

Original Article

Exposure to exogenous estrogen through intake of commercial milk produced from pregnant cows

Kazumi Maruyama, Tomoe Oshima and Kenji Ohyama

Interdisciplinary Graduate School of Medicine and Engineering, Department of Clinical Nursing and Pediatrics, University of Yamanashi, Yamanashi, Japan

Abstract *Background:* Modern genetically improved dairy cows continue to lactate throughout almost the entire pregnancy. Therefore, recent commercial cow's milk contains large amounts of estrogens and progesterone. With regard to the exposure of prepubertal children to exogenous estrogens, the authors are particularly concerned about commercial milk produced from pregnant cows. The purpose of the present study was therefore to examine concentrations of serum and urine sex hormones after the intake of cow milk.

Methods: Subjects were seven men, six prepubertal children, and five women. The men and children drank 600 mL/m² of cow milk. Urine samples were collected 1 h before the milk intake and four times every hour after intake. In men the serum samples were obtained before and 15, 30, 45, 60, 90 and 120 min after milk intake. Women drank 500 mL of cow's milk every night for 21 days beginning on the first day of the second menstruation. In three successive menstrual cycles, the day of ovulation was examined using an ovulation checker.

Results: After the intake of cow milk, serum estrone (E1) and progesterone concentrations significantly increased, and serum luteinizing hormone, follicle-stimulating hormone and testosterone significantly decreased in men. Urine concentrations of E1, estradiol, estriol and pregnanediol significantly increased in all adults and children. In four out of five women, ovulation occurred during the milk intake, and the timing of ovulation was similar among the three menstrual cycles.

Conclusions: The present data on men and children indicate that estrogens in milk were absorbed, and gonadotropin secretion was suppressed, followed by a decrease in testosterone secretion. Sexual maturation of prepubertal children could be affected by the ordinary intake of cow milk.

Key words cow milk, estrogen, prepubertal child, sexual maturation, sexual precocity.

During the 1960s and 1970s, with the worldwide spread of the Green Revolution,¹ the possibility of year-round global milk production was realized. Modern genetically improved dairy cows, such as the Holstein, continue to lactate throughout almost the entire pregnancy, extending the milk-producing period to 305 days per year.² Therefore, recent commercial cow's milk contains large amounts of estrogens and progesterone.^{3–5}

A dramatic increase in estrogen-dependent malignant diseases, such as ovarian, corpus uteri, breast, testicular and prostate cancers has been recognized.^{5–8} Ganmaa *et al.* investigated the incidence and mortality of testicular and prostate cancers in relation to dietary practices. Among various food items, cow's milk and cheese had the highest correlation with incidence and mortality rate of these cancers.^{5,7,9} They also investigated the correlation between food consumption and

incidence rates of breast, ovarian and corpus uteri cancers. The intake of milk, meat and cheese was closely correlated with those cancers.⁸

Among the exposure of humans, especially prepubertal children, to exogenous estrogens, we are particularly concerned with commercial milk produced from pregnant cows. In Japan, milk is produced predominantly by lactating cattle, and approximately 80% of this milk originates from pregnant cows. In prepubertal children there is little secretion of estrogens, and serum 17 β -estradiol (E2) concentration is undetectable (<2 pg/mL) in a conventional enzyme immunoassay.¹⁰ Therefore, exposure to small doses of estrogens may have adverse effects on growth and maturation in prepubertal children. But because measuring the concentration of estrogens in milk is considerably difficult, concentrations reported by several analysts range widely from low to extremely high.^{3–5,11–13}

In the present study we examined the concentration of estrogens and progesterone in the serum and urine of young men and prepubertal children after the intake of cow's milk. Moreover, we investigated the influence of daily milk intake on the menstrual cycles of healthy women. If the milk contains high

Correspondence: Kenji Ohyama, MD, 1110 Shimogatou, Chuou, Yamanashi, 409-3898, Japan. Email: kohyama@yamanashi.ac.jp

Received 23 July 2008; revised 22 April 2009; accepted 8 May 2009.

Table 1 Men: subject characteristics

	Height (cm)	Weight (kg)	Body surface (m ²)	Milk intake (mL)
1	177	73.0	1.90	1140
2	174	83.0	1.97	1182
3	161	66.0	1.69	1014
4	167	71.4	1.81	1086
5	162	59.0	1.62	972
6	175	60.0	1.73	1038
7	178	64.0	1.80	1080

concentrations of estrogens and progesterone, the timing of ovulation may be affected by successive milk intake.

