Pharmacokinetic and Biochemical Profiling of Sodium Dichloroacetate in Pregnant Ewes and Fetuses[S]

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ABSTRACT

Sodium dichloroacetate (DCA) is an investigational drug that shows promise in the treatment of acquired and congenital mitochondrial diseases, including myocardial ischemia and failure. DCA increases glucose utilization and decreases lactate production, so it may also have clinical utility in reducing lactic acidosis during labor. In the current study, we tested the ability of DCA to cross the placenta and be measured in fetal blood after intravenous administration to pregnant ewes during late gestation and labor. Sustained administration of DCA to the mother over 72 hours achieved pharmacologically active levels of DCA in the fetus and decreased fetal plasma lactate concentrations. Multicompartmental pharmacokinetics modeling indicated that drug metabolism in the fetal and maternal compartments is best described by the DCA inhibiting lactate production in both compartments, consistent with our finding that the hepatic expression of the DCA-metabolizing enzyme glutathione transferase zeta1 was decreased in the ewes and their fetuses exposed to the drug. We provide the first evidence that DCA can cross the placental compartment to enter the fetal circulation and inhibit its own hepatic metabolism in the fetus, leading to increased DCA concentrations and decreased fetal plasma lactate concentrations during its parenteral administration to the mother.

SIGNIFICANCE STATEMENT

This study was the first to administer sodium dichloroacetate (DCA) to pregnant animals (sheep). It showed that DCA administered to the mother can cross the placental barrier and achieve concentrations in fetus sufficient to decrease fetal lactate concentrations. Consistent with findings reported in other species, DCA-mediated inhibition of glutathione transferase zeta1 was also observed in ewes, resulting in reduced metabolism of DCA after prolonged administration.

Introduction

Defects in mitochondrial function have been associated with cardiomyopathies and disorders of skeletal muscle (Holmgren et al., 2003; Magida and Leinwand, 2014; Fatica et al., 2019). We previously found that sheep fetuses exposed to hypercortisolemia in late pregnancy suffered an increased rate of stillbirth and perinatal mortality (Keller-Wood et al., 2014); this effect of a chronic increase in cortisol is similar to the observation that the risk of late gestation stillbirth is increased in human pregnancies complicated by chronic maternal hypercortisolemia secondary to maternal stress (László et al., 2013; Silver and Ruiz, 2013) or by

maternal Cushing's syndrome (Brue et al., 2018). Transcriptomic and metabolic analyses of heart and skeletal muscle from the fetuses and newborns from our ovine model of gestational hypercortisolemia suggested that inhibition of mitochondrial metabolism (Richards et al., 2014; Walejko et al., 2019; Joseph et al., 2020) may be a predisposing factor in the development of bradycardia and altered ECG patterns at the time of delivery (Antolic et al., 2018). Excess cortisol exposure in utero is associated with overexpression of pyruvate dehydrogenase kinase (PDK) 4, encoding the predominant pyruvate dehydrogenase kinase isoform in cardiac and skeletal muscle (Pilegaard and Neufer, 2004). PDK inhibits the mitochondrial pyruvate dehydrogenase complex (PDC), which catalyzes the rate-determining step in the oxidation of glucose-derived pyruvate to acetyl CoA, thereby linking cytoplasmic glycolysis to the tricarboxylic acid cycle and oxidative phosphorylation (Stacpoole, 2017). Four isoforms of pyruvate dehydrogenase kinase (PDKs 1–4) phosphorylate and inhibit PDC, whereas pyruvate dehydrogenase phosphatases 1 and 2 dephosphorylate the complex and restore catalytic activity (Sugden and Holness, 2003). Inhibition of PDC activity decreases the utilization of pyruvate and molecules, such as lactate, in equilibrium with pyruvate.

The prototypic PDK inhibitor, dichloroacetate (DCA), has been used for many years as an investigational drug in the treatment of diverse congenital and acquired causes of PDC deficiency associated with


ABBREVIATIONS: CL, clearance; DCA, sodium dichloroacetate ; GSTZ1, glutathione transferase zeta1; MM, Michaelis-Menten; PCR, polymerase chain reaction; PDC, pyruvate dehydrogenase complex; PDK, pyruvate dehydrogenase kinase.

mitochondrial energy failure. DCA has been shown to have therapeutic efficacy in neonates, improving right ventricular carbohydrate utilization and function in neonatal piglets after pulmonary artery banding (Kajimoto et al., 2019) and reducing brain injury in neonatal mice after hypoxic ischemia (Sun et al., 2016). DCA was reported to be effective in treating a human newborn with lactic acidosis due to inherited mitochondrial disease (Bennett et al., 2020). Therefore, we hypothesized that DCA, administered intravenously to pregnant ewes, would cross the placenta and provide a therapy for treatment of lactic acidosis and mitochondrial dysfunction during labor and delivery. Our long-term goal is to determine if DCA can improve myocardial bioenergetics and thus mitigate or prevent fetal bradycardia and distress during labor in cortisol-exposed fetuses. The present studies tested the hypothesis that DCA can cross the placenta to the fetal circulation when administered to pregnant ewes and achieve pharmacologically active concentrations in the ovine fetus.

Effective therapy with DCA is dependent on DCA concentrations, which is limited by rapid metabolism of DCA to the inactive metabolite, glyoxylate, by the enzyme glutathione transferase zeta1 (GSTZ1) (Cornett et al., 1999). The enzyme is predominantly expressed in liver cytoplasm and mitochondria, although other tissues express much lower protein levels (James et al., 2017; Squirewell et al., 2020). Repeated doses of DCA can suppress the expression of GSTZ1, thereby decreasing the rate of DCA metabolism (Cornett et al., 1999). DCA metabolism by GSTZ1 is influenced by GSTZ1 haplotype in humans (Shroads et al., 2012; James et al., 2017) and also by age (Shroads et al., 2008) and liver chloride concentration (Jahn et al., 2018). The predicted ovine sequence is 84.0% similar to the human sequence (NM_145870: Homo sapiens glutathione S-transferase 1) and 79.3% similar to the rat sequence (NM 001109445.1) of GSTZ1. The ovine GSTZ1 sequence is homologous to the EGT haplotype of the human "fast" metabolizers (Shroads et al., 2012, 2015), predicting that sheep are rapid DCA metabolizers. However, in human fetal tissue, activity of GSTZ1 is very low (Li et al., 2012, Zhong et al., 2018), suggesting that the fetus might have little, if any, capacity for DCA metabolism. The present studies therefore modeled DCA pharmacokinetics to address the change in drug metabolism with repeated administration over time in the both the pregnant ewe and ovine fetus.

