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From the Department of Reproductive Physiology and Pathology,
Norwegian College of Veterinary Medicine, Oslo.

Serum Fructosamine Levels in Dairy Cows Related to Metabolic Status in Early Lactation

By *Erik Ropstad*

Ropstad, E.: Serum fructosamine levels in dairy cows related to metabolic status in early lactation. Acta vet. scand. 1987, 28, 291–298. – Serum levels of fructosamine were assayed in 57 samples from 23 Norwegian Red Cattle dairy cows in early lactation. The animals were assigned to a 2^2 factorial feeding experiment. The groups differed in energy and protein supply. All samples were collected before morning feeding.

The relationship between fructosamine levels and the plasma concentrations of glucose, acetoacetate, free fatty acids, total cholesterol, lipoproteins, triglycerides, total proteins, progesterone and to weight and energy balance was studied. The overall mean fructosamine level was 1.41 ± 0.20 mmol/l ranging from 0.92–1.92 mmol/l. Significant relationships were recorded between fructosamine and the concentrations of glucose, acetoacetate, free fatty acids, triglycerids, total cholesterol and energy and weight balance. Free fatty acids and cholesterol showed the best correlation with fructosamine (r_s = –0.67 and 0.70, respectively, $p < 0.001$). The levels of fructosamine increased significantly between 2 and 4 weeks and between 4 and 8 weeks after calving. Animals with a weight gain during 3 weeks prior to fructosamine measurement had higher fructosamine levels than those showing a weight loss (< –7 kg/week) ($p < 0.05$). The results indicate that serum fructosamine levels in cows may be of interest as an indicator of metabolic status in the early stages of lactation.

free fatty acids; cholesterol; glucose; acetoacetate; energy balance.

Introduction

Glycosylation of proteins occurs as a non-enzymatic modification directly dependent on the prevailing glucose concentration (*Bunn et al.* 1978). Consequently, the degree of glycosylation of haemoglobin (*Dunn et al.* 1979, *Goldstein & Parker* 1982) and serum proteins (*McFarland et al.* 1979, *Yue et al.* 1980, *Mahaffey et al.* 1984) has been correlated with indices of glycemia.

The assay called the fructosamine assay, is based upon the reduction of a tetrazolium-salt by 1-deoxy-fructose, the Amadori rearrangement product formed by the condensation of glucose and proteins. The assay has the advantage of technical simplicity, low cost, and ease of automation using standard laboratory equipment (*Johnson et al.* 1982). In humans the fructosamine level may serve as an index of intermediate (1 to 3 weeks) blood glucose control (*Baker et al.* 1983, *Hindle et al.* 1986), and the assay is of value as a screening test for diabetes mellitus (*Johnson et al.* 1982, *Baker et al.* 1983, *Baker et al.* 1984 and 1985, *Hindle et al.* 1986).

The present study was designed to measure serum fructosamine levels in dairy cows in early lactation and to evaluate the usefullness of this parameter as an index of metabolic control.

Materials and methods

Animals

Twenty-eight Norwegian Red Cattle dairy cows were randomly assigned to a 2^2 factorial feeding experiment. The cows were fed grass silage ad libitum and concentrates according to yield and experimental group. The groups differed in protein and energy levels. Two batches of concentrates were made, one with high and one with low protein content (17,5 %, digestible crude protein (DCP) and 12,5 % DCP, respectively), to provide two levels of protein supply. Within each protein content two levels of energy supply were used. Half of the animals were fed according to requirements and the others were fed approximately 3 feed units (FU) below requirements. Feed consumption was recorded daily, and milk production was recorded 3 days a week. All animals were weighed weekly and samples for estimation of feed quality were collected every second week.