Methods

The subjects included healthy young men aged 19–21 years (Table 1). All of the men drank a volume of 600 mL/m² (body surface) of milk within 10 min.

Seven prepubertal children were enrolled in the study (Table 2). Four of the seven children drank a volume of 600 mL/m² of milk, but two of them could only drink 61% and 73% of the milk volume. Another girl could not drink half of the volume. Six children except this girl took part in this study.

Five women who had regular menstruation were included in the study (Table 3). Four of the five women had menstrual cycles of 28 days, but one woman aged 36 years had a regular cycle of 36 days. Four of the women did not regularly drink cow's milk, and one woman had a cup of milk every morning.

The cow's milk used for the study was commercially available cow's milk containing more than 3.5% fat.

Procedure

Men

Intake of milk and dairy products was prohibited for 3 days prior to the study. All of the men drank 600 mL/m² of cow's milk within 10 min. Urine samples were collected 1 h before the milk intake and four times every hour after the intake. The volume of the urine samples was measured, and they were stored at -20°C for 2 days. Subsequently, urine concentrations of estrone (E1), E2, estriol (E3) and pregnanediol were measured. Urine excretion volume of these hormones every hour was calculated as follow; Urine excretion volume of hormone (ng or µg/h) = urine concentration of hormone (ng or µg/mL) × urine volume (mL/h). Urine data are expressed as excretion volume per hour. Serum

Table 2 Prepubertal children: subject characteristics

	Age Years : Months	Sex	Height (cm)	Weight (kg)	Body surface (m ²)	Body surface-based milk volume (mL)	Total milk intake (mL) (%)
1	8 : 8	M	127.0	26.0	0.96	580	580 (100)
2	7 : 3	M	119.8	19.5	0.82	490	490 (100)
3	8 : 8	M	125.0	24.0	0.92	550	340 (62)
4	7 : 6	F	122.0	22.0	0.92	550	550 (100)
5	8 : 8	F	122.2	21.8	0.87	520	520 (100)
6	9 : 9	F	129.6	32.6	1.07	640	470 (73)
7	7 : 7	F	117.6	19.6	0.81	490	180 (37)

Table 3 Women: subject characteristics

	Age (years)	BMI	Menstrual cycle (days)
1	19	17.7	29–35
2	19	21.8	25–27
3	31	21.7	29–35
4	32	20.3	24–29
5	36	23.4	37–39

BMI, body mass index.

samples were obtained before and 15, 30, 45, 60, 90 and 120 min after the milk intake. Serum concentrations of E1, E2, luteinizing hormone (LH), follicle-stimulating hormone (FSH) and testosterone were measured. Hormones levels of urine and sera were measured at Special Reference Laboratory (Hachioji, Tokyo, Japan). Serum E1 levels were measured on radioimmunoassay (RIA), and E2, testosterone and progesterone, on electrochemiluminescence immunoassay. Serum LH and FSH levels were measured on chemiluminescence immunoassay. Urine estrogens (E1, E2, E3) were measured on RIA, and pregnanediol on gas chromatography–mass spectroscopy.

Prepubertal children

Intake of milk and dairy products was prohibited for 3 days prior to the study. Intake of milk volume was 600 mL/m², but two out of the six children could drink only 61% and 73% of the volume within 10 min, respectively. In children, serum samples were not obtained, and urine samples were collected in a method similar to that of the adults, and the concentration of E1, E2, E3 and pregnanediol was measured.

Women

In three successive menstrual cycles, basal body temperature was measured, and the day of ovulation was examined on measurement of urine LH concentrations using an ovulation checker from 17 days before the expected first day of the next menstruation cycle. All of the women drank 500 mL of cow's milk every night for 21 days beginning on the first day of the second menstruation. The day of ovulation in the second menstrual cycle during which milk was consumed was compared with that of the first and third menstrual cycles.

