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Fatty Acid Profile in Milk from Goats, *Capra aegagrus hircus*, Exposed to Perchlorate and its Relationship with Perchlorate Residues in Human Milk

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Abstract Polyunsaturated fatty acids (PUFA) in milk are vital for normal growth and development of infant mammals. Changes in fatty acid composition were observed in milk fat from goats dosed with perchlorate (0.1 and 1 mg/kg body weight/day) for 31 days, but the effect was not persistent. Adaptation may be induced in these goats to compensate for the perchlorate effect. In an analysis of fatty acid composition in human milk samples, a weak negative correlation was observed between perchlorate concentrations and total PUFA in 38 human milk samples.

Keywords Perchlorate · Fatty acid profile · Milk

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Milk fat is very important for growing newborns. Fatty acids are an energy source for infant mammals as well as being involved in some other biological processes (German and Dillard 2006). Lipids are structural components of all tissues and fatty acids are indispensable for cell membrane synthesis. Among fatty acids, polyunsaturated fatty acids (PUFA) are also involved in the regulation of neurotransmitter release, synthesis of biologically active oxygenated derivatives, and gene expression at the transcriptional level during adipocyte differentiation (Alessandri et al. 2004). Lower PUFA intake through milk could arrest the development of the immune system, brain, and visual system in infants since milk is the only nutrient source for infants (Neville and Picciano 1997). Alteration of PUFA composition in membranes may also affect a variety of membrane functions such as effects on ion channels, transporters, protein binding, cellular and intracellular signal transduction, and neurotransmission.

Milk fat synthesis is a multiple step process involving many enzymes, such as fatty acid synthase (FAS), which is the key enzyme for de novo synthesis of short- and medium-chain fatty acids ($C \leq 14$) within mammary epithelial cells. Thyroid hormone (i.e., triiodothyronine) was reported to stimulate transcription of the fatty acid synthase gene (Kameda 1995). Therefore, as a thyroid hormone disruptor, perchlorate may affect de novo fatty acid synthesis in mammary glands. Hormones such as prolactin also play important roles in mammary gland development and milk synthesis and secretion. Prolactin has been postulated to regulate activation of LPL in the mammary gland (Neville and Picciano 1997), and may also affect the activity of FAS. Induction of prolactin requires thyroid hormones. Perchlorate may eventually have an influence on the fatty acid composition of milk fat through interference with prolactin and in turn, the function of LPL and (or) FAS.

Interruption in thyroid hormone production by perchlorate may also change the fatty acid profile in milk fat by affecting fatty acid de novo synthesis in other tissues such as the liver in addition to the mammary gland.

Perchlorate competitively inhibits iodide uptake by the thyroid gland, and as a result, interferes with normal thyroid hormone production. In mammals, this interference affects development, growth, and metabolism (Wolff 1998). Environmental contamination of perchlorate in the US appears to be widespread, especially in aqueous systems, because of its common use in military munitions and other industrial products, and also its natural occurrence (Dasgupta et al. 2005; Jackson et al. 2005b).

Perchlorate can be taken up into plants and animals (Jackson et al. 2005a; Sanchez et al. 2005; Yu et al. 2004). Forage crops (plants or edible vegetables) can accumulate perchlorate with bioconcentration factors ranging from 1.4 to 620 (Jackson et al. 2005a). Humans can also be exposed to perchlorate through drinking water or perchlorate-containing food (such as milk and lettuce). Reports indicate that perchlorate occurs in some supermarket milk samples and human milk, and that perchlorate can be distributed into dairy milk (Capuco et al. 2005; Kirk et al. 2003; Krynitsky et al. 2004). This suggests that perchlorate contamination in the US is much more widespread than originally thought and human exposure to perchlorate has likely occurred for several years, hypotheses supported by reports on the detection of perchlorate in human urine (Valentin-Blasini et al. 2005).

There is concern about the potential effect of perchlorate exposure on milk quality, particularly because perchlorate has been found in milk. Within an experiment designed to evaluate perchlorate excretion in milk from dairy goats, we also opportunistically examined milk fatty acid profiles in order to investigate possible effects of perchlorate exposure on fatty acid composition in milk fat. To support this study, we also investigated possible relationships between perchlorate residue levels and fatty acid levels in human breast milk samples collected locally as part of another study.

