PLASMA LEPTIN, INSULIN, GLUCOSE, AND UREA CONCENTRATIONS THROUGHOUT LACTATION IN DAIRY COWS

ABDULLAH ERYAVUZ, GULCAN AVCI¹, HACI AHMET ÇELIK², AND ISMAIL KUCUKKURT¹

Department of Physiology, ¹Department of Biochemistry, ²Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, Afyon Kocatepe University, 03200, Afyonkarahisar, Turkey
gulcanavci@hotmail.com

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Abstract

The investigations were performed on 12 Holstein cows at the age of 3-5 years. The blood samples were taken at the 2nd, 3rd, 5th, 7th, and 8th months of lactation. Plasma leptin levels were low during the early period of lactation and remained low until the 7th month and increased significantly (P<0.05) in the dry period. Plasma insulin levels were low at the 2nd and 3rd months and increased significantly (P<0.05) at the 5th month of lactation and remained high in the 7th and 8th months. Plasma glucose concentration was the highest at the 2nd month, whereas it was the lowest at the 8th month. Plasma urea concentrations were significantly (P<0.05) higher at the 3rd month and lower at the 8th month than that at other months of lactation. In conclusion, the plasma leptin, insulin, urea, and glucose concentrations were altered during the lactation period due to changes in the energy requirements of dairy cows.

Key words: cow, urea, lactation, leptin, insulin, glucose.

A low fertility in high-yielding dairy cows, which is a topic seriously emphasised by dairy farms and milking industry, has been considered as one of the most restrictive factors for successful dairy farms. Thus, a high fertility in dairy cows is necessary to ensure the continuance of their performances. Since the reproductive performance of dairy cows is negatively affected by a negative energy balance, this topic suggests the concept of energy balance and the question of how the energy balance affects the fertility of dairy cows. Dramatic changes in the energy metabolism throughout lactation are well recognised in dairy cows. Increasing feed intake, decreased insulin sensitivity, and hyperlipidaemia are major features during the early lactation period (13, 16). Since the demand for energy in dairy cows due to high milk yield is at a high-level in early lactation, the large deficit in energy intake in relation to energy requirements during early lactation period may not be met by the intake of feed (16). Consequently, the difference between the energy supplied with the feed and what is required for milk synthesis is generally referred to as the energy balance (21). Because the energy balance is often negative in the early lactation period, the high-yielding dairy cows are negatively affected by a negative energy balance (13). Therefore, body reserves have to be mobilised and animals try to compensate this energy deficiency by using their body fat depots. Block et al. (3) estimated that mobilisation of endogenous lipids meets approximately 33% of the cow’s energy requirements between parturition and the third week of lactation. However, dairy cows meet their energy requirements for both maintenance and milk synthesis from the feed intake during the progressive periods of lactation. Additionally, these animals compensate this energy loss initially by storing body fats during the last periods of lactation. Therefore, dairy cows are fed different diets according to the period of lactation because they have different energy and protein requirements (1).

The energy metabolism of domestic animals is under the control of hormonal factors (2). Leptin, which is a protein secreted by adipose tissues and informing the central nervous system (CNS) about body fat reserves also regulates the reproduction besides feed consumption and energy intake (2, 24). Numerous studies on the leptin and its physiological functions in both humans and animals have been carried out in recent years (24). The differences in body adipose tissues, due to the energy requirements for milk production in dairy cows through the lactation period, can be determined by the variations in plasma level of leptin secreted by the mentioned tissue (1, 10). There are rather a few studies demonstrating variations in plasma leptin level through the lactation period. In this study, our objective was to determine the plasma concentrations of leptin, insulin, urea, and glucose, and thus to define the variations in body metabolism during lactation period in dairy cattle.
Material and Methods

Materials. A total of 12 Holstein cows, between 3 and 5 years of age, having the same breeding and feeding conditions, weighing about 620 kg, were obtained from a private dairy farm in the Afyonkarahisar Province of Turkey. All the cows became pregnant approximately on the 75th d of lactation. The milk yield during 305 d of lactation was on average 6 870 l per cow. The animals were fed a compound concentrate diet and roughages containing corn silage, clover, barley straw, and malt pulp at early, mid, and late lactation stages. The chemical analysis of the rations is displayed in Table 1. On the 2nd, 3rd, 5th, 7th and 8th months of lactation blood samples were collected from the jugular vein to heparinised vacuum tubes before morning feeding.