Statistical analysis

The data were analyzed using SPSS II (SPSS, Chicago, IL, USA). Non-parametric Wilcoxon signed rank tests were

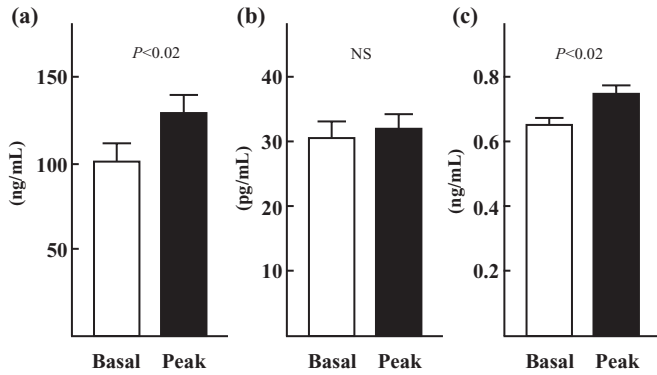


Fig. 1 Comparison between basal levels and peak levels of (a) serum estrone (E1), (b) estradiol (E2) and (c) progesterone before and after intake of cow milk in men ($n = 7$).

performed to examine the difference of hormone concentrations before and after the intake of milk. $P < 0.05$ was taken as significant.

Approval for the present study was obtained from the ethics committee of the University of Yamanashi School of Medicine.

Results

Men

Serum basal and peak concentrations of E1, E2 and progesterone during examination of the milk intake are shown in Figure 1. Serum E1 concentration was significantly increased and peaked 30–60 min after the intake of milk (mean \pm SE, before and peak: 102.3 ± 10.3 pg/mL and 128.9 ± 11.8 pg/mL, $P < 0.02$). Serum E2 concentration was unchanged during the 2 h examination (before and peak: 31 ± 4 pg/mL and 32 ± 4 pg/mL, NS). Serum progesterone concentration significantly increased and peaked 30–60 min after the intake of milk (mean \pm SE, before and peak: 0.66 ± 0.08 ng/mL and 0.75 ± 0.10 ng/mL, $P < 0.02$).

Serum basal and nadir concentrations of LH, FSH and testosterone before and after milk intake are shown in Figure 2. Serum LH and FSH concentration gradually decreased in six out of seven men, and reached a nadir 60–120 min after the intake of

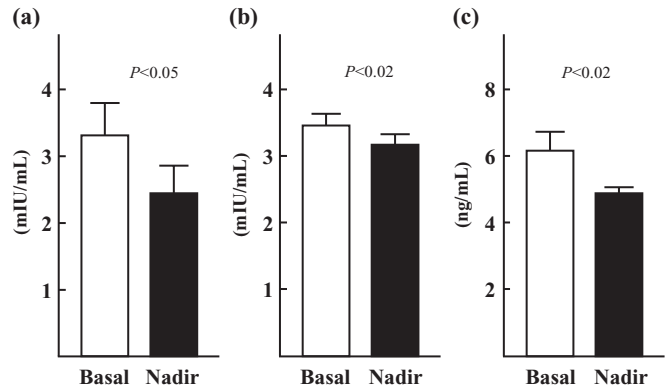


Fig. 2 Comparison between basal and nadir levels of (a) serum luteinizing hormone, (b) follicle-stimulating hormone and (c) testosterone before and after intake of cow milk in men ($n = 7$).

milk (before and lowest point: LH, 3.29 ± 0.49 mIU/mL and 2.48 ± 0.43 mIU/mL, $P < 0.05$; FSH, 3.43 ± 0.17 mIU/mL and 3.19 ± 0.15 mIU/mL, $P < 0.02$). Serum testosterone concentrations decreased considerably 120 min after intake in all subjects (before and lowest point: 6.04 ± 0.38 ng/mL and 4.94 ± 0.13 ng/mL, $P < 0.02$).

As shown in Figure 3, the volume per hour of urinary excretion of E1, E2, E3 and pregnanediol significantly increased after the intake of milk in all subjects ($P < 0.02$). Urine E1 excretion increased 1 h after intake, and reached a peak 4 h after intake in five out of seven men. Urine E2 excretion increased 1 h after the intake in six out of seven men. Peak excretion of E2 in urine was detected after 1 h in three subjects, and after 4 h in another three subjects. Urine E3 excretion also increased after 1 h in six out of seven men, and reached peak levels after 4 h in five men. Urine pregnanediol excretion peaked after 1 h in two out of seven men, and after 4 h in another four men.

