Androgens have benefits, such as promoting muscle growth, but also significant costs, including suppression of immune function. In many species, these trade-offs in androgen action are reflected in regulated androgen production, which is typically highest only in reproductive males. However, all non-reproductive Arctic ground squirrels, irrespective of age and sex, have high levels of androgens prior to hibernating at sub-zero temperatures. Androgens appear to be required to make muscle in summer, which, together with lipid, is then catabolized during overwinter. By contrast, most hibernating mammals catabolize only lipid. We tested the hypothesis that androgen action is selectively enhanced in Arctic ground squirrel muscle because of an upregulation of androgen receptors (ARs). Using Western blot analysis, we found that Arctic ground squirrels have AR in skeletal muscle more than four times that of Columbian ground squirrels, a related southern species that overwinters at approximately 0°C and has low pre-hibernation androgen levels. By contrast, AR in lymph nodes was equivalent in both species. Brain AR was also modestly but significantly increased in Arctic ground squirrel relative to Columbian ground squirrel. These results are consistent with the hypothesis that tissue-specific AR regulation prior to hibernation provides a mechanism whereby Arctic ground squirrels obtain the life-history benefits and mitigate the costs associated with high androgen production.

1. Introduction

In male vertebrates, testosterone plays a key role in life-history decisions, orchestrating a suite of co-evolved traits which trade-off survival and self-maintenance for reproduction [1,2]. Consequently, testosterone production is highest during the breeding season, and virtually absent in the non-breeding season [3]. Columbian ground squirrels (CGS) (*Urocitellus columbianus* Ord) follow this pattern, as do virtually all other hibernating rodents [4]. Arctic ground squirrels (AGS) (*U. parryii* Richardson) do not (note that both species were formerly in the genus *Spermophilus*). Both species live in North America and have a three to four month active season, with the post-breeding summer being devoted to intensively foraging to build up reserves for hibernation [4,5]. AGS live in the arctic tundra and, in the northwest, in alpine areas and forest meadows (latitudinal range ca 58–70°N); CGS live in the alpine and subalpine meadows of the western mountains (ca 43–55°N). AGS hibernate in frozen ground over permafrost (hibernacula temperatures down to −23°C) [6]; CGS hibernate at or just below the frost line (hibernacula temperatures approx. 0°C) as do other sciurids [7,8]. AGS of both sexes have levels of testosterone and related androgens that are 10–200 times those in other ground squirrel species [4,9,10] (e.g. mean levels in
pre-hibernating AGS males, adults = 6.90 ng ml⁻¹, juveniles = 6.21 versus CGS, adults = 0.26, juveniles = 0.31; female comparisons were similar; see figs 2 and 3 in [4]). The metabolic demands imposed on AGS by extreme cold during hibernation are met prior to hibernation by increasing both lipid and protein stores [5,11]; by contrast, CGS increase only lipid stores [4]. Gluconeogenesis resulting from muscle catabolism can provide sufficient glucose needed by AGS for maintenance of key tissues that pure lipid catabolism cannot [4,11]. Thus in AGS, high androgen production appears to be an adaptation that increases lean mass in summer [4] in preparation for its catabolism in winter while hibernating under deep freeze conditions.

This remarkable adaptation raises the question of how AGS avoid paying the costs of high androgen levels during the pre-hibernation period. For example, testosterone increases vulnerability of mice to parasitic infection, likely via actions in the lymph node system [12]. However, immunosuppression does not occur in pre-hibernating AGS males in the face of high androgen levels [10]. To reconcile these potentially positive and negative impacts of androgens, Boonstra et al. [4] proposed that there is an upregulation of androgen receptors (ARs) in muscle tissue to promote muscle growth and a down-regulation of AR in key tissues, such as those of the immune system, that could reduce survival. However, it is currently unclear the extent to which (or even whether) androgen-regulated traits can be selectively dissociated from the breeding to the non-breeding season during evolution. Adaptations that affect the tissue-specific presence of AR could be the means of achieving specificity of androgen action.