The blood plasma was separated by centrifugation at 3 000 rpm for 10 min at 4°C. Plasma urea and glucose concentrations were measured spectrophotometrically using commercial kits (BioSystems, Spain. Cat No. 11536; Teco Diagnostic, USA). Plasma leptin (XL-85K, multi-species leptin RIA kit, Linco Research, Inc., USA) and insulin (RI-13K, insulin RIA kit, Linco Research, Inc., USA) were measured using a radioimmuno-assay (RIA). Intra- and inter assay coefficients of leptin and insulin analyses were 4.3% and 6 % and 4.7 % and 8.3%, respectively.

Statistical analysis. Differences among leptin, insulin, urea, and glucose concentrations in different months of lactation were done by a paired Student–t test in SPSS software computer programme (20).

Results

Plasma levels of leptin and insulin through lactation period are displayed in Fig. 1. Although the difference in leptin concentrations between the 7th and 8th month of lactation were insignificant, plasma leptin was found to be significantly (P<0.05) higher at the 8th month as compared to other months. The insulin level was significantly (P<0.05) higher at the 5th month of lactation compared to the 2nd and 3rd months of lactation but insignificant in the 7th and 8th months compared to other months. Table 2 presents the mean values of plasma glucose and urea concentrations during lactation. Glucose concentrations were the highest in the 2nd month and the lowest in the 8th month of lactation. The glucose concentration in the 2nd month of lactation was significantly higher (P<0.05) than that in other months (Table 2). The urea concentration in the 3rd month of lactation was significantly higher (P<0.05), but its concentration in the 8th month was significantly lower (P<0.05) than that other months.

Table 1: Chemical compositions of diets given to dairy cows in various periods of lactation (% DM)

<table>
<thead>
<tr>
<th>Chemical analysis</th>
<th>Early lactation (&lt;month 4)</th>
<th>Mid lactation (4 to 5 month)</th>
<th>Late lactation (6 to 7 month)</th>
<th>Dry period (8 month)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter (DM) intake (kg/day)</td>
<td>23.29</td>
<td>22.18</td>
<td>17.1</td>
<td>11.92</td>
</tr>
<tr>
<td>Net energy lactation (Mcal/kg)</td>
<td>1.66</td>
<td>1.6</td>
<td>1.46</td>
<td>1.46</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>16.8</td>
<td>17</td>
<td>15.1</td>
<td>12</td>
</tr>
<tr>
<td>Ca/P</td>
<td>1.6</td>
<td>1.9</td>
<td>2.2</td>
<td>1.3</td>
</tr>
<tr>
<td>ADF (%)</td>
<td>20.5</td>
<td>23</td>
<td>26.6</td>
<td>29</td>
</tr>
<tr>
<td>NDF (%)</td>
<td>32.5</td>
<td>36</td>
<td>40</td>
<td>46</td>
</tr>
<tr>
<td>Crude cellulose (%)</td>
<td>16</td>
<td>18</td>
<td>20.7</td>
<td>22</td>
</tr>
<tr>
<td>NFC (%)</td>
<td>39.9</td>
<td>37</td>
<td>35.5</td>
<td>32</td>
</tr>
<tr>
<td>Concentrate (%)</td>
<td>41</td>
<td>34</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>Roughage (%)</td>
<td>59</td>
<td>66</td>
<td>80</td>
<td>70</td>
</tr>
</tbody>
</table>

ADF - acid detergent fibre; NDF - non-denatured fibre; NFC - non-fibre carbohydrate

Table 2: Plasma glucose and urea concentrations throughout lactation in dairy cows (n=12, ± SE)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>2nd month</th>
<th>3rd month</th>
<th>5th month</th>
<th>7th month</th>
<th>8th month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mg/dL)</td>
<td>36.75 ± 7.00b</td>
<td>59.05 ± 11.06a</td>
<td>45.18 ± 9.66b</td>
<td>43.80 ± 7.21b</td>
<td>18.82 ± 5.24c</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>92.36 ± 15.88a</td>
<td>80.93 ± 11.85b</td>
<td>80.93 ± 11.85b</td>
<td>84.85 ± 13.41ab</td>
<td>74.86 ± 12.15b</td>
</tr>
</tbody>
</table>

