutrient Compositional Changes and Residue in Eggs, Tissues, and Excreta Following Oral Administration of Atorvastatin to Laying Hens

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Laying hens were fed a control diet alone or with 0.06 g of atorvastatin, a synthetic 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, per 100 g of diet for 20 days. Compared to controls, egg yolks from treated hens contained greater amounts of amino acids and reduced levels of total fatty acids and cholesterol. In contrast, egg albumen amino acid contents were unaffected by dietary treatments. In a residue study, seven hens each received a single oral dose of ~20 μCi of [^{14}C]-atorvastatin. Approximately 71% of the radioactivity was recovered in the excreta and liver, whereas virtually no radioactivity was detected in kidney, heart, muscle, bile, plasma, or egg albumen at 15 days postdosing. Yolk radioactivity peaked at 4 days postdosing in six of the seven birds and was absent in eggs laid after day 10. Reminiscent of that of certain antibiotic drugs, the atorvastatin egg residue pattern appeared to coincide with the physiological pattern of daily yolk accretion within the ovary.

KEYWORDS: Atorvastatin; drug residues; egg nutrient composition; laying hens

INTRODUCTION

Chicken eggs are a concentrated source of dietary cholesterol. Until recently, recommendations to limit cholesterol intake, and thus egg intake, were based upon the premise that dietary cholesterol increases plasma cholesterol, which in turn increases heart disease risk (*1*). However, several epidemiological studies conducted during the past decade refuted this dogma and suggested that egg consumption and dietary cholesterol are not significant factors with regard to the risk of coronary heart disease (CHD) (reviewed in ref *1*). Nevertheless, the role of dietary cholesterol as a contributor to CHD risk for the general population remains controversial (*2*−*4*), and some groups (*5*) continue to recommend limiting egg consumption to help prevent CHD.

In contrast, there is little argument over the point that innate differences in responsiveness to dietary cholesterol exist in humans, and it has been demonstrated that between ~15 and 30% of the population lacks the ability to decrease cholesterol production in response to an increase in dietary cholesterol intake (*6*, *7*). However, the identification of true "hyper-responders" has been greatly hampered by both within-person fluctuations in serum cholesterol levels and the lack of a simple test to discriminate this subgroup from the rest of the population (*8*). Nevertheless, it is currently recommended that hyper-responders (suspected or confirmed) should monitor their intake of dietary cholesterol to reduce their risk of CHD (*9*).

Because eggs are an excellent source of many beneficial nutrients (*10*, *11*), a major reduction in the cholesterol content of eggs would yield a foodstuff of extremely high nutritional quality that could be more freely consumed by hyper-responders and others (e.g., patients with known CHD) who need to limit their intake of cholesterol. Unfortunately, most attempts to reduce the cholesterol content of eggs have been marginally successful at best (*12*, *13*). However, recent work in our laboratory (*14*−*16*) has shown that inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR), the key enzyme that controls the rate of cholesterol biosynthesis in the liver (*17*), are effective egg cholesterol-lowering agents when orally administered to laying hens. HMGR inhibitors, or "statins", were introduced in the late 1980s and rapidly became the therapeutic agents of choice in the treatment of hyperlipidemia in humans. In addition, new liver-selective statins, with greater atherogenic lipoprotein-lowering properties and good safety profiles, continue to be developed and evaluated in human clinical trials (*18*, *19*).

Similar to their effects in humans, statins vary in potency when orally administered to chickens on an equal weight basis. For example, in terms of maximal egg yolk cholesterol-lowering ability (percent reduction), lovastatin was shown to be least

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efficacious (−7%), atorvastatin to be most efficacious (−46%), and simvastatin to be of intermediate efficacy (−22%) when fed at 0.06 g/100 g of diet (*16*). Although the desired endpoints are different [e.g., serum low density lipoprotein (LDL) cholesterol lowering in humans and egg cholesterol reduction in chickens], they apparently are achieved by a common mechanism, namely, decreased hepatic very low density lipoprotein (VLDL) production and/or the up-regulation of hepatic LDL receptors (*20*). Although it has been demonstrated that a large and sustained reduction of egg cholesterol content can be achieved by the oral administration of atorvastatin to laying hens (*16*), two key concerns remain with regard to the possible future use of statins in the commercial production of low cholesterol eggs: (1) the effects of these agents on egg nutrient composition and (2) the potential transfer of drug to the egg. Thus, the present study was conducted to address these important issues, which would be expected to be of interest to consumers, egg producers, the pharmaceutical industry, and government regulatory agencies.

MATERIALS AND METHODS

Egg Nutrient Composition Study. Protocols for this experiment and the residue study (see below) were approved by the Purdue University Animal Care and Use Committee. Sixteen, 82-week-old, second-cycle White Leghorn hens were selected from the Purdue University laying flock. The hens were placed in individual 30 × 35 × 45 cm slant-back cages in an environmentally controlled room (24 °C and 16 h of light daily) and assigned to one of two dietary treatments: (1) a corn−soybean meal-based layer (control) ration (*14*) or (2) the control ration supplemented with 0.06 g of atorvastatin/100 g of diet. Atorvastatin, [*R*-(*R**,*R**)]-2-(4-fluorophenyl)-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1*H*-pyrrole-1-heptanoic acid calcium salt, was synthesized (*21*) and provided by Parke-Davis Pharmaceutical Research Division (Ann Arbor, MI). In the 8 days immediately preceding the experiment, the eight control birds averaged 92.2% hen-day egg production [(100 × number of eggs laid)/(number of hens × days)] and laid eggs weighing 63.5 g, whereas the eight hens assigned to the atorvastatin treatment averaged 92.2% hen-day egg production and laid eggs weighing 63.7 g. Hens were given free access to feed and water throughout the 20-day experiment, and feed intake, egg production, and egg weights were recorded daily on an individual basis.

Eggs were collected and weighed, and the yolks were separated as previously described (*16*). Beginning on day 0, pooled yolk and albumen samples were prepared every 2 days using one egg from each hen. Samples were lyophilized and subsequently analyzed (see below) for fatty acid (yolks only), cholesterol (yolks only), crude protein (yolks and albumens), and amino acid compositions (yolks and albumens). Unfortunately, due to unexplainable (and uncorrectable) errors in yolk weight measurements and lyophilization values during preparation of the 10th and 11th pools, egg composition data from days 18 and 20 are not presented.

Egg Cholesterol Analysis. Total cholesterol in the lyophilized egg yolk samples was determined according to AOAC Official Method 941.09 (*22*).

Egg Fatty Acid Analysis. One gram of each lyophilized egg yolk sample was homogenized with 15 mL of $CHCl_3$/MeOH (2:1) containing 50 mg of heptadecanoic acid (catalog no. N-17-A, Nu-Check Prep, Elysian, MN) as an internal standard for analytical calibration. The samples were then sonicated and filtered as previously described (*14*). One milliliter of each filtrate was transferred to a 15-mL screw-capped test tube, and the solvent was evaporated under a stream of nitrogen at 60 °C. Methyl esters of fatty acids were prepared using boron trifluoride (14% in methanol) (*23*) and analyzed by capillary gas−liquid chromatography. Briefly, the methyl esters were extracted in isooctane for chromatographic analysis by a Hewlett-Packard 6890 gas chromatograph equipped with a flame ionization detector and autosampler.

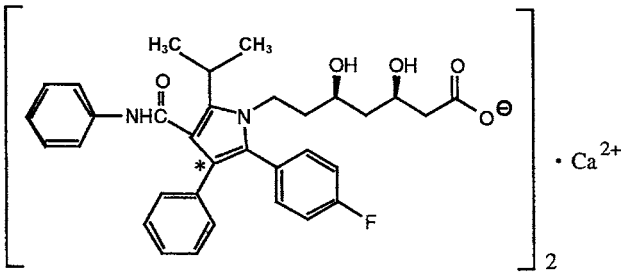

Figure 1. Structure of atorvastatin. The asterisk indicates the carbon-14 position.

Separations were achieved on a J&W DB-23 fused-silica capillary column (30 m × 0.25 mm i.d., film thickness = 0.25 μm) with a helium flow rate of 1.3 mL/min. The following temperature program was used: 50 °C for 2 min, 50−180 °C at 10 °C/min, 180 °C for 5 min, 180−240 °C at 5 °C/min, and 240 °C for 10 min. Samples (1-μL injection volume) were introduced by split injection (ratio = 100:1), with injector and detector temperatures maintained at 295 and 300 °C, respectively. An external reference standard containing known amounts of fatty acid methyl esters (Supelco 37 component FAME mix, catalog no. 4-7885-U) was used to obtain retention times and allow for the quantification of individual fatty acids, with the results adjusted for recovery of the internal standard.

