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Review

Yolk androgens as pleiotropic mediators of physiological processes: A mechanistic review

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ABSTRACT

Avian egg yolks are made up of complex mixtures of physiologically relevant substances. Androgens, in particular, accumulate in yolks in variable amounts based on social and environmental conditions experienced by laying females, and using experimental elevations of yolk androgen content, researchers have unveiled potent physiological and behavioral effects on offspring. These patterns and effects are exciting in an adaptive context, as the transfer of endogenously-produced substances such as androgens to egg yolks may allow fine manipulation of offspring phenotype to maximize reproductive success. However, to gain an in-depth understanding of how yolk androgens function in an adaptive context, we must first understand the complex entanglement of physiological and endocrine interrelationships that change after exposure to yolk androgens. Here, we take a comparative approach towards a discussion of how yolk androgens can simultaneously affect multiple body systems within developing birds, ultimately resulting in the large-scale phenotypic endpoints that may represent adaptive consequences of exposure to elevated levels of yolk androgens.

Contents

1. Introduction

The manipulation of offspring phenotype through exposure to physiologically relevant substances has become a hot topic in evolutionary and ecological literature. Over the past two decades, hormone-mediated maternal effects have become of great interest (Groothuis and Schwabl 2007; Groothuis et al., 2005; Gil, 2003) in particular, the potential use of yolk androgens by females birds to manipulate offspring quality. By depositing variable amounts of androgens into the eggs, females can preemptively set the trajectory for offspring phenotype before the developing oocyte has even been ovulated, according to changing environmental and social conditions. Further, this can be accomplished by simply depositing a seemingly inexpensive substance made naturally on a daily basis.

Embryonic development is a dynamic stage in an organism's lifetime during which physiological and behavioral systems are permanently imprinted. Direct actions of androgens on target tissues during this stage

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of development can have lasting effects on morphology and behavior, and androgens play a large part in the organization of several major hormonal and neural pathways within the body (Quinn and Ottinger 2006; McCormick et al., 1998; Arnold and Gorski 1984). As a result, exposure to androgens during this sensitive time can have wide-ranging, permanent effects on the offspring. It is still unclear whether these effects result from direct androgenic effects on tissues, or are mediated by changes in androgen receptor abundance or function that persist beyond hatch.

In the chicken, the vitilline system, a network of venules that carry nutrients from the yolk sac to the embryo, is sufficiently differentiated to permit the circulation of blood by the 2nd day of incubation (Romanoff, 1960). Thus, the developing embryo is exposed to the content of the yolk, and thus the maternal hormones deposited there, from that point on in development (Fig. 1). Work on the zebra finch has shown the presence of androgen receptors (AR) in the developing brain as early as embryonic day 7, and in the muscles that innervate the syrinx by embryonic day 10 (Godsave et al., 2002). While a complete survey for the presence of ARs and the ontogeny of androgen-dependent tissues remains to be done in an avian system, it seems likely that the major avian body systems are capable of responding to androgens early on in embryonic development, during a time when the neural and physiological axes are organized. The timing during which distinct aspects of offspring morphology are organized and the length of time that androgens are available in yolks for absorption determine which aspects of offspring phenotype are modulated by yolk hormones (Carere and Balthazart, 2007).

Of course, such possibilities are exciting in an adaptive context, as the deposition of yolk androgens may present a mechanism of fine control over offspring phenotype, and thus lifetime reproductive success as well. However, a full understanding of how yolk androgens may function in an evolutionary context requires a knowledge of the many ways in which they affect the developing offspring physiologically. To date, the surface of physiologically mechanistic knowledge regarding yolk androgens has only been scratched, and while the recent integration of the physiological, ecological, and evolutionary fields in an effort to answer such questions should be commended, there is much work to be done.

The purpose of this review is to examine the observed effects of yolk androgens on offspring physiology and behavior, and, by integrating previous work done in both mammalian and avian species, identify potential mechanisms by which yolk androgens may alter offspring phenotype. Yolk androgens exert many potential large-scale changes on offspring. The ones where most literature is available and potentially have the largest impact on offspring phenotype are alterations of growth and development (i.e. muscle development, skeletal growth, and weight gain) and immune function by exposure to yolk androgens. Despite the increasing numbers of studies examining how physiological doses of yolk androgens affect various aspects of offspring phenotype, many potential physiological targets of yolk androgens remain to be tested and clarified. Given the lack of mechanistic work done in wild birds, it becomes necessary to utilize data from experiments that test the effects of plasma hormones on various physiological systems, as well as studies in which pharmacological doses of androgens were used, to provide insight into future experiments that need to be done in a more natural context. We use documented effects on growth and immunity, resulting from both yolk exposure and adult exposure to androgens, as a springboard to discuss the complicated web of physiological and endocrinological effects that may result from exposure to yolk androgens during development. We show that the developmental effects of androgens are complex and multi-tiered, and that by depositing androgens into eggs, females are introducing offspring to a series of trade-offs that are modulated by both internal and external influences.

