

Original study

Glucose infusion response on some metabolic parameters in dairy cows during transition period

Enrico Fiore¹, Matteo Giancesella¹, Francesca Arfuso², Elisabetta Giudice², Giuseppe Piccione², Marcello Lora¹, Annalisa Stefani³ and Massimo Morgante¹

¹Department of Animal Medicine, Productions and Health (MAPS), University of Padua, Legnaro, Italy, ²Department of Veterinary Sciences, University of Messina, Polo Universitario dell'Annunziata, Messina, Italy, ³Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy

Abstract

The aim of this study was to evaluate the effect of glucose tolerance test (GTT) on glucose, insulin, non-esterified fatty acids (NEFA) and β -Hydroxybutyrate (BHB) concentration in dairy cows during prepartum and postpartum period. Forty Two clinically healthy Holstein cows were enrolled in two high producing dairy farms in the Northeast Italy. All animals were divided into two equal groups on the basis of the farm of origin: Group A (farm located in Vicenza) and Group B (farm located in Padua). Body condition score (BCS) was determined for both groups. GTT was performed 7 ± 5 days before and 7 ± 5 days after calving. Blood samples were collected from each animal before (T0) and after the administration of glucose at different times. Plasma concentration of glucose, insulin, NEFA and BHB was evaluated. Application of two way analysis of variance (ANOVA) showed no significant effect of farm and a significant effect of transition period (prepartum and postpartum) on BCS for both groups. Multivariate analysis of variance (MANOVA) was applied to determine significant effects of infusion glucose time, transition period and farm on glucose, insulin, NEFA and BHB. No significant difference was observed between Groups A and B and a significant effect of transition period and glucose infusion was found on all parameters studied. Results confirm that glucose is an important direct controller of metabolic response in dairy cows during the transition period because of the inappropriate energetic supply that characterizing this physiological phases.

Keywords: dairy cows, glucose tolerance test, prepartum and postpartum period, haematochemical parameters

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Corresponding author:

Giuseppe Piccione; email: gpiccione@unime.it

Department of Veterinary Sciences, Polo Universitario Annunziata, University of Messina. 98168, Messina, Italy

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Abbreviations: ADF: acid detergent fibre, AUC: area under curve, ANOVA: analysis of variance, BCS: body condition score, BHB: β -Hydroxybutyrate, GTT: glucose tolerance test, MANOVA: multivariate analysis of variance, NDF: neutral detergent fibre, NEB: negative energy balance, NEFA: non-esterified fatty acids, NFC: non fibre carbohydrates

Introduction

The transition period was defined as the change from a gestational non-lactating to a non-gestational lactating state (Morgante *et al.* 2012). It is characterized by changes in both metabolic and immune functions in animals (Piccione *et al.* 2012). Some of these alterations are related to increases in energy requirements driven by both fetal needs and lactogenesis. During this period, dairy cows showed a marked decrease in dry matter intake that is related to physical, behavioral, metabolic and hormonal changes around parturition (Grummer *et al.* 2004, Allen *et al.* 2005). As a result, dairy cows enter a negative energy balance (NEB) that is further aggravated by the nutrient prioritization towards the mammary gland (Leroy *et al.* 2008). The NEB status is characterized by alterations in blood metabolite and hormone profile (Piccione *et al.* 2012). Glucose metabolism takes on increased significance in the ruminants during pregnancy (Aldoretta & Hay 1999, Morgante *et al.* 2012). Increased glucose requirements of the gravid uterus during late pregnancy and even greater requirements of the lactating mammary glands necessitate major adjustments in glucose production and utilization in maternal liver, adipose tissue, skeletal muscle, and other tissues. During late pregnancy and early lactation hepatic glucose synthesis is increased to accommodate uterine or mammary demands even if the supply of dietary substrate is inadequate and glucose utilization by adipose tissue and muscle is reduced and glucose up-take by adipose tissue and muscle is reduced. Glucose demands during early lactation was greater than during the period of late pregnancy (Contreras & Sardoillo 2011). These responses, exaggerated by moderate undernutrition status in pregnant animals, are mediated by reduced tissue sensitivity and responsiveness to insulin, associated with decreased tissue expression of the insulin-responsive facilitative glucose transporter (GLUT4) (Bell & Bauman 1997). Peripheral tissue responses to insulin remain severely attenuated during early lactation but recover as the animal progresses through mid-lactation (Guesnet *et al.* 1991, Bell & Bauman 1997, Sasaki 2002). Thus, during the period of NEB, key hormone expression and tissue responsiveness alter to increase lipolysis and decrease lipogenesis, causing high levels of non-esterified fatty acids (NEFA) and β -hydroxybutyrate (BHB) concentration which are indicative of lipid mobilization and fatty acid oxidation (Sakha *et al.* 2006, Wathes *et al.* 2009). Excessive fat mobilization can induce an imbalance in hepatic carbohydrate and fat metabolism, which may result in ketosis (Herd & Emery 1992, Goff & Horst 1997). Ketosis is a metabolic disorder that primarily occurs 2-7 weeks after calving (Gillund *et al.* 2001) and is associated with increased concentrations of NEFA and decreased levels of glucose in blood (Itoh *et al.* 1998, Nielsen & Ingvarsen 2004). The ketone bodies, acetoacetate (AcAc) and acetone (Ac) can be reduced to BHB in an enzymatic reaction or decarboxylated to Ac in a spontaneous non-enzymatic reaction (Nielsen & Ingvarsen 2004). Ketosis causes economic losses to the dairy farmer due to treatment costs, decreased milk production, impaired reproduction efficiency and increased involuntary culling (Nielsen & Ingvarsen 2004).

