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ORIGINAL ARTICLE

The effect of subclinical ketosis on indices of insulin sensitivity and selected metabolic variables in transition dairy cattle

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Abstract Up to now, there have been several endeavors describing the extent of insulin resistance (IR) in dairy cows with particular emphasis on transition period, but it is not fully explicit what IR plays in the etiology of subclinical ketosis (SCK) in transition cows. The present study was therefore conducted on a commercial dairy herd with a stock population of 730 Holstein-Friesian cows to evaluate the extent of IR in transition cows with SCK. Blood samples were collected initially from each of the transition cows (n = 24) to estimate serum levels of β-hydroxybutyrate (BHBA). Cows with no clinical signs but having serum BHBA >1.20 to 2.9 mmol/l were considered to have SCK (n = 20), while those having serum BHBA concentrations <1.00 mmol/l were considered as controls (n = 4). Blood samples were then used for estimating values of serum non-esterified fatty acid (NEFA), glucose, insulin, and cortisol. Different surrogate indices for insulin sensitivity were also calculated. Our findings demonstrated that values of BHBA, NEFA, and insulin were significantly higher in cows with SCK than those of controls ($P < 0.05$), while values of cortisol and glucose, despite being higher in diseased cows than controls, did not reach statistical significance (P = 0.605 and 0.269). Indices of insulin sensitivity showed a significant decrease in revised quantitative insulin sensitivity check index (RQUICKI) (P = 0.047) and a significant increase in homeostasis model assessment (HOMA) in diseased cows compared with controls (P = 0.007). The elevated values of serum glucose, insulin, cortisol, NEFA, and BHBA are therefore suggestive of impaired whole-body insulin sensitivity and were consistent with decreased RQUICKI in the diseased cows. Further studies are needed to use prophylactic feed additives to enhance insulin sensitivity and to help mitigate the deleterious consequences of bovine ketosis during the transition phase.

Keywords Subclinical ketosis · Insulin resistance · Dairy cattle · Transition period

Introduction

The periparturient period, defined as 3 weeks around the time of parturition, is physiologically stressful and represents a critical time for dairy cattle (Sundrum 2015). During that time, many physiological, nutritional, metabolic, and immunological alterations can occur as the cow transits from a gestational non-lactating state to the period of copious milk production (Sordillo and Raphael 2013). The ability of a dairy cow to withstand these alterations is crucial to optimize herd management since the demands for milk production cannot be met solely by feed intake (Herdt 2000). Cattle unable to safely transit through this period are at a higher risk for metabolic disorders and decreased milk yield (Drackley 1999; Herdt 2000).

Among the commonly reported metabolic disorders that occurred during the transition phase, hyperketonemia is mostly prevalent and provokes a significant economic impact (Opsomer 2015). In that context, a working definition of SCK is that "a condition indicated by abnormal concentrations of circulating ketone bodies in the absence of detectable clinical signs of the disease" (Andersson 1988). In a dairy cattle herd, SCK can lead to serious economic loss involving a decrease in milk yield, decrease reproductive efficiency, and

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increase the risk of various diseases such as abomasal displacement and clinical ketosis (Andersson 1988; Opsomer 2015). Hence, early diagnosis of SCK can permit proper treatment and help mitigate further economic losses (Enjalbert et al. 2001; Geishauser et al. 2001). Because of its stability in blood, *beta*-hydroxybutyrate (BHBA) still considered the gold standard test for the diagnosis of SCK under field condition (Herdt 2000).

Insulin resistance (IR) has been defined as a state when a physiological level of insulin produces a lesser biological response than do normal (Kahn 1978). Other investigators have described IR as a condition in which high amount of insulin is required to produce a normal biological response (Berson and Yalow 1970). It has been reported that insulin could play a fundamental role in the physiological adaptation of dairy cows especially around the time of parturition (Zachut et al. 2013). The glucose metabolism in ruminants is often characterized by low peripheral glucose levels and a low insulin response of the peripheral tissues (Hayirli 2006). After calving, cows undergo a period of transient IR to prioritize the insulin-independent uptake of glucose by the mammary gland in order to favor milk production. Hence, the maintenance of blood glucose values within the normal physiologic ranges is of utmost importance during the transition phase (De Koster and Opsomer 2013).

