

# Clinical Utility of Plasma Fructosamine Concentration as a Hypoglycemic Biomarker during Early Lactation in Dairy Cattle

A.A. Megahed , M.W.H. Hiew, and P.D. Constable

**Background:** Plasma fructosamine concentration ([FRA]) is a widely used long term hyperglycemic biomarker in humans and dogs, but its clinical usefulness as a hypoglycemic biomarker in dairy cattle is uncertain.

**Objectives:** To evaluate the relationship between plasma [FRA] and glucose concentration ([gluc]) as well as indices of energy balance during early lactation in dairy cattle, and to characterize the influence of plasma total protein concentration ([TP]) and albumin concentration ([albumin]) on [FRA].

**Animals:** Convenience sample comprising 103 periparturient Holstein–Friesian cattle.

**Methods:** Plasma [gluc], [TP], [albumin], and other clinicopathologic indices of energy status were determined periodically from Day 4 postpartum. Body condition score (BCS) was assessed, and backfat thickness (BFT) and longissimus dorsi muscle thickness (LDT) were measured ultrasonographically. Plasma [FRA] was measured at approximately 28 days postpartum. Associations between plasma [FRA] and study variables were evaluated using Spearman's rho and stepwise forward linear regression. Statistical significance was declared at  $P < 0.05$ .

**Results:** A positive association was detected between plasma [FRA] and mean plasma [gluc] from Days 4–28 postpartum ( $r_s = +0.36$ ,  $P < 0.001$ ), and between plasma [FRA] and LDT ( $r_s = +0.28$ ,  $P = 0.007$ ), BCS ( $r_s = +0.23$ ,  $P = 0.029$ ), and BFT ( $r_s = +0.21$ ,  $P = 0.043$ ). Multivariable regression identified a positive association between plasma [FRA] and mean plasma [gluc] and [albumin] from Days 4–28 postpartum. Correcting plasma [FRA] for [albumin] improved the association ( $r_s = +0.46$ ,  $P < 0.001$ ) between plasma [FRA] and mean plasma [gluc].

**Conclusions and Clinical Importance:** Plasma [FRA] does not provide a clinically useful method for quantifying the magnitude of hypoglycemia or negative energy balance in dairy cows during early lactation.

**Key words:** glycated protein; hyperketonemia; hypoglycemia.

Dairy cattle undergo a period of negative energy balance during early lactation, primarily because their dietary energy intake is inadequate relative to the level of milk production.<sup>1,2</sup> The amount of glucose metabolized by dairy cattle during the periparturient period increases from approximately 0.5 kg of glucose per day before parturition to approximately 2.5 kg glucose per day during the first 3 weeks of lactation; the latter is at least 4 times the amount (0.6 kg/day) of glucose produced by the liver during gluconeogenesis when cattle are at maintenance intake.<sup>3–5</sup> A plasma glucose concentration ([gluc]) of less than 55 mg/dL

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## Abbreviations:

[albumin]	albumin concentration
[BHB]	whole blood $\beta$ -hydroxybutyrate concentration
[FRA]	fructosamine concentration
[gluc]	glucose concentration
[NEFA]	non-esterified fatty acids concentration
[TC]	total cholesterol concentration
[TP]	total protein concentration
BCS	body condition score
BFT	back fat thickness
LDT	longissimus dorsi muscle thickness
NBT	nitroblue tetrazolium
28M	milk production at 28 days postpartum
F305M	mature-equivalent fat production for 305 days of lactation
M305M	mature equivalent milk production for 305 days of lactation
P305M	mature-equivalent protein production for 305 days of lactation

(3.0 mmol/L) in early lactation has been suggested to provide an accurate method of identifying inadequate energy intake in dairy cattle for their level of milk production.<sup>6,7</sup>

However, plasma [gluc] may undergo diurnal variations in cows fed once or twice a day because of the circadian rhythm of hormones involved in the control of glucose metabolism and feed intake,<sup>8,9</sup> although the timing and magnitude of daily changes in plasma [gluc] in early lactation vary from study to study.<sup>10–15</sup> Accordingly, measurement of long-term indices of plasma [gluc], such as the plasma concentration of fructosamine ([FRA]), could potentially have clinical value in reflecting the magnitude of negative energy balance, similar to biochemical indices such as the plasma concentration of non-esterified fatty acids ([NEFA]), an index of the rate of fat mobilization and

$\beta$ -hydroxybutyrate ([BHB]), an index of the completeness of oxidation of mobilized fat in the liver. The clinical usefulness of plasma [NEFA] as a biomarker of negative energy balance suffers from a lack of specificity because plasma [NEFA] rapidly increases in response to sympathetic activation and stress, and is at its highest value before the morning feeding.<sup>10,12–15</sup> In addition, the test is expensive to run (usually >\$10US/test) and the availability of practical NEFA field tests is limited. Similarly, the clinical usefulness of a single measurement of blood, serum, or plasma [BHB] as a biomarker of negative energy balance does not have perfect specificity. This is because postprandial increases in plasma [BHB] occur in cattle fed grain once or twice a day,<sup>9–11</sup> or fed a total mixed ration (TMR) once or twice a day,<sup>12–14</sup> because of metabolism of absorbed butyrate by ruminal epithelium to BHB.<sup>12</sup> Postprandial increases in plasma [BHB] were observed in lactating dairy heifers fed ad libitum<sup>15</sup> but not in dairy cattle fed ad libitum in a large commercial herd in Germany.<sup>16</sup>