2. Yolk androgens and developmental growth

2.1. Muscular development

The formation of muscle during vertebrate embryogenesis is a product of multiple differentiation and diversification events that, in the chicken, begin as early as Hamilton stage 11 of embryonic development (Buffinger and Stockdale, 1994), which correlates approximately to 40 h of incubation (Boyd, 1962). Sexual dimorphisms in the sizes of various muscles are common in birds, and sex steroids have been implicated as the driving forces behind these sex differences (reviewed in Cooke et al., 1998), perhaps participating in key developmental events during embryogenesis. Thus, by depositing androgens into the yolks of eggs, female birds can manipulate the size and performance of the offspring muscular system. In fact, androgen injections into the yolks of eggs has been shown to increase the size of the *musculus complexus*, or the

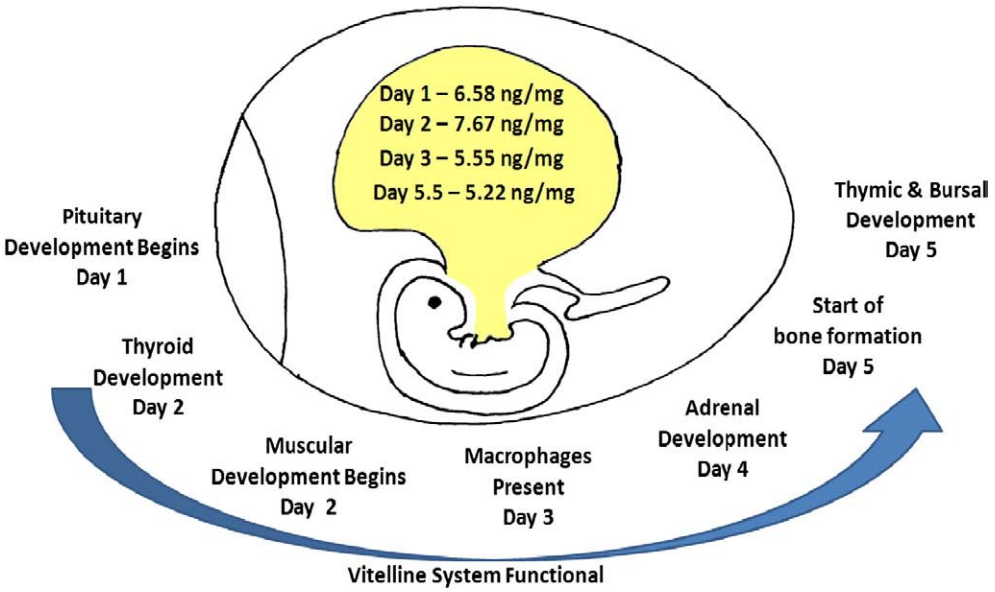

Fig. 1. Timing of systemic differentiation in relation to yolk androgen levels and vitelline system development. Yolk androgen levels represent the sum of androstenedione, testosterone, and dihydrotestosterone adapted from results published by Elf and Fivizzani (2002). All developmental timing and yolk androgen levels shown here were documented in a chicken model system.

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hatching muscle, which is responsible for breaking the shell during hatching and the flexion of the neck during begging in red-winged blackbirds (Lipar and Ketterson, 2000). These results were not supported by work in chickens, as androgen supplementation of chicken embryos *in ovo* had no effect on the muscle development of male embryos and decreased the development of the *Pectoralis superficialis* muscle in female embryos (Henry and Burke, 1999). Yet, treatment of adult turkeys with testosterone, 5aDHT, or 19-nortestosterone resulted in an increase in the weight of the right breast muscle (Fennell and Scanes, 1992). While this experiment has not been performed on turkeys during the embryonic period, the effect may be similar if androgen receptors are present in muscle at that time. More experiments examining the effects of androgen supplementation *in ovo* need to be done.

Studies on the effects of androgens on avian muscle tissue are scarce and conflicting. By extrapolating from mammalian studies, however, we can begin to hypothesize about the mechanisms by which yolk androgens may alter muscle sizes during avian embryogenesis. For example, in mammals, testosterone has been found to increase the rates of both the turnover and the synthesis of protein (Mauras et al., 1996) and also stimulates intracellular calcium release and mitogen-activated protein kinases in skeletal muscle cells (Estrada et al., 2003), contributing to the strength of muscle contraction. Androgen exposure also aids in nitrogen retention in skeletal muscle in mammals, leading to muscular hypertrophy (Mooradian et al., 1987), and induces proliferation of muscle cell precursors and myonuclei numbers, also leading to increases in muscle mass (Joubert and Tobin, 1995).

Finally, it has been hypothesized that androgens may be involved in the interactions between muscle cells and their neurons (reviewed in Staub and De Beer, 1997), a phenomenon that is becoming increasingly well-studied in avian systems. Work on the zebra finch embryo shows androgen receptors in the hindbrain, an area that contains motor nuclei that are important for the function of the hatching and begging muscles (Godsave et al., 2002) and also in the chick embryonic spinal cord in the motorneurons which innervate the limb muscles (Reid et al., 1981; Weil, 1986). Proper motorneuron function is vital for the proper development of the muscular system, and the exposure of avian embryos to maternal androgens could significantly alter processes associated with motorneuron, and thus muscular, development.