Factors that cause IR in humans are also related to those involved in the development of ruminant hepatic lipidosis and ketosis (Drackley et al. 1992). The mechanisms for IR are not completely understood and seem to be a combination of genetic, cellular, and environmental causes (Ferrannini and Mari 1998). To date, there have been several endeavors describing IR in dairy cows with emphasis on transition or lactation periods (Sano et al. 1993; Sternbauer and Luthman 2002; Terao et al. 2010; Jaakson et al. 2013) or in association with primary clinical ketosis in cows (Xu et al. 2014) or ketotic cows with primary displaced abomasum (Khalphallah et al. 2015). But, it is not fully explicit what IR plays in the etiology of SCK. In line with these considerations, the present study was designed to evaluate the extent of IR in cows with SCK during the transition period.

Materials and methods

Study area and animal population

The present study was conducted on a commercial herd with a stock population of 730 Holstein-Friesian cattle. The farm is located in the city of Ras El-Bar, Damietta Governorate, Egypt. The study has taken place during the period between August and October 2015 where the average annual temperature was 20.2 °C (26 °C in August and 23 °C in October) (http://en.climate-data.org/location/51100/). Cows on the farm had, in general, an average of 305 days normalized milk production, body weight of 550 ± 50 kg, and at 3–7 years of age and where economics played the main role in farm decisions. The animals were apparent healthy, of different parities, and were artificially inseminated. The cows milked twice a day with a record of milk production in an average of 20.0 ± 3.0 kg/head/day in summer season and had a range of body condition score at 3–3.5. The investigated cows were free from external and internal parasites and had no history of metabolic and production disease in that year. All animals were kept under identical conditions of housing and vaccination throughout the study period. The feed for all cows consisted of a base ration fed as a daily total mixed ration as well as corn silage and Alfa hay in summer as an alternative source of roughage. Water supply was offered to all animals ad libitum. Cows were dried off 60 days before the expected time of parturition.

Sampling and the criteria of animal selection

On the farm, there were 170 cows at different stages of lactation. Twenty-four primiparous cows (i.e., cows pregnant with their second calf), at 3–4 years of age, were only located at a late gestation time approximately 3 weeks before the expected time of parturition. For the initial screening of SCK, 10 ml of venous blood were drained from each of the transition cattle (n = 24), through coccygeal venipuncture into a plain tube, i.e., without anticoagulants. Blood was rapidly cooled on crushed ice and was transported to the laboratory to be centrifuged at 1400×g for 10 min to separate blood serum. Only clear non-hemolyzed serum was collected and then aliquoted for estimation of BHBA. Basically, cows with no remarkable clinical signs but having serum BHBA concentrations >1.20 to 2.9 mmol/l were considered to have SCK as previously mentioned by McArt et al. (2012) and Xu et al. (2014). At the meantime, cows with no clinical signs and serum BHBA concentration <1.00 mmol/l were considered to be negative controls. The collected serum samples were further used to estimate the following biochemical variables: non-esterified fatty acid (NEFA), glucose, insulin, and cortisol, according to the standard protocols of the manufacturers. Commercial kits were used for estimating serum BHBA (Ben Biochemical Enterprise, Italy; Cat No. HB8855), glucose (Spinreact, Spain; Cat No. 41011), insulin (Bovine Insulin ELISA Kits, ALPCO, USA; Cat No. 30-AS1011.1), and cortisol (Bovine cortisol ELISA Kit, MyBioSource, USA Cat No. MBS738051), while NEFA was measured chemically according to the method described by Schuster and Pilz (1979). In general, cows were included in the present study in the following conditions: (1) if their previous gestation period was more than 260 days, (2) if they were not treated for ketosis before BHBA testing, and (3) if they have had a previous normal calving altitude and were being clinically healthy following the parturition.