To test this hypothesis, we examined AR expression in brain, lymph nodes and muscle tissue of pre-hibernating AGS and compared this with that in CGS. If androgenic traits can be dissociated from one another in an adaptive manner, we predict that AR levels would be increased in AGS in muscle, but not in lymph nodes (an important component of the immune system); AR levels in CGS should remain low in all tissues.

2. Material and methods

Squirrels were trapped and dissected largely as described in [10]. We trapped CGS on 18 and 19 July 2009 on an open meadow near Barrier Lake, Alberta (51.1’ N, 115.1’ W) and AGS on 28 and 29 July 2009 on the grasslands of a ranch near Whitehorse, Yukon (60.7’N, 135’W—1632 km NW of the CGS site). One small Columbian juvenile male was excluded because we were not able to collect all tissues from it. We analysed only juveniles in AGS and a mixture of adults and juveniles in CGS. This was intentional as few adults were caught in AGS and previous studies indicated that androgen levels were extremely high, irrespective of age (figs 2–4 in [4]), and uniformly low in all age classes in CGS (figs 2 and 3 in [4]). Hence, the presence or absence of androgens at this stage of their life history is a generalized one, with age not being the relevant determinant. The brain (cerebrum), quadricep muscle and lymph nodes around the caecum were immediately collected using sterile technique and frozen on dry ice. On our return to the University of Toronto, all tissue was stored at −80°C.

Western blot analysis was carried out on frozen tissues largely as previously described [13]. The amount of protein loaded onto SDS-PAGE gels varied by tissue (60 µg of muscle, 30 µg of brain or 15 µg of lymph nodes) in a yoked fashion such that each sex and species was represented on gels for muscle, or brain or lymph nodes. Each sample was loaded into a separate gel for AR immunoblotting (10% acrylamide) and for actin immunoblotting (6% acrylamide) and protein was then transferred onto nitrocellulose membranes. Membranes were stained using primary antibodies for AR (SC-816; 1:500; Santa Cruz) or actin (sc-1616-HP; 1:1000; Santa Cruz) in conjunction with secondary antibody (sc-2004-HP; 1:1000, Santa Cruz) and the ECL Plus reagent. Relative optical density (ROD) measurements of resulting bands were made using a STORM phospho-imager. We analysed ROD of AR normalized to actin ROD for the same subject.

As we had small sample sizes and to avoid the assumptions of normality, we use the Mann–Whitney U-test for comparisons between groups. All data are presented as means ± s.e. We analysed for differences between species using normalized AR ROD data separately. The null hypothesis was rejected at p < 0.05.

3. Results

We collected 24 animals (13 AGS and 11 CGS). We attempted to obtain useable protein from all of them, but about half had protein deterioration and thus we analysed only a subset: seven AGS, all juveniles (body mass: four males—430.2 ± 20.4 g; three females—363.7 ± 25.7 g) and six CGS (four males [two adults: two juveniles]—468.5 ± 155.1; two females [one adult: one juvenile]—350.5 ± 164.5). From the entire sample collected, adrenal glands were more than two times heavier in AGS than CGS (z = −2.98, p = 0.03) (AGS: males—0.154 ± 0.029 g, N = 6; females—0.109 ± 0.008, N = 7; CGS: males—0.076 ± 0.018, N = 5; females—0.059 ± 0.015, N = 6).

To our knowledge, AR western blotting has not been previously reported in ground squirrels. Similar to other species, we observed a major band of approximately 110 kDa (figure 1a). We compared AR abundance in the three tissues separately in AGS (N = 7 skeletal muscle and brain, N = 6 lymph nodes) and CGS (N = 6). Compared with CGS, AGS had AR levels more than four times greater in muscle (z = −3.0, p = 0.003) and 1.6 times greater in brain tissue (z = −3.0, p < 0.003), and similar levels in lymph nodes (z = −0.80, p = 0.42) (figure 1b).

