Estrogens in consumer milk - is there a risk to human reproductive health?

A review

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Abstract

Possible effects of xenoestrogens on human health, in particular on male reproductive health, have attracted considerable attention in recent years. Cows’ milk was suggested in numerous publications as one of possible sources of xenoestrogens that could affect human health. Although milk has undoubtedly many beneficial health effects and could even have a role in reducing incidence of some cancers, concerns were raised about presumably high levels of estrogens in cows’ milk. In intensive farming, concentrations of estrogens in milk are higher due to long milking periods that today extend long into the pregnancy, when concentrations of estrogens in the cow body rise. Numerous studies examined potential effects of milk on reproductive health and endocrine related cancers in both experimental studies with laboratory animals, and in human epidemiological studies. In the present review article, we compiled a review of recently published literature about the content of estrogens in cows’ milk and potential health effects, in particular on reproductive system, in humans. Although results of published studies are not unequivocal, it seems that there is stronger evidence suggesting that amounts of estrogens in cows’ milk are too low to cause health effects in humans.
1. Introduction

In the last decades several studies suggested that there is an increase in the incidence of different reproductive disorders in general population, particularly in males, such as hypospadias, cryptorchidism and testicular cancer, which is now the most common cancer in young males (1). Similarly, the incidence of breast cancer is rising in females (2), and several studies reported a decline in semen parameters in males (3, 4, 5, 6, 7). Although some of these reports are being discussed or even disputed, in the western world the incidence of testicular cancer in males and breast cancer in females is undoubtedly rising (1, 2). The causes for these increases are not known, although environmental exposure to different chemicals with estrogenic-like activity or even estrogens themselves from different sources have been proposed to contribute to these effects (6, 8, 9).

Many studies hinted that various exogenous compounds (environmental pollutants) that could be present in low concentrations in food/feed and water could disrupt the development and function of the neuroendocrine system in vertebrates. Such compounds can act as agonists or antagonists of different hormonal receptors and could thus disrupt cell signaling and homeostasis (8, 10). Different studies suggest that some reproductive, developmental, neurologic, oncologic, cognitive and behavioral disorders in humans could be associated with the exposure to such endocrine disruptors (11, 12). The possibility that hormone mimicking xenobiotics could disrupt the development and function of the endocrine system in humans and animals (8, 10) also opened the question about potential effects of naturally present estrogens in cow milk on the endocrine system of people consuming milk. Sharpe and Skakkebaek (1993) (8) reviewed possible sources of estrogen exposure in humans. Beside residues of estrogenic contraceptives, chemical pollutants with estrogenic activity, mycoestrogens and phytoestrogens
from natural sources, they suggested that important source of estrogens could present food of animal origin, especially milk and dairy products.

Potential exposure to exogenous estrogens is especially important in regard to prenatal and prepubertal development of the reproductive system in children. Endogenous levels of estrogens in children are low and small amounts of exogenous estrogens could interact with their hormonal system and disrupt the development of the urogenital tract, mammary glands and the central nervous system (8, 11, 13). Some of these disruptions could become evident only in adult life, even though they have an origin in prenatal or early postnatal disturbances in hormonal balance. These include lower sperm count, which is correlated with the reduced number of Sertoli cells, risk for testicular cancer and possibly also risks for breast and prostate cancers (11, 13, 14). In this respect, milk as a potential source of exogenous estrogens is especially important as consumption of cows’ milk is usually the highest in children.

Exogenous estrogens can disrupt hypothalamic-pituitary-gonadal axis (HPG axis). They could interfere with the normal function of estrogen receptors in the hypothalamus and pituitary gland, and also in peripheral, estrogen responsive tissues (15). Schematic diagram showing hypothetical routes of exposure and health effects in human body are shown in figure 1.
The influence of exogenous estrogens on the endocrine system and cancer development was reported in numerous studies and reviewed in many publications (11, 16, 17). In this review we will focus on the presence of estrogens in consumers’ milk and their potential effect on human health. However, it should be noted that various pollutants, such as PCBs, with estrogenic properties, could also be present in milk in considerable concentrations. These xenoestrogens could pose an equally important risk for human health, but this is beyond the scope of this review article, which is focusing on endogenous milk estrogens.

2. Estrogens in cows’ milk

The positive effect of milk and dairy products in a diet is widely recognized, as milk is an important source of proteins and calcium. However, milk might also contain significant amounts of estrogens (18, 19, 20, 21, 22, 23, 24, 25, 26, 27) due to the fact that selection and
improved management of dairy cows in the last 100 years increased milk production per cow, and extended milking period into 7th or even 8th month of pregnancy (28). As production of estrogens by the placenta is rising throughout the pregnancy, this causes higher levels of estrogens in milk (19, 25).