Fructosamine is a stable keto-amine rearrangement product (1-deoxy-I-[lysyl-albumin]fructose) that results from the non-enzymatic irreversible linkage of free aldehyde groups of sugar molecules, such as glucose or fructose, and the amino group of protein molecules, usually albumin or other plasma proteins such as IgG.<sup>17,18</sup> Plasma [FRA] therefore depends on the average plasma [gluc] and total protein concentration ([TP]), plasma protein composition, and the rate of plasma protein turnover.<sup>19,20</sup> Because the mean lifespan of bovine albumin is 16.5 days,<sup>21</sup> it is generally accepted that plasma [FRA] reflects the average plasma [gluc] over the previous 2–3 weeks.<sup>20</sup> Plasma [FRA] will therefore increase with prolonged hyperglycemia or prolonged hyperproteinemia, and decrease with prolonged hypoglycemia or increased protein turnover.<sup>22</sup> The preferred plasma protein for glycation in humans is albumin, where 1.4–16.0% of albumin molecules are glycated and approximately 80% of fructosamine is glycated albumin.<sup>17</sup> The favored protein for glycation in domestic animals has not been established.<sup>19</sup>

Plasma [FRA] has been investigated as a clinicopathologic test for identifying the presence of long-term disturbances of glucose metabolism such as diabetes mellitus in dogs and cats,<sup>23,24</sup> hyperglycemia in horses,<sup>25</sup> hypoglycemia because of pregnancy toxemia in sheep,<sup>26,27</sup> and hypoglycemia because of excessive negative energy balance in lactating dairy cattle.<sup>8,28–30</sup> We hypothesized that plasma [FRA] would be a herd-level monitoring tool for predicting prolonged hypoglycemia and the magnitude of negative energy balance in lactating dairy cattle. Accordingly, the first objective of this study was to evaluate the relationship between plasma [FRA] and [gluc], as well as between plasma [FRA] and indices of energy balance, specifically NEFA, BHB, BCS, BFT, and longissimus dorsi muscle thickness (LDT), during early lactation in a large number of dairy cattle. The 2 previous cattle studies examining the association between plasma [FRA] and mean plasma [gluc] in early lactation utilized small sample sizes and produced conflicting results, in that a weak association was identified in a study of 23 Norwegian Red dairy cows in Norway ( $r_s = +0.29$ ,  $P < 0.01$ ),<sup>28</sup> but no association was observed in a study of 17 Holstein cows in Uruguay ( $P = 0.16$ ).<sup>29</sup> Moreover, the mean plasma [FRA] reported in the one study that did

identify a significant relationship between plasma [FRA] and mean plasma [gluc]<sup>28</sup> was more than 5 times greater than that reported in other studies in dairy cattle,<sup>8,29–31</sup> raising questions regarding assay specificity in that study. The second objective of this study was to characterize the association between plasma [FRA] and plasma [TP], between plasma [FRA] and plasma albumin concentration ([albumin]), and between plasma [FRA] and plasma globulin concentration ([globulin]) in order to identify the most likely plasma protein fraction (albumin or globulin) for glycation in cattle.

## Materials and Methods

All methods were evaluated and approved by the Purdue University Animal Care and Use Committee under protocol number 1201000598. The study reported here was part of a larger study investigating energy and potassium homeostasis in the periparturient period, and the prediction of parturition and dystocia in Holstein–Friesian cattle. These results have been published elsewhere.<sup>32–36</sup>

### Animals, Housing, and Feeding

An observational study was conducted using a convenience sample of 103 periparturient Holstein–Friesian cattle (32 primiparous, 71 multiparous) from the Purdue University Dairy Research and Education Center over a 10-month period between May 29, 2012 and March 29, 2013. Enrolled cows were moved from an outdoor dry lot to temperature controlled (16–27°C) individual box stalls (3.1 × 3.1 m) at 4 days before the estimated parturition date based on breeding records and pregnancy diagnosis at approximately 40 days after breeding. After calving, all cows were kept in the same temperature-controlled individual box stalls for 3 days or until they recovered from any postpartum health problems before being moved to a free stall barn. Parturition cows were fed an acidogenic TMR (dietary cation–anion difference [DCAD] = –18 mEq/100 g of dry matter {DM}), where DCAD = ([Na<sup>+</sup>] + [K<sup>+</sup>]) – ([Cl<sup>-</sup>] + [S<sup>2-</sup>]) based on formulations recommended by the National Research Council<sup>37</sup> for late gestation cows. After calving, all cows switched to a lactating cow TMR based on formulations recommended by the National Research Council<sup>37</sup> for fresh cows. All animals were deemed healthy based on daily routine physical examinations performed by members of the research team (all veterinarians) in the individual box stalls. Cows suspected by the herdsman to have ketosis were administered oral propylene glycol daily for 1–5 days after blood samples were collected; however, blood samples in these cows were always obtained > 20 hours after the previous treatment with propylene glycol. Cows were milked twice daily toward the end of each milking period in a milking parlor between 05:15 and 08:30 and between 16:00 and 19:00 hours. Daily milk, 305 d mature-equivalent milk (M305M), fat (F305M), and protein (P305M) yields were calculated after completion of lactation and recorded using the dairy's automatic recording software.<sup>8</sup> Therefore, these milk production indices represent actual and not projected values.

### Experimental Methods

Blood samples were obtained daily before the morning feeding in order to minimize any potential effect of diurnal variation in metabolite concentrations. Blood samples were collected at approximately 09:00 from the coccygeal vein or artery at weekly intervals from Days 4 to 35 lactation, with the day of calving being Day 0. The day of sampling was categorized as postpartum 7, 14, 21, or 28 days for statistical analysis based on the nearest weekly sample period (ie, samples obtained on postpartum Days 4–10 were categorized as Day 7, samples obtained on postpartum Days 11–17 were categorized as Day 14, samples obtained on postpartum Days 18–24 were categorized as Day 21, and samples obtained on postpartum Days 25–35 were categorized as Day 28; if 2 samples were collected during the latter period then the first collected

sample was retained for statistical analysis). Blood samples were collected using 20 G vacutainer needles, vacutainer holders, and 10 mL lithium heparin blood collection tubes.

