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. Acetoacetate 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

Table 2
Characteristics of cows at diagnosis of clinical ketosis.

	Treatment	
	PG + control	PG + glucocorticoids[a]
Number of cows	14	17
Primiparous	10	13
Multiparous	4	4
Days in milk (mean ± SD)	13 ± 7	20 ± 12
Milk production (kg, mean ± SD)[b]	23.3 ± 6.3	23.9 ± 6.3
Feed intake (proportion, mean ± SD)[c]	0.36 ± 0.25	0.59 ± 0.19
BFT in cm (mean ± SD)	0.86 ± 0.55	0.83 ± 0.46
Caesarean section (n)	9	11
Under treatment at diagnosis (n)[d]	4	6

Cows diagnosed with clinical ketosis in the first six lactation weeks were randomly allocated to treatments consisting of twice daily, orally administered PG for 3 days (250 mL/dose) and either a single intramuscular injection of sterile isotonic sterile isotonic saline solution (control) or a single intramuscular injection of glucocorticoids (dexamethasone-21-isonicotinate).
SD, standard deviation; BFT, back fat thickness measured by ultrasound.

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

[b] Milk production on the evening preceding the day of diagnosis + milk production on the morning of diagnosis.

[c] Pre-treatment feed intake at the morning of diagnosis. Feed intake was semi-quantitatively assessed by estimating the proportion of the offered maize silage-concentrate mixture consumed by the animals at approximately 2 h after feeding.

[d] Representing the number of cows being under treatment for a concurrent disease (retained placenta and/or subsequent endometritis, n = 9; enteritis, n = 1) at the time of diagnosis of clinical ketosis.

2 of the study than at diagnosis ($P < 0.05$). Greater BFT ($P < 0.05$) and fewer DIM ($P < 0.001$) were associated with higher NEFA concentrations in both groups.

No treatment effect on plasma 3-MH concentrations was observed. Mean 3-MH concentrations were lower on days 4–6 ($P < 0.01$) and in primiparous cows ($P < 0.05$). Cows with fewer DIM had higher 3-MH concentrations ($P < 0.001$). Plasma insulin concentrations were higher in glucocorticoid-treated cows on days 2–5 ($P < 0.05$) than in cows receiving PG treatment alone. Plasma insulin increased with DIM ($P < 0.05$). Mean plasma growth hormone (GH) concentrations were lower after treatment every day ($P < 0.05$) except on day 4; there were no differences between treatments. Cows with greater DIM at diagnosis had lower GH concentrations ($P < 0.001$).

Adipose tissue variables are shown in Fig. 2. Expression of HSL in adipose tissue did not differ between treatment groups, but was lower on day 2 than day 3 ($P < 0.05$). Neither treatment nor time influenced mRNA expression of GPR109a (BHBA receptor) or PPARγ in adipose tissue. GPR109a expression was greater when cows had greater DIM at diagnosis ($P < 0.05$).

Discussion

This study investigated the metabolic effects of glucocorticoids in cows with clinical ketosis that were treated with PG. Clinical ketosis was defined as a decrease in feed intake and milk production, and was confirmed by measuring urine acetoacetate.

