Plasma Yolk Precursor Dynamics during Egg Production by Female Greater Scaup (*Aythya marila*): Characterization and Indices of Reproductive State

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ABSTRACT

We characterized dynamics of the plasma yolk precursors vitellogenin (VTG), very-low-density lipoprotein (total VLDL-TG), and VLDL particle size distribution during egg production by female greater scaup (order: Anseriformes, *Aythya marila*). We also evaluated VTG and total VLDL-TG as physiological indices of reproductive state. Mean (± 1 SE) plasma concentrations of VTG and total VLDL-TG for females with nondeveloped ovaries were 0.58 ± 0.05 μg Zn mL^{-1} and 3.75 ± 0.29 mmol TG L^{-1}, respectively. Yolk precursor concentrations increased rapidly to maximum levels in association with small increases in ovary mass during rapid follicle growth. Mean concentrations of VTG and total VLDL-TG for females with a full ovarian follicle hierarchy were 3.38 ± 0.40 μg Zn mL^{-1} and 7.31 ± 2.56 mmol TG L^{-1}, respectively. Concentrations of VTG and total VLDL remained elevated throughout the laying cycle and decreased markedly by 3 d into incubation. Individual reproductive state (non-egg producing vs. egg producing) was more accurately identified by plasma profiles of VTG (90%) than by those of total VLDL-TG (74%). Greater scaup VLDL particle sizes during egg production were within the range for predicted yolk-targeted VLDL size (25–44 nm). We conclude that plasma profiles of VTG and total VLDL-TG can be used as nonlethal, physiological indices of reproductive state in greater scaup and should be of great utility to a variety of evolutionary, ecological, and applied conservation studies of reproduction in waterfowl.

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Introduction

Studies of cost of reproduction in birds have traditionally focused only on the chick-rearing stage of breeding (Monaghan and Nager 1997). More recent studies, wherein investment in egg production itself has been experimentally manipulated, have provided empirical evidence demonstrating costs of egg production in terms of decreased future fecundity, chick provisioning ability, and survival (Monaghan et al. 1995, 1998; Nager et al. 2001; Visser and Lessells 2001). However, we still know very little about the physiological mechanism(s) underlying such costs (Williams 2005; Harshman and Zera 2007; Zera et al. 2007). One putative physiological mechanism in egg-producing females involves the marked changes in lipoprotein metabolism, including variation in the types and absolute concentrations of circulating lipoproteins, with transient high plasma levels of yolk precursors, as well as the changes in the macromolecular structure of the predominant lipoproteins associated with yolk formation (Walzem 1996; Williams 2005). Recent studies in humans have suggested a link between lipoprotein phenotype and longevity (Barzilai et al. 2003), and in bees (*Apis mellifera*), the high-density lipoprotein vitellogenin (VTG) may play a role in regulatory control of aging (Amdam et al. 2004). Thus, changes in lipoprotein metabolism in laying birds might be linked to key components of the cost of reproduction such as survival, via as-yet-unknown pathways.

In birds, the two main plasma yolk precursors, VTG and yolk-targeted very-low-density lipoprotein (VLDLy), are produced by the avian liver in response to increasing plasma estrogen concentrations during reproduction (Deeley et al. 1975; Chan et al. 1976; Johnson 2000). The primary source of egg yolk protein is VTG, whereas VLDLy is the primary source of egg yolk lipid (Deeley et al. 1975; Chan et al. 1976). Consequently, circulation levels of both VTG and VLDLy are elevated in the domestic hen (order: Galliformes, *Gallus domesticus*) during egg production. Therefore, yolk precursors are readily sequestered during rapid follicle growth (RFG; the phase of avian egg production when ovarian follicles import large amounts of yolk precursors from circulation to accumulate yolk lipid and protein; for a review, see Johnson 2000) to provide

nutrients and energy for developing embryos (Deeley et al. 1975; Chan et al. 1976).

Studies of the domestic hen have shown that an increase in plasma triacylglycerol concentration during egg production is associated with an almost complete shift in VLDL synthesis; VLDLy particles, ranging in size from 20 to 55 nm, are produced instead of the larger (30 to >200 nm) generic VLDL particles that are supplied to the hen's tissues (Walzem 1996; Speake et al. 1998; Walzem et al. 1999). The VLDLy particle becomes the predominant circulating lipoprotein in egg-laying chickens because of its high rate of secretion (Williams et al. 2001), lipase resistance (Griffin and Hermier 1988; Boyle-Roden and Walzem 2005), and reliance on ovarian receptors for its clearance from circulation (Ho et al. 1974). Therefore, plasma concentrations of yolk-targeted VLDLy increase to specifically ensure energy availability for the developing embryo. Pore size of the granulosa basal lamina acts as a size-selective sieve, allowing only VLDLy particles with sizes in the range 25–44 nm (hereafter, sVLDLy range) to filter across the follicular wall (Perry and Gilbert 1979; Griffin and Perry 1985). Salvante et al. (2007) suggested that, for the nonpoultry zebra finch (order: Passeriformes, *Taeniopygia guttata*) and potentially for free-living species in general, aspects of lipoprotein metabolism during egg production differs from the domestic hen model such that nonpoultry females maintain production of larger diameter generic VLDL particles during egg production to meet their own energy requirements under naturally variable breeding conditions. Thus, yolk precursor or lipoprotein dynamics might provide the basis for a trade-off between self-maintenance in laying females, current reproduction, and egg or offspring quality.

