Gene expression and protein secretion of apolipoprotein B100 (ApoB100) in transition dairy cows under hot or thermoneutral environments

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ABSTRACT - The aim of the study was to investigate the effects of hot season on gene expression and protein secretion of $ApoB_{100}$ in transition dairy cows. Hot season strongly down-regulated $ApoB_{100}$ gene and protein expression. This condition and the higher circulating NEFA were responsible for the higher lipid accumulation in liver of heat-stressed transition cows.

Key words: Heat stress, Hot season, Liver $ApoB_{100}$, Transition cow.

Introduction - Requirements for energy and proteins increase dramatically during the periparturient period to support the onset of milk production. That period is also characterized by a reduction of dry matter intake (DMI). Under these conditions, energy requirements are satisfied, in part, by mobilization of fatty acids from adipose tissue, resulting in increased circulating concentrations of non-esterified fatty acids (NEFA). Although NEFA can be used by other tissues, the liver extracts NEFA in proportion to their concentration in the bloodstream. This may lead to hepatic lipidosis (Grummer, 1993), which has been supposed to explain reduced capacity of the liver to synthesize apolipoprotein B_{100} ($ApoB_{100}$). As well known, $ApoB_{100}$ is the essential core component of very low density lipoproteins (VLDL) and it is necessary for stabilizing the VLDL particles. High environmental temperature in periparurient dairy cows may exaggerate the decline in DMI, adversely affects milk production, and may increase the incidence of metabolic disorders (Lacetera et al., 1996). Previous studies (Collier et al., 1982; Ronchi et al., 1999) have shown that heat stress can lead to modification of important physiological and metabolic functions in cattle. In particular, it was reported that high temperatures are responsible for modifications of lipid metabolism and liver functionality in heat-stressed subjects (Ronchi et al., 1999).

To date, there is no information available concerning the possible relationship between heat stress and gene expression and secretion of $ApoB_{100}$. Therefore, the objective of the present study was to investigate the effects of hot season on gene expression and protein secretion of $ApoB_{100}$ in transition dairy cows.

Material and methods - The trial was carried out in a commercial dairy herd. Twelve multiparous Holstein cows were utilized in the study. Six cows gave birth in spring (SP: 28 March to 30 April). The remaining 6 cows gave birth in summer (SU: 15 June to 2 July). The two groups were monitored from -30 to 35 d relative to day of calving and were balanced for parity and body condition score (BCS). The 12 cows were fed the same total mixed ration. Values of air temperature (°C) and relative humidity (%) were recorded at 1 h intervals using two electronic data recorders (Skye Instruments, Llandrindod Wells, UK). Data for the air temperatures and relative humidity were utilized to calculate the temperature-humidity index (THI). Milk yield

was recorded by automatic electronic recorders. BCS of cows was scored weekly. Blood samples were taken at 08.00h from the jugular vein at -30±2, -18±2, and -4±1 d prepartum and 3, 17, and 35d postpartum. Blood samples were collected in *Vacutainer* tubes containing Li-heparin as anticoagulant agent and put in ice. Within 2 h blood samples were centrifuged and plasma was thus separated. Plasma was analyzed for NEFA (Wako Fine Chemical Industries, Dallas, TX), and ApoB100 [single radiant immunodiffusion (SRID) assay, Metabolic Ecosystem, Miyagi, Japan]. Liver biopsies were performed on d -30, 3, and 35 relative to day of calving after first locating the liver by ultrasound (Bernabucci et al., 2004). After collection an aliquot of liver tissue was put in a 10% neutered buffered formalin for histopathological examination. A second aliquot of liver tissue was rinsed in RNase-free water, frozen in liquid nitrogen and stored at -80°C until the quantitative real-time PCR (rt-PCR) and SRID assays. The degree of lipidosis was quantified on a percentage scale with estimation of the amount of liver parenchyma affected according to the size of lipid droplets (micro- and macro-vesicular), and distribution of affected cells (single cell, focal, perilobular, centrolobular or diffuse) using a 5-point scale. Gene expression of $ApoB_{100}$ was measured by quantitative rt-PCR. Total RNA was extracted by homogenizing liver tissue in QIAzol Lysis reagent (Qiagen, Chatsworth, CA) according to manufacturer's instructions. Total RNA (1µg) was reverse transcribed with ImProm-II reverse trascription system (Promega, Madison, WI). Aliquots of cDNA were subjected to rt-PCR using a Quantitect SYBR Green kit (Qiagen) and the following bovine primer pairs were used: ApoB100 (3' GCAGTTAGTTTCTCCTTGGCACTC; 5' AGAAAGAATTGAAGCGCACCCA), and RPS9 (3' CCTCGACCAAGAGCTGAAG; 5' CCTCCAGACCTCACGTTTGTTC) as internal control. rt-PCR was carried out with LightCycler 3.5 amplification system (Roche Applied Science, Indianapolis, IN) according to the manufacturer's instructions. Extraction of hepatic proteins was performed after the isolation of total RNA according to manufacturer's instructions. The resulting preparation was analyzed for total proteins (Pierce, Rockford, IL) and cellular $ApoB_{100}$ by SRID. Cellular $ApoB_{100}$ concentration was expressed as µg/µg of total proteins. Plasma $ApoB_{100}$ concentration was expressed as µg/mL. Data were analyzed as repeated measures using the GLM procedure of SAS® (SAS, 1999). Least squares means were separated with the PDIFF procedure of SAS®. Significance was declared at $P<0.05$.

