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Circulating 11-oxygenated androgens across species

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Abstract

The androgen precursors, dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS) are produced in high amounts by the adrenal cortex primarily in humans and a few other primates. The human adrenal also secretes 11-oxygenated androgens (11-oxyandrogens), including 11β-hydroxyandrostenedione (11OHA4), 11-ketoandrostenedione (11KA4), 11β-hydroxytestosterone (11OHT) and 11-ketotestosterone (11KT), of which 11OHT and 11KT are bioactive androgens. The 11-oxyandrogens, particularly 11KT, have been recognized as biologically important testicular androgens in teleost fishes for decades, but their physiological contribution in humans has only recently been established. Beyond fish and humans, however, the presence of 11-oxyandrogens in other species has not been investigated. This study provides a comprehensive analysis of a set of C_{19} steroids, including the traditional androgens and 11-oxyandrogens, across 18 animal species. As previously shown, serum DHEA and DHEAS were much higher in primates than all other species. Circulating 11-oxyandrogens, especially 11KT, were observed in notable amounts in male, but not in female trout, consistent with gonadal origin in fish. The circulating concentrations of 11-oxyandrogens ranged from 0.1 to 10 nM in pigs, guinea pigs and in all the primates studied (rhesus macaque, baboon, chimpanzee and human) but not in rats or mice, and 11OHA4 was consistently the most abundant. In contrast to fish, serum 11KT concentrations were similar in male and female primates for each species, despite significantly higher circulating testosterone in males, suggesting that 11KT production in these species is not testis-dependent and primarily originates adrenal-derived 11-oxyandrogen precursors.

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Key terms:

adrenal; steroids; 11-oxyandrogens; primates

1. Introduction

The adrenal secretion of abundant quantities of the androgen precursors, dehydroepiandrosterone (DHEA) and its sulfate (DHEAS), is a phenomenon limited to humans and some non-human primates [1–11]. The adrenal zona reticularis (ZR) of Old World primates [9] has an expression pattern of steroidogenic enzymes that makes it the site of DHEA and DHEAS biosynthesis in these species [3, 12, 13]. Notably, in adult humans, DHEAS circulates at prodigious concentrations, vastly exceeding the levels of other steroid hormones [14–16]. Past research has shown that the adrenal production of these 19-carbon (C19) steroids is negligible or absent in common laboratory and domestic animals such as mice, rats, hamsters, pigs, guinea pigs, dogs, sheep and cattle [4, 11, 17].

Recent liquid chromatography-tandem mass spectrometry (LC-MS/MS) studies have provided evidence for human adrenal production of 11-oxygenated androgens (11-oxyandrogens) and their potential role in androgen-related disorders such as premature adrenarche, congenital adrenal hyperplasia, castration-resistant prostate cancer and polycystic ovary syndrome [18–22]. These 11-oxyandrogens include derivatives of androstenedione (A4) and testosterone (T), including 11β-hydroxyandrostenedione (11OHA4), 11-ketoandrostenedione (11KA4), 11β-hydroxytestosterone (11OHT) and 11-ketotestosterone (11KT) [15, 23]. Of these, 11KT is a potent androgen, with bioactivity comparable to that of T [15, 19, 20, 24]. For over six decades 11KT has been recognized as a biologically important gonadal androgen in teleost fishes where it was shown to mediate induction of sexual male-type behavior, onset of spermatogenesis and female-to-male sex reversal [25–34]. In humans, however, the potential contribution of 11KT to physiology and disease, particularly in women and children, has only recently been documented [15, 20–22, 35]. Beyond fish and humans, the evolutionary phylogeny of 11-oxyandrogens across other species has not been studied. The goal of the current study was to provide a characterization of the 11-oxyandrogens in a variety of species, ranging from fish to humans, using LC-MS/MS.