Materials and Methods

Eighteen lactating Nubian goats from the Texas Tech University Agriculture Research Farm were assigned to three different perchlorate dosing groups chosen to simulate a range of environmentally relevan exposures: 0, 0.1, and 1 mg/kg body weight/day (i.e., six goats in each dosing group). Each group consisted of goats of similar weight (and presumably age). For example, four "heavier" (presumably older) goats were paired with two "light" (presumably younger) goats to form each group. Animals had accessed to creep and finishing diet, which contains 2.5% fat. The goats were orally dosed (gavage) with perchlorate from Monday to Friday of each week (19 May–21 June 2003). Milk was collected daily before perchlorate dosing (i.e., 24 h after previous dosing). Fatty acid analyses were conducted for milk samples collected on 20 May (day 2), 28 May (day 10), 4 June (day 17), 11 June (day 24), and 18 June (day 31). Because some samples were lost and unavailable for fatty acid analysis, n ranged from 3 to 6 for different groups at different times. The study was conducted according to a protocol approved by the Animal Care and Use Committee at Texas Tech University (Lubbock, TX).

Thirty-eight human milk samples were provided by the Department of Human Development and Family Studies, Texas Tech University. These samples were collected from healthy lactating mothers residing in Lubbock, TX or nearby counties (from July 2004 to June 2005) as part of an unrelated study evaluating the relationship between fatty acids in milk and cognitive abilities of young infants. Lactation stages were around either 6 weeks or 3 months. The study was conducted according to a protocol approved by the Institutional Review Board (IRB) and Animal Care and Use Committees at Texas Tech University (Lubbock, TX).

The fatty acid analysis was conducted following a procedure (Scopesi et al. 2001) with slight modifications. Identification and quantification of fatty acids in samples were based on a 37-component FAME mixture standard (AccuStandard Inc., New Haven, CT). Recovery of an internal standard (C19:0) was 84% ± 13% (mean ± SD). Interday coefficient of variation (cv) for analysis of fatty acid methyl esters in dairy milk were <15% after extraction, transesterification, and instrumental analysis during a week period.

Monounsaturated fatty acids (MUFA), PUFA, medium-chain fatty acids, and long-chain fatty acids were investigated as endpoints. MUFA consisted of C16:1n9, 18:1n9, 20:1n9, and 22:1n9. PUFA included ω-6 series (C18:2n6, 18:3n6, 20:3n6, and 20:4n6), and ω-3 series (C18:3n3, 20:3n3, 20:5n3, and 22:6n3). Medium-chain fatty acids were composed of fatty acids with carbons ≤14 (C8:0, 10:0, 12:0, and 14:0), and long-chain fatty acids included fatty acids with carbons ≥16 (C16:0, 16:1n9, 18:0, 18:1n9, 18:2n6, 18:3n6, 18:3n3, 20:0, 20:1n9, 20:3n3, 20:3n6, 20:4n6, 20:5n3, 22:0, 22:1n9, and 22:6n3). Fatty acid was expressed as a percentage of extracted lipid from the goat milk, and as a percentage of total fatty acids for the human milk samples.

Perchlorate in human milk was determined by ion chromatography coupled with an API 2000TM MS/MS system (IC-MS/MS) (El Aribi et al. 2006). Quality control samples included blanks, spiked matrix [with spiked perchlorate concentrations of 0.1 (n = 2) or 0.5 (n = 3) ng/

Table 1 *p* Values in two-way ANOVA test for fatty acid content in lactating goats dosed with perchlorate from Monday to Friday for 4.5 weeks

Test	Factor	Two-way ANOVA *p* value					
		MUFA	PUFA	ω-6	ω-3	MCFA	LCFA
Time × dose	Time	3.2E-8***	0.02*	0.04*	1.8E-5***	0.0002***	1.9E-5***
	Dose	0.01**	2.2E-6***	1.8E-5***	4.0E-4***	0.26	0.16
	t × d	0.59	0.17	0.22	0.23	0.40	0.58
Time + dose	Time	1.3E-8***	0.03*	0.05*	8.0E-6***	1.4E-4***	1.2E-5***
	Dose	0.01**	3.1E-6***	2.2E-5***	4.0E-4***	0.26	0.15