Immediately after blood collection, blood [BHB] was measured in a drop of non-heparinized blood from the tip of the vacutainer needle using Precision Xtra point-of-care meter.<sup>b</sup> Plasma [BHB] was calculated from the Precision Xtra reading for blood [BHB] in mmol/L ([BHB]<sub>b-meter</sub>) using the following equation developed by our laboratory<sup>34</sup>:

$$[\text{BHB}] = 0.62 \times [\text{BHB}]_{\text{b-meter}} + 0.20.$$

### **Body Condition Score and Ultrasonographic Determination of Backfat and Muscle Thickness**

Body condition score (BCS) and backfat thickness (BFT) were measured to assess fat mobilization and LDT was measured to assess muscle mobilization; these are well validated indices that assess the magnitude of negative energy balance in early lactation.<sup>6,34,35,38,39</sup> Periparturient dairy cows initially mobilize skeletal muscle in response to negative energy balance in order to provide amino acids for gluconeogenesis. Therefore, the rate of muscle mobilization is believed to play an important role in glucose metabolism during the first 4 weeks of lactation<sup>31,38</sup> and is likely to be associated with plasma [FRA].

BCS (on a 1–5 scale) was measured by 1 of 2 trained investigators (AM, MH) on approximately Day 28 postpartum using an established scoring system.<sup>40</sup> Ultrasonography was used to measure BFT at the sacral area adjacent to the first coccygeal vertebra and LDT at the level between the 12th and 13th rib as described elsewhere<sup>31,41</sup> at approximately Day 3 antepartum and 7, 14, 21, or 28 days postpartum relative to calving. Hair was clipped at the measurement points and a portable ultrasonographic unit (Exago 90–1119)<sup>c</sup> with a 5 MHz linear transducer was employed using the following settings: B-mode and 80 mm depth. The cow was placed in a normal, relaxed standing position and 70% alcohol and coupling gel was applied to maintain adequate acoustic contact. The transducer was positioned lightly and vertically to the fat and muscle interface to avoid compression of fat and muscle. The ultrasound gain was adjusted to as low as possible to avoid unwanted reflection artifacts and suitable images were stored and analyzed using ImageJ software.<sup>d</sup> The BFT and LDT were measured to the nearest 0.1 mm using the straight freehand line tool of ImageJ at the thickest section. Superficial and profound fascias were used as landmarks to measure the BFT, where the straight freehand line extended from the midpoint of the superficial fascia to the midpoint of the profound fascia at the thickest section. For LDT measurements, the straight freehand line extended between the 2 connective tissue layers ensheathing the muscle bundles to measure only the muscle tissue at the thickest section. The investigator (AM) measuring fat and muscle thickness did not have access to BCS information.

### **Plasma Biochemical Analysis**

Heparinized blood samples were centrifuged in a climate controlled laboratory area (temperature range, 21–24°C) that was adjacent to the animal housing area. The heparinized blood samples were centrifuged within 30 minutes of collection for 5 minutes at 1,400 × g. Plasma was harvested and [TP] was measured in triplicate using a handheld analog refractometer.<sup>c</sup> Plasma [gluc] was measured using a Precision Xtra point-of-care meter. Plasma [gluc] was calculated from the Precision Xtra reading for plasma [gluc] in mg/dL ([gluc]<sub>p-meter</sub>) using the following equation developed by our laboratory<sup>32</sup>:

$$[\text{gluc}] = 0.66 \times [\text{gluc}]_{\text{p-meter}} + 15.$$

Plasma was then transferred into polypropylene vials within 1 hour of centrifugation and stored at –20°C. Plasma [FRA] was determined

using a kinetic color reaction that reflects the ability of keto-amines to reduce nitroblue tetrazolium (NBT) to formazine in an alkaline medium; the rate of formazine production was measured spectrophotometrically<sup>f</sup> at the Veterinary Diagnostic Laboratory, College of Veterinary Medicine, University of Illinois at Urbana-Champaign and is proportional to the plasma [FRA].<sup>8</sup> All plasma samples were analyzed on the same day. The NBT analytical method has been validated in bovine serum<sup>8</sup>; the within-run coefficient of variation was 1.6–4.6%, the between-run coefficient of variation was 9.2–13.5%, and the detection limit was 9.1 μmol/L. The reference range for bovine serum from 19 clinically healthy lactating dairy cows was 213–265 μmol/L.<sup>8</sup> Human serum [FRA] is stable when stored at –20°C for at least 16 months.<sup>42</sup>

Plasma concentrations of serum biochemical variables were measured using the same spectrophotometer, including plasma albumin concentration ([albumin]) by bromocresol green, plasma [NEFA] by Acyl-CoA synthetase-Acyl-CoA oxidase, and plasma total cholesterol concentration ([TC]) by cholesterol dehydrogenase. Plasma globulin concentration ([globulin]) was calculated as the difference between plasma [TP] and plasma [albumin]. Plasma [NEFA] and [TC] were determined on a subset of the 103 animals, comprising 10 primiparous and 22 multiparous animals. The parity proportions of the subset reflected that of the sample population, and animals were selected using a random number generator followed by sorting<sup>g</sup> from lowest to highest within parity groups.

