Effect of dietary estrogens from bovine milk on blood hormone levels and reproductive organs in mice

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ABSTRACT

Cows are often milked until 60 d before their next expected calving. Milk from cows in the third trimester of pregnancy contains up to 20 times more estrogens than milk from nonpregnant cows. The aim of this study was to evaluate whether exposure to known doses of estrogens from bovine milk could affect blood hormone levels in mice and influence their reproductive organs. This study was performed with 30 intact male and 30 ovariectomized female mice. Mice of each sex were randomly divided into 3 experimental groups, each with 6 animals of each sex, and a control group with 12 animals of each sex. The first experimental group received 4 mL of milk each day from a pregnant cow with natural estrone (E1) and 17β-estradiol (E2) in concentrations 0.093 and 0.065 ng/mL, respectively. The second experimental group received 4 mL of the same milk each day, with an added 10 ng/mL of both E1 and E2. The third experimental group received 4 mL of the same milk each day, with an added 100 ng/mL of both E1 and E2. The control group received no milk. After 8 d of treatment, mice were euthanized, blood was collected, and the uteruses, testes, and seminal vesicles were weighed. The results of our study demonstrated that consumption of native milk from a pregnant cow did not affect plasma E1 and E2 levels in either sex; uterine weight in females; or testosterone levels and testes and seminal vesicle weights in males. Similarly, we found no changes in the group that received the milk with an added 10 ng/mL of E1 and E2. We did observe elevated plasma estrogens in both sexes, increased uterus weight in females, and decreased plasma testosterone levels in males from the group that received milk with an added 100 ng/mL of E1 and E2. However, concentrations in the third group exceeded the physiological concentration of milk estrogens by 1,000 times, so it would be extremely unlikely to find such concentrations in native cow milk.

Key words: cow milk, estrogens, lactation, mice

INTRODUCTION

In milk production, dairy cows are usually milked until the seventh or eighth month of pregnancy. The period between calving and first insemination is approximately 60 d, and cows are usually dried 2 mo before the next expected calving. During pregnancy, the concentration of estrogens in the body increases, mainly because of increased production in the placenta, and this increase is believed to play an important role in preparation for parturition (Davidson and Stabenfeldt, 2013). Pape-Zambito et al. (2008) have shown that estrogen levels in milk are correlated with estrogen levels in blood, suggesting that milk from cows in late pregnancy may contain increased estrogen concentrations. The average levels of estrone (E1) in milk from cows that are more than 6 mo pregnant could be up to 20 times higher than those in milk from nonpregnant cows. According to Pape-Zambito et al. (2008), levels of E1 and 17β-estradiol (E2) in the milk of cows in the third trimester of pregnancy are approximately 25 and 5 pg/mL, respectively. Malekinejad et al. (2006) reported even higher levels: up to 118 ± 17 pg/mL for E1 and 21 ± 3 pg/mL for E2. It has also been reported that levels of milk estrogens correlate with milk fat content (Pape-Zambito et al., 2007, 2010; Farlow et al., 2012; Macrina et al., 2012). These levels reportedly decrease with processing (Wolford and Argoudelis, 1979), although Pape-Zambito et al. (2010) did not find a reduction in milk E2 levels during pasteurization and homogenization.

The role of milk consumption in the incidence of some cancers in humans has been described in several epidemiologic studies, but the results have not been conclusive. Consumption of milk with relatively high levels of estrogens is a source of concern. It has been suggested that estrogens from bovine milk could contribute to some endocrine-related cancers and male...
reproductive disorders in humans (Ganmaa et al., 2001; Ganmaa and Sato, 2005), but this hypothesis has not been tested or proven experimentally. Some other experiments and meta-analyses have shown that milk consumption is not a risk factor for cancer development (Davoodi et al., 2013; Bernichtein et al., 2015; Lars-son et al., 2015), and some of them have even shown protective effects for milk (Cho et al., 2004; Elwood et al., 2008; Davoodi et al., 2013). However, Maruyama et al. (2010) have shown that the consumption of cow’s milk could significantly increase serum levels of E1 and progesterone in humans. Additionally, in the same experiment, the authors observed a significant decrease in levels of LH, FSH, and testosterone. Rich-Edwards et al. (2007) reported higher values of IGF-1 and growth hormone after long-term intake of milk in 1 of 2 tested groups of children. In rodent models, some studies have reported uterotrophic effects after milk intake in prepu-bertal ovariectomized rats (Ganmaa et al., 2006; Zhou et al., 2010), but other studies have not found significant effects of commercial milk consumption on female rats’ reproductive system (Li et al., 2005), uterotrophic activity in mice (Nielsen et al., 2009), or changes in be-havior and uterine weights in rats (Furnari et al., 2012).

