Genistein is an Efficient Estrogen in the Whole-Body throughout Mouse Development

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The widespread use of diets containing estrogenic compounds raises questions on how relevant the presence of phytoestrogens may be, in order to allow a correct development of the reproductive ability and sexual maturity in humans and animals. The isoflavone genistein is the most estrogenically active molecule present in soy. Here we show that genistein, through an estrogen receptor (ER) mediated action, modulates gene expression in the whole body of male mice in a dose- and time-dependent manner, at all ages. By luciferase bioassays, we show that genistein-induced ER activation is present in reproductive and nonreproductive organs of the transgenic mice Estrogen Responsive Element (ERE)-tK-LUC, although to an extent that is lower than what observed with the administration of estradiol. Peak activity was registered at genistein doses of 500–5000 μg/kg, at 12 h from the administration by gavage. In the liver, ER-α and ER-β messenger RNAs and two target genes, CYP17 and the progesterone receptor, were modulated by genistein. CYP17 and PR time-dependent induction was similar to that of luciferase. ER-α protein level followed an opposite regulation by genistein and estradiol. Genistein passed from the lactating mother to the suckling offspring at levels sufficient to activate gene expression in reproductive and nonreproductive tissues of the pups, with maximal upregulation at 16–24 h. We also followed responsiveness to genistein in the testis, from early development to adult age. Testis are well responsive to genistein as well as to estradiol already at day 14.5 of fetal development, as determined by exposing organotypic cultures from mouse fetus testis. Ovaries were not responsive under the same conditions. Activation of luciferase correlates with an activation of cell proliferation in testis, but not in the ovaries. Prolonged exposure (15 days) to genistein also decreases prostate weight like estradiol. In conclusion, our results show that genistein affects reproductive and nonreproductive organs of male mice in a dose- and time-dependent manner, at all developmental ages.

Key Words: phytoestrogens; estrogen receptors; estrogen responsive elements; reporter mice; reproduction.

It is now accepted that estrogens play a crucial role in the development and function of the male reproductive system (Couse et al., 1999, 2001; O’Donnell et al., 2001; Hess, 2003), as evidenced by the phenotype of estrogen receptors knockout mice (Eddy et al., 1996; Korach, 2000; Krege et al., 1998) and aromatase knockout mice (Robertson et al., 2002). The male reproductive tract—and probably the testis themselves—are responsive to estrogens from fetal life through adulthood, because they express estrogen receptors (ER-α and ER-β) and/or estrogen-related receptors from early stages of embryonal development (Nielsen et al., 2000). Recent studies indicate that in the mouse embryo, even the primordial germ cells, the precursors of oocytes and spermatogonia, and the somatic cells of the sex indifferentiated gonadal ridges, express ER-α and/or ER-β, respectively (Moe-Behrens et al., 2003; Mitsuaga et al., 2004). Thus, any compound that interacts with these receptors is potentially able to modulate and eventually interfere with the development of the reproductive system.

There are several chemicals produced by plants, present as natural constituent of food, that are able to bind to ERs (Cornwell et al., 2004). These compounds are called phytoestrogens and are present at high levels in certain foods such as soy. Although a great amount of research on the toxicological, nutritional, and pharmacological properties of phytoestrogens has been conducted, a sufficiently informative picture of their targets and biological effects is still unavailable.

People are exposed to these nutritional estrogens depending on the type and daily intake of vegetables. Asian populations may assume from 30 to 50 mg/day of isoflavones from soy (Adlercreutz et al., 1993; Kimira et al., 1998) equivalent to about 1 mg/kg/day of total genistein. These intakes produce blood levels of isoflavones ranging from 100 to 500 nanomolar (nM) (Setchell et al., 1998; Uehar et al., 2000). Because in previous experiments on mice we have found that blood levels of genistein in this concentration range exert estrogenic effects (Penza et al., 2006, 2007), it can be postulated that these dietary levels of genistein can reach estrogenically active levels also in humans. Finally, it must be taken into consideration that
human infants drinking soy-based formulas are exposed to much higher levels of genistein than adults on soy-containing diets. Their blood levels may reach 1–5 μM total genistein, ten times higher than the amount found in adults’ serum. Moreover, the infants show a much higher level of genistein aglycone, which is the active form of genistein (Chang et al., 2000; Doerge et al., 2002).