More studies examining the mechanisms of androgen action on muscle as well as the timing of maximal yolk androgen exposure in relation to developmental time scales must be completed in an avian model system to fully understand the actions of yolk androgens on embryonic muscle development. Additionally, the function of such changes in muscle mass and the duration of these changes into adulthood can help to elucidate the function of yolk androgens in an adaptive context. The potential immediate benefit of increasing muscle mass, particularly the *musculus complexus*, in avian embryos is clear. If androgenic effects on muscle extend into adulthood, life histories of individual species may determine the costs and benefits of androgenic influences on muscle. In many cases, high muscle mass is considered to be a benefit in adult birds, as mass-specific power available from muscles determines the range of speeds over which a bird can fly, with higher speed ranges requiring more mass-specific power (Ellington, 1991). The pectoral muscles in particular are important for flight, and may even provide energy during long flights and periods of fasting (Lindstrom et al., 2000). These benefits may only be relevant, however, when discussing birds that use flight as the major form of locomotion. Ratites and poultry, for example display little or no flight capabilities, and depend upon ground locomotion to escape predators. Heavier flight muscles could impair ground escape speed in these birds, and may thus serve as a detriment. Increased muscle production even causes physiological abnormalities and disorders such as cardiovascular disease, bone abnormalities (Julian, 1998) and decreased reproductive efficiencies (Decuypere et al., 2003) in broiler chickens. Thus, the life history traits within target species as well as the extent to which androgens stimulate muscle growth and how that growth affects physiological systems becomes important in terms of the cost/benefit analysis of yolk androgens. Future studies should examine long-term effects of yolk androgens on flight performance, escape speed, and agility in a variety of avian species.

2.2. Skeletal growth

Several recent studies examining the effects of yolk androgens on the development of avian offspring have documented increases in skeletal size of offspring that received an androgen treatment *in ovo* as compared to controls. For example, house finch nestlings hatching from eggs that received a testosterone injection had significantly larger tarsi on the day after hatch, suggesting a stimulation of skeletal growth during the embryonic period (Navara et al., 2006). Similarly, in black-headed gull nestlings one day after hatch, treatment of eggs with androgen was associated with a reduction of the size hierarchy within clutches in terms of tarsus length, suggesting that androgens had a stimulatory effect on bone growth in those nestlings (Eising et al., 2001). Finally, canary chicks hatching from eggs receiving a testosterone injection reached 50% of their final tarsus length more quickly during the nestling period than control chicks (Schwabl, 1996). In the case of the canary, size differences may be partially explained by androgen-related behavioral changes in offspring. In the house finch and the black-headed gull, however, size differences were found very early in development during which the yolk sac was likely still being absorbed and before offspring started begging for food, suggesting a difference in bone growth *in ovo*. While many developmental events occur simultaneously and may each have an effect on bone growth, yolk androgens may exert a direct effect on the skeletal system during embryogenesis.

As was the case with the mechanisms of androgen action on muscle development, the mechanistic basis for androgenic action on bone has not been well described in birds. In fact, previous work examining the effects of androgen treatment on adult chickens produced ambiguous results: In some cases, androgens stimulated skeletal growth while in others skeletal growth was suppressed by or was unaffected by androgen treatment (reviewed in Weppelman, 1984). Additionally, post-hatch androgen treatment impaired linear bone growth in turkeys (Wise and Ranaweera, 1975). It is possible that, in birds, the effects of androgen treatment before and after hatch differ, as was the case with black-headed gull chicks. When exposed to androgen treatment *in ovo*, skeletal growth was stimulated (Eising et al., 2001) while androgen treatment post-hatch resulted in the suppression of skeletal growth (Groothuis and Ros, 2005).

We can also attempt to form hypotheses and design future studies based on results produced in mammalian systems. In mice, for example, androgen treatment increased the concentration of transforming growth factor B in bone cells while increasing the sensitivity of bone cells to other growth factors (Kasperk et al., 1990), all of which could result in the stimulation of bone growth. Additionally, testosterone treatment increased calcium concentrations in male rat osteoblasts (Lieberherr and Grosse, 1994). Testosterone exposure also increases the rates of calcium retention and absorption (Mauras et al., 1996), adding to bone size and stability.

In addition to the direct effects that androgens may exert on bone growth during the embryonic period, there is also a large amount of evidence for an interaction between androgens and the hypothalamic–pituitary–growth hormone axis. For example, in mammals, testosterone stimulates the release of growth hormone releasing hormone (GHRH) at the hypothalamic level, ultimately stimulating the release of growth hormone from the anterior pituitary gland (Bondanelli et al., 2003). In the rat, there is a gender difference in patterns of GH secretion (Painson et al., 2000) resulting in vastly different patterns of somatic growth between the sexes (Robinson and Clark, 1987). In humans, androgenic hormones stimulate the production of GH, which stimulates the release of insulin-like-growth factor-1 (IGF-1) in the male (Mauras et al., 1996). IGF-1 has also been related to somatic growth

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(Behringer et al., 1990). Characterizations of avian GH using electrophoretic separations suggest that it is very similar to its mammalian counterpart (Farmer et al., 1979) and it is likely that similar interactions exist in birds. Therefore, yolk androgens may be able to stimulate avian growth indirectly, through the manipulation of growth hormone releasing patterns at the hypothalamic and pituitary level.