The aim of our study was to evaluate whether the consumption of milk with known doses of estrogens (both naturally presented and added in concentrations 100 and 1,000 times higher) could affect blood hormone levels and reproductive organs in mice.

MATERIALS AND METHODS

Animals and Housing

We used inbred BALB/c mice, reared in the Center for Animal Genomics at the Veterinary Faculty, University of Ljubljana. Animals were housed under standard conditions (22 ± 2°C, 12:12 h light:dark regimen) with regular rodent chow (phytoestrogen-free diet; Teklad, 2016, Harlan, Milan, Italy) and water ad libitum. All female mice were ovariectomized bilaterally at 60 d of age (after puberty) to eliminate fluctuations in endoge-nous gonadal steroids. The mice were anesthetized with a mixture of ketamine (100 µg/g of BW; Vetoquinol Biowet, Gorzow, Poland), acepromazine (2 µg/g of BW; Fort Dodge Animal Health, Fort Dodge, IA) and xylazine (10 µg/g of BW; Chanelle Pharmaceuticals Ltd., Loughrea, Ireland), and the gonads were excised through small incisions. The incisions were stitched with absorbable sutures (Braun, Tuttinglen, Germany) and mice received 2 injections of butorphanol (2 µg/g of BW; Fort Dodge Animal Health) after surgery to alleviate potential pain. Animals were allowed at least 14 d to recover from the ovariectomies. One week before starting the experiment, animals were placed in individual polycarbonate cages with a surface area of 300 cm². The age of the mice at the beginning of the experiment was 80 to 105 d. All animal experiments were approved by the Administration of the Republic of Slovenia for Food Safety, Veterinary, and Plant Protection, license number 34401–45/2011/6, and all experiments were done according to European Union directives and National Institutes of Health (Bethesda, MD) guidelines.

Assay Characteristics of E1 and E2 Measurements in Milk

Milk E1 and E2 concentrations were determined using an estrone ELISA kit and an estradiol-sensitive ELISA kit (catalog numbers DE4174 and DE4399; Demeditec Diagnostics, Kiel-Wellsee, Germany). Because these commercial ELISA kits were validated for the detection of E1 or E2 in serum or plasma, we performed partial validation for the detection of E1 and E2 in milk.

The milk sample (1.5 L) used for partial validation originated from a 201-d pregnant Holstein cow. Milk was collected during morning milking and immediately transported to the laboratory. Validation for E1 was performed the same day, and validation for E2 was performed the next day. The milk sample was stored at 4°C. We used native, unprocessed milk to obtain assay characteristics of E1 and E2 measurements. The assay procedure was performed following original instructions from the E1 and E2 ELISA kits. We used pure milk (without extractions) as a matrix for determining assay characteristics.

We assessed the accuracy of the test (recovery) by measuring known amounts of E1 and E2 added to milk; E1 (Fluka, St. Galen, Switzerland) and E2 (Sigma-Aldrich, St. Louis, MO) were separately dissolved in dimethyl sulfoxide (Merck, Darmstadt, Germany) in concentrations of 300 ng/mL. After that, 10, 20, 30, and 40 µL of dissolved E1 or E2 (as well as 50 and 60 µL for E1 only) were added to 100 mL of milk to reach concentrations of 0.03, 0.06, 0.09, and 0.12 ng of added E1 or E2 per mL of milk (as well as 0.15 and 0.18 ng/mL for E1 only). Every spiked sample was measured in 6 replicates.

