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

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ABSTRACT

We investigated the hypothesis that obesity in dairy cows enhanced expression of proteins involved in hepatic fatty acid uptake and metabolism. Sixteen Holstein-Friesian close-up cows were divided into 2 equal groups based on their body condition score (BCS) as optimal ($3.25 \leq BCS \leq 3.5$) and high ($4.0 \leq BCS \leq 4.25$). 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 non-esterified fatty acid (NEFA) levels were determined at each sample point. In addition, β-hydroxybutyrate and triglycerides levels were measured in the basal samples. The liver biopsies were analyzed for total lipid content and protein expression of insulin receptor beta (IRβ), fatty acid translocase (FAT/CD36) and sterol regulatory element-binding protein-1 (SREBP-1). Basal glucose and insulin were higher in high-BCS cows, which coincided with higher circulating triglycerides and hepatic lipid content. Clearance rate and AUC for NEFA during GTT were higher 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. These results suggest that increased expression of hepatic CD36 and SREBP-1 is relevant in the obesity-driven lipid accumulation in the liver of dairy cows during late gestation.

Overconditioning of dairy cows during the dry period is not advised, but is still observed, particularly among cows with longer calving intervals (Pires et al., 2007). Cows with a body condition score (BCS) greater than 4.0 at parturition are at increased risk of developing metabolic disorders mainly associated with fatty liver (Bobe et al., 2004). Although limited oxidative or transport capacities of ruminant liver may be an important contributing factors to fatty liver (Katoh, 2002; Murondoti et al., 2004), increased non-esterified fatty acid (NEFA) influx and/or capacity for esterification seems to be the primary cause of the higher hepatic concentrations of total lipid and triglycerides (TG) (Litherland et al., 2011).

Increasing evidence suggests that fatty acid transport proteins can regulate their transport and subsequent metabolism in many tissues, which is also proposed for dairy cows. Bovine liver cells have been shown to express at least three 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 et al., 2007) as they channel fatty acids toward β-oxidation, whereas NEFA taken up through CD36 are preferentially stored in hepatocytes (Bradford et al., 2009). Furthermore, the increase in hepatic TG accumulation observed in animal models of obesity could be ascribed to dysregulation of master transcriptional regulator of hepatic lipid metabolism, sterol regulatory element-binding protein-1 (SREBP-1) (Haas et al., 2012). Activation of SREBP-1 leads to fatty liver through stimulation of transcription of the network encompassing, at least in part, synthesis of fatty acids and TG (Shimano, 2001). It also mediates the induction of steatosis by insulin and tumor necrosis factor-alpha in hepatocytes (Endo et al., 2007; Haas et al., 2012). Despite the potential relevance of SREBP-1 in driving hepatic lipogenic pathways in transition dairy cows, there is a paucity of data regarding the role of SREBP-1 in ruminant liver in normal and pathological states (Deng et al., 2014).

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 cows depending on their BCS.

Sixteen multiparous Holstein-Friesian cows on a commercial farm were assigned to one of the two close-up dry groups according to their BCS. Cows were grouped as optimal ($3.25 \leq BCS \leq 3.5$; $n = 8$)

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Table 1
Basal metabolites, insulin, and response variables to GTT in high-BCS and optimal-BCS groups of cows at 10 days before parturition (mean ± SD).

Item	High-BCS	Optimal-BCS	P
Basal			
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
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
Glucose response to GTT			
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 response to GTT			
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 response to GTT			
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

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).

At 10 days before calving each animal involved in the study was subjected to an intravenous glucose tolerance test (GTT) as described by Prodanović et al. (2013). Blood samples were collected immediately before (basal samples) and at 5, 15, 30, 45, 60, 90, 120 and 180 min after glucose infusion, and analyzed for glucose, insulin and NEFA. Basal samples were additionally analyzed for β-hydroxybutyrate (BHBA) and TG. Glucose and BHBA were measured in whole blood enzymatically using commercial test strips (Abbott Diabetes Care Ltd., Oxon, UK). NEFA and TG were measured by spectrophotometry (BioSystems S.A., Barcelona, Spain) using the respective kits both from Randox Laboratories Ltd. (Crumlin, UK). Insulin concentration as determined by radioimmunoassay (INEP, Zemun, Serbia).

