Relationship between Insulin to Glucagon Ratio and Metabolic Parameters in Primiparous and Multiparous Dairy Cows in Transitional Period

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Abstract

In order to investigate the relationship between insulin to glucagon ratio and metabolic factors in transitional period of dairy cows, 28 cows heifers with body condition score of 3.25- 3.75 were selected. Dairy cows received close up diet, from 21 days prepartum until parturition and lactation diet according to nutritional requirements after parturition. Blood was sampled at 10 days before and 20 days after parturition. BCS was assessed again 20 days after parturition. Serum levels of insulin, glucagon, glucose, NEFA, BHBA, triglyceride, cholesterol and albumin were measured. Insulin, glucagon, insulin to glucagon ratio, cholesterol and albumin levels were not significant between pre and postpartum in primiparous and multiparous cows. Glucose, triglyceride and BCS were decreased at 20 days postpartum than 10 days before partum but NEFA and BHBA were increased in both groups. Serum levels of all parameters were not significantly different between primiparous and multiparous cows. No significant correlation between insulin to glucagon ratio and blood metabolites before and after parturition in primiparous and multiparous cows was observed. It seems that insulin to glucagon ratio decreases as a physiologic response in a transitional period for adaptation to increasing demands of glucose, but is not related to NEFA and BHBA. Therefore, although insulin to glucagon ratio decreased, supplying nutrient requirements of dairy cows can decrease negative energy balance.

Key words: Cow, Glucagon, Insulin, Transitional Period.

Introduction

The transition period of dairy cows is defined as almost 3 weeks before to 3 weeks after parturition (Grummer, 1995). A majority of metabolic diseases and health problems occur during this time and decrease profit potential of dairy cows in that lactation (Drackley, 1999). In this period, demand of nutrients is increasing while feed intake is insufficient to meet metabolic demands (Ingvartsen and Andersen, 2000). During three weeks before parturition, nutrient requirements of fetus and placenta are at maximum levels, yet DMI decrease by 10-30 % (Bell, 1995). Within three weeks of the onset of lactation, milk yield, milk proteins, fat, and lactose increase rapidly and

exceed feed intake (Bertoni *et al*. 2009). The imbalance between energy consumed and energy demand for milk synthesis causes negative energy balance (Drackley, 1999; Ingvartsen, 2006).

A number of metabolic hormone levels change physiologically to overcome negative energy balance in this period. The concentration of insulin declines, glucagon increases, and insulin to glucagon ratio decreases as homeorhetic adaptations (Radostits *et al*. 2007, Park *et al*. 2010). In addition to hormonal changes, muscle and adipose tissue becomes resistant to insulin (Bell, 1995; De Koster and Opsomer, 2013). These changes stimulated mobilization of long chain fatty acids from adipose tissue, increase hepatic gluconeogenesis and reduce the peripheral use of glucose (Bobe *et al*. 2004; Ingvartsen, 2006). During lipolysis, non-esterified fatty acid is released from lipid stores, serves as an alternative source of energy for other tissues to preserve glucose and is used by the mammary gland for lactose synthesis. Also NEFA uptake by the liver can oxidize to the produced energy, oxidize and produce ketone bodies or esterified to triglyceride (Drackley *et al*. 2001). As a result of accumulation of triglyceride in the liver, excessive rate of lipid mobilization can increased plasma concentrations of NEFA predispose the cow to the development of metabolic disorders, such as fatty liver and ketosis (Grummer, 1995; Drackley *et al*. 2001; Bobe *et al*. 2004).

The objective of this study was to determine the relationship between insulin to Glucagon ratio and blood metabolites in primiparous and multiparous dairy cows in a transitional period.

Material and methods

Animals

This study was undertaken in two dairy herds with 256 and 115 milking cows in Fars province, southern Iran. Management status was the same in two herds. Twenty-eight clinically healthy Holstein dairy cattle (14 primiparous and 14 multiparous) were selected according to expected calving time and body condition score (BCS). Body condition was scored at the beginning of the experiment, on a scale of 1 (thin) to 5 (obese), using increments of 0.25 (Wildman *et al*. 1982). Animals with body condition scores of 3.25- 3.75 were enrolled in the experiment. BCS was assessed again 20 days after parturition. The animals were housed in open sheds barn before parturition and in a free-stall barn after parturition.