We determined the precision of the test using intra- and interassay CV. Intraassay CV were determined by measuring natural and added E1 and E2 into milk. Interassay CV were determined by measuring native E1 and E2 in milk in 2 assays, with 8 replicates in each assay. Table 1 presents the details of assay characteristics.

The average recovery rates for E1 and E2 measurements were 100 and 107%, respectively. Interassay CV for E1 (n = 16) and E2 (n = 16) were 12.40 and 14.26%, respectively.
Native, unprocessed milk used for in vivo experiments was collected after morning milking. Milk was collected from 5 cows, pregnant between 178 and 221 d, to detect potential variability between individual cows. Three liters of milk from each cow were immediately transported to the laboratory. We measured E1 and E2 in 4 replicates. Table 2 presents basic data for the cows and milk E1 and E2 concentrations. As expected, levels of estrogens varied between individual cows, and milk with the highest estrogen concentrations (both E1 and E2) was chosen for subsequent in vivo experiments.

Pure E1 (Fluka) and E2 (Sigma-Aldrich), previously dissolved in dimethyl sulfoxide (Merck), were added in concentrations of 10 ng/mL (milk 10) and 100 ng/mL (milk 100) to the milk of cow 2. The final estrogen concentrations in milk used for subsequent experiments were 10.093 ng/mL of E1 and 10.065 ng/mL of E2 in milk 10 and 100.093 ng/mL of E1 and 100.065 ng/mL of E2 in milk 100. The milk with no added estrogens contained the same amount of dimethylsulfoxide (0.2 μL/mL) as the milk given to the control group. All milk samples were divided into aliquots of 30 mL and stored at −20°C until use.

**Experimental Protocol**

The experiment was performed on 30 intact male and 30 ovariectomized female mice (Table 3). Mice of each sex were randomly divided into 3 experimental groups, with 6 animals of each sex in each group, and a control group, with 12 animals of each sex. Although mice were not weighed during the study, they were all from our inbred colony, which has an average BW of 20.9 g ± 0.58 for males (n = 10; mean ± SE) and 17.3 g ± 0.52 (n = 10) for females. The first group of male and female mice received 4 mL per day of native milk without added estrogens (milk group); the second group received 4 mL per day of milk with 10.093 ng/mL of E1 and 10.065 ng/mL of E2 (milk 10 group); and the third group received 4 mL per day of milk with 100.093 ng/mL of E1 and 100.065 ng/mL of E2 (milk 100 group). The control group received only water containing the same amount of dimethylsulfoxide (0.2 μL/mL) as the milk given to the experimental groups. During the ex-

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**Table 1.** Assay characteristics of the measurements of milk estrone (E1) and 17β-estradiol (E2) using commercial ELISA plasma/serum kits