On the same day as the GTT, liver was sampled via percutaneous biopsies as previously described (Šamanc et al., 2010). Liver samples were fixed in 10% (*w*/*v*) neutral formalin for 24 h 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 et al., 1983), and presented as percentage (%). Additional liver samples were frozen immediately in liquid nitrogen for Western blot method. 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.

Tissue lysate preparation for Western blot analysis was performed according to previously described procedures (Stanišić et al., 2016). Hepatic proteins were determined using antibodies against insulin receptor beta subunit (IRβ), SREBP-1 and CD36 from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). β-Actin antibody was used as a loading control.

The glucose tolerance test measurements included basal levels of glucose, insulin and NEFA, area under the curve (AUC) for all parameters; clearance rates (CR) for glucose and NEFA; Peak (maximal post-infusion concentration) and half-life ($T_{1/2}$, the time to reach half maximal concentration) for glucose; Peak (maximal post-infusion concentration) and increment (ΔMax, difference between the basal and the maximal concentration) for insulin.

Clearance rate (for the first 45 min for glucose profile, the first 60 min for NEFA profile) and glucose half-life during the GTT were calculated according to the formulas:

$CR\ (\%/min) = \{(\ln[ta] - \ln[tb]) / (tb - ta)\} \times 100$ and $T_{1/2}\ (min) = (0.693 / CR) \times 100$, where [ta] and [tb] are the concentrations in time a and time b, respectively.

The area under the curve in response to glucose in the first 120 min was calculated using incremental change and trapezoidal rule (Kaneko, 1997; Schoenberg et al., 2012).

Data were evaluated with Student's *t*-test on the Statistica 6.0 program. A value of P <; 0.05 was considered statistically significant.

As expected, cows with high BCS had higher basal glucose, insulin and hepatic lipid concentrations during late gestation (Table 1; Fig. 1). Similar metabolic changes in association with body condition, or at least feeding intensities, have been observed as parturition approached in many other studies (Reid et al., 1986; Rukkwamsuk et al., 1998; Holtenius et al., 2003; Janovick et al., 2011). The observed signs resemble those of obesity-induced Type II diabetes in humans (Petersen and

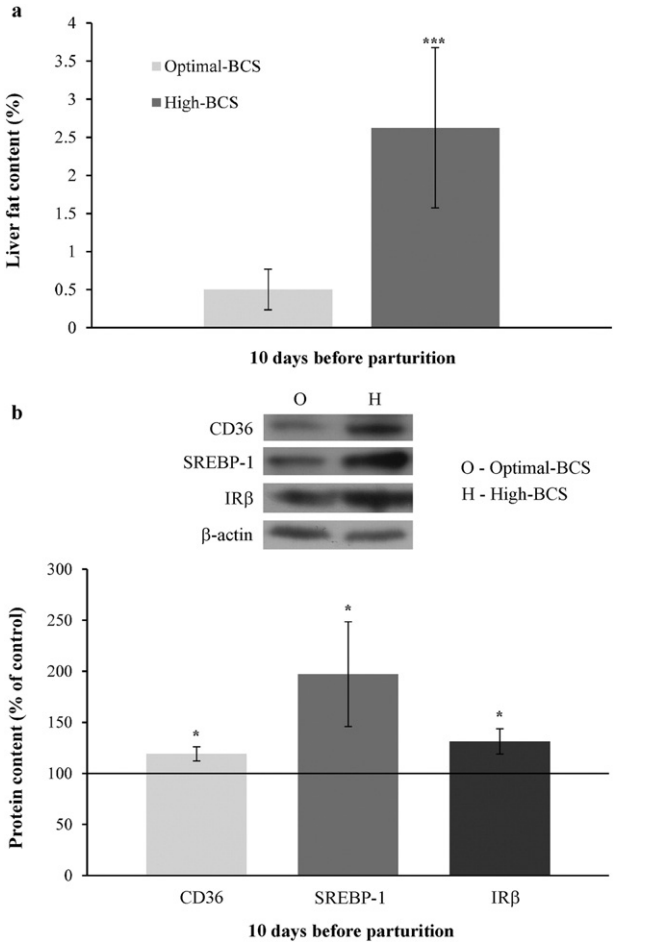

Fig. 1. Contents of total lipid (a), CD36, SREBP-1 and insulin receptor beta (IRβ) protein expression (b) in hepatocytes for high-BCS and optimal-BCS cows at 10 days before parturition. The total lipid content in the liver was determined by stereological method. The content of CD36, SREBP-1 and IRβ protein 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.