Sampling

Blood samples were collected from the jugular vein at 6 a. m. (before morning feeding) twice weekly from 2 weeks before until 12 weeks after calving. Samples for estimation of acetoacetate, glucose and free fatty acids, triglycerides, total cholesterol, total proteins and progesterone were collected into heparinized vacutainers and centrifuged immediately. Until analysis the plasma was stored at either –196°C for acetoacetate and glucose assays or at – 80°C for free fatty acids, triglycerides, total cholesterol, total proteins and progesterone assays. Samples for estimation of lipoproteins were collected into vacutainers with EDTA as the anticoagulant. After centrifugation the plasma was stored at +4°C and analyzed within 6 h. Samples for estimation of fructosamine were collected into vacutainers without anticoagulant. After separation, the serum was stored at – 80°C until analyzed.

Methods

Analyses for total cholesterol, triglycerides, free fatty acids and total proteins (the biuret method with albumin as the standard) were carried out in a centrifugal autoanalyzer (Gemsaec Fast Analyzer from Electro-nucleonics Inc.). Control sera or pooled sera were used to monitor the day to day repeatability. Plasma acetoacetate and glucose were estimated by the methods described by *Blom & Halse* (1975). The plasma concentrations of total cholesterol and triglycerides were measured using the appropriate test kits supplied by Boehringer. Free fatty acids were analyzed by the acyl CoA synthetase-acyl CoA oxidase method (reagents from Wako Chemicals). Lipoproteins were analyzed by the LIPO lipoprotein kit used with the Paragon Electrophoresis System provided by Beckman Instruments Inc. Plasma progesterone was measured by a method described by *Benjaminsen & Karlberg* (1981). Serum fructosamine concentrations were estimated as described by *Johnson et al.* (1982) at the Central Laboratory, Aker Hospital, Oslo. Energy balance was calculated from data on feed intake, milk production and feed quality.

Fifty-seven samples from 23 animals were assayed for fructosamine. These samples were collected 2, 4 or 8 weeks after calving.

Sixteen animals were sampled at all 3 stages of lactation, while 7 animals were sampled at 1 or 2 stages only. Plasma acetoacetate, glucose and progesterone were measured in all samples collected (i.e. twice weekly). The other parameters were measured once weekly. Mean values for the metabolic parameters, weight- and energy balance were calculated for a period of 2 and 3 weeks prior to fructosamine measurement. Means for 3 weeks were estimated only for samples collected 4 and 8 weeks after calving (n = 41).

Statistical analysis

Differences among means were assessed using the Wilcoxon Two Sample test and correlation analysis (Spearman correlation coefficients, r_s) was used to assess the relationship between fructosamine and other parameters.

Results

The results listed in Table 1 indicate a considerable variation among the observations. Correlations between single measurements and mean values for 2 and 3 weeks were generally high. For glucose and free fatty acids the correlation coefficients between single samples and means for 3 weeks were $r_s = 0.65$ ($p < 0.001$) and $r_s = 0.74$ ($p < 0.001$), respectively (not listed). This indicates a stable feed intake during the period investigated.

The overall mean fructosamine level was 1.41 ± 0.20 mmol/l ranging from 0.89–1.92 mmol/l. Serum fructosamine levels increased significantly ($p < 0.03$) from 2 weeks to 4 and 8 weeks after calving (means = 1.27 mmol/l, 1.39 mmol/l and 1.53 mmol/l, respectively, all means differed significantly) (Fig. 1).

Table 1. Means, standard deviation and ranges for variables included in the study.

Variables	Same day (n = 57)[1]		Mean 3 weeks (n = 41)[2]	
	Mean ± SD	Range	Mean ± SD	Range
Fructosamine, mmol/l	1.41 ± 0.20	0.89 – 1.92	–	–
Acetoacetate, mmol/l	0.15 ± 0.20	0.02 – 1.42	0.15 ± 0.19	0.02 – 1.05
Glucose, mmol/l	3.96 ± 0.32	2.69 – 4.81	3.98 ± 0.26	3.31 – 4.52
Free fatty acids, µmol/l	501 ± 260	103 – 1158	499 ± 217	150 – 1066
Total proteins, g/l	78.4 ± 6.5	66.0 – 90.0	77.1 ± 6.5	64 – 90.0
Triglycerides, µmol/l	197 ± 65	92 – 418	209 ± 51	110 – 357
Total cholesterol, mmol/l	3.88 ± 1.18	1.70 – 7.00	3.62 ± 1.18	1.90 – 7.50
Energy balance, feed units/day	–	–	–3.65 ± 1.89	–7.81 – –0.05
Weight balance, kg/week	–	–	–3.35 ± 5.58	–15.3 – 15.7

[1] Same day: Fructosamine and metabolic parameters measured in samples collected the same day.