Although many aspects of yolk precursor production have been well studied in captive galliforms (e.g., Deeley et al. 1975; Chan et al. 1976; Wang and Williams 1982; Walzem 1996; Walzem et al. 1999) and passerines such as the zebra finch (e.g., Williams 2000, 2001; Salvante and Williams 2002, 2003), relatively little is known about yolk precursor dynamics during egg production in free-living species (e.g., Vanderkist et al. 2000; Challenger et al. 2001). Furthermore, no study to date has examined yolk precursor dynamics in detail during egg production by an anseriform in the wild. Thus, we studied the dynamics of VTG and total VLDL-TG (i.e., generic and VLDLy) during the laying cycle in free-living female greater scaup (order: Anseriformes, *Aythya marila*; hereafter, scaup), a species that incurs particularly high energetic and nutritional costs of egg production in comparison with other waterfowl (Flint and Grand 1999), not only because of precocity but also because of large clutch and egg sizes (Alisauskas and Ankney 1992). In this study, we characterize circulating concentrations of both yolk precursors in relation to ovarian follicular development during egg production using surrogate markers of VTG and total VLDL-TG, and we predict that yolk precursor concentrations are tightly coupled with the egg production phase of the breeding cycle (sensu Vanderkist et al. 2000; Challenger et al. 2001). We also describe circulating yolk precursor concentrations among discrete reproductive states and examine the predictive utility of plasma profiles of VTG and total VLDL-

TG as nonlethal, physiological methods for determining reproductive state in female waterfowl. Additionally, we characterize VLDL particle size distributions among discrete reproductive states in female scaup to better understand lipoprotein metabolism during egg production in a free-living anseriform. On the basis of work by Salvante et al. (2007), we predict that there is a shift in VLDL particle size distribution associated with egg production that differs from the shifts in particle size distribution characterized for the domestic laying hen (Walzem 1996).

Material and Methods

Field Methods

This study was conducted on female scaup breeding along the lower Kashunuk River (60°20′N, 165°35′W), in the Yukon-Kuskokwim Delta in western Alaska, during the breeding seasons of 2002 and 2003. Our work was performed in accordance with permits from the U.S. Fish and Wildlife Service, the State of Alaska Department of Fish and Game, and the Yukon Delta National Wildlife Refuge. In addition, our study conformed to guidelines of the Canadian Committee on Animal Care (Simon Fraser University [SFU] Animal Care Permit 637B-02). Approximately two female scaup were collected each day between May 19 and June 21, 2002 ($n = 58$), and between May 15 and June 19, 2003 ($n = 54$), because these dates included the egg production phase of the breeding cycle for most individuals (Flint et al. 2006). In addition, three female scaup wintering along the British Columbia coast were collected (on March 8, 2003) in accordance with a permit from Environment Canada to obtain yolk precursor data for wintering birds. At the time of collection, a blood sample (≤ 5 mL) was immediately taken from the heart via a heparinized syringe. A 0.6-mL subsample of blood was expelled into an ethylenediaminetetraacetic acid (EDTA)-coated Vacutainer containing 30 μL of 0.5 mol L^{-1} disodium EDTA, while the remaining blood was transferred to a heparin-treated Vacutainer. Both blood samples were stored cool until centrifugation.

Reproductive organs were dissected within 24 h of collection. Ovaries were weighed and subsequently preserved in 10% formalin. Plasma for VTG and total VLDL-TG analyses was separated from heparin-treated blood via centrifugation within 24 h of collection and stored frozen. Plasma for VLDL particle size analysis was separated from EDTA-treated blood also via centrifugation within 24 h of collection and refrigerated in EDTA-coated tubes treated with 1 μL sodium azide per 100 μL plasma to prevent mold formation.

Laboratory Methods

Formalin-preserved ovaries were dissected as described by Gorman et al. (2007) to determine reproductive state for individual females. Reproductive states were defined as winter (females

collected in coastal British Columbia [$n = 3$]), nondeveloped (largest ovarian follicle, <9.36 mm and <0.26 g dry mass [$n = 32$]), RFG (largest ovarian follicle, ≥9.36 mm and ≥0.26 g dry mass, as well as no postovulatory follicles present [$n = 32$]), laying (≥1 postovulatory follicle and a preovulatory follicle >35.6 mm or the presence of an oviductal egg [$n = 30$]), and incubation (a hierarchy of regressing postovulatory follicles and the presence of a brood patch [$n = 10$]).

Plasma samples were assayed for vitellogenic zinc (Zn; zinc kit, Wako Chemicals) and total triglycerides (as surrogates for the yolk precursors VTG and total VLDL-TG, respectively; for 2002 samples: triglyceride E kit, Wako Chemicals; for 2003 samples: Glycerol Reagents A and B, Sigma) at SFU following the methods in Mitchell and Carlisle (1991). All assays were measured using a Biotek 340i microplate reader. Because two different kits were used to estimate plasma total VLDL-TG, we validated quantification by the Wako and Sigma kits using plasma obtained from captive, egg-producing female zebra finches and found no significant difference in the concentrations measured by each kit (Wako: 11.91 ± 1.11 mg mL^{-1}; Sigma: 12.79 ± 1.19 mg mL^{-1} [values are sample means ±1 SE]; $n = 29$; T. D. Williams, unpublished data). Intra-assay coefficients of variation for VTG, using a laying domestic hen plasma pool ($n = 10$), and for total VLDL-TG, using a 19-wk-old domestic hen plasma pool ($n = 40$), were 7.5% and 7.9%, respectively. Interassay coefficients of variation were 9.7% for VTG ($n = 15$) and 6.3% for total VLDL-TG ($n = 7$).