Egg Crude Protein and Amino Acid Analyses. Crude protein was determined in the lyophilized egg yolk and albumen samples according to AOAC Official Method 990.03 (LECO combustion analysis) (*22*), and amino acid profiles were determined according to AOAC Official Method 982.30 E (a,b,c) (*22*).

[^{14}C]Atorvastatin Residue Study. To ensure acclimation to a change in location, from the Purdue University Poultry Research Center to the Life Science Animal Building on campus, eight 70-week-old, second-cycle White Leghorn hens were housed in individual 30 × 35 × 45 cm slant-back cages in an environmentally controlled room (24 °C and 16 h of light daily) for 28 days prior to the initiation of a residue study. A corn−soybean meal-based layer ration (*14*) and water were provided for ad libitum consumption.

Radiolabeled atorvastatin, with the ^{14}C label at the 3-position of the pyrrole moiety (*24*), was synthesized and provided by Parke-Davis Pharmaceutical Research, Ann Arbor, MI, as the calcium salt (**Figure 1**; specific activity = 60.7 μCi/mg). Radiochemical purity was 99.6%, and chemical purity was 99.8%. Beginning at 1:00 p.m. on day 0, seven hens each were given a single oral dose of atorvastatin (19.87 μCi per animal) in 1 mL of 0.2% dimethylacetamide/polyethylene glycol 200 (DMA:PEG 200) (*25*) using a 12-cm^3 syringe and a 20-gauge × 7.62 cm animal intubation needle with a 2.25 mm ball (Popper and Sons, Inc., New Hyde Park, NY);while one hen (control) received 1 mL of the DMA:PEG200 vehicle only. The tip of the needle was placed in the cranial end of the esophagus of the hen before pushing the plunger of the syringe to administer the dose. Immediately following administration of the [^{14}C]atorvastatin dose or vehicle, each hen received an additional 1 mL of vehicle, followed by 2 mL of distilled water. The hens were then placed back in their cages.

Eggs were collected daily, and the yolks and albumens were separated and frozen. On day 15 postdosing, all hens were euthanized via carbon dioxide inhalation and the following tissues/fluids were collected: heart, kidney, liver, muscle (pectoralis major), blood, and bile. Total excreta was also collected from each hen. Egg yolks, egg albumens, entire tissues, and excreta were weighed, lyophilized, and finely ground, and their weights were recorded. Duplicate samples (50 mg each of egg albumen, muscle, kidney, or heart; 100 mg each of egg yolk or liver) or quintuplicate samples (100 mg each of excreta) were burned directly in a Packard Combusto-Cone for 1 min using a Packard (Downers Grove, IL) model 307 Tri-Carb sample oxidizer. Duplicate blood plasma (250 μL) and bile (125 μL) samples were pipetted into a Combusto-Cone lined with a Packard Combusto-Pad, and an equal volume of Packard Combustaid was added prior to combustion. The $^{14}CO_2$ formed during combustion was trapped in a

Table 1. Body Weights, Feed Consumption, Egg Production, and Egg Weights of Hens Fed a Control (CON) Diet Alone or with 0.06 g/100 g of Atorvastatin (AT) for 20 Days[a,b]

diet	body wt change[c] (g)	feed consumption (g)	hen-day egg production[d] (%)	egg wt[e] (g)
CON	31.9 ± 95.9a	118.8 ± 12.1a	87.50 ± 11.33a	62.04 ± 2.41a
AT	78.7 ± 111.3a	100.3 ± 12.3b	59.38 ± 14.25b	55.64 ± 2.63b

[a] Values are means ± SD of eight hens. [b] Means within a column with no common letter differ significantly ($P \leq 0.05$). [c] The mean initial body weights of the control and atorvastatin-fed hens were1855.6 and 1988.1 g, respectively. [d] Hen-day egg production was calculated as (100 × no. of eggs laid)/(no. of hens × days). [e] Mean values of 140 and 95 eggs from the control and atorvastatin-fed hens, respectively.

column filled with Carbo-sorb E (Packard), a carbon dioxide absorbent, and formed a carbamate (*26*), which was subsequently flushed into a vial with a scintillator (Packard Permafluor E) for ^{14}C. Radioactivity was measured by liquid scintillation counting in a Packard model 1600TR liquid scintillation analyzer. The counts obtained were corrected for background, quench, and counter efficiency (*27*) and the results expressed as dpm. Although very minimal, adjustments were also made in the net dpm of the egg components, tissues, physiological fluids, and excreta from the seven [^{14}C]atorvastatin-treated hens by subtracting any radioactivity detected in the respective samples from the control hen.

The limit of detection for total radioactive residue in egg yolk or liver was ~10 ppb (~1 ng equivalent of atorvastatin in 100 mg of combusted sample); because 50 mg of albumen, muscle, kidney, or heart was combusted, the limit of detection for these samples was ~20 ppb. Recovery of the oxidation products was determined by adding a known amount of the [^{14}C]atorvastatin solution to triplicates of each sample type (egg component, tissue, or physiological fluid) before and after oxidation. The respective average pre- and postburn recoveries (percent) were 91.0 and 98.7 (egg yolk), 92.4 and 99.5 (egg albumen), 95.1 and 98.7 (liver), 91.1 and 100.0 (muscle), 95.3 and 96.0 (kidney), 95.6 and 101.1 (heart), 95.7 and 92.6 (blood plasma), 95.3 and 103.7 (bile), and 97.4 and 95.6 (excreta).

Statistics. Analysis of variance (*28*) was performed on all data using the General Linear Models procedure of the SAS Institute (*29*). Arc sine transformations (*28*) were performed on all percentage data. However, because the statistical patterns were similar for both transformed and nontransformed results, only the latter are presented. Individual treatment differences were tested by the Student−Newman−Keuls multiple-range test (*28*).

RESULTS

Egg Nutrient Composition Study. As compared to control hens, atorvastatin-treated birds exhibited significantly reduced mean 20-day feed intakes, hen-day egg production, and egg weights (**Table 1**). Changes in body weight were variable and not different between treatments. The weights of eggs and egg components also did not differ between groups on day 0 but, by day 6, significant reductions in egg weights and yolk weights were observed in the atorvastatin-fed hens versus the control hens (**Table 2**); a similar reduction in albumen weights was noted by day 8. Absolute shell weight data followed a similar trend, but because the two-way interaction (diet × day) was

Table 2. Weights of Eggs and Egg Components from Hens Fed a Control (CON) Diet Alone or with 0.06 g/100 g of Atorvastatin (AT)[a]

	egg wt[b] (g)		yolk wt[b] (g)		albumen wt[b] (g)		shell wt (g)	
day	CON	AT	CON	AT	CON	AT	CON	AT
0	61.65 ± 2.92ab	63.69 ± 2.69a	16.76 ± 1.33ab	18.20 ± 0.94a	39.04 ± 1.58ab	39.29 ± 2.57ab	5.85 ± 0.56	6.19 ± 0.27
2	60.43 ± 2.52abc	60.88 ± 2.09abc	16.70 ± 1.66ab	17.75 ± 1.20a	38.00 ± 1.30ab	37.36 ± 2.34ab	5.73 ± 0.48	5.77 ± 0.33
4	61.54 ± 4.20ab	58.00 ± 2.59bdc	16.66 ± 2.02ab	16.89 ± 1.06ab	39.13 ± 2.61ab	35.80 ± 2.66bc	5.75 ± 0.58	5.32 ± 0.59
6	63.39 ± 3.52ab	56.36 ± 3.65cde	16.93 ± 1.49ab	14.34 ± 0.53c	40.18 ± 2.77a	36.33 ± 3.44abc	5.82 ± 0.45	5.69 ± 0.60
8	61.39 ± 2.77ab	54.50 ± 2.97de	16.85 ± 1.62ab	14.81 ± 0.69bc	38.84 ± 1.68ab	33.98 ± 2.56cd	5.70 ± 0.42	5.71 ± 0.48
10	61.00 ± 2.76abc	53.07 ± 3.55e	16.59 ± 1.32ab	14.13 ± 0.62c	38.55 ± 1.72ab	33.48 ± 3.06cd	5.86 ± 0.47	5.59 ± 0.59
12	60.91 ± 3.04abc	51.56 ± 4.08e	16.50 ± 1.23ab	13.88 ± 1.32c	38.65 ± 1.89ab	32.21 ± 2.83d	5.76 ± 0.43	5.46 ± 0.54
14	60.55 ± 2.71abc	52.93 ± 4.36e	16.40 ± 1.36ab	14.29 ± 1.88c	38.45 ± 1.55ab	33.23 ± 2.57cd	5.70 ± 0.47	5.40 ± 0.59
16	61.90 ± 3.68ab	51.65 ± 4.56e	16.81 ± 1.30ab	13.88 ± 1.89c	38.45 ± 2.60ab	31.76 ± 2.58d	6.65 ± 0.71	6.01 ± 0.78
Pr > *F*								
diet[c]	**		**		**		*	
day[c]	**		**		**		**	
diet × day[c]	**		**		**		NS	

