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Presence of ochratoxin A in human milk in relation to dietary intake.

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Abstract

Individual and geographical variations in ochratoxin A (OA) levels in human blood and milk samples may be due to differences in dietary habits. The purpose of this study was to examine the relationship between OA contamination of human milk and dietary intake. Human milk samples were collected from 80 Norwegian women. The usual food intake during the last year was recorded using a quantitative food frequency questionnaire. The concentration of OA in the human milk was determined by HPLC (detection limit 10 ng/l). Seventeen (21%) out of 80 human milk samples contained OA in the range 10 - 182 ng/l. The women with a high dietary intake of liver paste (liverwurst, liver pâté) and cakes (cookies, fruitcakes, chocolate cakes etc.) were more likely to have OA-contaminated milk. The risk of OA contamination was also increased by the intake of juice (all kinds). In addition, the results indicate that breakfast cereals, processed meat products, and cheese could be important contributors to dietary OA intake. OA contamination of the milk was unrelated to smoking, age, parity, and anthropometric data other than body weight.

Key words: ochratoxin, human milk, diet, mycotoxin
Introduction

Ochratoxin A (OA) is a mycotoxin produced by a number of *Aspergillus* and *Penicillium* species growing on cereals and other plant substrates. The toxin is a naturally occurring contaminant in food and animal feed particularly in temperate climates (IARC 1993, Speijers and Van Egmond 1993, Höhler 1998), and is frequently found in human blood (Hald 1991, IARC 1993, Scott et al. 1998, Ueno 1998, Palli et al. 1999) and human milk in many countries (Gareis et al. 1988, Breitholtz-Emanuelsson et al. 1993, Jonsyn et al. 1995, Micco et al. 1995, Skaug et al. 1998).

Animal studies have shown that OA has nephrotoxic, carcinogenic, immunosuppressive, and teratogenic effects (Kuiper-Goodman and Scott 1989, IARC 1993, Höhler 1998, SCF 1998). The wide spectrum of harmful effects of OA gives reason for concern about neonatal exposure to this contaminant. Neonates may be considerably more susceptible than adults. LD\(_{50}\) values for OA (oral route) is 20 –30.3 mg/kg body weight in adult rats, as compared to 3.9 mg/kg body weight in neonate rats (Kuiper-Goodman and Scott 1989). In humans, the OA concentration in foetal serum is reported to be twice the maternal one, indicating an active transfer of the toxin across placenta (Zimmerli and Dick 1995).

The high prevalence of OA contaminated human blood samples in Europe and Canada indicates a continuous and widespread exposure. Analyses of blood and milk samples show significant differences in OA levels between individuals and regions. Some of the heterogeneity of the OA levels found in biological fluids may be explained by differences in
the detection limit of the employed methods. However, variations in OA levels between individuals can also be due to differences in dietary habits. The present investigation was initiated to examine the relationship between OA contamination of human milk and dietary intake.

Materials and methods

Sampling

Human milk samples from 80 women were collected during July 1995 to September 1996. The milk donors were healthy women, between 19 and 35 years of age, living in the Oslo area, Norway. The samples were collected 4 weeks after they gave birth. Because milk composition fluctuates during the day and during a single feed, all the milk samples were taken from one morning feed (not the first one), 3-5 minutes after the baby started suckling. The milk was expressed manually into sterile plastic cups. The samples were kept in a home refrigerator until the next day when they were brought to the laboratory and frozen at -70 °C until extraction and HPLC analysis.

The study was approved by the Regional Ethic Committee.

Diet registration

All the milk donors answered a self-administered quantitative food frequency questionnaire (Nes et al 1992, Andersen et al 1996, Andersen et al 1999). The questionnaire wasphrased to obtain information about the usual food intake during the last year. The questionnaire asked about the frequency of use and portion size of about 180 food items, of which 34 food
commodities commonly used in the Norwegian diet were included in the present study. The portion sizes of the different food items were converted to weights mainly based on standard portions. For all the milk donors, anthropometric data (body weight, height, body mass index), and information on age, parity, and smoking habits, were obtained from their pregnancy journals.

