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# Effects of a single glucocorticoid injection on propylene glycol-treated cows with clinical ketosis

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ABSTRACT

This study investigated the metabolic effects of glucocorticoids when administered to propylene glycol-treated cows with clinical ketosis. Clinical ketosis was defined by depressed feed intake and milk production, and a maximal score for acetoacetate in urine. All cows received 250 mL oral propylene glycol twice daily for 3 days and were randomly assigned to a single intramuscular injection with sterile isotonic saline solution ($n = 14$) or dexamethasone-21-isonicotinate ($n = 17$). Metabolic blood variables were monitored for 6 days and adipose tissue variables for 3 days. β-Hydroxybutyrate (BHBA) concentrations in blood decreased in all cows during treatment, but were lower in glucocorticoid-treated cows. Cows treated with glucocorticoids had higher plasma glucose and insulin concentrations, whereas concentrations of non-esterified fatty acids, 3-methylhistidine and growth hormone were unaffected. mRNA expression of hormone-sensitive lipase, BHBA receptor and peroxisome proliferator-activated receptor type γ in adipose tissue was not affected. This shows that lipolytic effects do not appear to be important in ketotic cows when glucocorticoids are combined with PG. Plasma 3-methyl histidine concentrations were similar in both groups, suggesting that glucocorticoids did not increase muscle breakdown and that the greater rise in plasma glucose in glucocorticoid-treated cows may not be due to increased supply of glucogenic amino acids from muscle.



## Introduction

Cows with clinical ketosis often receive combined therapy with glucocorticoids and oral glucogenic supplements, such as propylene glycol (PG). Glucocorticoids decrease blood ketone concentrations in cows with clinical ketosis, and increase blood glucose concentrations in cows with clinical ketosis (Wierda et al., 1987; Shpigel et al., 1996) and in healthy cows (Baird and Heitzman, 1970; Jorritsma et al., 2004; Coffer et al., 2006). It has not been demonstrated whether this increase in blood glucose is due to stimulation of gluconeogenesis in the liver.

Stimulation of muscle breakdown by glucocorticoids could increase the flow of glucogenic amino acids from muscles to the liver to support gluconeogenesis, but effects in early lactation dairy cows are not known. Alternatively, it could be hypothesised that reduced utilisation of glucose by peripheral tissues, such as the mammary gland, might contribute to the rise in blood glucose concentrations. Glucocorticoid treatment reduces milk production in healthy dairy cows (Hartmann and Kronfeld, 1973; Wierda et al., 1987; Coffer et al., 2006), but not in ketotic cows (Philipp et al., 1991).

Lipolytic effects have been observed in healthy cows treated with glucocorticoids (Seifi et al., 2007), although effects of glucocorticoids on adipocyte lipolysis in clinically ketotic cows have not been investigated. We hypothesised that glucocorticoids would not increase lipolysis in the adipose tissue of cows with clinical ketosis, since increased release of non-esterified fatty acids (NEFA) would not be consistent with the clinical recovery that is generally observed following treatment.

A better understanding of the metabolic effects of glucocorticoid treatment in ketotic cows would support decisions in therapy of clinical ketosis. The aim of this study was to investigate the effects of a single glucocorticoid injection when administered to PG-treated cows with clinical ketosis by monitoring clinical recovery, metabolic blood variables and adipose tissue metabolism.

### Materials and methods

This study was performed from 2008 to 2010 at the clinic of the Faculty of Veterinary Medicine, Utrecht University. Cows enrolled in the study were healthy, late pregnant, mainly Holstein-Friesian dairy cows purchased for teaching. A Caesarean section was performed in approximately half of the cows solely for teaching purposes. Cows were kept in tie stalls, milked twice daily (06:00 and 19:00), and fed according to requirements (Product Board Animal Feed). A mixture of maize silage and concentrates (approximately 6 kg product per portion) and grass silage

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(ad libitum) was supplied twice daily at around 07:00 and 18.00. Drinking water was available ad libitum. All experimental procedures were approved by the Ethical Committee on Animal Experiments of Utrecht University (approval number 2008.III.04.034; date of approval 24 April, 2008).

