Dairy cows endure major adaptive changes during the transition from gestation to lactation. For instance, they undergo a period of negative energy balance, caused by a sudden increase of demand for energy to support the high milk yield and their inability to fulfill this demand by dry-matter intake in the periparturition period. In this respect, excessive hepatic uptake of mobilized nonesterified fatty acids of hydrolyzed adipose tissues from the bloodstream may cause fatty liver because of the innate inability of cows to return triglycerides to blood as very low-density lipoproteins.

Evaluation of the correlation between serum biochemical values and liver ultrasonographic indices in periparturient cows with different body condition scores

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Objective—To determine alterations of serum biochemical variables in relation to changes of near- and far-field mean grayscale histogram (MGSH) and attenuation rates in liver ultrasonograms of periparturient cows.

Animals—67 Holstein cows.

Procedures—Cows were allocated on the basis of body condition score into underconditioned (n = 21), moderately conditioned (23), and overconditioned (23) groups. Serum samples (obtained every 10 days from 30 days before to 30 days after calving) were analyzed for aspartate aminotransferase, alanine aminotransferase, and γ-glutamyltransferase activities and BUN, albumin, calcium, and inorganic phosphorus concentrations along with digital estimation of near- and far-field MGSH values of liver ultrasonograms and deep attenuation. Values were compared among groups and within each group, and their correlations were determined in the pre- and postpartum periods.

Results—Serum biochemical variables did not differ significantly among groups. Aspartate aminotransferase and γ-glutamyltransferase activities increased in the postpartum period. Fluctuations of alanine aminotransferase activity were not significant; BUN decreased significantly in the peripartum period. Albumin concentration decreased prior to parturition and remained low, but significantly increased after parturition. Calcium concentration decreased on day 10 but subsequently increased. Phosphorus concentration decreased stepwise until day 10 after calving. Postpartum biochemical variables had weak correlations with near- and far-field MGSH values in overconditioned cows. The highest levels of sound attenuation were found in overconditioned cows on calving day.

Conclusions and Clinical Relevance—Liver ultrasonographic features were poorly correlated with changes of serum biochemical variables. This suggests that liver ultrasonography is not a good technique for estimating functional liver abnormalities in periparturient cows.

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Cows with higher BCS usually have less of an increase in dry-matter intake after calving, and readily break down their large stores of body fat. There is also an increasing risk for overconditioned cows to develop fatty liver, which makes high BCS one of the major risk factors concerning metabolic disturbances.

The liver plays a major role in the transition from gestation to lactation by being the main target for mobilized fat and having the key role in keeping the blood glucose concentration high enough to support energy needs.
demands for production, reproduction, and health. 10–12 Accordingly, fat accumulation in hepatocytes and related consequences can alter both the liver function and its structure in this critical period. Fatty liver is also associated with different metabolic or metabolism-associated disorders. 1

There are several direct and indirect methods to measure fatty change of liver. 5,11 Liver biopsy is an invasive and often impractical method for commercial farm applications. Alternatively, blood constituents are highly influenced by liver function and hence are widely used as an indicator of metabolic status and liver function in single animals or groups of animals. 14–16 However, blood biochemical profiling cannot directly address the level of liver triglyceride accumulation and the associated structural changes of liver at population levels. Studies in cattle, 17,18 cats, 19 and humans 20 have determined that specific ultrasonographic features are a reliable indicator of hepatic lipidosis. Lower acoustic impedance of fat, compared with normal liver parenchyma, causes a higher echogenicity and greater attenuation of ultrasonographic waves in a fat-infiltrated liver. 17 Hepatic lipidosis results in increased hepatic size and rounding of liver margins. Furthermore, hepatic lipidosis increases the coarseness of echoes and echogenicity of the liver parenchyma near the abdominal wall followed by weakening of distant echoes from the abdominal wall, known as deep attenuation. Poor or no visualization of hepatic vessels may also be noticed in captured images. 18 Ultrasonography of liver followed by digital analysis of sonograms has promise as a non-invasive method for diagnosis of fatty liver and estimation of liver triglyceride content in dairy cattle. 17,21,22 We hypothesized that combined application of liver ultrasonography and blood biochemical profile, which are minimally invasive, might offer a noninvasive, practical method for concomitant monitoring of liver function and structural alterations in periparturient cow populations with different risks of developing accumulation of fat in the liver attributable to different prepartum BCSSs. Therefore, the aim of the study reported here was to determine whether the nMGSH and fMGSH values of liver ultrasonographic images and ultrasonographic attenuation are related to alterations in serum biochemical variables of AST, ALT, and GGT activities and BUN, albumin, calcium, and inorganic phosphorus concentrations in underconditioned, moderately conditioned, and overconditioned periparturient Holstein cows.