Treatment with PG, alone or in combination with glucocorticoids, reduced plasma BHBA concentrations and urine acetoacetate scores. Earlier studies have shown decreases in BHBA concentrations when ketotic cows were treated with either PG or glucocorticoids (Wierda et al., 1987), or when dexamethasone therapy was combined with IV glucose infusion (Shpigel et al., 1996). In our study, dexamethasone therapy was combined with 3 days of PG treatment to produce a more prolonged and gradual glucose supply than with glucose infusion. When PG supplementation was stopped, mean ketone body concentrations in blood and urine increased in the control group, but remained low in cows treated with glucocorticoids.
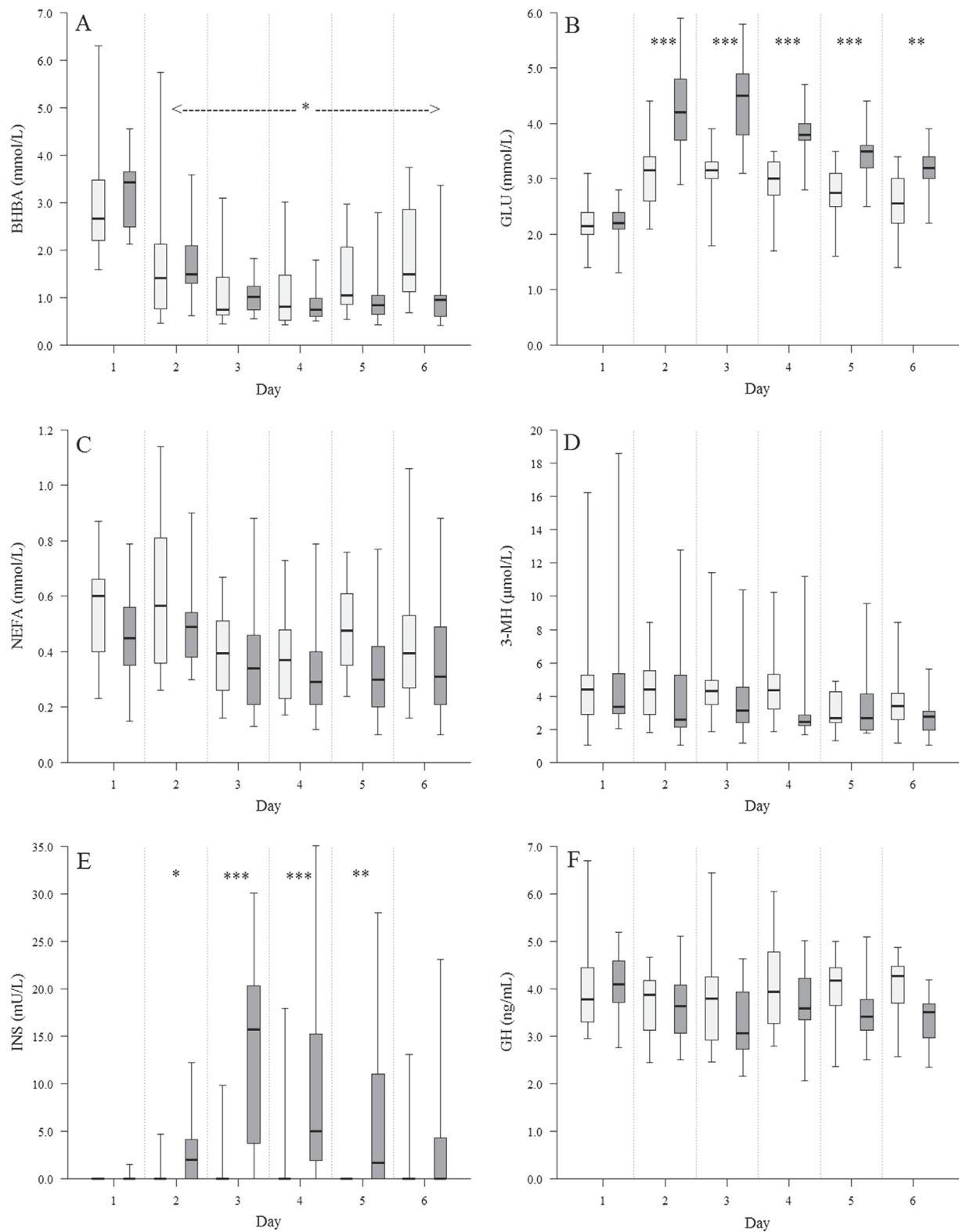
The study was conducted at a university clinic, where pregnant cows were purchased to teach Caesarean sections; thus, the majority of cows in the trial had a Caesarean section (67%). This is different from regular dairy practice, because these cows could have delivered naturally. The incidence of clinical ketosis was relatively high in the clinic (47% of cows), and allowed collection of sufficient clinical cases of ketosis in a controlled environment over a 2 year monitoring period. Randomisation of cows to treatment controlled for any changes over time.

