Estrone and Estrone Sulfate Concentrations in Milk and Milk Fractions

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ABSTRACT

Dairy products naturally contain estrogens, and some consumer groups contend these estrogens cause adverse health effects. The objectives of this research were to characterize estrone (E_1) and estrone sulfate (E_1S) concentrations in milk from a large number of individual cows, in skim and fat fractions of milk, and in retail milk to provide food and nutrition practitioners with information to estimate potential consumption. Milk was from Holstein cows. Data are presented as means and standard deviations. Analysis of variance was used to determine differences in E_1 and E_1S content of whole milk and its skim and fat fractions. Mean E_1 and E_1S concentrations (n=173 cows) were 7.0±12.7 and 46.7±62.1 pg/mL (25.89±46.96 and 172.74±229.71 pmol/L), respectively. Analysis of milk fractions (n=50 samples) demonstrated that 55% of E_1 and 14% of E_1S were associated with the fat fraction with the remainder associated with the skim fraction. Concentrations of E_1 and E_1S in pasteurized-homogenized whole milk (n=8) averaged 10.3±0.6 and 85.9±7.3 pg/mL (38.09±2.22 and 317.74±27.00 pmol/L), respectively. Production rates of E_1 plus estradiol in human beings range from 54,000 to 630,000 ng/day. US Food and Drug administration guidelines state that no physiologic effects occur when consumption is ≤1% of the endogenous quantities produced by the segment of the population with the lowest daily production. This threshold value for intake would be 540 ng/day. Estimated total E_1 intake from three servings of whole milk was 68 ng/day, which represents 0.01% to 0.1% of daily production rates in human beings. These findings support levels below the current guidelines for safe consumption.
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THERE IS CONCERN AMONG SOME CONSUMERS about the safety and potential adverse health effects of hormones in animal-based foods.[1] Dairy products, like other foods, contain bioactive substances, most of which are present in minute quantities. Recent reports have implicated estrogens in dairy products in development of breast, uterine, and ovarian cancers,[2-4] male reproductive disorders,[5,6] adolescent obesity,[7] and teenage acne.[8,9] These reports were based on epidemiologic studies, experiments with rodents, or were simply speculative (ie, no supporting data). Estrogens in particular often were cited as being causative for these adverse health effects.

During the 1970s, the first reports of estrone (E_1) and estradiol (E_2) concentrations in whole milk were published.[10-12] More recently, Pape-Zambito and colleagues[13] reported E_2 concentrations in whole milk from >200 cows and E_1 and E_2 concentrations in 13 cows during the course of pregnancy.[14] In that study, milk contained more E_1 than E_2 (14). Concentrations of E_1 increased from <1 pg/mL (3.70 pmol/L) during the first trimester of pregnancy to 27.1 pg/mL (100.24 pmol/L) by the end of pregnancy.[14] For E_2, Vicini and colleagues[15] reported mean concentrations ranging from 4.97 to 6.63 pg/mL (18.24 to 24.34 pmol/L) in 334 retail whole milk samples. Remesar and colleagues[16] stated that free E_1 concentrations in foods they analyzed were "extremely low" (values not reported), but that total E_1 content of whole milk was 1.7 μmol/kg (~460 ng/mL). Garcia-Pelaez and colleagues[17] reported free E_1 concentrations of 5.4 nmol/kg (1.5 ng/mL) for commercial whole milk, whereas Malenkinejad and colleagues[18] reported free E_1 concentrations of 20.0 pg/mL (73.98 pmol/L) and total estrone (free E_1+E_1S) concentrations of 202 pg/mL (747.20 pmol/L). Overall, most of these studies examined a small number of milk samples (ie, <20) for E_1 and its conjugates.

There are a number of studies using large numbers of animals and samples that report concentrations of E_2.[13,14,19] However, because of the wide discrepancies among reported E_1 values and the limited number of samples analyzed in most studies, there is a need for accurate quantification of E_1 and conjugated E_1 in milk even though E_1 is less biologically potent than E_2.[20,21] This is especially important considering the growing number of claims that have been made about the health effects of consuming estrone and its conjugates in milk. Some authors[16-18] report data on concentrations of E_1 in milk from a small number of individual cows or samples pooled from a few cows and use this information to make general recommendations to the consuming public. These recommendations should be based on a larger evidence base. For food and nutrition practitioners to respond to consumers' concerns, it would be helpful for them to know the normal

range of concentrations observed in milk from individual animals. It also would be helpful to know how these hormones are partitioned between the fat and skim fractions of milk to estimate the amount that would be provided in a typical serving of other dairy products for which estrogen concentrations are not available. It also is important to analyze milk that is packaged to be sold in a retail setting because most people do not consume milk from individual cows. In addition, it provides a comparison to the data on milk from individual cows. The objectives of our study were to quantify free E_1 and estrone sulfate (E_1S) concentrations in milk from a large number of cows, to quantify and compare E_1 and E_1S concentrations in whole milk and its skim and fat fractions, to determine concentrations of E_1 and E_1S in pasteurized-homogenized whole milk, and to provide food and nutrition practitioners with information that will enable them to estimate potential consumption of E_1 and E_1S content from dairy products. Three experiments were conducted to meet these objectives. The hypotheses tested were that the quantity of E_1 and E_1S present in the recommended daily intake of 3 glasses milk would be below the current US Food and Drug Administration (FDA) guidelines for safe consumption and that E_1 would primarily associate with the fat fraction of milk, whereas E_1S would primarily associate with the skim fraction.