Materials and Methods

Sixty-seven Holstein cows from a commercial dairy herd were allocated to 3 groups on the basis of BCS as follows: underconditioned (BCS ≤ 2.75; n = 21), moderately conditioned (BCS, 3 to 3.75; 23), and overconditioned (BCS ≥ 4; 23). Body condition scores were determined on a scale of 1 to 5. 23 The parity of the cows was assigned to be equally distributed among groups. Standard balanced feed was provided in a total mixed ration for all groups in accordance with established nutritional guidelines. 24 Written consent was obtained from the farm owners. The animal care committee of the University of Tehran Department of Basic Sciences also approved the sampling procedures to be in agreement with animal ethics codes.

Body condition scoring and blood collection were performed in 7 sequential instances on each cow, starting from 30 ± 4 days before the estimated date of calving and continuing every 10 days until 30 days of lactation. Because the mean values of actual prepartum sampling days were not significantly different from the estimated days, the samples were labeled as days −30, −20, −10, 0, 10, 20, and 30. Sample 0 was collected approximately 7 hours after parturition. Blood was obtained from the coccygeal vein into plain evacuated tubes. Sera were collected after centrifugation of blood at 1,500 × g for 15 minutes, and samples were stored at −80°C until analysis.

Activity of AST was measured on the basis of the colorimetric reaction of oxaloacetate formation from aspartate and subsequent decarboxylation of oxaloacetate resulting in formation of pyruvate, which makes a colored complex with 2,4-dinitrophenylhydrazine. Serum ALT activity was estimated on the basis of formation of pyruvate from alanine, which followed the procedure as described for the AST colorimetric reaction. 9 Serum GGT activity was measured on the basis of the colorimetric reaction of glycglycine and 5-amino-2-nitrobenzate complex with glutamate. 25 Concentration of BUN was calculated by means of the modified berthelot enzymatic method in which urease enzyme produces ammonium ion from urea, resulting in a colored complex in the presence of a chromogen. 26 Serum albumin concentration was measured on the basis of the colorimetric reaction with bromcresol-green. 9 Total calcium concentrations were quantified on the basis of formation of purple complex with ortho-cresolphthalein. 27 Albumin-adjusted calcium concentrations were calculated according to the following equation: albumin-adjusted calcium concentration = serum calcium concentration + 0.8 (4 − serum albumin concentration). 25 Serum phosphorus concentration was calculated on the basis of the colorimetric formation of phosphomolibdic acid complex from phosphorus concentration and molyblic acid. 28

In each session, ultrasonographic scanning of the liver was performed with the region of interest close to the recommended site for liver biopsy. 25 The right intercostal space between the 10th and 11th ribs was clipped 30 cm ventral to the transverse processes of the thoracic vertebrae. A microconvex transducer of 2 to 4 MHz attached to a portable base unit captured the images after the application of coupling gel. 4 The settings of the base unit and the region of interest were constant throughout scanning. The ultrasonograms were digitally analyzed for their histogram in grayscale mode (0 to 255 shades of gray, where 0 is solid black and 255 is solid white) via software. 7 In each image, the areas in depths of 5 cm (nMGSH) and then 12 cm (fMGSH) were targeted for evaluation. Two 32 × 32-pixel squares were placed in targeted areas, which had to cover only homogeneous parenchymal tissues without any vessels, lesions, artifacts, or heterogeneous densities. The targeted areas were not to be under a vessel or under a high or low echogenic mass. The MGS values were measured for each depth, then the difference between
nMGSH and iMGSH values was calculated as an indicator of ultrasonographic wave attenuation in liver parenchyma. Higher MGSH values were interpreted as higher echogenicity of liver parenchyma.

Statistical analysis—Mean ± SD values were compared between groups via 1-way ANOVA. When raw data or log transformation failed the normality test, ANOVA was performed on ranks with Kruskal-Wallis or Dunn tests. Within-group comparison of the variables was performed via repeated-measures ANOVA in terms of sampling days. The correlations between nMGSH and iMGSH with serum variables were determined by use of the Pearson correlation method in each group for the prepartum (–30, –20, and –10-day) and postpartum (10-, 20-, and 30-day) periods. Statistical analysis was performed with software. Values of $P < 0.05$ were considered significant.

