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Original Article

Effects of genistein and equol on human and rat testicular 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase 3 activities

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Abstract

The objective of the present study was to investigate the effects of genistein and equol on 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-hydroxysteroid dehydrogenase 3 (17β-HSD3) in human and rat testis microsomes. These enzymes (3β-HSD and 17β-HSD3), along with two others (cytochrome P450 side-chain cleavage enzyme and cytochrome P450 17α-hydroxylase/17-20 lyase), catalyze the reactions that convert the steroid cholesterol into the sex hormone testosterone. Genistein inhibited 3β-HSD activity (0.2 μmol L^{-1} pregnenolone) with half-maximal inhibition or a half-maximal inhibitory concentration (IC_{50}) of 87 ± 15 (human) and 636 ± 155 nmol L^{-1} (rat). Genistein's mode of action on 3β-HSD activity was competitive for the substrate pregnenolonrge and noncompetitive for the cofactor NAD^+. There was no difference in genistein's potency of 3β-HSD inhibition between intact rat Leydig cells and testis microsomes. In contrast to its potent inhibition of 3β-HSD, genistein had lesser effects on human and rat 17β-HSD3 (0.1 μmol L^{-1} androstenedione), with an $IC_{50} \geq 100$ μmol L^{-1}. On the other hand, equol only inhibited human 3β-HSD by 42%, and had no effect on 3β-HSD and 17β-HSD3 in rat tissues. These observations imply that the ability of soy isoflavones to regulate androgen biosynthesis in Leydig cells is due in part to action on Leydig cell 3β-HSD activity. Given the increasing intake of soy-based food products and their potential effect on blood androgen levels, these findings are greatly relevant to public health.

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1 Introduction

Hormonally active agents present in the environment (food, air and water) are classified as endocrine disruptors if they mimic the action of natural hormones, block the action of natural hormones, and/or interfere with the production, transport, metabolism or excretion of naturally occurring hormones [1]. Soy isoflavones

occur in soybeans and soy-based diets, and these supplements form an increasing non-dairy protein component of the human diet for their putative health benefits. For example, soybeans contain the glycoside conjugates genistein and daidzein, which have been demonstrated to prevent menopausal symptoms, osteoporosis, cancer and heart disease [2]. After ingestion, genistein and daidzein are converted by hydrolases in the gastrointestinal tract to form the aglycones genistein and daidzein. In a significant percentage of consumers (about 30%), daidzein is further metabolized to equol [3]. Soy isoflavones, classified as phytoestrogens, have structural similarities to the natural estrogen 17β-estradiol and are known to possess estrogenic properties. For example, there is experimental evidence that genistein and daidzein can transactivate estrogen receptors *in vitro* [4]. It has been suggested that equol has greater affinity for the estrogen receptor than its parent precursor daidzein, and has similar potency to genistein [5]. In particular, a significant number of infants are raised on soy-based infant formulas, which raises concern about the potential reproductive toxicity of genistein and other soy isoflavones when consumed at high levels and/or for prolonged periods.

The male reproductive tract expresses high levels of steroid hormone receptors, including the estrogen receptor. Following ingestion, soy isoflavones are known to reach the reproductive organs [6]. Thus, excessive exposure to agents that exhibit estrogenic activity may affect male reproductive tract development and function. With regard to this concern, it has been estimated that genistein and daidzein can reach high concentrations in infants who consume large amounts of soy-based products (1.5–2.5 μmol L^{-1}) [7]. Similarly, serum genistein levels in the adult can be as high as 10 μmol L^{-1} immediately following consumption of a phytoestrogen-rich meal [7]. Moreover, previous studies showed that genistein has the ability to suppress testosterone production in the rat Leydig cells *in vitro* and *in vivo* [8, 9].

Testosterone production in Leydig cell utilizes cholesterol as a substrate. Conversion of the cholesterol substrate to testosterone occurs in a series of reactions catalyzed by four enzymes: cytochrome P450 cholesterol side-chain cleavage enzyme, 3β-hydroxysteroid dehydrogenase (3β-HSD) isoform, cytochrome P450 17α-hydroxylase/17-20 lyase, and 17β-hydroxysteroid dehydrogenase 3 (17β-HSD3). Hydroxysteroid dehydrogenases (3β-HSD and 17β-HSD3) are localized to the smooth endoplasmic reticulum in Leydig cells. There are two 3β-HSD isoforms in humans, types 1 and 2. Type 1 is expressed in the placenta [10] and type 2 is expressed in the testis [11]. Previously, Le Bail *et al*. [10] reported that genistein was a potent inhibitor of placental 3β-HSD1 isoform. In the present study, we evaluated the effects of two isoflavones (genistein and equol) on testicular 3β-HSD and 17β-HSD3 enzyme activity, using microsomal protein fractions obtained from human and rat testes. For comparison, we used isolated rat Leydig cells in some assays. The results showed that genistein has a potent inhibitory effect on 3β-HSD activity, which is presumably linked to its ability to suppress androgen biosynthesis. In comparison with genistein, equol exerted a lesser inhibitory effect on human testis microsomes and had no effect on 17β-HSD3 activity in both the human and rat testis.