[2] Mean 3 weeks: Mean values for samples collected during 3 weeks prior to fructosamine measurement. Means were estimated only at 4 and 8 weeks after calving.

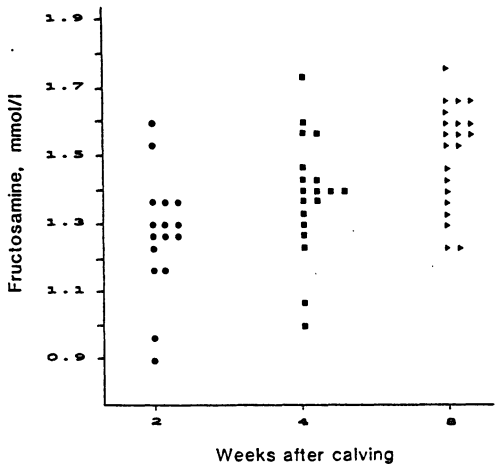

Figure 1. Frequency distribution of serum fructosamine levels related to stage of lactation. Fifty-seven samples from 23 dairy cows are included.
● = samples collected 2 weeks after calving.
■ = samples collected 4 weeks after calving.
▸ = samples collected 8 weeks after calving.

Serum fructosamine levels correlated negatively with free fatty acids ($r_s = -0.67$, $p < 0.001$), and acetoacetate ($r_s = -0.38$, $p < 0.01$). A positive correlation was seen between fructosamine and total cholesterol ($r_s = 0.70$, $p < 0.001$), glucose ($r_s = 0.29$, $p < 0.1$) and triglycerides ($r_s = 0.35$, $p < 0.01$). Serum fructosamine was not significantly correlated with the relative percentages of α- and β-lipoproteins or with total proteins. The correlations between fructosamine and metabolic parameters showed only minor changes when mean values for the metabolic parameters were used (Table 2, Figs. 2 and 3).

A positive correlation was observed between fructosamine and mean weight balance 3 weeks prior to fructosamine measurement ($r_s = 0.35$, $p < 0.1$) and with estimates of the mean energy balance for 2 and 3 weeks before fructosamine measurement

Table 2. Correlations between serum fructosamine levels and metabolic parameters, means of metabolic parameters and means of weight and energy balance. Observations from 23 dairy cows were included in the analysis.

	Spearman correlation coefficient, r_s [1]		
Parameter	Same day[2] (n = 57)	Mean 2 weeks[3] (n = 57)	Mean 3 weeks[3] (n = 41)
Acetoacetate	0.38[b]	-0.37[b]	-0.38[a]
Glucose	0.29[a]	0.22[a]	0.15
Free fatty acids	-0.67[c]	-0.66[c]	-0.73[c]
Triglycerids	0.35[b]	0.17	-
Total proteins	0.02	-	0.16
Total cholesterol	0.70[c]	-	0.59[c]
Weight balance	-	0.12	0.35[a]
Energy balance	-	0.23[a]	0.27[a]

[1] Correlation coefficients with superscripts are significantly different from zero
a: $p < 0.1$ b: $p < 0.01$ c: $p < 0.001$

[2] Same day: Fructosamine measured in samples collected the same day as the metabolic parameters.

[3] Mean 2 and 3 weeks: Mean values refer to measurements done on samples collected during 2 and 3 weeks prior to fructosamine measurement. Means for 3 weeks estimated only 4 and 8 weeks after calving (n = 41).