In cows, as in other mammals, estrogens are primarily synthesized in ovaries and placenta. Their production (and blood levels) importantly fluctuate with the stage of the estrous cycle or stage of the pregnancy and their levels in blood are consequently highly variable. Estrogens as lipophilic substances easily pass through the blood-milk barrier and their presence in milk is in direct correlation with their levels in blood (19). Malekinejad et al. (2006) (25) have thus shown that during pregnancy cumulative concentration of free and enzymatically deconjugated E1 increased from 7.9 ng/L in non-pregnant cows to 1266 ng/L in the third trimester of pregnancy while αE2 and βE2 concentration increased from 33 to 322 ng/L and from 18.6 to 51.2 ng/L, respectively. Additionally, the concentration of conjugated estrogen estrone sulphate increases up to 33-times during the pregnancy.

Therefore, as suggested by Ganmaa et al. (2001) (6), milk that we consume today is most likely very different from milk that was consumed 100 years ago, especially in regard to the concentration of different hormones, including estrogens. This is primarily due to improved selection and management of dairy herds that increased milk production per cow about 5-fold in comparison to 60 or 70 years ago (29). However, it was reported that milk estradiol concentration is negatively correlated with the milk yield (19), and this is most likely a consequence of increased blood flow through the liver due to higher feed intake in high yielding cows, resulting in increased metabolic clearance of estrogens, and also due to the dilution effect in milk (30). Therefore, the increase in estrogens content in milk does not linearly increase with the increased milk production, yet due to the milking in the late pregnancy, concentrations of estrogens in the milk are almost certainly much higher that they were 100 or more years ago.
Reports about estrogen concentrations in milk are numerous, yet very varied, and there is no conclusive data about the amount of estrogens present in commercial milk. Pape-Zambito et al. (2008) (19) reported that milk from the majority of cows in their study contained less than 2 pg/mL of E2 but other studies reported much higher levels of estrogens in milk (Table 1). These variations are likely due to differences in sampling, as concentrations of estrogens in individual cows vary, not only due to their physiological status (pregnant or non-pregnant) but also as a consequence of metabolic status and many other physiological parameters that could affect production and secretion of estrogens. Furthermore, differences between studies are most likely arising due to different sampling methods, different methods of measurement, different methods of extracting lipophilic steroid hormones from aqueous milk, and finally, some studies do not report whether they measured free or conjugated estrogens. As reported by Pape-Zambito et al. (21) estradiol in milk significantly correlates ($r = 0.20$) with the amount of milk fats what is understandable as steroid hormones are lipophilic substances and are easily dissolved in lipid droplets in milk, but could cause a major difference if the amounts of estrogens are estimated in full-fat or skimmed milk. However, heat treatment, a common practice in the dairy industry (pasteurization or sterilization of the milk), does not affect milk E1 and E2 concentrations and similarly, souring does not affect the amount of estrogens in milk (31). Therefore, commercially available dairy products (heat treated pasteurized or UHT milk, or soured products such as yogurt or sour milk) contain similar amounts of estrogens as native milk, and could present a source of estrogens entering the human body. Concentrations of estrogens in milk as was reported in several studies and are presented in Table 1.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Estrone (pg/mL)</th>
<th>Conjugated form (pg/mL)</th>
<th>17β-estradiol (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heap and Hamon</td>
<td>Estrone sulphate</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Study</th>
<th>Conditions</th>
<th>Data Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pape-Zambito et al. (2008)</td>
<td>Non pregnant: 30</td>
<td>1st trimester: 0.6</td>
</tr>
<tr>
<td></td>
<td>Pregnant: 1000</td>
<td>1st trimester: 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2nd trimester: 7.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2nd trimester: 0.9</td>
</tr>
<tr>
<td>Macrina et al. (2012)</td>
<td></td>
<td>3rd trimester: 27.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3rd trimester: 5.0</td>
</tr>
<tr>
<td>Pape-Zambito et al. (2007)</td>
<td></td>
<td>Estrone sulphate: 46.7 ± 62.1</td>
</tr>
<tr>
<td>Farlow et al. (2012)</td>
<td>Commercial milk</td>
<td>Total E1</td>
</tr>
<tr>
<td></td>
<td>Regular: 14.45 ± 1.49</td>
<td>Commercial milk</td>
</tr>
<tr>
<td></td>
<td>Organic: 9.74 ± 0.39</td>
<td>Regular: 5.84 ± 0.63</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Organic: 6.00 ± 0.46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total E2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Commercial milk</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Regular: 28.19 ± .26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Organic: 6.52 ± 11.84</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Conjugated E1: 368</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Conjugated E2: 85</td>
</tr>
<tr>
<td>Zhou et al. (2010)</td>
<td>Traditional milk:</td>
<td>Traditional milk:</td>
</tr>
<tr>
<td></td>
<td>98.5 ± 12.4</td>
<td>24.6 ± 3.0</td>
</tr>
<tr>
<td></td>
<td>Commercial milk:</td>
<td>Commercial milk:</td>
</tr>
<tr>
<td></td>
<td>150.2 ± 8.4</td>
<td>34.1 ± 2.0</td>
</tr>
<tr>
<td>Malekinejad et al. (2006)</td>
<td>Commercial milk:</td>
<td>Commercial milk:</td>
</tr>
<tr>
<td></td>
<td>8.2 – 20</td>
<td>βE2: 10.3 – 20.6</td>
</tr>
<tr>
<td></td>
<td>Raw milk</td>
<td>Raw milk</td>
</tr>
<tr>
<td></td>
<td>Not pregnant: 6.2</td>
<td>Non pregnant:</td>
</tr>
</tbody>
</table>
1st trimester: 9.2  αE2: 7.2  βE2: 5.6
2nd trimester: 57  1st trimester:
3rd trimester: 118  αE2: 12.0  βE2: 10.0