<table>
<thead>
<tr>
<th>Added concentration (ng/mL)</th>
<th>Expected concentration (ng/mL)</th>
<th>Mean measured concentration (ng/mL)</th>
<th>Recovery (%)</th>
<th>Intraassay CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (n = 8)</td>
<td>0.053</td>
<td>0.053</td>
<td>11.40</td>
<td></td>
</tr>
<tr>
<td>0.03 (n = 6)</td>
<td>0.083</td>
<td>0.089</td>
<td>107</td>
<td>14.06</td>
</tr>
<tr>
<td>0.06 (n = 6)</td>
<td>0.113</td>
<td>0.114</td>
<td>101</td>
<td>5.69</td>
</tr>
<tr>
<td>0.09 (n = 6)</td>
<td>0.143</td>
<td>0.141</td>
<td>99</td>
<td>8.58</td>
</tr>
<tr>
<td>0.12 (n = 6)</td>
<td>0.173</td>
<td>0.182</td>
<td>94</td>
<td>8.23</td>
</tr>
<tr>
<td>0.15 (n = 6)</td>
<td>0.203</td>
<td>0.192</td>
<td>93</td>
<td>2.93</td>
</tr>
<tr>
<td>0.18 (n = 6)</td>
<td>0.233</td>
<td>0.216</td>
<td>93</td>
<td>5.78</td>
</tr>
<tr>
<td>E2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (n = 8)</td>
<td>0.050</td>
<td>0.079</td>
<td>107</td>
<td>8.65</td>
</tr>
<tr>
<td>0.03 (n = 6)</td>
<td>0.080</td>
<td>0.079</td>
<td>99</td>
<td>8.65</td>
</tr>
<tr>
<td>0.06 (n = 6)</td>
<td>0.110</td>
<td>0.111</td>
<td>115</td>
<td>8.37</td>
</tr>
<tr>
<td>0.09 (n = 6)</td>
<td>0.140</td>
<td>0.160</td>
<td>115</td>
<td>9.52</td>
</tr>
<tr>
<td>0.12 (n = 6)</td>
<td>0.170</td>
<td>0.190</td>
<td>112</td>
<td>6.12</td>
</tr>
</tbody>
</table>

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**Table 2.** Days of pregnancy and lactation of cows, daily milk production, and milk estrone (E1) and 17β-estradiol (E2) concentrations

<table>
<thead>
<tr>
<th>Cow no.</th>
<th>Day of pregnancy</th>
<th>Day of lactation</th>
<th>Daily milk production1 (L)</th>
<th>Milk E1 concentration (ng/mL)</th>
<th>Milk E2 concentration (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>178</td>
<td>280</td>
<td>15.7</td>
<td>0.017</td>
<td>&lt;0.0075</td>
</tr>
<tr>
<td>2</td>
<td>196</td>
<td>282</td>
<td>12.2</td>
<td>0.093</td>
<td>0.065</td>
</tr>
<tr>
<td>3</td>
<td>191</td>
<td>266</td>
<td>9.1</td>
<td>0.076</td>
<td>0.022</td>
</tr>
<tr>
<td>4</td>
<td>189</td>
<td>265</td>
<td>12.3</td>
<td>0.056</td>
<td>0.040</td>
</tr>
<tr>
<td>5</td>
<td>221</td>
<td>291</td>
<td>15.1</td>
<td>0.041</td>
<td>0.033</td>
</tr>
</tbody>
</table>

1Daily milk production was determined during milk recording, 4 d before milk collection.
experiment, animals from all groups were given food and water ad libitum. Milk was given to animals in plastic conical centrifuge tubes measuring 17.1 × 120 mm, 15 mL (TPP, Technoplastic Plastic Products AG, Trasadingen, Switzerland). At the conus of the tube, a hole with a diameter of 1.7 mm was drilled and carefully sanded. The whole tube was thoroughly washed with distilled water and dried under UV light. Each mouse received 4 mL of test milk in the tube. We developed this procedure on 12 animals initially, to ensure that they could drink from the tubes, and that the tubes were not leaking. During the experiment, we observed the tubes for leaking during the first 10 min of exposure, but none of the tubes leaked. The amount of milk in the tubes was checked every 2 h. Mice were given milk for 8 d. Fresh milk was added every morning at 0800 h. All mice had consumed almost all of the milk by 1200 h, except on the first day of treatment, when some mice (18 altogether randomly across all groups) consumed 2 to 4 mL of milk.

On the eighth day of treatment (3 to 4 h after the mice consumed the daily amount of milk, milk 10, or milk 100), the mice were subjected to deep anesthesia with a mixture of ketamine (100 μg/g of BW; Vetquino1 Biowet), acepromazine (2 μg/g of BW; Fort Dodge Animal Health) and xylazine (10 μg/g of BW; Chanelle Pharmaceuticals Ltd.). After thoracotomy, blood was collected directly from the heart and blood plasma was stored at −20°C until hormone measurements were performed. The mice were thus euthanized by bleeding from the heart. Afterward, in females, 1 cm of the left uterine horn was dissected, blotted, and weighed; in males, testicles and seminal vesicles were isolated, blotted, and weighed (balance EW420-3NM, Kern, Balingen, Germany).