**Results**

Activities of AST, ALT, and GGT and concentrations of BUN, albumin, calcium, and inorganic phosphorus did not differ significantly among groups when evaluated via 1-way ANOVA. However, repeated-measures ANOVA revealed significant changes on different days within each group (Table 1).

Table 1—Descriptive (mean ± SD) values of serum biochemical profile variables in 3 groups of cows on various sampling days relative to the day of parturition (day 0).

<table>
<thead>
<tr>
<th>Variable (reference range)</th>
<th>Group</th>
<th>Day</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
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<tbody>
<tr>
<td>AST (78–132 U/L)</td>
<td>UC</td>
<td>–30</td>
<td>–20</td>
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<td></td>
<td>MC</td>
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<td>OC</td>
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<td>ALT (11–40 U/L)</td>
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<td>OC</td>
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<td>GGT (6.1–17.4 U/L)</td>
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<td>OC</td>
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<tr>
<td>BUN (6.0–27.0 mg/dL)</td>
<td>UC</td>
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<td>OC</td>
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</tbody>
</table>

*Significantly ($P < 0.05$) different from value at the previous time point.

MC = Moderately conditioned. OC = Overconditioned. UC = Underconditioned.

Table 2—Descriptive (mean ± SD) values of ultrasonographic variables in 3 groups of cows on various sampling days relative to the day of parturition (day 0).

<table>
<thead>
<tr>
<th>Variable Groups</th>
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<th>20</th>
<th>30</th>
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<td>nMGSH (5 cm)</td>
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<tr>
<td>UC</td>
<td></td>
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<tr>
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<td>OC</td>
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<tr>
<td>$P$ value</td>
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<tr>
<td>iMGSH (12 cm)</td>
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<td></td>
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<tr>
<td>UC</td>
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<tr>
<td>MC</td>
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<td>OC</td>
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<tr>
<td>1-way ANOVA</td>
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<tr>
<td>$P$ value</td>
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</table>

*Significantly ($P < 0.05$) different from value at day –20.

NS = Not significant.

See Table 1 for remainder of key.
Postpartum AST activities on day 20 were significantly increased, compared with the previous time point, for underconditioned (P = 0.004), moderately conditioned (P = 0.003), and overconditioned (P < 0.001) groups. Postpartum GGT activities on day 10 were significantly (P < 0.001) increased, compared with the previous time point, for all groups. Other than a significant (P = 0.001) decrease on day –20 in underconditioned cows, fluctuations of ALT activities were not significant. Concentrations of BUN decreased significantly on day –10 in the underconditioned (P = 0.003) and overconditioned (P = 0.002) groups; the decrease was not significant in the moderately conditioned group. Subsequently, BUN concentration increased significantly on day 20, compared with day 10, in the underconditioned (P = 0.004), moderately conditioned (P < 0.001), and overconditioned (P < 0.001) groups. Albumin concentration decreased significantly on day –20 in the underconditioned (P = 0.037), moderately conditioned (P < 0.001), and overconditioned (P < 0.001) groups and decreased significantly on day –10 in the overconditioned group (P = 0.021). Subsequently, albumin concentration increased significantly on day 20, compared with day 10, in the underconditioned (P < 0.001), moderately conditioned (P = 0.007), and overconditioned (P < 0.001) groups. Total calcium and albumin-adjusted calcium concentrations decreased significantly on day 10, compared with day 0, in the underconditioned (P = 0.002), moderately conditioned (P < 0.001), and overconditioned (P = 0.006) groups, which was followed by a significant (P < 0.001) increase on day 20 in all groups. Inorganic phosphorus concentrations significantly decreased on day –20 in the underconditioned (P < 0.001), moderately conditioned (P < 0.001), and overconditioned (P = 0.002) groups. Inorganic phosphorus concentrations significantly decreased again on day –10 in the underconditioned (P = 0.049), moderately conditioned (P = 0.01), and overconditioned (P = 0.001) groups. Minimum inorganic phosphorus concentrations were recorded on day 10. Afterward, there was a significant increase in inorganic phosphorus concentrations on day 20 in the underconditioned (P < 0.001), moderately conditioned (P = 0.018), and overconditioned (P = 0.027) groups.