The rations were examined by Spartan ration formulation/evaluation software for dry matter, fiber, energy, crude protein, calcium, and phosphorus. Both multiparous and primiparous cows consumed a diet balanced to meet requirements for cows in close up and early lactation at ad libitum intake. Both pre and postpartum diets were mixed daily and fed as TMR. Ingredient and Chemical composition of the diet is shown in table 1. The cows were milked three times a day and milk production was recorded at each milking.

Blood sample collection

Blood was sampled via puncture of a coccygeal vein with vacupuncture tube without anticoagulant, 10 days before and 20 days after parturition. Serum was harvested following

centrifugation 10 min at 3000 g and was stored at -20 ºC until analysis for hormones and metabolites.

Measurement of blood hormones and metabolites

Insulin and glucagon concentrations were measured in serum by immunoassay method using bovine kits (Korain Biotech Co., LTD, Shanghai, China). The standard curves were prepared at concentrations 7.5 to 240 mIU/l for insulin and 100 to 3200 ng/l for glucagon. The sensitivity of these methods was 0.27 for insulin and 5.24 for glucagon. Intra and inter-assay coefficients of variation (CV) were <8 and < 10 % for insulin and glucagon. BHBA and NEFA concentrations were measured with 3-HBDH-NAD+3-hydroxybutyrate dehydrogenase- NAD+ and Acyl-CoA synthetase & Acyl-CoA oxidase methods by available kits (Randox Laboratories Ltd., Crumlin, Co., Antrim, UK). Serum concentrations of glucose, triglyceride, cholesterol, and albumin were determined using commercial kits: (GOD-PAP, GPO-PAP/, CHOD-PAP, Bromocresol green methods by Pars Azmoon Co, Tehran, Iran). All measurements were performed using spectrophotometer (Schimadzu, 120-12).

Statistical analysis

The sigma state program was used for statistical evaluations. Values were presented as means± standard error of means (SEM). Differences were declared significantly when p-values were ≤ 0.05. Parameters were recorded two times before and after parturition and were analyzed by the paired-t-test. Independent t-test was used to compare between primiparous and multiparous cows before and after parturition. The correlations between insulin to glucagon ratio and other parameters pre and postpartum were assessed by Pearson's correlation test.

Result

Plasma metabolites and hormones concentration are presented in tables 2 and 3. Milk production in multiparous and primiparous cows was 39.0±1.05 and 33.0±0.626 kg per day, respectively; so, this difference was significant ($p<0.05$). Serum levels of insulin, glucagon, and insulin to glucagon ratio were remained stable pre and post parturition in both groups ($p>0.05$). These parameters were not different between primiparous and multiparous cows as well ($p>0.05$).

Primiparous and multiparous cows had lower glucose ($p<0.001$), greater NEFA ($p<0.001$) and BHBA concentration postpartum than prepartum ($p<0.01$). There was no significant effect of parity on glucose, NEFA, and BHBA ($p>0.05$).
BCS decreased at 20 day postpartum ($p<0.001$) but was similar in both groups. BCS loss was 0.5 and 0.7 in multiparous and primiparous cows, which was not significant ($p>0.05$). Serum cholesterol and albumin difference in both groups were not significant pre and postpartum ($p>0.05$) and no differences were seen between primiparous and multiparous cows ($p>0.05$).

TG at 20 day postpartum decreased compared to 10 day before parturition in both groups ($p<0.001$) while it was not affected by parity ($p>0.05$). The Correlation between insulin to glucagon ratio and blood metabolites, 10 days before and 20 days after parturition, is presented in table 4. No significant correlation was observed between insulin to glucagon ratio and blood metabolites before and after parturition in primiparous and multiparous cows ($p>0.05$).