Letters (a,b,c) indicate significant differences of plasma glucose and urea concentrations between different periods of lactation in same line (P<0.05).
Discussion

Cows with a high milk yield are exposed to major changes in the energy metabolism in the early lactation period and these changes lead up to hormonal changes as well (13). With the beginning of lactation and accession to a peak milk yield in the 1st and 2nd month of lactation, the demand for energy in dairy cows cannot be met by a sufficient high feed intake and this energy shortage initiates the mobilisation of fats in the adipose tissue (13). Leptin is a good indicator to determine the adipose tissue content in the body (1, 2, 10). In this study, the plasma leptin level remained low until the 7th month of the lactation period but started to rise and reached its highest level at the 8th month, when the animals were in the dry period. Our results were in agreement with reports showing that plasma leptin levels decreased in the early lactation period in dairy cows (3) and that this decrease continued until the 4th month of lactation and increased between the 4th and 6th months (1). These observations indicate that the animal uses up the energy reserved in the form of body fat to replace the energy lost due to high milk yield during the lactation period, and these animals regenerate the initially consumed fats by storing fats at the last stage of lactation. In conclusion, a reduction in the plasma leptin level is caused by a decrease in the leptin secretion by the adipose tissue in the early lactation period, because leptin signals from the periphery to the CNS inform about the total fat deposits of the body and control the feed intake and energy expenditure (2). Fricke (13) has stated that there is a positive correlation between milk production and feed consumption in dairy cows. Our results also indicate that the low plasma leptin level until the 7th month of lactation may contribute to an increase in the feed intake by the animals during lactation period by decreasing the signal of leptin to the CNS to reduce feed consumption (24). This statement is important to demonstrate the role of the adipose tissue in the physiological mechanism to continue lactation in dairy cows.

Insulin increases both glucose input especially to the cardiac and skeletal muscles and adipose tissues and their sensitivity to insulin (17). Therefore, there is a positive relationship between plasma leptin and insulin levels (1, 3). However, it has been emphasised in recent publications that an increase in the secretion of growth hormone and a decrease in the sensitivity of the skeletal muscles and adipose tissues to the insulin, have effects on the adaptation to the initiation of lactation in ruminants (3). The insulin level in the blood is low during the initiation period of lactation (1, 4). In the present study, the insulin levels were low parallel to the low leptin levels until the 5th month of lactation and displayed an increase starting from the 5th month. The increase in the plasma insulin levels enhances the synthesis of leptin in adipose tissue (1, 3), due to the availability of glucose stored in the adipose tissue (17). In fact, an increase in the leptin levels in the 7th month of lactation was observed in this study. This observation shows that the excessive energy intake with feed starting from the 5th month of lactation is stored in the adipose tissue and increases the sensitivity of adipose tissues to insulin. On the other hand, Choung et al., (7) reported that an increase in the plasma ammonia level caused a decreased insulin level in the blood of cows. If this report is taken into account, an increase in the blood ammonia level may be considered to have an effect on the occurrence of a low insulin level at the initiation stage of this study because animals were fed more the concentrate diet in early lactation period than in other lactation periods (Table 1).
Microbial protein synthesis in the rumen, supplies the majority of absorbable amino acids to the small intestine. The protein requirements of dairy cattle producing low to moderate amounts of milk can usually be met by microbial protein synthesised in the rumen. However, high producing dairy cows may have protein requirements that exceed the amount provided by microbial protein (9). Therefore, high producing dairy cows need energy- and protein-dense rations for both energy and protein requirements during the transition and early lactation periods (16). However, when the additional protein level in the diet of dairy cows is high, plasma urea concentration increases and affects fertility negatively (21). Thus, there is a relationship between the plasma urea concentration and reproductive performance in dairy cows (14). A rise in plasma urea concentration of dairy cows can negatively affect reproduction and embryonic development (6, 18).