### **Statistical Analysis**

Normally distributed data (as assessed by the Wilk–Shapiro test) were summarized as mean ± SD, non-normally distributed data were summarized as median and interquartile range.  $P < 0.05$  was assigned as statistically significant. Spearman's rho was used to characterize the association between plasma [FRA] and the actual plasma [gluc], [albumin], [total protein], [globulin], [NEFA], and [TC], as well as the actual blood [BHB], BCS, BFT, and LDT at approximately Day 28 postpartum, the cumulative milk production until 28 days postpartum, and M305M, F305M, and P305M. Spearman's rho was also used to characterize the association between plasma [FRA] at approximately Day 28 postpartum and the mean plasma [gluc], [albumin], [total protein], [globulin] from 4 to 28 days postpartum.

Stepwise forward multivariable linear regression was used to characterize the association between plasma [FRA] at approximately Day 28 postpartum and the mean plasma [gluc], [albumin], and [globulin] over the period of 4–35 days postpartum, and parity. The plasma [FRA] at postpartum Day 28 was selected as the dependent variable because plasma [FRA] is lowest at this time point.<sup>31</sup> Use of mean plasma concentrations accounts for weekly differences between analytes for their mean highest or lowest values. The postpartum period of Days 4–35 was selected to avoid the transient effects of parturition on some plasma biochemical concentrations. Parity (primiparous = 0; multiparous = 1) was tested as a covariate in the model. A  $P < 0.05$  was used for entry and exit in the stepwise multivariable regression procedure. An interaction term was added to the model for 2 or more independent predictors by centering the actual value for each predictor. Centering was accomplished by subtracting the actual value from the overall mean for each variable and multiplying the result. In addition, if an independent variable was found to be statistically significant (ie, albumin), then this variable was used to correct [FRA] when characterizing the association of [FRA] with metabolic variables of interest. A statistical software program<sup>h</sup> was used for analysis.

### **Results**

Cows in the study reported here were in negative energy balance, based on reductions in BFT (46%) and LDT (26%) since parturition.<sup>35,36</sup> Hypoglycemia (plasma [gluc] < 55 mg/dL) was very common during the study

**Table 1.** Spearman correlation coefficients between plasma fructosamine concentration, albumin-corrected plasma fructosamine concentration, and other indices of energy status for 103 Holstein–Friesian cattle (primiparous, n = 32; multiparous, n = 71) in early lactation.

Variable (units)	n	Mean ± SD or Median (Interquartile Range)	Plasma Fructosamine Concentration		Albumin-Corrected Plasma Fructosamine Concentration	
			$r_s$	P Value	$r_s$	P Value
Plasma [fructosamine] on Day 28 postpartum (µmol/L)	103	253 ± 20	ND	ND	ND	ND
Mean plasma [gluc] (4–28 days postpartum) (mg/dL)	101	56 ± 6	0.36	0.0002	0.46	<0.0001
Plasma [gluc] on Day 28 postpartum (mg/dL)	101	56 ± 7	0.38	<0.0001	0.47	<0.0001
Mean plasma [albumin] (4–28 days postpartum) (g/dL)	100	3.0 ± 0.4	0.12	0.24	ND	ND
Plasma [albumin] on Day 28 postpartum (g/dL)	32	3.3 ± 0.4	0.06	0.76	ND	ND
Mean plasma [TP] (4–28 days postpartum) (g/dL)	102	7.0 ± 0.5	0.12	0.23	0.07	0.49
Plasma [TP] on Day 28 postpartum (g/dL)	101	7.2 ± 0.5	0.25	0.011	0.22	0.033
Mean plasma [globulin] (4–28 days postpartum) (g/dL)	100	3.9 ± 0.6	0.01	0.93	0.16	0.10
Plasma [globulin] on Day 28 postpartum (g/dL)	32	3.3 ± 0.7	0.20	0.27	0.31	0.079
Mean blood [BHB] (4–28 days postpartum) (mmol/L)	56	0.8 ± 0.3	−0.24	0.079	−0.30	0.030
Blood [BHB] on Day 28 postpartum (mmol/L)	49	0.8 ± 0.3	−0.20	0.17	−0.24	0.10
Mean plasma [NEFA] (4–28 days postpartum) (mmol/L)	32	0.33 (0.18, 0.38)	0.08	0.67	−0.03	0.86
Plasma [NEFA] on Day 28 postpartum (mmol/L)	32	0.13 (0.10, 0.25)	0.16	0.37	0.08	0.69
Mean plasma [TC] (4–28 days postpartum) (mg/dL)	32	96 ± 26	−0.12	0.50	−0.25	0.18
Plasma [TC] on Day 28 postpartum (mg/dL)	32	110 ± 31	−0.13	0.49	−0.25	0.17
BCS on Day 28 postpartum (units)	92	2.6 ± 0.3	0.23	0.029	0.25	0.020
BFT on Day 28 postpartum (mm)	97	7.3 (5.0, 10.6)	0.21	0.043	0.20	0.053
LDT on Day 28 postpartum (mm)	91	37.4 ± 4.6	0.28	0.0065	0.25	0.019
Cumulative milk production for the first 28 days postpartum (kg)	102	906 ± 225	−0.09	0.38	−0.23	0.026
M305M (kg)	101	10,543 ± 1,636	−0.19	0.051	−0.22	0.027
F305M (kg)	101	431 ± 84	−0.11	0.26	−0.13	0.21
P305M (kg)	101	328 ± 48	−0.17	0.09	−0.22	0.031

ND, not determined; gluc, glucose; PP, blood BHB, plasma total protein; β-hydroxybutyrate; NEFA, non-esterified fatty acids; TC, total cholesterol; BCS, body condition score; BFT, backfat thickness; LDT, longissimus dorsi thickness; M305M, 305 days milk production; F305M, 305 days mature-equivalent fat yield; P305M, 305 days mature-equivalent protein yield.

period, being identified in 53% (172/327) of the plasma samples and 79% (81/103) of the cows. Hyperketonemia ([BHB] > 1.2 mmol/L) was uncommon during the study period, being identified in 8% (11/131) of the blood samples and 17% (9/54) of the cows. Twelve cows (12%) were treated for ketosis by the herdsman for 1–5 days based on clinical signs. Plasma [NEFA] > 0.7 mmol/L was rare during the study period, being identified in 2% (4/167) of the plasma samples and 4% (4/102) of the cows.