2 Materials and methods

2.1 Materials

[1,2-^{3}H]androstenedione and [^{3}H]pregnenolone, with specific activity (40 Ci $mmol^{-1}$), were purchased from Dupont-New England Nuclear (Boston, MA, USA). Unlabelled androstenedione, pregnenolone, progesterone and testosterone were purchased from Steraloids (Newport, RI, USA). Genistein (CAS# 529-59-9, purity > 99%) and (+/–) equol (CAS# 531-95-3, purity = 98%) were purchased from commercial sources (Indofine, Somerville, NJ, USA). Genistein and equol were prepared using ethanol as a solvent. Male Sprague-Dawley rats (250–300 g) were purchased from Charles River Laboratories (Wilmington, MA, USA). All animal procedures were approved by the Institutional Animal Care and Use Committee of Rockefeller University, and were performed in accordance with the Guide for the Care and Use of Laboratory Animals. Human male testes were obtained from the First Affiliated Hospital of Wenzhou Medical College, and were used under the guidance of the College Committee on Clinical Investigations.

2.2 Methods

2.2.1 Isolation of Leydig cells

Six 90-day-old Sprague-Dawley rats were killed by asphyxiation with CO_2 and the testis tissues were obtained. Leydig cells were isolated and purified as described previously [12]. The purity of Leydig cell fractions was evaluated by histochemical staining for 3β-HSD activity, using 0.4 mmol L^{-1} etiocholanolone

as the steroid substrate [13]. More than 95% of Leydig cells stained intensely for the steroidogenic enzyme marker. Three isolations were performed.

2.2.2 Preparation of microsomal protein

Microsomal preparations of human and rat testes were performed as described previously [14]. Briefly, testes were homogenized in cold 0.01 mmol L^{-1} phosphate buffered saline (PBS) containing 0.25 mmol L^{-1} sucrose and centrifuged at 700 × *g* for 30 min. The supernatants were then transferred to new tubes and centrifuged at 10 000 × *g* for 30 min. Thereafter, the supernatants were centrifuged at 105 000 × *g* for 1 h twice. Pellets were resuspended, and protein concentrations were measured using the Bio-Rad Protein Assay Kit (cat# 500-0006; Bio-Rad, Hercules, CA, USA), following the manufacturer's protocol. Microsomes were used for the analysis of 3β-HSD and 17-HSD3 activity.

2.2.3 3β-HSD assay

3β-HSD activity in testicular microsomes and Leydig cells was measured as described previously [15]. In brief, 3β-HSD activity assay tubes contained 0.2 μmol L^{-1} pregnenolone and 40 000 dpm [^{3}H]pregnenolone. The 30-min reactions were initiated by the addition of 10 μg human or rat testis microsomal proteins with 0.2 mmol L^{-1} NAD^+ in the presence of differing concentrations of genistein to determine the half-maximal inhibitory concentration (IC_{50}). In order to determine the mode of action, concentrations of pregnenolone ranging from 0.002 to 10 μmol L^{-1} (dissolved in ethanol with a final concentration of 0.2% in the reaction buffer) plus 0.2 mmol L^{-1} NAD^+ were added to a 0.5% Tween-20 PBS reaction mixture containing 10 μg rat testis microsome and differing concentrations of genistein or equol. Additional experiments were performed to determine whether genistein acts by competing with the cofactor NAD^+. Concentrations of NAD^+ ranging from 0.002 to 100 μmol L^{-1}, plus 0.2 μmol L^{-1} pregnenolone, were added to a 0.5% Tween-20 PBS reaction mixture containing 10 μg rat testis microsomes and 0.1–1 μmol L^{-1} genistein or equol. Separate experiments were performed using intact rat Leydig cells. Pregnenolone (0.2 μmol L^{-1}) was added to the assay tubes containing 0.02 × 10^6 Leydig cells in a 0.25-mL DMEM:F12 medium without exogenous NAD^+ and incubated for 30 min. The enzymatic assays were performed in the linear range of the reaction versus time and substrate concentration. All reactions were stopped by adding 2 mL ice-cold ether. The steroids were extracted, and the organic layer was dried under nitrogen. The steroids were separated chromatographically on thin layer plates in chloroform and methanol (97:3), and the radioactivity was measured using a scanning radiometer (System AR2000; BioScan Inc., Washington DC, USA). The percentage conversion of pregnenolone to progesterone was calculated by dividing the radioactive counts identified as progesterone by the total counts associated with pregnenolone plus progesterone.