Detection of Plasma E1, E2, and Testosterone

Determination of plasma hormone concentrations was performed using commercial ELISA kits (Demeditec Diagnostics) following the user’s manual (available at http://www.demeditec.com/). We performed control of measurements using intra- and interassay CV.

We determined E1 concentrations using an estrone ELISA kit (catalog number DE4174). The range of detection was 6.5 to 1,000 pg/mL (0.0065 to 1.0 ng/mL). Intra- and interassay CV were 8.72% and 12.88% for test plasma with 92 pg/mL, and 10.66% and 18.08% for test plasma with 328 pg/mL.

We determined E2 concentrations using an estradiol-sensitive ELISA kit (catalog number DE4399). The range of detection was 1.5 to 200 pg/mL (0.0015 to 0.2 ng/mL). Intra- and interassay CV were 11.38 and 16.70% for plasma with 5.88 pg/mL, and 12.52 and 16.85% for plasma with 93.28 pg/mL.

We determined testosterone concentrations using a testosterone ELISA kit (catalog number DE1559). The range of detection was 0.2 to 16 ng/mL. Intra- and interassay CV were 6.28 and 13.54% for plasma with 0.80 ng/mL, and 8.32 and 16.40% for plasma with 11.76 ng/mL.

Statistical Analysis

We used SPSS (version 21; IBM, Chicago, IL) for statistical analyses and the Shapiro-Wilk test to estimate data distribution. Because data were distributed normally, we used one-way ANOVA with the Fisher LSD post hoc test to evaluate statistically significant differences between groups. All results are presented as mean ± SE, and statistical significance was considered at P < 0.05.

RESULTS

Hormone Concentrations

Plasma E1 concentration did not differ between groups, with the exception of males drinking milk 100. In this group only, the concentration of plasma E1 was elevated (P < 0.05) compared to all other groups (Figure 1a).

Similarly, plasma E2 did not differ between groups, with the exception of females drinking milk 100. This group had elevated (P < 0.05) levels of plasma E2 compared to other groups (Figure 1b).

Table 3. Experimental groups and treatments with estrone (E1) and 17β-estradiol (E2)

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Milk</th>
<th>Milk 10</th>
<th>Milk 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of male mice</td>
<td>12</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>No. of female mice</td>
<td>12</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Treatment</td>
<td>Water + 0.2 μL/mL DMSO&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Native milk (0.093 ng/mL E1 and 0.065 ng/mL E2) + 0.2 μL/mL DMSO</td>
<td>Native milk + 10 ng/mL E1 and 10 ng/mL E2 + 0.2 μL/mL DMSO</td>
<td>Native milk + 100 ng/mL E1 and 100 ng/mL E2 + 0.2 μL/mL DMSO</td>
</tr>
</tbody>
</table>

<sup>1</sup>Dimethylsulfoxide.
Figure 1. Plasma concentrations of estrone (E1), 17β-oestradiol (E2), and testosterone in groups of mice drinking native milk (0.093 ng/mL E1 and 0.065 ng/mL E2, n = 6), milk 10 (10.093 ng/mL E1 and 10.065 ng/mL E2, n = 6), milk 100 (100.093 ng/mL E1 and 100.065 ng/mL E2, n = 6), or water (control group; n = 12). In male mice, E1 was significantly elevated (*\(P < 0.05\)) in the milk 100 group compared with the other 3 groups. In female mice, E2 was significantly elevated (*\(P < 0.05\)) in the milk 100 group compared with the other 3 groups. In male mice, testosterone was significantly reduced (*\(P < 0.05\)) in the milk 100 group compared with the other 3 groups. Results are presented as mean ± SE.
Plasma testosterone concentration was measured in male mice only. The group drinking milk with the highest concentration of estrogens (milk 100) had lower levels of testosterone ($P < 0.05$). We observed no significant differences among the other 3 groups (Figure 1c).