The evaluation of NEFA and BHB represents a strategy for the monitoring of subclinical ketosis and prepartum negative energy balance in dairy cows (Contreras *et al.* 2010). The intravenous Glucose tolerance test (GTT) was useful to studying the physiological adaptation of animals to transition period since it produced a specific insulin response path (Morgante *et al.* 2012).

The aim of this study was to evaluate the effective modifications of some metabolic parameters (glucose, insulin, NEFA and BHB) in Holstein cows during transition period after Glucose Tolerance Test.

Material and methods

Farm condition and animals

42 multiparous Holstein cows were selected from two high producing dairy farms in the Northeast Italy. The data were collected from October 2011 to February 2012.

The cows were divided into two equal groups on the basis of the farm of origin: Group A, represented by cows obtained from farm located in Vicenza, Italy (45° 33' N; 11° 34' E; 39 m above sea level) and Group B, represented by cows obtained from farm located in Padua (45° 24' N; 11° 52' E, 12 m above sea level). Both farms make a dry period of 60 days and a period of steaming-up of 15 days before calving. Farms were selected with the same milk production (about 10 000 kg for year); moreover milk yield quality was not different: both farms had an average of 3.7 of milk-fat %, and the same values (3.4) of milk protein %. All the animals were clinically healthy and free from internal and external parasites. Their health status was evaluated based on rectal temperature, heart rate, respiratory profile, appetite, fecal consistency, and hematologic profile. Each animal was kept under natural photoperiod and ambient temperature. Body condition score (BCS, 0 to 5 scale) was evaluated before the start of trial in each subject. Table 1 shows the chemical composition of diets used during steaming-up and subsequent early lactation. The value of NDF (neutral detergent fiber), ADF (acid detergent fiber), NFC (non fiber carbohydrates), starch and crude protein, are the same in both farm. Water was available ad libitum.

Table 1
Chemical composition of diets used during steaming-up and subsequent early lactation in both farms

Chemical composition, %	Group A		Group B	
	Steaming-up	Early lactation	Steaming-up	Early lactation
Crude protein	12.63	16.59	12.87	16.66
Ethreal extract	4.18	6.01	4.55	5.32
Ash	7.55	7.42	7.83	7.34
NDF	43.25	33.17	43.99	33.41
NFC	33.39	38.81	33.56	38.27
Dry matter degradable	58.58	68.48	59.68	68.22
ADF	24.66	20.37	24.46	20.12
Starch	16.71	25.46	16.49	25.94