2.2.4 17β-HSD3 assay

Activity of the 17β-HSD3 enzyme in testicular microsomes was measured as described previously [15]. Briefly, assay tubes contained 0.1 μmol L^{-1} androstenedione and 40 000 dpm [^{3}H]androstenedione in a 0.5% Tween-20 PBS reaction buffer. Androstenedione was dissolved in ethanol with a final concentration of 0.2% in the reaction buffer. To investigate the inhibition of enzyme activity, reactions were initiated by the addition of 50 μg human or rat testis microsomal protein to 0.2 mmol L^{-1} NADPH in the presence of 100 μmol L^{-1} genistein or equol for 90 min. The enzymatic assays were performed in the linear range of the reaction versus time and substrate concentration. Reactions were stopped by the addition of 2 mL ice-cold ether. The steroids were extracted, and the organic layer was dried under nitrogen. The steroids were then separated chromatographically on thin layer plates in chloroform and methanol (97:3), and radioactivity was measured using a scanning radiometer (System AR2000). The percentage conversion of androstenedione to testosterone was calculated by dividing the radioactive counts identified as testosterone by the total counts associated with androstenedione plus testosterone.

2.2.5 Statistics

Assays were repeated for two to four times. The IC_{50} was calculated with GraphPad (Version 4; GraphPad Software Inc., San Diego, CA, USA), using a nonlinear regression of curve fit with one-site competition model. Dixon and Lineweaver-Burk plots were used to determine the mode of inhibition. Data were further analyzed by one-way ANOVA followed by DUNCAN multiple comparison testing to identify significant differences between groups when three or more groups were compared. All data were expressed as mean ± SEM. Differences were regarded as significant at $P < 0.05$.

3 Results

3.1 Effects of genistein and equol on 3β-HSD and 17β-HSD3 activities

Levels of 3β-HSD, which catalyzes the conversion of pregnenolone to progesterone, were measured in human and rat testis microsomes. As shown in Figure 1, when the maximum concentration of inhibitors (100 μmol L^{-1}) was used, genistein inhibited 3β-HSD activity in both human (Figure 1A) and rat (Figure 1B) testis microsomes by 98% ($P < 0.001$) and 98.5% ($P < 0.001$), respectively, while equol demonstrated significant inhibition of human 3β-HSD by 42% ($P < 0.001$), with no effect on rat 3β-HSD activity. The IC_{50}s for inhibition of human and rat 3β-HSD by genistein were 87 ± 15 and 636 ± 156 nmol L^{-1} (mean ± SEM, Figure 1D, Table 1). Thus, genistein had a greater inhibitory effect on the human than rat 3β-HSD enzyme (Table 1). In agreement with our observations in testis microsomes, 10 μmol L^{-1} genistein inhibited 3β-HSD activity in intact Leydig cells by 98% ($P < 0.001$), but

Table 1. IC_{50} and Ki (app) for genistein inhibition of human and rat 3β-hydroxysteroid dehydrogenase activity.

Parameters	Human 3β -HSD	Rat 3β-HSD
IC_{50} (nmol L^{-1})	87 ± 15	636 ± 156
Ki (app) (nmol L^{-1})	67 ± 11	490 ± 120
V_{max} (nmol per mg protein per min)	91 ± 14	1 855 ± 6

Abbreviations: IC_{50}, half-maximal inhibitory concentration; Ki (app), inhibition constant; V_{max}, the maximum initial velocity of the enzyme; 3β-HSD, 3β-hydroxysteroid dehydrogenase activity. Values are shown as mean ± SEM, $n = 4$.