Vicini et al.  2nd trimester:

Goyon et al.  3rd trimester:
(2017) (27)  αE2: 47.0  βE2: 21.0

Organic milk: 6.40

Conventional milk: 4.97

Regular milk:

Abbreviations of detection methods:

RIA – radioimmunoassay
LC-MS/MS – liquid chromatography tandem mass spectrometry
HPLC – high-performance liquid chromatography
αE2 – 17α-estradiol
βE2 – 17β-estradiol

Footnote: Estriol (E3) was also found in some milk samples. Its milk concentrations vary between below LOD and 27 pg/mL (23, 25).

From the human health point of view, it is not only important that concentrations of estrogens in milk have most likely increased with the intensive farming, but also that consumption of milk and dairy products has increased in the last decades, and this could also contribute to the increased human exposure to estrogens from milk (32, 33).
It is important to note that in some studies estrogen levels were measured in native milk from individual cows, in some in pooled samples, and in some in processed milk available in the stores. Furthermore, methods of measurements differ between different studies, some studies do not report whether they measured free or conjugated estrogens, and all these factors account for the high variability between measurements.

3. Exposure to exogenous estrogens through milk consumption

Estrogens in milk are present in free and in conjugated forms as sulphates or glucuronates. Although the most potent endogenous estrogens estrone (E1) and 17β-estradiol (E2) are present in the milk, the main estrogen present in milk is estrone sulphate (18). Estrogens in conjugated forms are not biologically active but they could be deconjugated in the human (and animal) body by either bacterial or endogenous sulphatases and glucuronidases during digestion in the intestinal tract. Free, biologically active estrogens (E1 and E2) could thus be (re)formed from the estrone sulphate after consumption of cows’ milk (16, 34). Ingested estrogens are normally further metabolized in the liver where they could be conjugated (inactivated) or deconjugated (activated) during the metabolic processes. However, small amounts of orally ingested estrogens also escape enterohepatic digestive processes and reach the systemic circulation directly, without passing through the liver (16, 35).

It was suggested that during milk ingestion, only about 2% to 5% of milk estrogens enter the systemic circulation (16), yet this might be enough to cause potential harmful health effects if ingested concentrations of estrogens are high. However, studies reporting the intake of estrogens through milk are scarce, and even few that exist report controversial results.

Ganmaa et al. (2001) (6) reported that children in Japan receive up to 10 ng of 17β-estradiol daily by milk. Authors suggest that this amount might already have harmful effects, based on
the causative observation that milk consumption and incidence of some endocrine related cancers increased in the last decades, although this assumption has not been supported by any experimental studies. In contrast to suggestions by Ganmaa et al (2001) (6), Fritsche and Steinhart (1999) (36) suggested that ingestion of up to 100 ng of estrogens daily is negligible in comparison to the endogenous estrogen synthesis in humans. According to the FDA guidelines (37) no physiological effects of estrogens are expected to occur when consumption is less than 1% of endogenous quantities produced by the segment of population with the lowest daily production – prepubertal boys (37). Macrina et al. (2012) (20) suggested that no harmful health effect could be expected if daily intake of estrogens is less than 540 ng/day what would represent about 1% of daily production in segment of population with the lowest estrogen production (prepubertal boys). Concentrations of estrogens in milk in this particular study would therefore present only 0.01% to 0.1% of daily endogenous production rate and likely, no harmful effects can be expected (20). Even with other studies that reported higher concentrations of estrogens in milk, intake of both E1 and E2 would most likely not exceed 1% of the endogenous daily production and therefore should not present a risk for human health. This is based on the assumption that 1% or less exogenous estrogens interact with so low number of estrogen receptors that they could not influence endocrine function (38). However, from the consumer point of view it is important to note that milk and dairy products are not the only foodstuffs containing estrogens and therefore, a total amount of ingested estrogens (from milk and dairy products and other foodstuffs) must be taken into consideration when estimating potential health effects. Furthermore, certain types of diet, for example low fiber diet, might stimulate the absorption of estrogens from the digestive tract and this could further increase the amount of exposure (8).