The mean plasma [FRA] at approximately Day 28 postpartum, and mean plasma [gluc], [albumin], [globulin], [TP], [NEFA] and [TC], and blood [BHB] over the period of 4–28 days postpartum, and the actual plasma [gluc] on Day 28 postpartum, are presented in Table 1. Plasma [FRA] was similar ( $P = 0.24$ ) for cows treated for ketosis ( $246 \pm 28$  µmol/L) or untreated ( $253 \pm 18$  µmol/L). Mean plasma [gluc] was similar ( $P = 0.83$ ) for cows treated for ketosis ( $56 \pm 5$  mg/dL) or untreated ( $56 \pm 7$  mg/dL). Plasma [FRA] at approximately Day 28 postpartum was positively but weakly associated with mean plasma [gluc] over the period of 4–28 days postpartum, and BCS, BFT, and LDT at approximately Day 28 postpartum (Table 1; Fig 1A). The linear regression equation for the plasma [FRA]-[gluc] relationship was  $[FRA] = 198 + 0.96 \times [gluc]$  ( $R^2 = 0.10$ ,  $P = 0.0014$ ). Plasma [FRA] at approximately Day 28 postpartum was positively but weakly associated with plasma

[TP] at approximately Day 28 postpartum. Interestingly, plasma [FRA] at approximately Day 28 postpartum was similarly associated with mean plasma [gluc] over the period of 4–28 days postpartum and mean plasma [gluc] at approximately Day 28 postpartum.

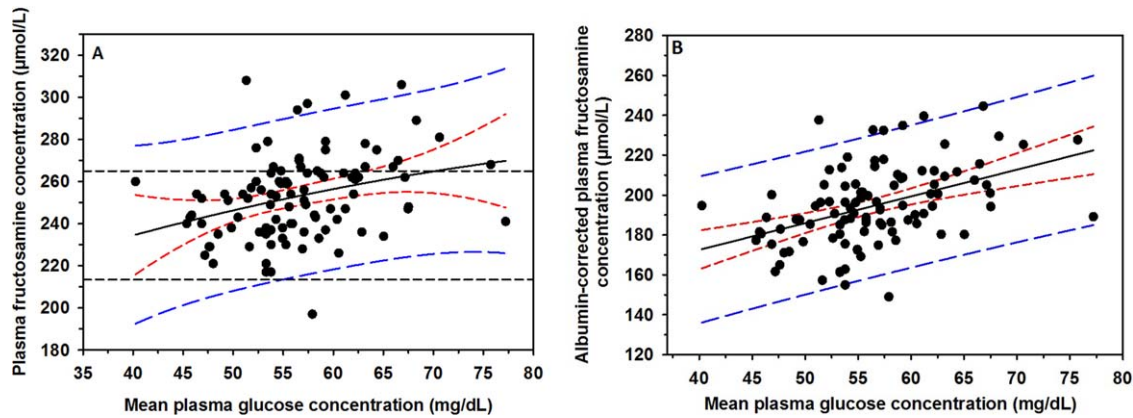
Stepwise regression analysis indicated that plasma [FRA] was dependent on the mean plasma [gluc] in mg/dL ( $P < 0.0001$ ;  $R^2 = 0.10$ ) and the mean plasma [albumin] in g/dL ( $P = 0.0007$ ,  $R^2 = 0.20$ ) over the period of 4–28 days postpartum, such that:

$$[FRA] = 119 + 1.34 \times [gluc] + 19.2 \times [albumin].$$

An equation to correct plasma [FRA] for mean plasma [albumin] in g/dL over the period of 4–28 days postpartum is therefore:

$$[FRA]_{\text{corrected}} = [FRA]_{\text{measured}} - 19.2 \times [albumin].$$

Plasma  $[FRA]_{\text{corrected}}$  at approximately Day 28 postpartum was  $195 \pm 20$  µmol/L (range, 149–245 µmol/L). Plasma  $[FRA]_{\text{corrected}}$  was similar ( $P = 0.46$ ) for cows treated for ketosis ( $191 \pm 28$  µmol/L) or untreated ( $195 \pm 19$  µmol/L). Plasma  $[FRA]_{\text{corrected}}$  at approximately Day 28 postpartum was positively but weakly associated with mean plasma [gluc] over the period of 4–28 days postpartum, negatively



**Fig 1.** (A) Scatterplot of the relationship between plasma fructosamine concentration ( $\mu\text{mol/L}$ ) at approximately Day 28 postpartum and plasma mean glucose concentration ( $\text{mg/dL}$ ) over the period of 4–28 days postpartum in 103 Holstein–Friesian cattle (32 primiparous, 71 multiparous). The horizontal dotted lines represent the upper and lower limits of the reference range for lactating dairy cows.<sup>8</sup> The thick solid black line is the regression line ( $y = 198 + 0.96 \times [\text{gluc}]$ ,  $R^2 = 0.10$ ,  $P = 0.0014$ ), the red short dotted lines are the 95% confidence interval for regression line, and the blue long dashed lines are the 95% confidence interval for prediction. (B) Scatterplot of the relationship between albumin-corrected plasma fructosamine concentration ( $\mu\text{mol/L}$ ) at approximately Day 28 postpartum and mean plasma glucose concentration ( $\text{mg/dL}$ ) over the period of 4–28 days postpartum. The thick solid black line is the regression line ( $y = 119 + 1.34 \times [\text{gluc}]$ ,  $R^2 = 0.19$ ,  $P < 0.0001$ ).

associated with mean blood [BHB] over the period of 4–28 days postpartum, positively but weakly associated with plasma [gluc], [TP], BCS, and LDT at approximately Day 28 postpartum, and negatively and weakly associated with cumulative milk production for the first 28 days postpartum, 305 days mature-equivalent milk production, and 305 days mature-equivalent protein yield (Table 1; Fig 1B).