Measurements of insulin sensitivity

Different surrogate indices were used to calculate insulin sensitivity as follows: revised quantitative insulin sensitivity check index (RQUICKI) = 1 / [log (glucose, mg/dl) + log (insulin, μU/ml) + log (NEFA, mmol/l)] (Xu et al. 2014); quantitative insulin sensitivity check index (QUICKI) = 1 / log (glucose, mg/dl) + log (insulin, μU/ml) (Katz et al. 2000); revised quantitative insulin sensitivity check index including BHBA ($RQUICKI_{BHB}$) = 1 / log (glucose, mg/dl) + log (insulin, μU/ml) + log (NEFA, mmol/l) + log (BHBA, mmol/l) (Balogh et al. 2008; Abuelo et al. 2016); homeostasis model assessment (HOMA) = insulin (μU/ml) × glucose (mmol/l) (Abuelo et al. 2016); log transformation of HOMA (log HOMA) = log (insulin (μU/ml) × glucose (mmol/l) (Abuelo et al. 2016); and reciprocal score of HOMA ($HOMA^{-1}$) = 1 / (insulin (μU/ml) × glucose (mmol/l) (Abuelo et al. 2016).

Statistical analysis

Data were statistically analyzed by using SPSS statistical software program (SPSS, version 15, USA). Independent samples *t* test was used to compare variables between ketotic and control groups. The means and standard deviation (SD) for each variable were calculated. At $P < 0.05$, results were considered statistically significant. Spearman correlation coefficient was also applied to emphasize the correlation among the tested variables and the indices of insulin sensitivity.

Results

An overview of biochemical alterations as well as surrogate indices of insulin sensitivity in dairy cattle with SCK during the transition period is presented in Tables 1 and 2. In the present study, SCK was diagnosed on the basis of serum levels of BHBA. Cows with serum BHBA >1.20 mmol/l were considered to have SCK ($n = 20$), while cows whose serum BHB < 1.00 mmol/l were served as controls ($n = 4$). Cows with SCK had no detectable clinical findings during the initial screening. Biochemically, values of serum BHBA, NEFA, and insulin were significantly higher in cows with SCK than those of controls ($P < 0.05$), while values of serum cortisol and glucose (despite being higher in diseased cows than those of controls) showed no significant changes between the two groups ($P = 0.605$ and $P = 0.269$, respectively) (Table 1).

Indices of insulin sensitivity showed a significant decrease in RQUICKI ($P < 0.047$) and a significant increase in HOMA ($P < 0.007$) in diseased cows compared with controls, while other surrogate indices showed no significant alteration between the two groups (Table 2). A positive correlation between BHBA and NEFA ($r = 0.44$, $P < 0.05$) was illustrated in Table 3. There were also statistically significant correlations among the estimated metabolic variables and indices of insulin sensitivity; the detailed results about these correlations are presented in Table 3.

Table 1 Means ± SD of selected metabolic profile in dairy cattle with subclinical ketosis compared with controls during the transition period

Variables	Control group ($n = 4$)	SCK group ($n = 20$)	*P* value
BHBA (mmol/l)	0.97 ± 0.112	1.8 ± 0.356	0.000
NEFA (mmol/l)	0.34 ± 0.04	0.50 ± 0.10	0.006
Glucose (mg/dl)	44.62 ± 4.85	53.16 ± 15.46	0.269
Insulin (μU/ml)	40.85 ± 4.94	53.72 ± 18.27	0.014
Cortisol (ng/ml)	12.58 ± 1.87	14.14 ± 5.79	0.605

BHBA β-hydroxy butyrate, *NEFA* non-esterified fatty acid, *SCK* subclinically ketotic group

Table 2 Means ± SD of insulin sensitivity indices in dairy cattle with subclinical ketosis compared with controls during the transition period

Index	Control group ($n = 4$)	SCK group ($n = 20$)	*P* value
RQUICKI	0.35 ± 0.013	0.32 ± 0.027	0.047
QUICKI	0.30 ± 0.009	0.29 ± 0.02	0.354
$RQUICKI_{BHB}$	0.34 ± 0.022	0.55 ± 0.36	0.224
HOMA	100.8 ± 19.9	161.8 ± 79.02	0.007
Log HOMA	1.95 ± 0.13	2.13 ± 0.26	0.200
$HOMA^{-1}$	0.0098 ± 0.002	0.0083 ± 0.006	0.630