4. **Milk consumption and health**
4.1 Milk consumption and reproductive disorders

The potential role of milk estrogens on reproductive health was studied in some experiments in animal models. Zhou et al. (2010) (24) reported uterotrophic effects of milk feeding in ovariectomized immature rats. Groups of rats fed with commercial (industrially prepared) or traditional (obtained directly from cows without any processing) milk for 7 days had increased uterine weights and increased uterine to body weight ratio in comparison to control group which was not fed with milk. Interestingly, the height of the uterine epithelium and endometrium was increased only in the group fed with commercial milk, but not in the control group or group fed with the traditional milk. This could be explained by differences in hormone content between the two types of milk as commercial milk had higher levels of estrogens. Interestingly, the largest difference between milks was in the levels of progesterone, which was about 20-times higher in commercial milk in comparison to the traditional milk, suggesting that observed effects were most likely due to the differences in progesterone, not estrogens. Similarly, Ganmaa et al. (2005) (39) reported an uterotrophic effect of low fat commercial milk in ovariectomized immature and young mature rats. Possibly due to higher sensitivity, this effect was stronger in immature rats than in young mature rats.

Nielsen et al. (2011) (40) studied the onset of puberty in rats drinking commercial milk and found that puberty, as estimated by the time of vaginal opening, occurred earlier in rats consuming milk, in comparison to rats not exposed to milk. Although rats in this study had also increased levels of serum E2 after consuming commercial milk, it is difficult to conclude that increased levels of estrogens were responsible for differences in the timing of vaginal opening. Milk has well-established growth promoting properties (23, 41) and therefore it is completely plausible to assume that young rats consuming milk would grow faster. Body weights at the time of puberty were not reported in this study, however this could trigger earlier onset of puberty.
In contrast to the above-mentioned studies, Li et al. (2005) (42) performed an experiment in a group of Wistar rats that were fed with regular commercial milk and made comparison to the group of rats that were fed artificial milk without estrogens (control group). Reproductive parameters (fertility index, gestation index, weights of uterus and ovary, days of vaginal opening and length of the estrus cycle), histological parameters and IGF-1 blood levels did not differ between groups, suggesting that estrogens present in commercial milk did not influence the reproductive function. Similarly, Furnari et al. (2012) (43) also examined possible uterotrophic effects in rats and did not find any such effects of commercial milk.

In our recent study we examined potential effects of milk with known (natural or spiked) content of estrogens (44). In this study, we did not detect any effect of estrogens from milk on plasma E1, E2 and testosterone levels in gonadally intact male and ovariectomized female C57Bl/6 mice, and there were no uterotrophic effects observed in female mice, or any effects on testis or seminal vesicles weights in mice drinking milk from cows in late pregnancy with relatively high levels of estrogens. Furthermore, adding estrogens to milk in concentrations exceeding endogenous concentrations by 100-fold also did not have any effect on hormone levels or reproductive organ weights. Only when milk was spiked with estradiol and estrone concentrations exceeding endogenous concentration by 1000-fold, we detected an increase in E1 and E2 blood levels in female mice, decrease in testosterone blood levels in male mice, and uterotrophic effects (increased uterine weights) in females. These results suggest that estrogens do pass from milk into the bloodstream, but to exert any physiological effects on reproductive tracts at least in mice, their levels must exceed endogenous concentration of estrogens in milk by more than 100-times. Therefore, although there are physiological differences between mice and humans, considering the safety margin of more than 100-fold (perhaps even 1000-fold), it
is plausible to conclude that endogenous estrogens from milk do not pose a risk for reproductive health.

A comprehensive two-generations reproductive study on milk consumption in rats was performed by Ganmaa et al. (2004) (23). In this study, rats were fed milk through two generations and neither in F1 nor in F2 generations reproductive effects were observed. Reproductive parameters such as fertility, gestation duration, litter size, sex ratio of the offspring, offspring survival rate, the length of the estrus cycle, FSH, LH, testosterone levels in males and FSH, LH, E2, progesterone and prolactin levels in females did not differ between group drinking milk and control group with the exception of anogenital distance that was slightly reduced in female, but not male pups in F2 generation. Furthermore, some rare skeletal abnormalities were recorded in pups from F2 generation in milk consuming group such as shortened tails, although there was no plausible explanation why milk consumption would cause these skeletal abnormalities and it is possible that they occurred by chance.