As expected, plasma  $[\text{FRA}]_{\text{corrected}}$  was dependent on the mean plasma [gluc] in  $\text{mg/dL}$  ( $P < 0.0001$ ;  $R^2 = 0.19$ ; Fig 1B), such that:

$$[\text{FRA}]_{\text{corrected}} = 119 + 1.34 \times [\text{gluc}].$$

## Discussion

The first major finding of this study was that plasma [FRA] on approximately Day 28 of lactation was positively but weakly correlated with mean postpartum [gluc] from Day 4 to 28 postpartum. The second major finding was that albumin appears to be preferred plasma protein for glycation in cattle, as in humans.

The weak positive association reported in this study between plasma [FRA] and mean plasma [gluc] ( $r_s = +0.36$ ,  $n = 101$ ,  $P = 0.0002$ ) was consistent with a previous report in 23 Norwegian Red dairy cows in Norway ( $r_s = +0.29$ ,  $P < 0.01$ ),<sup>28</sup> but differed from that in 17 Holstein cows in Uruguay ( $r = +0.15$ ,  $P = 0.16$ ).<sup>29</sup> The weak association is most likely because plasma [gluc] in dairy cattle is subject to tight homeostatic control and therefore has limited variability,<sup>43</sup> and because the relatively small decrease in plasma [gluc] that commonly occurs in early lactation in dairy cattle is insufficient to markedly alter plasma [FRA], when compared to the large increase in plasma [gluc] in hyperglycemic humans, dogs, and cats because of diabetes mellitus.<sup>18,20,23–25</sup> Additionally, 12% of animals in this study were administered propylene glycol as a treatment for hyperketonemia daily for 1–5 days. This

treatment was administered between Days 4 and 28 for its gluconeogenic effect. Ketosis treatment therefore had the potential to alter plasma [gluc] during the time period of study; however, oral drenching of propylene glycol causes a transitory increase in plasma [gluc] that peaks within 90 minutes of administration.<sup>44,45</sup> We would therefore not anticipate an impact of ketosis treatment on plasma [FRA] or mean plasma [gluc] when blood samples were collected approximately 20 hours after treatment. This was consistent with our findings that plasma [FRA] and  $[\text{FRA}]_{\text{corrected}}$  on Day 28 of lactation and mean plasma [gluc] for Days 4–28 of lactation were similar for cows treated for ketosis and untreated cows.

Another potential reason for the weak association between plasma [FRA] and mean plasma [gluc] is the presence of nonspecific reducing components in plasma that react with NBT.<sup>46,47</sup> It has been known for more than 30 years that these nonspecific reducing components approximate 67% of the measured FRA in human plasma.<sup>47,48</sup> Other studies reported that the nonspecific reducing components contribute approximately half of the measured plasma [FRA]<sup>49,50</sup>; these estimates were similar to the nonspecific binding estimate of 61% in the study reported here, where the intercept value of 119  $\mu\text{mol/L}$  provided an estimate of the amount of nonspecific reducing components in bovine plasma, which approximated 61% (119/195) of the mean measured plasma [FRA]. Therefore, the NBT method appears to be a nonspecific method for monitoring plasma [FRA] in dairy cattle.

Plasma  $[\text{FRA}]_{\text{corrected}}$  was weakly associated with a number of indices of energy balance in this study, including positive associations with mean plasma [gluc] from Days 4 to 28 postpartum as well as BCS and LDT on Day 28 postpartum, and a negative association with mean blood [BHB] from Days 4 to 28 postpartum. These findings confirmed our hypothesis that plasma [FRA] provides an index of energy balance in the early postpartum period. The negative association between plasma [FRA] and milk volume, milk

protein, and LDT reflects the large uptake of glucose and protein by the mammary gland in early lactation.<sup>7,51,52</sup> The increase in protein demand during early lactation was consistent with the observed decrease in BCS and LDT in the cows in this study.

We conclude that plasma [FRA] does not appear to provide a clinically useful method for quantifying the magnitude of hypoglycemia or negative energy balance in dairy cows during early lactation. Our conclusion is based on the weakness of the associations, the lack of association between plasma [FRA] and mean plasma [NEFA], and the weak association between plasma [FRA] and mean plasma [BHB] (both of which have been well-shown to be associated with excessive negative energy balance). Additional challenges with adopting plasma [FRA] as a test for evaluating negative energy balance is the relatively high cost of testing (\$US8/test) and the need to send plasma samples to a laboratory for testing with a resultant delay in obtaining test results.

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## Footnotes

- <sup>a</sup> PCDart software, Lancaster DHIA Ltd, Manheim, PA.  
<sup>b</sup> Precision Xtra; Blood Glucose and Ketone Monitoring System, Abbott Diabetes Care Inc, Alameda, CA.  
<sup>c</sup> Exago 90-1119, ECM Ltd, Angoulême, France.  
<sup>d</sup> ImageJ software (version 1.50i), National Institutes of Health, Bethesda, MD.  
<sup>e</sup> Analog Refractometer, MASTER-SUR/N $\alpha$ , Atago Inc, Bellevue, WA.  
<sup>f</sup> Hitachi 911, Roche Diagnostics, Basel, Switzerland.  
<sup>g</sup> Microsoft Excel 2010, Microsoft Corp, Redmond, WA.  
<sup>h</sup> SAS 9.3 software, SAS Inc, Cary, NC.
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*Conflict of Interest Declaration:* Authors declare no conflict of interest.