Particle size distribution analysis was conducted on lipoproteins isolated as the density $d < 1.020$ g mL^{-1} fraction of plasma exactly as described in Walzem et al. (1994). On the basis of studies of galliforms, this density fraction includes VLDL, VLDLy, and some hydrolytic products of VLDL such as intermediate-density lipoproteins, if present (Hermier et al. 1985, 1989). Lipoprotein particle diameters were measured by dynamic laser light-scattering analysis using a Microtrac series 250 ultrafine particle analyzer with a laser probe tip (UPA-250; Microtrac, Clearwater, FL) and Microtrac software (Honeywell, Washington, PA) as previously described (Hickman et al. 1994; Veniant et al. 2000). Particle size distribution was used throughout our study (Chen et al. 1999). In some cases, raw particle diameter distributions were converted to population percentiles to facilitate statistical comparisons (for details, see Walzem et al. 1994; Salvante et al. 2007).

Statistical Analyses

All statistical analyses were performed in SAS, version 8.0 (SAS Institute 1999). To describe yolk precursor concentrations as physiological indices of reproductive state, general linear models were used to examine variation in yolk precursor concentrations (VTG, total VLDL-TG) in relation to discrete reproductive state explanatory variables (winter, nondeveloped, RFG, laying, incubation; $n = 100$). Year was also included as an explanatory variable to assess interannual variation in yolk precursor dynamics. Information-theoretic methods were used to

direct model selection (Burnham and Anderson 2002). The set of candidate models for each yolk precursor included biologically plausible combinations of explanatory variable groupings, with and without the year term, as well as an equal means model, resulting in 34 candidate models to describe variation in VTG and total VLDL-TG concentrations individually (see Gorman 2005 for candidate model set). For each model, Akaike's Information Criterion (AIC) including a correction for small sample size (AIC$_c$) was calculated. Candidate models were compared using ΔAIC$_c$ values, which is the difference between the AIC$_c$ value for a particular model and the lowest AIC$_c$ value (i.e., the most parsimonious model) within the candidate model set. Models with ΔAIC$_c$ values ≤2 were considered to be well supported (Burnham and Anderson 2002). In addition, AIC$_c$ weight (AIC$_c$$W$) values were used to consider the likelihood that a particular supported model was the best model (Burnham and Anderson 2002). This modeling approach is analogous to performing a traditional ANOVA to determine differences in yolk precursor concentration among discrete groups (i.e., reproductive state), where AIC model selection criteria are used instead of an alpha level to determine which model(s) best describe variation in our data. Parameter estimation for the year term included calculation of model-averaged parameter estimates based on AIC$_c$$W$ values for all candidate models (Burnham and Anderson 2002). Standard error values for the year term were based on unconditional variances calculated across the same models.

We also tested the predictive utility of yolk precursors (VTG and total VLDL-TG individually) to identify nonreproductive females (i.e., females with nondeveloped ovaries as well as incubating females) and reproductive females (RFG and laying). Because year was not an important parameter in describing variation in VTG or total VLDL concentrations (see "Yolk Precursors as Physiological Indices of Reproductive State" in "Results"), our analysis first used yolk precursor data from 2002 to discern VTG and total VLDL-TG threshold concentrations (i.e., individuals with lower concentrations than the threshold value were classified as nonreproductive, whereas individuals with higher concentrations were classified as reproductive) that maximized the correct classification of reproductive state. This involved iteratively assigning yolk precursor data to nonreproductive or reproductive groups and comparing the predicted classification with the known classification for a particular individual. This threshold value was subsequently applied to 2003 data to test how well reproductive state was predicted on the basis of 2002 threshold values. Finally, we determined threshold concentrations of both VTG and total VLDL-TG that maximized the correct classification of reproductive state using pooled data from 2002 and 2003.

To characterize VLDL particle size distributions among discrete reproductive states, general linear models were used to examine variation in mean, median, and modal particle size, as well as the percentage of particles occurring within the sVLDLy range, in relation to discrete reproductive state explanatory variables (nondeveloped, RFG, laying, incubation; $n = 71$). Percentage data were arcsin transformed before anal-

Table 1: Characterization of vitellogenin (VTG), total very-low-density lipoprotein (total VLDL-TG), and mean, median, and modal VLDL particle sizes in female greater scaup

Yolk Precursor	Reproductive State				
	Winter	Nondeveloped	RFG	Laying	Incubation
VTG (μg Zn mL^{-1})	.13 $\pm$ .13	.58 $\pm$ .05	2.45 $\pm$ .24	3.33 $\pm$ .21	1.73 $\pm$ .66
Total VLDL-TG (mmol TG L^{-1})	1.10 $\pm$ .23	3.75 $\pm$ .29	6.76 $\pm$ .68	7.64 $\pm$ .67	4.85 $\pm$ 2.09
Mean VLDL particle size (nm)		41.65 $\pm$ 4.56	32.14 $\pm$ 1.25	44.56 $\pm$ 4.73	61.95 $\pm$ 19.35
Median VLDL particle size (nm)		36.67 $\pm$ 4.09	29.54 $\pm$ 1.07	36.53 $\pm$ 2.25	33.22 $\pm$ 3.89
Modal VLDL particle size (nm)		34.34 $\pm$ 4.74	29.36 $\pm$ 1.16	35.51 $\pm$ 2.32	30.36 $\pm$ 4.64
Percentage of VLDL particles within sVLDLy range (%)		48.16 $\pm$ 5.08	59.15 $\pm$ 2.93	63.91 $\pm$ 3.17	59.98 $\pm$ 8.63