2. Materials and Methods

2.1. Animal sera

Sera from the following animal classes were studied: *Osteichthyes* (trout), *Amphibia* (frog), *Reptilia* (alligator), *Aves* (chicken) and *Mammalia* (laboratory and domesticated animals, and primates). Sera from animals were obtained as detailed in Table 1. Additional sera from laboratory and domesticated animals, and non-human primates were also procured from the Unit for Laboratory Animal Medicine (ULAM), University of Michigan and commercially from BioIVT (Hicksville, NY). Animals used in this study, with the exception of alligators, were of reproductive age. The sera from ULAM were obtained after all procedures were approved by the Institutional Animal Care and Use Committees at the University of

Michigan. The protocols used for serum procurement followed the Public Health Service guidelines for the humane care and use of experimental animals. After approval from the institutional review boards at the University of Michigan, serum was obtained from men and women between ages 20–40 years who had morning (8am–10am) blood draws in an outpatient setting, as part of routine medical care for minor health condition or as part of annual exams (Table 1).

2.2. Steroid Quantitation by LC-MS/MS

We quantified 8 C19 steroids using LC-MS/MS: DHEA, DHEAS, A4, T, 11OHA4, 11KA4, 11OHT and 11KT. Unlabeled and deuterium-labeled steroids were obtained from Sigma-Aldrich, Cerilliant, C/D/N isotopes, Steraloids and The National Heart, Lung and Blood Institute RTI International Metabolite Standards Synthesis Center (Supplemental Table 1). Steroid extraction by liquid-liquid extraction and quantitation was carried out as previously described [20].

Samples (10 μL) were injected via autosampler and resolved with a pair of Agilent 1260/1290 binary pump HPLCs via 2D liquid chromatography, first on a 10 mm × 3 mm, 3 μm particle size Hypersil Gold C4 loading column (Thermo Scientific, Waltham, Massachusetts) followed by a Kinetex 50 mm × 2.1 mm, 2.6 μm particle size biphenyl resolving column (Phenomenex, Torrance, CA). The mobile phases consisted of 0.2 mmol/L aqueous ammonium fluoride (A) and methanol with 0.2 mmol/L ammonium fluoride (B). Steroids were eluted using gradient specifications as described in Supplemental Tables 2 and 3. The column effluent was directed into the source of an Agilent 6495 triple quadrupole mass spectrometer using electrospray ionization in positive ion mode for Δ4 (A4, T and 11-oxyandrogens) and Δ5 (DHEA) or negative ionization mode for steroid sulfates (DHEAS) and analyzed using multiple reaction monitoring (MRM) mode (Supplemental Table 1). Quantitation was accomplished by comparing ion currents for the monitored ions with weighted (1/x) 12-point linear external calibration curves (r^2 was >0.995) and corrected for specimen dilution and recovery of internal standards using ChemStation and MassHunter software (Agilent, Santa Clara, CA). Intra-assay and inter-assay coefficients of variation (CV) were assessed by measuring quality control pooled serum samples five times within a run and across five different runs, respectively, and were < 12% for all steroids. The lower limit of detection (LOD) and lower limit of quantitation (LOQ) for each steroid were defined by the minimum concentration achieving an extrapolated signal-to-noise ratio of 3 and 5, respectively. LOD ranged from 0.007 nM for A4 to 0.105 nM for DHEAS and LOQ ranged from 0.011 nM for A4 to 0.174 nM for DHEAS (Supplemental Table 1).

It should be noted that sex of the trout was not available on the receipt of the serum samples. The identification of the sex of the trout based on the 11KT concentrations after measurement by LC-MS/MS. Trout demonstrating substantial serum concentrations of 11KT were marked as ‘males’ on the basis of previous studies [25–34].

2.3. Statistical analysis

GraphPad Prism 7 (La Jolla, CA) was used for statistical analysis. Non-parametric Mann-Whitney *U* test or Wilcoxon matched-pairs signed rank test was used for two group

comparisons. Kruskal-Wallis test was used for multiple group comparison. Significance was accepted at an alpha level of 0.05.

3. Results

3.1. Circulating concentrations of the classic adrenal androgens DHEA and DHEAS across different species

Negligible concentrations of DHEA and DHEAS were found in species ranging from fish to birds, as well as in rodents and other common laboratory and domesticated animals (Fig. 1, Table 2). Large amounts of circulating DHEA and DHEAS were observed only in primates (Fig. 1) ($p < 0.001$, each primate species vs. other non-primate species). While rhesus monkeys demonstrated the highest DHEA concentrations among primates (64.7 ± 10.8 nM), maximal circulating DHEAS was seen in humans (5215 ± 320 nM). A sub-analysis by sex showed that men have higher levels of both DHEA and DHEAS compared to women ($p< 0.001$; data not shown), in concordance with previous reports [36–39].

