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The relationship of asprosin with β -hydroxybutyric acid and postpartum disorders in cows

MEHMET AKIF KILINC^{1*}  and ALI RISVANLI²

¹ Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, Bingöl University, 12000 Merkez/Bingöl, Turkey

² Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, Kyrgyz-Turkish Manas University, Bishkek, Kyrgyzstan

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RESEARCH ARTICLE



ABSTRACT

The aim of the present study was to determine asprosin levels in cows, the relationship of this hormone with postpartum disorders and β -hydroxybutyric acid, and also the potential of asprosin to be a marker for postpartum diseases. The study was designed as a two-stage trial. In the first stage, blood asprosin and β -hydroxybutyric acid levels of 20 healthy Simmental cows aged 3–4 years were measured at the time of calving, and on days 3, 6, 9, 12 and 15 postpartum. In the second stage, 200 cows were divided into two groups: (1) healthy ($n = 100$) and (2) diseased (placental retention, hypocalcaemia, metritis, lameness, abomasal displacement, mastitis; $n = 100$); asprosin and β -hydroxybutyric acid concentrations of the blood were assessed on day 15 postpartum. In conclusion, the asprosin level was found to be at measurable levels in cows, and a negative correlation with β -hydroxybutyric acid was found. According to these findings, the data obtained from this study could be used for the prevention, control and treatment of some postpartum disorders associated with ketosis and for developing novel hypotheses concerning the actions of this hormone. It was concluded that further studies are required to reveal the associations between asprosin and postpartum disorders.

KEYWORDS

cow, β -hydroxybutyric acid, postpartum disorders, asprosin, ELISA

INTRODUCTION

In several studies with cows, elevated levels of β -hydroxybutyric acid and non-esterified fatty acids (NEFA) were reported to lead to reproductive disorders, such as placental retention, metritis, endometritis, purulent vaginal discharge, impaired cyclic activity, and infertility (Kaufmann et al., 2010; Ospina et al., 2010; Abdelli et al., 2017). These disorders were also proposed to result in huge economic losses due to prolongation of the interval to first oestrus, increase in insemination numbers per pregnancy, prolongation of the duration of becoming pregnant after delivery, increase in genital infections, and risk of ovarian cysts (Geishauser et al., 2001).

The transition period is the time when metabolic and infectious disorders occur most frequently (Ingvarsen, 2006), and clinical and subclinical ketosis, fatty liver, abomasal displacement, placental retention, metritis, and mastitis are reported to be the most common problems (Grummer, 1995; Drackley, 1999). Ketosis, abomasal displacement, and placental retention can develop due to negative energy balance (NEB) resulting from increased energy demand and decreased feed intake (Duffield et al., 2002). Energy deficiency may also affect the immune system, playing a key role in the emergence of infections, such as metritis and mastitis (Dohoo and Martin, 1984; Kremer et al., 1993).

*Corresponding author. Tel.: +90 424 237 0000; fax: +90 424 238 8173.
E-mail: makilinc@bingol.edu.tr

Many significant hormones that regulate metabolism and energy balance are synthesised by the white adipose tissue, which is reported to be a vital structure due to its regulating role in the balance between glucose and insulin. Abnormal release of hormones from the white adipose tissue leads to development and progression of Type II diabetes in humans (Booth et al., 2016). Asprosin, one of the hormones derived from fat tissue in mammals, has been described in recent studies (Romere et al., 2016). Asprosin is a peptide hormone that stimulates glucose release from hepatic tissue during fasting. This hormone causes an increase in glucose synthesis of the liver by activating the glucose-protein-cAMP-protein kinase A pathway. Furthermore, recombinant asprosin injection causes an elevation in plasma glucose and insulin hormone levels (Alan et al., 2019). During fasting, elevated concentration of asprosin penetrating the blood-brain barrier is reported to trigger the appetite by stimulating the hypothalamic nutrition centre, subsequently resulting in the decrease of the asprosin level after feeding (Duerschmid et al., 2017). Asprosin initiates liver activity during fasting and has a critical role in normal neurological functions, too. Therefore, a systemic co-ordination between fasting, appetite, and asprosin-mediated hepatic glucose release via different hypothalamic and hepatic mechanisms has been supposed (Romere et al., 2016). It has also been reported that several studies are focused on the relationship between asprosin and Type II diabetes as most of the hormones synthesised in the white adipose tissue play a role in the prevention of Type II diabetes (Zhang et al., 2019).