Alterations in nMGSH and fMGSH along with attenuation values were summarized (Table 2). The nMGSH values slightly decreased on the day of calving, but this decrease was only significant in the underconditioned group. There was a significant (P = 0.016) difference between nMGSH values in underconditioned and overconditioned cows at calving day. For fMGSH values, a significant decrease was evident in each group on the day of calving. The fMGSH values of underconditioned and moderately conditioned cows were different on days –30 (P = 0.05), –10 (P = 0.017), and 0 (P = 0.017). The highest attenuation levels of moderately conditioned and overconditioned cows were recorded on the day of calving, and the values were significantly higher than on day –20 (P = 0.002) in the overconditioned group. However, the alterations of attenuation values in underconditioned and moderately conditioned cows were not significant among sampling days. Attenuation values were significantly higher in overconditioned cows, compared with underconditioned cows, on each sampling day except day –20. Moreover, underconditioned
between liver dysfunction and the structural changes associated with MGSH values in overconditioned cows were attributed to a concomitant increase during the postpartum period. It also could be suggestive of an association between liver dysfunction and the structural changes caused by lipid accumulation in overconditioned cows. However, this correlation was not seen in other groups, and confirmation requires further investigation.

Reduced BUN concentration near the time of calving was observed in each group. This reduction may be associated with decreased ureagenesis during this period, which is compromised because of the liver’s functional shift to augment energy pathways. Also, BUN concentration in the peripartal period is influenced by ruminal protein metabolism and overall proteolysis. This reduction may also be associated with reduced total ammonia absorption from the rumen. The weak positive correlation between BUN concentration and MGSH values of overconditioned cows was the result of the concomitant increase in both of these variables during the postpartum period. We conclude that there was a plausible association between peripartum changes of liver function and some fatty liver–associated structural changes in the liver images.

Albumin concentration is considered a good indicator of liver function because it is only synthesized by hepatocytes. In the present study, fluctuations in concentrations of serum albumin concentration were observed in which the values were closest to the lower reference limit from days –20 through 20. This is in agreement with previous reports in periparturient cows. Low albumin concentrations may also reflect an increase in protein consumption in tissues. It has been reported that low plasma albumin concentration during the last 3 to 4 weeks of gestation may be attributed to repartitioning of labile protein stores to mammary glands in preparation for lactation or simply decreased hepatic synthesis. There is also a report of decreased albumin concentrations in early postpartum cows. In the present study, the significant decrease in albumin concentration from day –30 to –20 was greater than expected, but was not likely clinically relevant because the concentrations remained in the reference range. The postpartum positive correlation between MGSH and albumin concentration in overconditioned cows could presumably be attributable to gradual regaining of normal liver function and structure through progression of lactation.

In agreement with the present findings, a postpartum decrease in serum calcium concentration has been reported in cattle. This decrease is attributed to increasing milk yield and inability of cows to fulfill the need for calcium from body reserves. There is ample evidence of associations between alterations of calcium and energy metabolism. In this respect, reduced calcium concentration could be associated with abnormalities of energy metabolism in recently calved cows. Considering that fatty liver represents a major disturbance of energy metabolism, it could be correlated with reduced calcium concentrations as well.

Calcium is an important part of the signaling system that relays information to the CNS regarding liver energy status; therefore, intracellular calcium concentration may be a factor associated with energy status during peripartal periods, and liver energy is reduced in cows with fatty liver. Also, available historic data indicate that high nonesterified fatty acids concentrations and fatty liver are related to hypocalcemia.

Serum AST, GGT, and ALT are considered liver-associated enzymes that may leak into the bloodstream because of liver damage, although serum ALT activity is considered a nonspecific marker for bovine liver damage. Increased AST and GGT activities have been reported in cows during early lactation and also in ketotic cows. In the present study, postpartum AST and GGT activities significantly increased in all groups. The increased activity in the early postpartum period might be attributed to liver damage caused by fat accumulation in hepatocytes. Despite the significant increase, however, the population mean did not exceed the reference range; therefore, the amount of liver damage was not considered extensive at the population level. Given that no specific clinical sign could be associated with the increased activity of liver enzymes, the increase was perceived as a subclinical alteration in liver function. The positive correlations of AST and GGT activities with MGSH values in overconditioned cows were attributed to a concomitant increase during the postpartum period. It also could be suggestive of an association between liver dysfunction and the structural changes.
rats, calcium deficiency modifies polyunsaturated fatty acids metabolism by reducing liver desaturase activity. During gestation, fetal skeletal rhus is a component of phospholipids, phosphoproteins, and ATP. Postpartum overconditioned cows. Inorganic phosphorus concentration and fMGSH values in the present study started from day –30 and reached a minimum value on day 10. Concentrations, which were beyond the scope of this study, in serum albumin and total and ionized calcium concentrations were observed in the present study starting with the measured value of the accumulated fat in the liver and the amount of synthesized albumin, as well as alterations in serum albumin and total and ionized calcium concentrations, which were beyond the scope of this study.

