

Effects of oral androstenedione on steroid metabolism in liver of pregnant and non-pregnant female rats

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Abstract

It is unknown whether androstenedione, a steroidal dietary supplement taken to enhance athletic performance, can affect physiological hormone levels by altering liver enzyme activities that metabolize steroid hormones. Altered hormone levels could be especially devastating during pregnancy. Mature female rats were gavaged with 0, 5, 30 or 60 mg/kg/day androstenedione beginning two weeks prior to mating and continuing through gestation day 19. Non-pregnant female rats were gavaged over the same time frame with 0 or 60 mg/kg/day androstenedione. Livers were removed from dams on gestation day 20 and from non-pregnant rats after five weeks' treatment. Liver microsomes were incubated with 200 μ M testosterone, and the reaction products were isolated and analyzed by HPLC. In pregnant rats, formation of 6 α -, 15 β -, 7 α -, 16 β -, and 2 β -hydroxytestosterone was increased significantly vs. control at the highest dose level only. Formation of 6 β -hydroxytestosterone increased significantly at both the 30 and 60 mg/kg/day dose levels. In non-pregnant rats, 60 mg/kg/day androstenedione significantly increased formation of 15 β -, 6 β -, 16 β -, and 2 β -hydroxytestosterone. The data suggest that high oral doses of androstenedione can induce some female rat liver cytochromes P450 that metabolize steroid hormones and that the response to androstenedione does not differ between pregnant and non-pregnant female rats. Published by Elsevier Ltd.

Keywords: Androstenedione; Steroids; Liver; Endocrine disruption; Cytochromes P450

1. Introduction

Androstenedione is one of many naturally-occurring steroidal compounds in common use as dietary supplements. These supplements are used for their alleged ability to enhance athletic performance by virtue of their metabolic conversion to testosterone. In vivo, androstenedione is the immediate biosynthetic precursor of both testosterone and estrone. Supraphysiological levels of androgenic steroids are known to have adverse health

consequences in humans including: endocrine disruption (e.g., masculinization in females), hepatotoxicity (peliosis hepatitis, cholestatic jaundice, hepatocellular adenomas), and cardiovascular toxicity (e.g., decreased HDL). Because of these potential health risks, the US Food and Drug Administration mandated that products containing androstenedione could no longer be sold and distributed as dietary supplements (FDA, 2004). Although frank hepatotoxicity in humans is associated with both the therapeutic and illicit use of 17- α -alkylated anabolic-androgenic steroids (Ishak and Zimmerman, 1987), no clear association with hepatotoxicity has been established for non-alkylated steroids such as androstenedione.

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The liver is the major catabolic organ for steroid hormones. Steroids are first hydroxylated to less active metabolites in phase I reactions catalyzed by cytochromes P450 (CYP). The hydroxylated metabolites are then conjugated in phase II reactions to polar metabolites that are more readily excreted. Therefore, any exposure that alters phase I or phase II activities in liver has the potential to cause endocrine disruption through either increased or decreased rate of steroid hormone catabolism (You, 2004). CYP activities in rat liver have a distinct sexual dimorphism (Waxman et al., 1985). Some CYP specific activities in female rat liver decrease during pregnancy (Borlakoglu et al., 1993; Kim, 1995; Kuriyama et al., 2000; Czekaj et al., 2000).

Leder et al. (2002) studied the effects of single oral doses of androstenedione on serum levels of androstenedione and sex steroids in healthy postmenopausal women. After androstenedione administration, serum levels of estrone over 12 h were elevated by up to 115% at the 100 mg dose relative to control. Testosterone levels over 12 h were elevated approximately 450% relative to control at the 100 mg androstenedione dose. Estradiol levels were not elevated relative to placebo. Kicman et al. (2003) studied the effect of a single 100 mg dose of androstenedione on plasma testosterone over 24 h in 10 healthy young women in a two-period crossover study. Plasma testosterone levels integrated over 24 h were 16-fold greater than control. The authors also noted that the plasma testosterone concentrations observed were similar to those encountered in abuse of testosterone for anabolic purposes. These authors expressed concern about the potential adverse effects of altered sex hormone levels in women taking androstenedione. More recent studies in young women have confirmed these earlier observations and shown that acute ingestion of 100–300 mg androstenedione increases serum testosterone (5–10 times) and serum estradiol (1.5–2 times) at 4 h after dosing (Brown et al., 2004).

Because of the exacting hormonal balances needed both to become pregnant and to sustain normal pregnancy, endocrine disruption through altered steroid hormone metabolism could be especially devastating in women of child-bearing age. Exposure of pregnant rats to anabolic steroids during pregnancy has been shown to have an adverse impact on both the dams and their developing fetuses (Wolf et al., 2002). A preliminary report (Shrier et al., 2001) showed that androstenedione administration (1.25 mg/day) to pregnant rats increased maternal plasma estrone but not estradiol levels.

The effects of oral androstenedione on pregnant female rats have been evaluated in our laboratory in a series of studies (Sprando et al., 2004, *in press*; Sahu et al., 2005). In the present study, activities of phase I enzymes that metabolize steroid hormones were measured in livers obtained from pregnant dams at gestation day 20. These same enzyme activities were also measured in

livers obtained from non-pregnant female rats exposed to the same amount of androstenedione for the same period in order to assess whether pregnancy alters the response of female rat liver to androstenedione.

2. Materials and methods

2.1. Materials

The test compound, androstenedione, and some hydroxylated testosterone HPLC standards (all >99% pure) were obtained from Steraloids (Newport, RI). HPLC grade solvents were obtained from J.T. Baker (Phillipsburg, NJ). Finasteride was obtained from US Pharmacopeia (Rockville, MD). All other reagents were obtained from Sigma (St. Louis, MO).

2.2. Animals

Full details on animal husbandry are provided in Sprando et al. (*in press*). Briefly, CD-CRL:BR/VAF rats were obtained from Charles River Laboratories (Wilmington, MA). Animals had *ad lib.* access to food (Purina Rodent Chow 5002, Purina Mills, Richmond, IN) and water. The pregnant females were dosed by gavage with 0, 5, 30 or 60 mg/kg/day androstenedione in corn oil beginning two weeks prior to mating and continuing through to gestation day 19. Dams were euthanized on gestation day 20. For the present liver steroid metabolism component, six animals were selected randomly from each treatment group, and their livers were excised, weighed, and processed as described below. Non-pregnant, nulliparous female rats, 8 per treatment group, were dosed by gavage with 0 or 60 mg/kg/day androstenedione in corn oil for five weeks. Animals were then euthanized, and their livers were collected and processed as for pregnant animals.

2.3. Preparation of microsomes

Livers were homogenized 1:4, w/v in 1.15% KCl in 10 mM phosphate buffer (pH 7.4), filtered through gauze and centrifuged at $10,000 \times g$ for 10 min. The supernatant was removed and centrifuged at $105,000 \times g$ for 70 min. The microsomal pellet was washed once, resuspended in 50 mM phosphate buffer (pH 7.4) containing 1 mM EDTA, then stored at -80°C until assayed. Total microsomal protein was determined using the micro BCA assay (Pierce Biotechnology, Rockford, IL).

2.4. Cytochrome P450 assay

Multiple cytochrome P450 activities were determined by measuring testosterone hydroxylation as described by

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