The presence of asprosin in humans has recently been determined. This hormone becomes active during fasting and leads to an elevation in plasma glucose and insulin hormone. In the light of these data, the aim of this study was to investigate the presence of a relationship between β -hydroxybutyric acid, which increases in NEB in cows, and asprosin, and also between postpartum disorders, which are more likely to develop as a result of NEB, and whether this hormone could be used for the early diagnosis of ketosis.

MATERIALS AND METHODS

Animals and samplings

The animal experiments were approved by the Ethics Committee Report (01/11/2019–3819) obtained from the Experimental Animals Local Ethics Committee of Bingöl University.

First stage of the study

In the first stage, 20 healthy Simmental cows aged between 3 and 4 years were kept in an animal enterprise located in Bingöl province in the eastern part of Turkey. Cows that had undergone 1–2 lactations and were within the first 15 days of lactation were used in the study. The animals were fed year-round in semi-open pastures and a free-roaming barn with concentrate feed containing barley and a ration

consisting of dry meadow grass, corn silage, alfalfa and hay. Cows included in the study were milked twice a day (DeLaval, Turkey), and their average lactation milk yield was 5,500 L.

At this stage, blood samples were obtained from 20 such cows, which had clinically healthy body condition scores varying between 3.5 and 3.8 and completed their normal gestation period, at calving and on days 3, 6, 9, 12 and 15 postpartum. The study aimed to reveal the relationship between the following two parameters by measuring β -hydroxybutyric acid and asprosin levels in the blood samples.

Second stage of the study

In the second stage of the study, 200 cows of different ages and breeds raised in Bingöl and its surroundings were used. Data were collected regarding the age, breed, number of lactations, lactation period, daily milk yield, dry period, milking method, whether they had a previous disease, whether they went to the pasture, whether any hygiene process was applied before and after milking, and the nutrition regime of the animals. The animals were divided into two groups at this stage: (1) Cows that had a normal birth and did not develop any problems in the first 15 days postpartum were allocated to Group 1 ($n = 100$) and (2) cows that had a postpartum problem within the first 15 days of normal birth were designated as Group 2 ($n = 100$). The cows included in the second group were selected from the animals that had experienced at least one of several problems, including uterine prolapse, placental retention, septic metritis, puerperal metritis, foot disease, hypocalcaemia, hypomagnesaemia, ketosis, downer cow syndrome, tympany, and clinical mastitis, among others.

A blood sample was obtained from cows in both groups on postpartum day 15. The levels of β -hydroxybutyric acid and asprosin were measured in the blood samples obtained from animals in both groups, and the groups were compared with regard to these levels. In-group comparisons of Group 2 were made for blood asprosin and β -hydroxybutyric acid values after considering the various types of postpartum disorders.

In addition, the animals in Groups 1 and 2 were divided into two subgroups: (1) those with β -hydroxybutyric acid concentration of $<1.2 \text{ mmol L}^{-1}$ (without ketosis) and (2) $\geq 1.2 \text{ mmol L}^{-1}$ (with subclinical ketosis), and the asprosin levels were compared.

β -hydroxybutyric acid measurements

β -hydroxybutyric acid concentrations of the cows were measured with a ketometer (TaiDoc, Hasvet, Turkey). After the test strips were placed in the ketometer, less than one drop of (0.1 mL) blood obtained from the tail vein was dropped onto the terminal part of the strip and the results were read after 15 s (Baştan and Gürbulak, 2012).

Based on previous studies, it was reported that the ketometer device is suitable for use in farms for monitoring the level of β -hydroxybutyric acid (Bach et al., 2016; Fiorentin et al., 2017; Leal Yepes et al., 2018).



Asprosin measurements

Asprosin levels in the blood sera were determined using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Sunred Bovine Asprosin Kit, Shanghai) and an ELISA reader (Bio Tek Instruments, USA) as described by Ugur and Aydin (2019).

Statistical analysis

Following suggestions from statisticians, in the first stage of the study, the appropriate number of animals to be used was determined by performing a power analysis. As a result of the power analysis, this number was reached with a 98% confidence interval. The data were presented as mean \pm standard deviation (SD). A value of $P < 0.05$ was considered significant. Statistical analyses were performed using the SPSS statistical program (22.0, Chicago, IL, USA). While the Shapiro–Wilk test or skewness and kurtosis values were used to determine the normality of data distributions, the variance homogeneity of the groups was determined by the Levene test. The independent t -test or one-way analysis of variance (ANOVA) was used for data fulfilling the parametric assumptions, followed by Tukey's *post hoc* test for multiple comparisons. For the analysis of data that did not fulfil the parametric assumptions, the Kruskal–Wallis and then the Mann–Whitney U test were used for multiple comparisons. The Spearman rank correlation test was done to demonstrate the relationship between asprosin and β -hydroxybutyric acid levels. Changes in asprosin and β -hydroxybutyric acid levels according to the measurement time were determined using the method of repeated measurements in the general linear model (GLM) procedure. Mauchly's test of sphericity was used to check the sphericity assumption. The Greenhouse–Geisser test was used for the parameters for which the assumption of sphericity was not met.