A stepwise decrease in inorganic phosphorus concentration was observed in the present study starting from day –30 and reaching a minimum value on day 10. Moreover, there was a weak correlation between inorganic phosphorus concentration and IMGSH values in postpartum overconditioned cows. Inorganic phosphorus is a component of phospholipids, phosphoproteins, nucleic acids, and ATP. During gestation, fetal skeletal development could withdraw up to 10 g of inorganic phosphorus/d from maternal inorganic phosphorus pools. In addition, postparturient hypophosphatemia has been associated with an increasing fatty infiltration of the liver. It has been suggested that hypophosphatemia could occur secondary to a metabolic derangement, most likely subclinical ketoacidosis in the prepartum period caused by submaintenance feeding of a pregnant animal. Provision of phosphorus and vitamin B12 can improve energy metabolism of periparturient dairy cows, which signifies the role of inorganic phosphorus during this period. In the present study, the correlation between inorganic phosphorus and MGSH in overconditioned cows was also suggestive of a possible link between inorganic phosphorus concentrations and fatty liver–associated features of liver ultrasonograms.

Changes in the echotexture of the liver may be associated with underlying structural alterations of various causes. Fatty infiltration may occur in various degrees in periparturient cows via mobilization from fat depots, causing the described specific features in ultrasonograms. In this respect, BCS is known as a major risk factor for fat mobilization. In addition, maximum triglyceride accumulation in hepatocytes typically happens in the first 10 days after calving. The nMGSH values generally had nonsignificant changes during the sampling period. However, fMGSH values were decreased significantly on the day of calving in each group, which explained the finding of higher attenuation rates at calving in moderately conditioned and overconditioned groups. However, variation among individual cows in attenuation values at calving was evident. Overall, population-level mean values revealed that underconditioned cows had significantly lower attenuation than moderately conditioned and overconditioned cows at calving, and this was consistent with their limited capacity to accumulate fat in hepatocytes because of having fewer fat depots to mobilize. In contrast to expectations, MGSH values decreased at calving, compared with values obtained before calving. Some speculations were made for this unpredicted finding, as follows. Blood flow is known to reduce the echogenicity of the parenchymal tissues, and liver blood flow increases from the prepartum to postpartum periods, which might reduce the echogenicity. Other factors that might have contributed to the reduced MGSH values obtained at calving could be the anatomic change in liver location (ie, its distance from the abdominal wall) caused by delivery of the fetus and the variation in the degree of ruminal fill caused by reduced intake among cows that have recently calved.

The observed pattern in changes of attenuation levels and the difference between groups suggested that this variable could be a more sensitive indicator for fatty liver–associated changes in ultrasonographic images of recently calved cows, when MGSH values are compared on a population basis. This finding is in agreement with previous research results, which indicate a higher sensitivity for the attenuation variable, when echogenicity changes in individual cows with fatty liver are compared.

Serum biochemical analysis revealed the expected peripartum alterations attributable to adaptations that influenced the metabolism and liver function in each group of cows. However, the overall intensity of the changes was not critical for the investigated groups. Therefore, the probability of high incidence of severe forms of fatty liver was not high, and no clinical evidence of this was found. The MGSH revealed minor structural changes in association with fatty liver. However, the attenuation value could discriminate the BCS groups, which were at different risks of developing fatty liver, and this fact makes the attenuation variable more valuable for population monitoring. Moreover, the ultrasonographic pattern had a correlation with serum biochemical variables only in overconditioned cows, and such observations were not made in the underconditioned and moderately conditioned cows. Even though a strong correlation was not expected in this study, the lack of any correlation was attributed to insufficient sensitivity of MGSH to detect minor structural changes. More sensitive and applicable ultrasonographic variables, which could reveal the milder forms of fatty liver, could enable bovine practitioners to implement liver ultrasonography as an efficient monitoring modality for metabolic disorders in dairy cow populations. The divergence between the measured serum variables and ultrasonographic variables suggests that assessment of liver function and structure requires separate and sensitive methods to categorize the liver’s health status in periparturient dairy cows.

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b. Pars-azmoon Chemical Co, Tehran, Iran.
References