Time × dose includes potential interaction in the statistical test. Time + dose does not include potential interaction in the statistical test
*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$

mL], and check standards (0.2 ng/mL, n = 5). A spiked milk sample, a check standard, and a blank were analyzed after every nine samples (a sub-batch) during the IC-MS/MS analyses. One sample per sub-batch was analyzed in duplicate. Perchlorate recovery of the check standard was assured between 80% and 120%. Coefficient of variation for all duplicates ranged from 0.5% to 9.6%; 90%–118% perchlorate was recovered in the spiked matrix.

Statistical analyses were conducted using R software (R 2.0.1 vision, Free Software Foundation, Boston, MA). Two-way ANOVA was conducted on data from the goat study to test the effects of two factors: dosage and dosing time, and/or their interaction. Differences in fatty acid composition of milk among the three treatments at either exposure interval in goats were tested using one-way ANOVA followed by Tukey's multiple comparison. A linear regression analysis between perchlorate content and total PUFA levels in the human milk samples was also conducted. Student's *t* test was used to test the difference in fatty acid profiles between two different lactation stages in humans (6 week and 3 month). An $\alpha = 0.05$ was used for all tests.

Results and Discussion

Results of the two-way ANOVA for the goat study are shown in Table 1. No significant effect was found in terms of the interaction of dosage and dosing time regarding all end-points. Time had a significant effect on fatty acid profiles in terms of all end-points, and dose had a significant effect on the fatty acid profile (PUFA, ω-6, ω-3, and MUFA content). Total PUFA content and ω-6 fatty acids in goat milk were significantly reduced at day 10 ($p = 0.005$ and 0.011, respectively), day 17 ($p < 0.001$), and day 24 ($p < 0.05$), but not at days 2 and 31 in the high dose treatment (Figs. 1, 2). ω-3 fatty acids showed no apparent dose-dependent response during perchlorate exposure (data not shown). MUFA content was significantly reduced only at day 17 ($p = 0.013$) in the high dose treatment. Although

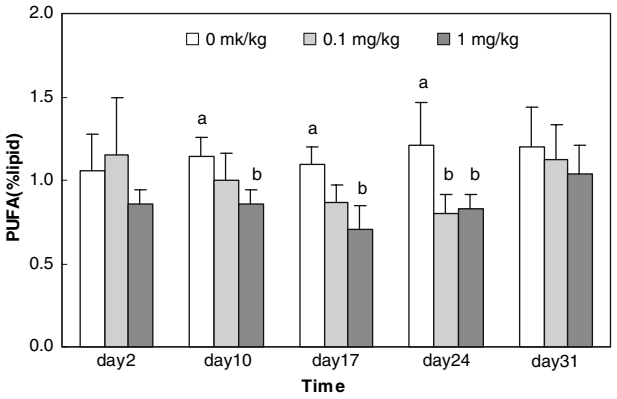

Fig. 1 Content of total polyunsaturated fatty acids (PUFA) in goat milk during perchlorate exposure (mean ± SD) (n = 3 and 4 at 0.1 mg/kg at days 17 and 24, respectively, n = 3 at 1 mg/kg at day 24, and n = 5 or 6 at all other time points). *Different letters* indicate a significant difference at the 0.05 level

dose did not show a significant effect on medium-chain and long-chain fatty acids (MCFA and LCFA) in milk (Table 1), differences in both MCFA and LCFA among the three treatments were still tested using one-way ANOVA. Significant reductions in medium- and long-chain fatty acids were observed at day 24 only ($p = 0.043$ and 0.010, respectively). No significant difference was observed over the dosing time in terms of PUFA and ω-6 fatty acids in both control and low dose groups (Fig. 3), but there was a significant difference between day 17 and 31 regarding both PUFA and ω-6 in the high dose group.

In the current experiment, total PUFA was decreased at days 10, 17, and 24 for both perchlorate exposure scenarios, with a particularly significant reduction occurring in the 1 mg/kg/day treatment (Fig. 1). This indicates that perchlorate exposure depressed total PUFA in milk of lactating goats, causing a deficiency in PUFA available to the young. Maternal PUFA is the major contributor of the PUFA in milk for younglings during early development.