3.2. Circulating concentrations of the 11-oxyandrogens across different species

As observed with DHEA and DHEAS, all primates had >1 nM circulating concentrations of A4, 11OHA4 and 11KA4 (Supplemental Fig.1, Fig. 2), and lower amounts of the 11-oxygenated derivatives of A4 were detected in dogs, cattle, sheep and horses (Fig. 2). Among lower species, only serum from pigs and guinea pigs exhibited substantial amounts (0.1–1 nM) of 11-oxyandrogens. Circulating concentrations of 11OHA4 and 11KA4 in guinea pigs (5.5 ± 1.2 nM; 0.9 ± 0.2 nM, respectively) were similar to those seen in humans (5.6 ± 0.4 nM; 1.1 ± 0.1 nM respectively) (Fig. 2).

Amongst tetrapods, primates, particularly chimpanzees and humans, produced both 11OHT and 11KT (Fig. 3). Interestingly, with the exception of pigs and guinea pigs, these bioactive 11-oxyandrogens were negligible or absent in the other tetrapods studied. While pigs exhibited high 11OHT (0.4 ± 0.1 nM) with concentrations comparable to those observed in humans (0.4 ± 0.1 nM), guinea pigs demonstrated 11KT concentrations (0.4 ± 0.1 nM) similar to those seen in the Old World monkeys (rhesus and baboon) (~0.2 nM). These 11KT values were, however, significantly lower than in humans (0.9 ± 0.1 nM) ($p < 0.001$) (Fig. 3). Notably, unlike T, serum concentrations of 11OHT and 11KT were similar between sexes across tetrapods (Fig. 4 and Supplemental Fig.2).

The maximal concentrations of 11KT across species were observed in the male trout, with levels 59-fold higher than those seen in men (0.8 ± 0.1 nM) ($p < 0.001$); however, male trout had lower circulating T concentrations than men ($p < 0.05$) (Fig. 5B, Table 2). In contrast to humans and other primates, female trout produced negligible amounts of 11KT (Fig. 5A, Table 2).

Female primates produced similar quantities of 11KT and T (Fig. 5A). While male trout synthesized substantially higher concentrations of 11KT as compared to T (258-fold, $p< 0.05$), T was elevated than 11KT in all male primates (Fig. 5B). The concentrations of 11KT in males and female primates, however, were not significantly different ($p = 0.205$).

4. Discussion

Adrenal steroidogenesis varies among species based on differences in the expression and activities of steroidogenic enzymes in the different adrenocortical zones. This is particularly the case for adrenal production of androgens and their precursors. In primates, adrenal biosynthesis of DHEA and DHEAS occurs within the ZR, a zone which exhibits abundant cytochrome b5 (CYB5) and sulfotransferase type 2A1 expression, but which lacks 3β-hydroxysteroid dehydrogenase type 2 (HSD3B2) [6, 7, 13, 40, 41]. CYB5 is an allosteric regulator known to enhance the 17,20-lyase activity of CYP17 (17α-hydroxylase/17,20-lyase) and to facilitate the conversion of 17α-hydroxypregnenolone to DHEA in the ZR in primates via the Δ5 pathway [7, 41, 42]. The low expression of HSD3B2 in the ZR limits the enzymatic competition with CYP17, thus promoting the flow of substrates towards DHEA and DHEAS.

