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Milk and Plasma Glucocorticoid Alterations After Injections of Hydrocortisone and Adrenocorticotropin (39714)

F. C. GWAZDAUSKAS, M. J. PAAPE, AND M. L. McGILLIARD

Department of Dairy Science, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061 and Animal Physiology and Genetics Institute, Agricultural Research Center, ARS, USDA, Beltsville, Maryland 20705

Physiological relationships between blood and milk concentrations of estrogens and progesterone have been shown during the bovine estrous cycle and during pregnancy (1, 2). Blood concentrations have been reported to be of lower magnitude than milk concentrations. Glucocorticoid concentrations in milk during lactation (3) and after adrenocorticotropin (ACTH) injection (4) have been reported in two recent abstracts. However, the relationships between blood and milk glucocorticoid concentrations were not evaluated. In several clinical conditions, natural glucocorticoids, synthetic glucocorticoids, and ACTH are administered therapeutically. The concentrations of glucocorticoids in milk and plasma after treatment with adrenal product substitutions or adrenal-stimulating compounds should therefore be determined.

The objective of this study was to determine changes in plasma and milk glucocorticoid concentrations after intramuscular injection of hydrocortisone and ACTH.

Materials and methods. Eighteen Holstein cows, free from intramammary mastitis pathogens were used. Ten cows, producing an average of 16 kg of milk/day, were injected intramuscularly with 1600 mg of Δ^4-pregnen-11β,17α,21-triol-3,20-dione (hydrocortisone; Sigma Chemical Co., St. Louis, Mo.) after the 0800-hr milking. Eight cows, producing an average of 18 kg of milk/day, were injected im immediately after and 7 hr after the 0800-hr milking with either 250 (n = 6) or 100 (n = 2) IU of ACTH (porcine ACTH, Sigma Chemical Co., St. Louis, Mo.).

Foremilk samples (80 ml) were collected aseptically from each mammary quarter before the 0800-hr milking and 15 hr later. Mammary quarter samples were pooled immediately, and a 40-ml aliquot was poured off and designated as the whole milk fraction. The rest of the sample (280 ml) was centrifuged at 5000g for 30 min at 5°, and the skim milk fraction was removed. Milk fractions were frozen and stored at −20°.

Immediately after collection of the foremilk samples, tail vein blood samples were collected via vena puncture in heparinized syringes. Blood was placed immediately into an ice bath and later centrifuged at 5000g for 30 min at 5°. The plasma was harvested and stored at −20°.

Total glucocorticoids in milk and plasma were quantified using the competitive protein binding assay (5) as modified by Gwazdauskas *et al.* (6). During the analysis of glucocorticoids in milk, approximately 1500 cpm of radioactive cortisol [1,2-^{3}H(N)]hydrocortisone (New England Nuclear) was added to each 20-ml glass culture tube as an internal standard to correct for procedural losses. Samples of milk (1-ml or 0.5-ml) were extracted twice, each with five volumes of iso-octane. These extracts were discarded. The rest of the milk residue was then extracted by vigorous shaking for 1 min with methylene chloride. After freezing, the organic residue was poured into a test tube and evaporated to dryness in a water bath (40°) under air. The sample was then reconstituted with 2 ml of methylene chloride:methanol (9:1). A 0.2-ml aliquot was taken to estimate recovery. Aliquots of 0.1 and 0.2 ml or 0.3 and 0.6 ml were dried and assayed for total glucocorticoids (6). The duplicate estimates on each sample satisfied the dose-response relationships. Validity of this system for determination of milk glucocorticoids was shown by the addition of known amounts of nonradioactive cortisol to aliquots of milk and extracting and assaying as described (Table I).

Statistical evaluations of factors influencing final glucocorticoid concentrations in plasma, whole milk, and skim milk were by

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least-squares procedures. Each model included effects of treatments and covariates on preskim milk, prewhole milk, and preplasma concentrations of glucocorticoids.

Results and discussion. Results in Table II show the means and standard errors for glucocorticoids in plasma, whole milk, and skim milk before and after hydrocortisone or ACTH injections. Injecting 1600 mg of hydrocortisone resulted in 2.7-, 8.0-, and 5.0-fold increases in glucocorticoids in plasma, whole milk, and skim milk, respectively. Paape *et al.* (7) infused hydrocortisone (1600 mg) into the mammary gland of lactating dairy cows and reported plasma glucocorticoid concentrations of 127 ng/ml 3 hr later. The concentration of glucocorticoid in whole milk from the infused mammary quarters was over 400 ng/ml at 4 to 9 hr after infusion. The concentration in mammary quarters not infused was only 14 ng/ml at 4 hr after infusion, but basal concentrations were not reached until 48 hr after the infusion. Glucocorticoid concentrations in plasma before injection in the present study are similar to plasma concentrations reported by Paape *et al.* (7) (12.5 vs 7.2 ng/ml). However, the basal concentration of glucocorticoids in whole milk were lower in this study (0.9 vs 3.4 ng/ml).