**Determination of OA**

Extraction of OA from the milk and HPLC determination were carried out according to the method of Breitholtz-Emanuelsson et al. 1993, with slight modifications. Details of the procedure have been described previously (Skaug et al. 1998). A volume of 2 ml milk was extracted with acidic methanol and chloroform, and purified on silica gel cartridge. The sample extracts were analysed by HPLC ion-pair technique, at alkaline pH, with fluorescence detection (380 nm excitation wavelength, 450 nm emission wavelength). Separations were carried out on a Spherisorb S3ODS2 (C-18) column, 4.6 x 150 mm, with 3 µm particles. Flow rate was 0.8 ml/min.

**Analytical quality control**

A solution of OA (10 µg/ml in methanol) was calibrated spectrophotometrically at 333 nm using the value of 6640 for the extinction coefficient (Bauer and Gareis, 1987). The OA solution was diluted to 10⁻⁸ M in methanol and stored at – 20 °C. Working standard solutions for calibration were prepared every day by dilution of the 10⁻⁸ M OA stock solution with HPLC mobile phase. For quantitation, peak heights were measured by a Merck Hitachi integrator. The calibration curves used for quantitation were calculated by the least-squares method. The detection limit (signal-to-noise ratio of 3) for standard solutions was 1.5 pg OA.
The quantitation limit (signal-to-noise ratio of 10) for spiked milk samples was calculated to be 10 ng/l. Negative milk samples spiked with OA (range 10-500 ng/l) were used for recovery experiments. Mean recovery for the entire procedure was 75 % (range 64-83 %) with a coefficient of variation of 13 % (n=14). Results were not corrected for recovery.

Qualitative confirmation of positive samples were performed in two ways: 1) As a routine all sample extracts shown to contain OA during initial HPLC analysis were analysed in duplicate, with a direct spiking of the second aliquot. This was performed by adding an amount of OA stock solution to the aliquot. The chromatograms of the unspiked and the spiked sample extract were then compared. 2) Derivatization of OA through methylation of the extracts with subsequent HPLC analysis was also used for confirmation of positive samples (Zimmerli and Dick 1995): A 200 µl aliquot of the purified sample extract was evaporated to dryness, and the residue was dissolved in 2.5 ml methanol and 0.1 ml conc. HCl. The mixture was kept overnight at room temperature. After evaporating the mixture to dryness, the residue was dissolved in 200 µl mobile phase. A volume of 50 µl was injected into the chromatograph and analysed (disappearance of the OA peak, and, in samples containing more than 40 ng/l OA, appearance of a new peak with the same retention time as that of the methyl ester).

Statistics

The association between OA contaminated samples and intake was tested using the chi-squared test, and logistic regression. Fisher’s exact test and a Mann-Whitney test were used for testing relationship between OA contamination and smoking, and season. The most highly contaminated group was compared with milk donors with lower or non-detectable amounts of
OA using the t-test. Data were analysed by SPSS software program. A p-value < 0.05 was considered statistically significant.

**Results**

Seventeen out of 80 milk samples (21%) were found to contain OA in the range 10 - 182 ng/l. The median OA concentration of positive samples was 16 ng/l. The mean OA concentration of positive samples was 30 ng/l.

The mean daily intakes (g/day) of selected foods are given in table 1. The prevalence of OA contaminated (≥ 10 ng/l) milk samples was compared between the milk donors with low (defined as 1. tertile, i.e. below the 33th percentile), medium (2. tertile), and high (3. tertile, i.e. above the 66th percentile) dietary intake of each food item (table 1). The frequency of OA contaminated milk samples was significantly different between the three intake groups for cakes (cookies, fruitcakes, chocolate cakes etc.) (p = 0.010), and liver paste (liverwurst, liver pâté) (p = 0.035). Figure 1 shows the frequency of OA contaminated milk samples among women with low, medium and high intake of cakes, and liver paste. The risk of OA contaminated milk was five times higher among individuals with medium intake of liver paste and 11 times higher in the high intake group as compared with individuals in the low intake group (estimated by logistic regression).

Concerning juices, the OA contamination frequency was not significantly different between the three intake categories. However, testing by logistic regression showed that intake of juice was a significant risk factor of OA-contaminated milk (odds ratio 1.7 (glass/day), p=0.046).
Forty-eight women (60%) reported no intake of wine. The frequency of OA contaminated milk was 14.6% in this group, as compared with 31.3% among the wine drinking milk donors (not significant).

No association was found between OA contamination of the milk and the intake of other foodstuffs, such as cereals, pork meat, and coffee.

Fifteen women (19%) reported daily smoking. No association was found between OA contamination of the milk and smoking habits, age, parity, or anthropometric data other than body weight.