*Monitoring and diagnosis of clinical ketosis*

All peripartum cows ($n = 83$) at the clinic were monitored every morning for clinical ketosis during the first 6 weeks of lactation. Milk production and feed intake were recorded twice daily. Feed intake was assessed semi-quantitatively by estimating the proportion of the maize silage-concentrate mixture consumed after feeding for ~2 h. Urine samples (spontaneous or collected after manual stimulation) were analysed for acetoacetate every morning using urinalysis test strips (Labstix, Siemens). Cows were defined as clinically ketotic based on the combination of three clinical signs: feed intake reduction (≤75% of the maize silage-concentrate mixture consumed), milk production reduction by at least 10%, and a maximal score (++++) for acetoacetate in urine.

*Experimental design*

Upon diagnosis, cows were all treated with 250 mL PG (Eurovet Animal Health) orally twice daily for 3 days and randomly allocated to one of the following single intramuscular injections: (1) 1 mL/50 kg bodyweight sterile isotonic saline solution (negative control; Braun Melsungen); or (2) 1 mg/50 kg body weight dexamethasone-21-isonicotinate (Voren Suspension; Boehringer Ingelheim). Researchers involved in enrolment of cows were blinded to treatment until the data analysis was completed.

The experimental scheme is shown in Table 1. After diagnosis and enrolment, cows were followed for 6 days. Feed intake and milk production were recorded twice daily and urine acetoacetate was measured once daily. Jugular blood samples were collected daily in the morning (before PG administration) into heparin, ethylene diamine tetraacetic acid (EDTA) and sodium fluoride tubes. Samples were centrifuged at 2800 *g* for 10 min and plasma aliquots were frozen at –20 °C. Adipose tissue biopsies were collected on days 1, 2 and 3 from the ischiorectal fossa, alternately on the left and right side of the tail base (van der Drift et al., 2013). Adipose tissue samples were frozen in liquid nitrogen and stored at –80°C until analysis.

Ultrasound measurement of back fat thickness (BFT) in the pelvic region was assessed weekly with a scanner 100 (Pie Medical, Maastricht, The Netherlands) and linear transducer (5.0 MHz) as described by Schröder and Staufenbiel (2006). The last measurement prior to diagnosis was used to indicate the body condition of cows. Any treatments for concurrent diseases (retained fetal membranes and/or endometritis, $n = 9$; enteritis, $n = 1$) at diagnosis were recorded, as was recurrence of clinical ketosis after the study.

*Plasma*

EDTA-treated plasma samples were analysed for β-hydroxybutyrate (BHBA) (kinetic enzymatic method; Ranbut kit; Randox Laboratories), NEFA (colorimetric method; FA 115 kit; Randox Laboratories), insulin (solid-phase $^{125}$I radioimmunoassay, Coat-A-Count Insulin, Siemens) and growth hormone (Taverne et al., 1988, with modifications for a final antibody dilution of 1:15,000 and a standard curve of 0.05–12.5 ng/mL). Heparinised plasma samples were analysed for 3-methylhistidine (3-MH) (Houweling et al., 2012). Sodium fluoride-treated plasma samples were analysed for glucose (oxygen rate method; GLUCm, Synchron test kit, Beckman Coulter).

**Table 1**
Experimental protocol.

| | Day | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| Morning (chronological order) | | | | | | |
| Feed and milk recording | x | x | x | x | x | x |
| Urinalysis acetoacetate (test strip) | x | x | x | x | x | x |
| Blood sampling | x | x | x | x | x | x |
| Fat biopsy | x | x | x | | | |
| IM injection control or glucocorticoids[a] | x | | | | | |
| PO propylene glycol 250 mL | x | x | x | | | |
| Evening | | | | | | |
| Feed and milk recording | x | x | x | x | x | x |
| PO propylene glycol 250 mL | x | x | x | | | |

Cows diagnosed with clinical ketosis in the first 6 weeks of lactation were randomly allocated to treatments consisting of twice daily, orally administered propylene glycol for 3 days (250 mL/dose) and either a single intramuscular injection of sterile isotonic saline solution (control) or a single intramuscular injection of glucocorticoids (dexamethasone-21-isonicotinate).

[a] Dose 1 mg dexamethasone-21-isonicotinate per 50 kg body weight.