More studies examining the mechanistic basis behind the androgenic effects on bone must be done on an avian model system before we can fully understand how yolk androgens alter embryonic bone growth. As with the muscular effects, it is also important to understand the duration of these effects into adulthood, and how androgenic effects on bone relate to life histories of avian species. In ground-dwelling birds, a quick flight escape is essential for survival of predation events, and species with longer femur lengths exhibit faster escape speeds (Burns and Ydenberg 2002; Swaddle and Lockwood, 1998). Larger birds that begin flight by soaring from above may not require such benefits of bone length. Additionally, body size is often important in both competitive and sexual selection contexts. However, there are often potential detriments associated with raising larger, faster growing offspring, particularly in an environment in which resources are limited. Because resource availability is finite, there is a balance between the number of offspring that can be produced and the size of those offspring (Smith and Fretwell, 1974). Perhaps a large number of small offspring would reach adulthood compared to a similar number of large offspring. Thus, future studies examining the long-term effects of yolk androgens on rearing costs, escape speed, inter and intra-specific competitive ability, and sexual selection processes are also necessary.

2.3. Weight gain and metabolic function

Exposure to androgens *in ovo* can also have both immediate and long-term effects on weight gain and metabolic processes. For example, canary and starling chicks that had received a testosterone injection *in ovo* were heavier than control chicks early in development (Schwabl, 1996; Pilz et al., 2004). Additionally, treatment of eastern bluebird eggs with testosterone resulted in chicks that gained more weight over the nestling period as compared to controls (Navara et al., 2005). In such cases it is hard to tease apart the behavioral and physiological causes for the androgen-related effects on weight gain in offspring because embryonic exposure to androgens has been shown to increase begging behavior in several species, including the canary (Schwabl, 1996) and the black-headed gull (Eising et al., 2001) (discussed in detail in the next section). In both the canary and the starling, however, the treatment differences on offspring mass occurred very early in the nestling period (at 22 h and 2 days post-hatch respectively). Thus, androgens may exert direct effects on the physiological mechanisms involved in weight gain in canary and gull nestlings. Additionally, while starling nestlings treated with testosterone *in ovo* were heavier than controls early in the nestling period, testosterone-treated nestlings did not beg more than controls at this time, suggesting that embryonic exposure to T may affect weight gain independently of begging behavior. Finally, male and female chickens exhibit sexual size dimorphisms as early as the embryonic period (Henry and Burke, 1999b). In this section we will focus on the direct physiological effects that androgens can exert on weight gain as opposed to the indirect behavioral effects that may produce similar results.

In general, androgens are considered anabolic hormones (Weppelman 1984). To our knowledge, only two studies to date report on the effects of yolk androgens on metabolic processes in developing birds. In one case, experimentally elevated levels of yolk testosterone increased resting metabolic rate in zebra finch nestlings (Tobler et al., 2007) while yolk steroid supplementation did not affect the resting metabolic rate of great tit nestlings (Eising et al., 2003). While work examining the effects of androgens on metabolic processes in avian embryos is sparse and conflicting, there is a good amount of evidence that androgens play a role in the modulation of metabolic processes in adult birds. For example, high testosterone levels are associated with decreases in metabolic rate in white-crowned sparrows (Wikelski et al., 1999), and testosterone treatment causes a significant decrease in body temperature in male Japanese quail (Hannsler and Prinzinger 1979), indicative of a lower metabolic rate. If AR abundance is similar in the avian embryo as in the adult bird, androgens may exert a suppressive effect on metabolic function, which could extend through the early period of nestling development, thus increasing weight gain. Such a mechanism argues for a direct effect of androgens on physiological mechanisms associated with weight gain in developing offspring. On the other hand, however, androgen treatment has also been linked to increases in metabolic processes in some avian species. For example, in adult house sparrows, increases in testosterone levels were associated with increases in basal metabolic rate (Buchanan et al., 2001). Likewise, in adult dark-eyed juncos, energy metabolism is lower in castrates than in intact males, and mean body temperature in castrates is also higher than in intact males during the day (Lynn et al., 2000). Androgens have also been shown to modulate food intake and fattening in adult birds (Deviche, 1995). Such a mechanism argues for a more indirect effect of androgens on weight gain in developing birds, through behavioral alterations on food intake.

Finally, androgens also exert indirect effects on weight gain in adult birds and mammals through the modulation of related endocrine pathways. For example, in a study on adult white-crowned sparrows, testosterone was associated with the release of prolactin from the anterior pituitary gland, which immediately induced fat deposition (Yokoyama, 1976). Androgens have also been known to interact with the hypothalamic–pituitary–thyroid axis in mammals, which is a major controlling factor of metabolic pathways (Pathak and Chandola-Saklani 1988; Esposito et al., 2002). Finally, in a study on adult dark-eyed juncos, testosterone supplementation elevated circulating levels of corticosterone (Ketterson et al., 1991), which is responsible for the stimulation of fat deposition into the central areas (Griffin, 1996). Thus, through a variety of indirect targets, yolk androgens may exert potent effects on weight gain throughout the embryonic and nestling periods in developing birds. In addition, since exposure to androgens can imprint the function of several major brain axes (McCormick et al., 1998), androgen-related effects on weight gain and metabolism could potentially extend throughout adulthood in birds exposed to high levels of androgens *in ovo*.

Future studies should address whether yolk androgens stimulate weight gain in the long-term. These studies are particularly important, as weight gain through the deposition of fat comes with a series of benefits and detriments, including increases in energy availability, insulation during winter conditions, and size differences that may function in competitive and sexual selection contexts, but also decreases in flight speed ranges, increases in predation risk, and increased prevalence of fat-related pathologies (Witter and Cuthill, 1993). The relationships between the longevity of the effects resulting from yolk androgen exposure with the life history traits of the birds and the costs/benefits associated with weight gain in those birds will help to determine the adaptive significance of the stimulation of offspring weight gain using yolk androgens.