**Organ Weights**

We measured uterine weights in female mice from all 4 groups, and we observed a significant increase in uterine weight in mice that drank milk with the highest concentration of estrogens (milk 100). We observed no significant differences among the other 3 groups (Figure 2).

We measured the weight of testes (both testes together) and seminal vesicles in male mice from all 4 groups. We observed no significant differences among the groups.

**DISCUSSION**

We examined the possible effects of estrogen consumption via cow milk on blood steroid hormone levels and the weight of reproductive organs. In milk production, cows are milked up to the seventh or eighth month of pregnancy, when levels of estrogens in blood significantly increase, mainly due to production in the placenta (Shah et al., 2006). This could be a cause for concern for human health, because doses in milk do correlate with doses in the bloodstream, and levels of E1 might exceed 0.1 ng/mL in milk from cows in the third trimester of pregnancy (Malekinejad et al., 2006).

In this study, the highest E1 and E2 concentrations in native milk from pregnant cows were 0.093 and 0.065 ng/mL, respectively, similar to a previous report by Malekinejad et al. (2006), although in that study, milk from different cows was pooled and the results did not account for individual variability. Concentrations of E1 and E2 in our study were higher than those in a study by Pape-Zambito et al. (2008), but this could be explained by lower milk production in cows from our study (Table 2). Another possibility for the discrepancies between studies could be potential cross-reactivity of the assay used in our study with cholesterol, the influence of triglycerides during measurements of hormone concentrations, or both. However, we performed validation of the assays used for measuring E1 and E2 in milk samples, and we obtained very good recovery rates, suggesting a small effect of cholesterol cross-reactivity, if any. Nevertheless, it is known that estrogens can have physiological effects even in very low doses, so, we examined whether concentrations in milk could affect steroid hormone levels and reproductive organ weights in mice. Milk containing natural estrogens did not change physiological parameters in mice, suggesting that doses were not high enough to produce any undesirable effects in mice (Figures 1, 2, and 3). This might be partially caused by the metabolism of estrogens; it has been reported before that large amounts of estrogens can be inactivated by the gastrointestinal and hepatic systems (Moore et al., 1982). Parodi (2012) reported that only 5% of E2 from milk reaches the systemic circulation. These data indicate that only a relatively high oral dose of E2 would provide a biologically active concentration in peripheral blood. Therefore, in our study, it is likely that plasma E1 and E2 did not increase in mice drinking pregnant cow’s milk because the estrogens in the milk were at low enough levels to be metabolized during first liver passage and did not reach the systemic circulation.

Furthermore, intake of pregnant cow milk did not cause effects characteristic of estrogenic action. Uterine weights, which are strongly influenced by estrogens (Davidson and Stabenfeldt, 2013), did not differ between the milk and control groups (Figure 2). This finding was in contrast to those of Zhou et al. (2010) and Ganna et al. (2006), who described uterotrophic effects after 7 d of milk intake in rats. However, in both these studies the amount of estrogens in the milk was not reported, so the exposure to estrogens is unknown. The difference between studies could also be caused by species-specific responses, or even by age: Zhou et al. (2010) and Ganna et al. (2006) used young, mostly prepubertal rats, but we tested adult female mice. Therefore, we cannot rule out the possibility that
the concentrations of estrogens in milk from our study would not have affected the development of the reproductive system in the prepubertal period or even during fetal development. However, the results of our study are in line with those of Nielsen et al. (2009) and Furnari et al. (2012), who also found no uterotrophic activity after milk intake in rodent models. The absence of change in uterine weight is additional confirmation that milk estrogens did not reach the systemic blood circulation in concentrations that could cause biological effects.

