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Phytoestrogens alter the reproductive organ development in the mink (*Mustela vison*)

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Abstract

The aim of the present study was to examine the reproductive effects of two perorally applied phytoestrogens, genistein (8 mg/kg/day) and β-sitosterol (50 mg/kg/day), on the mink (*Mustela vison*) at human dietary exposure levels. Parental generations were exposed over 9 months to these phytoestrogens and their offspring were exposed via gestation and lactation. Parents and their offspring were sampled 21 days after the birth of the kits. Sex hormone levels, sperm quality, organ weights, and development of the kits were examined. The exposed females were heavier than the control females at the 1st postnatal day (PND). The control kits were heavier than the exposed kits from the 1st to the 21st PND. Phytoestrogens did not affect the organ weights of the adult minks, but the relative testicular weight of the exposed kits was higher than in the control kits. The relative prostate weight was higher and the relative uterine weight lower in the β-sitosterol-exposed kits than in the control kits. Moreover, the plasma dihydrotestosterone levels were lower in the genistein-exposed male kits compared to the control male kits. This study could not explain the mechanisms behind these alterations. The results indicate that perinatal phytoestrogen exposures cause alterations in the weight of the reproductive organs of the mink kits.

Keywords: Genistein; Mink; Reproduction; Sex hormones; β-Sitosterol

Introduction

Genistein is a soy isoflavone that is structurally similar to vertebrate estradiol (E_2; Fig. 1a–b) and it binds weakly to estrogen receptors (Kostelac et al., 2003; Setchell and Cassidy, 1998). β-Sitosterol is structurally related to animal cholesterol (Fig. 1c–d) and can possibly act as a precursor of sex steroids (Moghadasian, 2000). Both compounds are released to the aquatic environment from pulp and paper mill and sewage treatment plant effluents (Kiparissis et al., 2001; Spengler et al., 2001) causing serious problems to reproduction of several fish species (Lehtinen et al., 1999; MacLatchy and van der Kraak, 1995; van der Heuvel et al., 2002).

Recently there has been increasing interest in phytoestrogens also due to their beneficial effects on human health. Phytoestrogens are natural constituents of many plant-based food items (Frische and Steinhart, 1999). They are added to margarines and many other food items to lower elevated total and low-density lipoprotein cholesterol levels (Drexler et al., 1981). Clinical studies indicate that phytoestrogen supplements can prevent many diseases such as cancer, cardiovascular diseases, or osteoporosis (Moghadasian, 2000; Moreau et al., 2003; Setchell and Cassidy, 1998). However, in animal studies, it has been observed that phytoestrogens can also have undesirable effects (Klein, 1998; Malini and Vanithakumari, 1991). For instance, it has been shown in rats that exposure to genistein or β-sitosterol

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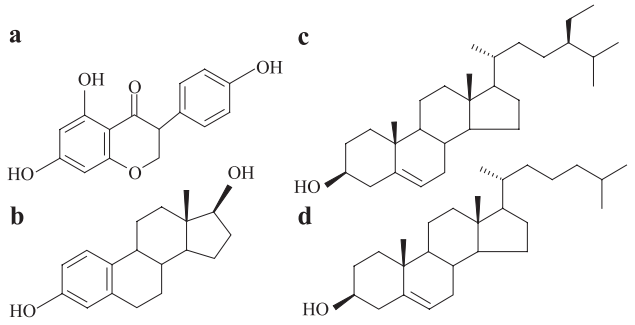

Fig. 1. Molecular structures of genistein (a), estradiol (b), β-sitosterol (c) and cholesterol (d).

can cause reproductive tract abnormalities and changes in reproductive organ weights at doses (0.5–50 mg/kg/day) similar to those that humans can be exposed to when using phytoestrogen-enriched food products daily (Malini and Vanithakumari, 1991; Nagao et al., 2001; Wisniewski et al., 2003).