*Adipose tissue*

RNA isolation and cDNA synthesis: mRNA expression of hormone sensitive lipase (HSL), niacin receptor (GPR109a, receptor for BHBA), peroxisome proliferator-activated receptor type γ (PPARγ) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in the adipose tissue of cows was assessed on days 1–3. Total RNA was isolated from adipose tissue using the RNeasy Lipid Tissue Mini Kit (Qiagen). After RNA isolation, cDNA synthesis was performed on isolated RNA (iScript cDNA Synthesis Kit, Bio-Rad Laboratories). The cDNA was stored at –20 °C until further analysis.

SYBR green quantitative PCR: For each target gene, the quantitative PCR (qPCR) master mix consisted of 6 μL DNase and RNase free water, 10 μL SYBR green Premix Ex Taq (RR041A, Lonza) and 1 μL of both forward and reverse target specific primers (Isogen Life Science; GAPDH from Invitrogen; 200 nM final concentration; see Appendix: Supplementary material). Two microlitres cDNA solution was added to 18 μL of each target specific master mix in separate qPCR plate wells. The qPCR was performed on a Bio-Rad iQ5 platform. Specific gene amplification was evaluated by DNA melting point analysis following amplification. Relative expression ratios were calculated as described by Pfaffl (2001).

*Statistical analysis*

Thirty-one cows that developed ketosis were enrolled in the study; 17 were treated with glucocorticoids and 14 were included in the control group. The NEFA result from one cow was missing on day 1 (analytical error). Insulin concentrations in plasma were low; when below the detection limit of the test kit (<1.0 mIU/L), sample values were arbitrarily set at 0.9 mIU/L for data analysis. Adipose tissue samples were missing on days 2 ($n = 1$) and 3 ($n = 2$). The qPCR failed for one adipose tissue sample on day 3 and analysis of HSL expression failed for two samples on days 1 and 2.

Blood and adipose tissue variables were analysed as dependent variables in linear mixed-effects models. Cow was always included as a random effect because of repeated measurements per cow. Random intercepts per cow were used for all variables, with the exception of plasma insulin, since nearly all insulin values were below the detection limit at diagnosis. For all blood variables, a random time effect was included (random coefficient for each cow) to model time-dependent correlations between observations within cows. Plasma 3-MH and insulin concentrations were log-transformed to obtain normally distributed model residuals. Fixed effects of time (days 1–6, where day 1 represents diagnosis of clinical ketosis; Table 1), parity (1 or >1), Caesarean section (yes/no) and treatment for concurrent diseases (yes/no), and the covariates days in milk at diagnosis (DIM) and BFT at diagnosis, as well as time × treatment, time × parity, time × BFT, BFT × treatment and treatment × Caesarean section interactions were investigated.

Model parameters were estimated with the maximum likelihood method. Stepwise, backwards model reduction was performed with Akaike's information criterion used for model selection. Models were visually checked by Q–Q plots of residuals (normality) and predicted values versus residuals (linearity, constant variance). Differences in recurrence of ketosis after the study between treatments were analysed using univariate logistic regression analysis. Data were analysed using the statistical package R (version 2.14.2, R Foundation for Statistical Computing).

## Results

Characteristics of cows from both treatment groups are shown in Table 2 and metabolic blood variables are shown in Fig. 1. Acetoactetate scores in urine decreased in all cows during treatment, but scores remained lower in glucocorticoid-treated cows up to day 6 of the experimental period (see Appendix: Supplementary material). Plasma BHBA concentrations decreased after treatment ($P < 0.01$) in both groups, but were lower in glucocorticoid-treated cows ($P < 0.05$). Greater BFT at diagnosis was associated with higher plasma BHBA concentrations on days 5 and 6 ($P < 0.01$) in both treatment groups. Clinical ketosis recurred in 9/14 control cows and 5/17 cows treated with glucocorticoids ($P = 0.06$).

Neither previous Caesarean section nor treatment for concurrent disease affected blood or adipose tissue variables. Plasma glucose concentrations increased after treatment in both groups ($P < 0.05$ on days 2–6), but were higher in glucocorticoid-treated cows ($P < 0.01$ on days 2–6). For all cows, glucose concentrations were lower with greater parity ($P < 0.01$), greater BFT ($P < 0.01$) and fewer DIM at diagnosis ($P < 0.01$).

No treatment effect on plasma NEFA concentrations was observed. Mean plasma NEFA concentrations were lower on days 3–6 ($P < 0.01$) than at diagnosis in primiparous cows, but not in multiparous cows; the latter had higher NEFA concentrations on day