Materials and Methods

The pregnant sheep is a commonly used model of human pregnancy, as many aspects of ovine fetal development mirror those in humans (Barry and Anthony, 2008; Morrison et al., 2018). In addition, the model allows for placement of catheters for simultaneous blood sampling of both mother and fetus (Barry and Anthony, 2008). Two groups of animals were studied: in Study I ewes were treated with DCA as boluses every 12 hours for a total of five injections; in Study II, ewes were treated with a bolus followed by infusion of DCA. Adult, time-dated pregnant Dorset-cross ewes were used in both Studies I and II. Ewes used in Study I ($n = 4$) were obtained from the University of Florida Animal Sciences Department, and ewes in Study II ($n = 10$) were obtained from Advanced Ovine Solutions (Attica, NY). Throughout the duration of the study, animals were housed in a facility with a light-controlled (12-hour light, 12-hour dark cycle) and temperature-controlled room. Ewes were maintained on a diet of pelleted feed and were supplemented with limited hay. The University of Florida Institutional Animal Care and Use Committee approved all animal use.

Animal Surgery. Surgery was performed on animals in late gestation under isoflurane anesthesia (Study I: 131 ± 1-day gestation, $n = 4$; Study II: 126 ± 1-day gestation, $n = 10$; term in these sheep is 146–148 days gestation). Catheters were placed ipsilaterally in a maternal femoral artery and vein and in the fetal tibial artery (Jensen et al., 2002). The catheters were exteriorized through an exit site on the flank. The animals were administered flunixin meglumine during surgery (intravenous dose) and meloxicam (oral dose) postoperatively. Postsurgical care also included monitoring rectal temperatures and treatment with ampicillin

(Polyflex; Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO), intramuscularly, for 5 days and daily cleaning of the catheter exit site with povidone-iodine tincture.

Experimental Details. In Study I, the pharmacokinetics study of DCA (TCI America, Portland) was performed in late gestation at least 5 days after surgery. The drug was dissolved in sterile isotonic 0.9% NaCl solution. Each dose was administered over 5 minutes to ewes in five doses of 25 mg/kg every 12 hours. This dose was chosen based on the effective dosing regimen in studies in adult humans with primary mitochondrial disease (James et al., 2017) and assuming that there would be distribution of DCA based on combined fetal and maternal body weight. After the first and fifth doses (at 0 and 48 hours), maternal and fetal blood samples (~2 ml) were collected at −5, 0, 5, 10, 15, 20, 30, 60, 120, 240, 360, 480 and 720 minutes. Samples were placed immediately on ice before centrifuging to separate plasma, which was stored at −80°C until assayed.

In Study II, DCA was administered to the ewe over a 24-hour period during labor, which was induced by infusions of oxytocin (4.1 mU/kg oxytocin infused over 5 minutes every 30 minutes) (Shinozuka et al., 1999). Oxytocin treatment began 48 hours prior to the administration of DCA. Because of the low plasma concentrations in the fetus in Study I with maternal injection at 12-hour intervals and the observed decrease in metabolism with repeated injections of DCA, the dosing regimen was altered for the subsequent study. In Study II, the drug was administered as a bolus followed by infusions of decreasing rates over the next 24 hours. The first dose of DCA was administered to the ewe as an intravenous infusion of 25 mg/kg over 3 minutes; this was followed by an infusion of 12.5 mg DCA/kg per hour for 8 hours and an infusion of 6.25 mg DCA/kg per hour for the next 16 hours. This cohort of animals included ewes that either had been treated with cortisol (1 mg/kg per day) starting on day 115 of pregnancy or that received no DCA. Two animals treated with DCA delivered either before or immediately after the last sample at 24 hours of DCA. These samples were not included in the analysis of DCA concentrations or of plasma glucose and lactate. Plasma was collected for measurement of glucose and lactate during the periods of DCA treatment, and an aliquot was stored at −80°C until for analysis of DCA concentrations by gas chromatography–mass spectrometry (Yan et al., 1997).

Determination of DCA Concentration in the Plasma. The DCA levels in the blood samples were measured after derivatization and extraction, as previously described (Yan et al., 1997). Plasma samples (200 μl) and an internal standard of 2-ketohexanoic acid (10 μg) were mixed with 500 μl of boron trifluoride–methanol complex in a glass tube and heated for 15 minutes at 110°C. Upon cooling, 1 ml of methylene chloride and 1 ml of water were added to the reaction mixture, then vortexed for 5 minutes and allowed to stand for 10 minutes. After centrifugation at 3000 rpm, the methylene chloride layer was transferred to a glass vial for gas chromatography–mass spectrometry analysis.

Pharmacokinetic Modeling of DCA in Maternal and Fetal Plasma. Plasma concentration-time values of DCA in both ewes and fetuses in Study I were subjected to noncompartmental and multi-compartmental analyses (Phoenix 64; Certara USA, Inc.). Noncompartmental analysis on day 1 and day 3 concentration-time data was performed separately for ewes and fetuses, both to understand the extent of accumulation and to develop a combined pharmacokinetics model of DCA in mother and fetus. The area under the curve for ewes and the fetuses were calculated separately using the linear trapezoidal method for day 1 and day 3. Clearance (CL) and volume of distribution were calculated for both day 1 and day 3 for ewes (Phoenix WinNonlin version 7.1).

Plasma concentration-time data for both ewes and fetuses were further subjected to multicompartmental modeling (Phoenix NLME version 7.1). Population pharmacokinetics models with two or three disposition compartments, first-order, Michaelis-Menten (MM), parallel first-order, parallel MM, or parallel first-order, parallel, and MM elimination were evaluated to best describe the concentration-time data. Residual error was described by a combined error (proportional and additive) model. A semimechanistic multicompartmental model describing the autoinhibition of DCA was developed to obtain the best fit for concentration-time data of both mothers and fetuses. The model included an intercompartment between the mother (A_1) and fetus (A_3), which physiologically could be considered the placental compartment (A_2). To describe the decreased clearance and increased exposure of DCA from day 1 to day 3, a model describing potential autoinhibition (INH_M and INH_F) of clearances of both ewes (CL) and fetuses (CL_F) was implemented as described in eqs. 1–5 (Bulitta et al., 2013).

$$dA_1/dt = -((1 - INH_M)*CL*C) - (A1*K_{12} - A_2*K_{21}) \quad (1)$$

$$dINH_M/dt = K_p*((I_{max}*C)/(IC_{50}+C)) - INH_M \quad (2)$$

$$dA_2/dt = (A_1*K_{12} - A_2*K_{21}) - (A_2*K_{23}$$
$$-A_3*K_{32}) - ((1 - INH_F)*CL_F*C_2) \quad (3)$$

$$dA_3/dt = (A_2*K_{23} - A_3*K_{32}) \quad (4)$$

$$dINH_F/dt = K_p*((I_{max}*C_2)/(IC_{50}+C_2)) - INH_F \quad (5)$$

Here, K_p, I_{max}, and IC_{50} are turnover rate constants for inhibition, maximum inhibition, and concentration causing 50% of I_{max}, respectively. Micro constants, K_{12}, K_{21}, K_{23}, and K_{23}, are intercompartmental distribution rate constants.