Prepubertal children

Changes in urinary excretion volumes of E1, E2, E3 and pregnanediol are shown in Figure 4. Urinary excretion patterns were similar among these four hormones. Peak excretion volume of

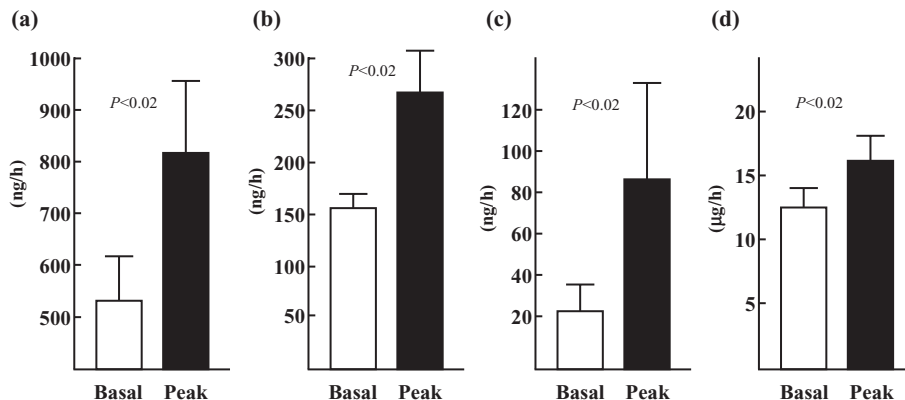


Fig. 3 Comparison between basal excretion volumes (basal) and maximum excretion volumes (peak) of (a) urine estrone, (b) estradiol, (c) estriol and (d) pregnanediol before and after intake of cow milk in men ($n = 7$).

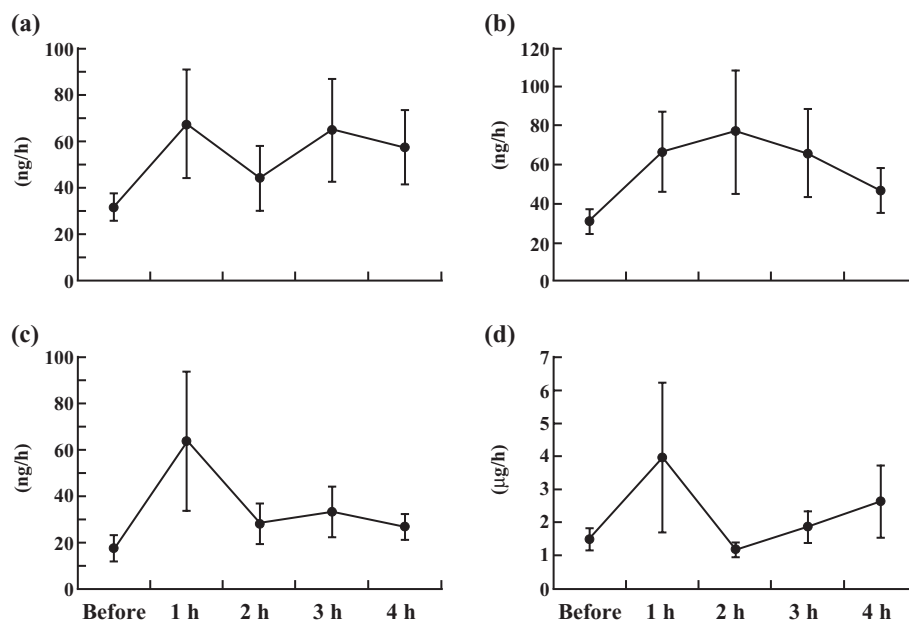


Fig. 4 Changes in mean urine excretion volume of (a) estrone, (b) estriol, (c) estradiol and (d) pregnanediol after intake of cow milk in prepubertal children (mean \pm SE, $n = 6$).

the hormones was detected at 1 h in three children, at 3 h in three children, and at 4 h in one child. The net increase in volume of urine excretion of E2 at 4 h after the intake of milk ranged from 39 to 109 ng. Urinary basal and peak excretion volumes of E1, E2, E3 and pregnanediol before and after milk intake are shown in Figure 5. Urinary excretion of these hormones significantly increased after intake ($P < 0.02$).