A further concern arises from the knowledge that both ER-α and ER-β receptors or ER-β only depending on the cell type, are expressed in human ovary and testis, respectively, during fetal life (Gaskell et al., 2003; Vaskivuo et al., 2005). Very controversial are, however, the studies reported on the effects of phytoestrogens in male, particularly for their effect on development and the reproductive health following perinatal exposure. In the offspring, phytoestrogens might potentially affect both the germ and somatic testicular cells with consequences involving a complex array of pathological changes, collectively known as the testicular dysgenesis syndrome (for a review see Sharpe, 2001).

At present, there are some questions about the estrogenic effects of isoflavones that still need to be clarified. First, it is yet not clear whether the estrogenic effect observed with genistein on the whole physiology and in particular on the reproductive tract are mainly due to the activation of ER-mediated pathways. Second, it is yet not clear what are the active estrogenic concentrations of genistein, because in few studies genistein effects were observed only at pharmacological concentrations (Nagao et al., 2001). Third, what is the most sensitive period of life where exogenous estrogens may alter tissue-specific gene expression profiles and development.

In this work we have analyzed, by a whole-body approach, the estrogenic dose-dependent effect of genistein on several tissues of adult estrogen reporter transgenic male mice engineered to express a reporter of ER transcriptional activity (ERE-tK-LUC mouse), a model that provides a functional picture of the map of ER activation (Ciana et al., 2001, 2003; Maggi et al., 2004; Penna et al., 2004; Villa et al., 2004). The doses of genistein used were representative of the intake typical of western diets (low intake, from 5 to 50 κg/kg/day) and eastern diets (high intake, from 50 to 500–1000 μg/kg/day), and the intake of infants fed with soy-based formulas and soy-based nutritional supplements (high intake, from 500 to 5000 μg/kg/day) (Setchell et al., 1997). We used this mouse model also to analyze the effect of genistein exposure of lactating mothers on suckling estrogen reporter pups. Finally, we used in vitro culture system to investigate whether genistein exerts estrogenic action on the gonads of fetus, adults, and developing mice.

MATERIALS AND METHODS

Experimental animals. The procedures involving animals and their care were conducted in accord with institutional guidelines, which comply with national and international laws and policies (National Institutes of Health, Guide for the Care and Use of Laboratory Animals, 1996 (7th ed.) [Washington, D.C.]; National Academy Press, National Research Council Guide, www.nap.edu/readingroom/books/labrats). ERE-tK-LUC transgenic mice were kept in animal rooms maintained at a temperature of 23°C, with natural light/dark cycles. For the experiments, we used heterozygous littermates, obtained by mating our founders with C57BL/6J wild-type mice. Heterozygous transgenic male mice were screened by PCR analysis for the presence of the transgenic cluster. Before treatments, heterozygous male mice (2 months old) were put 4 days on an estrogen-free diet (Piccioni, Italy). Genistein was given by oral gavage, which is its normal route of assumption, estradiol by ip injection. Vegetal oil was used as vehicle and negative control. The animals were sacrificed by cervical dislocation and the tissues dissected and immediately frozen on dry ice. Tissue extracts were prepared by homogenization in 500 μl of 100 mM K2PO4 lysis buffer (pH 7.8) containing 1mM dithiothreitol, 4mM ethylene glycol tetraacetic acid, 4mM ethylendiaminetetraacetic acid (EDTA), and 0.7mM phenylmethylsulfonylfluoride, with three cycles of freezing–thawing and 30 min of microfuge centrifugation at maximum speed. Supernatants, containing luciferase, were collected, and protein concentration was determined by Bradford’s assay (Bradford, 1976).

