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Obesity-driven prepartal hepatic lipid accumulation in dairy cows is associated with increased CD36 and SREBP-1

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Abstract:	We investigated the hypothesis that obesity in cows enhanced expression of proteins involved in hepatic fatty acid uptake and metabolism. Sixteen Holstein-Friesian close-up cows were assigned to optimal or high body condition score (BCS) groups. Intravenous glucose tolerance test (GTT) and liver biopsies were carried out at day 10 before calving. Blood samples were collected before (basal) and after glucose infusion, and glucose, insulin and nonesterified fatty acid (NEFA) levels were determined at each sample point. In addition, β-hydoxybutyrate, triglycerides and estradiol levels were measured in the basal samples. The liver biopsies were analyzed for total lipid content and protein expression of fatty acid translocase (FAT/CD36), sterol regulatory element-binding protein-1 (SREBP-1) and insulin receptor beta (IRβ). Basal glucose and insulin were higher in high-BCS cows, which coincided with greater circulating triglycerides and hepatic lipid content. High-BCS cows had lower RQUICKI, while clearance rate and AUC for NEFA during GTT were greater in optimal-BCS cows. The development of insulin resistance and fatty liver in obese cows was paralleled by increased hepatic expression of the IRβ, CD36 and SREBP-1. Our findings suggest that hyperinsulinemia likely plays a causative role in the obesity-driven CD36 and SREBP-1 overexpression and hepatic lipid accumulation.

Obesity-driven prepartal hepatic lipid accumulation in dairy cows is associated with increased CD36 and SREBP-1

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Abstract

We investigated the hypothesis that obesity in cows enhanced expression of proteins involved in hepatic fatty acid uptake and metabolism. Sixteen Holstein-Friesian close-up cows were assigned to optimal or high body condition score (BCS) groups. Intravenous glucose tolerance test (GTT) and liver biopsies were carried out at day 10 before calving. Blood samples were collected before (basal) and after glucose infusion, and glucose, insulin and nonesterified fatty acid (NEFA) levels were determined at each sample point. In addition, β-hydoxybutyrate, triglycerides and estradiol levels were measured in the basal samples. The liver biopsies were analyzed for total lipid content and protein expression of fatty acid translocase (FAT/CD36), sterol regulatory element-binding protein-1 (SREBP-1) and insulin receptor beta (IRβ). Basal glucose and insulin were higher in high-BCS cows, which coincided with greater circulating triglycerides and hepatic lipid content. High-BCS cows had lower RQUICKI, while clearance rate and AUC for NEFA during GTT were greater in optimal-BCS cows. The development of insulin resistance and fatty liver in obese cows was paralleled by increased hepatic expression of the IRβ, CD36 and SREBP-1. Our findings suggest that hyperinsulinemia likely plays a causative role in the obesity-driven CD36 and SREBP-1 overexpression and hepatic lipid accumulation.

Key words: prepartum, dairy cows, fatty liver, CD36, SREBP-1.

Introduction

In dairy cows, the transition from late lactation to dry period is associated with a decrease in the energy requirement due to a drop in milk production (Bell 1995). At this time, if levels of corn silage or grains are not reduced accordingly, a positive energy balance may result in excess adipose mass and obesity. Although overconditioning during the dry period is not advised, it is still observed, particularly among cows fed ad libitum and/or cows with longer calving intervals (Šamanc and others 2010). It is widely accepted that dairy cows with a body condition score (BCS) greater than 4.0 at parturition are at increased risk of developing metabolic disorders mainly associated with increase in circulating levels of non-esterified fatty acids (NEFA) and decrease in dry matter intake (Kim and Suh 2003, Pires and others 2007). Moreover, it has been observed that obese cows mobilize body fat prepartally, earlier and more extensive than cows that are in optimal body condition (Kokkonen and others 2005, Šamanc and others 2015). Thus, due to adipose tissue dysfunction and/or increased insulin resistance, the former cows may be challenged by prepartal hepatic fat accumulation (Rukkwamsuk and others 1998, Holtenius and others 2003). Additionally, changes in regulatory mechanisms controlling hepatic glucose and lipid metabolism that occur during the transition period may strongly contribute to the accumulation of fat in liver cells (Drackley and others 2001, Ji and others 2012).