Blood sampling and analysis

Glucose tolerance test was carried out 7 ± 5 days before and 7 ± 5 days after calving. All animals were received sterile 50% glucose solution at a dosage of 0.25 g/kg body weight. Polyethylene catheters were aseptically inserted into both external jugular veins (one for the injection and the second for blood sampling) one the day prior to the experiment. Blood samples were collected into vacuum tubes (BD Vacutainer Systems, Preanalytical Solutions, Plymouth, UK) containing lithium-heparin. Blood samples were taken at T0 (pre-administration), and at T2, T5, T10, T15, T20, T30, T40, T60, T80, T100, T120, T140, T160 (2, 5, 10, 15, 20, 30, 40, 60, 80, 100, 120, 140, 160 minutes post-glucose administration). Samples were refrigerated and they were processed in the laboratory for analyses. The tubes were centrifuged (Labfuge 400, Heraeus) at 1750g for 10 min and the obtained plasma were stored at -18°C one hour after blood collection. All samples have been tested for glucose with a Cobas C 501 analyser (Roche Diagnostics, Mannheim, Germany). On Cobas C 501 were performed also NEFA and BHB analysis: NEFA concentrations were determined by using a colorimetric method, NEFA RX Monza test (kit no. FA 115, Randox, Crumlin, UK) while BHB concentrations were determined by RANBUT RX Monza test (kit no. RB 1007, Randox, Crumlin, UK). Free-insulin concentration in each sample was quantified with a commercial ^{125}I -IRMA kit developed for human samples (BI-Ins IRMA kit; CIS Bio International Ltd.) and previously validated for bovine plasma samples (Kerestes *et al.* 2009).

Statistical analysis

Two way analysis of variance (ANOVA) was applied to determine significant effect of different period (prepartum and postpartum) and farm on BCS. Multivariate analysis of variance (MANOVA) was applied to determine significant effects of infusion glucose time, transition period (prepartum and postpartum) and farm effect on glucose, insulin, BHB and NEFA. P -value <0.05 was considered statistically significant. Bonferroni's multiple comparisons test was used for post hoc comparisons. Area under curve (AUC) of total glucose, insulin, NEFA and BHB in the prepartum and postpartum period were calculated.

All statistical analyses were performed using Statistica 7.0 (StatSoft Inc., Tulsa, OK, USA). All the results were expressed as Mean Values \pm Standard Deviation ($M \pm SD$).

Results

The results showed no significant effect of farm ($P=0.32$) and a significant effect of transition period ($P<0.05$) on BCS.

Higher BCS values in prepartum period respect to postpartum period were found in both Groups (Figure 1).

Statistical effect of glucose infusion and transition period on glucose, insulin, BHB and NEFA was found ($P<0.001$), while no statistical effect of farm was found ($P>0.05$) (Figure 2).

Table 2 shows mean values of AUC \pm standard deviation of total glucose, insulin, NEFA and BHB in the prepartum and postpartum period.

Table 2

Mean values of AUC \pm standard deviation of total glucose, insulin, NEFA and BHB in the prepartum and postpartum period

Metabolic parameters	Prepartum period	Postpartum period
Glucose AUC, mmol/l	795.74 \pm 76.68	752.06 \pm 80.22
Insulin AUC, pmol/l	26412.46 \pm 2532.21	13973.52 \pm 1381.47
NEFA AUC, mEq/l	30.99 \pm 2.79	102.47 \pm 8.63
BHB AUC, mmol/l	82.71 \pm 9.31	109.54 \pm 11.57