[a] Values are means ± SD of one egg from each of eight hens per diet collected during consecutive 2-day periods. [b] Dietary means with no common letter differ significantly ($P \leq 0.05$). [c] NS = $P > 0.05$; * = $P \leq 0.05$; ** = $P \leq 0.01$.

Table 3. Relative Percentages of Components of Eggs from Hens Fed a Control (CON) Diet Alone or with 0.06 g/100 g of Atorvastatin (AT)[a]

	yolk (%)		albumen (%)		shell (%)	
day	CON	AT	CON	AT	CON	AT
0	27.16 ± 1.35	28.62 ± 1.81	63.35 ± 0.89	61.65 ± 1.92	9.49 ± 0.73	9.73 ± 0.29
2	27.60 ± 1.88	29.18 ± 2.05	62.91 ± 1.54	61.33 ± 2.30	9.49 ± 0.79	9.49 ± 0.69
4	27.03 ± 2.26	29.18 ± 2.51	63.62 ± 1.74	61.66 ± 2.21	9.35 ± 0.90	9.16 ± 0.84
6	26.92 ± 2.12	25.51 ± 1.26	63.83 ± 1.88	64.37 ± 2.09	9.25 ± 0.44	10.12 ± 1.09
8	27.41 ± 1.79	27.22 ± 1.48	63.29 ± 1.30	62.30 ± 2.01	9.30 ± 0.81	10.48 ± 0.67
10	27.18 ± 1.33	26.64 ± 1.46	63.20 ± 0.75	62.86 ± 1.39	9.62 ± 0.89	10.50 ± 0.80
12	27.07 ± 1.06	26.94 ± 1.63	63.46 ± 0.48	62.45 ± 1.67	9.47 ± 0.75	10.61 ± 0.78
14	27.05 ± 1.35	26.92 ± 1.74	63.52 ± 0.66	62.84 ± 1.96	9.43 ± 0.86	10.23 ± 1.02
16	27.16 ± 1.47	26.80 ± 1.86	62.10 ± 1.45	61.57 ± 2.20	10.74 ± 1.03	11.63 ± 1.21
Pr > *F*						
diet[b]	NS		**		**	
day[b]	*		*		**	
diet × day[b]	NS		NS		NS	

[a] Values are means ± SD of one egg from each of eight hens per diet collected during consecutive 2-d periods. [b] NS = $P > 0.05$; * = $P \leq 0.05$; ** = $P \leq 0.01$.

Table 4. Albumen Indispensable Amino Acid Contents (As Is Basis) of Eggs from Hens Fed a Control (CON) Diet Alone or with 0.06 g/100 g of Atorvastatin (AT)[a]

		amino acid (mg/g of albumen)									
day	diet	Arg	His	Ile	Leu	Lys[b]	Met	Phe[b]	Thr[b]	Trp[b]	Val[b]
0	CON	6.11 ± 0.20	2.57 ± 0.12	5.56 ± 0.04	9.20 ± 0.10	7.13 ± 0.05ab	3.87 ± 0.01	6.62 ± 0.18ab	4.68 ± 0.02abcd	1.89 ± 0.01ab	7.20 ± 0.10abcde
	AT	6.62 ± 0.96	2.51 ± 0.10	5.54 ± 0.05	9.19 ± 0.24	7.02 ± 0.04ab	3.83 ± 0.11	6.52 ± 0.07ab	4.72 ± 0.05abc	1.99 ± 0.04a	7.02 ± 0.18bcdef
2	CON	5.99 ± 0.03	2.47 ± 0.03	5.39 ± 0.24	9.16 ± 0.32	6.91 ± 0.12ab	3.79 ± 0.15	6.41 ± 0.05ab	4.65 ± 0.13bcd	1.79 ± 0.06ab	6.89 ± 0.03def
	AT	5.97 ± 0.06	2.51 ± 0.04	5.51 ± 0.14	9.15 ± 0.17	6.97 ± 0.05ab	3.83 ± 0.07	6.47 ± 0.00ab	4.66 ± 0.05bcd	1.80 ± 0.10ab	7.05 ± 0.06bcdef
4	CON	6.09 ± 0.06	2.51 ± 0.05	5.50 ± 0.15	9.30 ± 0.29	7.07 ± 0.08ab	3.88 ± 0.08	6.56 ± 0.04ab	4.78 ± 0.07abc	1.98 ± 0.07a	6.98 ± 0.04bcdef
	AT	5.83 ± 0.07	2.42 ± 0.02	5.35 ± 0.22	8.88 ± 0.12	6.75 ± 0.07b	3.77 ± 0.07	6.32 ± 0.02b	4.54 ± 0.03d	1.81 ± 0.05ab	6.79 ± 0.10ef
6	CON	6.12 ± 0.02	2.50 ± 0.01	5.51 ± 0.10	9.32 ± 0.35	7.06 ± 0.11ab	3.88 ± 0.11	6.48 ± 0.11ab	4.80 ± 0.03abc	1.89 ± 0.07ab	7.09 ± 0.06bcdef
	AT	5.85 ± 0.03	2.40 ± 0.00	5.36 ± 0.21	9.06 ± 0.22	6.79 ± 0.12b	3.78 ± 0.09	6.30 ± 0.15b	4.62 ± 0.03cd	1.72 ± 0.09b	6.68 ± 0.05f
8	CON	6.23 ± 0.04	2.58 ± 0.02	5.62 ± 0.21	9.51 ± 0.23	7.22 ± 0.15a	3.87 ± 0.20	6.63 ± 0.19ab	4.84 ± 0.01ab	1.95 ± 0.03a	7.33 ± 0.16abc
	AT	6.12 ± 0.02	2.54 ± 0.00	5.64 ± 0.13	9.48 ± 0.27	7.12 ± 0.13ab	4.02 ± 0.09	6.68 ± 0.12ab	4.79 ± 0.06abc	1.86 ± 0.03ab	7.15 ± 0.02abcde
10	CON	6.01 ± 0.02	2.49 ± 0.01	5.48 ± 0.11	9.26 ± 0.21	6.74 ± 0.12b	3.83 ± 0.10	6.43 ± 0.14ab	4.73 ± 0.01abc	1.85 ± 0.03ab	7.06 ± 0.12bcdef
	AT	6.08 ± 0.02	2.53 ± 0.02	5.62 ± 0.17	9.26 ± 0.39	6.84 ± 0.13ab	3.94 ± 0.10	6.60 ± 0.13ab	4.75 ± 0.02abc	1.85 ± 0.04ab	7.13 ± 0.21abcde
12	CON	6.06 ± 0.09	2.47 ± 0.04	5.26 ± 0.10	9.17 ± 0.20	6.75 ± 0.05b	3.88 ± 0.09	6.41 ± 0.02ab	4.79 ± 0.00abc	1.93 ± 0.02ab	6.92 ± 0.04cdef
	AT	6.24 ± 0.06	2.58 ± 0.05	5.84 ± 0.15	9.63 ± 0.27	6.97 ± 0.02ab	4.03 ± 0.04	6.78 ± 0.07a	4.85 ± 0.02a	1.96 ± 0.08a	7.50 ± 0.22a
14	CON	6.06 ± 0.19	2.48 ± 0.12	5.55 ± 0.08	9.24 ± 0.04	6.75 ± 0.19b	3.84 ± 0.06	6.44 ± 0.06ab	4.72 ± 0.09abc	1.89 ± 0.04ab	7.24 ± 0.03abcd
	AT	6.05 ± 0.13	2.50 ± 0.04	5.39 ± 0.10	9.09 ± 0.51	6.70 ± 0.11b	3.94 ± 0.13	6.55 ± 0.02ab	4.75 ± 0.02abc	1.85 ± 0.02ab	7.02 ± 0.05bcdef
16	CON	6.12 ± 0.01	2.54 ± 0.04	5.54 ± 0.01	9.34 ± 0.14	6.84 ± 0.01ab	3.82 ± 0.06	6.50 ± 0.08ab	4.74 ± 0.03abc	1.81 ± 0.00ab	7.34 ± 0.05abc
	AT	6.26 ± 0.03	2.59 ± 0.00	5.74 ± 0.09	9.28 ± 0.57	6.99 ± 0.07ab	4.00 ± 0.06	6.74 ± 0.11a	4.82 ± 0.02ab	1.88 ± 0.07ab	7.39 ± 0.18ab
Pr > *F*											
diet[c]		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
day[c]		NS	NS	NS	NS	**	NS	*	**	**	**
diet × day[c]		NS	NS	NS	NS	*	NS	*	**	*	**