The current study demonstrates that circulating DHEA and DHEAS are an order of magnitude lower in non-primate species as compared to Old World monkeys, apes and humans, which is in agreement with previous reports [4, 10, 11, 17, 43, 44]. The negligible levels of DHEA and DHEAS in the common laboratory animals, including rats and mice, have been attributed to the lack of adrenal expression of CYP17 [35, 45–47]. The low 17,20-lyase adrenal activity in rabbit and dog as compared to primates, and the preferential hydroxylase activity of the hamster CYP17 enzyme over the lyase reaction might explain the low secretion of DHEA in these species [48, 49]. Hornsby et al. demonstrated that HSD3B2 activity was 10-fold higher in bovine adrenocortical cells than in fetal human adrenocortical cells, diverting the Δ5 steroid flux into the Δ4 pathway at the pregnenolone → progesterone step, thus obstructing the synthesis of DHEA and DHEAS [50, 51]. The retention of HSD3B2 in the inner cortical zone in these species might also contribute to low DHEA concentrations [52, 53]. The current study confirms that efficient adrenal production of DHEA/DHEAS is limited to primates, all of which exhibit a steroidogenically unique ZR, with an enzyme profile that promotes the Δ5 androgenic pathway.

These findings also suggest that amongst non-primate tetrapods, only pigs and guinea pigs are capable of synthesizing 11-oxyandrogens. Early studies indicated that in pig, both the testis and the adrenal can produce 11OHA4 and the testis alone synthesizes 11OHT [54–56]. Porcine CYP17 is able to catalyze both the Δ4- and Δ5- lyase reactions without the need for CYB5, promoting the synthesis of A4 and 11OHA4 at the expense of DHEA [56–60]. Unlike human CYP17, guinea pig lyase activity of CYP17 preferentially metabolizes 17α-hydroxyprogesterone, a Δ4 steroid, to A4 [61–65], which is then rapidly converted into 11OHA4 by 11β-hydroxylase [64, 66]. 11OHA4 can be further metabolized to the other 11-oxyandrogens as previously described [67, 68]. Interestingly, we found that the concentrations of 11OHA4 and 11KA4 in guinea pig sera are comparable to those seen in primates. This phenomenon could perhaps be attributed in part to the decreased sensitivity of the guinea pig glucocorticoid receptor to glucocorticoids, leading to enhanced adrenocorticotropic hormone (ACTH) [69–72]. Elevated ACTH, in turn, stimulates the adrenal output of not only cortisol but also of C_{19} steroids, including 11-oxyandrogens [15, 22].

Recent reports have highlighted the production 11-oxyandrogens in humans [15, 18, 20, 22, 73]. Our data demonstrate that other primates, such as Old World monkeys and apes, also synthesize these steroids. In primates, the 11-oxyandrogens are likely adrenal-derived, because their synthesis depends on the 11β-hydroxylation of A4 and T via 11β-hydroxylase, an enzyme which is almost solely expressed in the adrenal gland in these species [23, 74]. Although it has been previously proposed that 11KT might be a direct product of the gonad in humans [75], the low gonadal expression of 11β-hydroxylase suggests that the contribution of gonads to the synthesis of 11-oxyandrogens is minimal. Moreover, 11OHA4 is a poor substrate for 17β-hydroxysteroid dehydrogenase type 3, the testicular isoenzyme responsible for conversion of A4 to T [21]. To gauge the origin of 11KT in primates, we compared the concentrations of T and 11KT between sexes. As previously found in humans [22], 11KT concentrations were similar in both sexes in all primate species, despite significantly higher circulating T in males. Importantly, 11KT and T circulated at comparable levels in females. Collectively, this data suggests that in primates, 11KT is likely synthesized from the peripheral metabolism of adrenal-derived 11OHA4 as suggested previously [15, 23, 68]. This contrasts with the teleost fishes, where 11-oxyandrogens represent the principal testicular androgen because of the gonadal expression of 11β-hydroxylase [76–81].

The clinical importance of 11-oxyandrogens warrants identifying potential animal models to further study their production and role in physiology. Herein, we have demonstrated that 11-oxyandrogens are produced in multiple primate species. Using primates as research models, however, limits most mechanistic studies owing to the costs and availability of these animals. Of note, amongst non-primate animals, pigs and guinea pigs synthesize 11-oxyandrogens, have circulating levels similar to those seen in primates, and therefore might serve as suitable models. Further investigations are, nonetheless, required to ascertain the utility of these animals as appropriate model systems to better understand the biosynthesis, regulation and the physiological role of the 11-oxyandrogens.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Steroid Abbreviations:

11-oxyandrogens	11-oxygenated androgens
DHEA	Dehydroepiandrosterone
DHEAS	DHEA sulfate
A4	Androstenedione
T	Testosterone
11OHA4	11β-Hydroxyandrostenedione
11KA4	11-Ketoandrostenedione
11OHT	11β-Hydroxytestosterone
11KT	11-Ketotestosterone

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Highlights

- 11-oxygenated androgens, as well as DHEA and DHEAS were quantified using LC-MS/MS in 18 animal species.
- The serum concentration of the classic adrenal androgens DHEA and DHEAS in sexually mature non-primate mammals are an order of magnitude lower than that found in Old World monkeys, apes and humans.
- Other than trout, only guinea pigs, pigs and primates had significant levels of circulating 11-oxygenated androgens.
- Although circulating concentration of testosterone was significantly higher in male vs. female primates, circulating 11KT levels were comparable between the sexes in all primates.
- Testosterone and 11KT circulated at similar concentrations in reproductive age women.

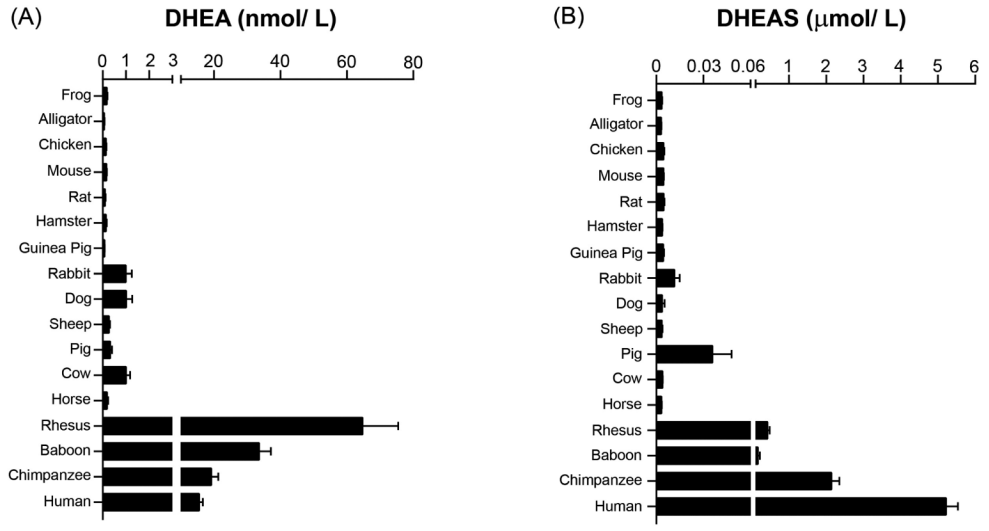

Fig. 1. Circulating concentrations of the classic adrenal androgens in adult animals across different species.

LC-MS/MS was utilized to quantify the serum concentrations of DHEA and DHEAS in 17 species. Males and females were pooled. Only primates were shown to produce high amounts of these androgen precursors. Data are denoted as mean ± SEM. Concentrations of DHEA and DHEAS are expressed in nmol/L (nM) and μmol/L (μM) respectively. DHEA, dehydroepiandrosterone; DHEAS, DHEA sulfate.