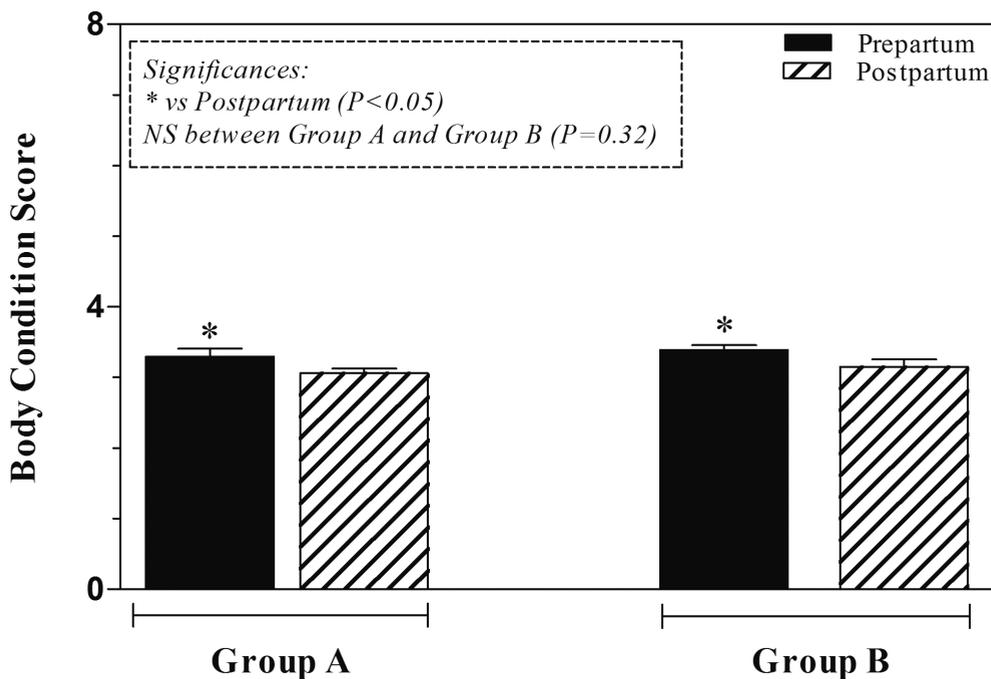


Figure 1
Farming condition and transition period effect on BCS

Discussion

The transition period affects significantly the metabolic profile and this can justify the variation of studied parameters during the prepartum and postpartum period. Metabolic changes included alterations in the energy balance that lead to increased lipid mobilization with consequent increase of plasma NEFA concentrations (Contreras *et al.* 2010).

In the present study, glucose, insulin, BHB and NEFA were significantly affected by the physiological periods considered (prepartum and postpartum) and by GTT both in Group A and Group B. In both groups all parameters showed the same trend in pre- and postpartum period after glucose infusion. Glucose and insulin values were higher in the prepartum period respect to postpartum period before glucose infusion (T0) and for all different timing following