Fatty liver develops when the rate of NEFA influx in the liver exceeds all possible disposal routes (Katoh 2002). Although limited oxidative or transport capacities of ruminant liver may be an important contributing factors to fatty liver (Katoh 2002, Murondoti and others 2004), increased NEFA influx and/or capacity for esterification seems to be the primary cause of the higher hepatic concentrations of total lipid and triglycerides (TGs) (Litherland and others 2011). It is well known that NEFA, due to its hydrophobic nature, freely crosses plasma membranes by passive diffusion. In recent years, increasing evidence suggests that fatty acid transport proteins can regulate their transport and subsequent metabolism in many tissues and it is also shown in dairy cows. Bovine liver cells express several types of these proteins: fatty acid translocase (FAT/CD36), fatty acid transporter protein 2 (FATP2) and fatty acid binding protein 3 (FABP3). Latter two can play important roles in the adaptation of metabolism to energy deficiency (Loor and others 2007) as they channel fatty acids toward β-oxidation, whereas upregulated CD36 increases NEFA uptake and storage of TGs (Bradford and others 2009).

Sterol regulatory element-binding protein-1 (SREBP-1) is the key regulator of hepatic lipid metabolism. Activation of SREBP-1 increases hepatic lipogenesis under conditions of excessive dietary intake and leads to fatty liver through stimulation of transcription of the network encompassing *de novo* fatty acids synthesis and esterification processes (Shimano and others 1999, Sone and others 2002). SREBP-1 affects liver metabolism directly, as well as acting indirectly through peroxisome proliferator-activated receptor-γ (PPARγ) and PPARγ ligands (Kim and others 1998, Nassir and Ibdah 2014). Furthermore, it mediates the induction of lipid biosynthesis by insulin in hepatocytes (Haas and others 2012). Despite the potential relevance of SREBP-1 in driving hepatic lipogenic pathways in transition dairy cows, there is a paucity of data regarding expression of SREBP-1 in ruminant liver in normal and pathological states.

Obese and thin cows respond differently to the metabolic demands of late gestation. The molecular mechanism behind these differences is still unclear, especially for a regulatory mechanism that drives prepartal lipid accumulation in the liver. As CD36 and SREBP-1 have been shown to be expressed in ruminant liver as well, we examined their possible involvement in NEFA uptake and lipid accumulation in hepatocytes of close-up dry cows depending on their BCS.

Materials and Methods

Animals, diets, and housing

The animal-related component of the study was approved by the Ethical Committee of the Faculty of Veterinary Medicine, University of Belgrade in accordance with the National Regulation on Animal Welfare. In this study, sixteen multiparous Holstein-Friesian cows on a commercial dairy were assigned to one of the two close-up dry groups according to their BCS. Cows were grouped as optimal ($3.25 \leq BCS \leq 3.5$; $n = 8$) and high ($4.0 \leq BCS \leq 4.25$; $n = 8$) using a 5-point scale system recommended by Elanco Animal Health Bulletin AI 8478. Diversity in BCS of cows kept under the same dietary regimen was a consequence of differences in previous intercalving intervals (data not shown). During the dry period, cows were fed with diets in the form of total mix ration (TMR) that differs in ingredients and chemical composition for far-off dry cows (weeks 8 to 3 before calving) and close-up dry cows (wk 3 to 0 before calving) (Table 1). Diets were fed at 0630 and 1700 h. Cows were housed in free stall barn and had free access to water.

Table 1. Ingredients and chemical composition of the far-off and close-up diets

	Diet	
Item	Far-off[1]	Close-up[1]
Ingredient		
Corn silage, g/kg of DM	518	496
Grass silage, g/kg of DM		79
Straw, g/kg of DM	212	190
Hay, g/kg of DM	114	
Ground shelled corn, g/kg of DM	22	34
Tritical, g/kg of DM	22	34
Barley, g/kg of DM	22	34
Sunflower meal, g/kg of DM	38	36
Soybean meal, g/kg of DM	43	48
Dried beet pulp, g/kg of DM		18
Minerals, g/kg of DM	10	19
Propylene glycol, g/kg of DM		12
Chemical analysis		
Crude protein, g/kg of DM	126	138
Crude fat, g/kg of DM	31	28
NEL, MJ/kg of DM	5.7	6.7
ADF, g/kg of DM	270	241
NDF, g/kg of DM	438	377

[1]The far-off period was from week 8 to 3 before calving, and the close-up period was from week 3 to 0 before calving.