Note. Values are reproductive state group means ($\pm$1 SE). Mean ($\pm$1 SE) percentage of yolk-targeted VLDL particles with sizes in the range 25–44 nm (sVLDLy range) for reproductive state groups. RFG = rapid follicle growth.

ysis. However, nontransformed data were used for descriptive and graphical purposes (i.e., Table 1; Fig. 4). Inadequate amounts of lipoproteins were recovered from winter samples for accurate measurement of VLDL particle size distributions; therefore, we were unable to include this group in these analyses. Year was also included as an explanatory variable to assess interannual variation in mean, median, and modal VLDL particle sizes, as well as percentage of particles occurring within the sVLDLy range. Information-theoretic methods were again used to direct model selection (Burnham and Anderson 2002) and the set of candidate models (18) was a reduced set based on the candidate model set used to describe variation in VTG and total VLDL-TG concentrations. The candidate model set for VLDL particle size distribution analyses included all models described in Gorman (2005) that did not include the winter term; therefore, any redundant models that resulted were also excluded.

Results

Characterization of Yolk Precursor Dynamics during the Laying Cycle

Circulating concentrations of VTG and total VLDL-TG surrogates were positively correlated within individuals ($r^2 = 0.33$). Concentrations of both yolk precursors increased rapidly as total ovary mass increased during RFG before laying (Fig. 1). We described the relationship between total ovary mass and concentrations of both yolk precursors before laying as a hyperbolic function (yolk precursor concentration = [$a \times$ total ovary mass]/[b + total ovary mass]; VTG: $r^2 = 0.71$; VLDL: $r^2 = 0.27$; Fig. 1). Mean ($\pm$1 SE) concentration was low in females with nondeveloped ovaries and increased to 1.62 $\pm$ 0.29 μg Zn mL^{-1} VTG and 5.91 $\pm$ 1.20 mmol TG L^{-1} total VLDL-TG in females with one follicle in RFG ($n = 11$). Yolk precursor concentrations continued to increase through RFG; in females with ovarian development at a full follicle hierarchy ($n = 4$), mean ($\pm$1 SE) concentration was 3.38 $\pm$ 0.37 μg Zn mL^{-1} VTG and 7.31 $\pm$ 2.56 mmol TG L^{-1} total VLDL-TG (Fig.

1). Yolk precursor concentrations remained high throughout the laying cycle and decreased markedly by day 3 of incubation (Fig. 2).

Yolk Precursors as Physiological Indices of Reproductive State

Mean ($\pm$1 SE) VTG and total VLDL-TG concentrations for winter, nondeveloped, RFG, laying, and incubation reproductive states are presented in Table 1 and Figure 3. Two models of reproductive state groupings received support for describing variation in VTG concentration (ΔAIC$_c \leq 2$; Table 2). The most
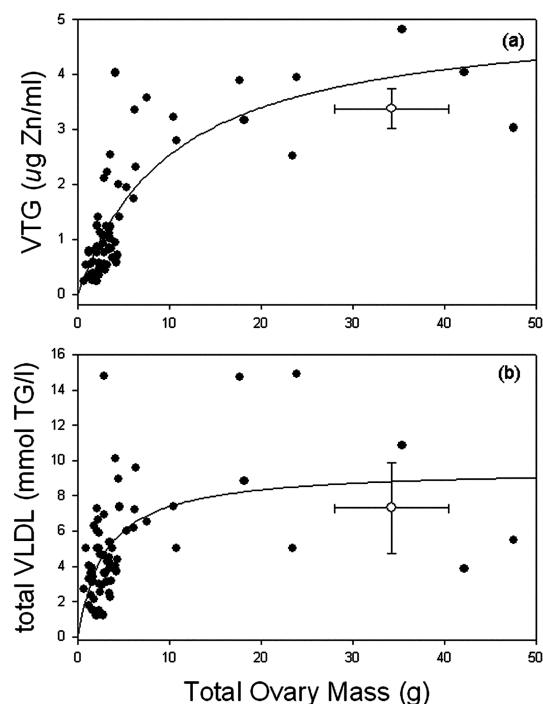