3. Yolk androgens and immune function

Androgens have a wide range of physiological effects on developing and adult birds, only a fraction of which have been truly addressed. Some effects have the potential to act as a detriment to developing offspring, creating trade-offs that could place limits on the optimal amount of yolk androgens for offspring survival. One such effect is the modulation of the immune system, because androgens are potently immunomodulatory in nature, and when deposited into yolks, have been shown to suppress the immune systems of developing birds (Andersson et al., 2004; Groothuis et al., 2005; Navara et al., 2005; Müller et al., 2005; but see Tschirren et al., 2005).

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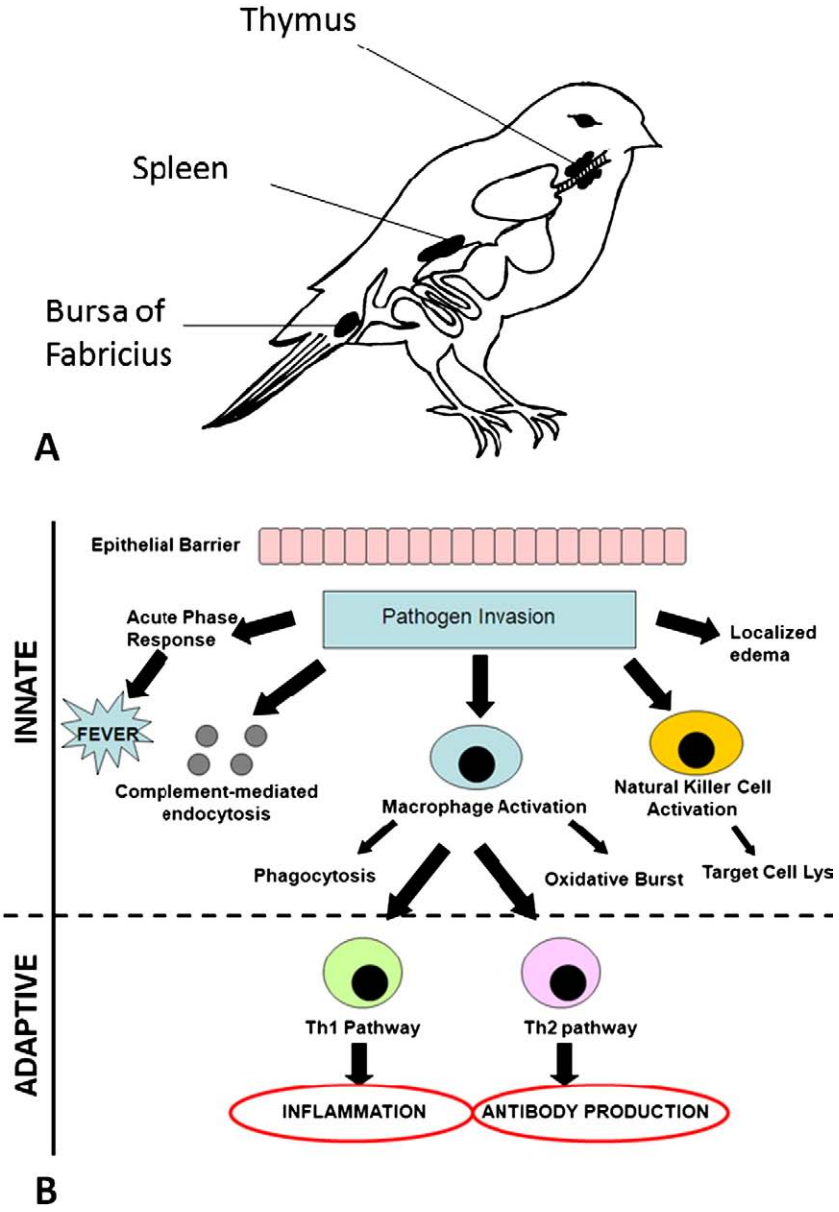

Fig. 2. Potential immunological targets for yolk androgens. (A) The locations of the thymus, bursa, and spleen during early development. These are the three major sites of immunological development during the embryonic period. The Bursa of Fabricius is the origination site for B-cells, the thymus is the origination site for T-cells, and the spleen contains both cell types, serving as an organizational center. (B) The immune system is complex and involves the interaction of multiple cell types. Macrophages initially recognize and respond to pathogen exposure through phagocytosis and the production of cytokines that stimulate febrile responses, natural killer cell activation, complement-mediated endocytosis, localized edema, and the differentiation of T-cells into either a TH1 pathway that results in T-cell mediated inflammation or a TH2 pathway that results in B-cell activation and antibody production. The red circles indicate immunological aspects that have been tested in response to yolk androgen exposure.