Glucose and Lactate Concentrations. Maternal and fetal glucose and lactate concentrations were measured from plasma samples collected into tubes containing potassium oxalate and sodium fluoride (YSI Model 2700 glucose/lactate analyzer, Yellow Springs, OH). The samples were maintained on ice prior to analysis, and all readings were done in duplicate.

GSTZ1 Genotyping. We tested for haplotypes of the GSTZ1 gene in several commonly used breeds of sheep. DNA was extracted from the lung tissue of six sheep fetuses (two each from Suffolk, Rambouillets, and Dorset cross breeds) using the QIAamp DNA Mini Kit (Qiagen, MD) on approximately 250 mg of tissue. The concentration of DNA was determined on a nanodrop spectrophotometer (ThermoFisher, Waltham, MA). Primers for polymerase chain reaction (PCR) and sequencing were designed for the predicted GSTZ1 gene (ensemble ID: ENSOARG00000002420) using Primer 3 software, from 10 exons (Supplemental Table 1). Designated DNA fragments were amplified by PCR, using a HotStar Taq Master Mix kit (Qiagen) on a thermocycler. Multiple PCR conditions with different annealing temperatures and times were tried until single sharp bands with the right DNA fragment size were obtained. The PCR amplicons were verified on agarose gel. The PCR amplicons were then sequenced by Sanger sequencing, and the obtained sequences were analyzed by comparing with published predicted sheep GSTZ1 sequence (Shroads et al., 2012).

Protein Expression of GSTZ1. Ewes were sacrificed 24 hours after the last dose of DCA, and tissues were collected. Samples of liver, 2–3 g, were homogenized in a volume of buffer 1 (0.25 M sucrose, 0.05 M Tris-Cl pH 7.4, 5 mM EDTA, 1 mM dithiothreitol (DTT), 0.2 mM phenylmethylsulfonyl fluoride (PMSF) equal to four times the tissue weight. The homogenates were centrifuged at 600g for 10 minutes to sediment nuclei and unbroken cells, at 13,300g for 20 minutes to sediment mitochondria, and at 145,000g for 45 minutes to sediment microsomes (Smeltz et al., 2019). The 145,000g supernatant was the cytosol fraction. The mitochondrial pellet was suspended in buffer 1 and resedimented at 13,300g for 20 minutes. The washed pellet was resuspended in a volume of buffer 1 equal to half the weight of tissue. The protein concentration of each subcellular fraction was determined with a bicinchoninic acid reagent (Thermo-Fisher, Waltham, MA). Liver mitochondrial and cytosolic fractions were flushed with nitrogen and stored in aliquots at −80°C until use.

For quantification of GSTZ1 protein expression, the liver cytosol samples, 80 μg protein, as well as a single rat liver cytosol sample, 5 μg (as reference), were separated by sodium dodecyl sulfate polyacrylamide gel-electrophoresis with 12% polyacrylamide gels, then electrophoretically transferred onto nitrocellulose membranes (Bio-Rad Laboratories, Hercules, CA). Once transferred, the membrane was allowed to block in low-fat milk for 1 hour, then overnight in primary antibody (custom-prepared rabbit-anti-rat GSTZ1) (Smeltz et al., 2019) at a 1:200 antibody to milk ratio. After washing, membranes were treated with horseradish peroxidase–labeled goat anti-rabbit secondary antibody, 1:5000 (GE Healthcare, Chalfont St. Giles, UK) and incubated for 1 hour. The membrane was washed, then exposed to the ECL Plus Western Blotting detection reagents (Thermo Fisher Scientific, Waltham, MA), and protein signals were quantitated on the ChemiDoc MP System (Bio-Rad Laboratories, Hercules, CA). Preliminary studies showed that polyclonal antibodies raised in rabbits to rat and human GSTZ1 full-length protein cross-reacted with a sheep protein at the expected molecular weight of GSTZ1. The intensities of bands in the sheep liver cytosol samples were compared with the intensity of the band from the rat liver cytosol, adjusted for total protein loaded, to give relative expression levels.

Statistical Analysis. Maternal and fetal glucose and lactate values in Study I on day 1 and day 3, and values over the 24 hours of treatment in Study II, were analyzed by two-way analysis of variance with repeated measures across time (Sigmaplot version 13.0). Between-day comparisons at multiple time points were performed by post hoc tests, using the Bonferroni t test method. In case of failed

conditions of normality, a nonparametric alternative was used. Maternal lactate and fetal glucose to lactate ratios between day 1 and day 3 were analyzed by Friedman's repeated measures analysis of variance on ranks due to the failed normality test. In Study II, plasma glucose was analyzed by one-way ANOVA corrected for repeated measures; plasma lactate values were not normally distributed and were analyzed by Friedman's repeated analysis of variance on ranks. Data from fetuses that were delivered before the end of the 24 hours sampling period were excluded; therefore samples from only eight ewes and fetuses are included in this analysis. Differences in GSTZ1 protein expression between maternal and fetal livers and between control and DCA-treated sheep were evaluated by one-way ANOVA, with multiple comparisons.