RESULTS

Cows excluded from the study

In the second stage of the study, the β -hydroxybutyric acid level ($n = 95$) of five cows from the diseased animal group (Group 2) and the asprosin level ($n = 99$) of one cow from the healthy animal group (Group 1) were not included in the statistical analysis due to high out-of-range values.

First stage of the study

There was no correlation detected after the Spearman correlation test performed between β -hydroxybutyric acid levels measured at the time of birth (day 0), on postpartum days 3, 6, 9, 12, and 15 and asprosin levels ($P > 0.05$, for all time points).

When the β -hydroxybutyric acid and the asprosin levels were compared on the days that the blood samples were obtained, no statistically significant differences were found between the days ($P > 0.05$) as shown in Table 1.

Second stage of the study

As a result of measurement of blood samples obtained from 200 cows using the ketometer, the β -hydroxybutyric acid levels were found to be higher in postpartum diseased animals in Group 2 compared to Group 1. When the groups were compared with regard to asprosin levels, asprosin levels were found to be higher in the diseased cows in Group 2 than in the healthy ones, as shown in Table 2. In addition, a weak negative correlation was found between β -hydroxybutyric acid levels and asprosin levels ($r = -0.194$, $P < 0.01$) on the basis of the Spearman correlation test, as shown in Fig. 1.

When the β -hydroxybutyric acid and the asprosin levels of cows with disease were compared separately with those of the healthy cows, the β -hydroxybutyric acid levels were found to be higher in cows with placental retention than in healthy cows. The asprosin levels of animals with clinical hypocalcaemia, puerperal metritis and lameness were found to be higher than those of the healthy animals as shown in Table 3.

No difference was found by the in-group comparison of the diseased animal group (Group 2) with regard to β -hydroxybutyric acid levels. When the animals were compared with regard to asprosin levels, the asprosin level was found to be lower in cows with metritis and higher in cows with lameness, as compared to cows with other diseases (vaginal prolapse, abomasal displacement, theileriosis, mammary oedema, pneumonia, and acute rumen acidosis) as shown in Table 4.

In the second stage of the study, the animals in Groups 1 and 2 were divided into two subgroups: cows with β -hydroxybutyric acid levels below 1.2 mmol L^{-1} (without ketosis, $n = 176$) and higher than 1.2 mmol L^{-1} (with subclinical ketosis, $n = 18$). The mean asprosin level was found to be higher in the group without ketosis compared to cows with subclinical ketosis, as shown in Table 5.

Table 1. β -hydroxybutyric acid (BHBA) and asprosin levels at the time of birth and on days 3, 6, 9, 12 and 15 postpartum

Parameter	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15	P
BHBA (mmol L^{-1})	0.78 ± 0.46	0.71 ± 0.35	0.61 ± 0.26	0.74 ± 0.46	0.92 ± 0.78	0.66 ± 0.23	0.242 ¹
Asprosin (ng mL^{-1})	1.69 ± 0.64	1.57 ± 0.53	1.72 ± 0.56	1.72 ± 0.62	1.69 ± 0.67	1.73 ± 0.45	0.889 ²

The data are presented as the mean values of the measurements on different days \pm standard deviation.

¹Repeated measurements ANOVA, Greenhouse–Geisser.

²Repeated measurements ANOVA, Sphericity Assumed.



Table 2. β -hydroxybutyric acid (BHBA) and asprosin levels of cows in Group 1 and Group 2

Parameter	Group 1 (<i>n</i> = 99)	Group 2 (<i>n</i> = 95)	<i>P</i>
BHBA (mmol L ⁻¹)	0.66 ± 0.31	0.77 ± 0.31*	0.022 ¹
Asprosin (ng mL ⁻¹)	0.86 ± 0.27	0.99 ± 0.41*	0.038 ²

The data are presented as mean ± standard deviation.

¹Independent *t*-test.

²Mann–Whitney *U* test.