There is a physiological mechanism called “ruminohepatic nitrogen cycle” to preserve nitrogen in ruminants (5). Plasma urea occurs by both conversion of ammonia into urea in the liver in order to generate energy from proteins by gluconeogenesis and absorbing ammonia made up as a result of microbial degradation of nitrogen-containing components in the rumen that exceeds its bacterial utilisation (19). In this study, plasma urea concentrations determined between the 3rd and 7th months of lactation were higher than the interval levels of 26-39 mg/dL (12-18 mg/dL for BUN) reported by Harris (14), which did not negatively affect the reproduction in dairy cows, while the plasma urea concentration in the 8th month, when the cows reach the dry period, was lower than the values reported by Harris (14). Our results show that the increase in energy requirement to meet high milk production from the 2nd to the 8th month of lactation leads to an increase in the ammonia concentration occurring due to the amino acid degradation in the liver, and consequently causes a rise of the urea concentration in the plasma. However, Zhu et al., (23) suggested that triglyceride accumulation in the liver as a result of excessive fat mobilisation from the adipose tissue, impaired the conversion of ammonia into urea. Therefore, in this study, the fat mobilisation from adipose tissue may affect plasma urea concentration in the early lactation period. Harris (14) reported that plasma urea concentration below 26 mg/dL is due to a lack of low crude protein (CP) or rumen digestible protein (RDP) in the ration, while plasma urea concentration above 39 mg/dL may be a result of an excessive amount of CP or DPR in the ration or insufficient amount of fermentable energy in the rumen. Referring these reports to results of our study, the composition of the diet consumed by animals through lactation period seems to influence the plasma urea concentration. Westwood et al. (22) found that, although the plasma urea concentration increased in postpartum cows fed two different diets containing the same CP but different RDP levels, the increase in plasma urea concentration in cows fed diets containing high RDP level was remarkably higher.

Huntington et al. (15) reported that in cattle consuming high-concentrate diets, plasma glucose comes approximately at 44% from organic acid absorption from the rumen (predominantly propionate) and subsequent conversion to glucose in the liver, at 33% from postruminal glucose absorption, and at 23% from other carbon sources such as amino acids and subsequent conversion to glucose in the liver. In this study, plasma glucose concentration was significantly higher in the samples collected in the second month of lactation, compared to the samples taken at other stages of lactation. However, the lowest plasma glucose concentration was measured in the samples collected in the 8th month. Because growth hormone releases fatty acids into the circulation by mobilising them from fat stores and increases glucose synthesis in the liver (17), the increase in the growth hormone can cause a rise in the blood glucose concentration and fatty acids during the initiation period of lactation (1). Our results showed that plasma glucose concentration increased in parallel to the rise of milk yield at the beginning of lactation, but it was reduced towards the end of lactation, when the cows reached the dry period. High concentrations of both glucose and urea measured in all samples except the 8th month has indicated an increase in the utilisation of amino acids for the production of glucose in gluconeogenetic pathway in the liver through the lactation period. This result is consistent with a report suggesting that more than 15 kg of protein can be mobilised in the body during the first two months of lactation (23). On the other hand, ammonia that occurs by the degradation of the nitrogen-containing components in the rumen and exceeds bacterial utilisation is absorbed from the rumen and converted into urea in the liver (11). Since the transformation of ammonia into urea causes a 15% energy loss (19) at the same time, it may require an extra load to the energy consumed. An excessive amount of ammonia produces glutamate in the Krebs cycle in the mitochondria of the liver cells via transamination reaction of α-ketoglutarates. This reaction reduces oxidative phosphorylation by leading to α-ketoglutarate loss; consequently, numerous ketone bodies develop (17). Actually, it has been reported that hyperammonaemia increases both the glutamine (23) level in the blood of cows and ketone bodies (12). Eryavuz et al., (11) found an increase in plasma glucose concentration, corresponds with an increase in plasma urea concentration in sheep when the animals were fed diet containing urea. When high-yielding dairy cows are fed a diet containing high protein because of their high protein requirements, ammonia increases in the rumen due to microbial fermentation of protein and is absorbed to a high degree to the blood (22). In this study, an increase in plasma urea and glucose concentrations observed through lactation period except for dry period may originate from the high levels of nitrogen-containing components or insufficient amount of fermentable energy in the diets (14).

In conclusion, the results of this study demonstrate that leptin and insulin levels in the plasma of dairy cows change throughout the lactation period, which suggests that they have a physiological role in the dairy cow’s energy metabolism during lactation. Plasma
glucose and urea concentrations are influenced by lactation period.

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