Table 1: Ingredient composition and chemical composition of the diet offered prepartum (-21 to parturition) and postpartum

	prepartum		**postpartum**	
Herds	**A**	**B**	**A**	**B**
Ingredient composition (% of DM)				
Alfalfa hay	6.42	23.45	2.71	14.25
Corn silage	27.42	25.04	34.37	28.54
Wheat straw	23.66	10.59	5.35	1.07
Beet pulp molasses	3.22	3.36	3.4	4.08
Barely	10.98	9.53	11.47	13.15
Corn	10.63	11.07	15.87	15.28
Wheat bran	3.84	5.41	2.67	3.61
Soybean meal	3.84	4.85	5.35	6.77
Cotton seed meal	3.90	4.93	5.43	1.8
Canola meal	3.89	-	6.5	-
Meat meal	0.20	-	0.85	-
Corn gluten meal	0.74	0.72	1.56	2.5
Lipid	0.43	0.42	1.2	1.16
Vitamin supplementation	0.63	0.41	0.59	0.57
Mineral supplementation	0.21	0.21	0.3	0.28
Sodium bicarbonate	-	-	0.9	0.87
Calcium carbonate	-	-	0.3	0.29
Salt	-	-	1.19	0.86
Chemical composition				
fNDF (%DM)	34.9	31.3	23.7	23
NDF (%DM)	44.3	40.5	34.8	31.8
NEL (mcal/kg)	1.49	1.59	1.55	1.56
CP (%DM)	13.23	15.01	16.15	16.82
MP (%DM)	9.19	9.56	11.21	11.50
RUP (%CP)	33.4	29.04	40	38.5
EE (%DM)	2.94	2.86	4	3.66
NFC (%DM)	36.3	38.2	40.1	41.5
Starch (%DM)	20.73	20.01	25.86	25.45
Ca (%DM)	0.44	0.55	0.8	0.79
P (%DM)	0.39	0.39	0.47	0.42
Ca: P (%DM)	1.12	1.41	1.7	1.71
Forage ratio (%DM)	57.5	59.08	42.43	43.85
DCAD (meq/kg)	100	140	208	192

DM: dry matter; fNDF: forage neutral detergent fiber; NDF: neutral detergent fiber; NEl: net energy for lactation; CP: crude fiber; MP: metabolizable protein; RUP: rumen undegradable protein; EE: ether extract; NFC: non-fibrous carbohydrates; DCAD: dietary cation- anion difference.

Table 2: serum hormone, metabolite concentrations and BCS (Mean± SEM) of primiparous and multiparous cows before and after parturition

	Primiparous			**Multiparous**		
	prepartum	**postpartum**	**p-value**	**Prepartum**	**postpartum**	**p-value**
Insulin (ng/l)	4172.67±263.80	3995.12±259.72	**0.37**	4585.05±294.14	4320.43±300.61	**0.18**
Glucagon (ng/l)	1249.68±68.83	1120.44±84.95	**0.22**	1293.65±58.17	1100.86±70.56	**0.07**
INS:GC (ng/ng)	3.45±0.24	3.79±0.24	**0.52**	3.59±0.22	3.74±0.133	**0.24**
Glucose (mmol/l)	3.29±0.12	2.42±.13	**<0.001**	3.63±0.18	2.29±0.07	**<0.001**
NEFA (mmol/l)	0.255±0.00	0.476±0.04	**<0.001**	0.256±0.00	0.390±0.02	**<0.001**
BHBA (mmol/l)	0.299±0.02	0.413±0.01	**0.002**	0.368±0.02	0.488±0.03	**0.005**
TG (mmol/l)	0.15±0.01	0.11±0.00	**<0.001**	0.14±0.01	0.08±0.00	**<0.001**
Cholesterol (mmol/l)	1.96±0.07	2.25±0.14	**0.08**	2.04±0.13	2.27±0.13	**0.307**
Albumin(g/l)	35.11±0.44	33.75±10	**0.28**	34.00±0.60	31.00±1.10	**0.16**
Bcs	3.41±0.03	2.65±0.09	**<0.001**	3.38±0.03	2.81±0.06	**<0.001**

Table 3: comparison of serum hormone, metabolite concentrations and BCS (Mean± SEM) between primiparous and multiparous cows in before parturition and after parturition