We also analyzed the effect of genistein exposure of lactating mothers on suckling estrogen reporter pups at 4 days of age. Eventually, we investigated whether genistein exerts estrogenic action on fetal testis of the transgenic mice. Fetal testis were then dissected from embryos obtained by breeding ERE-tK-LUC with C57BL/6J or where indicated from CD-1 mice. The day of plug was considered as 0.5 days postcoitum (dpc).

Chemicals. Estradiol (17β-E2) and genistein were purchased from Sigma (Pomezia, Italy); β-benzene-hexachloride was purchased from Superchrom (Milan, Italy).

Enzymatic assay. Luciferase enzymatic activity was measured, as reported by De Vet et al. (1987), in tissue extracts at a protein concentration of 1 mg/ml. The light intensity was measured with a luminometer (Digen Diagnostics, Gathersburg, USA) over 10 s and expressed as relative light units/mg proteins.

Protein isolation and Western analysis. Tissues were excised and homogenized in lysis buffer (1M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid [pH 7.9]; 2M MgCl2; 10% [vol/vol] glycerol; 5M NaCl; 0.5M EDTA; 0.1% Triton-X 100; 14.2M mercaptoethanol; 100mM phenylmethylsulphonylfluoride) supplemented with a protease inhibitor cocktail. Homogenized was centrifuged for 25 min at 13209 in cold room. Protein concentration was determined by the Bradford’s assay. Equal amount of proteins from each treatment group were boiled in 1× Laemmli buffer (50mM Tris–HCL, 2% sodium dodecyl sulfate (SDS), 0.1% bromophenol blue, 175mM β-mercaptoethanol). Proteins were separated by SDS–10% polyacrylamide gel electrophoresis, and electrophoresed to polyvinylidene difluoride membrane. Membranes were blocked in Blotto (5% milk, Tris-buffered saline (10mM Tris–HCL, pH 8.0, 150mM NaCl), and 0.05% Tween 20) and probed with primary antibodies ER-α (R21) (1:1000) and ER-β (CO1531) (antibodies were a gift from Dr. Greene, Chicago, IL). Following incubation with peroxidase-conjugated secondary antibody, immunoglobulins were visualized using the enhanced chemiluminescence detection system (Pierce, Milan, Italy). Densitometric quantitation of ER-α levels relative to actin levels was performed using the Matrix software program (Quantavision, Canada).

Messenger RNA quantification by real-time reverse transcription–PCR. Total RNA was extracted from 10 to 50 mg of tissue using the Nucleospin RNA II kit (BD Bioscience, San José, CA) and following the manufacturer’s instruction. RNA for each sample was reverse transcribed using the High capacity complementary DNA Archive Kit (Applied Biosystems, Foster City, CA). Quantitative PCR was performed using the Assay on Demand kit based on TaqMan chemistry (Applied Biosystems). Reverse transcription–PCR (RT-PCR) reactions were performed on an ABI PRISM 7000 Sequence Detection System instrument and data analysis was done with the ABI PRISM
RESULTS

Map of ER Activation by Genistein in Mouse Organs

The ERE-mediated transcriptional activity of genistein was tested in vivo in the transgenic ERE-tK-LUC reporter mouse line. A dose response analysis was determined in several tissues of male mice orally treated with 0, 5, 50, 500, 5000 μg/kg of the compound. These amount of genistein gave a serum concentration respectively of 30 ± 4; 35 ± 6; 77 ± 8; 86 ± 8; 92 ± 9nM 12 h after the gavage.

The response was quantified by assaying the enzymatic activity of the transgenic marker luciferase (LUC). In parallel, mice were also treated with estradiol as a reference compound. As reported in Figure 1 genistein showed the ability to modulate the ERs, although at different concentrations and in a tissue preferential manner. Maximal increase in ER action was almost always evident at a concentration between 500 and 5000 μg/kg (Fig. 1) (500 μg/kg are equivalent to the intake of 30 mg for a 60 kg person). Liver and thymus responded to the compound already at a concentration of 50 μg/kg. Genistein was a less efficient inducer in the pancreas, eye, and cerebellum. Peak activity was registered at 12 h and started to decrease at 24 h in all the tissues being more evident in the liver (Fig. 2).