Similar to the response of total PUFA upon perchlorate exposure, ω-6 fatty acids were reduced at days 10, 17, and 24 for both perchlorate treatments (Fig. 2). ω-6 fatty acids

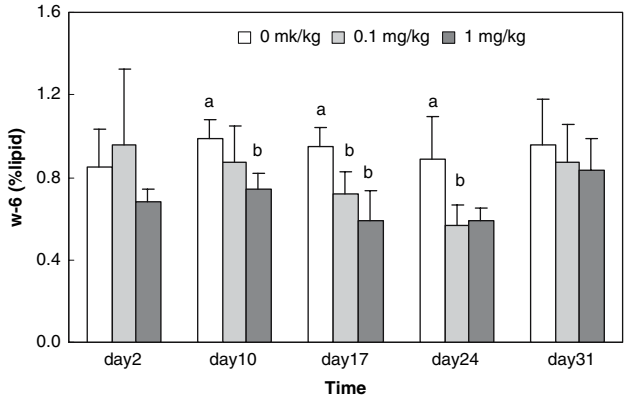

Fig. 2 Content of ω-6 fatty acids in goat milk during perchlorate exposure (mean ± SD) (n = 3 and 4 at 0.1 mg/kg at days 17 and 24, respectively, n = 3 at 1 mg/kg at day 24, and n = 5 or 6 at all other time points). *Different letters* indicate a significant difference at the 0.05 level

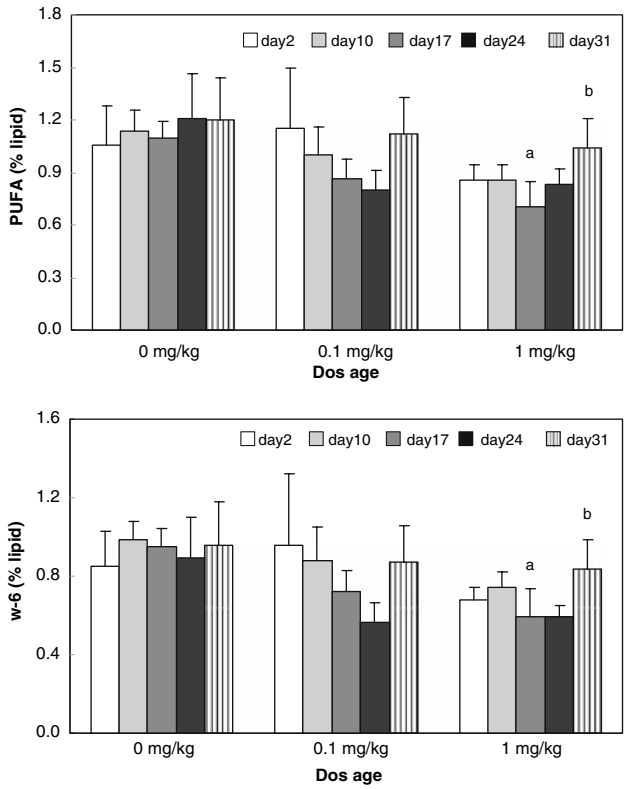

Fig. 3 Changes in PUFA (*top*) and ω-6 fatty acid (*bottom*) content (% of lipid) in goat milk with time in three different dosing groups. *Different letters* indicate a significant difference between day 17 and 31 in the high dose group (0.05 level)

account for a major portion of total PUFA in goat milk. In the current study, a significant linear correlation was found between ω-6 fatty acids and total PUFA content in all goat milk samples (r^2 = 0.99) at day 17. Therefore, ω-6 fatty acids and total PUFA in milk responded similarly to perchlorate exposure. Among the ω-6 fatty acids examined in the current study, linoleic acid (LA, C18:2n6) was the dominant fatty acid. Also, a significant linear correlation existed between the LA content and total PUFA content within all perchlorate treatments (r^2 = 0.99) at day 17. LA is a precursor for arachidonic acid (ARA, C20:4n6), while linolenic acid (C18:3n3) is a precursor for docosahexaenoic acid (DHA, C22:6n3). Both ARA and DHA are indispensable nutrients for early development and growth of the brain and retina (Alessandri et al. 2004). In the current study, ARA and DHA in the high dose group decreased to 79% ± 13% of the control at day 24 and 63% ± 17% of the control at day 31, respectively. This is probably due to the decrease in the precursors of long-chain PUFA during exposure; LA decreased to 61% ± 16% of the control at day 17 and 65% ± 6% of the control at day 24, and linolenic acid decreased to 75% ± 10% of the control at day 17 and 73% ± 14% of the control at day 24.