RQUICKI revised quantitative insulin sensitivity check index, *QUICKI* quantitative insulin sensitivity check index, $RQUICKI_{BHB}$ revised quantitative insulin sensitivity check index including BHBA, *HOMA* homeostasis model assessment, *Log HOMA* log transformation of HOMA, $HOMA^{-1}$ reciprocal score of HOMA, *SCK* subclinically ketotic group

Discussion

In the present study, cows with SCK showed a statistically significant higher values of serum insulin, BHBA, and NEFA than those of controls, while values of serum glucose and cortisol showed no significant alterations between the two groups. Unlike the results obtained in the present study, some authors have found hypoinsulinemia and hypoglycaemia in several clinical entities including SCK in cows (Tehrani-Sharif et al. 2012), clinical ketosis in cows (Kerestes et al. 2009; Xu et al. 2014), clinical ketosis in buffalo (Teli and Ali 2007), or physiologically during the transition phase (Abuelo et al. 2016). Nevertheless, some authors have found normal glucose concentrations in cows with SCK (Grohn et al. 1983; Sakha et al. 2006, 2007; Forslund et al. 2010). The enormous glucose drain toward the udder and the distinctive features of the transition period in dairy cows make the

Table 3 The correlations among the metabolic variables and indices of insulin sensitivity in dairy cattle with subclinical ketosis during the transition period

	BHBA	NEFA	Glucose	Insulin	Cortisol	RQUICKI	QUICK	$RQUIKI_{BHB}$	HOMA	Log HOMA	$HOMA^{-1}$
BHBA	1	0.44*	−0.06	0.03	0.07	−0.06	0.24	−0.34	0.01	−0.09	0.09
NEFA		1	−0.22	0.06	−0.06	−0.22	0.26	−0.33	−0.05	−.20	0.49*
Glucose			1	0.25	0.31	−0.55**	−0.62**	−0.22	0.78**	0.71**	−0.47*
Insulin				1	0.30	−0.85**	−0.75**	0.12	0.78**	0.82**	−0.53**
Cortisol					1	−0.27	−0.22	−0.47*	0.38	0.26	0.03
RQUICKI						1	0.82**	−0.01	−0.89**	−0.89**	−0.49*
QUICKI							1	−0.22	−0.81*	−0.93**	0.69**
$RQUICK_{IBHB}$								1	−0.06	0.12	−0.41*
HOMA									1	0.94**	−0.61
Log HOMA										1	−0.71**
$HOMA^{-1}$											1

BHBA β-hydroxy butyrate, *NEFA* non-esterified fatty acid, *RQUICKI* revised quantitative insulin sensitivity check index, *QUICKI* quantitative insulin sensitivity check index, $RQUICKI_{BHB}$ revised quantitative insulin sensitivity check index including BHBA, *HOMA* homeostasis model assessment, *Log HOMA* log transformation of HOMA, $HOMA^{-1}$ reciprocal score of HOMA

*Correlation is significant at 0.05 level, **correlation is significant at the 0.01 level

glucose metabolism an example of how intensive genetic selection can drive metabolism to extremes. However, it becomes apparent that glucose could have a low sensitivity and cannot be a good criterion for the diagnosis of SCK, and it does not appear to be useful for monitoring SCK. Similar findings were previously obtained by Sakha et al. (2006). It has also been stated that the periparturient period in dairy cows could be associated with alterations in insulin action particularly in the peripheral tissues and the extent of insulin secretion prepartum has been found to correlate with higher insulin action (Zachut et al. 2013).

In the current investigation, BHBA was found to correlate significantly with NEFA ($r = 0.44$, $P < 0.05$). However, the negative correlation between BHBA and glucose concentrations was not observed here. These findings were coincided with those previously mentioned by Asl et al. (2011), but unlike those obtained by several authors elsewhere (Sakha et al. 2007; Tehrani-Sharif et al. 2012). In some reports, it has been suggested that plasma BHBA concentrations above 1.4 mmol/l combined with plasma glucose values of less than 55 mg/dl could be used to classify cows as having poor energy status (Whitaker et al. 1993).