We did not notice any differential effect in ERs activation in tissues expressing different levels of the ERs, as the liver versus the lung. The response to genistein never approached that of estradiol and is delayed with respect to the natural estrogen, which peaks at 6 h. Measurement of luciferase in tissues of animals treated with vehicle alone showed a stable basal activity of the transgenic marker.

Reproductive tissues were also weighed after 15 days of exposure. We noticed a significant decrease in seminal vesicles and testis weight of mice exposed 6 days after weaning (4 weeks old) to estradiol but not genistein. The prostate weight significantly decreases with both compounds. Body weight was unaffected by genistein, whereas it significantly decreased with estradiol. The effect of genistein was dose dependent and inversely correlated with the reporter upregulation (Figs. 3A–D).

Comparison between the Regulation of Endogenous Genes and Luciferase

To verify if luciferase induction reflected modulation of estrogen-dependent gene expression by genistein, we determined in the liver of genistein exposed mice the level of the progesterone receptor (PR) and the steroidogenic P450c17alpha (CYP17) messenger RNAs (mRNAs), known targets of the estrogens in this organ. PR and CYP17 mRNAs were quantified by real-time RT-PCR in mice treated for 1, 3, 6, 12, 24 h with 500 μg/kg genistein or 5 μg/kg estradiol. As shown in Figure 4, PR and CYP17 mRNAs induction was maximal at 6–12 h, a kinetics that appear a little early, compared with luciferase upregulation, which is maximal at 12–24 h. Both genes were also upregulated by estradiol (Fig. 4A).

The modulation of ER-α and ER-β mRNAs by genistein and estradiol in the liver was also evaluated. ER-α and ER-β mRNAs were upregulated by genistein following a time course shorter than that of luciferase (maximal level at 3 h). In estradiol treated mice, however, only the ER-β mRNA was upregulated. Interestingly ER-α protein level was downregulated in the liver by genistein (Fig. 4B) indicating that the regulation of transcription or mRNA synthesis/stabilization does not follow the same trend of the ligand-dependent protein turnover, possibly due to a regulatory effect on the proteasome degradation pathway (Marsaud et al., 2003). ER-β protein was at undetectable levels (not shown). ER proteins are modulated by genistein also in the testis and lung of 2 months old mice further confirming that ERs modulation by nutritional doses of genistein is an effect occurring in different body systems. In the testis ER-α modulation inversely correlates with the induction of luciferase being ER-α maximally downregulated at the time and doses where induction of luciferase was at the highest levels (500 μg/kg; 6–12 h) (Fig. 4C). A strong decrease of ER-β occurred in testis at 24 h, whereas in the lung it was maximal already at 3 h, respectively later and much earlier than luciferase increase in the same organs.
(Chen and Rogan, 2004), we used ERE-tK-LUC pups to investigate whether genistein exerts estrogenic action in breast-fed pups fed by genistein exposed mothers. Lactating mothers were given a single dose of genistein (50 mg/kg) by oral gavage at day 4 after delivery. This amount produced in the dam a serum genistein level of 550 ± 109nM (not shown). ERE-tK-LUC suckling pups were killed at 0, 6, 16, and 24 h after the mothers’ treatment and luciferase activity was measured in the liver, lung, heart, thymus, testis, and brain (Fig. 5). Mothers’ treatment resulted in an increased luciferase activity in all pup organs examined, which resemble the expression observed in the adults, although it appears more consistent in the lung and testis. This indicates that genistein passes from the mother to the milk at concentrations sufficient to exert estrogenic actions on reproductive and nonreproductive tissues of breast-fed newborns.