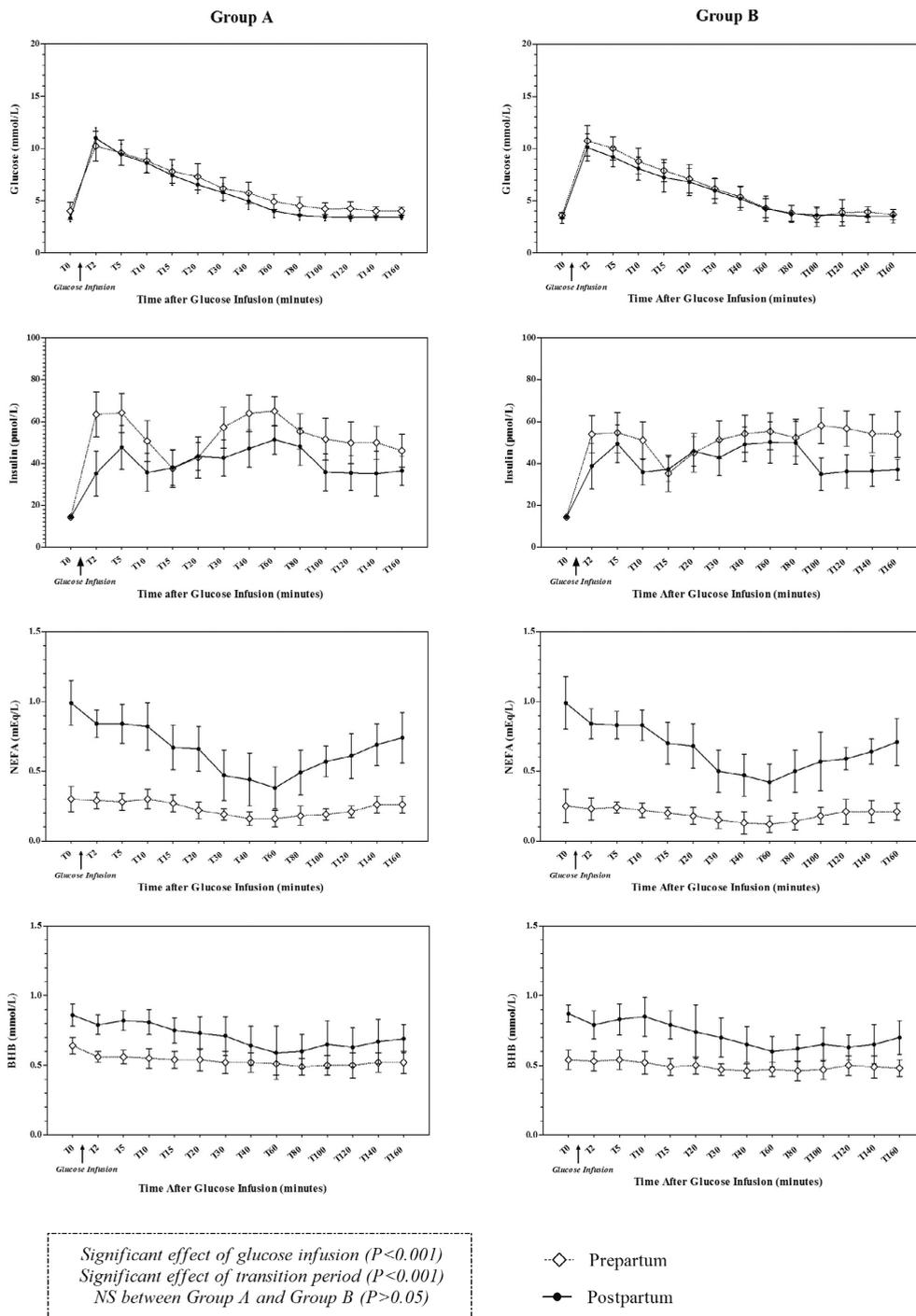


Figure 2
 Trend of glucose, insulin, NEFA and BHB before (T0) and after glucose infusion of Group A and Group B during prepartum and postpartum period

glucose infusion in both groups. The rate of glucose and insulin during GTT showed a rapid increase two minutes after glucose infusion and a progressive decline to starting values after 160 min (T160) in both periods. Glucose and insulin trends obtained from this study reflected the metabolic adaptation to postpartum phase, according to previous results (Drackley 1999, Zhao & Keating 2007). During this physiologic phase insulin resistance inhibits glucose use in insulin-dependent tissue, such as muscle and adipose tissues: glucose can be deviated mainly to non-insulin dependent tissues such as the mammary gland for milk production (Kerestes *et al.* 2009, Contreras & Sardillo 2011). Lower glucose and insulin levels found during postpartum period respect to prepartum period, before and after glucose infusion, may be due to a decreased responsiveness of pancreatic β -cells to a state of hyperglycemia, caused by factors which inhibit the release of insulin, such as the increase of NEFA.

NEFA values showed an opposite trend respect to glucose and insulin. NEFA levels were higher in postpartum respect to prepartum before glucose infusion (T0) and for all different timing following glucose infusion in both groups. These results indicated the activation of lipid mobilization that represented another metabolic mechanism of adaptation to postpartum period (Piccione *et al.* 2012). In fact, it is well known that low insulin concentration and reduced insulin sensitivity of the tissues around parturition increase lipid mobilization and induces further rises in plasma NEFA concentrations (Hayirli 2006). The use of fat reserves and the activation of lipid mobilization during postpartum period were demonstrated by the lower BCS found in this period respect to prepartum. About response to GTT, NEFA values showed a decrease until 60 min after glucose infusion (T60) and a subsequent increase with return to starting values at T160 both in prepartum and in postpartum period. The early decrease of NEFA values after the glucose administration demonstrates that lipid mobilization of fatty acids is reduced in positive energetic balance condition. The plasma BHB profile followed the same pattern of NEFA concentration.

According to Grummer *et al.* 2004, the increase of plasma NEFA concentration led to the increase of ketogenesis by hepatocytes. BHB levels were higher in postpartum respect to prepartum before glucose infusion (T0) and for all different timing following glucose infusion in both groups. BHB concentrations recorded at T0 and for all different timing following glucose infusion in prepartum and postpartum both in Group A and in Group B were within the reference range (0.00-0.86 mmol/l, Radostits *et al.* 2007) and not exceeded the 1.00 mmol/L. These values indicated no subclinical ketosis (Geishauser *et al.* 2000). Moreover, the early decrease of BHB levels in response to GTT, demonstrate that, following glucose infusion, the production of ketone bodies was reduced.

The results of this study demonstrated that glucose infusion influenced metabolism in ruminants modifying glucose, insulin, NEFA and BHB profile. These variations indicated that glucose is an important direct controller of metabolic interactions and responses in dairy cow during transition period. The decrease of glucose and the increase of NEFA and BHB found in our study, showed the difficulty of dairy cows to cope with the energy demand characterizing the transition period. These data suggest that reference ranges for diagnostic interpretation of blood metabolite concentrations should be adjusted to time periods relative to calving. Interactions between time period and health status suggest prepartum blood metabolite concentrations may provide some indication to postpartum disease risk and can be useful as a herd monitoring tool.

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