Although difficult to perform, some epidemiological studies attempted to determine possible effects of milk consumption on the human endocrine system. Afeiche et al. (2013) (45) analyzed the ejaculates of young males and potential correlation with the consumption of dairy products. They reported that young males who frequently consumed full fat dairy products had lower ratio of progressive motile sperms and more frequent alterations in sperm morphology. They also reported that higher blood FSH levels were correlated with the regular consumption of full fat dairy products, whereas LH, E2 and testosterone concentrations were not correlated with the intake of dairy products (44). Maruyama et al. (2010) (46) measured concentrations of different hormones in sera from 7 adult men. They reported that in men who drank around 1 L of milk, serum E1 and progesterone significantly increased and peaked 30 – 60 minutes after the milk intake, while E2 concentrations did not change significantly. Moreover, serum FSH, LH and
testosterone concentrations significantly decreased after milk intake and were found to be at the lowest levels 60 - 120 minutes after milk intake. These results suggest that hormones from milk did activate the negative feedback loop and suppressed gonadotropins secretion, although it is not known how prolonged this effect was, as they measured hormone levels only up to 120 minutes after the milk consumption. Additionally, in the same study, urinary levels of E1, E2, E3 and pregnanediol significantly increased 60 – 120 minutes after milk intake in prepubertal children.

Rich-Edwards et al. (2007) (47) performed two separate experiments in prepubertal children who were drinking whole and low fat milk in two geographically distant locations. In Ulaanbaatar in Mongolia school children aged 10 or 11 years were drinking 710 mL of whole milk daily for a month, while in Boston (USA), school children (6 – 8 years old) were drinking low fat milk for a week. After the milk consumption period, children from Ulaanbaatar had significantly higher plasma IGF-1, IGF-1/IGFBP-3 ratio and GH levels in comparison to children that did not drink the same amount of milk. In children from Boston higher levels, but not significantly different from controls, of IGF-1, IGF-1/IGFBP-3 ratio and GH were also observed. This suggest the effects of milk on growth hormone axis, yet it is difficult to directly compare both groups of children as they were from different cultural and social backgrounds including differences in diets, they were living in very different geographic locations, gender of subjects was different (only girls in Boston, mixed group in Mongolia) as well as age of subjects (6-8 years in Boston, 10-11 years in Mongolia).

There are no conclusive results whether estrogens or other sex steroid hormones are present in the milk in concentrations high enough to exert physiological effects on the reproductive system. However, studies suggesting that estrogens are not present in sufficient amounts to cause physiological effects on the reproductive tract in adult life in experimental animals seem
to be more numerous and more convincing. Nevertheless, with the exception of a study by
Ganmaa (2004) (23), all other studies examined possible effects of milk after relatively short
exposure and therefore looked only at acute effects in adult life. Considering some
aforementioned studies in humans that do suggest the effect of milk consumption on HPG axis,
additional studies with longer exposure to cows’ milk with known amount of estrogens (or
progesterone), and especially studies with exposure to the cows’ milk during prepubertal and
pubertal periods should be performed, as younger animals are likely more sensitive to the
effects of estrogens.

4.2 Milk consumption and endocrine related cancers

Several cancers are closely connected with the endocrine system. Some cancers could affect
endocrine glands such as adrenal, testicular or ovarian cancers, while in some others, cancerous
cells are responsive to hormones, in particular to sex steroid hormones, such as some forms of
breast cancers that are stimulated by estrogens, or prostate cancer that is stimulated by
testosterone. This has raised a concern about possible negative effects of milk consumption on
the development or progression of such cancers.

4.2.1 Mechanistic connections between milk and dairy products and cancers

The evidence that exposure to exogenous estrogens from various sources could trigger cancer
development in experimental animals is convincing (48). The carcinogenic effect of estrogens
and some estrogens’ metabolites is associated with an activation of estrogen receptors alpha
and beta that stimulate transcription of genes promoting cell proliferation (49). This
physiological effect of estrogens during estrous cycle, pregnancy and lactation could also result
in cancerous transformation of cells. Increased DNA replication could lead to increase in
mutations occurring during DNA synthesis and estrogens could directly stimulate proliferation
of different cells. Also, estrogens could stimulate the synthesis of various growth factors, which could stimulate proliferation of different cells through endocrine or paracrine mechanisms (50).