**P* < 0.05 shows the significance level of the differences between mean values of the groups in the same line.

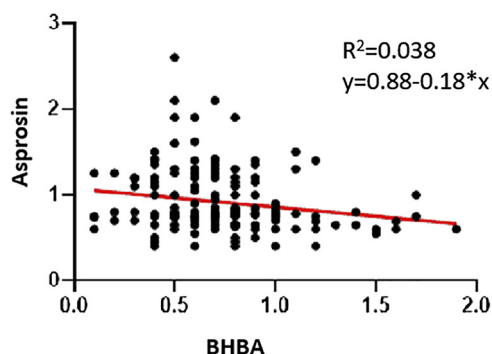


Fig. 1. The correlation between β -hydroxybutyric acid (BHBA) and asprosin levels measured at the second stage of the study

The animals without ketosis (*n* = 176) were divided into two subgroups, i.e. healthy (*n* = 90) and diseased (*n* = 86). The mean asprosin level was found to be higher in diseased cows than in healthy cows as shown in Table 6.

No statistically significant difference was found between ‘without postpartum clinical problems’ (*n* = 9) and ‘with postpartum clinical problems’ (*n* = 9) cows in the subclinical

Table 5. Asprosin levels in animals with subclinical ketosis or without ketosis

Parameter	Subclinical ketosis (BHBA ≥ 1.2 mmol L ⁻¹) (<i>n</i> = 18)	Without ketosis (BHBA < 1.2 mmol L ⁻¹) (<i>n</i> = 176)	<i>P</i>
Asprosin (ng mL ⁻¹)	0.71 ± 0.21	0.95 ± 0.35*	0.006 ¹

BHBA (β -hydroxybutyric acid).

The data are presented as mean ± standard deviation.

¹Independent *t*-test.

**P* < 0.05 shows the significance level of the differences between the mean values of the groups in the same line.

Table 6. Asprosin levels in healthy and diseased animals without ketosis

Parameter	Without ketosis Diseased (<i>n</i> = 86)	Without ketosis Healthy (<i>n</i> = 90)	<i>P</i>
Asprosin (ng mL ⁻¹)	1.02 ± 0.41*	0.88 ± 0.28	0.007 ¹

The data are presented as mean ± standard deviation.

¹Independent *t*-test.

**P* < 0.05 shows the significance level of the differences between the mean values of the groups in the same line.

ketosis (BHBA ≥ 1.2 mmol L⁻¹) group (*n* = 18), as shown in Table 7.

DISCUSSION

As a result of 16 different prevalence studies conducted in dairy cattle farms in the United States since 1984 by

Table 3. β -hydroxybutyric acid (BHBA) and asprosin levels of cows with disease, as compared to those of healthy cows

Parameter	Clinical hypocalcaemia (<i>n</i> = 9)	Mastitis (<i>n</i> = 34)	Puerperal metritis (<i>n</i> = 20)	Placental retention (<i>n</i> = 18)	Lameness (<i>n</i> = 5)	Other diseases (<i>n</i> = 9)	Healthy (Group 1) (<i>n</i> = 99)
BHBA (mmol L ⁻¹)	0.64 ± 0.22 ¹	0.75 ± 0.28 ¹	0.79 ± 0.26 ¹	0.90 ± 0.4** ¹	0.54 ± 0.05 ¹	0.76 ± 0.41 ¹	0.66 ± 0.31
Asprosin (ng mL ⁻¹)	1.08 ± 0.34* ²	0.91 ± 0.30 ¹	1.03 ± 0.42* ¹	0.90 ± 0.37 ¹	1.84 ± 0.51** ²	0.81 ± 0.30 ¹	0.86 ± 0.27

The data are presented as mean ± standard deviation.

¹Independent *t*-test.

²Mann–Whitney *U* test.

Asterisks (**P* < 0.05 and ***P* < 0.01) indicate statistical difference compared to the healthy group.

Table 4. Comparison of β -hydroxybutyric acid (BHBA) and asprosin levels in Group 2 (diseased animals)

Parameter	Clinical hypocalcaemia (<i>n</i> = 9)	Puerperal metritis (<i>n</i> = 20)	Mastitis (<i>n</i> = 34)	Placental retention (<i>n</i> = 18)	Lameness (<i>n</i> = 5)	Other diseases (<i>n</i> = 9)	<i>P</i>
BHBA (mmol L ⁻¹)	0.77 ± 0.30	0.76 ± 0.41	0.66 ± 0.31	0.90 ± 0.4	0.54 ± 0.05	0.76 ± 0.41	0.183 ¹
Asprosin (ng mL ⁻¹)	1.00 ± 0.41 ^b	0.81 ± 0.30 ^b	0.86 ± 0.27 ^b	0.90 ± 0.37 ^b	1.84 ± 0.51 ^a	0.81 ± 0.30 ^b	0.024 ¹

The data are presented as mean ± standard deviation.