Baseline characteristics of cows were similar, except for the number of DIM at diagnosis, which differed between treatment groups despite randomisation ($P = 0.04$, Welch two sample t test). Therefore, all models were corrected for DIM to distinguish between the effects of treatment and stage of lactation. For plasma BHBA, glucose and insulin concentrations, treatment effects were significant even after correction for DIM in the models. We analysed plasma GH concentrations to investigate whether lower GH levels could provide an explanation for the metabolic effects of glucocorticoids, which are known to have antagonistic effects on GH secretion and actions in humans (Mauras, 2009), GH concentrations in neonatal calves (Sauter et al., 2003) and insulin-like growth factor 1 and 2 concentrations in cows (Maciel et al., 2001). However, there was no difference in GH concentrations between treatments after correction for DIM.

Glucose concentrations at diagnosis were low in clinically ketotic cows. Insulin concentrations were also low in these animals and, in most cases, could not be quantified using the test kit. Mean plasma glucose concentrations increased in all ketotic cows, but significantly more in cows treated with glucocorticoids. In both groups, the increase in blood glucose concentrations may result from enhanced gluconeogenesis, since glucogenic precursors were supplemented. Detailed measurement of feed intake was not feasible in our study, so the possibility cannot be excluded that study outcomes were influenced by differences in feed intake between treatment groups, since glucocorticoids may stimulate feed intake.
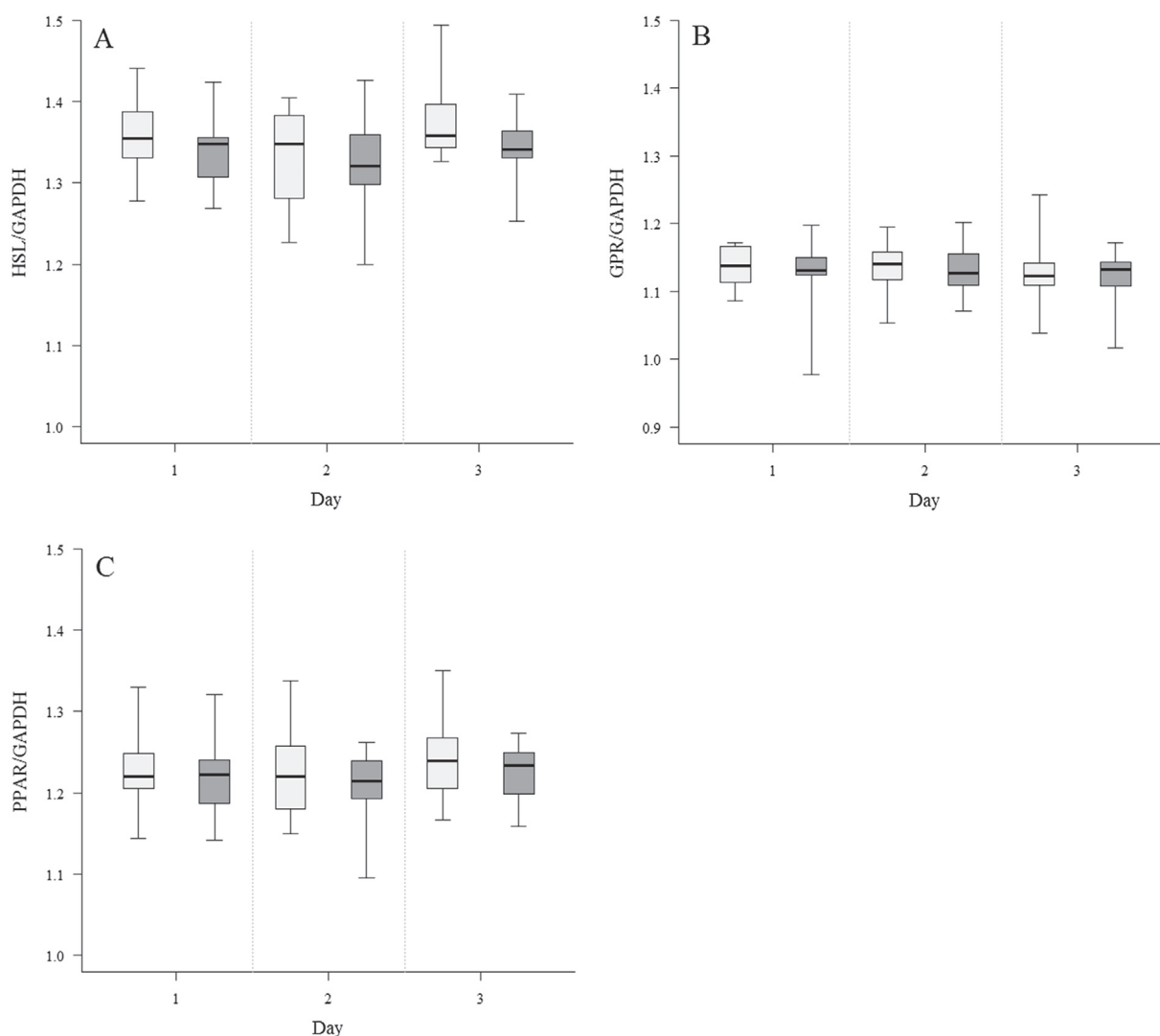
Plasma 3-MH concentrations were similar, suggesting equal muscle breakdown in both groups and a similar supply of glucogenic amino acids from muscle to liver for gluconeogenesis. In previous studies, glucocorticoids did not increase or decrease the activity of the gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PEPCK) in the livers of healthy cows (Baird and Heitzman, 1970; Butler and Elliot, 1970), calves (Hammon et al., 2005) and sheep (Filsell et al., 1969; Franko et al., 2007). It remains to be elucidated whether the greater rise in plasma glucose concentrations in glucocorticoid-treated ketotic cows was due to a stimulation of liver gluconeogenesis by a greater feed intake, or to reduced peripheral use of glucose.