Figure 1. Relationship between total ovary mass and (a) plasma vitellogenin (VTG) and (b) plasma total very-low-density lipoprotein (total VLDL-TG) concentration in female greater scaup before the onset of laying. Circles, mean ovary mass and yolk precursor concentration ($\pm$1 SE) in females with a full follicle hierarchy.
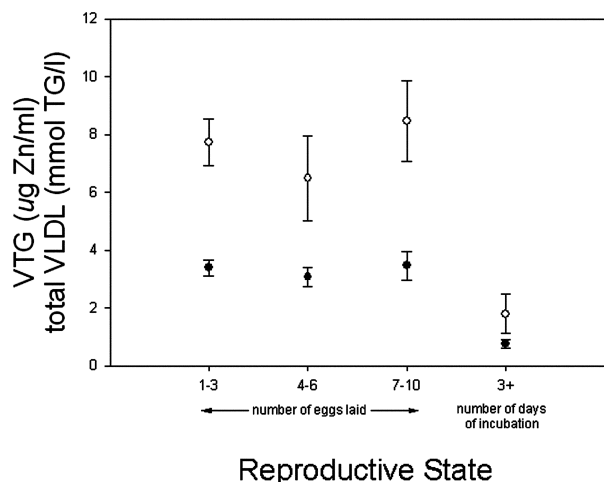
Figure 2. Variation in plasma concentration of vitellogenin (VTG; *solid circles*) and total very-low-density lipoprotein (total VLDL-TG; *open circles*) through laying and early incubation of female greater scaup. Values are group means (± 1 SE). Sample sizes for reproductive state groups were 1–3 eggs laid ($n = 13$), 4–6 eggs laid ($n = 7$), 7–10 eggs laid ($n = 10$), and 3+ d incubation ($n = 7$).

parsimonious model grouped winter and nondeveloped states together and indicated that this group was different in mean VTG concentration from all other reproductive states. Mean VTG concentrations of RFG, laying, and incubation reproductive states were also different from all other reproductive states. The most parsimonious model had a substantially higher AIC_cW value than the next best model (Table 2), in which all reproductive states were categorized separately. There was no indication that the year term was an important parameter for describing variation in VTG concentration; the weighted parameter estimate for year was 0.01 with an unconditional SE of 0.06.

Eight models of reproductive state groupings received support for describing variation in total VLDL-TG concentration ($\Delta AIC_c \leq 2$; Table 2). There was a lack of a clearly higher-weighted model (i.e., AIC_cW values) among the eight models receiving support, indicating a high degree of model uncertainty (Table 2). The most parsimonious model grouped non-egg-producing states (winter, nondeveloped, and incubation) together and fecund states (RFG and laying) together. All models with ΔAIC_c values ≤ 2 suggested that fecund females had a different mean total VLDL-TG concentration than the other groups (Table 2). Although the year term occurred in two of the eight models receiving ΔAIC_c values ≤ 2, the weighted parameter estimate was small (0.19), with a relatively large associated unconditional SE (0.26), suggesting that year was not an important explanatory variable for total VLDL-TG concentration.

Yolk Precursors as Physiological Predictors of Reproductive State

For predicting nonreproductive and reproductive states of females, VTG performed better than total VLDL-TG. A VTG

concentration of 1.4 μg Zn mL^{-1} best identified reproductive state using 2002 data; 92% of individuals were correctly identified. Applying this threshold value to 2003 data resulted in the correct identification of 88% of females. In comparison, a threshold value of 5.4 mmol TG L^{-1} total VLDL-TG, based on 2002 data, correctly identified 72% of females. When applied to 2003 data, this threshold value correctly identified 74% of individuals. Using pooled data from both 2002 and 2003, threshold values that best predicted nonreproductive and reproductive states were 1.4 μg Zn mL^{-1} for VTG (90% correct identification) and 5.3 mmol TG L^{-1} for total VLDL-TG (74% correct identification).

Characterization of VLDL Particle Size Distributions during the Laying Cycle

Average (± 1 SE) mean, median, and modal VLDL particle sizes for nondeveloped, RFG, laying, and incubation reproductive states are presented in Table 1. Four models of reproductive state groupings received support for describing variation in mean VLDL particle size (Table 2). AIC_cW and r^2 values were low for all models, suggesting a high degree of model uncertainty (Table 2). The most parsimonious model grouped nondeveloped, RFG, laying, and incubation states separately (Table 2). The weighted parameter estimate $\pm$ associated unconditional SE of the year term was 3.79 $\pm$ 3.18, suggesting that year was not an important parameter for describing variation in mean VLDL particle size.

Four models of reproductive state groupings also received support for describing variation in median VLDL particle size (Table 2). Again, AIC_cW and r^2 values were low for all models, indicating a high degree of model uncertainty (Table 2). The most parsimonious model grouped nondeveloped and incubation reproductive states together and RFG and laying states
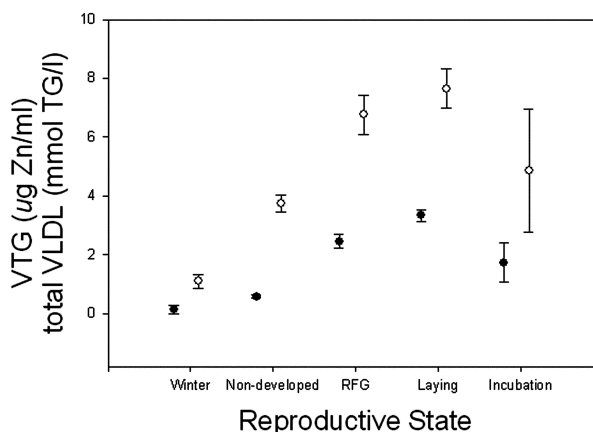

Figure 3. Mean yolk precursor concentration of vitellogenin (VTG; *solid circles*) and total very-low-density lipoprotein (total VLDL-TG; *open circles*) for discrete reproductive states of female greater scaup. Values are group means ± 1 SE. Sample sizes for reproductive state groups were winter ($n = 3$), nondeveloped ($n = 32$), rapid follicle growth (RFG; $n = 25$), laying ($n = 31$), and incubation ($n = 9$).