[a] Values are means ± SD of duplicate analyses of pooled samples consisting of one egg from each of eight hens per diet collected during consecutive 2-day periods. [b] Dietary means with no common letter differ significantly ($P \leq 0.05$). [c] NS = $P > 0.05$; * = $P \leq 0.05$; ** = $P \leq 0.01$.

Table 5. Albumen Dispensable Amino Acid Contents (As Is Basis) of Eggs from Hens Fed a Control (CON) Diet Alone or with 0.06 g/100 g of Atorvastatin (AT)[a]

		amino acid (mg/g of albumen)							
day	diet	Ala[b]	Asp[b]	Cys	Glu	Gly	Pro	Ser	Tyr[b]
0	CON	6.30 ± 0.14abc	10.62 ± 0.05ab	3.04 ± 0.04	13.68 ± 0.19	3.68 ± 0.03	3.99 ± 0.17	6.30 ± 0.51	4.33 ± 0.06ab
	AT	6.33 ± 0.08abc	10.64 ± 0.02ab	3.06 ± 0.10	13.68 ± 0.28	3.67 ± 0.05	4.03 ± 0.05	6.44 ± 0.64	4.28 ± 0.00ab
2	CON	6.24 ± 0.02bc	10.43 ± 0.17bc	3.00 ± 0.15	13.47 ± 0.52	3.62 ± 0.10	3.84 ± 0.10	6.40 ± 0.69	4.21 ± 0.10ab
	AT	6.36 ± 0.15abc	10.56 ± 0.01ab	2.99 ± 0.19	13.64 ± 0.35	3.67 ± 0.04	3.81 ± 0.06	6.39 ± 0.51	4.24 ± 0.05ab
4	CON	6.37 ± 0.04abc	10.70 ± 0.06ab	3.05 ± 0.10	13.80 ± 0.46	3.72 ± 0.11	3.97 ± 0.04	6.58 ± 0.65	4.28 ± 0.03ab
	AT	6.10 ± 0.01c	10.20 ± 0.03c	2.87 ± 0.13	13.47 ± 0.15	3.52 ± 0.10	3.83 ± 0.03	6.44 ± 0.16	4.12 ± 0.03b
6	CON	6.32 ± 0.07abc	10.74 ± 0.05ab	3.07 ± 0.12	13.92 ± 0.22	3.72 ± 0.10	4.05 ± 0.10	6.84 ± 0.14	4.30 ± 0.09ab
	AT	6.12 ± 0.07c	10.25 ± 0.05c	2.86 ± 0.15	13.52 ± 0.22	3.56 ± 0.07	3.92 ± 0.08	6.70 ± 0.11	4.13 ± 0.11ab
8	CON	6.43 ± 0.06ab	10.91 ± 0.08a	3.19 ± 0.01	14.18 ± 0.27	3.77 ± 0.10	4.19 ± 0.10	6.86 ± 0.04	4.38 ± 0.12ab
	AT	6.44 ± 0.02ab	10.71 ± 0.08ab	3.06 ± 0.02	14.23 ± 0.23	3.70 ± 0.07	4.13 ± 0.13	6.95 ± 0.18	4.34 ± 0.10ab
10	CON	6.26 ± 0.03bc	10.63 ± 0.04ab	3.10 ± 0.02	13.86 ± 0.22	3.69 ± 0.07	3.89 ± 0.01	6.78 ± 0.01	4.23 ± 0.10ab
	AT	6.35 ± 0.11abc	10.66 ± 0.10ab	3.05 ± 0.03	14.15 ± 0.24	3.66 ± 0.15	3.96 ± 0.06	6.85 ± 0.04	4.31 ± 0.07ab
12	CON	6.30 ± 0.04abc	10.77 ± 0.16ab	3.12 ± 0.04	13.84 ± 0.11	3.64 ± 0.01	3.90 ± 0.22	6.65 ± 0.26	4.20 ± 0.01ab
	AT	6.58 ± 0.07a	10.91 ± 0.02a	3.07 ± 0.02	14.66 ± 0.30	3.80 ± 0.08	4.11 ± 0.04	6.99 ± 0.01	4.40 ± 0.05a
14	CON	6.30 ± 0.02abc	10.63 ± 0.21ab	3.07 ± 0.08	13.96 ± 0.03	3.66 ± 0.05	3.93 ± 0.13	6.67 ± 0.06	4.25 ± 0.05ab
	AT	6.34 ± 0.04abc	10.74 ± 0.14ab	2.98 ± 0.10	14.07 ± 0.09	3.61 ± 0.05	3.91 ± 0.21	6.66 ± 0.25	4.25 ± 0.05ab
16	CON	6.39 ± 0.06abc	10.74 ± 0.07ab	3.04 ± 0.05	14.09 ± 0.25	3.72 ± 0.01	4.02 ± 0.04	6.70 ± 0.07	4.26 ± 0.04ab
	AT	6.50 ± 0.17ab	10.87 ± 0.07a	3.13 ± 0.09	14.50 ± 0.38	3.73 ± 0.11	4.08 ± 0.01	6.87 ± 0.05	4.39 ± 0.03ab
Pr > *F*									
diet[c]		NS	*	*	NS	NS	NS	NS	NS
day[c]		**	**	NS	**	NS	*	NS	*
diet × day[c]		**	**	NS	NS	NS	NS	NS	*

[a] Values are means ± SD of duplicate analyses of pooled samples consisting of one egg from each of eight hens per diet collected during consecutive 2-day periods. [b] Dietary means with no common letter differ significantly ($P \leq 0.05$). [c] NS = $P > 0.05$; * = $P \leq 0.05$; ** = $P \leq 0.01$.

not significant, comparisons among all 18 means were not made. When expressed on a relative percentage basis and compared to day 0 values, the yolk content of eggs from atorvastatin-fed hens tended to decrease (although $P > 0.05$), whereas albumen and shell values generally increased beginning on day 6 (**Table 3**).

Albumen indispensable amino acid contents were not affected ($P > 0.05$) by dietary treatment (**Table 4**), although a significant main effect of day and a significant two-way interaction (diet × day) were observed for Lys, Phe, Thr, Trp, and Val. Similarly, with the exceptions of Asp and Cys, albumen dispensable amino acid contents were also unaffected ($P > 0.05$) by dietary treatment (**Table 5**). Significant day effects were noted for Glu and Pro, whereas a significant two-way interaction (diet × day) was noted for Ala, Asp, and Tyr. However, the overall biological significance of the above changes is questionable, as the absolute albumen amino acid contents at days 0 and 16 were generally very similar for both dietary treatments. In contrast, marked increases ($P \leq 0.05$) in the absolute content of all yolk indispensable amino acids, except Met and Trp, were noted by day 8 in eggs from statin-fed hens, with Met levels elevated by day 14 (**Table 6**). In addition, significant day effects