**Estrogenic and Proliferation Action of Genistein on Fetal Testis**

We then investigated whether genistein can exert estrogenic action directly on fetal gonads.
To this aim, testis and ovaries of 14.5 dpc mouse embryos were chosen because at this age there is evidence for expression of both ER mRNAs and proteins in the developing testis and ovary (Greco et al., 1991, 1993; Jefferson et al., 2000; Lemmen et al., 1999; Nielsen et al., 2000).

Gonads were dissected from 14.5 dpc ERE-tK-LUC embryos and cultured in the presence of 1 \mu M genistein, 10nM estradiol or vehicle (ethanol) for 6 h at 37°C, before running the luciferase assay. Figure 6A shows that luciferase was induced twofold in testis exposed to genistein or estradiol and the addition of the antiestrogen ICI-182780 inhibited this response. Ovaries did not show any significant response to either genistein or estradiol (Fig. 6B).

We then tested whether genistein and estradiol were able to affect the proliferation of testicular and ovarian cells using a 3H-thymidine incorporation assay. Both compounds appeared to stimulate testicular cell proliferation as revealed by a significant twofold increase of 3H-thymidine incorporation in cultured testis, which is inhibited by the antiestrogen ICI-182780 (Fig. 7A). Ovaries were unresponsive to the same treatments (Fig. 7B).
DISCUSSION

We used the ERE-tK-LUC transgenic mice as a model to test the hypothesis that genistein, at doses found in diets containing various amount of isoflavones, has estrogenic effects on the whole body, including reproductive organs.

We show here that genistein doses, as present in a single meal containing moderate to high amounts of soy, or in the assumption of a common isoflavone-containing food supplement (30 mg, equal to 500 l g/kg), produce in many tissues (liver, heart, thymus, spleen, testis, cortex) a response that is equal or very close to the maximal response observed with the 5000 l g/kg dose. The peak in reporter induction in adult mice was detectable at 12 h and is compatible with the time-dependent profile of...
genistein seen in the plasma of human subjects after assumption of a single dose of genistein (King and Bursill, 1998). Reporter activation reflected, although with a slightly different kinetic, the regulated expression of target endogenous genes (CYP17, PR, ER-α, and ER-β). The action of genistein was always less potent than estradiol, except for the PR gene that was induced almost at the same level.

ER levels are modulated by genistein in the liver, testis, and other organs such as the lung, proving that their involvement/recruitment in genistein action occurs in the whole body. ERs modulation by genistein was observed to be tissue specific. In the liver genistein was efficient in inducing ER-α mRNA, and downregulating the ER-α protein, an effect not elicited by estradiol, which showed an opposite action on ER-α protein. Similarly to estradiol, genistein induced upregulation of ER-β mRNA. Previous reports (Shupnik et al., 1998) showed that a ligand induces upregulation of ER-α protein, that occurs in the liver, but not in other tissues. Here we confirm this finding, but we also show that it is specific for the endogenous ligand, because genistein always downregulated ER-α protein. In this and other organs, on the contrary, genistein upregulated ER-α mRNA, whereas estradiol caused a significant mRNA downregulation.

It is known that the ER levels may determine the cellular response to ER ligands (Meegan and Lloyd, 2003). On the other hand ER stability is influenced by specific ligands (Wijayaratne and McDonnell, 2001), which may condition the interaction of the receptor with tissue-specific coregulators.

**FIG. 5.** Estrogenic action of genistein in tissues of 4-day-old suckling estrogen reporter pups of genistein treated mothers. Lactating mothers were treated by oral gavage with a single bolus of 50 mg/kg body weight of genistein. Controls were treated with vegetal oil. Luciferase activity was measured in the liver, lung, heart, thymus, testis, and brain at 0, 6, 16, 24 h after mother treatment. Bars represent the average ± SEM of three individual experiments, each performed in duplicate. *p < 0.05, as compared with the control.