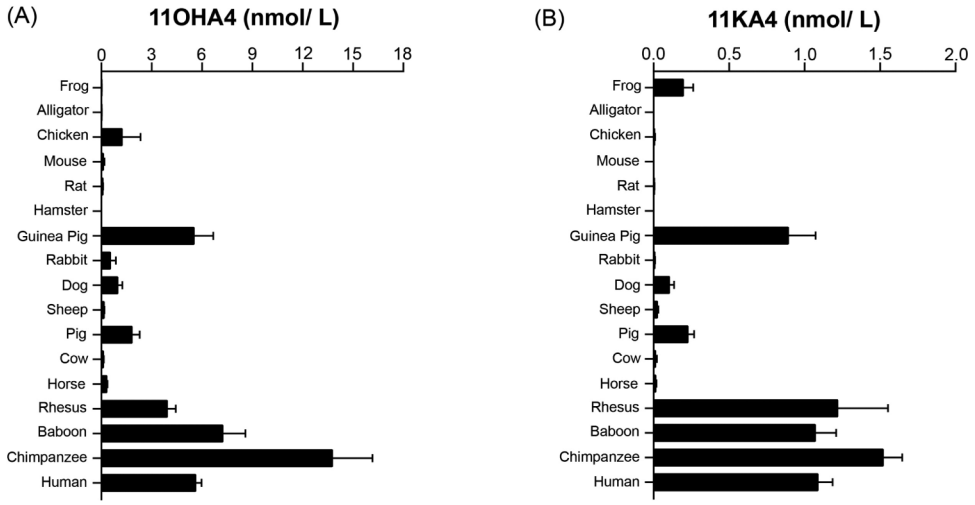

Fig. 2. Circulating concentrations of the 11-oxygenated derivatives of androstenedione in adult animals across different species.

LC-MS/MS was utilized to measure the serum concentrations of 11OHA4 and 11KA4 in 17 species. Males and females were pooled. In addition to all primates, guinea pigs exhibited significant serum amounts of both 11OHA4 and 11KA4. Data are denoted as mean ± SEM. Steroid concentrations are expressed in nmol/L (nM). 11OHA4, 11β-hydroxyandrostenedione; 11KA4, 11-ketoandrostenedione.

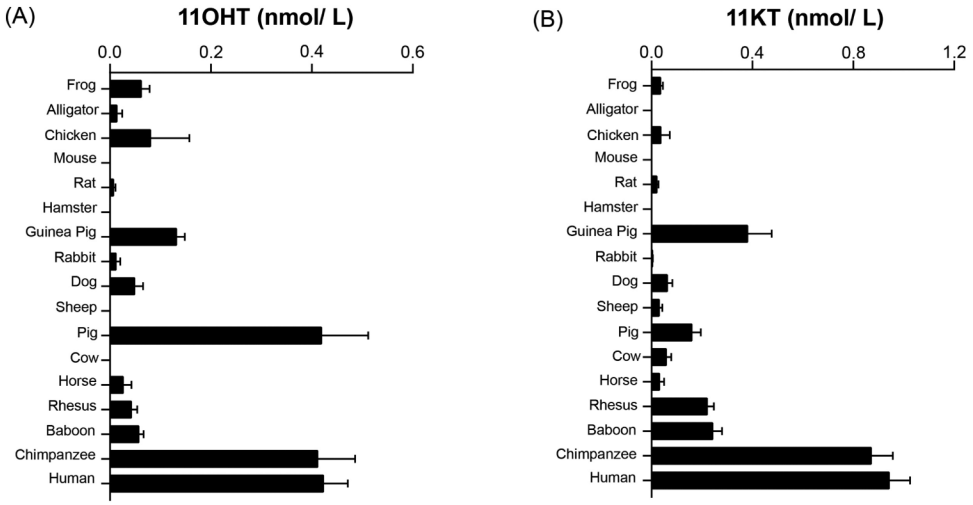

Fig. 3. Circulating concentrations of the bioactive 11-oxyandrogens in adult animals across different species.

LC-MS/MS was utilized to measure the serum concentrations of 11OHT and 11KT in 17 species. Males and females were pooled. Primates, pigs and guinea pigs demonstrated substantial serum concentrations of 11OHT and 11KT. Data are denoted as mean ± SEM. Steroid concentrations are expressed in nmol/L (nM). 11OHT, 11β-hydroxytestosterone; 11KT, 11-ketotestosterone.