4. Discussion

We investigated the hypothesis that during the pre-hibernation period, AGS use tissue-specific regulation of AR levels to maximize the benefit and minimize the costs associated with high androgen levels. We made three major findings. First, our previous research [4] indicated that the adrenals were the site of production of the high pre-hibernation androgen levels. Our evidence here is concordant with this, with adrenal mass of AGS being about twice that of CGS. We speculate that this increase is caused by hypertrophy of the zona reticularis, the site of production of adrenal androgens in other mammals [4]. Second, we found that AR levels were markedly elevated in skeletal muscle in AGS relative to CGS, but that AR levels in the lymph nodes were similar in the two species. This would encourage muscle growth in AGS under the influence of high androgen levels [4,5]. It would not suppress their immune function and, indeed, the immune response in pre-hibernating, non-breeding male AGS to a standardized challenge is much greater than in breeding males [10]. Third, we found a modest increase in brain AR in AGS, possibly facilitating aggressive or metabolic functions of androgens. AGS may thereby maximize the benefit of high circulating
Blots were stained for AR or pan-actin (actin). (muscle, brain and lymph node tissue from four individual Arctic (A) and Columbian (C) ground squirrels, two males (1,2) and two females (3,4) from each species. Muscle mass in some fibre types, and more robust effects of indicates a modest role for AR in myocytes in promoting differentiation of muscles within individuals, and the degree to which muscles are sensitive to androgen appears to be related to AR within myocytes, rather than other cell types in muscle [15]. Genetic manipulation of mice and rats 
androgen production in summer linked to environment severity in winter. 
Androgens are well known to promote muscle mass in males of a variety of vertebrate species and it appears that differences in androgen action on skeletal muscle between species may be specified at the receptor level. For example, increased AR expression is observed in skeletal muscles of golden-collared manakin (Manacus vitellinus), which exhibits hypertrophic courtship muscles, relative to zebra finches (Taenopygia guttata) or ochre-bellied flycatchers (Mionectes oleaginous), which do not, despite similar circulating androgen levels in these species [14]. Interestingly, androgens also differentially regulate muscles within individuals, and the degree to which muscles are sensitive to androgen appears to be related to AR within myocytes, rather than other cell types in muscle [15]. Genetic manipulation of mice and rats indicates a modest role for AR in myocytes in promoting muscle mass in some fibre types, and more robust effects of androgens [4] to promote muscle anabolism and metabolism and to promote aggressive behaviour, while also minimizing the cost on immune function.

Androgens well known to promote muscle mass in males of a variety of vertebrate species and it appears that differences in androgen action on skeletal muscle between species may be specified at the receptor level. For example, increased AR expression is observed in skeletal muscles of golden-collared manakin (Manacus vitellinus), which exhibits hypertrophic courtship muscles, relative to zebra finches (Taenopygia guttata) or ochre-bellied flycatchers (Mionectes oleaginous), which do not, despite similar circulating androgen levels in these species [14]. Interestingly, androgens also differentially regulate muscles within individuals, and the degree to which muscles are sensitive to androgen appears to be related to AR within myocytes, rather than other cell types in muscle [15]. Genetic manipulation of mice and rats indicates a modest role for AR in myocytes in promoting muscle mass in some fibre types, and more robust effects of AR in myocytes in regulating energy production and storage in skeletal muscle and energy balance systemically, including loss of fat mass (e.g., [16]). Consistent with an evolutionary constraint on AR action on muscle, sufficient overexpression of AR in myocytes is associated with perinatal male mortality and neuromuscular atrophy [13] and also loss of fat mass [16], which remains an important energy source in AGS.

**Figure 1.** Western blot analysis of AR in Arctic and Columbian ground squirrels. (a) Representative images of Western blots obtained from samples of skeletal muscle, brain and lymph node tissue from four individual Arctic (A) and Columbian (C) ground squirrels, two males (1,2) and two females (3,4) from each species. Blots were stained for AR or pan-actin (actin). (b) ROD (mean ± 1 s.e.) of Arctic (filled bars, n = 7) and Columbian (open bars, n = 6) ground squirrels. AR ROD measurements were normalized to actin RODs for each subject, then the relative multiple increase of the tissue (i.e. fold change in gene expression studies) was determined by taking the ratio of normalized AR RODs for each subject and the Columbian group average normalized AR ROD. Asterisks (*) represent statistical significant Mann–Whitney U-test between species for that tissue type.

**References**


