Insulin Resistance in Different Physiological States of High Producing Holstein Dairy Cows

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ABSTRACT

Background: Alterations in energy demands during different physiological states of dairy cows predispose them to metabolic disorders. Insulin as a main metabolic hormone has a key role to maintain homeorhesis in dairy cows. Insulin resistance phenomenon can expose these animals to metabolic dysfunctions. Information regarding insulin resistance at each physiological state of high producing dairy cows can assist veterinarians to control and prevent the metabolic disorders at herd levels.

Materials, Methods & Results: This research was carried out at winter 2014 on 25 multiparous Holstein dairy cows from a high producing industrial dairy farm. The total mixed rations were formulated and prepared for all animals according to National Research Council (NRC) requirements. At this farm, a dry period of 60 days has been considered. Milk production was about 10,000 kg for year, an average of 3.6 of milk fat %, and 3.3 of milk protein %. All the animals were clinically healthy and body condition score (BCS) was estimated based on 0 to 5 system. Cattle were divided into 5 equal groups containing early, mid and late lactations, far-off and close-up dry periods. A blood sample was taken immediately after catheterization, and dextrose 50% was administered at 500 mg/kg, 10 mL/kg/h, subsequently. Blood samples were collected from all cows through the fixed catheter prior to and 1, 2, 3 and 4 h after dextrose 50% infusion in plain tubes. After sera separation, glucose and insulin were detected in all samples. Significant and rapid elevation of serum glucose and insulin concentrations were seen in all studied animals at 1st h after dextrose administration ($P < 0.05$). Decreasing the glucose level near to base line levels was seen at h 4 in late lactation and far-off dry cows. The glucose level at this time was remained significantly higher than h zero in other groups ($P < 0.05$). In early, mid and late lactation and close-up dry cows, insulin levels at h 4 were remained significantly higher than base line values ($P < 0.05$).

Discussion: Cows during the early far-off dry period are experiencing relatively low metabolic demands as fetal growth is just beginning to accelerate and cows are no longer lactating. Fetal and uterine tissues are insulin-independent and so as energy demands increase with days of pregnancy, maternal peripheral tissues will become more insulin resistant in order to support fetal growth. These changes in the role of insulin have effects on energy metabolism in dry cows. Although pregnant dairy cows had essentially the same basal insulin levels as the lactating and non-pregnant non-lactating dairy cows, the amount of insulin secreted during infusion of glucose or propionate was higher in pregnant cows than in the lactating and non-pregnant non-lactating cows. The significant increasing patterns of insulin and glucose were seen after dextrose infusion in early lactation dairy cows ($P < 0.05$). In the mid-lactation group the glucose at 4th h was significantly remained at higher levels than base line values. These findings indicate the presence of insulin resistance in early and mid lactation dairy cows. It was reported that insulin clearance rates had a tendency to decrease during the postpartum period. The reduced clearance of glucose observed at post partum was due to a greater degree of insulin resistance which gave rise to more pronounced net lipolysis from the adipocytes. These qualitative changes in metabolism occurring during lactation are necessary to support a homeostatic state. Despite the presence of high concentrations of insulin, high levels of glucose were seen in early and mid-lactation and close-up dry cows. The insulin resistance was found in early and mid lactation and close-up dry cows. It could be concluded that high energy demands to lactogenesis and suffering from the negative energy balance are the main reasons of insulin resistance in high producing Holstein dairy cows.

Keywords: insulin resistance, lactogenesis, fetal growth, Holstein dairy cows.
INTRODUCTION

Recent increases in bovine milk production and reproductive capacities are considered to be a result of selecting the cows which utilize more energy to production than entry to their body reserves [1,3,20,26]. Dairy cows enter to negative energy balance, which is characterized by alterations in blood metabolite and hormone profile, in some of the physiological states during life [18]. These changes determine their health, productivity, and profitability. These changes normally are exquisitely coordinated by hormonal and metabolic changes to support the new physiological state, the concept known as homeorhesis [2].

Insulin is one of the most important metabolic hormones which can affect the cow’s homeorhesis during different physiological states from dry to lactation periods. The tissue sensitivity to insulin is of special interest in lactating animals [23]. Both the responsiveness of insulin to glucose and the tissue responsiveness to insulin can change during different physiological states of dairy cows. These changes define as insulin resistance and sensitivity phenomena [8,11,17].