Glucocorticoids have been shown to reduce milk production in healthy dairy cows in most (Hartmann and Kronfeld, 1973; Wierda et al., 1987; Coffer et al., 2006) but not all (Jorritsma et al., 2004) studies. Hartmann and Kronfeld (1973) demonstrated reduced mammary uptake of glucose following administration of dexamethasone. A reduced glucose drain for lactose production by the mammary gland would provide a plausible explanation for the rise in blood glucose concentrations. However, in cows with clinical ketosis, a reduction in milk production was not observed after treatment with glucocorticoids (Philipp et al., 1991). There was no indication of a marked reduction in milk yield in cows treated with glucocorticoids in this study. Milk production data (see Appendix: Supplementary material) were not tested statistically, because clinical ketosis depresses milk yield and expected milk production for cows as a reference was lacking; the difference in DIM at diagnosis would have interfered with any existing treatment effects and a relatively large measurement error was expected due to the lack of automated milk recording in this study.

Fig. 1. Plasma concentrations of (A) β-hydroxybutyrate (BHBA), (B) glucose (GLU), (C) non-esterified fatty acids (NEFA, $n = 13$ on day 1 for control group), (D) 3-methylhistidine (3-MH), (E) insulin (INS) and (F) growth hormone (GH) of cows with clinical ketosis randomly allocated to treatments consisting of twice daily, orally administered PG for three days (250 mL/dose) and either a single IM injection of sterile isotonic saline solution (control, light grey boxes, $n = 14$) or a single intramuscular injection of dexamethasone-21-isonicotinate (glucocorticoids, dark grey boxes, $n = 17$). Boxes represent median and interquartile range; whiskers include all cases. Day 1 represents diagnosis of clinical ketosis (pre-treatment values). Asterisks indicate significant differences between treatments at the time points (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; for BHBA, the asterisk indicates a significant effect of treatment but no significant treatment × time interaction).