All these effects of estrogens could lead to cancer development, and the risk for cancer development increase with doses and length of exposure (49, 51) in both animals and humans.

Several female cancers such as breast cancer, ovarian cancer and uterine cancer are well known to be responsive to estrogens and in such cancers, one can easily imagine the connection between increased milk consumption and progression of the disease, although as mentioned before, it is questionable whether the levels of estrogens in milk, and especially ingested levels, are high enough to cause such effects. Some studies also suggest that exposure to estrogens during fetal and early postnatal life could increase the risk for testicular cancer in adult life (52, 53) and high milk consumption during these periods could increase the total amount of estrogens in the body.

Prostate cancer, on the other hand, is clearly dependent on testosterone, so direct connection to the consumption of milk or dairy products is less clear. Nevertheless, prostate cells do express estrogen receptors in addition to androgen receptors (54), and estrogens can bind, albeit with lower affinity, to androgen receptors. Therefore, estrogens from milk could potentially also directly stimulate the growth of prostate neoplastic cells. Furthermore, similar to testicular cancers, rodent studies have suggested that prenatal or postnatal exposure to estrogens could promote development of prostate cancer later in life (55) and this would be in agreement with human epidemiological studies suggest a connection between adolescent consumption of milk and later increased risk for both prostate and testicular cancer (55, 56, 57).

Different possible mechanism contributing to prostate cancer development and/or progression is calcium metabolism. Namely, milk and dairy products are an abundant source of calcium and high consumption of dairy products contribute to higher absorption of calcium from intestinal tract. However, high availability of calcium in the body reduces the levels of vitamin D2, which
has been shown to have negative effects on cell proliferation and thus progressive development of neoplastic cells (58). However, milk often also contains large amounts of vitamin D, especially full fat milk, and calcium itself has been shown in some studies to have a protective role in many cancers. Therefore, this hypothesis about increased risk for cancer development due to excessive calcium from milk seems less plausible.

Melnik et al. (2012) (59) suggested yet another mechanism possibly connecting milk consumption and prostate cancer. They suggest that certain peptides and amino acids, derived from whey proteins, could influence mTORC-signaling pathways and permanently alter cell metabolism in prostate gland. mTORC signaling pathways are involved in the regulation of cell growth and proliferation, and suppresses autophagy, and activation of this pathways by aminoacids from cow’s milk could have oncogenic properties. Yet, this hypothesis has not been truly tested and remains speculative.

In addition to sex steroid hormones, milk could contain several carcinogenic environmental pollutants such as dioxins, furans, pesticide residua, heavy metals and feed contaminants (phytoestrogens, mycotoxins (aflatoxin, zearalenol)), which can also contribute to cancer development, however, the role of these pollutants is beyond the scope of this article. Nevertheless, there is a potential synergistic effect of milk estrogens and environmental pollutants, yet this dynamic has not been addressed experimentally.

4.2.2 Milk consumption and breast cancer

Ganmaa and Sato (2005) (60) correlated incidence rates of breast, ovarian and uterine cancers with food intake in 40 countries. High levels of milk intake were reported to be associated with the incidence of ovarian, corpus uteri and breast cancers. However, Moorman and Terry (2004) (61), in contrary, reported that epidemiology data on consumption of dairy products and breast cancer does not suggest a correlation between these two. Similarly, analysis of 8 cohort studies
by Missmer et al. (2002) (62) showed no correlation between milk consumption and breast cancer risk. Meta-analysis by Dong et al. (2011) (63) even suggests that intake of low fat milk reduces the risk for breast cancer development. Yet, in the same study, high fat milk was not observed as being protective and thus the role of milk estrogens (present in higher amount in full fat milk) in breast cancer development was not excluded. Similarly, meta-analysis of cohort studies by Zang et al. (2015) (64) reported that consumption of low-fat dairy products reduces the risk for breast cancer while high-fat dairy products do not, although the consumption of high fat dairy products also doesn’t increase the risk for breast cancer according to this study. Thus, it is possible that milk does offer a protection against the development of breast cancer, but estrogens in high fat milk might counteract these protecting mechanisms.