There are literatures about insulin resistance in dairy cows commonly with emphasis on transition or lactation periods [16,21,23,24]. But there are no comprehensive study regarding the insulin resistance during different physiological states of the productive life of dairy cows. Hence, the present experimental study was carried out in early, mid and late lactations, far-off and close-up dry periods of dairy cows to determine the insulin resistance phenomenon by using intravenous glucose tolerance test (IVGTT) in each state and compare them together.

MATERIALS AND METHODS

Animals

This research was carried out at winter 2014 on 25 multiparous Holstein dairy cows from a high producing industrial dairy farm around Shiraz, Southwest Iran. Cows were housed in open-shed barns with free access to water and shade. The total mixed rations were formulated and prepared for all animals according to National Research Council (NRC) requirements. At this farm, a dry period of 60 days has been considered. Milk production was about 10,000 kg for year, an average of 3.6 of milk fat %, and 3.3 of milk protein %. All the animals were clinically healthy, had no history of debilitating disease, and free from internal and external parasites due to routine antiparasitic programs. Body condition score (BCS) was estimated based on 0 to 5 system. Cattle were divided into 5 equal groups containing early (30.2 ± 5.7 days after calving, with 3.25 ± 0.25 BCS), mid (108.1 ± 8.4 days after calving, with 3.25 ± 0.25 BCS) and late lactations (184.5 ± 5.7 days after calving, with 3.5 ± 0.25 BCS), far-off (281.9 ± 5.4 days after calving, 228.4 ± 8.6 days of pregnancy, with 3.5 ± 0.25 BCS) and close-up dry periods (312.1 ± 8.3 days after calving, 255.6 ± 6.3 days of pregnancy, with 3.5 ± 0.25 BCS).

IVGTT

All experiments were done 1 h after morning meal, at 6 AM, approximately. A 14-gauge 5.1-cm catheter was secured in the right jugular vein and used for blood samplings and dextrose infusions. A blood sample was taken immediately after catheterization, and dextrose 50% (Zoopha®) was administered at 500 mg/kg, 10 mL/kg/h, subsequently [8]. Blood samples were collected from all cows through the fixed catheter prior to and 1, 2, 3 and 4 h after dextrose 50% infusion in plain tubes.

Serological assays

Immediately after blood collections, sera were separated by centrifugation for 10 min at 3,000 g and stored at -22°C until assayed. Glucose was assayed by an enzymatic (glucose oxidase) colorimetric method (ZistChem®)[2]. Insulin was measured by bovine insulin ELISA kit (Cusabio®)[3] [Specificity 100%, and precision: intra-assay and inter-assay CV < 8% and 10%, respectively].

Statistical analysis

All data are presented as mean ± standard deviation (SD). Differences between the averages of concentrations of different serological factors at each h after dextrose infusion in the different experimental groups were analyzed by one-way ANOVA and the least significant difference (LSD) test was used to find differences. The paired samples t test was used to compare the concentrations of different serological factors at two different times in each group. Repeated Measures ANOVA was used to evaluate the changing patterns of glucose and insulin in each group using SPSS software (SPSS for Windows, version 20, SPSS Inc, Chicago, IL, USA). The level of significance was set at P < 0.05.
RESULTS

Serum concentrations of insulin and glucose at different times in the experimental groups are presented in Tables 1 and 2. The changing pattern of insulin and glucose after dextrose infusion in all experimental groups are shown in Figures 1 and 2. Significant and rapid elevation of serum glucose concentration was seen in all studied animals at 1st h after dextrose administration (P < 0.05). Decreasing the glucose level near to base line levels was seen at h 4 in late lactation and far-off dry cows. The glucose level at this time was remained significantly higher than h zero in other groups (Figures 1 and 2; Table 2; P < 0.05).

Significant increases of insulin levels were seen in all studied groups from zero to 2nd h after dextrose administration. In early, mid and late lactation and close-up dry cows, insulin levels at h 4 were remained significantly higher than base line values (Figures 1 and 2; Table 1; P < 0.05). The serum insulin levels in far-off dry cows were decreased to base line levels at 4th h.

Despite the presence of high concentrations of insulin, high levels of glucose were seen in early and mid-lactation and close-up dry cows (Figures 1 and 2; Tables 1 and 2). The results of the present study showed that, serum insulin level at h zero in early lactation group was significantly lower than other groups. Its concentrations at 4th h in late lactation and close-up dry cows were significantly higher than other cows (P < 0.05; Table 1). The highest glucose level at 4th h was seen in early lactation and its lowest concentration in late lactation cows (P < 0.05; Table 2).