As illustrated in Figs. 1 and 2, the maximum reduction of both PUFA and ω-6 fatty acids in the 0.1 mg/kg perchlorate treatment occurred later than in the 1 mg/kg treatment. The delayed response of goats to the lower perchlorate treatment indicates that the higher perchlorate concentrations appear to act on and alter PUFA and ω-6 fatty acid content with greater efficiency.

The lack of a significant change in the endpoints evaluated for all exposure scenarios at day 2 (Figs. 1, 2) indicates that a 2-day exposure to perchlorate, up to 1 mg/kg, was not sufficient to alter the fatty acid profile in goat milk. This was not completely unexpected because milk fat biosynthesis is a complex process in which many molecular-level activities are involved and some time is needed for an effect to be manifested. The reduction in most fatty acids that occurred at days 10, 17, and/or 24 was reversed; no significant difference was found when compared with the control at day 31 (Figs. 1, 2). This is possibly due to adaptation mechanisms which might be induced to compensate for the effect of perchlorate exposure, such that the goats increased the removal of perchlorate via milk and/or urine. It was found that the perchlorate content in the same goat milk 24 h after perchlorate dosing was significantly reduced when compared with levels at 2 h post-dose (perhaps because less perchlorate was available for excretion with time). Average perchlorate concentration in the milk at 2 h post-dose on the first exposure day was 172 and 120 ng/mL in the high and low dosing groups, respectively, and it decreased to 6.3 and 3.2 ng/mL at 24 h after dosing, respectively. Average weekly perchlorate concentration in goat milk collected 24 h after dosing decreased after week 1, particularly for the 1 mg/kg/day treatment (7.2 ng/mL in week 1 and 2.5 ng/mL in week 2). Excretion of perchlorate via urine is a major pathway for mammals following perchlorate exposure. Perchlorate excretion via urine in deer mice, either based on perchlorate concentration or mass

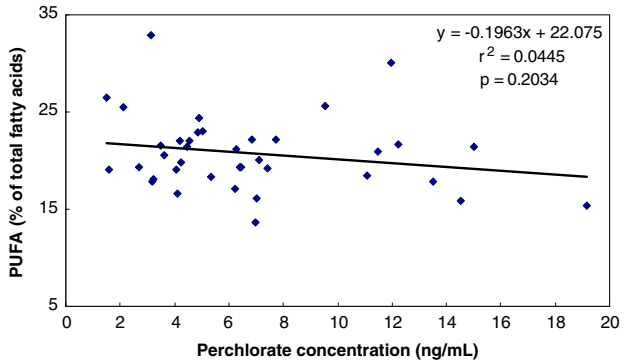

Fig. 4 Correlation ($r^2 = 0.04$; $p = 0.20$) between polyunsaturated fatty acid (PUFA) and perchlorate concentrations in human breast milk from mothers in Lubbock, TX or nearby counties (July 2004–June 2005). PUFA levels were expressed as percentage of total fatty acids

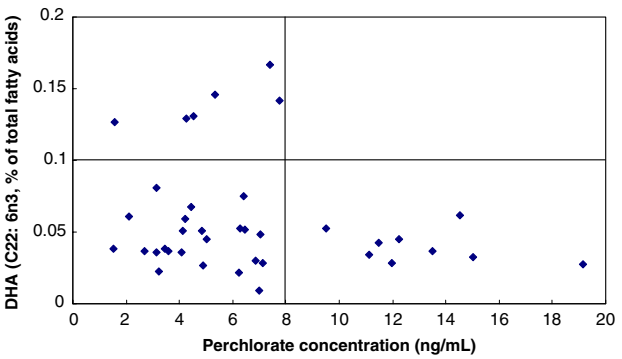

Fig. 5 Docosahexaenoic acid (DHA; C22:6n3) as a function of perchlorate concentration in human milk from Lubbock, TX or nearby counties (collected from July 2004 to June 2005). DHA levels were expressed as a percentage of total fatty acids. Four *quadrants* were obtained when dividing the ranges of the observed DHA and perchlorate into two