The avian immune system is both complex and integrative; it both affects and is affected by several other body systems. Starting with the recognition of a potential pathogen by circulating macrophages, many simultaneous and diverse immunological events occur, including the production of fever, non-specific cell lysis and endocytosis events, phagocytosis, and the stimulation of T-cells to act in one of two immunological directions. The first (TH1) results in inflammation and the second (TH2) stimulates an activation of an antibody response by B-cells (Fig. 2). To date, the effects of yolk androgens on immunity have only been considered in terms of two aspects of immunological defense: the T-cell mediated inflammatory response to PHA and the B-cell mediated production of antibodies during an antigen challenge (Fig. 3). It would be helpful to determine which other aspects of the complex immune response are altered by exposure to yolk androgens.

There is evidence in birds that three of the major modulatory cell types of the immune system begin developing during early embryonic stages. Macrophages, for example, are present and active as early as day 3 of development in quail embryos (Cuadros et al., 1992). To our knowledge, the effects of androgens on these cells during development have not yet been tested. T-cells originate in the thymus, a lymphoid organ located along each side of the jugular veins (Glick, 2000), which begins differentiating on day 5 of embryonic development in the chick (Le Douarin and Jotereau, 1975). B-lymphocytes originate in the avian bursa of Fabricius (Grossman, 1984; Sharma 1998), which also begins to form on day 5 of chick embryonic development (Romanoff, 1960). Studies on the chicken demonstrated the presence of active immune tissue in both the bursa and the thymus during the embryonic period (Sharma, 1998) making both lymphoid organs potential target tissues

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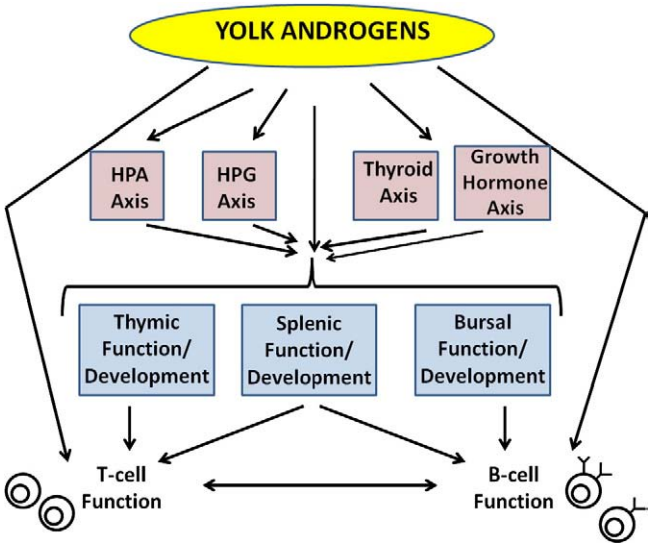

Fig. 3. Integrated effects of yolk androgens on immune function. Androgens have documented suppressive effects on these tissues, but can also affect them via interactions with the hypothalamic–pituitary–adrenal (HPA), the hypothalamic–pituitary–gonadal (HPG), the thyroid, and the growth hormone axes.

for androgen exposure. Further, aspects of both immunological defense systems interact with circulating testosterone, and ARs have been found on both B and T-lymphocytes as well as in the thymus and the bursa of Fabricius (Wunderlich et al., 2002; Viselli et al., 1995; Sullivan and Wira, 1979).

3.1. T-cell-mediated immunity

A growing body of literature suggests that androgens deposited in yolk significantly alter the T-cell-mediated immune response in developing birds. For example, *in ovo* injections of testosterone suppressed the cell-mediated immune response to phytohemagglutinin (PHA) in developing eastern bluebird (Navara et al., 2005) and black-headed gull chicks (Groothuis et al., 2005). These results are consistent with many other studies on adult birds in which testosterone treatment suppressed T-cell immunity: In adult chickens, testosterone treatment resulted in a decrease in gamma–delta-T-lymphocytes, which comprise a large circulating T-cell subset in birds (Arstila and Lassila, 1993). Testosterone treatment of adult European Starlings and dark-eyed juncos suppressed the T-cell response to PHA (Duffy et al., 2000; Casto et al., 2001). On the other hand, however, yolk testosterone treatment did not alter the response of great tit nestlings to PHA (Tschirren et al., 2005), and house finch chicks that received a testosterone injection *in ovo* exhibited a larger cell-mediated immune response to PHA as compared to controls (Navara et al., 2006). While, to our knowledge, such an androgen-related stimulation of T-cell immunity has not been previously demonstrated in other avian species, Siberian hamsters treated with testosterone exhibited a larger cell-mediated response to PHA compared to controls (Bilbo and Nelson, 2001). These studies suggest that yolk androgens exert a significant effect on T-cell mediated immunity in developing birds, but the nature of these effects is clearly species-specific, and perhaps context and/or dose-specific as well.

Very little mechanistic work examining androgen-related immunological effects on T-cell mediated immunity has been done in birds. Using a large body of mammalian literature on the subject, however, we can hypothesize about the mechanisms involved in androgen-driven immunomodulation in avian embryos. In adult mammals, there are several phenotypic thymocyte subtypes, including immature thymocytes, mature thymocytes that are positively selected to become helper or cytotoxic T-cells, and mature thymocytes destined for apoptosis. Androgen receptors have been found in the mammalian thymus in comparable amounts to those found in the reproductive tract (Viselli et al., 1995), and androgen exposure causes a shift in the thymic cell population towards apoptosis (Olsen and Kovacs 1996). It has been suggested that, overall, androgens generally suppress cytotoxic/suppressor T-cell function in mammals, but the effects seen in the peripheral immune system are most likely due to alterations within the thymocyte population (Olsen and Kovacs, 1996). However, recent evidence suggests that testosterone acts non-genomically through receptors on the surface of T-cells and macrophages to induce immunosuppression (Wunderlich et al., 2002). Additionally, in mice, androgen downregulates the production of various cytokines, including interleukin-4, interleukin-5, and gamma-interferon (Araneo et al., 1991). Thus the effects of androgen on T-cell mediated immunity can occur through several different targets working in combination with one another. Identifying the presence of ARs in specific immune-responsive tissues of the avian embryo are crucial before the mechanistic actions of yolk androgens on T-cell-mediated immunity can be elucidated.