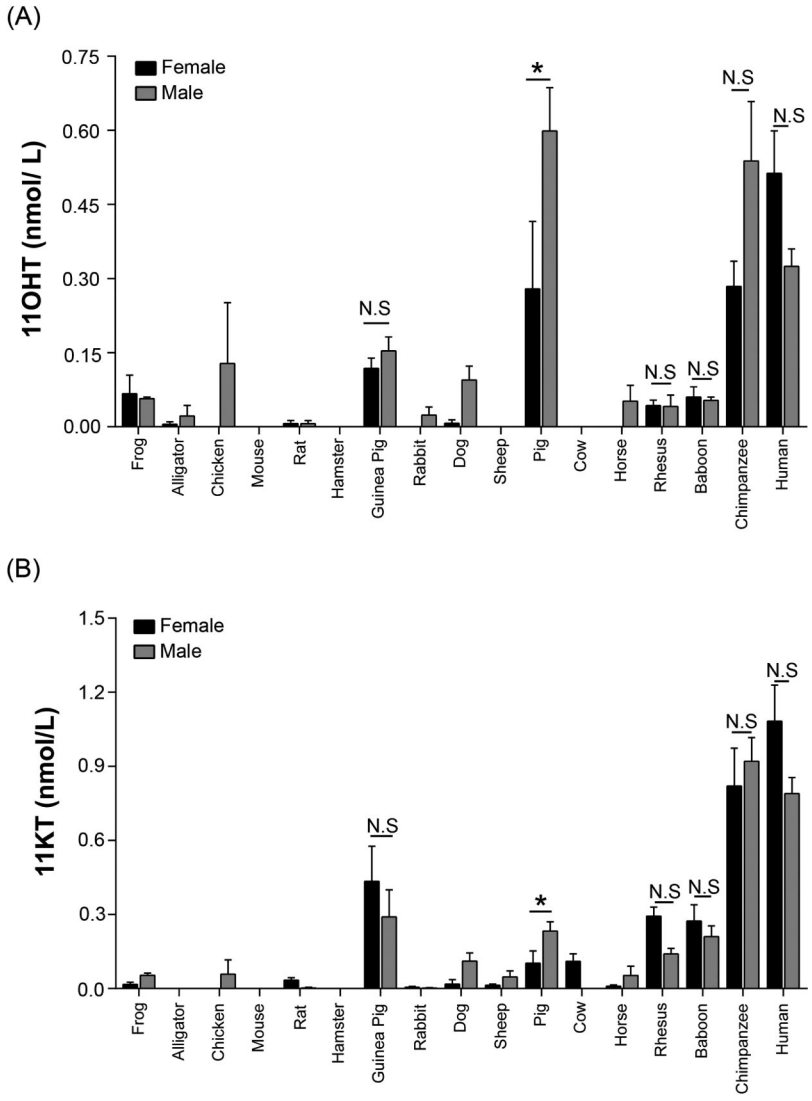

Fig. 4. Circulating concentrations of the bioactive 11-oxyandrogens in adult females and males of 17 species.

Data are denoted as mean ± SEM. Steroid concentrations are expressed in nmol/L (nM). 11OHT, 11β-hydroxytestosterone; 11KT, 11-ketotestosterone. Non-parametric Mann-Whitney U test was used to compare the steroid concentrations between females and males. *P<0.05; N.S, not significant.

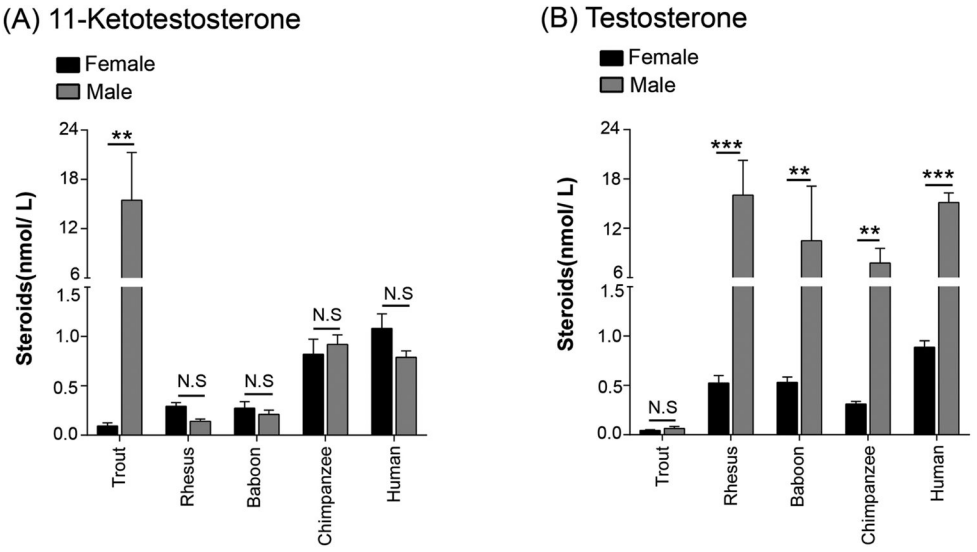

Fig.5. Comparison of circulating levels of (A) 11KT and (B) Testosterone in females vs. males for trout and primates.