Table 2: Candidate models describing variation in vitellogenin (VTG), total very-low-density lipoprotein (total VLDL-TG), and mean, median, and modal VLDL particle size in female greater scaup

Response Variable, Model Number	Explanatory Variables	No. Parameters	AIC_c	AIC_cW	r^2
VTG (μg Zn mL^{-1}):					
1	(W = ND) ≠ RFG ≠ L ≠ I	5	.00	.46	.83
2	W ≠ ND ≠ RFG ≠ L ≠ I	6	1.74	.19	.83
Total VLDL-TG (mmol TG L^{-1}):					
1	(W = ND = I) ≠ (RFG = L)	3	.00	.13	.76
2	W ≠ (ND = I) ≠ (RFG = L)	4	.10	.12	.76
3	(W = ND = I) ≠ (RFG = L), Y	4	1.03	.08	.76
4	(W = ND) ≠ (RFG = L) ≠ I	4	1.06	.08	.76
5	(W = ND = I) ≠ RFG ≠ L	4	1.23	.07	.76
6	W ≠ (ND = I) ≠ RFG ≠ L	5	1.36	.06	.77
7	W ≠ ND ≠ (RFG = L) ≠ I	5	1.55	.06	.77
8	W ≠ (ND = I) ≠ (RFG = L), Y	5	1.57	.06	.77
Mean VLDL particle size (nm):					
1	ND ≠ RFG ≠ L ≠ I	5	.00	.17	.13
2	ND ≠ RFG ≠ L ≠ I, Y	6	.41	.14	.15
3	(N = I) ≠ RFG ≠ L, Y	5	.69	.12	.12
4	(N = I) ≠ RFG ≠ L	4	1.51	.08	.08
Median VLDL particle size (nm):					
1	(N = I) ≠ RFG ≠ L	4	.00	.21	.07
2	ND = RFG = L = I	2	.65	.15	.00
3	(N = I) ≠ RFG ≠ L, Y	5	1.35	.11	.08
4	ND ≠ RFG ≠ L ≠ I	5	1.96	.08	.07
Modal VLDL particle size (nm):					
1	ND = RFG = L = I	2	.00	.30	.00
2	(N = I) ≠ RFG ≠ L	4	1.65	.13	.04
Percentage of particles in sVLDLy range (%):					
1	ND ≠ (RFG = L = I), Y	5	.00	.32	.28
2	ND ≠ RFG ≠ L ≠ I, Y	6	.73	.23	.29
3	ND ≠ RFG ≠ (L = I), Y	6	.73	.22	.29

Note. Models presented are those best supported, as well as with $\Delta AIC_c \leq 2$. W = winter; ND = nondeveloped; RFG = rapid follicle growth; L = laying; I = incubation; Y = year.

separately (Table 2). The next-best-supported model grouped all reproductive states together (i.e., equal-means model; Table 2). There was no evidence that the year term was an important parameter for describing variation in median VLDL particle size; the weighted parameter estimate ± unconditional SE was 0.94 ± 1.15.

Two models of reproductive state groupings received support for describing variation in modal VLDL particle size (Table 2). The most parsimonious model was the equal-means model, which grouped nondeveloped, RFG, laying, and incubation states together. This model received approximately three times the support of the next supported model (Table 2). Again, the weighted parameter estimate ± unconditional SE for the year term was 0.37 ± 0.87, indicating that this variable was not an important parameter for describing variation in modal VLDL particle size. Overall, these results suggest a high degree of model uncertainty with respect to reproductive state groupings that best described variation in mean, median, and modal VLDL particle size. Given the low associated AIC_cW and r^2 values for all models with ΔAIC_c values ≤2 and the fact that

the equal-means model was supported in median and modal VLDL particle analyses, we conservatively conclude that VLDL particle sizes do not shift during egg production by female scaup (see also Fig. 4).