Results

Pharmacokinetic Analysis of DCA in the Mother and Fetus. Repeated administration of DCA to the ewe increased DCA concentrations in both the ewe and the fetus. In Study I, in which DCA was administered over 3 days, an increase in trough (dosing interval) concentrations of DCA was observed in both mothers and fetuses with time (Fig. 1), consistent with the inhibition of metabolism of DCA by GSTZ1 from repeated drug dosing. Accordingly, pharmacokinetics parameters derived from noncompartmental analysis of the DCA concentrations in Study I revealed marked increases in the area under the plasma DCA concentration-time curve on day 3 relative to day 1 (10.5-fold and 147-fold for ewes and fetuses, respectively). To describe the autoinhibition of the metabolism of DCA in both ewes and fetuses and to account for ewe-to-fetus transfer of DCA, a best fit multicompartmental pharmacokinetic model was developed (Fig. 2) best described by maternal (central), placental, and fetal compartments. The best fit model was selected, based on the residual sum of squared error, Akaike's information criterion, visual predictive checks (Supplemental Fig. 1), diagnostic plots (Fig. 3; Supplemental Fig. 2), and bootstrapping. The concentration-time data were best described by the
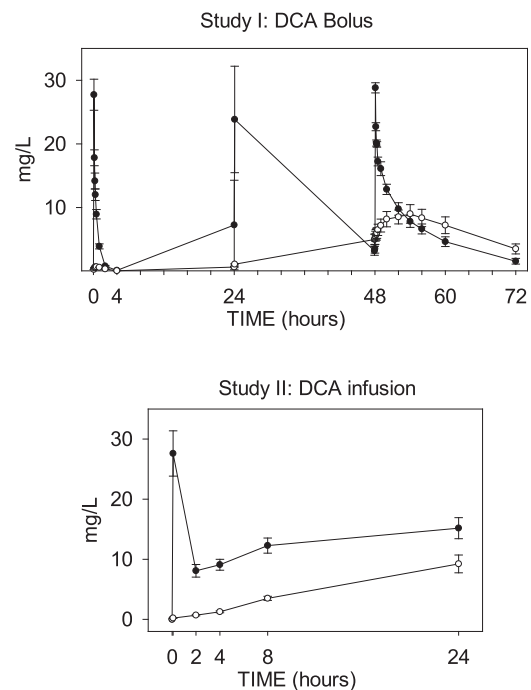

Study I: DCA Bolus

Study II: DCA infusion

Fig. 1. Mean plasma concentration-time profile of DCA in fetus and mother in Study I (upper panel) after maternal intravenous bolus doses of DCA (25 mg/kg every 12 hours) in pregnant ewes or in Study II (lower panel) after maternal bolus and infusion of DCA (25 mg/kg at 0 hour, followed by an infusion of 12.5 mg DCA/kg/h for 8 hours and an infusion of 6.25 mg DCA/kg/h from 8 to 24 hours). Closed circles indicate maternal values, and open circles indicate fetal values. Error bar represents S.E.M.
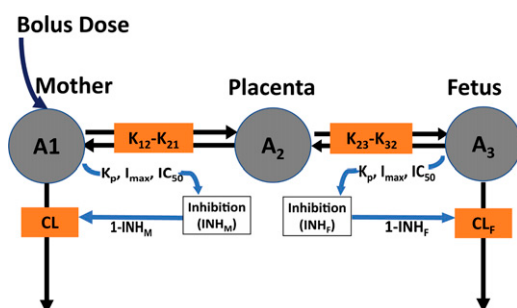
Fig. 2. Pharmacokinetic model for mother and fetus receiving 25 mg/kg DCA over five doses in 3 days using a multicompartmental model. A1, A2, and A3 are maternal, placental, and fetal compartments, respectively. Micro constants, K_{12}, K_{21}, K_{23}, and K_{32} are intercompartmental distribution rate constants. Autoinhibition (INH_M and INH_F) of clearances of both ewes (CL) and fetuses (CL_F) are described using K_p (turnover rate constant for inhibition), I_{max} (maximum inhibition), and IC_{50} (concentration causing 50% of I_{max}).

autoinhibition of mother and fetal clearances. Clearances of DCA without autoinhibition in ewes and fetuses were 195.7 ± 1.2 and 122.4 ± 28.2 l/h, respectively, suggesting lower expression of GSTZ1 in the fetus, compared with the mother (Table 1). Apparent volumes of distribution of both mother (63.3 l) and fetus (51.6 l) were greater than the total blood volume, reflecting extravascular distribution and accumulation in tissues. The maximum extent of autoinhibition was found to be 74%, with an IC_{50} of 0.04 mg/L. The developed model accommodated the complex pharmacokinetics of DCA in both ewes and fetuses simultaneously, including the DCA-mediated inhibition of GSTZ1. The DCA concentration versus time in ewes and fetuses in Study II was also consistent with this model (Fig. 3).

Expression of GSTZ1 in the Sheep. The sequences obtained from the six animals of different strains of sheep were analyzed for single nucleotide polymorphisms, and no single nucleotide polymorphisms were identified in the sheep *GSTZ1* gene.

Immunoblot analysis of the maternal and fetal control livers showed higher levels of expression of GSTZ1 in the untreated ewe compared with the untreated fetus. In both ewe and fetus, DCA treatment reduced the protein expression of GSTZ1 in liver cytosol (Fig. 4; Supplemental Fig. 3). There was no difference in GSTZ1 protein expression between the DCA-treated maternal and fetal samples. GSTZ1 expression was also detected in liver mitochondrial extracts from control sheep, but expression was lower than in cytosol (data not shown). Preliminary studies examined GSTZ1 expression in kidney cytosol and mitochondria and in placental cytosol, but expression was very low in kidney and not detected in placenta, so no further studies were done with these tissues.
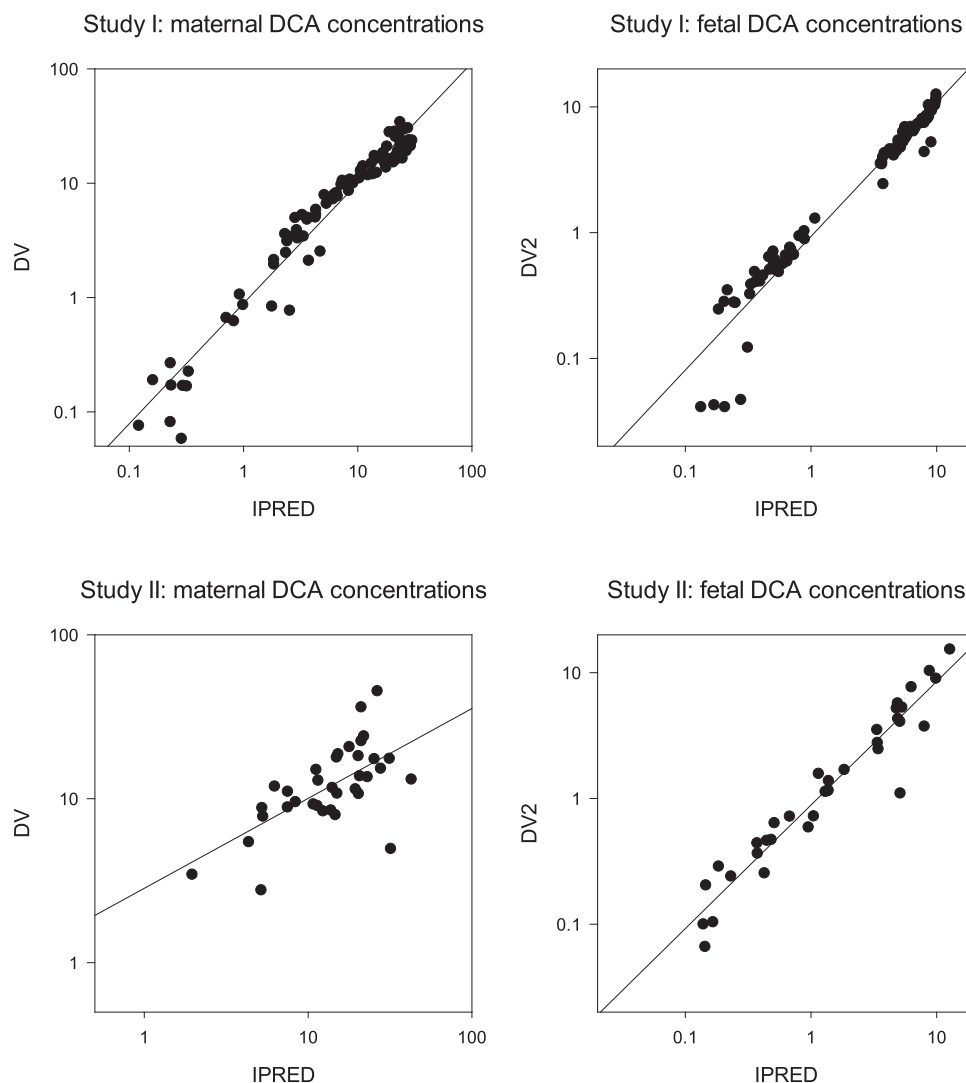