Glucose tolerance test

At 10 days before the expected date of calving each animal involved in the study was subjected to an intravenous glucose tolerance test (GTT). Cows were fasted 1 h before and during GTT that was conducted at approximately 10:00 h for an average of 6 min. Glucose was warmed to body temperature and administered intravenously via the jugular vein as a 50% solution (Hemofarm, Serbia) in the dose of 500 mg of D-glucose/kg of BW. Mean actual day of glucose tolerance testing was the same as target day, with standard deviation of 1.8 d.

Blood sampling and analyses of metabolites and hormones

Immediately before infusion (basal samples) and at 5, 15, 30, 45, 60, 90, 120 and 180 min after finishing the glucose infusion, blood samples were obtained from the jugular vein of the opposite side. Blood samples were drawn into evacuated serum separator tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ). Following blood collection, the whole blood sample was immediately tested on the FreeStyle Precision meter (Abbott Diabetes Care Ltd., Oxon, UK). The remainder of the blood was allowed to clot, then centrifuged at 1800 × *g* for 10 min, and aliquoted into 2-mL microfuge tubes. Aliquots of serum were stored at -20^{o}C until analysis. Each blood sample was analyzed for glucose, insulin and NEFA, while basal samples were analyzed for β-hydroxybutyrate (BHBA), TG and estradiol. Glucose and BHBA were measured in whole blood enzymatically using commercial test bands FreeStyle Precision (Abbott Diabetes Care Ltd., Oxon, UK). Serum metabolites (NEFA and TG) were measured using the respective kits both from Randox Laboratories Ltd. (Crumlin, UK). Analyses were performed automatically by spectrophotometry (A15; BioSystems S.A., Barcelona, Spain). Insulin and estradiol concentrations were determined by radioimmunoassay technique using a commercially available RIA kits (INEP, Zemun, Serbia) according to the manufacturer's guidelines.

Liver sampling and analyses

Liver biopsy was adjusted so that it occurred on the same day as the GTT. Liver was sampled via percutaneous biopsies from cows under local anesthesia at 1600 h (before afternoon feeding) following the method previously described (Šamanc and others 2010). Approximately 1 g of liver was placed in fixation buffer that contained neutral 10% formaldehyde solution and

used for histopathological analysis of lipid contents. Sections obtained using a freezing microtome (LEICA 1850, Jung Tissue Freezing Medium) were specifically stained with Sudan III. The liver lipid contents were semi-quantified through computer image analysis (Software Q Win) made using the appliance (LEICA Q 500 MC). Lipid content in the hepatocytes was evaluated using stereological method (Gaal and others 1983), and presented as percentage (%). Approximately 2 g of liver was frozen immediately in liquid nitrogen to be used for determination of hepatic fatty acid transporter CD36, transcription factor SREBP-1 and insulin receptor beta (IRβ) protein expression.

Tissue preparation

Protein was extracted from liver samples by homogenization with Ultra-Turrax homogenizer in modified RIPA buffer (50 mmol/l Tris-HCl, pH 7.4, 150 mmol/l NaCl, 1% Triton X-100, 0.2% Na-deoxycholate, 0.2% SDS, 1 mmol/l EDTA, protease inhibitors, phosphatase inhibitors). Liver homogenate was centrifuged at 15000 x g for 30 min at 4°C and the supernatant was refered to cell lysate. Protein concentration was determined by BCA Protein Assay Reagent (Pierce, Rockford, IL, USA) according to manufacturer's instruction. After that the cell lysate samples were prepared in Laemmli sample buffer, boiled for 5 minutes at 100°C, and frozen for use in Western blot analysis.

SDS polyacrylamide electrophoresis and Western blot

Lysate proteins (75 μg) were resolved on 10% SDS polyacrylamide gels and transferred onto PVDF membranes (Millipore, Bedford, MA, USA). The membranes were blocked with 5% w/v nonfat dried skimmed milk and incubated overnight at 4° C with specific antibodies against IRβ (sc-711), SREBP-1 (sc-366) or CD36 (sc-9154). After washing 5 x 5 min in TBST buffer, membranes were incubated with the corresponding horseradish peroxidase anti-rabbit IgG-HRP as secondary antibody (s.c.-2004), diluted in TBST 1:10.000. The immunoreactive proteins were detected with enhanced chemiluminescence. To ensure that protein loading was equal in all lysate samples, blots were striped and reprobed with the β-actin antibody (sc-1616R). Band intensities were scanned and quantified using the Image J software.