	Prepartum			**postpartum**		
	primiparous	**multiparous**	**p-value**	**primiparous**	**multiparous**	**p-value**
Insulin (ng/l)	4172.67±263.80	4585.05±294.14	0.29	3995.12±259.72	4320.43±300.61	0.42
Glucagon (ng/l)	1249.68±68.83	1293.65±58.17	0.627	1120.44±84.95	1100.86±70.56	0.85
INS:GC (ng/ng)	3.45±0.240	3.59±0.22	0.679	3.79±0.24	3.74±0.13	0.85
Glucose (mmol/l)	3.29±0.12	3.63±0.18	0.149	2.42±.13	2.29±0.07	0.36
NEFA (mmol/l)	0.255±0.006	0.256±0.005	0.825	0.476±0.044	0.390±0.02	0.10
BHBA (mmol/l)	0.299±0.02	0.368±0.02	0.074	0.413±0.01	0.488±0.03	0.06
TG (mmol/l)	0.15±0.01	0.14±0.01	0.540	0.11±0.00	0.08±0.00	0.08
Cholesterol (mmol/l)	1.96±0.07	2.04±0.132	0.590	2.25±0.14	2.27±0.13	0.92
Albumin (g/ l)	35.11±0.44	34.00±0.60	0.12	33.75±10	31.00±1.10	0.13

Table 4: Correlation between insulin to glucagon ratio and blood metabolites at 10 days before and 20 days after parturition

	Glucose	NEFA	BHBA	TG	Cholesterol	albumin
multiparous						
prepartum	-0.120	-0.310	-0.176	-0.132	-0.296	-0.116
postpartum	-0.210	0.451	-0.634	-0.209	0.076	0.061
primiparous						
prepartum	0.101	0.250	-0.027	0.202	-0.445	-0.138
postpartum	-0.406	0.264	-0.307	0.268	-0.143	-0.097

Discussion

In dairy cows, the transition period from the nonlactating to the lactating state is associated with increasing requirements of glucose for milk production (Bell *et al*. 1995). These rapid changes in energy demands require quick increase in hepatic gluconeogenesis and decrease in the peripheral use of glucose facilitated by physiological adaptations as "insulin declines, glucagon increases" resulting in decreases in insulin to glucagon ratio (Park *et al*. 2010).

Serum levels of Insulin, glucagon, and insulin to glucagon ratio were remained stable pre and post parturition in both groups ($p>0.05$). These parameters were not different between primiparous and multiparous cows as well ($p>0.05$). Insulin decreased after parturition as physiological changes to permit the direct use of glucose for milk synthesis, while glucose utilization and oxidation in non-mammary tissue are reduced (Bauman *et al*. 1988; Chilliard *et al*. 1999). In the study of Cavestany *et al*. (2009), insulin concentration remained stable during postpartum as a result of increased propionate production by corn base diet. Insulin has a positive correlation with energy intake (Chilliard, 1999). Increased propionate production, as a result of increased NFC in diet, results in an increased secretion of insulin (Brockman and Laarveld, 1986). The Previous studies (Holtenius *et al*. 2003; Rabelo *et al*. 2005) have also demonstrated a greater insulin concentration in high-energy than lower energy diets. In the current study, higher NFC after parturition delivered more glucogenic precursor to the liver and promoted insulin secretion. In the study of Selim *et al*. (2015), 7 days after parturition compared to 14 days before parturition, insulin was significantly lower, glucagon did not change and glucagon to insulin ratio was significantly higher. Other studies (Park *et al*. 2010, Smith *et al*. 2008) reported lower insulin

to glucagon ratio and greater NEFA and BHBA levels in early lactation than prepartum. A low insulin: glucagon ratio stimulates lipolysis in adipose tissue and ketogenesis in the liver (Radostits *et al*. 2007). In the present experiment, insulin to glucagon ratio at, 10 days before parturition and 20 days after parturition, was not significantly different, because insulin and glucagon concentrations had not a significant difference. As shown in the study of the Smith *et al*. (2008), at 21 days postpartum, this ratio was low and therefore was not correlated with other variables. Schwalm and Schultz (1976), determined the significant negative correlation between insulin and NEFA and positive correlation between insulin and triglyceride in ketotic cows. In the study of Whates *et al*. (2007), insulin in primiparous cows was negatively related to NEFA before and after parturition but no significant correlation was observed with BHBA. There was a negative correlation in multiparous cows with NEFA only before calving. However, a positive correlation to BHBA was observed between the first two weeks after parturition.