Experiment in rats performed by Qin et al. (2004) (65) showed increased incidence of mammary tumors induced by 7,12-dimethylbenz(A)anthracene-induced (DMBA) in Sprague Dawley rats which were fed low fat milk in comparison to rats which were fed artificial milk or did not receive milk. Similarly, consumption of estrone sulphate increased mammary tumor incidence in the same study, suggested that estrone sulphate from milk (which is water soluble and therefore present in low fat milk also) might be causing increased incidence of mammary tumors. However, Nielsen et al. (2011) (40) reported increase in time between DMBA administration and mammary tumor occurrence in Sprague Dawley rats, which were fed with milk before puberty (between 14 to 35 days of age) in comparison to controls. Additionally, tumor incidence and total number of tumor buds were lower in milk treated group, even though the serum E2 concentration was higher in milk-fed rats. These two studies cannot be compared directly since milk treatment in the study by Nielsen et al. (2011) (40) was performed before the onset of puberty and rats were fed milk only for 11 days while in the study by Qin et al. (2004) (65) rats were treated with milk from 42 days of
age for 20 weeks. Designs of these two studies are different and thus show different (controversial) influences of milk estrogens on mammary carcinogenesis. Qin et al. (2004) (65) demonstrated that long-term exposure to milk estrogens, including metabolite estrone sulphate, are involved in mammary cancer development, while Nielsen et al. (2011) (40) showed that the protective effect of milk feeding might be due to increased prepubertal estrogentic exposure, which reduces risk for mammary cancer development.

4.2.3 Milk consumption and prostate cancer

Prostate cancer is a cancer that is closely related to the exposure to sex steroid hormones, in particular, testosterone. Ganmaa et al. (2001) (6) reported that in Japan milk consumption increased 20-times between 1950 and 1997 and this does correlate with the increased incidence of prostate cancer. However, this connection is purely speculative as the authors did not provide any evidence about the direct link between milk consumption and the risk for developing prostate cancer, and increased incidence of prostate cancer could have many other origins. Nevertheless, some epidemiological studies do provide stronger evidence for the connection between milk consumption and prostate cancer risk. A study by Torfadottir (2012) (13) reported increased risk for prostate cancer in Icelandic men who consumed more dairy products in adolescence, but, interestingly, no such correlation was found between consumption of dairy products in adult life and prostate cancer risk, highlighting the potential importance of adolescent exposure to sex steroids from milk. Milk consumption has also been associated with prostate cancer recurrence especially in obese men (66) and progression of cancer growth and increased mortality in patients with localized prostate cancer (14, 67). In a recent meta-analysis, Aune et al. (2015) (68) also found an increased risk for prostate cancer in men with higher intake of dairy products. However, in contrast to these studies, Andersson et al. (1995) (69) did not find a connection between diet in childhood and prostate cancer incidence in Danish
patients, therefore, there is no conclusive evidence whether milk and in particularly sex steroid hormones from milk, have any role in the development of prostate cancer in humans.

Qin et al. (2006) (70) studied whether estrogens in milk present a possible risk for increased incidence of prostate cancer in rats. In their experimental study, rats were fed with 25 g of milk per day and they found increased incidence of some neoplastic lesions in rats fed with milk and treated with amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP). It should be noted that consumption of milk in this study was very high, about two thirds of daily rat diet presented milk, and milk consumed presented about 10% of their body weight (25 g) per day, what is incomparable to the daily consumption of milk in humans, and therefore does not provide a direct evidence about potential link between milk consumption and prostate cancer in men.

4.2.4 Milk consumption and testicular cancer

Another male cancer, testicular cancer, has been associated with higher dairy products consumption during adolescence (71). However, there are discrepancies in different studies whether full fat milk or other dairy products present a higher risk. A case control study by Davies et al. (1996) (56) in East Anglia suggested that milk consumption during puberty positively correlates with the incidence of testicular cancer, while a study by Garner et al (2003) (57), performed in Canada, found a positive correlation between the incidence of testicular cancer and high consumption of cheese, but not milk. Similarly, a study by Ganmaa et al (2002) (14) reported that higher cheese consumption is correlated with the incidence of testicular cancer. However, there were important differences between these studies. While studies by Davies et al. (1996) (56) and Garner et al. (2003) (57) were both case control studies, they report consumption of milk or dairy products either in adolescence (56) or in adult life (57) and this could explain the differences in conclusions between studies. Furthermore, it is quite plausible that males who consumed more cheese in adult life and had increased incidence of prostate
cancer (Canadian cohort) did consume more milk during adolescence, and this might be connected with the higher incidence of prostate cancer, rather than the direct influence of cheese consumption. In contrast, the epidemiological study by Ganmaa et al. (2002) (14) was not based on individual patient dairy consumption, but rather on general incidence of different cancers in different countries, and general dietary habits in these countries. This study is therefore much less specific and thus less informative.