Calculations and statistical analyses

All results are expressed as the means ± standard deviation (if not otherwise stated). The patterns of parameters during GTT were analyzed according to Kaneko (1997) and Schoenberg and others (2012). The variables calculated for the GTT data included the area under the curve (AUC; trapezoidal method) for all parameters; clearance rates (CR) for glucose and NEFA; the highest concentration (Peak) and the time required for the concentration to fall by one-half (half-life; $T_{1/2}$) for glucose; the highest concentration (Peak) and increment (insulin peak-basal insulin; ΔMax) for insulin. The Revised Quantitative Insulin Sensitivity Check Index (RQUICKI) was calculated by Holtenius and Holtenius (2007) .

Data analyses were performed using STATISTICA version 6. software package (StatSoft, Inc., Tulsa, Ok, USA). All of the above mentioned parameters were tested for normality using the Kolmogorov-Smirnov test statistic. Because almost all of these parameters were normally distributed, the paired *t*-test statistic was used to evaluate the significance of differences among the measurements. Differences were considered to be significant when the value $P < 0.05$.

Results

Basal blood samples

Table 2. Blood glucose and BHBA, serum NEFA, TG, insulin and estradiol concentrations, and RQUICKI for cows with high-BCS and optimal-BCS at 10 days before parturition

Item	High-BCS	Optimal-BCS	P
Glucose (mmol/L)	3.18±0.2	2.62±0.5	0.011
Insulin (μIU/ml)	17.33±4.90	10.24±5.04	0.02
NEFA (mmol/L)	0.43±0.14	0.46±0.20	0.75
RQUICKI	0.39±0.02	0.46±0.04	0.001
BHBA (mmol/L)	0.55±0.17	0.45±0.12	0.19
TG (mmol/L)	0.49±0.16	0.36±0.12	0.04
Estradiol (pg/mL)	239.9±97.11	163.9±74.61	0.07

Table 2 shows that basal glucose and insulin concentrations were higher in high-BCS cows than in optimal-BCS cows ($P = 0.011$ and $P = 0.02$, respectively), whereas the

concentration of basal NEFA did not differ between groups (P = 0.075) at 10 days before parturition. As expected, the RQUICKI value was lower in high-BCS cows compared with optimal-BCS cows (P = 0.001). No significant differences were observed for blood BHBA (P = 0.19) between groups, but high-BCS cows had greater circulating TG (P = 0.04). Mean serum estradiol concentrations tended to be lower in optimal-BCS compared to high-BCS cows (P = 0.07).

Glucose tolerance test

Table 3. Response variables to an intravenous glucose tolerance test in high-BCS and optimal-BCS groups of cows at 10 days before parturition

Item	High-BCS	Optimal-BCS	P
Glucose			
Basal (mmol/L)	3.18±0.2	2.62±0.5	0.011
CR_{45} (%/min)	1.63±0.33	1.80±0.46	0.38
$T_{1/2}$ (min)	44.12±8.9	41.22±13.51	0.62
Peak (mmol/L)	10.97±1.12	11.67±0.85	0.18
AUC_{120}(mmol/l×min)	877.87±79.21	869.12±69.77	0.82
Insulin			
Basal (µIU/ml)	17.33±4.90	10.24±5.04	0.02
Peak (µIU/mL)	112.39±51.66	134.9±31.41	0.33
ΔMax (µIU/mL)	102.15±49.72	116.95±26.62	0.47
AUC_{120} (µIU/ml×min)	9604.36±3534.44	7593.87±2679.90	0.22
NEFA			
Basal (mmol/L)	0.43±0.14	0.46±0.20	0.75
CR_{60} (%/min)	3.53±0.40	6.47±0.81	0.01
AUC_{120} (mmol/l×min)	-10.97±1.37	-25.50±11.80	0.004

As presented in Table 3, no glucose-associated insulin resistance was evident in any of the glucose infusion response variables calculated, although basal glucose and insulin concentrations were significantly different between examined groups. Consistent with this, peak insulin concentration, insulin increment (ΔMax) and insulin AUC did not differ between groups

($P = 0.33$, $P = 0.47$ and $P = 0.22$, respectively). In contrast to the glucose and insulin profiles, differences for the two groups were found to be significant in serum levels of NEFA during the GTT. NEFA clearance rate (CR_{60}) and NEFA AUC in response to glucose infusion were lower for cows that had excessive body fat than for cows with optimal body condition ($P = 0.01$ and $P = 0.004$, respectively).