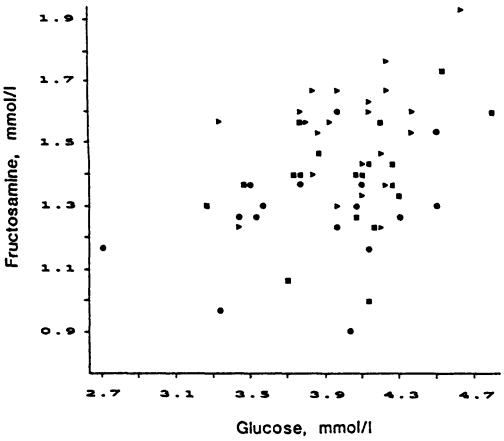

Figure 2. Correlation between prefeeding morning levels of serum fructosamine and plasma glucose in 57 samples from 23 dairy cows in early lactation ($r_s = 0.29$, $p < 0.01$).
● = samples collected 2 weeks after calving.
■ = samples collected 4 weeks after calving.
▶ = samples collected 8 weeks after calving.

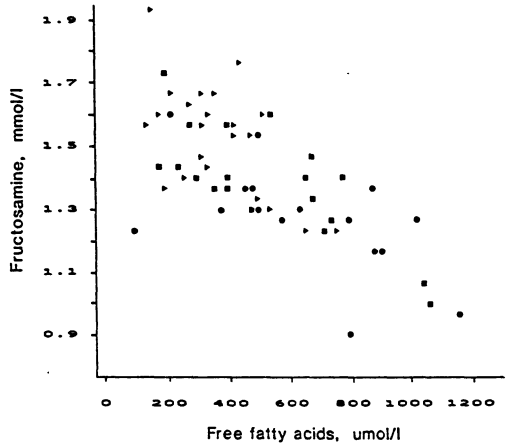

Figure 3. Correlation between prefeeding morning levels of serum fructosamine and levels of free fatty acids in 57 samples from 23 dairy cows in early lactation ($r_s = -0.67$, $p < 0.001$).
● = samples collected 2 weeks after calving.
■ = samples collected 4 weeks after calving.
▶ = samples collected 8 weeks after calving.

Table 3. Fructosamine levels and means of metabolic parameters related to average weight balance during 3 weeks prior to fructosamine measurement.

		Mean ± SD[1]			
			Mean 3 weeks[2]		
Weight balance, kg/week, mean 3 weeks	n	Fructosamine, mmol/l	Acetoacetate, mmol/l	Glucose, mmol/l	Free fatty acids, µmol/l
⩾ 0	10	1.56 ± 0.19[a]	0.07 ± 0.04[a]	4.12 ± 0.23[a]	326 ± 130[a]
0 – –7	18	1.47 ± 0.16[ab]	0.10 ± 0.10[a]	4.03 ± 0.23[a]	527 ± 222[b]
⩽ –7	13	1.33 ± 0.20	0.35 ± 0.30[b]	3.80 ± 0.25[b]	590 ± 196[b]

[1] a, b Means within columns with different superscript differ significantly, $p < 0.05$.
[2] Means of samples collected during 3 weeks prior to fructosamine measurement.

(r_s = 0.23 and 0.27, respectively, $p < 0.1$) (Table 2).

When grouping the observations according to mean weight balance 3 weeks before fructosamine measurement, it appeared that animals with a weight gain had significantly higher fructosamine levels ($p < 0.05$) than animals with a marked weight loss (<–7 kg/week) (Table 3).

No significant correlations were observed between serum fructosamine levels and number of days to first plasma progesterone level above 1 ng/ml.

Discussion

The present study on morning samples taken prior to feeding revealed a rather low correlation coefficient between glucose and fructosamine concentrations ($r_s = 0.29$, $p < 0.1$) (Fig. 1). The explanation for this may be that the diurnal variations strongly influence the fructosamine levels in dairy cows. A correlation between mean daily glucose and fructosamine might possibly give a higher correlation coefficient, as observed in humans by *Baker et al.* (1985).

Fructosamine assays in humans have been undertaken during studies of the control of hyperglycemia in diabetic patients. However, the present study comprises animals with normal and subnormal glucose levels. This difference could be of importance when examining the correlation between fructosamine and glucose levels. Correlation plots based on human samples seem to reveal a less significant relationship between the two parameters in samples with normal glucose levels than in samples with high glucose levels (*Johnson et al.* 1982, *Baker et al.* 1983). This may be because blood glucose levels are kept rather stable under physiological conditions.