Figure 1. The changing pattern of insulin and glucose following intravenous glucose tolerance test (dextrose 50%, at 500 mg/kg, 10 mL/kg/h) in early (A), mid (B) and late (C) lactating high producing Holstein dairy cows.
Table 1. Serum insulin levels (mean ± SD) following intravenous glucose tolerance test (dextrose 50%, at 500 mg/kg, 10 mL/kg/h) at different physiological states of high producing Holstein dairy cows during the winter 2014, Shiraz, Southwest Iran.

<table>
<thead>
<tr>
<th>Group</th>
<th>Hour 0</th>
<th>Hour 1</th>
<th>Hour 2</th>
<th>Hour 3</th>
<th>Hour 4</th>
</tr>
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<tbody>
<tr>
<td>Early lactation</td>
<td>29.02 ± 0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.91 ± 0.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.98 ± 0.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.74 ± 0.63&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.90 ± 0.33&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Mid lactation</td>
<td>29.80 ± 0.54&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.82 ± 0.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.93 ± 0.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.90 ± 0.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.98 ± 0.40&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Late lactation</td>
<td>29.83 ± 0.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.55 ± 0.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.81 ± 0.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.72 ± 0.63&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.54 ± 0.50&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Far-Off dry</td>
<td>29.95 ± 0.34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.49 ± 0.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.02 ± 0.54&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.32 ± 0.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.38 ± 0.41&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Close-Up dry</td>
<td>29.56 ± 0.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.49 ± 0.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.26 ± 0.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31.78 ± 0.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.34 ± 0.42&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> Different letters indicate significant differences at each column (<i>p</i> < 0.05).

Table 2. Serum glucose levels (mean ± SD) following intravenous glucose tolerance test (dextrose 50%, at 500 mg/kg, 10 mL/kg/h) at different physiological states of high producing Holstein dairy cows during the winter 2014, Shiraz, Southwest Iran.

<table>
<thead>
<tr>
<th>Group</th>
<th>Hour 0</th>
<th>Hour 1</th>
<th>Hour 2</th>
<th>Hour 3</th>
<th>Hour 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early lactation</td>
<td>88.75 ± 19.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>292.01 ± 81.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>253.02 ± 26.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>210.23 ± 14.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>190.87 ± 9.95&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Mid lactation</td>
<td>75.15 ± 11.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>288.01 ± 35.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>230.98 ± 24.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>190.20 ± 15.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>153.51 ± 9.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Late lactation</td>
<td>91.79 ± 50.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>255.40 ± 87.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>89.01 ± 19.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>71.34 ± 41.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50.84 ± 35.40&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Far-Off dry</td>
<td>80.45 ± 50.63&lt;sup&gt;a&lt;/sup&gt;</td>
<td>310.59 ± 69.72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>110.76 ± 26.54&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100.56 ± 25.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>90.71 ± 47.63&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Close-Up dry</td>
<td>90.47 ± 34.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>256.00 ± 84.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>220.34 ± 61.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>180.73 ± 57.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>150.27 ± 48.32&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> Different letters indicate significant differences at each column (<i>p</i> < 0.05).
DISCUSSION

Insulin is a protein hormone secreted by the β-cells of the pancreas which stimulates translocation of glucose transporters, resulting in glucose uptake by tissues. In ruminants, volatile fatty acids from the gastrointestinal tract are the major energy source rather than direct sources of glucose. Thus, insulin plays a slightly different role in ruminants vs. non-ruminants, though many aspects of insulin metabolism are the same. Insulin resistance involves changes in sensitivity (the amount of hormone required to illicit a response) or responsiveness (the maximum response to a hormone). In ruminants as in humans, insulin resistance can involve changes in sensitivity, responsiveness, or both [17].

In the present experimental study, the IVGTT was used to evaluate insulin resistance. IVGTT is one of the practical methods to assess insulin resistance in dairy cows. After the intravenous infusion of a glucose bolus, blood samples are taken at regular intervals to determine glucose and insulin concentrations. Blood glucose levels during an IVGTT are derived from the glucose bolus, endogenous glucose production in liver and kidney, glucose uptake by the intestines, renal glucose excretion, glucose uptake by the mammary gland and/or the gravid uterus, and glucose uptake by the insulin-sensitive tissues such as skeletal muscles and adipose tissue [6,19]. Blood insulin levels during an IVGTT are the result of insulin secretion by the pancreas in response to the glucose bolus, and the elimination of insulin by the liver. The combination of these physiologic processes results in the typical profile of insulin and glucose concentrations during an IVGTT. Clearance rate of glucose and insulin is one of the parameters which used to determine the insulin resistance during this test. Insulin resistance can be identified when glucose clearance is low [8].