Hepatic lipid and protein contents

There were differences between the two groups observed for contents of hepatic total lipid, IRβ, CD36 and SREBP-1 protein expression. As illustrated in Figure 1, high-BCS cows had higher liver lipid content than optimal-BCS cows ($P = 0.001$) 10 days before parturition. Expression of all analyzed hepatic proteins, IRβ, CD36 and SREBP-1, in cows in the high versus optimal body condition were significantly higher ($P = 0.04$, $P = 0.015$ and $P = 0.012$, respectively).

Discussion

Alteration in hepatic regulation of lipid metabolism may be responsible for the prepartal lipid accumulation in the liver. However, limited information exists in cows with high BCS because most studies have focused on how dry cow diets can alter metabolic activities in liver tissue. This study was performed to investigate the hypothesis that obesity leads to increased liver´s capacity to take up and convert fatty acids to esterified products in prepartum dairy cows. As expected, extended lactation was associated with a strong positive energy balance at 10 days before calving resulting in overcondition and increased serum glucose and insulin as well as hepatic total lipid concentrations. Similar metabolic changes in association with body condition, or at least feeding intensities, have been observed as parturition approached in many other studies (Reid and others 1986, Rukkwamsuk and others 1998, Holtenius and others 2003, Janovick and others 2011). The observed signs resemble those of obesity-induced Type II diabetes in humans (Petersen and Shulman 2006) and might reflect metabolic changes toward gluconeogenesis and/or peripheral insulin resistance. In accordance with Holtenius and Holtenius (2007), our findings based on RQUICKI calculation indicate on lower insulin sensitivity in obese cows. Since there are inconsistent results in literature related to reliability of RQUICKI as insulin

sensitivity indicator in dairy cows (Kerestes and others 2009), we also used a glucose tolerance test (GTT) to detect differences in insulin resistance. Recently, Schoenberg and others (2012) reported that measurements of changes in insulin resistance obtained by GTT are comparable with those from hyperinsulinemic-euglycemic clamp (HEC), which is the gold standard for the evaluation of tissue sensitivity to insulin. In the present study, the variables generated from the glucose and insulin profiles did not differ between groups, suggesting no significant differences in glucose-associated insulin resistance. Hence, the higher circulating levels of glucose and insulin in obese cows could be due to, at least in part, decreased insulin´s effect on restricting glucose production and/or to an increased hepatic gluconeogenesis (Hammon and others 2009). Nevertheless, more apparent differences were observed in responses of NEFA to GTT. Despite having the similar dynamics of insulin during GTT, obese cows had lower NEFA clearance rate and lower NEFA AUC following glucose infusion. These findings suggest that obese cows had greater insulin resistance related to lipid metabolism and may indicate that these cows were much less sensitive to an inhibition of lipolysis. The lower insulin sensitivity of cows in the high-BCS group may result in greater body fat mobilization and, consequently, increased plasma concentrations of NEFA. Although serum NEFA levels were similar for cows in the optimal-BCS and high-BCS groups, those in the high-BCS group experienced more intense accumulation of lipid in the liver prepartum.

The basis for hepatic lipid accumulation is complex and is thought to reflect the imbalance between synthetic and degradative pathways. In dairy cows, this process is often viewed as a function of reduced apolipoprotein availability and very low lipoprotein (VLDL) secretion (Katoh 2002). However, VLDL assembly and secretion are enough to keep up with NEFA supply in late gestation, suggesting that other abnormalities are playing key roles. In this context, it is of interest that cows in the high-BCS group showed greater TG concentrations in blood serum. Furthermore, increased fatty acids availability for incorporation into VLDL as well as for accumulation within the hepatocytes could also be a consequence of decreased fatty acids oxidation in the liver. Since we did not measure β-oxidation enzymes, we can not claim the different hepatic β-oxidation status in, although it should be noted that cows in the high-BCS and optimal-BCS groups had comparable NEFA and BHBA concentrations. In addition, detailed enzyme analyses in prepartum cows showed that state of energy balance did not influence capacity for β-oxidation (Murondoti and others 2004). It could be argued that this would