There was a trend (not significant) towards increased frequency of OA contaminated milk, and higher OA levels in milk samples collected during the winter season (October – April) as compared with the summer season (May – September).

To examine if high levels of OA in the milk were related to specific characteristics, the nine milk donors with the highest OA concentrations in the milk (above median of positive samples) were compared with those 71 donors with lower or not detectable amounts of OA in the milk. The highest contaminated individuals were characterized by having a significantly higher intake of cakes (p=0.046), processed meat products (p=0.019), breakfast cereals (cornflakes, oat, muesli, etc.) (p=0.043), and cheese (p=0.039); and higher body weight (p=0.031) as compared with milk donors with lower or not detectable amounts of OA in the milk (table 2).
Discussion

The prevalence and OA concentration range found in the present study correspond to a previous investigation of 115 human milk samples collected from three different areas in Norway, where 15 – 58% of the samples contained 10 – 130 ng/l OA (Skaug et al. 1998). Both studies show significant differences in OA contamination between individuals. We wanted to examine whether OA contamination of the milk was related to high intake of selected common food commodities in the Norwegian diet. If OA in human milk is a biomarker of the dietary exposure of the mother, the occurrence of OA in the milk should be related to the intake of contaminated food commodities. OA has been detected in a number of food categories, e.g. cereals, bread, pork and poultry meat, coffee, beer (IARC 1993, Speijers and van Egmond 1993, van der Stegen et al 1997, Jørgensen 1998), wine, red grape-juice (Zimmerli and Dick 1996), and cow’s milk (Breitholtz-Emanuelsson et al. 1993, Skaug 1999). Consequently, several food commodities can contribute to the total dietary intake of OA.

In the present study, OA was detected in 17 out of 80 human milk samples. As the distribution of OA concentration in the milk samples was skewed, the relationship between dietary intake and OA contamination was tested by logistic regression, and by comparing differences in prevalence of OA contamination between milk donors with low, medium and high dietary intake, using Chi-square test. As shown in table 1 and figure 1, the frequency of OA contaminated milk samples was significantly higher among individuals with high intakes of cakes, and liver paste. In addition, it was found that intake of juice (all kinds) increased the risk of OA contamination. The intake of processed meat products was also found to increase
the frequency of OA contaminated milk, from 11.1 % in the low intake group, 23.1 % in the medium group, to 29.6 % in the high intake group, although the differences were not statistically significant.

Pork products (meat, kidney, blood, and liver) are frequently contaminated with OA (Kuiper-Goodman and Scott 1989, Speijers and van Egmond 1993). Tissue distribution of OA in pigs appears to follow the order blood > kidney > liver > muscle > fat (Kuiper-Goodman and Scott 1989, Höhler 1998). Consequently, foods containing pork liver and blood or plasma, e.g. liver paste and processed meat products, could thus be important dietary sources of OA. This is consistent with the findings in the present study.

Fruit juice and liver products are foods commonly consumed by infants and small children. Food consumption surveys conducted in United Kingdom show that young children have an average level of consumption of soft drinks, mainly fruit juice concentrates, which is 16 times the equivalent adult figure (Lawrie 1998). Data on occurrence of OA in juice and liver products in the Norwegian market are scarce. Our findings suggest that occurrence of OA in juice and liver products should be evaluated, in particular foods for children.

The reason why individuals with high intake of cakes (cookies, fruitcakes, chocolate cakes, etc.) had a significantly increased risk of OA contamination is not clear. It could be due to use of contaminated ingredients, or mould growth during storage of the cakes.

OA has been detected in red wine, and to a lesser extent in white wine, and intake of wine can thus be a source of OA (Zimmerli and Dick 1996, Höhler 1998). The intake of wine was
skewed in our study group; 48 out of 80 (60%) reported no use of wine at all. However, the frequency of OA contamination of the milk was clearly higher among wine drinking milk donors than non-drinkers, supporting the assumption that wine can contribute to OA exposure. No association was found between presence of OA in milk, and intake of beer, tea, or coffee in this study.