**FIG. 6.** Estrogenic action of genistein on fetal gonads. At 14.5 dpc testis (A) and ovaries (B) obtained from estrogen reporter embryos were exposed to 1µM genistein, 10nM estradiol, or vehicle (ethanol), in culture for 6 h and subjected to the luciferase assay as reported in “Materials and Methods.” Bars represent the average ± SEM of two individual experiments, each performed with at least three gonads per group. *p < 0.05, as compared with the control.

**FIG. 7.** Effect of genistein and estradiol on fetal gonadal cell proliferation. 

3H-thymidine incorporation in 14.5 dpc testis (A) and ovaries (B) obtained from CD-1 mice and cultured for 24 h in the presence of 0.1 and 1µM genistein, 10nM estradiol, or vehicle (ethanol). The experiments were repeated twice, with a total of at least six gonads per group. Bars represent the average ± SEM of two individual experiments, each performed in triplicate. *p < 0.05, as compared with the control.
(Nilsson et al., 2001) and ER-associated proteins (Saceda et al., 1998) or with the proteasome degradation pathway (Marsaud et al., 2003). Lonard et al. (2004) reported that ER-α bound by selective estrogen receptor modulators 4-hydroxytamoxifen and raloxifene, increases the steady-state levels of ER-α protein itself and of ER-coactivators in a cell type-specific way, and that this event has functional consequences on the transcriptional activity of ERs.

We show here that although the modulation of ER-α, which is the only isoform detectable in the liver at protein level, differs in genistein and estradiol, the ERE-dependent activation of luciferase is similar and reflects what occurs in classical targets of estrogens, such as the PR and the steroidogenic enzyme CYP17. Besides the fact that this strengthens our data on the modulation of gene expression analyzed with reporter mice, it also indicates that genistein, although less potent than estradiol, may have functional consequences on estrogen synthesis and activity by strongly inducing the CYP17 gene and on the response of mouse tissues to progestinic compounds by upregulating the PR (Scippo et al., 2004).

Infants’ daily intakes of phytoestrogens from human milk are calculated to be 5–10 μg/kg. This amount is very low if compared with the amount provided by soy-based infant formulas (6000–11,000 μg/kg) (Setchell et al., 1997, 1998). Thus, it seems that there is no reason for concern about the maternal–infant transfer of phytoestrogens from human breast milk during breast feeding. On the basis of the weak estrogenic activity of isoflavones it is unlikely that the dietary intake from human milk is sufficient to exert significant estrogenic effects. This may be different for diets rich in phytoestrogens, such as those of people consuming soy supplement or soy-containing diets. Several authors showed that suckling rats are exposed to active levels of genistein passed from the mother during lactation (Doerge et al., 2001; Fritz et al., 1998; Hughes et al., 2004). They showed by assaying genistein levels in the mother and in suckling pups sera, that lactational transfer of genistein occurs, although exposure is generally lower than that measured in the fetal period. Here we report for the first time data proving that nutritional doses of genistein given to the dam and that produced blood levels in the dam of 550 ± 109 nM, passed to the pups during lactation in an amount sufficient to exert estrogenic actions in 4-day-old suckling mice. The six tissues analyzed, including the brain and reproductive organs, were all well responsive to genistein at 16–24 h after the mother treatment.

In other experiments Doerge et al. (2006) also showed that genistein crosses the rat placenta and can reach the fetus. Although fetal and neonatal serum concentration of total genistein were approximately 20-fold lower than maternal concentrations, the biologically active genistein aglycone concentration was only fivefold lower. Fetal brain contained predominately genistein aglycone at levels similar to those in the maternal brain, thus indicating that genistein can reach all tissues in the pups.