Women

In four out of five women, ovulation occurred during milk intake in the second menstrual cycle, and the timing of ovulation was similar among the three menstrual cycles. In these four women, the third menstruation and ovulation occurred regularly. In one woman, however, aged 36 years who had a menstrual cycle of

37–39 days, ovulation did not occur during the intake of milk. She ovulated 7 days after stopping milk intake.

Discussion

Toxicological and epidemiological studies have indicated that E2 could be categorized as a carcinogen.¹⁴ Milk is considered to be a rich source of estrogens. Indeed, E2 concentration is higher in mammary drainage than in the peripheral circulation in high-yielding cows.¹² Pregnant cows are under the control of relatively high levels of estrogens, and milk produced from pregnant cows contains correspondingly high concentrations of estrogens. Estrogen concentration in milk has been measured since the 1970s, mainly as an indicator of pregnancy.^{15,16} Concentration of E1 sulfate increases from 30 pg/mL in non-pregnant cows to

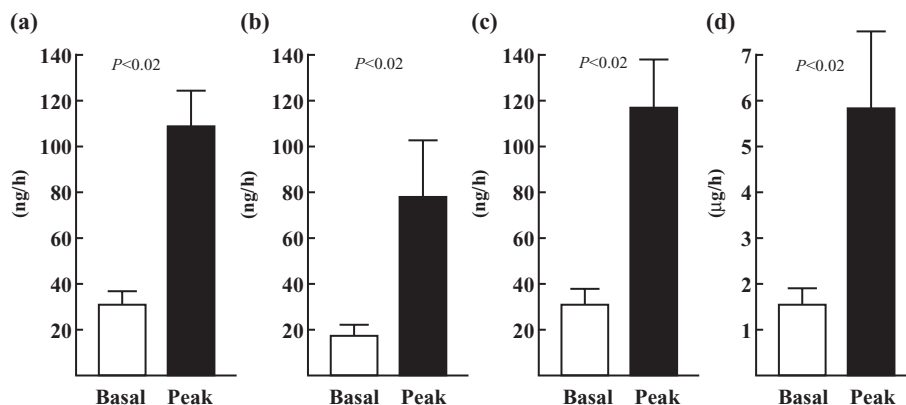


Fig. 5 Comparison between basal excretion volumes (basal) and maximum excretion volumes (peak) of (a) urine estrone, (b) estradiol, (c) estriol and (d) pregnanediol before and after intake of cow milk in prepubertal children ($n = 6$).

151 pg/mL in pregnant cows at 40–60 days of gestation, and to a maximum level of 1000 pg/mL in cows at 220 days of gestation.¹⁶ Recently, according to Malekinejad *et al.*, the concentration of estrogens in cow's milk under various conditions was measured using liquid chromatography–tandem mass spectrometry after enzymatic deconjugation.⁴ Their results indicated that processed milk with 3.5% fat contains high concentrations of E1 and E2. Qin *et al.* also reported high concentrations of estrogen in pregnant cow's milk.⁵ Moreover, milk and milk products contain high amounts of progesterone, which accumulate with increasing milk-fat content.³

In the present study in men, concentrations of serum E1 and progesterone increased after intake of cow's milk, concentrations of serum LH, FSH and testosterone significantly decreased 2 h after intake, and the volume of urine excretion of E1, E2 and E3 significantly increased. These results suggest that estrogens in milk were absorbed, and gonadotropin secretion was suppressed, followed by a decrease in testosterone secretion. The activation of a negative feedback mechanism due to exogenous estrogens in cow's milk indicates that men are affected by intake of commercial cow's milk. Because the main estrogen in milk is E1, and E2 concentration is relatively low compared to E1, serum E2 levels do not change for 2 h after the intake, but instead urine excretion of E2 significantly increases, suggesting that the conversion from E1 to E2 progresses slowly.

In prepubertal children, excretion volumes of estrogens and pregnanediol significantly increased 1–3 h after intake. The net increase of E2 excretion from the basal level (E2 in urine before the intake) was 39–109 ng/4 h in the present study.