Cereals and cereal products are generally regarded as the most important dietary sources of OA (IARC 1993, Jørgensen et al 1996, Jørgensen 1998). We found that the most highly contaminated milk samples were collected from individuals characterized by having a significantly higher intake of breakfast cereals, cakes, processed meat products, and cheese as compared with milk donors with lower or uncontaminated milk samples (table 2). The occurrence of OA in cheese has not been well established. However, OA has been detected in cow’s milk samples from Scandinavia (Breitholtz-Emanuelsson et al. 1993, Skaug 1999). In addition, fungi capable of producing OA can be isolated from raw cow's milk (Škrinjar 1995) and from the surface of cheese (Jesenská 1993). High intake of cheese might therefore be a source of OA exposure.

There was a trend towards increased prevalence and contamination level of OA in milk samples collected during winter season (Oct. - April) as compared with the summer season (May – Sep.). Dietary and drinking habits vary between seasons, and between countries according to tradition and cultural differences. In addition, fluctuations in mould growth and contamination level of cereals (Jørgensen 1998) may result in seasonal variations in dietary exposure to OA. A strong seasonal association was found in an Italian study, with higher OA values in human blood samples collected during summer as compared with autumn. The
authors reported a positive correlation between OA levels in blood and height, but not to weight, body mass index, age, or smoking (Palli et al 1999). In our study group, OA contamination of the milk was unrelated to smoking habits, age, parity, body mass index, and height. However, the donors with the most highly contaminated milk differed from those with less or not contaminated milk by having a significantly higher body weight (table 2).

Only a few studies have previously been carried out on the relationship between OA exposure and dietary habits. No correlation could be established between OA contamination of human milk and diet in a study in Italy of 111 lactating women (Micco et al 1995).

If the toxin occurs at low levels in a number of different food commodities, a clear correlation between diet and OA exposure is not to be expected. The concentration of OA in milk is assumed to reflect the concentration of OA in blood. However, the mechanism of OA transport into milk and the distribution ratio between blood and human milk is not known. Variation in OA levels in milk may be due to individual differences in bioavailability, distribution, and excretion of OA. Furthermore, intake of contaminated food may not be the only route of exposure to OA. Fungal spores can contain mycotoxins and inhalation exposure may thus contribute to the total intake, e.g. in people exposed to mould-contaminated indoor environments (Di Paolo et al. 1993, Hendry and Cole 1993).

Lactating women are not representative for the general population regarding eating and drinking habits, age, or smoking (Johansson et al 1997). Moreover, since the route of the excretion of OA in milk is not yet elucidated the correlation found in this study is not general. Nevertheless, examination of the relationship between dietary intake and OA contamination
of human milk is important in order to trace the sources of OA in milk. The OA levels found in the present study confirm previous data (Skaug 1998) that breastfed infants can be exposed to OA doses exceeding a proposed tolerable daily intake (TDI) of 5 ng/kg body weight (Nordic Working Group 1991, SCF 1998). Exposure to OA during the foetal and neonatal period characterized by rapid growth and development may be potentially harmful and should therefore be kept at the lowest achievable level.

Conclusion

The results of this study show that women with a high dietary intake of liver paste and cakes were more likely to have OA contaminated milk. Furthermore, the risk of OA contamination was increased by the intake of juice. In addition, it seems that breakfast cereals, processed meat products, and cheese could be important contributors to dietary OA intake.

Acknowledgements

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References


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Table 1.

Mean dietary intake and frequency of OA contaminated (≥ 10 ng/l) human milk samples among 80 women, divided into three groups according to intake.