The correlations within each parameter between single values and mean values during 2 and 3 weeks were generally high. This should be borne in mind when interpreting correlations between fructosamine and means of other parameters. Therefore, the high correlations seen in this study could be influenced by the interrelationship between single and mean values. In humans, however, there is considerable evidence that serum fructosamine levels reflect the mean levels of glucose during 1 to 3 weeks prior to measurement (*Hindle et al.* 1986).

The level of total proteins found in this study was within normal limits ($\bar{X} = 78.4 \pm 6.5$ g/l, range: 60.0–90.0). No significant relationship was seen between fructosamine and total protein levels (Table 2). This is supported by *Baker et al.* (1983) who found that serum fructosamine concentrations in humans are not influenced by small changes in total protein levels.

The highly significant relationship observed between free fatty acids, total cholesterol and fructosamine (Table 2, Fig. 2) was somewhat unexpected since the correlation to glucose was rather low. It is possible that fasting levels of free fatty acids and total cholesterol are good indicators of energy or carbohydrate balance and thereby also indicators of the mean daily glucose levels. Another possibility is that blood lipids may interfere with the binding of glucose to proteins. However, the chemical basis for this is uncertain. In humans there is conflicting evidence for a relationship between fructosamine and blood lipids (*Duncan & Heiss* 1984, *Lapolla et al.* 1985).

The stage of lactation significantly influenced the fructosamine levels (Fig. 1). The reason for this effect was not explained by this study. The possible influence of other factors connected with metabolic changes in early lactation needs further evaluation.

Despite of a low and insignificant correlation between fructosamine and the mean glucose level during 3 weeks, a positive relationship was observed between the mean weight and energy balance during the 3 weeks and fructosamine values (Tables 2 and 3). Animals showing a marked weight loss (< -7 kg/week) had significantly lower fructosamine levels than animals with a weight gain, as also indicated by the mean values of acetoacetate, glucose and free fatty acids.

In conclusion, serum fructosamine values seem to be influenced by metabolic changes. Decreased levels of fructosamine were in this study associated with increased levels of

free fatty acids and acetoacetate and with decreased levels of glucose. In the search for useful indicators of metabolic status in the dairy cow, serum fructosamine therefore seems to be of interest. However, further studies into the sources of variation should be carried out to assess its clinical usefulness.

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Sammendrag

Serumnivåer av fruktosamin hos melkekyr i tidlig laktasjon.

Fruktosamin ble målt i serum i 57 prøver fra 23 NRF-kyr i tidlig laktasjon. Dyrene deltok i et 2^2 faktorielt fôringsforsøk hvor gruppeforskjellene framkom ved å gi forskjellige nivåer av energi og protein. Alle prøver ble tatt før morgenfôring.

Fruktosaminnivåene ble relatert til konsentrasjonen av glukose, acetoacetat, frie fettsyrer, total

kolesterol, lipoproteiner, triglycerider, total protein og progesteron i plasma og til vekt- og energibalanse.

Gjennomsnittlig fruktosaminnivå var 1.41 ± 0.20 mmol/l, med variasjon fra 0.92 til 1.92 mmol/l. Signifikante relasjoner ble påvist mellom fruktosaminnivå og konsentrasjonene av glukose, acetoacetat, frie fettsyrer, triglycerider og kolesterol og mellom fruktosaminnivå og vekt- og energibalanse.

Høyest korrelasjon ble funnet mellom fruktosamin og frie fettsyrer og kolesterol, henholdsvis r_s = –0.67 og 0.70, ($p < 0.001$). Nivåene av fruktosamin økte signifikant fra 2 til 4 og 8 uker etter kalving ($p < 0.05$).

Resultatene indikerer at målinger av fruktosamin i serum kan være av interesse som en indikator på stoffskifteforandringer i tidlig laktasjon hos ku.

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Reprints may be requested from: Erik Ropstad, Norwegian College of Veterinary Medicine, P. O. Box 8146 Dep, N-0033 Oslo 1, Norway.