¹One-way ANOVA, Tukey's *post hoc* test.

The difference between the mean values of the groups that carry different letters (a-b) in the same line is significant at *P* < 0.05 level.



Table 7. Asprosin levels in animals with subclinical ketosis (BHBA ≥ 1.2 mmol L⁻¹) with postpartum clinical problems and without postpartum clinical problems

Parameter	Subclinical ketosis Clinical problems (n = 9)	Subclinical ketosis Without clinical problems (n = 9)	P
Asprosin (ng mL ⁻¹)	0.69 \pm 0.29	0.73 \pm 0.12	0.73 ¹

BHBA (β -hydroxybutyric acid).

The data are presented as mean \pm standard deviation.

¹Independent *t*-test.

evaluating β -hydroxybutyric acid levels in the early postpartum period, postpartum metabolic diseases were shown to be associated with β -hydroxybutyric acid levels (LeBlanc et al., 2005; Duffield et al., 2009; Ospina et al., 2010; Chapinal et al., 2011). In one study by Duffield et al. (2009), serum β -hydroxybutyric acid levels that were ≥ 1.2 mmol L⁻¹ in the first postpartum week were associated with the development of abomasal displacement and metritis. However, no association could be found between clinical mastitis and elevated serum β -hydroxybutyric acid levels. According to the studies investigating the relationship between ketosis and diseases, the threshold value of serum β -hydroxybutyric acid was found to be ≥ 1.1 mmol L⁻¹ for clinical ketosis, ≥ 1.7 mmol L⁻¹ for abomasal displacement, ≥ 1.4 mmol L⁻¹ for metritis, and ≥ 1.1 mmol L⁻¹ for lameness (LeBlanc et al., 2005; Duffield et al., 2009). According to a study conducted with almost 6,000 cows in ten European countries (Suthar et al., 2013), cows with elevated serum β -hydroxybutyric acid levels (≥ 1.4 , ≥ 1.1 and ≥ 1.7 mmol L⁻¹) within the first 15 days postpartum were found to have a 1.7-, 10.5- and 6.9-fold greater risk for metritis, clinical ketosis, and abomasal displacement, respectively, compared to cows having lower serum β -hydroxybutyric acid levels. The threshold value for serum β -hydroxybutyric acid has been reported to be ≥ 0.3 mmol L⁻¹. Similar results were found concerning the risk of abomasal displacement in a different study (LeBlanc et al., 2005). In the study of Duffield et al. (2009), the risk for metritis and abomasal displacement was also reported to increase in a manner similar to the previously described studies, a finding which was also described by Ospina et al. (2010). In these previous studies, it has been reported that β -hydroxybutyric acid can be used as an important marker for some postpartum diseases (LeBlanc et al., 2005; Duffield et al., 2009). However, although the β -hydroxybutyric acid level was found to be higher in diseased animals than in healthy animals, in the present study and in another study (Suthar et al., 2013) no relationship was found between the level of β -hydroxybutyric acid and disease occurrence. It was concluded that this difference was due to the fact that many factors, such as metritis, mastitis, placental retention and hypocalcaemia, play a role in the development of postpartum diseases, and that β -hydroxybutyric acid levels are also affected by various factors.

In a study conducted by Maylem et al. (2021) to explore the relationship between asprosin and polycystic ovary

syndrome (PCOS) in women, heifer ovaries collected from the slaughterhouse were used as test material. It was shown that asprosin receptors were expressed in the follicles of the collected ovaries. This study is the only one in the literature investigating asprosin in cattle. In the present study, we determined that asprosin hormone was present in measurable levels in the blood serum of cows, and the asprosin levels were found to be higher in cows with postpartum diseases than in healthy cows. In the postpartum period, a reverse correlation was found between β -hydroxybutyric acid and asprosin levels. In the first 15 days of the postpartum period, the asprosin level did not change among days. In the cows with postpartum diseases, the asprosin levels were found to be higher in animals without ketosis compared to the subclinical ketosis group. In addition, the asprosin level was found to be higher in diseased animals without ketosis compared to healthy animals without ketosis.

It can be concluded that asprosin, a hormone which has been shown to play an important role in energy metabolism and to be associated with insulin resistance in studies conducted in humans and mice, could be partially associated with ketosis cases related to many metabolic disorders in cows. According to these findings, our data could be used in the prevention, control and treatment of some postpartum disorders associated with ketosis, as well as for the development of novel hypotheses concerning the mode of action of this hormone. We also concluded that more comprehensive studies are required for better understanding the role of the asprosin hormone in cows and other animals and to determine whether or not this hormone could be used for the early diagnosis of ketosis.

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