Milk cortisol concentrations as an indicator of stress in lactating dairy cows

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ABSTRACT

The aim of this study was to compare cortisol concentrations in plasma (PCC) and milk (MCC) following administration of exogenous $ACTH_{1-24}$ or activation of the hypothalamo-pituitary-adrenal axis by a stressor (transport) in cows. Fifteen cows in mid-lactation were assigned to treatments with $ACTH_{1-24}$ (0.05 mg Synacthen™ iv at 1300h), transport (trucking for two 45 min periods from 1300h) or control (sampling only) in a 3x3 Latin square design. Blood samples were collected hourly from 1300-1500h and a composite milk sample was obtained at routine milking at 1515h.

There was a 6-fold elevation in PCC by 60 min following both ACTH treatment and transportation. At 120 min, PCC was two-fold higher in ACTH treated cows but remained at levels similar to those at 60 min in the transported animals, indicating that the adrenocortical response had been maintained during transport. Mean MCC was 12-fold greater after the transport treatment and a factor of 2 higher after ACTH treatment, compared to the control treatment ($P<0.01$). Values of MCC and PCC at the 120 min sampling were highly correlated ($r = 0.80$; $P<0.001$). This suggested that MCC may be a useful indicator of adrenocortical response to stressors, provided samples are obtained during the period of elevated PCC.

Keywords: Plasma cortisol; milk cortisol; ACTH, transport; dairy cattle.

INTRODUCTION

Increases in plasma cortisol concentrations (PCC) are a reliable indicator of adrenocortical response to an acute stressor (Tarrant *et al.*, 1992). Cortisol moves freely from plasma to milk and increases in milk cortisol concentrations (MCC) have been used to measure responses of the hypothalamo-pituitary-adrenal (HPA) axis of lactating cows to acute stress (Bremel and Gangwer, 1978). This method for assessing the level of stress due to a management procedure or condition is advantageous, because sampling is non-invasive, with minimal disturbance of the cows. Although the confounding effects of the stress of blood-sampling are eliminated, limitations may arise because of the dynamic equilibrium of cortisol concentrations between plasma and milk compartments (Fox *et al.*, 1981). The aim of this experiment was to assess these limitations by measuring changes in MCC and PCC during three treatments which would each produce a characteristic PCC-time curve. The treatments used were transportation, representing peak PCC maintained for the duration of the experiment (Tarrant *et al.*, 1992), injection of a synthetic adrenocorticotropic hormone (ACTH) which would produce a predictable peak followed by a decline so that PCC were at intermediate levels at milk sampling (Verkerk *et al.*, 1994), and a control treatment which would produce persistent basal PCC. Variation in the degree and duration of activation of the HPA axis following these treatments will allow assessment of MCC as a measure of response to stress.

MATERIALS AND METHODS

Animals - Fifteen multiparous dairy cows, aged between 3 and 5 years (Holstein-Friesian, n = 8; Jersey, n = 7), which were in mid-lactation, were randomly assigned to 3 treatment groups. All cows were in the first trimester of pregnancy at the time of the experiment.

Treatments - Each treatment was administered to each cow in a 3x3 Latin square design, with at least 72 h between treatments. Composite milk samples (obtained by constant sampling during the entire milk let-down period) were collected at the morning milking of each treatment day (0700 h; am milking) and animals were returned to pasture until 1300 h. Blood samples were obtained hourly by coccygeal venipuncture from 1300 -1500 h. A composite milk sample was collected from each animal during normal milking at 1515h (pm milking).

The only procedure used for the control treatment was blood sampling. The ACTH treatment was based on an adrenal response test previously validated for use in cattle (Verkerk *et al.*, 1994). An intravenous injection of 0.05 mg ACTH (Synacthen™, CIBA-Geigy, Switzerland) was administered into the coccygeal vein immediately following the blood sampling at 1300 h. Cows in the control and ACTH treatment groups were grazed near to the yards, and moved there for blood sampling at the appropriate times. The first blood sample from each cow in the transport treatment group was collected at 1300h. Cows were then loaded onto a truck (density 1.0 m^2 per cow) which was driven over a designated route for 45 min. At this point cows were unloaded and a further blood sample was collected before repeating the procedure.

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Blood samples were stored in iced water, and centrifuged (2500 rpm for 15 min) within 60 min of collection. Plasma was aspirated and stored at -20°C until analysed. Milk samples were de-fatted by centrifugation (2500 rpm for 15 min), and the skim milk fraction stored at -20°C until analysed.

Assays - Total cortisol concentrations in milk and plasma samples were measured in duplicate using an ^{125}I radio-immunoassay with PEG separation following extraction with ethyl acetate. This assay was developed at AgResearch Ruakura, and utilised an antibody raised in sheep (Ingram *et al.*, 1994). All plasmas were measured in one assay, and all milk samples in a second assay. The mean intra-assay coefficient of variation was 9.5%.

Statistical analyses - Results are reported as mean ± sem. The ratio of MCC at the pm milking to PCC at 120 min after the start of treatment was calculated for each cow. These ratios were also used as response variables. Data were examined by analyses of variance (ANOVA) using a general linear model (GLM) with Minitab (version 8.2; Minitab Statistical Software, State College, Pennsylvania). The model examined for effects associated with treatment, cow and day of treatment. Data were subjected to linear regression analyses using Minitab (version 8.2) to determine equations of best-fit for relationships between cortisol concentrations in milk and plasma.