Fig. 3. Observed plasma concentrations vs. individual prediction by final model of DCA. Dependent (observed) concentration (DV) and individual predicted concentration (IPRED) for data from Study I (upper panels; $r^2 = 0.95$ in ewe, left, and 0.94 in fetus, right) and Study II (lower panels; $r^2 = 0.41$ in ewe, left, and 0.93 in fetus, right).

TABLE 1
Estimated pharmacokinetic parameters for DCA derived from multicompartmental pharmacokinetic analysis

Parameter	Units	Estimate (% CV)	95% CI
Volume of central compartment (V)	l	63.3 (10.8)	54.4–72.7
Clearance without inhibition (CL)	l/h	195.7 (12.2)	158.9–220.1
Distribution rate constant from central to placental compartment (K_{12})	l/h	0.18 (22.3)	0.16–0.27
Distribution rate constant from placental to central compartment (K_{21})	l/h	0.16 (21.6)	0.15–0.17
Distribution rate constant from placental to fetal compartment (K_{23})	l/h	3.08 (35.1)	2.7–5.8
Distribution rate constant from fetal to placental compartment K_{32}	l/h	2.2 (20.3)	2.0–3.3
Clearance in fetus without inhibition (CL_F)	l/h	122.4 (28.2)	105.3–196.4
Fetal volume of distribution (V_2)	l	51.6 (12.2)	48.9–65.7
Concentration for 50% inhibition (IC_{50})	mg/l	0.04 (11.2)	0.03–0.05
Maximum inhibition (I_{max})		0.74 (5.2)	0.74–0.86
Turnover rate constant of inhibition (K_p)		1.29 (5.4)	1.13–1.29

CI, confidence interval.

Effect of DCA on Plasma Glucose and Lactate Levels. Maternal glucose was significantly decreased on day 3 compared with day 1 ($P = 0.04$) in Study I, but there was no significant time effect or interaction between day and time (Fig. 5). There was an overall significant decrease in maternal lactate levels ($P < 0.001$). There was a significant effect of day of treatment ($P = 0.03$) and time ($P = 0.005$) but no significant interaction of day and time on fetal glucose concentrations, and there were an overall effect of day ($P = 0.003$), effect of time ($P = 0.02$) and an interaction of day and time ($P = 0.010$) on fetal plasma lactate concentrations. In both the ewe and the fetus, the glucose-to-lactate ratio was significantly increased ($P = 0.007$ and <0.001 respectively) on day 3 as compared with day 1 (data not shown).

In Study II, the infusion of DCA significantly decreased both maternal and fetal plasma lactate concentrations ($P < 0.002$) but did not significantly change maternal or fetal plasma glucose concentrations (Fig. 6).

Discussion

DCA has long been investigated for its effects on intermediary metabolism and mitochondrial bioenergetics (James et al., 2017). Phase I–III clinical trials have evaluated its therapeutic effects in patients with cancer, diabetes, hyperlipoproteinemia, cardiac and pulmonary diseases, and primary mitochondrial disorders causing congenital lactic acidosis. Our study suggests that DCA can also be given in pregnancy to achieve metabolically significant effects in both mother and fetus.
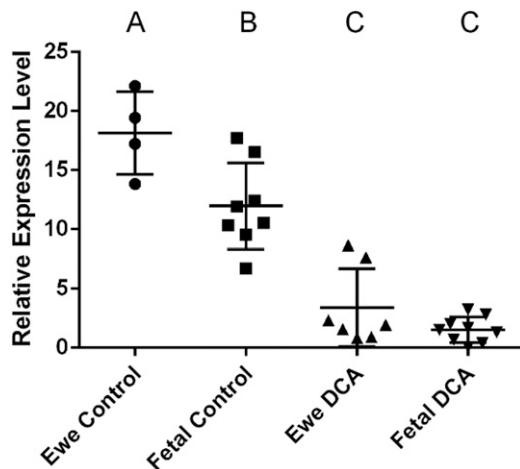

Fig. 4. DCA administration reduced the relative expression of GSTZ1 in fetal and maternal liver cytosol. Individual data points, means, and S.D. are shown for each group. Different letters, A, B, C, indicate groups with significantly different expression ($n = 4$–8 per group).

One of the major limitations of transplacental therapy is the inability of drugs to cross the placental barrier and reach the fetus in effective concentrations. The DCA anion is a small, water- and lipid-soluble molecule with a size of 128.9 KDa (151 KDa as the sodium salt) that can readily enter cells through convective (paracellular) absorption. In addition, the structural similarity of DCA to pyruvate and lactate enables its transport via the pyruvate and lactate transporters, SLC16A4 (MCT4) and SLC5A8 (SMCT1). These transporters are also present in the placenta (Dimmer et al., 2000; Babu et al., 2011), providing a mechanism by which DCA crosses to the fetus.