4.2.5 Milk consumption and non-hormone related cancers
Numerous studies have examined potential effects of dairy products consumption and development of different cancers, yet there is no conclusive evidence for either protective or promoting influences of dairy products on development of various cancers. This topic has been widely studied and discussed in numerous publications (72, 73, 74). Several large studies have investigated possible connections between consumption of dairy products and different cancers. A large cohort study by Park et al. (2009) (75) in which 36,965 and 16,605 cancer cases in men and women were analyzed, respectively, showed lower risk for cancers of digestive system in persons who regularly consume dairy products. The study focused on calcium intake by dairy products and demonstrated that the risk for cancers was decreasing with the increase intake of calcium through dairy products up to 1300 mg of calcium per day, but there was no further risk reduction with increasing intake of calcium. This study strongly suggests that calcium intake is associated with lower risk for all types of cancers in the digestive system, but especially for colorectal cancer. Ma et al. (2001) (76), in a case control study, reported moderate, not significantly reduced risk for colorectal cancer in consumers of low fat milk, however, in a subpopulation of patients with high blood levels of IGF-3, which is a known mitogen and inhibitor of apoptosis, consumption of low fat milk was significantly associated with the lower risk for colorectal cancer. A recent case control study by Chen et al.
(2017) (77) performed in non-smokers and non-alcohol drinkers also demonstrated lower risk for oral cancer in milk consumers, especially in males younger than 60 years.

In general, it seems that the protective effect of milk is mainly observed in consumers of low fat milk. A cohort study by Mettlin et al. (1990) (78) suggested that the risk for cancer occurrence depends on the type of consumed milk. In this study, there was an increased risk for cancers of the oral cavity, stomach, colon, rectum, lung, bladder, breast and cervix in people who regularly consumed whole milk in comparison to people who rarely consumed milk. However, there was also a reduced risk for cancers of oral cavity, stomach, rectum, lung and cervix in people who regularly consumed low fat milk in comparison to people who rarely consumed milk, suggesting that low fat milk could lower the risk for various cancers, while high fat milk could promote the incidence of various cancers. At the present it is not known what could be the reason for these differences between whole fat milk and skimmed milk, although one possible explanation could be a very simple one: people who consume low fat milk are usually more health conscious and have in general healthier lifestyle, and this could perhaps contribute to the lower incidence of certain cancers.

It is very likely that protective effects of milk are not associated with the absence (or presence) of estrogens or other sex steroid hormones in the milk. Most likely anti-carcinogenic effects of milk are associated with the effects of other milk ingredients. Namely, calcium reduces proliferation and enhances differentiation of mammary cells, and inhibit enteral absorption of some carcinogenic compounds (bile acids, saturated fatty acids) (79, 80). Calcium can also modulate some intracellular pathways that could impact differentiation of healthy cells and trigger apoptosis in transformed cells. Additionally, vitamin D, lactoferrin, casein and whey proteins reduce cellular proliferation and enhance cellular differentiation, poses apoptotic, anti-inflammatory and anti-oxidative properties and therefore express an anti-carcinogenic effect (17).
It seems that milk contains both carcinogenic and anti-carcinogenic compounds. The content of these compounds in the milk may vary widely and this could explain large differences between different studies that suggest protective, neutral or even harmful effects of dairy product consumption in regard to cancer risks, even without taking into account individual differences in both risk for cancer development, and possible individual differences in metabolism of milk and contents of milk. The role of milk consumption in cancer occurrence is not clear and since there is no general conclusion this question remains the subject of debate. Further observations of long-term exposure in animals and humans in combination with more accurate estimations of estrogen exposure from different sources and estimations of dose-response steps are necessary to better address these questions, although general answers would be difficult to obtain due to aforementioned individual differences in susceptibility for cancer, variations between milk consumed and differences in metabolism of milk and dairy products in individuals.

5. Conclusion

Estrogens could be present in milk in relatively large quantities due to intensive farming and milking of cows late into the pregnancy, when secretion of estrogens increases. With the exception of skimming, which removes fat-soluble sex steroid hormones, other manipulations of milk do not reduce the content of estrogens in milk. Commercial milk and dairy products, bought in the shops therefore do contain similar amounts of estrogens as native milk. Yet, it seems that these concentrations are too low to present a risk for reproductive health or development of different endocrine related cancers in adult humans (and animals). However, as suggested by numerous studies about health effects of estrogens/xenoestrogens, perinatal period might be an especially vulnerable period in both humans and animals, and health effects
of milk consumption during these critical periods have not been studied as extensively as adult effects, therefore, such studies should be performed in the future.

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The authors have no conflict of interest that could be perceived as prejudicing the impartiality of this manuscript.

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Table and figure legends:

**Figure 1:** Hypothetical potential mechanisms of disruptive effects of estrogens from milk and dairy products in the human body
Table 1: Concentrations of different estrogens in milk and dairy products as reported in different studies. Detection methods, what kind of milk (native, commercial...) or diary products was analyzed, and pregnancy status of cows is noted where it was mentioned in original article.