Average (±1 SE) percentage of VLDL particles falling within the sVLDLy range for nondeveloped, RFG, laying, and incubation reproductive states are reported in Table 1. Three models of reproductive state groupings received support for describing variation in percentage of particles within the sVLDLy range (Table 2). The most parsimonious model received an AIC_cW value that was approximately 1.5 times better than the support of the second-best model and an r^2 value of 0.28, grouping RFG, laying, and incubation reproductive states together and nondeveloped differently from all other states (Table 2). Year also had an effect on the percentage of particles within the sVLDLy range; the weighted parameter estimate ± unconditional SE for the year term was −0.09 ± 0.02. Thus, despite an overall lack of a reproductive state effect on VLDL particle distribution, there is some evidence that a greater percentage
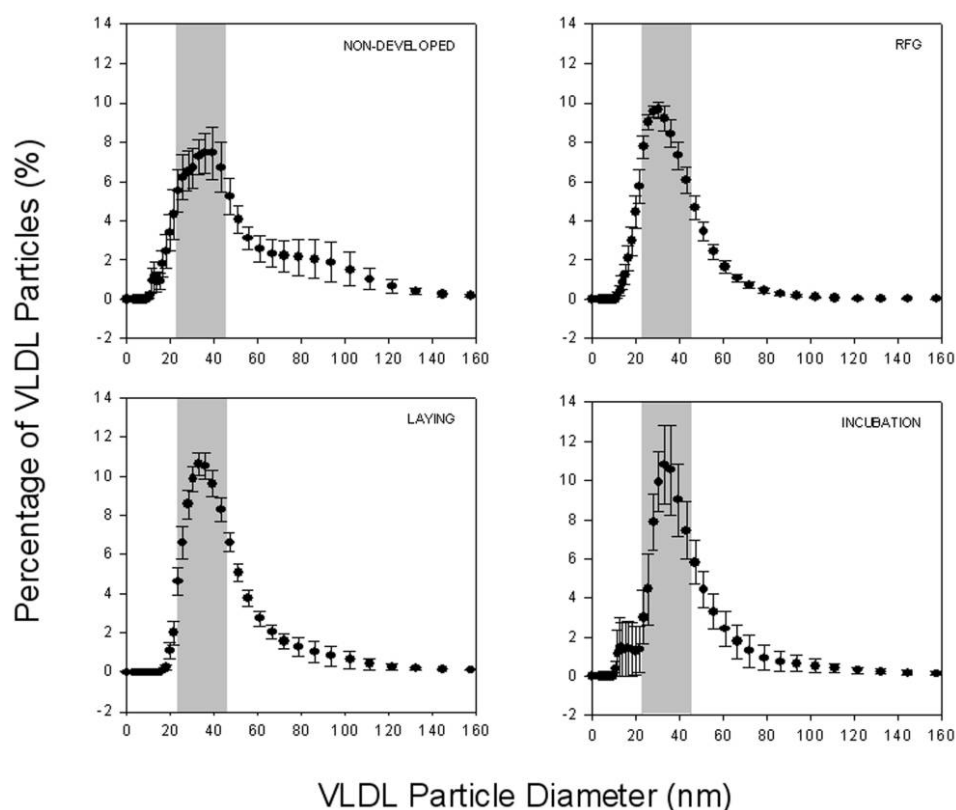
Figure 4. Percentage of very-low-density lipoprotein (VLDL) particles in each size class for discrete reproductive states of female greater scaup. Sample sizes for reproductive state groups were nondeveloped ($n = 18$), rapid follicle growth (RFG; $n = 22$), laying ($n = 26$), and incubation ($n = 5$). Gray shaded area indicates range of yolk-targeted VLDL particles with sizes in the range 25–44 nm.

of particles fall within the sVLDLy range during egg production and incubation.

Discussion

We found that changes in circulating yolk precursor concentrations were tightly coupled with timing of ovarian follicular development during egg production in scaup. Plasma concentrations of both VTG and total VLDL-TG were low in nondeveloped females and increased rapidly to maximum levels in association with increases of only a few grams of ovary mass during initiation of RFG. Concentrations of VTG and total VLDL-TG remained high through the laying cycle and decreased rapidly to low concentrations within 3 d of the onset of incubation. Analyses of VTG and total VLDL-TG concentrations allowed for accurate identification of reproductive (egg-producing) versus nonreproductive (non-egg-producing) individuals. In marked contrast, shifts in VLDL diameter were not definitive and differed from the pattern reported for galliforms such as chickens, quail, and turkeys.

VTG and total VLDL-TG dynamics in female scaup are similar to those reported for free-living passerines and charadriforms in terms of the general onset, maintenance, and termination of yolk precursor production during the laying cycle (e.g., for Cassin's auklet, Vanderkist et al. 2000; for European

starling, Challenger et al. 2001; for zebra finch, Salvante and Williams 2002). Basal VTG concentrations were generally similar among species; however, maximum VTG concentrations showed more variability: for scaup, basal and maximum means ± 1 SE were 0.13 ± 0.13 and 3.38 ± 0.40 μg Zn mL^{-1}, respectively; for Cassin's auklet, basal and maximum means ± 1 SD were 0.73 ± 1.26 and 1.66 ± 1.97 μg Zn mL^{-1}, respectively; for European starling, basal and maximum means ± 1 SE were 0.02 ± 0.02 and 4.01 ± 0.23 μg Zn mL^{-1}, respectively; and for zebra finch, basal and maximum means ± 1 SE were ~ 0.0 and 1.43 ± 0.51 μg Zn mL^{-1}, respectively. However, maximum total VLDL-TG ± 1 SE concentrations in scaup (7.31 ± 2.56 mmol TG L^{-1}) were approximately one-third of those in starling females (19.40 ± 1.40 mmol TG L^{-1}); a similar pattern was reported in Vanderkist et al. (2000), wherein maximum mean total VLDL-TG concentration ± 1 SE in breeding female Cassin's auklets was 7.40 ± 11.53 mmol TG L^{-1}. Scaup incur particularly high energetic and nutritional costs of egg production compared with other waterfowl (Flint and Grand 1999), the energy costs of which are primarily driven by a large clutch size and an energy-dense egg composition as a result of precocity. Such interspecific differences in yolk precursor concentrations highlight the importance of properly characterizing these physiological parameters in order to use them as a method for discerning reproductive state. The extent to which our data

are applicable to other waterfowl species remains unknown. However, given the limited interspecific data available on yolk precursor dynamics in free-living birds, our data suggest that VTG dynamics are more conserved across taxa, whereas variation in total VLDL-TG dynamics may be driven by developmental mode (i.e., altricial vs. precocial), evolutionary history (i.e., galliform vs. anseriform), dietary habits, or other as-yet-unappreciated differences.