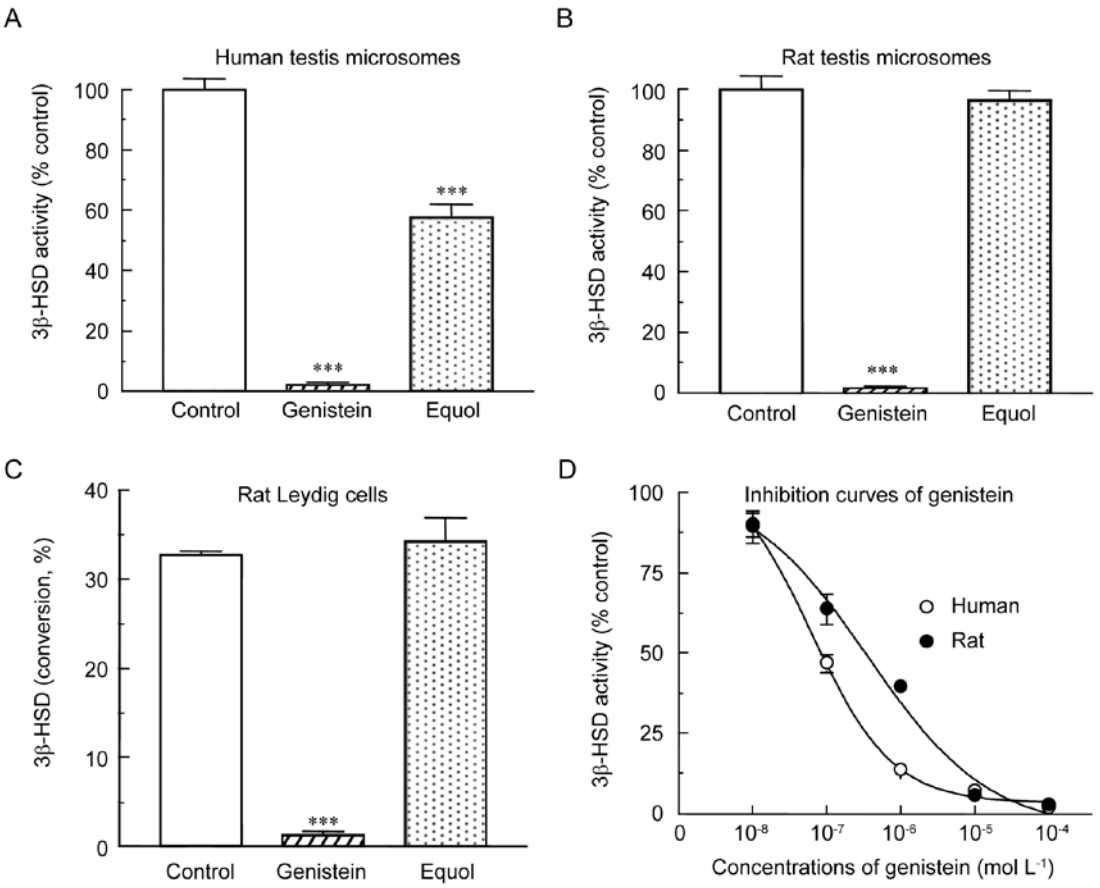

Figure 1. Effects of genistein and equol on human and rat 3β-hydroxysteroid dehydrogenase (3β-HSD) activity in human and rat testis microsome fractions and rat Leydig cells. 3β-HSD activity was measured by the conversion of pregnenolone (0.2 μmol L^{-1}) to progesterone in the presence of 100 μmol L^{-1} inhibitor (A–C) and various concentrations (mol L^{-1}) of genistein for half-maximal inhibitory concentration (IC_{50}, D). Mean ± SEM, $n = 4$. $^{***}P < 0.001$, compared with control (no inhibitor).

equol had no effect (Figure 1C). Assays of 17β-HSD3 activity indicated that genistein has a differential effect on steroidogenic enzymes. Compared with 3β-HSD, a high concentration of genistein (that is, 100 μmol L^{-1}) had a lesser inhibitory effect on both human and rat 17β-HSD3 activity, with only 35%–40% inhibition ($P < 0.001$). Equol did not affect human or rat 17β-HSD3 (Figures 2A and B). Similarly, only 100 μmol L^{-1} genistein inhibited 17β-HSD3 activity in the rat Leydig cells (35% inhibition) ($P < 0.001$), whereas equol had no effect (Figure 2C).

3.2 Mechanism explaining the effect of genistein on 3β-HSD activity

Genistein's mechanism of action for the regulation of 3β-HSD activity was investigated by enzyme kinetic analysis. The results of our Dixon plot analysis showed that genistein was a competitive inhibitor of 3β-HSD in both human (Figures 3A and B) and rat testis microsomes (Figures 3C and D). In order to detect whether genistein also binds to the cofactor-binding site of the 3β-HSD, various cofactor (NAD^+) concentrations were used. The Lineweaver-Burk plot showed, however, that genistein was an uncompetitive inhibitor of the cofactor for both human and rat 3β-HSDs (Figure 4). Taken together, these observations indicate that genistein inhibits 3β-HSD by competing with the steroid substrate on the enzyme.