RESULTS

Milk cortisol concentrations at the am milking and PCC at 0 min were similar for all treatments (Table 1).

At 60 min following the start of treatments, average PCC were elevated to a similar level in the ACTH-treated and transported groups (63.7 ± 2.9 and 65.9 ± 3.7 ng/ml, respectively), and were greater ($P<0.001$) than in the control cows (6.6 ± 0.9 ng/ml). By 120 min, PCC had fallen to 19.8 ± 2.3 ng/ml in the ACTH treated group, whereas in the transported group the levels were similar to those measured at 60 min (72.4 ± 2.9 ng/ml at 120 min vs. 65.9 ± 3.7 ng/ml at 60 min).

Concentrations of cortisol in milk samples collected at the pm milking were significantly influenced by the treatments ($P<0.001$), being greatest following transportation (12.03 ± 1.15 ng/ml). They were moderately elevated with the ACTH treatment (2.41 ± 0.42 ng/ml), and least with the control treatment (1.07 ± 0.12 ng/ml). Basal cortisol levels (control treatment) were higher in milk samples at the pm milking than in the am milking (1.07 ± 0.12 vs. 0.72 ± 0.13 ng/ml; $P<0.01$).

Across all treatments, there was a significant linear relationship between PCC at 120 min and MCC at the pm milking ($P<0.001$; $r = 0.80$). The ratios of MCC at the pm milking to PCC at 120 min were similar for the control and transported groups (0.195 ± 0.016 and 0.166 ± 0.016 respectively), but this ratio differed for the ACTH treated cows (0.125 ± 0.017; $P<0.05$).

DISCUSSION

Activation of the hypothalamo-pituitary-adrenal axis in lactating dairy cows by transportation and injection of ACTH was evaluated by measurement of changes in PCC and MCC. This was done to assess the time-frame and reliability of MCC as an indicator of response to stress since cortisol concentrations in milk and plasma are in dynamic equilibrium (Fox *et al.*, 1981). The results of this experiment support the hypothesis that MCC can be an indicator of activation of the HPA axis in lactating dairy cows. There was a strong correlation between post-treatment MCC and PCC in samples taken 120 min after treatment commenced ($r = 0.80$).

The mean adrenocortical response to the administration of 0.05 mg $ACTH_{1-24}$ appeared to follow a similar pattern to that seen previously in non-lactating cows when the mean peak PCC of 62.3 ng/ml occurred 50.5 min after injection (Verkerk *et al.*, 1994). In the present studies this treatment resulted in moderate elevations of PCC by 120 min after treatment, with corresponding moderate increases in MCC above control levels. In contrast, PCC was high at 60 min after treatment in the transported group, and remained elevated at 120 min, with post-treatment MCC also being much higher following this treatment than the others. This implies that adrenocortical activation was maintained during transportation, and suggests that this treatment may represent a severe acute stressor.

Although MCC reflected PCC in the range of values examined, the ratios of MCC to PCC were not consistent. They were similar for both control and transport treatments, but varied for the ACTH treatment. Samples from the two former treatments were collected following a period in which cortisol concentrations were relatively constant, i.e., a state of equilibrium would have been reached for cortisol movement between milk and plasma compartments. Samples for the ACTH treatment were collected as PCC was declining. The variance in the ratio with this latter treatment suggests that a period of time (as yet unknown) is required for PCC and MCC to reach equilibrium. The ratio of free cortisol concentrations has

TABLE 1: Plasma and milk cortisol concentrations (ng/ml) in samples taken at various intervals in relation to treatment. Values are given as mean (sem). Different superscripts indicate results which are significantly different within a column (i.e. between treatments; $P<0.001$).

	Plasma cortisol concentration			Milk cortisol concentration	
	0 min	60 min	120 min	am	pm
Control	9.1 (1.1)	6.6 (0.9)[a]	6.4 (0.9)[a]	0.72 (0.13)	1.07 (0.12)[a]
ACTH	9.8 (1.7)	63.7 (2.9)[b]	19.8 (2.3)[b]	0.70 (0.11)	2.41 (0.42)[b]
Transport	11.0 (1.4)	65.9 (3.7)[b]	72.4 (2.9)[c]	0.54 (0.06)	12.03 (1.15)[c]

been reported to be constant under steady state conditions between milk and plasma, although the ratio of total cortisol concentrations may vary (Shutt and Fell, 1985). This aspect of the relationship between PCC and MCC requires further investigation.

The increase in mean MCC between am and pm milking in the control cows might be attributed either to either the increased handling associated with the experiment, or to heat stress and/or fly annoyance, since the experiment was carried out during summer. This demonstrates the need to account for diurnal variation in measures of stress.

If animals are exposed to a moderate stressor but then have a recovery period of sufficient length before milking, MCC may not be a reliable indicator of the degree of response. Under the conditions of the experiment, mean MCC reflected mean PCC in samples taken 15 min before milking. When peak PCC occurred about 60 min before milk samples were collected (i.e., with the ACTH treatment), MCC did not reflect the size of the previous peak. This suggests that MCC may be a useful indicator of adrenocortical response to stressors, provided samples are obtained during the period of elevated PCC. The dynamics of the relationship between changes in milk and plasma cortisol concentrations require further clarification.

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