3.2. B-cell mediated immunity

The production of antigen-specific antibodies is a very important aspect of the immune response, and studies suggest that exposure to androgens during the embryonic period can have significant effects on the production of antibodies by developing birds. For example, black-headed gull nestlings that received a testosterone injection *in ovo* exhibited a significantly smaller antibody response to a challenge with a standard solution of lipopolysaccharide as compared to controls (Groothuis et al., 2005). Additionally, dipping chicken eggs into a solution containing testosterone propionate (TP) severely reduced the growth of the bursa of Fabricius, and resulting antibody production (Glick, 1961). In another study, treatment of chicken embryos with high doses of TP also resulted in a decrease in bursal weight (Norton and Wira, 1977), possibly mediated by ARs found in the bursal epithelium (Sullivan and Wira, 1979).

The observed suppressive effects of embryonic exposure to androgens on humoral immunity are consistent with findings in adult birds: Adult European Starlings exhibited a negative relationship between circulating concentrations of plasma testosterone and antibody

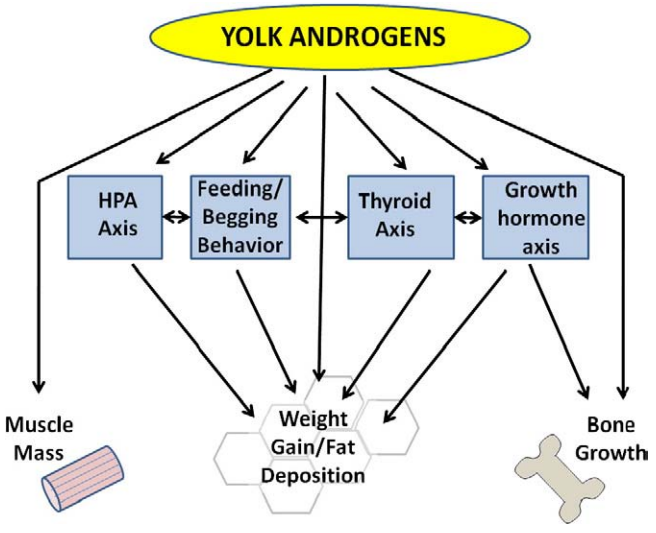

Fig. 4. Integrated effects of yolk androgens on body size. Observed large-scale effects of yolk androgens on body weight gain, muscle development, and skeletal growth may result either from direct effects of androgens on fat, muscle, and skeletal tissue, or through effects on the hypothalamic–pituitary–adrenal (HPA axis), the thyroid axis, the growth hormone axis, or through motivational effects on food intake and begging behavior. Many of these effects may interact with one another to produce the phenotypic endpoint as well.

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response to keyhole limpet haemocyanin (KLH) (Duffy and Ball, 2002). Likewise, in adult dark-eyed juncos, males that received testosterone implants displayed suppressed antibody production in response to a challenge with sheep red blood cells (SRBCs), and direct actions of testosterone resulted in an increase in antibody responsiveness in adult house sparrows challenged with SRBCs (Evans et al., 2000).

It is important to note that young birds differ from adult birds in the presence of thymic and bursal tissue: The thymus and bursa of Fabricius regress at the onset of sexual maturity (reviewed in Sharma, 1998). Thus much of the androgen-related immunosuppression in developing birds may occur organizationally, as a result of effects on the initial development of the thymic and bursal tissue, whereas effects seen in adulthood likely result from direct effects of androgens on T and B-cell populations in plasma and other remaining lymphoid tissue (reviewed in Grossman, 1984). Thus, exposure to androgens during the embryonic period may permanently imprint how birds respond to pathogens they encounter throughout their lifetimes.

3.3. Indirect actions of androgens on immunity

Because androgens have a multitude of effects on other endocrine systems, the actions of androgens can also affect immunity indirectly (Fig. 3). For example, injections of testosterone into American kestrel eggs resulting in offspring that had significantly higher levels of corticosterone in circulation (Sockman and Schwabl, 2001). Similarly, adult dark-eyed juncos implanted with testosterone displayed elevated levels of circulating corticosterone (Ketterson et al., 1991). Glucocorticoids are potent immunomodulators, generally exerting immunosuppressive effects overall in birds (El-Lethey et al., 2003). In chickens, elevated glucocorticoid levels induced thymocyte cell death (Compton et al., 1990) and direct treatment of adult chickens with adrenocorticotropic hormone (ACTH — an upstream stimulator of corticosterone release), resulted in a decreased antibody response (Thaxton et al., 1968). Further, treatment of adult birds with high dose glucocorticoids results in atrophy of lymphoid tissue (Marsh, 1992) and glucocorticoids inhibit the production of several cytokines (Daynes and Araneo, 1989; Araneo et al., 1991). Because androgens have the ability to permanently imprint the hypothalamic–pituitary–adrenal axis (McCormick et al., 1998), exposure to yolk androgens may alter not only the adrenal hormone milieu, but may also permanently imprint immune system function as well.