Table 6. Yolk Indispensable Amino Acid Contents (As Is Basis) of Eggs from Hens Fed a Control (CON) Diet Alone or with 0.06 g/100 g of Atorvastatin (AT)[a]

		amino acid (mg/g of yolk)									
day	diet	Arg[b]	His[b]	Ile[b]	Leu[b]	Lys[b]	Met[b]	Phe[b]	Thr[b]	Trp	Val[b]
0	CON	10.36 ± 0.10f	3.65 ± 0.14c	7.71 ± 0.31e	12.38 ± 0.10b	11.37 ± 0.03de	3.65 ± 0.14d	6.27 ± 0.10cd	7.14 ± 0.31c	3.24 ± 0.24	8.84 ± 0.20de
	AT	10.40 ± 0.03ef	3.67 ± 0.03c	7.68 ± 0.14e	12.32 ± 0.17b	11.36 ± 0.10de	3.63 ± 0.10d	6.27 ± 0.10cd	7.13 ± 0.24c	3.10 ± 0.71	8.93 ± 0.07de
2	CON	10.57 ± 0.24def	3.74 ± 0.14c	7.83 ± 0.34de	12.69 ± 0.48b	11.60 ± 0.17de	3.74 ± 0.07d	6.42 ± 0.28cd	7.39 ± 0.14c	2.94 ± 0.38	9.02 ± 0.31de
	AT	10.44 ± 0.03ef	3.69 ± 0.03c	7.83 ± 0.10de	12.39 ± 0.00b	11.42 ± 0.27de	3.62 ± 0.07d	6.27 ± 0.00cd	7.18 ± 0.27c	3.08 ± 0.27	8.99 ± 0.10de
4	CON	10.27 ± 0.14f	3.62 ± 0.03c	7.46 ± 0.51e	12.24 ± 0.14b	11.28 ± 0.07e	3.55 ± 0.07d	6.19 ± 0.07d	7.22 ± 0.31c	2.78 ± 0.54	8.57 ± 0.31e
	AT	10.43 ± 0.14ef	3.69 ± 0.03c	7.63 ± 0.20e	12.46 ± 0.14b	11.40 ± 0.07de	3.62 ± 0.14d	6.30 ± 0.10cd	7.37 ± 0.31c	3.45 ± 0.44	8.81 ± 0.17de
6	CON	10.31 ± 0.14f	3.63 ± 0.07c	7.52 ± 0.10e	12.29 ± 0.17b	11.31 ± 0.07de	3.56 ± 0.17d	6.20 ± 0.14d	7.16 ± 0.14c	2.82 ± 0.34	8.64 ± 0.00de
	AT	10.58 ± 0.17def	3.76 ± 0.07c	7.66 ± 0.20e	12.55 ± 0.10b	11.42 ± 0.07de	3.66 ± 0.14d	6.34 ± 0.17cd	7.40 ± 0.17c	2.43 ± 0.03	8.91 ± 0.14de
8	CON	10.80 ± 0.31def	3.79 ± 0.10c	7.63 ± 0.07e	12.92 ± 0.24b	11.72 ± 0.31de	3.94 ± 0.44cd	6.45 ± 0.03cd	7.56 ± 0.31c	3.14 ± 0.48	8.89 ± 0.34de
	AT	12.16 ± 0.24c	4.32 ± 0.10b	8.48 ± 0.07cd	14.40 ± 0.14a	12.90 ± 0.17c	4.39 ± 0.28bcd	7.18 ± 0.17b	8.31 ± 0.10b	2.42 ± 0.21	9.92 ± 0.07c
10	CON	11.04 ± 0.17de	3.93 ± 0.14c	8.06 ± 0.14cde	12.96 ± 0.21b	11.82 ± 0.10de	4.08 ± 0.21bcd	6.70 ± 0.14cd	7.40 ± 0.10c	2.77 ± 0.07	9.22 ± 0.14de
	AT	12.64 ± 0.32b	4.62 ± 0.14a	8.64 ± 0.07bc	14.32 ± 0.95a	13.18 ± 0.18bc	4.62 ± 0.00abc	7.45 ± 0.14ab	8.59 ± 0.00ab	2.98 ± 0.00	10.20 ± 0.18bc
12	CON	11.06 ± 0.10de	3.91 ± 0.10c	7.90 ± 0.03de	13.11 ± 0.38b	11.82 ± 0.21de	3.87 ± 0.31cd	6.71 ± 0.07cd	7.59 ± 0.07c	3.77 ± 0.52	9.02 ± 0.03de
	AT	13.08 ± 0.14a	4.75 ± 0.04a	9.08 ± 0.32ab	15.01 ± 0.32a	13.53 ± 0.28ab	4.55 ± 0.32abc	7.69 ± 0.14a	8.85 ± 0.07a	4.15 ± 0.32	10.54 ± 0.18ab
14	CON	11.17 ± 0.17d	3.99 ± 0.07c	8.13 ± 0.14cde	13.10 ± 0.28b	11.93 ± 0.21d	3.92 ± 0.31cd	6.74 ± 0.17c	7.47 ± 0.10c	3.43 ± 0.03	9.30 ± 0.14d
	AT	13.38 ± 0.25a	4.89 ± 0.18a	9.36 ± 0.04a	15.09 ± 0.56a	13.88 ± 0.11a	5.16 ± 0.28a	7.82 ± 0.18a	8.94 ± 0.07a	2.83 ± 0.63	10.95 ± 0.18a
16	CON	11.09 ± 0.21de	3.92 ± 0.07c	7.82 ± 0.10de	13.31 ± 0.07b	11.76 ± 0.21de	3.82 ± 0.34cd	6.66 ± 0.10cd	7.60 ± 0.00c	2.59 ± 0.38	8.93 ± 0.03de
	AT	13.39 ± 0.35a	4.88 ± 0.18a	9.12 ± 0.07ab	14.82 ± 0.84a	13.78 ± 0.07a	4.71 ± 0.28ab	7.78 ± 0.21a	9.00 ± 0.04a	3.74 ± 0.46	10.68 ± 0.25a
Pr > *F*											
diet[c]		**	**	**	**	**	**	**	**	NS	**
day[c]		**	**	**	**	**	**	**	**	*	**
diet × day[c]		**	**	**	**	**	**	**	**	NS	**

[a] Values are means ± SD of duplicate analyses of pooled samples consisting of one egg from each of eight hens per diet collected during consecutive 2-day periods. [b] Dietary means with no common letter differ significantly ($P \leq 0.05$). [c] NS = $P > 0.05$; * = $P \leq 0.05$; ** = $P \leq 0.01$.

Table 7. Yolk Dispensable Amino Acid Contents (As Is Basis) of Eggs from Hens Fed a Control (CON) Diet Alone or with 0.06 g/100 g of Atorvastatin (AT)[a]