4 Discussion

The present study demonstrates that genistein is a potent inhibitor of 3β-HSD activity, and more so in human testis microsomes than in the rat. In this regard, the estimated IC_{50} required to suppress half (50%) of human 3β-HSD activity was about 87 nmol L^{-1}, a level that can be readily attained in the blood of individuals consuming reasonable amounts of soy-based food products [7]. The inhibitory action of genistein on steroidogenic enzyme activity was replicated in experiments using isolated rat Leydig cells with full steroidogenic capacity. Interestingly, the estimated IC_{50} for genistein action in the rat testis microsomes was much higher (about 636 nm) than in the human testis microsomes. A previous report estimated an IC_{50} for genistein inhibition of 3β-HSD in bovine adrenal cortical tissue at 1 μmol L^{-1} [16]. Together, these observations indicate that there may be inter-species variability in sensitivity to genistein action in the testis. Neverthe-

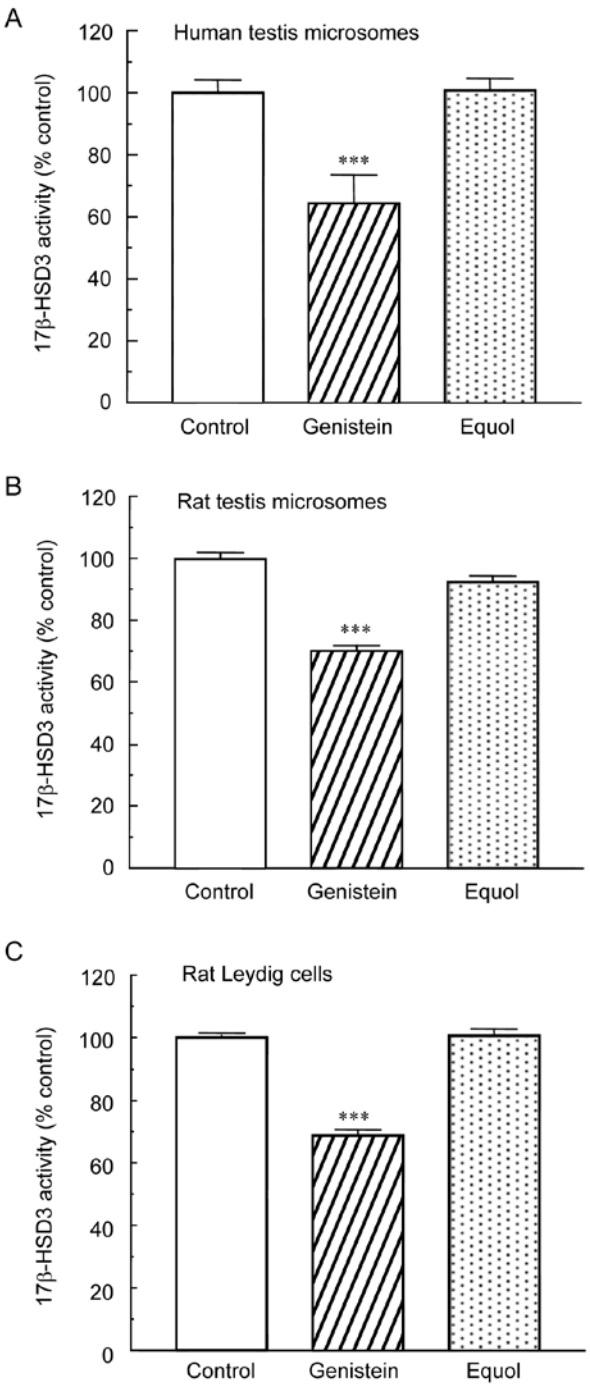

Figure 2. Effects of genistein and equol on 17β-hydroxysteroid dehydrogenase 3 (17β-HSD3) activity in human and rat testis microsome fractions and rat Leydig cells. 17β-HSD was measured by conversion of androstenedione (0.1 μmol L^{-1}) to testosterone in the presence of 100 μmol L^{-1} of inhibitor. (A): human testis; (B): rat testis; (C): rat Leydig cells. Mean ± SEM, $n = 4$. $^{***}P < 0.001$, compared with the control (no inhibitor).