Our findings showed that serum cortisol despite being higher in cows with SCK than those of controls, its values did not reach statistical significance ($P = 0.605$). Nonetheless, serum cortisol was found to correlate positively with NEFA ($r = 0.63$; P 0.01) and glucose ($r = 0.42$; P 0.05). The insignificant elevation of circulating cortisol besides the decrease in the sensitivity of peripheral tissue to insulin (as demonstrated in this study) could result in a state of hyperglycemia. Nevertheless, the obtained results were away from that obtained by Forslund et al. (2010) who stated that cows with ketosis had significantly low levels of cortisol attributed that to the difficulties in synthesizing cortisol when their energy demands increase in peak lactation.

The sensitivity of the peripheral tissue to insulin has been assessed through the calculation of various surrogate indices according to recently published report (Abuelo et al. 2016). The widely accepted gold standard method for assessing peripheral insulin sensitivity is the use of hyperinsulinemic euglycemic clamp test, but it has been found to be time-consuming and being an invasive procedure; hence, it has not been recommended for use either under field conditions or on a large-scale epidemiological investigations (Muniyappa et al. 2008).

In the current investigation, cows with SCK showed a significant low RQUICKI index and high HOMA compared with controls. High HOMA level could indicate an increase in serum values of glucose, insulin, or both, thereby could suggest a low insulin sensitivity and low glucose tolerance, while low RQUICKI and $RQUICKI_{BHB}$ could reflect the high concentration of insulin, glucose, BHBA, and NEFA and hence could suggest a low insulin responsiveness. The obtained findings were in parallel with the recently published reports in periparturient dairy cows (Abuelo et al. 2016). Similarly, some authors have found that values of RQUICKI were lower in ketotic cows than those in controls (Xu et al. 2014). Likewise, indices of log HOMA, $HOMA^{-1}$, and QUICKI were not significantly changed between the diseased and control group. These findings were a little bit away from the results obtained by several other authors (Abuelo et al. 2014, 2016), probably due to the low number of animals being studied.

Several other researchers have recently highlighted the role of NEFAs in insulin sensitivity of dairy cattle. In that context, an increase of circulating NEFAs has been detected following a fasting period or by the intravenous administration of a tallow

infusion and has caused an impairment of insulin-stimulated glucose uptake by insulin-sensitive tissues (Oikawa and Oetzel 2006; Pires et al. 2007; Schoenberg et al. 2012). In the same line, it has been reported that cows with elevated serum values of BHBA (i.e., more than 1.0 mmol/l 2 days before calving until 7 days post calving) were likely to have higher IR in the glucose metabolism at their peripheral tissues than cows with normal values of BHBA during that time (Kelpe et al. 2003). In the same study, a higher concentration of NEFAs was significantly correlated with a lower insulin secretion. These findings could suggest a significant role of NEFAs in the development of IR. Besides NEFAs, other potential (unknown) factors originating from the adipose tissues could probably affect insulin-stimulated glucose uptake.

Conclusion

The results herein demonstrated that dairy cows could be vulnerable to the development of NEB and SCK during the transition phase. The correlation among the metabolic variables and indices of insulin sensitivity could indicate that dairy cows undergo massive metabolic adaptations during the transition period and could imply a potential role of IR in the development of SCK in dairy cows. Of particular interest, the elevated values of serum glucose, cortisol, insulin, NEFA, and BHBA are suggestive of impaired whole-body insulin sensitivity and were consistent with decreased RQUICKI values in these animals. Further studies are needed to use medications or feed supplements to enhance insulin sensitivity and help mitigate the deleterious consequences of bovine ketosis during the transition time.

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Authors' contributions MAY and MRE designed and coordinated the study. MRE is responsible for data collection and analysis, besides writing and reviewing the manuscript, and is responsible for all correspondence with the journal. MSY is responsible for clinical examinations, participated in sample collection, and took part in the writing of the manuscript. All authors approved the final version of the manuscript for publication.

Compliance with ethical standards All procedures performed were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The farm owner was asked to sign a consent for agreeing to the proposed testing and was given a document that contains an information about the disease definitions, its economic impact, and the potential clinical consequences.