Fig. 2. Adipose tissue expression of (A) hormone sensitive lipase (HSL), (B) niacin receptor GPR109a (GPR) and (C) peroxisome proliferator-activated receptor type γ (PPARγ) relative to the house keeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in cows with clinical ketosis randomly allocated to treatments consisting of twice daily, orally administered PG for 3 days (250 mL/dose) and either a single intramuscular injection of saline (control, light grey boxes, $n = 14$) or a single intramuscular injection of dexamethasone-21-isonicotinate (glucocorticoids, dark grey boxes, $n = 17$). Boxes represent median and interquartile range; whiskers include all cases. Day 1 represents diagnosis of clinical ketosis (pre-treatment values). Missing values for all adipose tissue variables were at days 2 (glucocorticoids, $n = 1$) and 3 (control, $n = 1$; glucocorticoids, $n = 2$). Missing values for HSL expression were at days 1 (glucocorticoids, $n = 2$) and 2 (control, $n = 1$; glucocorticoids $n = 1$).

Lipolytic effects of glucocorticoid therapy would be unfavourable in cows with clinical ketosis. Injection of a glucocorticoid (isoflupredone) increased serum NEFA and BHBA concentrations, and increased the risk for subclinical ketosis in healthy postpartum cows (Seifi et al., 2007). In our study, glucocorticoids did not appear to induce lipolysis. We observed a decrease in mRNA expression of HSL in adipose tissue of cows in both groups on day 2, which may result from the increased concentration of insulin, which is anti-lipolytic (Degerman et al., 1997; Berggreen et al., 2009).

We hypothesised that glucocorticoids could influence the negative feedback of BHBA on adipocytes via expression of the BHBA receptor. Stimulation of this receptor by BHBA inhibits in vitro lipolysis in postpartum dairy cows (Metz and van den Bergh, 1972; Rukkwamsuk et al., 1998; van der Drift et al., 2013). In our ketotic cows, expression of the BHBA receptor was not influenced by treatment. Adipocyte PPARγ expression was assessed to investigate whether glucocorticoids influenced net fatty acid release via PPARγ mediated stimulation of glyceroneogenesis and subsequent fatty acid re-esterification in adipocytes. PEPCK is the key enzyme in adipocyte glyceroneogenesis (Reshef et al., 2003) and glucocorticoids decrease PEPCK mRNA in murine adipocytes (Franckhauser et al., 1995). However, glucocorticoids did not influence PPARγ expression in adipocytes of cows in this study.

Glucocorticoids are known to have marked effects on muscle metabolism in humans by inducing muscle breakdown, reducing glucose and amino acid uptake, and inhibiting muscle protein synthesis (Vegiopoulos and Herzig, 2007; Schakman et al., 2008). Reduced uptake of glucose by skeletal muscle was demonstrated in rats, where glucocorticoids inhibited insulin-stimulated recruitment of the glucose transporter GLUT4 to the cell surface (Weinstein et al., 1998). Increased muscle proteolysis did not appear to occur in our glucocorticoid-treated cows. Muscle protein stores may already have been largely depleted, since cows mobilise substantial amounts of protein in the periparturient period (Tamminga et al., 1997; van der Drift et al., 2012). Reduced glucose and amino acid uptake and protein synthesis in muscle may have occurred in glucocorticoid-treated cows, and could have contributed to their higher plasma glucose concentrations. Similarly, glucose uptake in adipose tissue

may have been decreased, as has been demonstrated in rats (Burén et al., 2002, 2008).

Conclusions

Cows with clinical ketosis had lower plasma BHBA concentrations for 6 days after treatment when glucocorticoids and glucogenic supplements were combined. Greater muscle breakdown did not occur and cannot explain the greater increase in plasma glucose and insulin concentrations in glucocorticoid treated cows. Lipolytic effects do not appear to be important in ketotic cows when glucocorticoids are combined with PG.

Conflict of interest statement

This study was partly funded by Boehringer Ingelheim, The Netherlands, marketing authorisation holder of the dexamethasone-21-isonicotinate product used for the glucocorticoid treatment. The study sponsor was not involved in the study design, collection, analysis or interpretation of the data from this study, nor in the writing of the manuscript.

Acknowledgements

Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.tvjl.2015.01.016.

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