Based on our findings, the glucose levels in far-off dry cows increased significantly after dextrose infusion and insulin also increased parallel to glucose (Figure 2). Serum concentration of glucose was decreased near to base line levels at h 4 which indicate the effect of insulin on clearance of glucose. Since the high levels of glucose were cleared after increasing insulin values, it can be concluded that there were not insulin resistance in far-off dry cows.

Cows during the early far-off dry period are experiencing relatively low metabolic demands as fetal growth is just beginning to accelerate and cows are no longer lactating. Fetal and uterine tissues are insulin-independent and so as energy demands increase with days of pregnancy, maternal peripheral tissues will become more insulin resistant in order to support fetal growth [4]. These changes in the role of insulin have effects on energy metabolism in dry cows.

According to our results, despite increasing insulin levels in close-up dry cows, glucose concentrations were not reached to base line levels at 4h h. The glucose and insulin levels at this time were significantly higher than base line values (P < 0.05) which indicate that insulin has failed to clear the glucose. Hence, insulin resistance was seen in close-up dry cows.

Recently it was reported that cows fed a higher energy level during the dry period had a greater degree of insulin resistance before and after calving, which induced higher plasma non-esterified fatty acid (NEFA) concentrations compared to those in cows fed below requirements [15]. Although pregnant dairy cows had essentially the same basal insulin levels as the lactating and non-pregnant non-lactating dairy cows, the amount of insulin secreted during infusion of glucose or propionate was higher in pregnant cows than in the lactating and non-pregnant non-lactating cows [22]. In rabbits, insulin sensitivity of insulin-sensitive tissues decreased during late pregnancy [13]. This may be associated with a decrease in the number of insulin receptors during late pregnancy [25].

The significant increasing patterns of insulin and glucose were seen after dextrose infusion in early lactation dairy cows (P < 0.05; Figure 1). In the mid-lactation group the glucose at 4th h was significantly remained at higher levels than base line values. These findings indicate the presence of insulin resistance in early and mid lactation dairy cows.

Also it was reported that reduced insulin response to glucose infusion during the post partum compared to the pre partum periods [15]. Based on this finding and upon our results, it seems that the responsiveness of insulin secretion to glucose and likewise insulin sensitivity in peripheral tissues might be decreased during early lactation. It was reported that insulin clearance rates had a tendency to decrease during the postpartum period [7]. The reduced clearance of glucose observed at post partum was due to a greater degree of insulin resistance which gave rise to more pronounced net lipolysis from the adipocytes [15,19]. These qualitative changes in metabolism occurring
during lactation are necessary to support a homeostatic state [14]. As a cow transits into early lactation, the mammary gland requires up to 80% of total body glucose turnover [2]. In the transition from late pregnancy to early lactation, not only do plasma insulin levels fall, but adipocytes also become insulin resistant [5]. After calving, the initiation of milk synthesis and rapidly increasing milk production greatly increases demands for glucose for milk lactose synthesis, at a time when feed intake has not reached its maximum [9]. Because much of the dietary carbohydrate is fermented in the rumen, little glucose is absorbed directly from the digestive tract. Consequently, dairy cows rely almost exclusively on gluconeogenesis (synthesis of glucose) from propionate in the liver to meet their glucose requirements [10]. Limited feed intake during the early post partum period means that supply of propionate for glucose synthesis also is limited [12].

In late lactation dairy cows, glucose levels were declined to base line values at the final sampling which could be due to decreasing the energy demands to lactogenesis. Furthermore, in these cows, the dry mater intake is parallel to low milk production. Despite the high level of glucose in mid-lactation and close-up dry cows at 4th h, the serum glucose level in early lactation cows was significantly higher than other ones.

CONCLUSION

Based on our findings, the highest degree of insulin resistance was seen in early lactation dairy cows. According to the clearance of serum glucose concentrations at 4th h after dextrose administration, it could be stated that there was insulin resistance in early and mid lactation and close-up dry cows. Finally, it concluded that high energy demands to lactogenesis and suffering from the negative energy balance are the main reasons of insulin resistance in high producing Holstein dairy cows.

MANUFACTURERS

1Zoopha Parnian Pars Co. Tehran, Iran.
2ZistChem Co. Tehran, Iran.
3Cusabio Biotech Co. Ltd. Wuhan, Hubei Province, China.

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Ethical approval. The present experiment was performed after being approved by the Ethics Committee of the School of Veterinary Medicine, Shiraz University.

Declaration of Interest. The authors declare that there is no conflict of interest.

REFERENCES


14 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. Veterinary Research Communications. 30(7): 749-774.