Testosterone production in the testes is controlled by LH, and estrogens usually suppress LH secretion via a negative feedback loop, resulting in a decrease of testosterone secretion. In accordance with this, Maruyama et al. (2010) have described a decrease in plasma testosterone levels in men after milk consumption. However, the results of our study showed no drop in testosterone production in male mice who drank native milk (Figure 1c), suggesting that plasma levels of estrogens after milk consumption were not high enough to affect the pituitary-gonadal axis. Again, differences between the study by Maruyama et al. (2010) and our study could reflect species-specific differences or different estrogen concentrations in the milk: Maruyama et al. (2010) did not report estrogen concentrations, so the studies could not be compared directly.

In this study, the results were the same even when the concentration of estrogens in the milk was increased. Concentrations of 10 ng/mL of each E1 and E2 did not cause physiological effects, either on hormone blood levels or on weight of reproductive organs (Figures 1, 2, and 3), suggesting that even doses 100 times higher than those found in native milk do not cause physiological effects in mice. However, consumption of milk with an added 100 ng/mL of E1 and 100 ng/mL of E2 (milk 100) did cause several physiological effects in both sexes, suggesting that the dose at which estrogens cause physiological effects in mice is somewhere between 10 and 100 ng/mL (approximately between 2,000 and 20,000 ng/kg of BW per day). Curiously, these high doses of estrogens caused a significant increase in plasma E1 in males but not in females, and a significant increase in plasma E2 in females but not in males. Similar to this, Maruyama et al. (2010) found an increase in plasma E1 but not E2 in men after milk intake, which they explained by saying that E2 concentrations in milk were too low to affect blood levels. We found no publications reporting differences in the kinetic properties of estrogens between males and females. Because there are differences between sexes in the activity of some enzymes included in steroidogenesis (Genuth, 2006) we presume that some differences might also exist in metabolic and elimination pathways, leading to differences in blood levels of estrogens in males and females, but this should be explored in future studies. Additionally, in the milk 100 group, uterine weights

![Figure 3](image-url) - Weights of testes and seminal vesicles in male mice drinking native milk [0.093 ng/mL estrone (E1) and 0.065 ng/mL 17β-estradiol (E2), n = 6], milk 10 (10.093 ng/mL E1 and 10.065 ng/mL E2, n = 6), milk 100 (100.093 ng/mL E1 and 100.065 ng/mL E2, n = 6), or water (control group, n = 12). No significant differences were observed between the groups. Results are presented as mean ± SE.
were significantly higher in comparison to the control, milk, and milk 10 groups, and testosterone concentration in the male milk 100 group was significantly lower. Estrogenic effects in the milk 100 groups were evident in both sexes.

To the best of our knowledge, this was the first study to examine the effects of known amounts of consumed estrogens from milk. We believe that our results, which show that estrogen levels in milk must exceed naturally present levels by more than 100 times to affect hormonal status and reproductive organs, are a new contribution to our understanding of the eventual disrupting effects of milk.

Because the present study was performed in mice, it cannot confirm with certainty that milk consumption has no disrupting effects on hormonal systems in humans, because there are important species-specific differences in the effects of steroid hormones and metabolism. However, the results of our study show that daily doses of 17.70 and 12.38 ng/kg BW (males) or 21.50 and 15.03 ng/kg BW (females) of E1 and E2 in native bovine milk had no physiological effects in mice. Additionally, we observed no effects in mice that were treated with milk 10, receiving doses of 1,900 (males) or 2,300 (females) ng/kg BW of E1 and E2. Therefore, even if humans are more sensitive to estrogens from milk, it is unlikely that naturally occurring estrogens could cause harmful effects, especially because we observed no changes in E1, E2, and testosterone concentrations and no uterotrophic effect even when doses were 100 times higher than native cow milk (milk 10 group). The potential for extrapolation of findings from the present study to humans should be determined in future studies.

CONCLUSIONS

The results of our study demonstrate that in mice, consumption of milk from pregnant cows did not affect plasma E1 and E2 levels; uterine weight in females; or testosterone levels, testicle, and seminal vesicle weights in males. We found elevated plasma estrogens and estrogenic effects in both sexes if milk with added 1,0065 ng/mL of E2) did not cause any physiological effects in the present study.

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