<table>
<thead>
<tr>
<th>Foods</th>
<th>Mean intake g/day (SD)</th>
<th>Frequency of OA contaminated milk samples (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>low intake group (1. tertile)</td>
</tr>
<tr>
<td>Cereals</td>
<td>48 (26)</td>
<td>7/26 (26.9)</td>
</tr>
<tr>
<td>Breakfast cereals</td>
<td>12 (16)</td>
<td>3/26 (11.5)</td>
</tr>
<tr>
<td>Bread</td>
<td>149 (50)</td>
<td>6/26 (23.1)</td>
</tr>
<tr>
<td>Rice</td>
<td>10 (23)</td>
<td>7/26 (26.9)</td>
</tr>
<tr>
<td>Pasta</td>
<td>12 (11)</td>
<td>8/29 (27.6)</td>
</tr>
<tr>
<td>Cakes</td>
<td>30 (22)</td>
<td>3/27 (11.1)</td>
</tr>
<tr>
<td>Coffee</td>
<td>102 (127)</td>
<td>6/27 (22.2)</td>
</tr>
<tr>
<td>Pork meat</td>
<td>8 (8)</td>
<td>5/27 (18.5)</td>
</tr>
<tr>
<td>Meat</td>
<td>51 (23)</td>
<td>6/27 (22.2)</td>
</tr>
<tr>
<td>Processed meat products</td>
<td>37 (18)</td>
<td>3/27 (11.1)</td>
</tr>
<tr>
<td>Black pudding, liver (a)</td>
<td>1 (5)</td>
<td>14/64 (21.9)</td>
</tr>
<tr>
<td>Liver paste</td>
<td>6 (5)</td>
<td>1/22 (4.5)</td>
</tr>
<tr>
<td>Chicken</td>
<td>8 (8)</td>
<td>7/28 (25.0)</td>
</tr>
<tr>
<td>Eggs</td>
<td>14 (9)</td>
<td>4/26 (15.4)</td>
</tr>
<tr>
<td>Milk, cream, -products</td>
<td>551 (286)</td>
<td>7/26 (26.9)</td>
</tr>
<tr>
<td>Cheese</td>
<td>24 (15)</td>
<td>7/26 (26.9)</td>
</tr>
<tr>
<td>Juice</td>
<td>109 (136)</td>
<td>5/27 (18.5)</td>
</tr>
<tr>
<td>Soft drinks, sweetened with sugar</td>
<td>84 (123)</td>
<td>5/26 (19.2)</td>
</tr>
<tr>
<td>Fruit and berries, fresh</td>
<td>127 (98)</td>
<td>3/26 (11.5)</td>
</tr>
<tr>
<td>Fruit and berries, preserved (b)</td>
<td>23 (17)</td>
<td>2/14 (14.3)</td>
</tr>
<tr>
<td>Nuts (c)</td>
<td>1 (3)</td>
<td>11/50 (22.2)</td>
</tr>
<tr>
<td>Chocolate</td>
<td>12 (13)</td>
<td>8/30 (26.7)</td>
</tr>
<tr>
<td>Herbal tea (d)</td>
<td>48 (98)</td>
<td>8/41 (19.5)</td>
</tr>
<tr>
<td>Tea, black</td>
<td>191 (253)</td>
<td>9/43 (20.9)</td>
</tr>
<tr>
<td>Beer (e)</td>
<td>18 (37)</td>
<td>10/45 (22.2)</td>
</tr>
<tr>
<td>Wine (f)</td>
<td>4 (8)</td>
<td>7/48 (14.6)</td>
</tr>
<tr>
<td>Total daily intake</td>
<td>2701 (662)</td>
<td>4/27 (14.8)</td>
</tr>
</tbody>
</table>

a) 64 reported no intake, b) n=38 women, c) 50 reported no intake, d) 41 reported no intake,
e) 45 reported no intake, f) 48 reported no intake
* \( p = 0.010 \) (chi-square)
** \( p = 0.035 \) (chi-square).

Table 2.

Characteristics of the highest contaminated milk donors as compared to the milk donors with lower or not detectable amounts of OA in the milk.

<table>
<thead>
<tr>
<th></th>
<th>High contaminated group ([OA]_{milk} \geq 16 \text{ ng/l} \ n = 9)</th>
<th>Low/not contaminated group ( n = 71)</th>
<th>T-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily intake of breakfast cereals</td>
<td>21.6 g</td>
<td>10.5 g</td>
<td>( p = 0.043 )</td>
</tr>
<tr>
<td>Daily intake of cakes</td>
<td>52.3 g</td>
<td>27.1 g</td>
<td>( p = 0.046 )</td>
</tr>
<tr>
<td>Daily intake of processed meat products</td>
<td>50.8 g</td>
<td>35.7 g</td>
<td>( p = 0.019 )</td>
</tr>
<tr>
<td>Daily intake of cheese</td>
<td>34.0 g</td>
<td>22.9 g</td>
<td>( p = 0.039 )</td>
</tr>
<tr>
<td>Body weight</td>
<td>71.1 kg</td>
<td>63.2 kg</td>
<td>( p = 0.031 )</td>
</tr>
</tbody>
</table>
Figure 1.

Frequency (%) of OA contaminated (≥ 10 ng/l) human milk samples among women in the low, medium, and high intake group for: a) liver paste (liverwurst, liver pâté), and b) cakes (cookies, fruitcakes, chocolate cakes etc.).

a) Intake of liver paste (p = 0.035, chi-square test).

b) Intake of cakes (p = 0.010, chi-square test).