Due to the integrated thyroid–gonad relationship, and the complex effects of androgens on the thyroid axis in birds (Gupta and Thapliyal, 1984; Thapliyal et al., 1984), the effects of thyroxine on immune function must also be considered. Thyroxine appears to have a generally stimulatory effect on immune function. For example, thyroidectomy of young chickens has been associated with decreases in bursal, thymic, and splenic sizes (Marsh, 1992). Further, decreasing thyroid levels has been associated with a decrease in circulating lymphocyte numbers (Marsh, 1992). Thus, androgens could have an indirect stimulatory effect on immune function through the organization of the hypothalamic–pituitary–thyroid axis, which could potentially explain the stimulatory effect of yolk androgens on house finch nestlings mentioned above.

Finally, we must also consider the interactions of androgens with the growth hormone pathways. As mentioned earlier, testosterone stimulates the release of growth hormone from the anterior pituitary gland in mammals (Bondanelli et al., 2003). In adult birds, growth hormone exerts a stimulatory effect on the growth of lymphoid organs (Marsh, 1992). When young chickens were treated with bovine growth hormone, bursal growth was stimulated and the antibody response to a challenge with SRBCs was enhanced (Marsh, 1992). Additionally, the same treatment prevented the suppressive effects of corticosterone on the bursa in young ducks (Glick, 1960) and young pigeons (Bates et al., 1962). Thus, while the direct effects of androgens during development may be immunosuppressive, the indirect effects can be immunoenhancing, providing another controlling mechanism over immune function during development.

4. Conclusions

The expression of a phenotypic endpoint usually represents the cooperative integration of many simultaneous physiological changes. The stimulation of body weight gain by yolk androgens, for example, could represent adjustments of several major endocrine axes, including the growth hormone axis, the thyroid hormone axis, and the adrenal axis in addition to behavioral changes affecting hunger, food intake, and begging behavior (Fig. 4). Adjustments of these axes can have effects on other major body systems, such as the immune system, which may result in changes in disease resistance depending on which aspects of the immune system are functionally altered, and how they interact to form overall immunological changes. Some effects appear beneficial while others appear detrimental. To add another layer of complexity not discussed in depth here, the physiological responses to high levels of yolk androgens are likely sex-specific, as several recent studies have shown that males and females exhibit different physiological and behavioral changes in cases where yolk androgens were experimentally elevated (Saino et al., 2006; von Engelhardt et al., 2006).

It seems that exposure to yolk androgens during embryonic development exerts long-term effects on offspring that last at least throughout the nestling period. Because yolk androgen levels decline sharply within the first two days of embryonic development, such effects must result from distinct organizational changes in offspring physiology. In mammals, fetal testosterone exposure determines the number and location of androgen receptors present in different brain areas during development and adulthood (Resko and Roselli, 1997). Future experiments in birds should explore the number, location, and function of hormone receptors and their role in creating the phenotypes we see in response to yolk androgen exposure. In addition, it is also important to explore dose–responses related to yolk androgen exposure, because even doses within the physiological range can have variable effects on offspring phenotype. Navara et al. (2005) showed that eastern bluebird nestlings hatching from eggs that received a physiologically low dose of testosterone tended to have larger tarsi, while those hatching from eggs that received a physiologically high dose did not. Because androgens can exert such potent, large-scale effects, it becomes necessary to perform dose–responses for each physiological change that is measured to determine what measured quantities of yolk androgens truly mean in a physiological context.

While the recent integration of ecological, evolutionary, and physiological fields to answer questions about how yolk androgens affect offspring should be commended, we must now take the next step to understand the many mechanistic changes which occur in developing birds when exposed to high levels of yolk androgens, and importantly, how those changes interact with one another. Overall, we need to understand how these changes function in different environmental contexts. For example, if androgens stimulate growth but depress immune function simultaneously, the pathogen-richness of the environment into which offspring hatch will determine whether the overall result is positive or negative. In this example, only two physiological effects were taken into account (growth and immune function). Given the complexity of the physiological changes resulting from yolk androgen exposure, there are many more scenarios that must be addressed, for example, changes in stress responsiveness and long-term effects associated with metabolism, growth, and secondary sexual traits.

While experiments addressing the phenotypic effects of yolk androgens on offspring are beginning to take more mechanistic approaches, the number potential physiological targets of yolk androgens that have gone untested is enormous. Currently, we must draw evidence from studies that examine the effects of plasma androgens on adult phenotype, and others that utilize pharmacological *in ovo* androgen treatment to document effects on the development of individual body

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systems. However, these studies give us direction in which to pursue more naturalistic studies that will eventually allow for a full understanding how yolk androgens affect offspring. Additionally, we need to determine whether the effects of yolk androgens permanently organizational or transiently activational, whether androgens affect offspring directly, or whether they alter androgen receptor number and function throughout the lifetime of the offspring, and how long-term effects of androgens may be differentially expressed by species with different life history traits. By answering more of these questions, we can formulate precise predictions concerning how yolk androgens function in an adaptive context.

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