The change in glucose, NEFA, BHBA concentrations and BCS in the present study was parallel with the previous studies (Rabelo *et al*. 2005; Holtenius *et al*. 2003; Janovick *et al*. 2011). Maybe the fall in glucose level, in the first weeks of postpartum, is the consequence of the high demands for lactose synthesis because energy losses could not be compensated completely by energy intake (Greenfield *et al*. 2000; Ohgi *et al*. 2005; Weber *et al*. 2013). The rise in NEFA and BHBA reflects more negative energy balance and mobilization of NEFA from adipose tissue to provide energy for lactogenesis (Ingvarsten & Andersen 2000; Cavestany *et al*. 2005; Janovick *et al*. 2011). The higher postpartum NEFA together with the same insulin and insulin to glucagon ratio pre and postpartum may reflect insulin resistance. Insulin resistance ensures that mobilization of body reserves occurs as homeorhetic changes to support the lactation (Janovick *et al*. 2011, De Koster and Opsomer, 2013)

Serum concentrations of NEFA and BHBA, in the present study, elevated after parturition but were lower than cut-point levels at pre and postpartum (NEFA: milker < 0.7, dry cow< 0.4; BHBA: milker < 1.00, dry cow < 0.6 mmol/l (Radostits *et al*. 2007)). Although insulin to Glucagon ratio was low, a balanced diet with sufficient NFC pre and postpartum can prevent high elevation of NEFA and BHBA. These results show that low insulin to glucagon ratio in the transition period is not associated with abnormalities of energy indicators when glucogenic diet is consumed and nutrient requirements are supplied.

The difference in insulin concentration between primiparous and multiparous cows was not observed. Rabelo *et al*. 2005 and Wathes *et al*. 2007 also studied both multiparous and primiparous cows but did not observe differences in insulin concentration between parity. However, in the study of Moyes (2004), it was lower in primiparous cows. Blood glucose, NEFA, and BHBA concentrations were a similarity in multiparous and primiparous cows. In the studies of Meikle *et al*. (2004); and Cavestany *et al*. 2005, primiparous cows had higher NEFA and BHBA levels than multiparous cows. Greater adipose tissue mobilization was related to the requirement for growth, the demand of lactation and lower feed intake in primiparous cows. The

results of the present study show that energy intake based on each group requirements can compensate more production in multiparous cows, growth and lower feed intake in primiparous cows.

No difference was observed in albumin and cholesterol levels pre and postpartum and between parity. Albumin and cholesterol is an indicator of liver function whose low concentration is attributed to fatty liver disease in dairy cattle. In fatty liver, triglyceride is accumulated in the liver which reduces albumin and cholesterol production (Bertoni *et al.* 2008; Bobe *et al.* 2004; Loor *et al.* 2007). Albumin also may be diverted to glucose synthesis in support of milk production when metabolic reserves are limited (Roche *et a.*, 2013). The other situation that caused to diminished albumin is inflammation, because albumin is negative acute phase protein (Burke *et al*. 2010, Bertoni *et al*. 2008).

Serum triglycerides are an important source of long chain fatty acid for milk synthesis, which explains significant triglyceride decreases at the onset of lactation (Blum *et al*. 1983, Kessler *et al*. 2014). Also, it may be due to the compromised export rate from the liver (Marc Van den Top *et al*. 2005, Roche *et al*. 2013). In this study, lower triglyceride level after parturition was not accompanied with lower albumin and cholesterol levels. These results indicated that liver function does not impair and triglyceride decreases are due to transferring to milk.

Conclusion

Insulin to glucagon ratio decreases in a transition period as homeorhetic adaptation. Fat mobilization is not related only to insulin to glucagon ratio. Although insulin to glucagon ratio is similar pre and postpartum, lipid mobilization is greater at postpartum related to milk synthesis. Dairy cows normally mobilize lipid store in transition period regardless of energy intake, but sufficient energy intake can decrease negative energy balance and fat mobilization in a transition period.

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