		amino acid (mg/g of yolk)							
day	diet	Ala[b]	Asp[b]	Cys	Glu	Gly[b]	Pro	Ser[b]	Tyr[b]
0	CON	7.09 ± 0.03d	12.18 ± 0.24c	2.74 ± 0.20	12.90 ± 0.03	3.96 ± 0.03c	4.97 ± 0.24	10.00 ± 0.75c	6.20 ± 0.00g
	AT	7.06 ± 0.14d	12.15 ± 0.48c	2.74 ± 0.20	12.85 ± 0.51	3.99 ± 0.07c	5.16 ± 0.03	9.87 ± 0.58c	6.22 ± 0.03g
2	CON	7.20 ± 0.14d	12.40 ± 0.07c	2.80 ± 0.17	13.01 ± 0.52	4.06 ± 0.03c	5.03 ± 0.17	10.48 ± 0.38c	6.42 ± 0.21efg
	AT	7.16 ± 0.10d	12.29 ± 0.55c	2.77 ± 0.31	12.92 ± 0.48	4.05 ± 0.14c	5.01 ± 0.07	9.93 ± 0.61c	6.24 ± 0.03g
4	CON	7.01 ± 0.14d	12.07 ± 0.44c	2.69 ± 0.20	12.65 ± 0.44	3.94 ± 0.07c	4.97 ± 0.03	10.37 ± 0.75c	6.24 ± 0.07g
	AT	7.12 ± 0.10d	12.29 ± 0.44c	2.78 ± 0.31	13.02 ± 0.31	4.06 ± 0.07c	5.10 ± 0.17	10.51 ± 0.79c	6.30 ± 0.03fg
6	CON	6.97 ± 0.07d	12.00 ± 0.44c	2.70 ± 0.24	12.58 ± 0.37	3.91 ± 0.07c	4.96 ± 0.07	10.24 ± 0.30c	6.23 ± 0.10g
	AT	7.18 ± 0.07d	12.29 ± 0.48c	2.75 ± 0.27	12.98 ± 0.44	4.07 ± 0.10c	5.23 ± 0.17	10.65 ± 0.48c	6.34 ± 0.10fg
8	CON	7.51 ± 0.51cd	13.26 ± 1.54bc	3.04 ± 0.41	14.78 ± 2.60	4.25 ± 0.41bc	5.70 ± 0.68	11.38 ± 1.06bc	6.52 ± 0.00def
	AT	8.26 ± 0.38bc	14.63 ± 0.66ab	3.33 ± 0.45	17.04 ± 2.06	4.83 ± 0.28ab	6.19 ± 0.31	12.88 ± 0.70ab	7.18 ± 0.03c
10	CON	7.52 ± 0.34cd	13.54 ± 0.62bc	3.13 ± 0.38	15.53 ± 1.78	4.34 ± 0.24bc	5.78 ± 0.62	10.73 ± 0.07c	6.58 ± 0.10de
	AT	8.76 ± 0.18ab	15.42 ± 0.18a	3.72 ± 0.49	18.52 ± 0.42	5.09 ± 0.11a	6.88 ± 0.53	13.38 ± 0.18a	7.42 ± 0.11b
12	CON	7.59 ± 0.28cd	13.59 ± 0.45bc	2.99 ± 0.31	15.61 ± 1.58	4.35 ± 0.17bc	5.76 ± 0.45	11.40 ± 0.17bc	6.66 ± 0.07d
	AT	8.98 ± 0.25a	15.72 ± 0.39a	3.50 ± 0.32	18.41 ± 1.21	5.15 ± 0.18a	7.02 ± 0.60	13.93 ± 0.71a	7.72 ± 0.04a
14	CON	7.67 ± 0.17cd	13.73 ± 0.41bc	2.99 ± 0.31	15.78 ± 1.17	4.41 ± 0.17bc	5.87 ± 0.59	10.98 ± 0.52c	6.69 ± 0.03d
	AT	9.14 ± 0.35a	15.99 ± 0.56a	3.97 ± 0.14	18.77 ± 1.61	5.31 ± 0.28a	7.35 ± 0.77	13.75 ± 0.00a	7.80 ± 0.00a
16	CON	7.60 ± 0.21cd	13.58 ± 0.45bc	2.95 ± 0.27	15.66 ± 1.47	4.38 ± 0.17bc	5.83 ± 0.51	11.55 ± 0.31bc	6.73 ± 0.07d
	AT	9.07 ± 0.28a	15.94 ± 0.46a	3.64 ± 0.25	18.62 ± 1.44	5.33 ± 0.25a	7.46 ± 0.88	14.13 ± 0.14a	7.86 ± 0.11a
Pr > *F*									
diet[c]		**	**	**	**	**	**	**	**
day[c]		**	**	**	**	**	**	**	**
diet × day[c]		**	*	NS	NS	**	NS	**	**

[a] Values are means ± SD of duplicate analyses of pooled samples consisting of one egg from each of eight hens per diet collected during consecutive 2-day periods. [b] Dietary means with no common letter differ significantly ($P \leq 0.05$). [c] NS = $P > 0.05$; * = $P \leq 0.05$; ** = $P \leq 0.01$.

and two-way (diet × day) interactions were observed for all amino acids except Trp. A similar pattern was observed for the yolk dispensable amino acid contents, with the exception that the two-way interaction (diet × day) was not significant for Cys, Glu, and Pro (**Table 7**).

The lack of effect of atorvastatin on albumen amino acid contents, and its marked influence on yolk amino acid levels, are reflected in the crude protein contents of these egg components (**Figure 2**). Moreover, as yolk amino acid and crude protein contents increased, concomitant reductions in several fatty acids [myristoleic (C14:1), palmitic (C16:0), palmitoleic (C16:1), linoleic (C18:2), and linolenic (C18:3)] (**Table 8**) and total fatty acids (**Figure 2**) were observed. In addition, atorvastatin-associated increases in stearic acid (C18:0), arachidonic acid (C20:4), and lignoceric acid (C24:0) were also noted; however, on an absolute basis, changes in the amounts of the latter two fatty acids were fairly minor (**Table 8**). Although significant, the effect of atorvastatin on oleic acid (C18:1) was somewhat confounded by the fact that, for an unknown reason, there was a 4 mg/g of yolk difference between dietary groups at day 0. In addition, eicosenoic acid (C20:1) values (<1 mg/g of yolk) were highly variable and difficult to interpret.

Table 8. Fatty Acid Content (As Is Basis) of Egg Yolks from Hens Fed a Control (CON) Diet Alone or with 0.06 g/100 g of Atorvastatin (AT)[a]

		fatty acid (mg/g of yolk)[b]									
day	diet	C14:1[c]	C16:0[c]	C16:1[c]	C18:0[c]	C18:1[c]	C18:2[c]	C18:3[c]	C20:1[c]	C20:4[c]	C24:0[c]
0	CON	2.28 ± 0.05	78.24 ± 0.22c	9.97 ± 1.44ab	21.55 ± 0.12g	101.22 ± 0.85	37.90 ± 0.48d	1.22 ± 0.06ab	0.51 ± 0.02abc	6.11 ± 0.00	0.84 ± 0.05c
	AT	2.21 ± 0.04	79.32 ± 0.09b	9.28 ± 0.00ab	23.70 ± 0.16f	105.59 ± 1.03	37.15 ± 0.13d	1.21 ± 0.06ab	0.61 ± 0.04ab	6.64 ± 0.06	1.20 ± 0.30c
2	CON	2.22 ± 0.03	79.23 ± 0.08b	10.00 ± 1.44ab	22.10 ± 0.07g	102.85 ± 0.43	37.83 ± 0.02d	1.20 ± 0.06ab	0.60 ± 0.05ab	6.29 ± 0.05	0.80 ± 0.00c
	AT	2.20 ± 0.02	80.21 ± 0.09a	9.32 ± 0.06ab	23.97 ± 0.04f	106.90 ± 1.58	37.83 ± 0.33d	1.24 ± 0.07ab	0.56 ± 0.06abc	6.77 ± 0.07	0.97 ± 0.01c
4	CON	2.15 ± 0.09	76.07 ± 0.16ef	9.68 ± 0.86ab	22.23 ± 0.59g	102.82 ± 1.84	38.26 ± 0.56d	1.63 ± 0.58ab	0.85 ± 0.41a	6.38 ± 0.20	0.74 ± 0.02c
	AT	2.08 ± 0.03	74.95 ± 0.48g	8.94 ± 1.50ab	26.07 ± 0.47e	105.46 ± 0.25	35.43 ± 0.60e	1.47 ± 0.59ab	0.53 ± 0.06abc	7.49 ± 0.67	1.27 ± 0.16c
6	CON	2.13 ± 0.04	75.52 ± 0.10fg	9.59 ± 0.77ab	22.13 ± 0.62g	101.53 ± 1.90	40.44 ± 0.66c	1.32 ± 0.07ab	0.59 ± 0.00ab	6.61 ± 0.56	0.86 ± 0.22c
	AT	1.88 ± 0.03	70.22 ± 0.12h	8.15 ± 0.97ab	29.31 ± 0.01c	105.43 ± 0.01	29.88 ± 0.03f	1.17 ± 0.58ab	0.48 ± 0.04abc	7.88 ± 0.74	1.35 ± 0.19c
8	CON	2.06 ± 0.01	77.40 ± 0.98cd	8.86 ± 0.60ab	22.27 ± 0.49g	100.55 ± 0.90	42.29 ± 0.39a	1.46 ± 0.06ab	0.56 ± 0.03abc	6.67 ± 0.35	0.96 ± 0.27c
	AT	0.88 ± 1.25	68.30 ± 0.05i	6.99 ± 1.39ab	32.63 ± 0.45a	106.02 ± 0.14	28.82 ± 0.11g	0.65 ± 0.00bc	0.22 ± 0.31bc	8.42 ± 0.68	1.84 ± 0.20b
10	CON	2.08 ± 0.02	76.61 ± 0.48de	9.75 ± 1.32ab	21.12 ± 0.02g	97.43 ± 0.04	42.18 ± 0.88a	1.91 ± 0.61a	0.51 ± 0.02abc	5.87 ± 0.19	0.78 ± 0.03c
	AT	1.64 ± 0.06	64.39 ± 0.37j	6.02 ± 1.38b	33.23 ± 0.85a	103.24 ± 1.93	23.43 ± 0.05h	nd[d] c	nd c	3.84 ± 5.43	2.27 ± 0.18a
12	CON	2.16 ± 0.04	76.70 ± 0.58de	9.93 ± 1.33ab	21.01 ± 0.02g	97.67 ± 1.65	41.80 ± 0.17ab	1.47 ± 0.03ab	0.55 ± 0.02abc	5.93 ± 0.01	0.80 ± 0.06c
	AT	1.57 ± 0.02	63.31 ± 0.01k	5.58 ± 1.85b	31.16 ± 0.44b	103.61 ± 0.31	22.04 ± 0.07i	nd c	0.44 ± 0.00abc	7.52 ± 0.04	2.31 ± 0.28ab
14	CON	2.17 ± 0.01	77.82 ± 0.14c	11.27 ± 0.09a	21.17 ± 0.04g	97.62 ± 0.83	41.12 ± 0.18bc	1.50 ± 0.05ab	0.25 ± 0.35bc	5.90 ± 0.05	0.83 ± 0.01c
	AT	1.47 ± 0.05	62.37 ± 0.67k	5.24 ± 2.15b	29.01 ± 0.72c	101.83 ± 2.18	20.53 ± 0.06j	nd c	0.48 ± 0.08abc	7.45 ± 0.10	2.18 ± 0.02ab
16	CON	2.23 ± 0.04	78.26 ± 0.04c	11.47 ± 0.07a	20.93 ± 0.02g	96.52 ± 0.65	40.37 ± 0.02c	1.50 ± 0.06ab	0.54 ± 0.02abc	5.61 ± 0.10	0.82 ± 0.02c
	AT	1.54 ± 0.01	63.23 ± 0.02k	5.39 ± 1.89b	27.62 ± 0.08d	100.90 ± 0.38	21.43 ± 0.01i	nd c	nd c	7.30 ± 0.00	2.40 ± 0.27a
Pr > *F*											
diet[e]		**	**	**	**	**	**	**	**	NS	**
day[e]		*	**	NS	**	**	**	**	**	NS	**
diet × day[e]		NS	**	*	**	NS	**	**	*	NS	**