PDC catalyzes the rate-determining step in the aerobic oxidation of glycolytically derived pyruvate and of molecules, such as lactate and alanine, in equilibrium with pyruvate. DCA maintains PDC in the dephosphorylated and active state by inhibiting PDKs. High circulating concentrations of lactate are characteristic of fetal distress (Eguiluz et al., 1983; Smith et al., 1983) and other obstetric complications (Vannuccini et al., 2016) that, in turn, lead to decelerations in fetal heart rate. In clinical trials of severe lactic acidosis (Stacpoole et al., 1983, 1992; Stacpoole, 1993), myocardial ischemia or failure (Bersin and Stacpoole, 1997; Michelakis et al., 2017), and pulmonary arterial hypertension (Michelakis et al., 2017), DCA reduced circulating lactate and improved cardiac efficiency, likely by shifting cardiac metabolism from fat to carbohydrate oxidation. DCA has also been similarly effective in adult rodent models of ischemia/reperfusion injury (Bersin and Stacpoole, 1997) and in hemorrhagic and septic shock (Subramani et al., 2017; McCall et al., 2018). To our knowledge, DCA has not previously been tested in a fetal model, although it has been shown to be effective in pediatric models of disease (Sun et al., 2016) and in human pediatric cases of congenital mitochondrial disease (Stacpoole et al., 2008), including in a newborn with severe lactic acidosis (Bennett et al., 2020).

Pharmacokinetic modeling of the DCA drug concentrations across time in the maternal and fetal blood suggested that it crossed the placenta and attained metabolically effective concentrations in the fetus. Sustained dosing of the drug increased its concentration in the fetus and produced significant decreases in fetal lactate levels on day 3. Using this information, we administered DCA to the ewe as a rapid infusion, followed by a prolonged infusion to elicit sustained, pharmacodynamically effective concentrations.

DCA is a mechanism-based inhibitor of GSTZ1, resulting in reduced expression as well as reduced activity of the enzyme and decreased plasma DCA clearance upon repeat exposure. The inhibition of GSTZ1 protein expression is thought to occur secondary to protein degradation caused by formation of adducts (Anderson et al., 1999; Tzeng et al., 2000); incubation of GSTZ1 with DCA and glutathione has been shown to cause adduct formation (Cornett et al., 1999; Anderson et al., 2002).
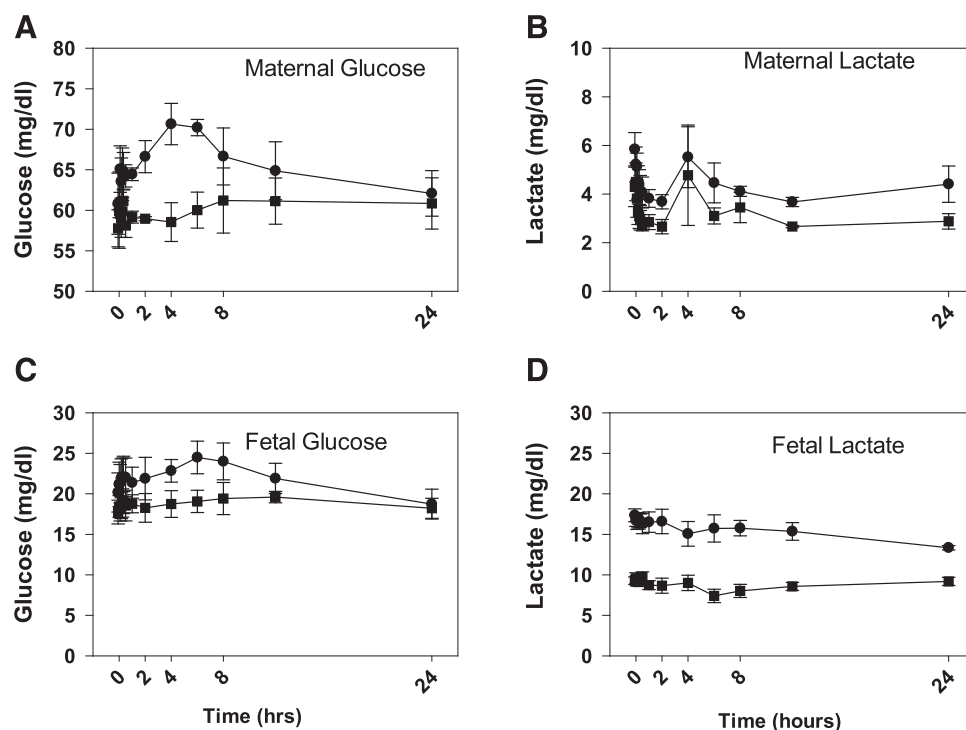
Fig. 5. Maternal (A and B) and fetal (C and D) concentrations of glucose (A and C) and lactate (B and D) after the first dose (day 1, circles) or fifth dose (day 3, squares) in Study I (*n* = 4).

Consistent with these effects, we found that the expression of GSTZ1 was decreased in tissues in both the pregnant ewe and in fetuses receiving DCA. Moreover, our pharmacokinetic modeling of the kinetics data in the pregnant ewes and fetuses also indicates that DCA-mediated GSTZ1 inhibition occurred. Studies in humans show that the metabolism of DCA is affected by the haplotypes of the *GSTZ1* gene in the general population, dichotomizing individuals into "slow" and "fast" DCA metabolizers (Shroads et al., 2004, 2012; Langaee et al., 2018). The sheep sequence corresponds to the sequence in the EGT haplotype, which facilitates the most rapid metabolism of the drug in humans. Our

pharmacokinetic analysis also demonstrates that the ewe rapidly metabolizes DCA. Our genotyping of the limited number of samples from various breeds of sheep failed to identify any single nucleotide polymorphisms in the gene in this species.