Our results show that VTG concentrations varied more discretely among reproductive states than did total VLDL-TG concentrations. The best-fitting model for VTG suggested that circulating concentrations were tightly associated with a prereproductive state (i.e., winter and nondeveloped), as well as with the RFG, laying, and incubation states. In contrast, the best-fitting models describing variation in total VLDL-TG concentrations grouped non-egg-producing (i.e., winter, nondeveloped, incubation) and fecund (i.e., RFG, laying) reproductive states. Our results for the predictive capacities of VTG and total VLDL-TG corroborate our conclusion that VTG is a more reliable index of reproductive state: a VTG threshold value correctly classified 90% of females, whereas total VLDL-TG classified 74% of females as either nonreproductive or reproductive. These results corroborate those of Vanderkist et al. (2000), which demonstrated VTG as a more reliable index of egg production in seabirds.

Observed differences in the predictive utility between VTG and total VLDL-TG are likely the result of the physiological roles of each yolk precursor. The yolk-targeted VLDLy components of total VLDL-TG and VTG are absent in male and immature and nonbreeding female chickens; however, following estrogen treatment or the onset of egg production, VTG and VLDLy are readily synthesized by the liver (Walzem 1996; Speake et al. 1998). Conversely, the generic VLDL component of total VLDL-TG is present in circulation of male and nonlaying female chickens, serving as an energy transport mechanism to somatic tissues (Walzem 1996). Given that we measured total VLDL-TG, our data include both yolk-targeted VLDLy and the generic VLDL portion that is used by females for maintenance metabolism during breeding. Therefore, total VLDL-TG is likely not as specific of an index of egg production as VTG is. Some interindividual variation in total VLDL-TG concentration could be related to individual variation in nutritional state or could be due to individual females maintaining production of generic VLDL during egg production (Salvante et al. 2007). Nevertheless, because we demonstrated that VTG and total VLDL concentrations are correlated within individuals, our study suggests that measurement of VLDL can be informative, particularly in studies where volume of plasma is insufficient for VTG analysis, such as those of small-bodied species (i.e., amount of plasma required per sample for VTG analysis is 25 times greater than that required for analysis of total VLDL-TG).

Our analyses conservatively suggest that scaup VLDL particle size did not shift during egg production; however, there appears to be an area of increased variability within the 60–110-nm range in nondeveloped birds (Fig. 4). Although our analyses did not detect an effect of reproductive state on this variability, particles of this size are likely lipolyzed by muscle tissue for energy. We could not include VLDL particle size data on wintering scaup in our analyses because there was not enough lipoprotein present in the samples to obtain an accurate distribution. Interestingly, the paucity of lipoprotein in plasma of wintering scaup is consistent with the idea that wintering birds are energetically stressed. In the passerine zebra finch (Salvante et al. 2007) and the domesticated Tsaiya duck (Lien et al. 2005), there is a shift to larger VLDL particle sizes during egg production. These data suggest that general changes in VLDL metabolism during laying in nonpoultry species might differ from those documented for galliforms (Walzem 1996; Speake et al. 1998; Walzem et al. 1999). In the laying hen, only VLDL particles 25–44 nm in diameter are observed distal to the granulosa basal lamina of the ovary during yolk formation and are thus able to reach the plasma membrane of the enlarged oocyte of developing ovarian follicles (Perry and Gilbert 1979; Griffin and Perry 1985). These studies have led to the suggestion that pores in the granulosa basal lamina act as size-selective sieves, allowing only VLDL particles of certain diameters to filter into the ovary (Perry and Gilbert 1979; Griffin and Hermier 1988). Thus, there is an almost exclusive shift to smaller VLDL particle sizes in the hen within this narrow or monodisperse range of VLDL diameters (Walzem 1996), perhaps related to strong selection for continuous laying. For the zebra finch, although mean VLDL particle size was smaller in nonlaying birds (presumably because more VLDL metabolites [e.g., intermediate-density lipoprotein] than VLDL per se were present in circulation), laying birds had a greater proportion of VLDL particles in the range 25–44 nm, which would allow VLDL particles to pass through the basal lamina and access the developing oocyte (Salvante et al. 2007). Similar to the zebra finch and laying hen, our data confirm that, during egg production by scaup, VLDL particle size is also within the specific sVLDLy range. Thus, despite the disparity in results for shifts in VLDL particle size distribution during egg production by galliforms, passerines, and anseriforms, it appears that all taxa studied to date maintain a large portion of VLDL particles that are within the sVLDLy range during egg production. Studies examining the determinants of alternative shifts in lipoprotein metabolism during reproduction would be informative for a better understanding of the molecular mechanisms underlying costs of egg production in birds.

Recent conservation concerns over declining North American scaup populations (i.e., *Aythya marila* and *Aythya affinis*) have prompted research in a number of areas, including reproductive ecology (Austin et al. 2000). In conjunction with radiotelemetry and behavioral studies, yolk precursor analysis can be used to more accurately discern where in the breeding cycle failed reproduction occurs (i.e., breeding propensity, failed nesting, or clutch loss due to predation or environmental conditions; e.g., Peery et al. 2004; Bond et al. 2008). Furthermore, yolk precursor analysis likely has important utility in studies of contaminant effects on reproduction in waterfowl. In summary, we conclude that plasma yolk precursor analysis is a

useful technique that should be applicable to a variety of research in evolutionary, ecological, and especially applied conservation studies of reproduction in waterfowl (see also Carey 2005).

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