The effects of genistein on reproductive development and functions are still controversial. Several reports have shown that genistein exposure during gestation and lactation has no adverse effects on reproductive organs, fertility, and embryonic development (Fielden et al., 2002, 2003; Fritz et al., 2003; Jung et al., 2004; Kang et al., 2002; Lee et al., 2004; Roberts et al., 2000). Other studies, on the contrary, have reported adverse effects (Delclos et al., 2001; Lewis et al., 2003; Nagao et al., 2001; Strauss et al., 1998), at doses comparable to those administered in the present study. These effects consist in a decreased fertility and estrous cycle changes in female and male rats, although these effects were observed only at the pharmacological dose of 100 mg/kg/day (Nagao et al., 2001). Wisniewski (Wisniewski et al., 2003) also reported that perinatal exposure to genistein resulted in alterations in masculinization of the reproductive system in male rats, an effect probably due to the estrogenic action of genistein. In other studies, genistein acted on adult mice as an estrogen at nutritional doses, but it was effective only at pharmacological doses when mice were exposed during perinatal life (Strauss et al., 1998). In a more recent work Jung et al. (2004) postulated that the severe impairment observed in the male reproductive system of rats exposed to genistein during perinatal life, might be due to its estrogenic action. This was further supported by the work of Nam et al. (2003) in which the author, reported that the expression of genes target of estradiol were upregulated by the estradiol and tamoxifen in the testis and prostate of mice. Many tests have shown that genistein inhibits the induction of acrosomal exocytosis and binding of spermatozoa to the zona pellucida, without affecting sperm motility parameters (Hirsch et al., 2000). Fielden et al. (2003) and Jung et al. (2004) reported that exposure to genistein doses higher than those used in this study, significantly increased the in vitro fertilizing ability of epididymal sperm by increasing their motility.

Taken together, these different studies on the effects of genistein on reproduction produced conflicting results. The data reported in the present paper show that genistein at a dose that may be found in serum of people on soy-containing diets (1 μM) is estrogenic in reproductive organs of adults mice and in the male fetus, at the gestational age of 14.5 days. The testis, but not the ovaries responded to genistein as well as to estradiol, by upregulating the reporter twofold through an ER-mediated mechanism. This effect was accompanied by a testicular increase in cell proliferation which was also ER mediated. It is not surprising that only the testis responded to estrogens at the developmental age of 14.5 dpc because in the mouse ovaries there is no detectable expression of ERs, whereas both ER-α and ER-β are expressed in the testis (Delbès et al., 2004; Greco et al., 1993; Jefferson et al., 2000).

Sensitivity to natural and synthetic estrogens varies among species and strains (Long et al., 2000). In this regard, it is interesting to note that significant estrogenic effects of genistein on cell proliferation resulted from exposure of 14.5-day-old...
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mouse testis of CD-1 mice, a strain which is among the least sensitive to natural estrogens (Spearow et al., 2001).

The observed effect on cell proliferation, in addition to the modulation of the ERE-dependent luciferase, suggests that prenatal exposure to genistein may directly alter developmental process of male gonads, possibly compromising male fertility later in life. Although indirect effects have also been reported on the testis through the modification of the function of the hypothalamic-pituitary-gonadal axis (Hilakivi-Clarke et al., 1999).

Models of the molecular activity of estrogens in mouse tissues have revealed new and unexpected roles for ER ligands, such as genistein. These models, beyond the ones of targeted gene disruption such as ER-α and ER-β knockouts (Eddy et al., 1996; Korach, 2000; Krege et al., 1998) or ER-α/ER-β double knockouts (Islander et al., 2003), include estrogen reporter mice (ERE-tK-LUC) (Ciana et al., 2001). We have shown here and in recent works (Penza et al., 2006, 2007) that ERE-tK-LUC mice represent a suitable model for assessing the estrogenicity of genistein in the whole body and its activity in a tissue dependent way, during development.

Our results may highlight an important effect of genistein in reproductive and nonreproductive organs of male mice. The effect of this phytoestrogen may differ depending on the dose administered and on the age at the time of administration, although at all age mice seem to be able to respond to this compound. Further examination of the effects of genistein should strengthen our understanding of the etiology of the incidence of reproductive diseases in humans on diets rich in phytoestrogens. In turn, this should help nutritionists to determine more accurately the benefit or the risk of estrogen containing diets during pregnancy and development.

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