In prepubertal boys, serum E2 level measured on ultrasensitive recombinant cell bioassay is 0.08 ± 0.2 pg/mL.¹⁰ Based on these data, the E2 production rate is 40 ng/day in prepubertal boys.¹⁷ The E2 in urine may be equal to or more than the daily E2 production rate in prepubertal boys. Reliable data on the daily production rate of E2 are still lacking in prepubertal children. Andersson and Skakkebaek reported that the conventional E2 production rate, according to the JECFA 1988 report, was presumably highly overestimated.¹⁷ Sheehan tested the hypothesis that no threshold exists when estradiol acts through the same mechanism as an active endogenous estrogen, and he found evidence that contradicted the threshold assumption and low-dose safety.¹⁸ Premature thelarche, gynecomastia, and pubertal growth spurt occur at very low or undetectable serum E2 levels, suggesting that prepubertal children are highly sensitive to estrogens.¹⁹ Serum E2 level (0.6 ± 0.6 pg/mL) in prepubertal girls was significantly greater than the level (0.08 ± 0.2 pg/mL) in prepubertal boys.¹⁰ Although this gender difference is extremely small in absolute figures, the higher level of E2 in girls may explain their earlier pubertal onset and growth spurt. Growth-promoting effects of very low doses of estrogen (25 ng/kg per day of ethinylestradiol) have also been observed in Turner syndrome.²⁰ Even small changes in serum E2 concentrations within the extremely low prepubertal range may, therefore, have significant biological implications. The present data on men and children indicate that the intake of estrogens from 600 mL/m² of cow's milk may correspond to the daily estrogen production rate in prepubertal

boys, and height growth and sexual maturation of prepubertal children could be affected by normal intake of cow's milk.

Recent surveys on the timing of pubertal onset show an alarming trend of earlier sexual maturation in girls.²¹ Anderson *et al.* reported a drop of approximately 2.5 months in the average age of menarche between 1963–1970 and 1988–1994 in US girls, and referred the relationship of earlier menarche to increased body mass index (BMI).²² Several reports concerning puberty in girls suggest a positive correlation between the timing of breast development or menarche and that of increases in BMI.^{23–25} Because adipose tissue is a source of estrogens, the cause of earlier sexual maturation may not only be a change in nutritional status, but also an increase in estrogen secretion from adipose tissue. Exposure to exogenous estrogens through intake of commercial milk produced from pregnant cows has spread around the world since the 1970s. We think that the intake of pregnant cow's milk is one of the major causes of early sexual maturation in prepubertal children.

The menstrual cycle in women is controlled by relatively high levels of E2 and progesterone. Commercial milk produced from pregnant cows contains not only estrogens but also progesterone.³ The prolonged intake of estrogen and progesterone compounds may affect the timing of ovulation. We examined the effect of the intake of cow's milk for 21 consecutive days from the start of the last menstruation to ovulation. The timing of ovulation in four of five women was not affected by the intake of milk. These four women were healthy young women, but the fifth woman suffered from oligomenorrhea of a 37–39 day cycle. Her ovulation occurred 7 days after the discontinuation of milk. These results suggest that ovulation in women with subclinical hypogonadism might be affected by an abundant intake of milk, although normal menstrual cycles are not influenced.

Since 1985, daily intake of cow's milk has been extensively recommended, especially to prepubertal children in Japan. The average age of menarche in girls living in metropolitan Tokyo occurred at 12 years 5 months in 1987, and 12 years 3 months in 1993.²⁶ These findings and the present data indicate that the intake of cow's milk may cause earlier sexual maturation. The relationship between estrogens in pregnant cow's milk and sexual maturation in children must be acknowledged as an important theme.

References

- 1 Brown LR. *Seed of Change. The Green Revolution and Development in the 1970s*. Praeger Publishers, New York, 1970.
- 2 Ganmaa D, Li X-M, Wang J *et al.* The experience of Japan as a clue to the etiology of testicular and prostatic cancers. *Med. Hypotheses* 2003; **60**: 724–30.
- 3 Hartmann S, Lacorn M, Steinhart H. Natural occurrence of steroid hormones in food. *Food Chem.* 1998; **62**: 7–20.
- 4 Malekinejad H, Scherpenisse P, Bergwerff AA. Naturally occurring estrogens in processed milk and raw milk (from gestated cows). *J. Agric. Food Chem.* 2006; **54**: 9785–91.
- 5 Qin LQ, Wang PY, Kaneko T, Hoshi K, Sato A. Estrogen: One of the risk factors in milk for prostate cancer. *Med. Hypotheses* 2003; **62**: 133–42.
- 6 Forman D, Moller H. Testicular cancer. *Cancer Surv.* 1994; **19**: 20: 323–41.