(A) While male trout synthesized substantially higher concentrations of 11KT as compared to female, no clear sex differences were observed for 11KT. (B) Testosterone, on the other hand, was synthesized in significantly elevated concentrations in male primates vs. females. Additionally, we observed that female primates produced similar quantities of 11KT and Testosterone. Data are denoted as mean ± SEM. Steroid concentrations are expressed in nmol/L (nM). 11KT, 11- ketotestosterone. Non-parametric Wilcoxon matched-pairs signed rank test was used to compare 11KT vs. Testosterone in the same sex. **P<0.01; ***P<0.001; N.S, not significant.

Table 1A.

Sources of animal sera

Source	Institution	Species	Number of animals provided	
			Female	Male
Irene Salinas	University of New Mexico	Trout	13	3
Michael Criscitiello	Texas A&M University	Frog	3	3
Ruth Elsey	Rockefeller Wildlife Refuge	Alligator	6	6
Catherine VandeVoort; John Capitanio	California Primate Research Center	Rhesus macaque	6	5
Anne Dorrance	Michigan State University	Rat	8	8
ULAM	University of Michigan	Rat	4	0
		Mouse	5	5
Alan Conley	University of California-Davis	Dog	10	9
		Horse	4	0
Bret McNabb; Joan Rowe	University of California-Davis	Cow	7	6
		Sheep	6	6
Barry Ball	University of Kentucky	Horse	0	4
Trish Berger; Russell Hovey	University of California-Davis	Pig	6	8
Adina Turcu	University of Michigan	Human	25	23
William Rainey	University of Michigan	Human	15	14

Additional sera from adult laboratory and domesticated animals, and non-human primates were also purchased from BioIVT (Hicksville, NY).

Table 1B.

Total number of animals included in the study

Species	Species Name	Female	Male
Trout	*Oncorhynchus mykiss*	13	3
Frog	*Xenopus laevis*	3	3
Alligator	*Alligator mississippiensis*	6	6
Chicken	*Gallus gallus*	3	5
Mouse	*Mus musculus*	5	5
Rat	*Rattus norvegicus domesticus*	11	8
Hamster	*Mesocricetus auratus*	5	3
Guinea Pig	*Cavia porcellus*	5	3
Rabbit	*Oryctolagus cuniculus*	6	6
Dog	*Canis lupus familiaris*	10	9
Sheep	*Ovis aries*	9	9
Pig	*Sus scrofa domestica*	9	7
Cow	*Bos taurus*	10	9
Horse	*Equus ferus caballus*	11	11
Rhesus macaque	*Macaca mulatta*	11	10
Baboon	*Papio hamadryas*	3	3
Chimpanzee	*Pan troglodytes*	5	5
Human	*Homo sapiens*	40	37

Table 2.

C_{19} Steroid concentrations in Trout.

Steroid	Female (n=13)	Male (n=3)	p value
DHEA	0.14 ± 0.01	0.24 ± 0.11	0.618
DHEAS	1.74 ± 0.22	3.11 ± 1.10	0.182
A4	0.32 ± 0.04	0.45 ± 0.12	0.230
T	0.04 ± 0.01	0.06 ± 0.02	0.409
11OHA4	0.65 ± 0.14	0.09 ± 0.06	0.029
11KA4	0.53 ± 0.13	0.54 ± 0.28	0.880
11OHT	0.02 ± 0.01	0.04 ± 0.04	0.116
11KT	0.09 ± 0.03	15.5 ± 5.79	0.004

Data are denoted as mean ± SEM and concentrations are expressed in nmol/L (nM). Statistical significance was determined by nonparametric Mann–Whitney U test. $P < 0.05$ was considered statistically significant.