implicate more changes in hepatic fatty acids esterification than oxidation in obese cows (Litherland and others 2011). In other words, a central issue in the regulation of hepatic lipid metabolism in this period is the mechanism by which hepatocytes take up and store fatty acids. In accordance with the hypothesis of this study, we observed for the first time that obesity leads to an increase of the expression of CD36 and SREBP-1 in the liver of cows at 10 days before calving. These results suggest a potential role for aberrant CD36 expression in enhanced liver fatty acid uptake during obesity, which could contribute to the increase in hepatic triglyceride synthesis and VLDL production. This suggestion is supported by studies in rodents and humans in which increased expression of lipogenic pathway is seen in livers of obese, insulin resistant subjects and is implicated in nonalcoholic steatohepatitis (Koonen and others 2007, Haas and others 2012, Nassir and Ibdah 2014). In contrast to non-ruminants, there is not enough studies so far, aimed to define the specific regulative role of CD36 and SREBP-1 in cattle liver. However, a recent study in mid lactation cows injected with tumor necrosis factor-a (TNFα) (Bradford and others 2009) showed that CD36 is functional in cattle liver and mediates TNFα effects on liver TG accumulation. Most of the studies in rodent models of obesity have shown that increased hepatic CD36 expression is sufficient to alter hepatic metabolism and promote liver TG storage (Koonen and others 2007). Nevertheless, with respect to the findings in obese rodents (Haas and others 2012), we assume that prepartal lipid accumulation in the liver of obese cows was also the result of an increased capacity of liver tissues to convert fatty acids to esterified products. In line with this assumption, in the present study, we observed increased level of hepatic SREBP-1, which suggests enhanced lipogenic potential promoting lipid synthesis and storage in cows with higher BCS.

Besides changes in the hormonal sensitivity of tissues, changes of the concentrations of hormones themselves may influence the metabolic fate of fatty acids within the liver. One of the factors linking obesity and/or insulin resistance with prepartal hepatic lipid accumulation appears to be elevation in blood insulin. In contrast to observed alterations in glucose metabolism, due to fact that insulin receptor expression is enhanced in the livers of obese cows, the pathways through which insulin regulates lipid metabolism would be expected to be enhanced. This is consistent with concept of selective insulin resistance in obesity suggested by Brown and Goldstein (2008). This idea is supported by the higher expression of the fatty acids transporter CD36 and transcription regulator SREBP-1, which are well-established drivers of the insulin-

induced lipogenic response in non-ruminants (Foufelle and Ferre 2002). While there are limited numbers of studies related to activation of CD36 and SREBP-1 in cattle, studies in other species have clearly shown that CD36 and SREBP-1 expressions were positively regulated by insulin in liver and in hepatocytes cultures (Shimomura and others 1999, Steneberg and others 2015). As insulin is also acting as an agonist of lipogenic pathways in bovine liver (Vernon 2005), it seems justified to speculate that high concentrations of insulin in obese cows during late gestation, together with elevated expression of hepatic insulin receptor, could explain an increase of CD36 and SREBP-1 presence in the liver contributing to lipid accumulation.

In addition, a chronic increase in circulating insulin have resulted in increased circulating estradiol in cows (Butler and others 2004), which could be another link between obesity and the increase in liver lipid content prepartum. A number of studies on hepatocytes have described direct and indirect roles for estrogen in the regulation of lipid metabolism. Estrogen is thought to act by interfering with growth hormone signaling via repression of signal transducer and activator of transcription 5 (STAT5) gene expression. STAT5 regulates several key enzymes or genes involved in NEFA uptake, hepatic *de novo* lipogenesis and their intracellular trafiking (Lichanska and Waters 2008). Whether estrogen acts in the same manner in dairy cows is currently unknown. Anyway, Grummer and others (1990) reported increased liver lipid and TG content when nonlactating, nonpregnant Holstein cows were injected with estrogen. In this study, serum estradiol levels tended to be higher in obese cows than in cows with optimal condition, but this was not statistically significant. Taken together, these results argue against a primarily role of estradiol in the observed lipid accumulation in obese cows.

