Serum apolipoprotein B100 concentrations in obese Holstein heifers

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SUMMARY

The purpose of this study was to examine the changes of serum apolipoprotein B100 (apo B100) concentrations and to evaluate the liver steatosis in obese Holstein heifers. In this study, 10 clinically healthy (with body condition scores (BCS) comprised between 2.75-3.50) and 10 obese (with BCS of 5), Holstein heifers were used. Serum concentrations of apo B100 were assayed by single radial immunodiffusion method. Hepatic biopsy samples were obtained from liver under local anesthesia with percutaneous biopsy needle for histopathological examination. Our results indicated that serum apo B100 concentrations were not significantly decreased (P = 0.39) in the obese heifers, and the degree of fat infiltration in liver was comparable to controls. It was concluded that the fatty liver disease was absent in the obese heifers and the changes in apo B100 concentrations should be carefully evaluated together with hepatic biopsy analysis.

Keywords: Fatty liver - apolipoprotein B100 - obesity - heifer.

Introduction

Special apolipoproteins, which constitute with lipids the lipoproteins, regulate lipoprotein metabolism [19]. Apo B100, an apolipoprotein, is synthesised in liver, and participates to the delivery of hepatic triglycerides to mammary glands, ovaries and adrenal cortex, and transportation of cholesterol to steroidogenic tissues. Serum concentrations of apo B100 are dependent on different physiological parameters such as age, breed, sex, parity and stage of lactation in cattle [13]. Moreover, KATOH et al. [15] reported that excessive lipid mobilisation due to calving stress, endocrine changes and negative energy balance caused the decrease of serum apo B100 concentrations. Abnormal serum concentrations of lipid and lipoprotein (especially apo B100 [31]) are generally related to liver dysfunction and diseases [21]. Many researcher groups reported that a decrease of the serum apo B100 concentrations is the obvious sign for spontaneous [13, 20] and also experimentally induced [15, 29] fatty liver diseases. Apolipoprotein synthesis in liver is regulated by metabolic and hormonal mechanisms [31]. MARCOS et al. [20] reported that steatosis, a metabolic disorder, leads to damage of granular endoplasmic reticulum and golgi apparatus in hepatocytes resulting in dysfunction of protein synthesis, but concerned mechanisms are not fully understood [10]. Obesity is a risk factor for metabolic diseases especially for development of fatty liver [26]. In this regard, researchers conducted in human revealed that fatty liver disease often existed in obese people [16]. Similarly, GILBERT et al. [9] and STRANG et al. [28] reported that there is a high incidence of fatty liver in obese cattle, sheep and rodents. Many researchers point out that excessive body condition is an important factor causing fatty liver [4, 25, 30]. On the other hand, fatty liver is one of the principal causes of infertility and production losses [14, 24]. Although the fatty liver disease is usually observed in dairy cows during the peri-parturient period [9, 30] and the incidence of fatty liver would be lower in heifers than in dairy cows [3, 24], some cases have been also described in heifers with excessive feeding [11]. However, some studies [6] recommend the suspicion of hepatosteatosis in heifers. Whereas variations of serum apo B100 concentrations were evidenced during fatty liver disease in dairy cows, this apolipoprotein was not yet measured in obese heifers according to our knowledge. Consequently, the aims of the present study are to investigate the occurrence of stetatosis in liver of obese heifers and to determine the diagnosis value of the serum apo B100 concentrations for fatty liver.

RÉSUMÉ

Concentrations sèricières en apolipoprotéine B100 chez des génisses Holstein obèses. Par T. CIVELEK, H.A. ÇELIK, F.M. BIRDANE, A. YAGCI, S.M. PANCARCI et M. KABU.

Les objectifs de cette étude étaient d’évaluer les modifications des concentrations sèricières en apolipoprotéine B100 (apoB100) ainsi que la présence d’une stéatose hépatique chez des génisses Holstein obèses. Dix femelles cliniquement en bonne santé, dont les scores d’état d’engraissement (BCS) étaient compris entre 2.75 et 3.50 et 10 femelles obèses avec des BCS de 5 ont été utilisées dans cette étude. Les concentrations sèricières en apo B100 ont été mesurées par une méthode d’immunodiffusion radiale simple et le degré d’infiltration graisseuse des biopsies hépatiques obtenues après ponction transcutanée sous anesthésie locale a été déterminé par histologie. Nos résultats montrent que les concentrations sèricières en apo B100 n’ont pas été significativement diminuées chez les génisses obèses (P = 0.39) et que le degré d’infiltration graisseuse du foie a été identique à celui des contrôles. En conclusion, d’une part, il n’y a pas eu de surcharge lipidique chez les génisses obèses et d’autre part, il convient d’interpréter conjointement les variations des concentrations sèricières en apo B100 et les résultats des biopsies hépatiques.