The doses used in this study were chosen based on the effective concentrations in adult humans and in children. In studies in adults, doses of 25 mg/kg per day have been found to be effective in primary mitochondrial disease and well tolerated in treatment of cancer (James et al., 2017). DCA can cause a reversible sensory-motor peripheral neuropathy in chronically treated adults and, rarely, in children who are exposed for
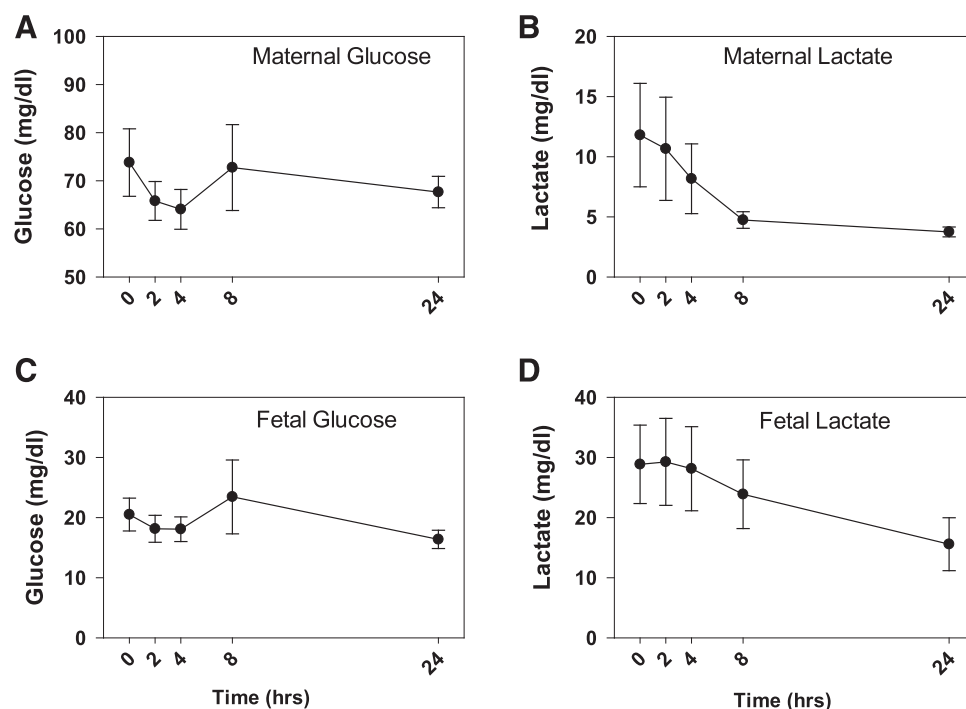
Fig. 6. Maternal (A and B) and fetal (C and D) concentrations of glucose (A and C) and lactate (B and D) during maternal DCA treatment in Study II (*n* = 8).

weeks or months to the drug (Stacpoole et al., 2019). Doses of 25 mg/kg per day were not found to be toxic even in long-term studies in children with mitochondrial disease (Berendzen et al., 2006; Abdelmalak et al., 2013), despite evidence that long-term treatment in adults with mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes resulted in peripheral neuropathy (Stacpoole et al., 2019).

Studies on human fetal liver samples have indicated that there is no appreciable expression of GSTZ1 in the human fetus and that GSTZ1 activity increases with postnatal age (Li et al., 2012; Zhong et al., 2018). The greater expression of GSTZ1 observed in term fetal sheep liver suggests that at full term, the ovine liver is more mature in this regard than that of human fetuses at term and leads to lower concentrations of DCA after the initial dose, which suggests that lower doses of DCA might be needed in human pregnancy. In the one report in a newborn, a dose of 30 mg/kg per day given in two to three divided doses was needed to prevent lactic acidosis (Bennett et al., 2020), suggesting that there is some ability to metabolize DCA in term infants. If one assumes no further clearance of the drug in the human fetus at term, then the doses used in this study would be expected to produce initial concentrations 2–3-fold higher than produced in our study in fetal sheep. We would expect the concentrations would approach the levels produced in the ovine fetus at 24 hours of treatment but would be less than those produced in the mother during treatment.

A potential caveat based on our results is that lactate is a major substrate for vital fetal organs, in particular the heart; hence, excessive oxidative removal of lactate by the placental compartment and the fetal liver by activation of PDC could decrease lactate as a nutrient to the heart. We did not observe any obvious toxicity of DCA in the mother or fetus. However, future studies should determine whether in utero administration of DCA is associated with side effects in newborn lambs. Most previous studies of DCA toxicity used high doses for long periods (Donohue et al., 2003; Stacpoole, 2011). A study in pregnant rats administered 0, 14, 140, or 400 mg/kg per day on gestation days 6–15 showed a not-observed effect limit of 14 mg/kg per day, although much higher doses did produce cardiovascular anomalies (Smith et al., 1992).

These studies are the first to explore the pharmacological effects of DCA in pregnancy. They demonstrate that DCA can cross the placenta and achieve concentrations that significantly reduce fetal lactate concentrations in plasma. Thus, DCA might have therapeutic utility in situations of fetal distress associated with increased lactate, metabolic acidosis, and cardiac distress. Future studies are required to evaluate DCA's therapeutic potential in such conditions.

Authorship Contributions

Study design: Joseph, Langaee, James, Stacpoole, Keller-Wood.

Conducted experiments: Joseph, Horne, Wood, James, Keller-Wood.

Performed data analysis: Joseph, Sharma, Horne, James, Keller-Wood.

Wrote or contributed to the writing of the manuscript: Joseph, Sharma, Langaee, James, Stacpoole, Keller-Wood.

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Supplemental Material for manuscript: DMD-AR-2020-000330

Title: Pharmacokinetic and biochemical profiling of Sodium Dichloroacetate in pregnant ewes and fetuses