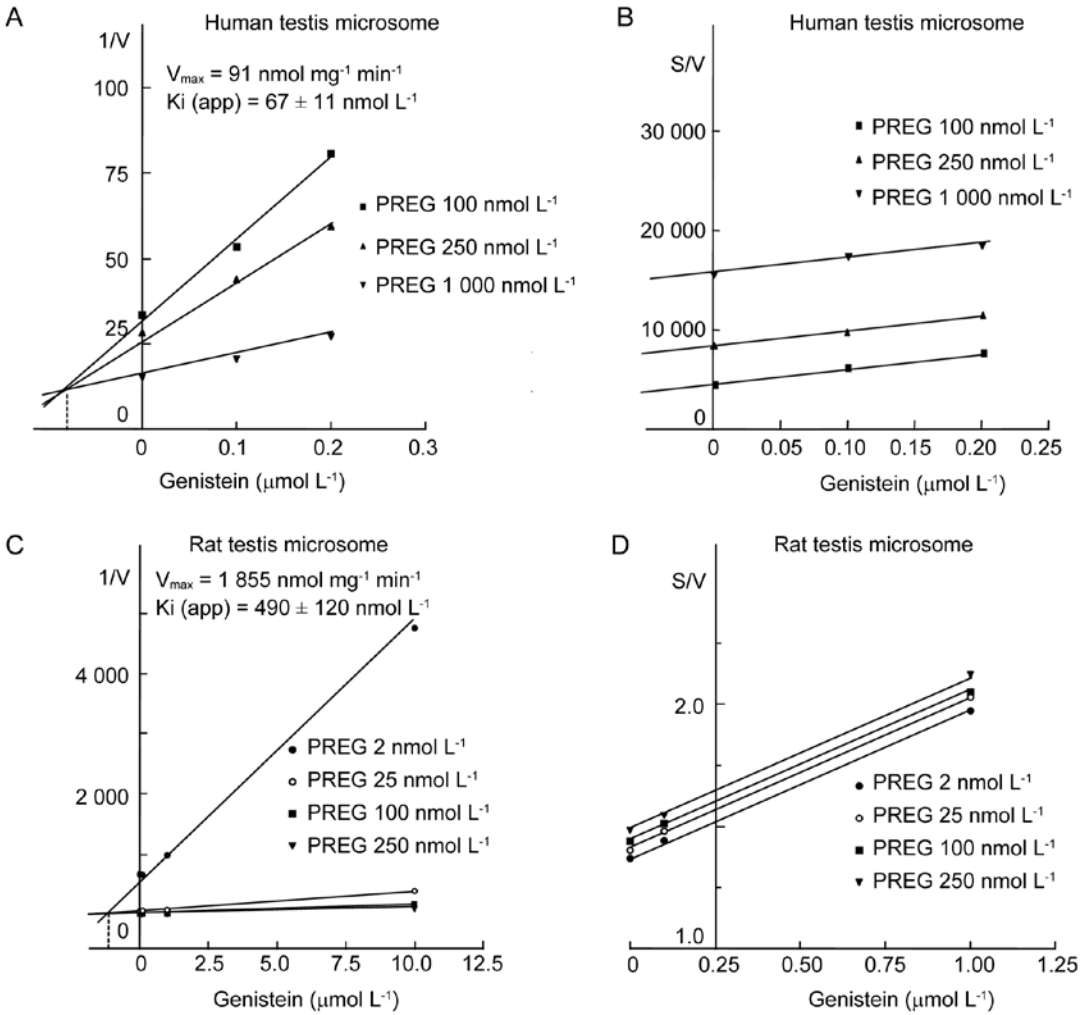

Figure 3. Dixon plots of human and rat testicular 3β-hydroxysteroid dehydrogenase (3β-HSD) activity in the presence of genistein. 3β-HSD activity was measured by conversion of pregnenolone (PREG) to progesterone in 10 μg rat microsomal protein for 30 min in the presence of varying concentrations of the substrate pregnenolone. (A) and (B): human testis microsomal 3β-HSD; (C) and (D): rat testis microsomal 3β-HSD. The experiments were repeated twice. One representative dataset was plotted. 1/V, reciprocal of initial velocity; S/V; substrate concentration over initial velocity. V_{max}, the maximum initial velocity of the enzyme; Ki (app), inhibition constant.

