During avian egg production, oestrogen mediates marked increases in hepatic lipid production and changes in the diameter of assembled very-low density lipoprotein (VLDL). A nearly complete shift from generic VLDL (~70 nm in diameter), which transports lipids to peripheral tissues, to yolk-targeted VLDL (VLDLy) (~30 nm), which supplies the yolk with energy-rich lipid, has been observed

Introduction

During avian egg production estrogens stimulate the liver to produce the egg-yolk precursors, yolk-targeted, very-low density lipoprotein (VLDLy) and vitellogenin (VTG), which provide embryos with the energy and nutrients required for growth and development (Gruber, 1972; Bergink et al., 1974; Deeley et al., 1975; Neilson and Simpson, 1973; Chan et al., 1976; Wallace, 1985; Walzem, 1996; Williams, 1998). As a result of increased total hepatic lipid production, plasma lipid concentrations increase from about 3 mg neutral lipid ml^{-1} plasma in non-laying turkeys ( ) to 21 mg in laying turkeys (Bacon et al., 1974). Similarly, plasma triacylglyceride concentration increased from 0.5–1.5 μmol ml^{-1} plasma in non-laying chickens ( ) to 20–50 μmol ml^{-1} plasma in laying chickens (Griffin and Hermier, 1988). Furthermore, data for egg-laying chickens, turkeys and quail (*C*) show that there is an oestrogen-dependent shift in VLDL synthesis from the production of generic VLDL, which ranges in size from 30 to >200 nm, to smaller, yolk-targeted VLDL, which ranges in diameter from 15 to 55 nm in domestic fowl (Chapman, 1980; Griffin, 1981; Walzem et al., 1994; Walzem, 1996; Speake et al., 1998; Chen et al., 1999; Walzem et al., 1999). Furthermore, whereas generic VLDL has at least six associated apolipoproteins (including apoA-I, apoB and apoC), VLDLy has only two associated apolipoproteins, apoB and apoVLDL-II, of which the latter is thought to be responsible for the decrease in VLDLy diameter (Chan et al., 1976; Kudzma et al., 1979; Griffin, 1981; Dashti et al., 1983; Lin et al., 1986; Schneider et al., 1990; Speake et al., 1998; Walzem et al., 1999). Consequently, the presence of circulating VLDLy in egg-producing females represents a dramatic shift in lipid

Poultry Nutrition, 1994), and were provided with 15 h of light per day. Ambient house temperature varied from 7–29°C. All animal husbandry and experimental procedures were conducted in accordance with a protocol approved by the

96-well microplates and were measured at 540 nm using a Biotek 340i microplate reader.

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Whole plasma contains a variety of different lipoprotein classes, e.g. VLDL, low density lipoprotein (LDL), high density lipoprotein (HDL). Therefore, plasma VLDL was isolated as the <1.020 g ml^{-1} fraction of plasma from zebra finches and chickens. The volume of each zebra finch plasma sample (approximately 100 μl) was measured and transferred into Beckman Ultra-Clear ultracentrifuge tubes (13×64 mm, #344088; Beckman Coulter, Fullerton, CA, USA), and NaCl density solution (=1.0063; equivalent salt density of undiluted plasma) was added until a final volume of 1 ml was reached. Alternatively, a sub-sample (1 ml) from each chicken plasma 0 cs 0 b1 gsBT/T.081m0 ce33da8c3c 10 1.51zaBr592.0013 T4 NaCl den5a sub-sample (1

(Walzem, 1996)]. By contrast, the sVLDLy range was based on the observation in domestic fowl that the only VLDL particles observed distal to the granulosa basal lamina of the ovary during yolk formation, and thus able to reach the plasma membrane of the enlarging oocyte of the developing ovarian follicles, ranged from 25 to 44 nm in diameter (Perry and Gilbert, 1979; Griffin and Perry, 1985; Griffin and Hermier, 1988). These studies suggested that pores in the granulosa basal lamina act as selective sieves, allowing only VLDL particles of certain diameters to filter into the ovary (Perry and Gilbert, 1979; Griffin and Perry, 1985; Griffin and Hermier, 1988).

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Whereas laying and non-laying female zebra finches did not differ in body mass at the time of blood sampling (>0.2; Table 1), laying zebra finches had higher plasma triacylglyceride levels than non-laying females (Wilcoxon rank-sum test: =–4.008,

distribution. On average, VLDL particle diameter distributions of laying chickens at 29-weeks of age were narrow, and peaked at small particle diameters (Fig. 4B), while non-laying chickens possessed wider, less peaked distributions (Fig. 4B) (range: =4.816, <0.0001; Table 1). Laying chickens had a larger

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As predicted, female zebra finches exhibited less dramatic shifts in lipid metabolism during egg production. However, this was mainly due to the unexpected finding that the majority of VLDL particles of non-laying zebra finches were very small in diameter (57% of particles had diameters less than 30 nm). Consequently, the diameter distributions of laying zebra finches actually shifted towards larger VLDL particles compared to the distributions of non-laying females. Furthermore, the diameter distributions of both laying and non-laying zebra finches peaked at small VLDL particles, and therefore overlapped considerably. Similar results have been reported for comparisons between growing (i.e. immature) and egg-producing Tsaiya ducks, *A* (Lien et al., 2005). When provided with access to food, domesticated Tsaiya ducks had VLDL particle diameter distributions, as assessed by transmission electron microscopy, that included more larger particles (range: 50–75 nm) and had larger mean VLDL particle diameters during egg production at 30 weeks of age (61.57±1.98 nm) than while actively growing at 12 weeks of age (range: 35–60 nm; mean diameter: 47.67±2.37 nm) (Lien et al., 2005). This is in stark contrast to the data from chickens, wherein less than 10% of the VLDL particles measured at the peak of egg laying had diameters larger than 51 nm (cf. nearly 60% of particles in non-laying chickens), resulting in very little overlap between the VLDL distributions of laying and non-laying chickens.