Authors: Joseph S, Sharma A, Horne LP, Wood CE, Langaee T, James MO, Stacpoole PW, and Keller-Wood M
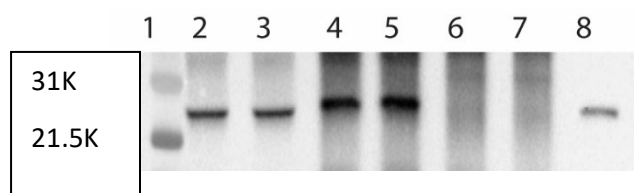
Journal: Drug Metabolism and Disposition
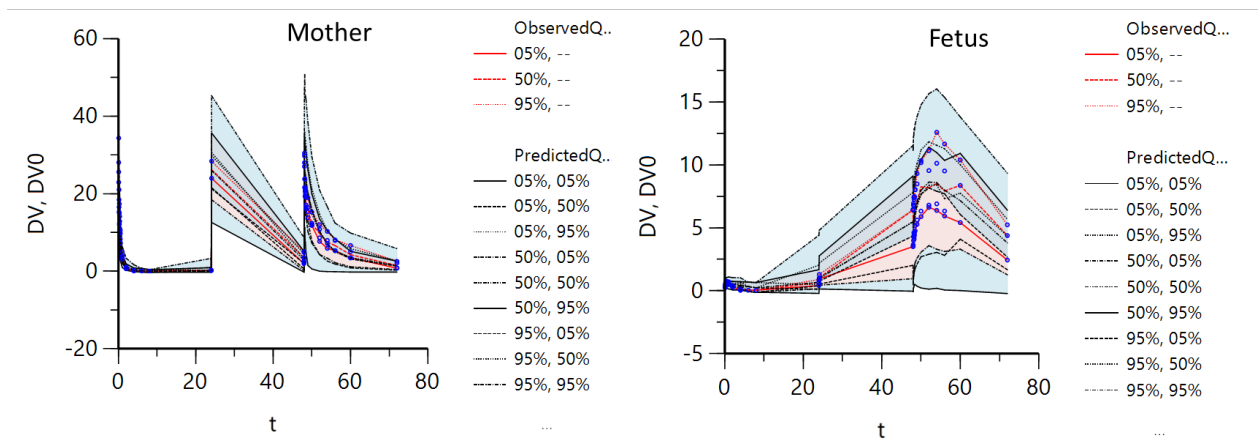
Supplemental Figure 1: Visual predictive checks (VPC) of the final model for DCA in mother and fetus
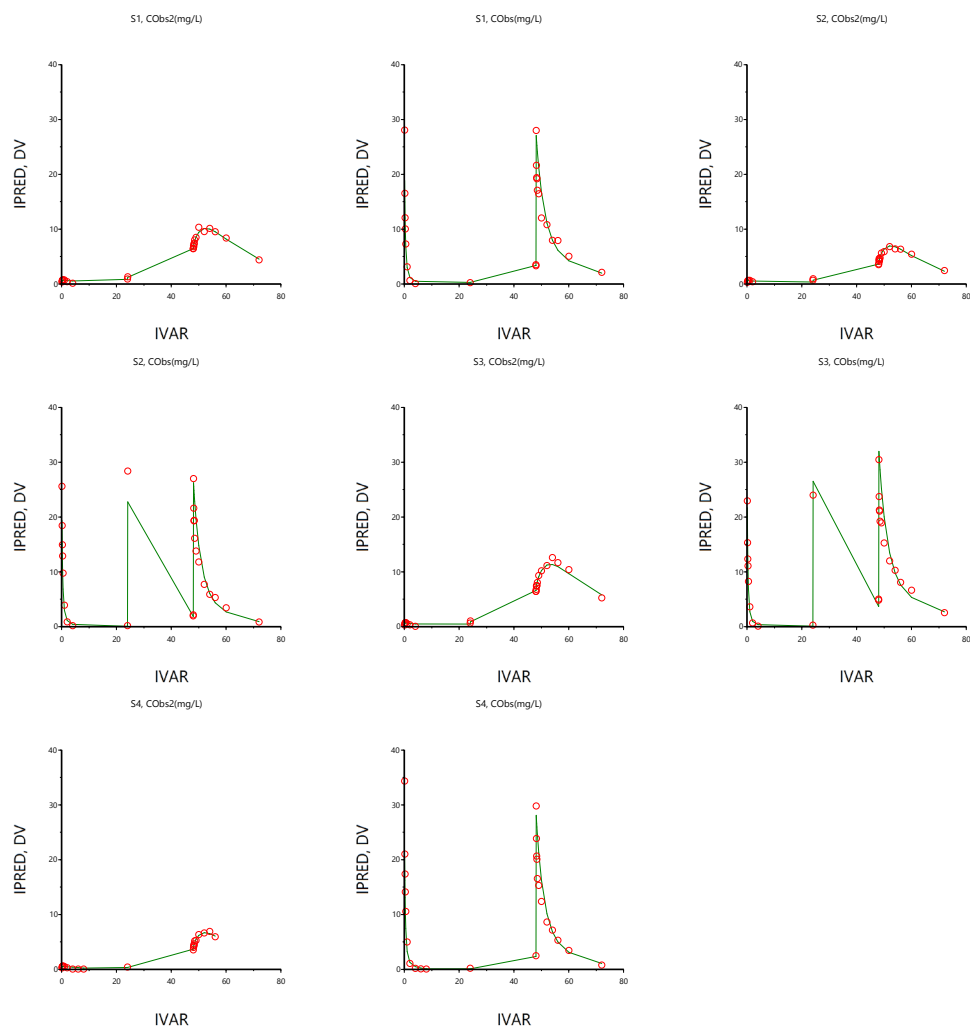
Supplemental Figure 2: Observed concentrations and individual predictions versus time (CObs, mother and CObs2, fetus) using data in Study I

	1	2	3	4	5	6	7	8

31K

21.5K

1. Mol wt markers (31 and 21.5 kDaltons)
2. Maternal control, 80 µg protein
3. Maternal control, 80 µg protein
4. Maternal control, 80 µg protein
5. Maternal control, 80 µg protein
6. Fetal control, 80 µg protein
7. Fetal control, 80 µg protein
8. Rat liver cytosol, 5 µg protein

Supplemental Figure 3: Representative figure showing GSTZ1 bands from maternal and fetal mitochondrial samples.

Supporting Table 1. Primer sequences used in PCR and sequencing of the ovine GSTZ1 gene

EXON	primer	Sequence	GC%	Tm	Extension and Annealing conditions
1	FP	ACCCGAGACCTTGGGGCGCA	70	69.3	95°- 30s, 62°-30s (40 cycles), 72°(1min)
	RP	GCGCCTCAGTGTTTCTAAGCAGC	56.5	63.2	
2	FP	TTCAGCAGTTGCTCCCTCTT	50	60.13	95°- 30s, 58°-45s (50 cycles), 72°(1min)
	RP	GCTCTTTGTGTGGTTCAGCA	50	60.13	
3	FP	GACCCCCAAGGCCTCTATC	55.1	59.32	95°- 30s, 56 °-30s (40 cycles), 72°(1min)
	RP	CCAAGGTGGACATGTCTTCA	55	59.59	
4	FP	TGGCGCAGAGAAAGAAGATT	45	60.1	95°- 30s, 58°-45s (50 cycles), 72°(1min)
	RP	TGTCCCATGAGGGAGAGC	61.1	59.7	
5	FP	TTCCTGCTTGCTCCTTTCTG	50	60.65	95°- 30s, 58°-45s (50 cycles), 72°(1min)
	RP	CTCCTCACTTCCCAAAGCAA	50	60.37	
6	FP	GTGTTGGAAGTGGAGGAGGA	55	60.09	95°- 30s, 58°-45s (50 cycles), 72°(1min)
	RP	CTGGGGGATGGGTATTTTCT	50	60.01	
7	FP	GGTCCAGGGTAGGACAGGTG	65	61.75	95°- 30s, 58°-45s (50 cycles), 72°(1min)
	RP	GACCACTGTCAAGGCCAAAG	55	60.69	
8	FP	ATTCCCTCCAGCACACACTC	55	60.12	95°- 30s, 60°-30s (45 cycles), 72°(1min)
	RP	CACCACAGCTGCCTCCTC	66.7	60.58	
9	FP	GCTACCCACAGAGCTGGTT	60	60.85	95°- 30s, 58°-45s (50 cycles), 72°(1min)
	RP	AGGAGGAAGCTGGGTGTTTT	50	60.11	

10	FP	GCAGGGCCACCTTCCTAC	66.7	60.62	95°- 30s, 62°-30s (50 cycles), 72°(45s)
	RP	GCTCACTTCTAGGCAAGTTCAG	50	58.42	