Conflict of interest The authors declare that they have no conflict of interest.

References

Abuelo A, Hernandez J, Benedito JL, Castillo C (2014) A comparative study of the metabolic profile, insulin sensitivity and inflammatory response between organically and conventionally managed dairy cattle during the periparturient period. Animal 8:1516–1525

Abuelo A, Hernandez J, Benedito JL, Castillo C (2016) Association of oxidative status and insulin sensitivity in periparturient dairy cattle: an observational study. J Anim Physiol Anim Nutr (Berl) 100:279–286

Andersson L (1988) Subclinical ketosis in dairy cows. Metabolic diseases of ruminant livestock. Vet Clin North Am Food Anim Pract 4:233–251

Asl AN, Nazifi S, Ghasrodashti AR, Olyaee A (2011) Prevalence of subclinical ketosis in dairy cattle in the southwestern Iran and detection of cutoff point for NEFA and glucose concentrations for diagnosis of subclinical ketosis. Prev Vet Med 1(100):38–43

Balogh O, Szepes O, Kovacs K, Kulcsar M, Reiczigel J, Alcazar JA, Keresztes M, Febel H, Bartyik J, Fekete SG, Fesus L, Huszenicza G (2008) Interrelationships of growth hormone AluI polymorphism, insulin resistance, milk production and reproductive performance in Holstein–Friesian cows. Vet Med (Praha) 53:604–616

Berson SA, Yalow RS (1970) Insulin antagonist and insulin resistance. In: Ellenberg M, Rifkin H (eds) Diabetes mellitus: theory and practice. McGraw-Hill, New York, pp. 388–412

De Koster JD, Opsomer G (2013) Insulin resistance in dairy cows. Vet Clin North Am Food Anim Pract 29:299–322

Drackley JK (1999) Biology of dairy cows during the transition period: the final frontier? J Dairy Sci 82:2259–2273

Drackley JK, Richard MJ, Beitz DC, Young JW (1992) Metabolic changes in dairy cows with ketonemia in response to feed restriction and dietary 1, 3-butanediol. J Dairy Sci 75:1622–1634

Enjalbert F, Nicot MC, Bayourthe C, Moncoulon R (2001) Ketone bodies in milk and blood of dairy cows: relationship between concentrations and utilization for detection of subclinical ketosis. J Dairy Sci 84:583–589

Ferrannini E, Mari A (1998) How to measure insulin sensitivity. J Hypertens 16(7):895–906

Forslund KB, Ljungvall OA, Jones B (2010) Low cortisol levels in blood from dairy cows with ketosis: a field study. Acta Vet Scand 52(1):31

Geishauser T, Leslie K, Kelton D, Duffield TF (2001) Monitoring for subclinical ketosis in dairy herds. Compend Contin Educ Vet 23: S65–S71

Grohn Y, Lindberg LA, Bruss ML, Farvar TB (1983) Fatty infiltration of liver in spontaneously ketotic dairy cows. J Dairy Sci 66:2320–2328

Hayirli A (2006) The role of exogenous insulin in the complex of hepatic lipidosis and ketosis associated with insulin resistance phenomenon in postpartum dairy cattle. Vet Res Commun 30:749–774

Herdt TH (2000) Variability characteristics and test selection in herd-level nutritional and metabolic profile testing: metabolic disorders of ruminants. Vet Clin North Am Food Anim Pract 16:387–403

Jaakson H, Ling K, Samarütel J, Ilves A, Kaart T, Kärt O, Ots M (2013) Blood glucose and insulin responses during the glucose tolerance test in relation to dairy cow body condition and milk yield. Vet Med Zoot 62:28–35

Kahn CR (1978) Insulin resistance, insulin sensitivity, and insulin unresponsiveness: a necessary distinction. Metabolism 27:1893–1902

Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, Quon MJ (2000) Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab 85:2402–2410

Kelpe CL, Moore PC, Parazzoli SD, Wicksteed B, Rhodes C, Poitout V (2003) Palmitate inhibition of insulin gene expression is mediated at the transcriptional level via ceramide synthesis. J Biol Chem 278: 30015–30021