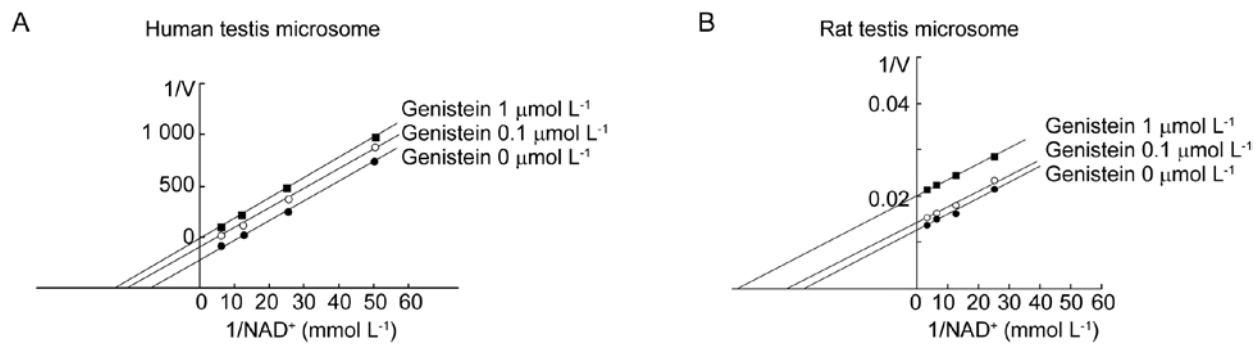

Figure 4. Lineweaver-Burk plot of 3β-hydroxysteroid dehydrogenase (3β-HSD) activity in human and rat testis microsomes in the presence of genistein. 3β-HSD was measured by the conversion of 0.2 μmol L^{-1} pregnenolone to progesterone in 10 μg protein for 30 min in the presence of varying concentrations of the cofactor NAD^+. (A): human testis microsomal 3β-HSD; (B): rat testis microsomal 3β-HSD. The experiments were repeated twice. One representative dataset was plotted. 1/V, reciprocal of initial velocity; S/V; substrate concentration over initial velocity.

less, the ability of genistein, the predominant isoflavone in soybeans, to affect steroidogenic enzyme activity at doses as low as 10 nm in the human testis has implications for testicular steroidogenesis [17].

An interesting observation from the present study was the differential action of genistein and equol in the human and rat testis microsomes. Genistein caused significant inhibition of 3β-HSD and 17β-HSD3 activity in both human and rat testis microsomes. Genistein also weakly inhibited 17β-HSD in intact Leydig cells. 17β-HSD type 5, which catalyzes the reduction of androstenedione to testosterone, is expressed in the rat and human testis [18]. It also remains to be determined whether genistein has a mitogenic effect in Leydig cells, which may augment serum androgen levels. However, the genistein-mediated inhibition was weak but significant at the highest concentration (100 μmol L^{-1}) tested. On the other hand, equol inhibited human 3β-HSD to a lesser degree compared with genistein and had no effect on rat testicular 3β-HSD and 17β-HSD3 activity. However, the mechanism of enzyme inhibition in the present study may be different from receptor-mediated action.

Results from the present study show that inhibition of human and rat 3β-HSD by genistein was competitive for the substrate. As 3β-HSD has two binding sites, one for steroid (pregnenolone) and the other for cofactor NAD^+, we further assessed whether genistein competes with the cofactor NAD^+ by investigating the effects of different concentrations of the cofactor in the presence of varying concentrations of the inhibitor. Genistein was non-competitive with the cofactor NAD^+ in its inhibition of 3β-HSD (Figure 3). We propose that genistein possibly blocks the substrate (pregnenolone) from its active site on the 3β-HSD enzyme.

Our observations lend support to the ongoing debate on the potential reproductive toxicity of soy-based diets. The levels of genistein affecting steroidogenic enzyme activity in the present study approximate levels that have been measured in individuals consuming soy products [7]. The present results are in agreement with our previous findings of genistein acting directly in rat Leydig cells to cause inhibition of androgen biosynthesis [8, 9]. Changes in androgen biosynthesis, occasioned by exposure to endocrine disruptors, have the potential to interfere with androgen-stimulated reproductive tract development [19, 20]. Besides the potent competitive inhibition of 3β-HSD, low concentrations of genistein also interfere with the coupling of transmembrane luteinizing hormone (LH) receptors with G proteins. The uncoupling of LH receptors from G proteins by genistein adversely affects adenylate cyclase function, thus blocking LH-induced stimulation of steroidogenesis in Leydig cells [9].

Structurally, genistein, daidzein and equol are very similar. It has been demonstrated that genistein and daidzein can bind to and activate estrogen receptor α *in vitro* [4]. Equol is also thought to have greater affinity for the estrogen receptor than its parent precursor daidzein, with a potency similar to genistein [5]. However, the estrogen receptor–isoflavone binding interaction may be different from the isoflavone–3β-HSD interaction, as genistein is over 1 000 times more potent than equol in the inhibition of 3β-HSD (Figure 1).

In conclusion, the present findings demonstrate that genistein is a potent inhibitor of 3β-HSD which is involved biochemical reactions that culminate in androgen biosynthesis. These findings, along with previous observations indicating that genistein interferes with LH signalling in rat Leydig cells [8, 9], suggest that genistein exerts multiple mechanisms to affect testicular steroidogenesis. LH binding stimulates steroidogenic enzyme activity, which enhances androgen biosynthesis. The present results demonstrate that genistein acts directly to interfere with enzyme activity. Thus, the inhibitory action of genistein, and perhaps other isoflavones, on androgen biosynthesis is possibly the result of multiple mechanisms of action. Population-based risk assessment will rely on better identification of the mechanisms of phytoestrogen action. Thus, additional studies to assess the testicular toxicity of dietary soy are warranted.