percentage of the total intake through dosed drinking water, increased and achieved a steady state soon (24 h) after perchlorate exposure (unpublished data from our laboratory). In addition, perchlorate could be lost through anaerobic metabolism in the intestine and cecum by microbial activity as suggested by Capuco et al. (2005) and data on perchlorate excretion in deer mice from out laboratory. Therefore, increased removal of perchlorate through milk and/or urine, and possible loss by anaerobic metabolism can result in less perchlorate in certain target tissues and thus compensate for the effect of perchlorate on fatty acid profiles.

The reverse effect described earlier may also be caused by the increased production of thyrotropin-releasing hormone (TRH), resulting from decreased thyroid hormone negative feedback. As a potent prolactin-releasing factor, TRH can stimulate prolactin secretion from the pituitary and as a result, alter the fatty acid profile in milk by reversing the effect of perchlorate. Prolactin plays important roles in mammary gland development and milk synthesis and secretion.

Diet has a major influence on fatty acid composition in milk, but milk and (or) milk fat composition also changes with lactation stage. In the current study, the goats had access to the same food and water, but their stage of lactation was unknown (i.e., they may not have been at the same stage of lactation despite the food, water, and weight grouping). Therefore, differences in lactation stage could have been a confounding factor in the altered fatty acid profile in milk observed in this study.

No significant statistical difference was observed between the 6-week and 3-month lactation stages in terms of either each fatty acid, total saturated fatty acids, MUFA, PUFA, ω-3, ω-6, medium-chain fatty acids ($C \leq 14$), or long-chain fatty acids ($C \geq 16$) (t test, $p > 0.05$). A weak negative relationship ($r^2 = 0.05$, $p = 0.20$) between perchlorate concentrations and total PUFA levels in the 39 human milk samples was observed (Fig. 4). As was the case for the goat milk samples, there were good linear relationships between linoleic acid (C18:2n6) and total PUFA, and ω-6 and total PUFA ($r^2 = 0.96$ and 0.98, respectively). The difference in total PUFA among samples resulted mainly from differences in linoleic acid. However, no significant relationship (either positive or negative with $p < 0.05$) was found between ARA, DHA, MUFA, medium- or long-chain fatty acids and perchlorate concentrations. When the ranges of observed perchlorate and DHA were divided into two groups, four quadrants were formed (Fig. 5); not a single datum with high DHA (>0.1%) – high perchlorate (>8 ng/mL) was found for these samples. Given an average body weight of 5.8 kg and an average daily breast milk intake of 712 mL for a 1 to 4-month old baby, 8 ng/mL perchlorate would result in 0.98 µg/kg/day intake of perchlorate, which is slightly higher than the reference dose of 0.7 µg/kg/day recommended by the National Academy of Sciences (NAS 2005).

Perchlorate was detected in all human milk samples, ranging from 1.5 to 19 ng/mL with an average of 6.8 ng/mL. During the first 6 months of lactation with exclusive breast-feeding, 24 h milk production from each breast is 454 ± 201 g (Kent et al. 1999). Assuming 5% of the perchlorate taken up by a 50-kg lactating mother was excreted via milk, then the perchlorate exposure level to the mother would be about 3.48 µg/kg body weight/day with a maximum of 5.02 µg/kg/day (corresponding to average and high 24 h milk production with the maximum perchlorate residues in milk, i.e., 19 ng/mL). The fact that the predicted perchlorate exposure level in humans was much lower than that administered in the goat dosing study (0.1 and 1 mg/kg body weight/day) could explain why no significant relationship was found between either endpoint and perchlorate concentrations in the human milk. In

addition, nutritional and physical factors which can influence fatty acid composition in milk were not incorporated into the data analyses. An epidemiological study with large sample sizes would contribute more to evaluating the potential risk of perchlorate on pregnant and/lactating women and their infants.

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