*Off-Label Antimicrobial Declaration:* Authors declare no off-label use of antimicrobials.

*Institutional Animal Care and Use Committee (IACUC) or Other Approval Declaration:* Authors declare that IACUC approval was obtained and that no other approval was needed.

## References

- Ingvarsen KL, Andersen JB. Integration of metabolism and intake regulation: A review focusing on periparturient animals. *J Dairy Sci* 2000;83:1573–1597.
- Hayirli AR, Grummer R, Nordheim EV, Crump PM. Animal and dietary factors affecting feed intake during the prefresh transition period in Holsteins. *J Dairy Sci* 2002;85:3430–3443.
- Overton TR. Substrate utilization for hepatic gluconeogenesis in the transition dairy cow. The Cornell Nutrition Conference for Feed in Manufacturers, Cornell University, Ithaca, NY, 1998.
- Reynolds CK, Aikman PC, Lupoli B. Splanchnic metabolism of dairy cows during the transition from late gestation through early lactation. *J Dairy Sci* 2003;86:1201–1217.

- Wathes DC, Cheng Z, Bourne N. Differences between primiparous and multiparous dairy cows in the interrelationships between metabolic traits, milk yield and body condition score in the periparturient period. *Domest Anim Endocrinol* 2007;33:203–225.

- Weber C, Hametner C, Tuchscherer A, et al. Variation in fat mobilization during early lactation differently affects feed intake, body condition, and lipid and glucose metabolism in high-yielding dairy cows. *J Dairy Sci* 2013;96:165–180.

- Whitaker DA, Smith EJ, da Rosa GO, Kelly JM. Some effects of nutrition and management on the fertility of dairy cattle. *Vet Rec* 1993;133:61–64.

- Jensen AL, Petersen MB, Houe H. Determination of fructosamine concentration in bovine serum samples. *Zentralbl Veterinarmed A* 1993;40:111–117.

- Eicher R, Liesegang A, Bouchard E, Tremblay A. Effect of cow-specific factors and feeding frequency of concentrate on diurnal variations of blood metabolites in dairy cows. *Am J Vet Res* 1999;60:1493–1499.

- Blum JW, Jans F, Moses W. Twenty four-hour pattern of blood hormone and metabolite concentrations in high-yielding dairy cows: Effects of feeding low or high amounts of starch, or crystalline fat. *Transbound Emerg Dis* 1985;32:401–418.

- Sutton JD, Hart IC, Morant SV. Feeding frequency for lactating cows: Diurnal patterns of hormones and metabolites in peripheral blood in relation to milk-fat concentration. *Br J Nutr* 1988;60:265–274.

- Nielsen NI, Ingvarsen KL, Larsen T. Diurnal variation and the effect of feed restriction on plasma and milk metabolites in TMR-fed dairy cows. *J Vet Med A Physiol Pathol Clin Med* 2003;50:88–80.

- Plaizier JC, Fairfield AM, Azevedo PA, et al. Effects of monensin and stage of lactation on variation of blood metabolites within twenty-four hours in dairy cows. *J Dairy Sci* 2005;88:3595–3602.

- Nikkhah A, Furedi CJ, Kennedy AD, et al. Effects of feed delivery time on feed intake, milk production, and blood metabolites of dairy cows. *J Dairy Sci* 2008;91:4249–4260.

- Wylie AR, Woods S, Carson AF, McCoy M. Periprandial changes in metabolite and metabolic hormone concentrations in high-genetic-merit dairy heifers and their relationship to energy balance in early lactation. *J Dairy Sci* 2008;91:577–586.

- Mahrt A, Burfeind O, Heuwieser W. Effects of time and sampling location on concentrations of  $\beta$ -hydroxybutyric acid in dairy cows. *J Dairy Sci* 2014;97:291–298.

- Arnbruster DA. Fructosamine: Structure, analysis and clinical usefulness. *Clin Chem* 1987;33:2153–2163.

- Kawamoto M, Kaneko JJ, Heusner AA, et al. Relation of fructosamine to serum protein, albumin, and glucose concentrations in healthy and diabetic dogs. *Am J Vet Res* 1992;53:851–855.

- Mosca A, Carenini A, Zoppi F, et al. Plasma protein glycation as measured by fructosamine assay. *Clin Chem* 1987;33:1141–1146.

- Pattullo KM, Kidney BA. Exploring fructosamine beyond diabetes mellitus. *J Am Vet Med Assoc* 2014;244:1268–1277.

- Cornelius CE, Baker NF, Kaneko JJ, Douglas JR. Distribution and turnover of I-131-tagged bovine albumin in normal and parasitized cattle. *Am J Vet Res* 1962;23:837–842.

- Bernstein RE. Nonenzymatically glycosylated proteins. *Adv Clin Chem* 1987;26:1–78.

- Reusch CE, Liehs MR, Hoyer M, Vochezer R. Fructosamine. A new parameter for diagnosis and metabolic control in diabetic dogs and cats. *J Vet Intern Med* 1993;7:177–182.

- Jensen AL. Glycosylated blood proteins in canine diabetes mellitus. *Vet Rec* 1995;137:401–405.

- Knowles WJ, Menzies-Gow NJ, Mair TS. Plasma fructosamine concentrations in horses with pituitary pars intermedia dysfunction with and without laminitis. *Equine Vet J* 2014;46:249–251.

- Cantley EL, Ford CM, Heath MF. Serum fructosamine in ovine pregnancy toxemia: A possible prognostic index. *Vet Rec* 1991;128:525–526.