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References

1 Stoker TE, Parks LG, Gray LE, Cooper RL. Endocrine-disrupting chemicals: prepubertal exposures and effects on sexual maturation and thyroid function in the male rat. A focus on the EDSTAC recommendations. Endocrine Disrupter Screening and Testing Advisory Committee. Crit Rev Toxicol 2000; 30: 197–252.
2 Kurzer MS, Xu X. Dietary phytoestrogens. Annu Rev Nutr

1997; 17: 353–81.
3 Setchell KD, Brown NM, Lydeking-Olsen E. The clinical importance of the metabolite equol-a clue to the effectiveness of soy and its isoflavones. J Nutr 2002; 132: 3577–84.
4 Martin PM, Horwitz KB, Ryan DS, McGuire WL. Phytoestrogen interaction with oestrogen receptors in human breast cancer cells. Endocrinology 1978; 103: 1860–7.
5 Muthyala RS, Ju YH, Sheng S, Williams LD, Doerge DR, *et al*. Equol, a natural estrogenic metabolite from soy isoflavones: convenient preparation and resolution of R- and S-equols and their differing binding and biological activity through oestrogen receptors alpha and beta. Bioorg Med Chem 2004; 12: 1559–67.
6 Sharpe RM. The roles of oestrogen in the male. Trends Endocrinol Metab 1998; 9: 371–7.
7 Setchell KD, Zimmer-Nechemias L, Cai J, Heubi JE. Exposure of infants to phyto-oestrogens from soy-based infant formula. Lancet 1997; 350: 23–7.
8 Akingbemi BT, Braden TD, Kemppainen BW, Hancock KD, Sherrill JD, *et al*. Exposure to phytoestrogens in the perinatal period affects androgen secretion by testicular Leydig cells in the adult rat. Endocrinology 2007; 148: 4475–88.
9 Hancock KD, Coleman ES, Tao YX, Morrison EE, Braden TD, *et al*. Genistein decreases androgen biosynthesis in rat Leydig cells by interference with luteinizing hormone-dependent signaling. Toxicol Lett 2009; 184: 169–75.
10 Le Bail JC, Champavier Y, Chulia AJ, Habrioux G. Effects of phytoestrogens on aromatase, 3β and 17β-hydroxysteroid dehydrogenase activities and human breast cancer cells. Life Sci 2000; 66: 1281–91.
11 Simard J, Ricketts ML, Gingras S, Soucy P, Feltus FA, *et al*. Molecular biology of the 3β-hydroxysteroid dehydrogenase/delta5-delta4 isomerase gene family. Endocr Rev 2005; 26: 525–82.
12 Salva A, Klinefelter GR, Hardy MP. Purification of rat leydig cells: increased yields after unit-gravity sedimentation of collagenase-dispersed interstitial cells. J Androl 2001; 22: 665–71.
13 Payne AH, Wong KL, Vega MM. Differential effects of single and repeated administrations of gonadotropins on luteinizing hormone receptors and testosterone synthesis in two populations of Leydig cells. J Biol Chem 1980; 255: 7118–22.
14 Ge RS, Hardy DO, Catterall JF, Hardy MP. Opposing changes in 3alpha-hydroxysteroid dehydrogenase oxidative and reductive activities in rat leydig cells during pubertal development. Biol Reprod 1999; 60: 855–60.
15 Hu GX, Zhou HY, Li XW, Chen BB, Xiao YC, *et al*. The (+)- and (-)-gossypols potently inhibit both 3beta-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase 3 in human and rat testes. J Steroid Biochem Mol Biol 2009; 115: 14–9.
16 Wong CK, Keung WM. Bovine adrenal 3β-hydroxysteroid dehydrogenase (E.C. 1.1.1. 145)/5-ene-4-ene isomerase (E.C. 5.3.3.1): characterization and its inhibition by isoflavones. J Steroid Biochem Mol Biol 1999; 71: 191–202.
17 Penning TM. Molecular endocrinology of hydroxysteroid dehydrogenases. Endocr Rev 1997; 18: 281–305.
18 Azzarello J, Fung KM, Lin HK. Tissue distribution of human AKR1C3 and rat homolog in the adult genitourinary system. J Histochem Cytochem 2008; 56: 853–61.
19 Akingbemi BT, Hardy MP. Oestrogenic and antiandrogenic chemicals in the environment: effects on male reproductive health. Ann Med 2001; 33: 391–403.
20 Ge RS, Chen GR, Tanrikut C, Hardy MP. Phthalate ester toxicity in Leydig cells: developmental timing and dosage considerations. Reprod Toxicol 2007; 23: 366–73.