Mots-clés : Foie gras - apolipoprotéine B100 - obésité - génisse.
Materials and methods

ANIMALS AND SAMPLES

A total of 20 cycling, 2 year-old Holstein heifers were used in this study and were fed with alfalfa hay (30.9%), barley hay (51.6%) and concentrates (17.5%). The compositions of the diet and of the concentrates were given Table I. The body condition scores (BCS) were evaluated by the same person as described by EDMONSON et al. [7] based on the palpation of traverse processes of loin vertebrae, cranial coccygeal vertebrae and tuber ischii. Scores were assigned using a five point scale (0 = very thin to 5 = grossly fat). According to their BCS, heifers were divided into 2 groups: Heifers with an optimal BCS (comprised between 2.75 and 3.50) constituted the control group (n = 10), whereas animals with high BCS (5) were included in the obese group (n = 10). The average ages were similar in the 2 groups: 2.06 ± 0.20 years in the control group and 2.16 ± 0.25 years in the obese group.

To determine the pubertal status of dairy heifers, they were examined transrectally with ultrasonograph for evidencing the corpus luteum once a week prior to initiation of the experiment. Nulliparous dairy heifers in control group were not inseminated, whereas, nulliparous dairy heifers in obese group were inseminated at least three times, and they were not pregnant.

Blood samples were collected from jugular vein, and after coagulation at room temperature (22-24°C) samples were centrifuged at 1073 g for 15 minutes at room temperature, then serum samples were stored at -20°C until apo B100 assays were performed.

### DIETS COMPOSITION

<table>
<thead>
<tr>
<th>Percentage</th>
<th>Ingredient</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2 %</td>
<td>Milk premix</td>
</tr>
<tr>
<td>15%</td>
<td>Barley</td>
</tr>
<tr>
<td>19%</td>
<td>Corn</td>
</tr>
<tr>
<td>12.1 %</td>
<td>Cotton seed meal</td>
</tr>
<tr>
<td>15 %</td>
<td>Sunflower seed meal</td>
</tr>
<tr>
<td>30 %</td>
<td>Wheat bran</td>
</tr>
<tr>
<td>7.5 %</td>
<td>Molasses</td>
</tr>
<tr>
<td>0.17 %</td>
<td>Calcium carbonate</td>
</tr>
<tr>
<td>0.5 %</td>
<td>Dicalcium phosphate</td>
</tr>
<tr>
<td>0.53 %</td>
<td>Salt</td>
</tr>
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**Calculated Analyses**

<table>
<thead>
<tr>
<th>Percentage</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.93 %</td>
<td>Crude ashes</td>
</tr>
<tr>
<td>12.64 %</td>
<td>Moisture</td>
</tr>
<tr>
<td>18.85 %</td>
<td>Crude protein</td>
</tr>
<tr>
<td>3.45 %</td>
<td>Crude fat</td>
</tr>
<tr>
<td>11.67 %</td>
<td>Crude fiber</td>
</tr>
<tr>
<td>1.25 %</td>
<td>Calcium</td>
</tr>
<tr>
<td>0.96 %</td>
<td>Phosphorus</td>
</tr>
<tr>
<td>2973</td>
<td>ME (kcal/kg)</td>
</tr>
</tbody>
</table>

Table 1. — Composition of diets and concentrates.

BIOCHEMICAL AND HISTOLOGICAL ANALYSES

Serum apo B100 concentrations were determined by using commercially available bovine apo B100 plate SRID test kit (Bovine apolipoprotein measurement kit, Code no: P0116-1) according to manufacturer’s instructions.

Hepatic biopsy samples were obtained from liver under local anesthesia (L-Anestin® (Lidocain HCL 20 mg/ml, Alke, Istanbul, Turkey, 1 ml / cm²) with percutaneous biopsy needle (14 - 16 g) using ultrasonography [27]. The samples were fixed in formaldehyde-calcium solution at + 4°C in the dark for a day and then were delivered to laboratory for histopathological examination. Prepared cross-section samples (12 µm) were stained with Sudan Black [5] and Oil Red O [18]. After preparation, percentage of hepatic lipidosis was determined using ocular square micrometer (Olympus) in the slides (µm² / 100µm² x 1000 magnification).