[a] Values are means ± SD of duplicate analyses of pooled samples consisting of one egg from each of eight hens per diet collected during consecutive 2-day periods. [b] Dietary means within a column with no common letter differ significantly ($P \leq 0.05$). [c] C14:1, myristoleic acid; C16:0, palmitic acid; C16:1, palmitoleic acid; C18:0, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; C18:3, linolenic acid; C20:1, eicosenoic acid; C20:4, arachidonic acid; C24:0, lignoceric acid. [d] Not detected. [e] NS = $P > 0.05$; * = $P \leq 0.05$; ** = $P \leq 0.01$.

Table 9. Total Cholesterol Contents of Eggs from Hens Fed a Control (CON) Diet Alone or with 0.06 g/100 g of Atorvastatin (AT)[a,b]

	cholesterol (mg/g of yolk)		cholesterol (mg/yolk)	
day	CON	AT	CON	AT
0	10.53 ± 0.10i	12.18 ± 0.09cde	176.5 ± 1.7g	221.7 ± 1.6a
2	12.57 ± 0.08a	12.10 ± 0.08cdef	210.0 ± 1.3c	214.7 ± 1.5b
4	12.07 ± 0.06def	11.93 ± 0.07f	201.1 ± 1.0e	201.5 ± 1.2e
6	11.70 ± 0.03g	11.02 ± 0.06h	198.1 ± 0.5f	158.0 ± 0.9h
8	12.05 ± 0.07ef	10.61 ± 0.04i	203.0 ± 1.1e	157.1 ± 0.6h
10	12.21 ± 0.02bcde	10.17 ± 0.12j	202.6 ± 0.3e	143.7 ± 1.7ij
12	12.39 ± 0.07b	10.46 ± 0.07i	204.4 ± 1.1de	145.1 ± 1.0ij
14	12.27 ± 0.02bcd	10.20 ± 0.06j	201.3 ± 0.4e	145.8 ± 0.9i
16	12.29 ± 0.02bc	10.26 ± 0.02j	206.6 ± 0.3d	142.4 ± 0.2j
Pr > *F*				
diet[c]		**		**
day[c]		**		**
diet × day[c]		**		**

[a] Values are means ± SD of duplicate analyses of pooled samples consisting of one egg from each of eight hens per diet collected during consecutive 2-day periods. [b] Among the 18 means within each diet–day combination, values with no common letter differ significantly ($P \leq 0.05$). [c] NS = $P > 0.05$; * = $P \leq 0.05$; ** = $P \leq 0.01$.

Reductions in yolk cholesterol contents, which were noted as early as day 2 in eggs from the atorvastatin-fed hens, appeared to reach a nadir by day 10, with a maximal cholesterol lowering of ~35% (**Table 9**). For some unknown reason, the day 0 value for the control group (10.53 mg/g of yolk, 176.5 mg/yolk) was artificially low. Although reanalysis of the sample confirmed the abnormally low value (data not shown), it was nonetheless considered to be artifactual, on the basis of over a decade of work by one of the authors (R.G.E.) in the area of egg lipid modification (*14*−*16*, *30*, *31*).

[^{14}C]Atorvastatin Residue Study. Fifteen-day postdosing mean hen-day egg production, egg weights, egg component weights, and egg component relative percentages were fairly similar between the seven [^{14}C]atorvastatin-treated hens and the control hen (**Table 10**). This effect was not unexpected, as the hens were of similar ages and similar production status, were fed the same control diet, and received the same amount of vehicle, with or without [^{14}C]atorvastatin.

Table 10. Mean 15-Day Egg Production, Egg Weights, and Egg Component Weights and Relative Percentages from Control (CON) and [^{14}C]Atorvastatin (AT)-Treated Hens

treatment	hen-day egg production[a] (%)	egg wt[b] (g)	yolk wt[b]	albumen wt[b]	shell wt[b]
				in grams	
CON	73.33	63.39 ± 2.62	19.30 ± 0.61	38.01 ± 2.54	6.08 ± 0.33
[^{14}C]AT	77.14 ± 16.26	62.08 ± 3.67	18.60 ± 1.02	38.01 ± 2.68	5.46 ± 0.42
				as % of egg wt	
CON			30.49 ± 1.41	59.91 ± 1.71	9.60 ± 0.66
[^{14}C]AT			29.98 ± 0.97	61.21 ± 1.19	8.81 ± 0.67

[a] Hen-day egg production was calculated as (100 × no. of eggs laid)/(no. of hens × days) for one control hen and seven [^{14}C]AT-treated hens. [b] Values are means of 11 eggs from one CON hen and 81 eggs from seven [^{14}C]AT-treated hens.

In concert with the metabolism of atorvastatin in mammals, most of the radioactivity was recovered in the excreta and liver (70.67 and 0.51% of the total dose, respectively) of the [^{14}C]-atorvastatin-treated hens (**Table 11**). In contrast, <0.01% of the total dose was found in kidney, heart, muscle, bile, plasma, or egg albumen (data not shown). Yolk radioactivity, which peaked at 4 days postdosing in six of the seven birds and was absent in eggs laid after day 10 (**Figure 3**), cumulatively accounted for 0.07% of the total dose (**Table 11**). Moreover, no more than 0.03% of the total dose (11881 dpm; equivalent to ~89 ng of radioequivalents) was detected in any one yolk. Hen 3, which failed to lay an egg on day 4, had peak yolk radioactivity values on day 5 (**Figure 3**).

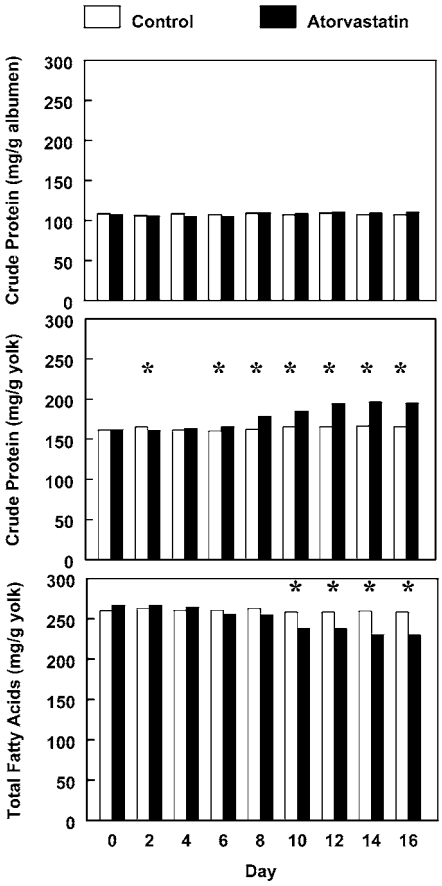

Figure 2. Influence of atorvastatin on egg albumen and egg yolk crude protein contents and egg yolk total fatty acid contents. Values are means of duplicate analyses of pooled samples consisting of one egg from each of eight hens per diet collected during consecutive 2-day periods. Asterisks indicate significant differences ($P \leq 0.05$) between treatment means within a 2-day period.