TABLE I. Precision of Glucocorticoid Determinations in Bovine Whole and Skim Milk Using Competitive Protein-Binding Quantification.

Cortisol (ng)			
Added	Measured	N^a	SE^b
Whole milk			
0	1.4	4	0.07
5	5.7	4	0.51
10	9.5	4	0.29
20	20.7	4	1.11
50	51.5	4	1.89
Skim milk			
0	1.2	4	0.22
5	4.9	4	0.52
10	12.5	4	1.48
20	19.9	4	1.26
50	58.1	4	1.76

[a] Number of samples assayed.
[b] Standard error.

Injection of 250 or 100 IU of ACTH resulted in a 7.4- and 5.2-fold increase in plasma glucocorticoids, respectively. In whole milk, the increase was 23.4- and 8.2-fold and, in skim milk, the increase in glucocorticoids was 27.3- and 11.0-fold. Two injections of ACTH, 7 hr apart, elevated the plasma concentration of glucocorticoids 2-fold higher than did hydrocortisone injections (Table II). Except for the plasma glucocorticoid concentrations, the concentrations of glucocorticoids found in skim or whole milk after ACTH injection were related to the dose of ACTH injected. Milk glucocorticoid concentration was 2-fold higher after 250 IU of ACTH was injected than after 100 IU of ACTH was injected. The greater plasma glucocorticoid concentration in cows given 100 IU of ACTH may have resulted from additional stress and endogenous ACTH release in these two cows at the time of blood collection. This stress would not have been manifested in the milk because milk samples were collected before collection of blood samples.

Univariate analyses of variance detected highly significant differences ($P < 0.01$) for treatments within each fluid category. Using preinjection concentrations as covariates in each model resulted in no significant preinjection concentration influence ($P > 0.10$) on the final glucocorticoid measurements. Preinjection concentrations of glucocorticoids in skim milk were correlated ($P < 0.05$) with the concentrations in whole milk and plasma before treatment (Table III).

TABLE II. Glucocorticoid Concentration in Bovine Plasma, Whole Milk, and Skim Milk Before and After Hydrocortisone or ACTH Injection.[a]

Treatment and dose	Number of cows	Plasma		Whole milk		Skim milk	
		Before injection	After injection	Before injection	After injection	Before injection	After injection
Hydrocortisone, 1600 mg	10	12.5 ± 2.4	34.3 ± 6.0	0.7 ± 0.2	5.6 ± 1.2	0.7 ± 0.2	3.5 ± 0.6
ACTH, 250 IU	6	9.5 ± 2.3	70.6 ± 13.0	1.0 ± 0.3	23.4 ± 5.7	0.9 ± 0.3	24.6 ± 7.6
ACTH, 100 IU	2	20.9 ± 6.9	109.1 ± 11.4	1.4 ± 0.2	11.5 ± 1.4	1.3 ± 0.2	14.3 ± 3.8

[a] Expressed in nanograms per milliliter ± SE.

TABLE III. CORRELATIONS OF GLUCOCORTICOIDS IN PLASMA, WHOLE, AND SKIM MILK.

	Whole milk before injection	Plasma before injection	Skim milk after injection	Whole milk after injection	Plasma after injection
Skim milk before injection	0.89[a]	0.54	0.53	0.44	0.56
Whole milk before injection		0.36	0.61	0.58	0.54
Plasma before injection			−0.04	−0.17	0.49
Skim milk after injection				0.95	0.68
Whole milk after injection					0.57

[a] $|r| > 0.45$ differs from 0 ($P < 0.05$).

Also, the final fluid concentrations of glucocorticoids were correlated to preinjection concentrations. These results suggest that glucocorticoid concentrations in mammary secretions reflect blood concentrations.

Although the pattern of milk glucocorticoids followed that of plasma glucocorticoids, the concentration of this steroid in both fluids was the reverse of that found for progesterone and estrogens (1, 2); there, progesterone and estrogen were found in higher concentrations in milk than in plasma. Our data suggest that although the mammary gland may actively take up and/or synthesize progesterone (2) and estrogens (1), it does not function the same with glucocorticoids.

The similarity of glucocorticoid concentrations in skim milk and whole milk suggests that glucocorticoids are not associated with milk fat, as has been shown with progesterone (2).

Summary. Intramuscular injections of hydrocortisone (1600 mg) or ACTH (250 or 100 IU) elevated the concentration of glucocorticoids in plasma and milk. The concentration of glucocorticoids in plasma was highly correlated with the concentration in whole milk and skim milk, both before and after either hydrocortisone or ACTH injections. The similarity of glucocorticoid concentrations in whole milk and skim milk fractions suggests that glucocorticoids are not primarily associated with milk fat.

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