27. Sorondo ML, Cirio A. Evaluation of the serum fructosamine test to monitor plasma glucose concentration in the late-pregnant sheep. *Anim Prod Sci* 2011;51:662–666.
28. Ropstad E. Serum fructosamine levels in dairy cows related to metabolic status in early lactation. *Acta Vet Scand* 1987;28:291–298.
29. Sorondo ML, Cirio A. Evaluation of the serum fructosamine test to monitor plasma glucose concentration in the transition dairy cow. *J Dairy Res* 2009;76:173–178.
30. Mostafavi M, Seifi HA, Mohri M, Jamshidi A. Evaluation of fructosamine as a new biomarker for diagnosis of hepatic lipidosis in dairy cows. *Anim Prod Sci* 2015;55:1005–1010.
31. Bruckmaier RM, Gregoretto L, Jans F, et al. Longissimus dorsi muscle diameter, backfat thickness, body condition scores and skinfold values related to metabolic and endocrine traits in lactating dairy cows fed crystalline fat or free fatty acids. *Transbound Emerg Dis* 1998;45:97–410.
32. Megahed AA, Hiew MWH, Townsend JR, et al. Evaluation of an electrochemical point-of-care meter for measuring glucose concentration in blood from periparturient dairy cattle. *J Vet Intern Med* 2015;29:1718–1727.
33. Hiew MW, Megahed AA, Townsend JR, et al. Clinical utility of calf front hoof circumference and maternal intrapelvic area in predicting dystocia in 103 late gestation Holstein-Friesian heifers and cows. *Theriogenology* 2016;85:384–395.
34. Megahed AA, Hiew MH, Townsend JR, Constable PD. Characterization of the analytical performance of an electrochemical point-of-care meter for measuring  $\beta$ -hydroxybutyrate concentration in blood and plasma from periparturient dairy cattle. *Vet Clin Pathol* 2017;46:314–325.
35. Megahed AA, Hiew M, Constable PD. Ultrasonographic determination of longissimus dorsi muscle thickness and intramuscular fat percentage in periparturient dairy cattle. *J Vet Intern Med* 2017;31:1360.
36. Megahed AA, Hiew M, Constable PD. Utilization of muscle tissue in periparturient dairy cattle as assessed by ultrasonographic measurement of muscle thickness and plasma creatinine concentration. *Proceedings. 48th Annu Conf Am Assoc Bov Pract, Ashland OH, 2015.*
37. National Research Council. *Nutrient Requirements of Dairy Cattle*, 7th revised version ed. Washington, DC: Proceedings of the National Academy of Sciences of the United States of America; 2001.
38. Van der Drift SG, Houweling M, Schonewille JT, et al. Protein and fat mobilization and associations with serum  $\beta$ -hydroxybutyrate concentrations in dairy cows. *J Dairy Sci* 2012;95:4911–4920.
39. Pires JA, Delavaud C, Faulconnier Y, et al. Effects of body condition score at calving on indicators of fat and protein mobilization of periparturient Holstein-Friesian cows. *J Dairy Sci* 2013;96:6423–6439.
40. Ferguson JD, Galligan DT, Thomsen N. Principal descriptors of body condition score in Holstein cows. *J Dairy Sci* 1994;77:2695–2703.
41. Schröder UJ, Staufenbiel R. Invited review: Methods to determine body fat reserves in the dairy cow with special regard to ultrasonographic measurement of backfat thickness. *J Dairy Sci* 2006;89:1–14.
42. Koskinen P, Irjala K. Stability of serum fructosamine during storage. *Clin Chem* 1988;34:2545–2546.
43. Ingvarsen KL, Dewhurst RJ, Friggens NC. On the relationship between locational performance and health: Is it yield or metabolic imbalance that cause production diseases in dairy cattle? *Livest Prod Sci* 2003;83:277–308.
44. Nielsen NI, Ingvarsen KL. Propylene glycol for dairy cows: A review of the metabolism of propylene glycol and its effects on physiological parameters, feed intake, milk production and risk of ketosis. *Anim Feed Sci Tech* 2004;115:191–213.
45. Mann S, Yepes FL, Behling-Kelly E, McArt JA. The effect of different treatments for early-lactation hyperketonemia on blood  $\beta$ -hydroxybutyrate, plasma nonesterified fatty acids, glucose, insulin, and glucagon in dairy cattle. *J Dairy Sci* 2017;100:6470–6482.
46. Johnson RN, Metcalf PA, Baker JR. Fructosamine: A new approach to the estimation of serum glycosylprotein. An index of diabetic control. *Clin Chim Acta* 1983;127:87–95.
47. Suhonen L, Stenman UH, Koivisto V, Teramo K. Correlation of HbA1C, glycated serum proteins and albumin, and fructosamine with the 24-h glucose profile of insulin-dependent pregnant diabetics. *Clin Chem* 1989;35:922–925.
48. Smart LM, Howie AF, Young RJ, et al. Comparison of fructosamine with glycosylated hemoglobin and plasma proteins as measures of glycemic control. *Diabetes Care* 1988;11:433–436.
49. Howey JEA, Browning MC, Fraser CG. Assay of serum fructosamine that minimizes standardization and matrix problems: Use to assess components of biological variation. *Clin Chem* 1987;33:269–272.
50. Schleicher ED, Mayer R, Wagner EM, Gerbitz KD. Is serum fructosamine assay specific for determination of glycated serum proteins?. *Clin Chem* 1988;34:320–323.
51. Bell AW, Slepets R, Ehrhardt RA. Growth and accretion of energy and protein in the gravid uterus during late pregnancy in Holstein cows. *J Dairy Sci* 1995;78:1954–1961.
52. Ingvarsen KL. Feeding- and management-related diseases in the transition cow: Physiological adaptations around calving and strategies to reduce feeding-related diseases. *Anim Feed Sci Technol* 2006;126:175–213.