Table 11. Mean Total Egg Yolk, Liver, and Excreta Radioactivity and Dose Recovery in Hens Following Administration of a Single Oral Dose of [^{14}C]Atorvastatin[a,b]

sample	radioactivity (total dpm)	recovery (% of dose)
egg yolks	32,773 ± 7,237[c]	0.07 ± 0.02
liver[d]	225,092 ± 38,919	0.51 ± 0.09
excreta[e]	31,172,683 ± 2,019,258	70.67 ± 4.58

[a] Values are means ± SD of seven hens. [b] Each bird received 19.87 μCi (44,111,400 dpm) of [^{14}C]atorvastatin via gavage. [c] Value represents the mean of the total dpm detected in the yolks of all eggs laid by each of the seven hens during the 15-day postdose period. [d] Entire livers from each hen were obtained on day 15 postdose. [e] Total excreta collected for 15 days postdose.

DISCUSSION

The present study is the first to examine the changes in relative component percentages and yolk and albumen macronutrient contents of eggs from statin-fed hens. In addition, the distribution of ^{14}C residues in eggs, tissues, and excreta of laying hens administered a single oral dose of radiolabeled atorvastatin is described for the first time. Associated changes in plasma and liver lipids, hepatic expression of key genes regulating cholesterol synthesis and VLDL assembly, and immunoreactive HMGR levels will be reported elsewhere (Elkin et al., unpublished data).

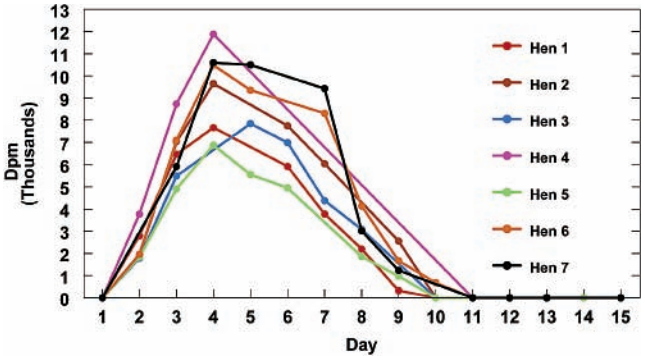

Figure 3. Total ^{14}C residue in egg yolks from seven hens each administered a single oral dose of [^{14}C]atorvastatin. Each data point represents the mean of duplicate determinations. Hens 1–7 laid 14, 11, 11, 7, 14, 13, and 11 eggs, respectively, during the 15-day postdosing period; radioactivity was found only in the yolks of 7, 6, 6, 3, 7, 8, and 6 eggs, respectively, all of which were laid between days 2 and 10.

Atorvastatin markedly altered the cholesterol, amino acid/crude protein, and fatty acid/crude fat contents of egg yolk, but had little effect on egg albumen amino acid/crude protein levels. This was not unexpected, as previous work from our laboratory showed that atorvastatin, as well as an atorvastatin analogue, modified the composition and secretion of hepatically synthesized, cholesterol- and triglyceride-rich VLDL (*15*, *16*), the main yolk precursor macromolecule (*32*). In contrast, even if atorvastatin or an active metabolite reached the oviduct, albumen composition would be expected to be unchanged because (1) albumen is virtually cholesterol-free and fat-free and (2) HMGR, the key enzyme of the cholesterol biosynthetic pathway that is inhibited by atorvastatin, is not involved in albumen synthesis. The above compositional changes are, for the most part, explainable and, from a human nutrition standpoint, very favorable because the eggs from atorvastatin-treated hens were lower in cholesterol and fat and relatively richer in high-quality protein. However, an explanation is lacking for the inconsistent response in individual yolk fatty acid contents (i.e., reductions in myristoleic, palmitic, palmitoleic, linoleic, and linolenic acids, with concomitant increases in the levels of yolk stearic, arachidonic, and lignoceric acids; **Table 8**).

All statins undergo varying degrees of metabolism in humans, primates, rodents, and dogs (reviewed in refs *24* and *33*); however, we are unaware of any previous reports in birds. Oxidation via the hepatic cytochrome P450 system, which produces ortho- and para-hydroxylated derivatives, is the predominant metabolic route in rats, dogs, and humans (*20*, *24*, *33*). Other biotransformation pathways include lactonization (of statin hydroxy acids), β-oxidation of the dihydroxyheptanoic or heptenoic side chain, and glucuronidation (*33*). The mouse is the only species to extensively use the β-oxidation pathway (*34*). Animal mass balance studies with HMGR inhibitors, including atorvastatin, have shown that these drugs and their metabolites are mainly excreted in the feces, following absorption, metabolism, and preferential excretion of unchanged drug and metabolites into the bile (*24*). Moreover, biliary recycling is an important component in the metabolism of atorvastatin and its excretion profile (*24*).

Atorvastatin calcium is only very slightly soluble in distilled water or pH 7.4 phosphate buffer (*35*). Thus, the detection of radioactivity in egg yolks in the present study was not totally unexpected, because the lipid solubility of a drug generally influences its deposition into lipid-rich egg yolk (*36*) and most likely resulted from the oocytic uptake of hepatically synthesized

VLDL particles containing atorvastatin residues in their hydrophobic cores. In addition, the atorvastatin egg residue pattern appeared to coincide with the physiological pattern of daily yolk accretion within the ovary and was reminiscent of that of certain antibiotic drugs (*37*, *38*). Moreover, the present findings also support the suggestion of Donoghue et al. (*37*, *38*) that the relative pattern of residue uptake in developing yolks may be somewhat universal and irrespective of the class of drug. However, factors, such as absorption, distribution, biotransformation, and excretion, which vary according to the drug type, as well as route of application, dose, duration of exposure, and species of bird, would be expected to affect the absolute amounts of residue present in laid eggs (*39*). Thus, although the atorvastatin ^{14}C residue pattern observed herein in laid eggs was very similar to the pattern of oxytetracylcine and ampicillin uptake by developing follicles observed by Donoghue et al. (*37*), other drugs may produce different residue patterns in laid eggs.

Previous attempts in our laboratory to detect statin residues in eggs using HPLC were unsuccessful (*14*, *15*). However, these methods had estimated detection limits of ~10−15 ng per injection, which were ~1 order of magnitude greater than that for ^{14}C in the present work (1 ng of [^{14}C]atorvastatin equated to ~134 dpm). Last, because ~30% of the total dose was not accounted for, it is possible that atorvastatin accumulated in additional tissues/organs besides those examined (e.g., adrenal, adipose, lung, bone marrow, and brain), although in a [^{14}C]-atorvastatin (CI-981) distribution study in rats, these tissues generally took up <1 μg of radioequivalents/g of tissue (*25*). Alternately, atorvastatin may have been metabolized via an alternate, as yet unreported, pathway unique to avians that resulted in the radiolabeled carbon atom being expired as radioactive carbon dioxide. Further studies will be necessary to address these possibilities and to identify the yolk and liver atorvastatin metabolites.

In contrast to these very positive nutrient compositional changes imparted by atorvastatin, egg production and egg weights were negatively affected, which agreed with previous work from our laboratory (*16*). However, this would not necessarily preclude the future use of atorvastatin, one of its analogues, or another statin by the egg industry. In addition to the obligate garnering of government regulatory approval for use of statins in laying hen diets, the cost of the compound(s) and the market premium received for the resulting "designer eggs" will be the other main factors that dictate whether this technology will be commercially adopted.

ABBREVIATIONS USED

CHD, coronary heart disease; DMA:PEG 200, dimethylacetamide/polyethylene glycol 200; HMGR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; LDL, low density lipoprotein; VLDL, very low density lipoprotein.

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