The aim of this study was to investigate the chronic effects of phytoestrogens on mink (*Mustela vison*) reproduction, sex steroid concentrations and on the development of the kits. Because the mink is a top predator, it is widely used in environmental contaminant exposure and ecosystem health studies (Beckett et al., 2002; Smits et al., 1995). The species is an excellent bioindicator, as it is very susceptible to various ecotoxicologically relevant compounds such as bleached-kraft mill effluents and oil (Beckett et al., 2002; Smits et al., 1995). In nature, the mink can encounter β-sitosterol and genistein through its diet containing, for example, freshwater fish, amphibians, waterfowl, and aquatic invertebrates.

Materials and methods

Animals and exposure. Sixty juvenile (age 100 ± 0.6 days) wild-type minks born in May 2002 were selected for the study. The animals were paired and housed in standard wire cages (85 × 31 × 45 cm) with wooden nest boxes with straw (27 × 31 × 38 cm). The male minks were resettled to the own cages at the end of December due to their aggressive behavior. They were mated using standard fur farm procedures in March and after mating the males were removed and placed into their own cages. The cages were suspended above the ground in a barn at the Juankoski Research Station of the University of Kuopio (63°N; 28°E). The animals were kept in natural photoperiod and temperature. They were assigned into three experimental groups as follows: (1) control, (2) genistein, and (3) β-sitosterol. The control group was given regular fur animal feed (Ylä-Karjalan Rehu Oy, Valtimo, Finland; approximately 4300 kcal/kg dry matter) and the other groups received the same feed mixed either with genistein at 8 mg/kg/day (LClaboratories, Woburn, MA, USA) or β-sitosterol at 50 mg/kg/day (Sigma-Aldrich Co, St. Louis, MO, USA) from August 18th 2002 to May 27th–June 7th 2003. The genistein dose was similar to the dose consumed by infants using soy-based formulas (Setchell et al., 1997). The β-sitosterol dose was based on previous dose-response studies on mustelids (Nieminen et al., 2003). It is also comparable to the estimated daily dose (43 mg/kg/day) used for the treatment of hypercholesterolemia (Moreau et al., 2003). The Animals' Care and Use Committee of the University of Joensuu approved all procedures.

Sampling. Blood samples were taken from a hind leg nail cut with aseptic scissors on August 13th, 2002 before the feeding experiment was started and on January 13th, 2003 before the mating season. The blood samples were collected into test tubes containing 5% EDTA and centrifuged at 3000 rpm for 20 min. The plasma was removed, frozen in liquid nitrogen and stored at −30 °C. The adult females and all the kits were weighed at the 1st, 7th, 14th, and 21st postnatal days (PND). The health and survival of the animals were examined daily.

The adult minks were electrocuted according to the current EU regulations (Council of the European Union 1993) between May 27th and June 7th 2003 when their kits were 21 days of age. The kits were stunned rapidly with diethyl ether. All the animals were weighed, their lengths measured, and the final blood samples obtained by heart punctures with sterile needles, placed into test tubes containing 5% EDTA and centrifuged at 3000 rpm for 20 min. Plasma was removed, frozen in liquid nitrogen and stored at −30 °C. The testes of male minks were dissected, weighed, frozen in liquid nitrogen, and stored at −30 °C. The liver, kidneys, and spleen were dissected and weighed. The carcasses were frozen at −20 °C, thawed at a later date and the adrenal glands, thyroid glands, prostate glands, ovaries, and uteri were dissected and weighed.

Semen analysis. Sperm samples were collected with electroejaculation (Electrojac IV, Minitüb GmbH, Tiefenbach, Germany) on March 25th, 2003 from 18 male minks (6/group) that had copulated 8.7 ± 1.0 days earlier. The animals were anaesthetized with a combination of xylazin (10 mg/kg) and ketamine (15 mg/kg) injected intramuscularly. The samples were diluted to 1:50 with a buffer solution (2.4 g Tris, 1.4 g citrate, 0.8 g glucose, 20 ml egg yolk, 3 ml glycerol/100 ml H_20; pH 6.86). A small amount of the diluted sample was taken in a microcuvette and the sperm concentration was quantified (Sperma Cue, Minitüb GmbH). The remaining sample was frozen gradually to −196 °C with liquid nitrogen. At the same time, blood samples were taken from the males for the testosterone (T) assay and the length and width of the testes were measured with an accuracy of 1 mm.

The sperm samples were thawed at +37 °C. Sperm morphology was examined on a wet preparation at 400×