METHODS

Sample Collection

In Experiment 1, milk samples from individual Holstein cows (n=173) were collected at a single morning milking at the Penn State University Dairy Farm (objective 1). It was important to sample as many cows as possible to increase the accuracy of the estimates. At the time of this experiment, there were 173 lactating cows producing milk for retail sale. These cows were free from clinical mastitis and were ≥5 days postcalving.

In Experiment 2, milk samples were collected from 50 Holstein cows at a single morning milking at the Penn State University Dairy Farm. These cows were free from clinical mastitis, ≥5 days post-calving, and their milk was later processed for retail sale. From prior testing, it was determined that at least 20 samples were needed to detect a difference in E_1 concentrations between whole and skim milk. Milk was subsampled; one portion remained as whole milk, and the remainder was separated into skim and fat fractions by centrifugation at 2,000*g* for 30 minutes at 4°C. The fat supernatant was removed with a spatula and the skim fraction was poured into a 50-mL centrifuge tube.

For Experiment 3, half-pint containers of pasteurized-homogenized whole milk (n=8) were obtained from the Penn State Berkey Creamery for eight different processing days. Milk from each processing day was not mixed with milk collected on other days, and this milk was destined for retail sale. These samples contained milk from approximately 200 cows during a 2-day period. The fat content of milk was not adjusted at any step during processing. The number of samples analyzed was based on variance estimates from our study (objectives 1 and 2) and published reports[14,22] for estrogens in milk. The sampling was designed to yield accurate estimates of E_1 and E_1S concentrations in samples of whole milk.

Cows in the above experiments received bovine somatotropin (Posilac, Monsanto Co) every 2 weeks beginning at ≥60 days of lactation. Fluid samples from all experiments were analyzed for fat, protein, and lactose content on a 4400 Combi Foss Analyzer (Foss Analytical A/S). After the initial processing described above, all samples were frozen and stored at −20°C until analyzed for E_1 and E_1S.

Sampling procedures for all experiments were approved by the Pennsylvania State University Institutional Animal Care and Use Committee.

Sample Extraction and Radioimmunoassay

The procedures for extracting estrogens from milk and milk fat were similar to those published by Pape-Zambito and colleagues.[14] Fat fractions were heated in a water bath (40°C) for 30 minutes until they were liquefied. E_1S was subjected to cleavage by sulfatase enzyme (from *Helix pomatia*, Sigma, catalog No. S9751) based on the procedure described by Kensinger and colleagues.[23] This enzyme preparation contained glucuronidase as well as sulfatase activity, so it also cleaved any E_1 glucuronide present in the samples. Hereafter, this will be referred to as E_1S, recognizing that it is the sum of E_1S plus E_1 glucuronide. Fractions from both the free E_1 and E_1S extraction procedures were dried under nitrogen and subjected to further extraction, LH-20 column chromatography, and radioimmunoassay as previously described.[14] All hormone data were corrected for percent recovery determined from internal tritiated E_1 or tritiated E_1 sulfate standards added to milk and milk fractions in each set of extractions. Radioimmunoassay intra- and interassay coefficients of variation were 4.8% and 14%, respectively. For Experiment 1, limit of detection and limit of quantification (LOQ) were 0.11 and 1.43 pg/mL (0.41 and 5.29 pmol/L), respectively. For Experiments 2 and 3, limit of detection and LOQ for fluid milk products were 0.04 and 0.45 pg/mL (0.15 and 1.66 pmol/L), respectively, and for fat were 0.13 and 1.66 pg/g, respectively.

Statistical Analyses

The Statistical Analysis System (version 9.1, 2003, SAS Institute Inc) was used for statistical analyses. Concentration values below the LOQ were assigned the LOQ value. Means and standard deviations were calculated using PROC MEANS, and correlations were computed by PROC CORR. In Experiment 2, data for E_1 and E_1S were not normally distributed and were transformed using $1/\ln[E_1]$ and $1/\ln[E_1S]$ before analysis of variance. PROC MIXED was used on transformed data to determine whether concentrations of free E_1 and E_1S were different among whole milk and its skim and fat fractions. Cow and sample type (ie, whole, skim, or fat) were class variables, and cow was designated as a random variable. The concentration values presented are means of the original data as the back-transformed means were one-third to one-half the value of the raw means. Therefore, the means presented here are high estimates of the E_1 and E_1S concentrations in the samples analyzed.

RESULTS AND DISCUSSION

Free E_1 concentrations in milk from 173 cows (Experiment 1) averaged 7.1±12.7 pg/mL (26.26±46.98 pmol/L) and ranged from 1.6 to 84.6 pg/mL (5.92 to 312.94 pmol/L). E_1S concentrations were 46.7±62.1 pg/mL (172.74±229.71 pmol/L) and ranged from 17.4 to 321.0 pg/mL (64.36 to 1,187.4 pmol/L). E_1