STATISTICAL ANALYSIS

Data was analysed with two-sample student t-test in Minitab 12 statistical program for Windows. Comparison between two groups were determined significant at p<0.05 level.

Results

CLINICAL EXAMINATION

The feed intake was higher in the obese heifers than in the controls. This high consumption of feed in these obese heifers could be caused by competition due to free-stall barns. Moreover, despite at least three artificial inseminations, transrectal ultrasound examination revealed no pregnancy in obese heifers.

SERUM APO B100 DETERMINATION AND LIVER HISTOLOGY

As shown in Table II, the serum apo B100 concentrations were slightly decreased in the obese heifers but the difference with the controls was not statistically significant because of the great dispersion of the values. Some hepatic lipidosis foci characterised by several cells engulfed by lipid vacuoles were observed in the both groups but the rate of fat infiltration of the liver remained weak and similar in the obese and control heifers.

There was no significant correlation between serum apo B100 concentrations and the degree of liver fat infiltration (p = 0.404, r = -0.297).

Discussion

In the present study, although the difference was not significant compared to control, a slight decrease of serum apo B100 concentrations was observed in the obese heifers. However, the degree of fat infiltration in liver was similar in the 2 groups (normal and obese heifers) indicating the
absence of fatty liver disease in obese animals. Previously, GAAL et al. [8] considered less than 20% lipidosis level as normal. Our study also produced similar data. Besides, no significant correlation was evidenced between serum apo B100 concentrations and the degree of steatosis in the liver. The increase of the food intake in obese heifers was probably due to an inactive leptin production, further leading to competition for ration [17] in this group, whereas control heifers were more rapidly satisfied.

These results are contradictory with previous studies that reported a link between obesity and the occurrence of fatty liver [16, 28]. Indeed, obesity is characterised by excessive adiposity, dyslipidemia and also hepatosteatosis [2] and RUKKWAMSUK et al. [24] and BOGIN et al. [3] reported that an excessive body weight (i.e. obesity) could lead to fatty liver disease. Studies conducted on humans revealed that the high incidence of fatty liver disease observed in obese people is associated with insulin resistance, hyperlipidemia, and also hepatosteatosis [2] and liver [16, 28]. Indeed, obesity is characterised by excessive adiposity, dyslipidemia and also hepatosteatosis [2] and RUKKWAMSUK et al. [24] and BOGIN et al. [3] reported that an excessive body weight (i.e. obesity) could lead to fatty liver disease. Studies conducted on humans revealed that the high incidence of fatty liver disease observed in obese people is associated with insulin resistance, hyperlipidemia, and also hepatosteatosis [2].

In our study, the lack of association between obesity and liver dysfunction evidencing by low serum apo B100 concentrations, would be related to the low fat accumulation into the liver of heifers. Similarly, REID and ROBERTS [23] reported that values of hepatic fat in heifers were below 10% at one week after calving. The another reason would be the age of animals. The relationship between the age and the development of fatty liver is well known [13] and in this regard, REICHEL et al. [22] demonstrated that the incidence of fatty liver was increased with the number of calving.

In conclusion, in spite of the obesity there is no significant alteration of serum apo B100 concentrations and the degree of fat accumulation of the obese heifers was similar to controls. The changes in apo B100 concentrations should be interpreted carefully together with hepatic biopsy analysis for the obese heifers.

### References


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**Table II.** — Serum apo B100 concentrations and liver histology in the obese and control heifers and statistical importance.

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 10)</th>
<th>Obese (n = 10)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apo B100 (µg/mL) (Mean±SD)</td>
<td>242.5 ± 40.3</td>
<td>222.0 ± 60.3</td>
<td>0.39</td>
</tr>
<tr>
<td>% (Mean±SEM)</td>
<td>3.70 ± 1.1</td>
<td>6.20 ± 1.4</td>
<td>0.18</td>
</tr>
<tr>
<td>Median</td>
<td>3.50</td>
<td>6.00</td>
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</tbody>
</table>

**Figure 1.** — Cellular damage observed in liver in the obese heifers (x160, stained with Sudan Black).