Kerestes M, Faigl V, Kulcsár M, Balogh O, Földi J, Fébel H, Chilliard Y, Huszenicza G (2009) Periparturient insulin secretion and whole-

body insulin responsiveness in dairy cows showing various forms of ketone pattern with or without puerperal metritis. Domest Anim Endocrinol 37:250–261

Khalphallah A, Aamer AA, AbdelAll T, Katoh H, Oikawa S, Nakada K, Elmeligy E (2015) Assessment of insulin and insulin resistance in dairy cattle with displaced abomasum pre and post-surgery. Scholar's Adv Anim Vet Res 2:162–176

McArt JAA, Nydam DV, Oetzel GR (2012) Epidemiology of subclinical ketosis in early lactation dairy cattle. J Dairy Sci 95:5056–5066

Muniyappa R, Lee S, Chen H, Quon MJ (2008) Current approaches for assessing insulin sensitivity and resistance in vivo: advantages, limitations, and appropriate usage. Am J Physiol Endocrinol Metab 294:E15–E26

Oikawa S, Oetzel GR (2006) Decreased insulin response in dairy cows following a four day fast to induce hepatic lipidosis. J Dairy Sci 89: 2999–3005

Opsomer G (2015) Interaction between metabolic challenges and productivity in high yielding dairy cows. Jpn J Vet Res 63:S1–S14

Pires JA, Souza AH, Grummer RR (2007) Induction of hyperlipidemia by intravenous infusion of tallow emulsion causes insulin resistance in Holstein cows. J Dairy Sci 90:2735–2744

Sakha M, Ameri M, Rohbakhsh A (2006) Changes in blood ß-hydroxybutyrate and glucose concentrations during dry and lactation periods in Iranian Holstein cows. Comp Clin Pathol 15:221–226

Sakha M, Ameri M, Sharifi H, Taheri I (2007) Bovine subclinical ketosis in dairy herds in Iran. Vet Res Commun 31:673–679

Sano H, Narahara S, Kondo T, Takahashi A, Terashima Y (1993) Insulin responsiveness to glucose and tissue responsiveness to insulin during lactation in dairy cows. Domest Anim Endocrinol 10:191–197

Schoenberg KM, Ehrhardt RM, Overton TR (2012) Effects of plane of nutrition and feed deprivation on insulin responses in dairy cattle during late gestation. J Dairy Sci 95:670–682

Schuster HG, Pilz K (1979) Colorimetric micro method for the determination of no esterified long-chain fatty acids in serum. Z Med Lab Diagn 20:212–217

Sordillo LM, Raphael W (2013) Significance of metabolic stress, lipid mobilization, and inflammation on transition cow disorders. Vet Clin North Am Food Anim Pract 29:267–278

Sternbauer K, Luthman J (2002) Insulin sensitivity of heifers on different diets. Acta Vet Scand 43:107–114

Sundrum A (2015) Metabolic disorders in the transition period indicate that the dairy cows' ability to adapt is over stressed. Animals 5:978–1020

Tehrani-Sharif M, Hadadi M, Noughabi HH, Mohammadi A, Rostami F, Sharifi H (2012) Bovine subclinical ketosis in dairy herds in Nishaboor, Iran. Comp Clinical Pathol 21: 1637–1641

Teli SA, Ali SL (2007) Plasma glucose and insulin profiles in ketotic buffaloes. Vet Scan 2, Article 19

Terao H, Fujita M, Tsumagari A, Sugino T, Bungo T (2010) Insulin dynamics in transition dairy cows as revealed by intravenous glucose tolerance testing. J Animal Vet Advances 9:2333–2337

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

Xu C, Shu S, Xia C, Wang B, Zhang HY (2014) Investigation on the relationship of insulin resistance and ketosis in dairy cows. J Vet Sci and Technol 5:2162

Zachut M, Honig H, Striem S, Zick Y, Boura-Halfon S, Moallem U (2013) Periparturient dairy cows do not exhibit hepatic insulin resistance, yet adipose-specific insulin resistance occurs in cows prone to high weight loss. J Dairy Sci 96:5656–5669