Laying zebra finches, like laying chickens, had higher circulating triacylglyceride levels and more particles within the sVLDLy and cVLDLy ranges than non-laying females, despite the fact that laying zebra finches had fewer very small VLDL particles, more large VLDL particles, and wider diameter distributions than non-laying females. Furthermore, the VLDL particle diameter distributions of zebra finches and chickens shifted towards similar modal and median VLDL particle diameters during egg production. These results suggest that, regardless of the direction that VLDL particle diameter distributions have to shift, specific changes in lipid metabolism (e.g. increased lipid production and maintenance of a large proportion of small VLDL particles of specific diameters) may be essential for egg production in both domesticated and non-domesticated birds. However, data on reproductive status and VLDL particle diameter distribution from more domesticated and free-living avian species are required to confirm the relationship between changes in lipid metabolism and avian egg production.

The differences in VLDL particle diameter distribution between non-laying chickens and zebra finches observed in this study may be due to differences in rates of lipid turnover due to variation in metabolic rate. Based on allometric scaling of metabolic rate (for reviews, see Calder, 1981; Taylor, 1987), smaller passerine songbirds have higher mass-specific metabolic rates than larger chickens (Lasiewski and Dawson, 1967; Reynolds and Lee, 1996; McKechnie and Wolf, 2004). Consequently, passerine songbirds also have higher rates of lipid turnover. When VLDL particles undergo lipoprotein lipase-mediated metabolism, triacylglycerol is removed by hydrolysis, and surface lipids and apolipoproteins (e.g. apo-A, apo-C) are transferred to other lipoprotein particles (e.g. high density lipoproteins) (for reviews, see Eisenberg, 1986; Walzem, 1996). This results in a decrease in VLDL particle size and an increase in particle density, and the eventual conversion to intermediate density lipoproteins (IDL) and then low density lipoproteins (for reviews, see Eisenberg, 1986; Walzem, 1996). Hermier et al. (Hermier et al., 1985) reported that IDL particles from immature chickens had an average diameter of 20.0 nm. Therefore, the abundance of very small VLDL particles observed in non-laying zebra finches (57% under 30

In contrast to domesticated birds that have undergone directional selection for specific traits, such as continuous egg production or rapid growth, the selective pressures on laying zebra finches, and on non-domesticated birds in general, are generally focused on maintaining traits that maximize the trade-off between current reproductive effort and future fecundity and survival (Williams, 1966; Stearns, 1992; Bernardo, 1996). The increased LPL-resistance of VLDLy may result in selection for the maintenance of larger, potentially generic, VLDL particles in non-domesticated birds during egg production, as observed in zebra finches in this study (19%) and Tsaiya ducks (~100%) (Lien et al., 2005), to ensure that laying females have an ample supply of VLDL that can be metabolized in case their own energetic demands increase during egg production due to changes in environmental conditions. Data on VLDL particle diameter distribution during egg production in many more free-living avian species, including other gallinaceous and passerine birds, are needed to determine whether the differences between chickens and zebra finches observed in this study are, in fact, due to differences in selective pressures on these birds, or to phylogenetic differences that are unrelated to inter-specific differences in adaptations to egg production.

In addition to egg production, reproduction in non-domesticated species generally involves broody behaviour, i.e. incubation and post-hatching parental care (e.g. provisioning and brooding of young). This is in contrast to many breeds of domesticated chickens, whose reproductive activity is limited to egg production as a result of commercial practices (e.g. photoperiod manipulation, egg removal) and decreases in broodiness, hatchability and fertility as a consequence of selection for increased egg production (Emmerson et al., 1991; Nestor et al., 1996; Sewalem et al., 1998) (reviewed by Romanov, 2001). Therefore, given that non-domesticated, laying females must ensure that they have adequate resources to perform post-laying parental behaviours, they may limit lipid allocation to current egg production in exchange for allocating more energy towards self-maintenance (i.e. maintaining larger VLDL particles) to enhance their chances for survival through the current reproductive period and beyond, thus maximizing current and potentially future reproductive effort. Further studies are needed that will assess the relationships between variation in VLDL particle diameter distribution during egg production in free-living avian species and both current and future reproductive success, and maternal survival and longevity.

List of abbreviations

VLDL	very low density lipoprotein
VLDLy	yolk-targeted VLDL
VTG	vitellogenin
LPL	lipoprotein lipase
cVLDLy	chicken VLDLy
sVLDLy	avian-ovary sieved VLDLy
zVLDLy	zebra finch VLDLy

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