- 7 Ganmaa D, Li X-M, Wang J, Qin LQ, Wang PY, Sato A. Incidence and mortality of testicular and prostatic cancers in relation to world dietary practices. *Int. J. Cancer* 2002; **98**: 262–7.
- 8 Ganmaa D, Sato A. The possible role of female sex hormones in milk from pregnant cows in the development of breast, ovarian and corpus uteri cancers. *Med. Hypotheses* 2005; **65**: 1028–37.
- 9 Ganmaa D, Wang PY, Qin LQ, Hoshi K, Sato A. Is milk responsible for male reproductive disorders? *Med. Hypotheses* 2001; **57**:510–14.
- 10 Klein KO, Baron J, Coll MJ, McDonnell DP, Cutler GB Jr. Estrogen levels in childhood determined by an ultrasensitive recombinant cell bioassay. *J. Clin. Invest.* 1994; **94**: 2475–80.
- 11 Pape-Zambito DA, Magliaro AL, Kensinger RS. Concentrations of 17 β -estradiol in Holstein whole milk. *J. Dairy Sci.* 2007; **90**: 3308–13.
- 12 Janowski T, Zdunczyk S, Malecki-Tepicht J, Baranski W, Ras A. Mammary secretion of oestrogens in the cow. *Domest. Anim. Endocrinol.* 2002; **23**: 125–37.
- 13 Lopez H, Bunch TD, Shipka MP. Estrogen concentrations in milk at estrus and ovulation in dairy cows. *Anim. Reprod. Sci.* 2002; **72**: 37–46.
- 14 Liehr JG. Is estradiol a genotoxic mutagenic carcinogen? *Endocr. Rev.* 2000; **21**: 40–54.
- 15 Wolford ST, Argoudelis CJ. Measurement of estrogens in cow's milk, human milk, and dairy products. *J. Dairy Sci.* 1979; **62**: 1458–63.
- 16 Heap RB, Hamon M. Oestrone sulfate in milk as an indicator of a viable conceptus in cows. *Br. Vet. J.* 1979; **135**: 355–63.
- 17 Andersson AM, Skakkebaek NE. Exposure to exogenous estrogens in food: Possible impact on human development and health. *Eur. J. Endocrinol.* 1999; **140**: 477–85.
- 18 Sheehan DM. No-threshold dose-response curves for nongenotoxic chemicals: Findings and applications for risk assessment. *Environ. Res.* 2006; **100**: 93–9.
- 19 Aksglaede L, Juul A, Leffers H, Skakkebaek NE, Andersson AM. The sensitivity of the child to sex steroids: Possible impact of exogenous estrogens. *Hum. Reprod. Update* 2006; **12**: 341–9.
- 20 Cutler GB. The role of estrogen in bone growth and maturation during childhood and adolescence. *J. Steroid Biochem. Mol. Biol.* 1997; **61**: 141–4.
- 21 Parent AS, Teilmann G, Juul A, Skakkebaek NE, Toppari J, Bourguignon JP. The timing of normal puberty and the age limits of sexual precocity: Variations around the world, secular trends, and changes after migration. *Endocr. Rev.* 2003; **24**: 668–93.
- 22 Anderson SE, Dallal GE, Must A. Relative weight and race influence average age at menarche: Results from two nationally representative surveys of US girls studied 25 years apart. *Pediatrics* 2003; **111**: 844–50.
- 23 Kaplowitz PB, Slora EJ, Wasserman RC, Pedlow SE, Herman-Giddens ME. Earlier onset of puberty in girls: Relation to increased body mass index and race. *Pediatrics* 2001; **108**: 347–53.
- 24 Wang Y. Is obesity associated with early sexual maturation? A comparison of the association in American boys versus girls. *Pediatrics* 2002; **110**: 903–10.
- 25 Himes JH. Examining the evidence for recent secular changes in the timing of puberty in US children in light of increases in the prevalence of obesity. *Mol. Cell. Endocrinol.* 2006; **254**:255: 13–21.
- 26 Ashizawa K. Skeletal and sexual maturation and growth in Tokyo girls: Longitudinal observations. *Clin. Pediatr. Endocrinol.* 1993; **2** (Suppl. 1): 5–8.