Conclusions

The present study shows for the first time that hepatic expressions of proteins involved in fatty acids transport and subsequent metabolism in dairy cows are increased during obesity. An up-regulation of these proteins could contribute to increased capacity of liver tissue to take up and storage fatty acids in late gestation observed in this and previous studies.

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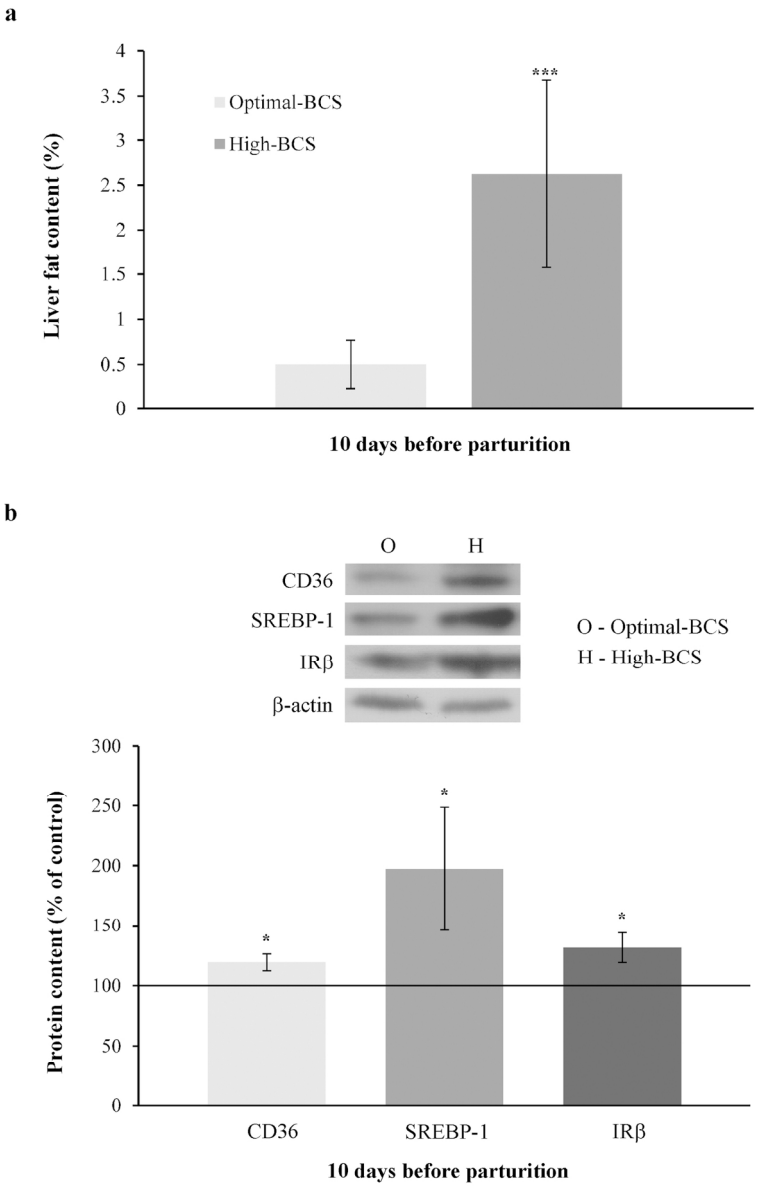
Figure 1. Contents of total lipid (a), CD36, SREBP-1 and insulin receptor beta (IRβ) protein expression (b) in hepatocytes for high-BCS and optimal-BCS cows at 10 days before parturition.

The total lipid content in the liver was determined by stereological method. The content of CD36, SREBP-1 and IRβ protein was determined by Western blot in hepatic cell lysate as described in Materials and Methods. Beta-actin was used as loading control. Results are expressed as mean ± SE, presented as percentage of the control and representative blots are placed above protein content histograms. Comparisons between high-BCS and optimal-BCS group were made by paired Student's t-test. Asterisks indicate significant differences: *$p<0.05$; ***$p<0.001$. Abbreviations: SREBP-1- sterol regulatory element-binding protein 1; IRβ – beta subunit of insulin receptor.



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