Results and conclusions - During SP, either the day (9-20 h) or night (21-8 h) THI was below 72, which represents the upper critical THI established for dairy cows. In SU, the mean daily values of THI were 79.5±2.9 during the day, and 70.1±4.7 during the night. Furthermore, during summer, three heat waves (a period of at least 3 consecutive days during which recovery hours were less than 10) were detected. Results are reported in table 1. Compared with SP cows, the milk yield was lower in SU cows. BCS began to decrease approximately 15d before calving only in SU cows, whereas in SP cows BCS started to decline after calving. Around calving NEFA were higher in SU cows, and started to increase before calving only in SU cows. $ApoB_{100}$ gene expression decreased after calving and showed lower values in SU cows compared with SP. Cellular $ApoB_{100}$ had the lowest values 3 d after calving in both groups. SU cows had lower values at -30 and 35d from calving when compared with SP cows. Plasma $ApoB_{100}$ concentration significantly decreased at parturition in both groups. Fat accumulation in liver was higher in SU cows before and after calving. Down-regulation of liver synthesis and a reduction of $ApoB_{100}$ secretion in the periparturient dairy cows (Gruffat et al., 1997; Miyamoto et al., 2006) has been reported. In our previous paper (Bernabucci et. al., 2004) we reported a down-regulation of $ApoB_{100}$ at transcriptional level in the early postpartum period, and suggested that this decrease could be consistent with decreased synthesis and/or secretion of VLDL from liver during the periparturient period. The decrease of BCS, the high NEFA and the presence of liver lipidosis before calving are not common in dairy cows. Those changes would indicate a severe alteration of metabolic status before calving which is not only explainable by heat stress conditions. In both seasons, cellular $ApoB_{100}$ paralleled changes of $ApoB_{100}$ mRNA only comparing data before calving (-30d) with data after calving (3 or 35 DIM). In contrast, after calving (3 *vs*. 35 DIM) cellular $ApoB_{100}$ did not correspond with its mRNA expression. Data clearly indicate that the regulation of $ApoB_{100}$ would take place both at transcriptional and post-transcriptional levels. The present study provides the first evidence on the effect of hot season on $ApoB_{100}$ gene and protein expression. Hot season strongly affected lipid metabolism. The higher NEFA and

the lower $ApoB_{100}$ gene and protein expression were likely responsible for the higher lipid accumulation in liver of SU cows. These conditions can make SU cows more susceptible to metabolic diseases.

Table 1. Lsmeans and pooled S.E. of mean (SEM) for milk yield, body condition score (BCS), NEFA, $ApoB_{100}$ mRNA, liver $ApoB_{100}$ protein (LP), plasma $ApoB_{100}$ protein (PP), and degree of liver lipidosis (DL) of transition dairy cows during spring (SP) and summer (SU) period.

Item			Days from calving						SEM
			-30	-18	-4	3	17	35	
Milk yield	l/d/head	SP				26.4^{C}	34.9^{B}	40.2^{A}	1.7
							**	**	
		SU				22.8^{b}	21.5^{b}	25.5^{a}	
BCS		SP	3.2^{a}	3.3^{a}	3.3^{a}	3.2^{a}	2.6^{b}	2.2^{b}	0.1
					**	**	**		
		SU	3.1^{a}	3.0^{a}	2.7^{b}	2.5^{b}	2.1^{c}	1.9^{c}	
NEFA	mmol/l	SP	0.203^{c}	0.197^{c}	0.469^{b}	0.721^{a}	0.384^{b}	0.211^{c}	0.167
					**	*	*		
		SU	0.502^{c}	0.466^{c}	1.120^{a}	1.125^{a}	0.848^{b}	0.468^{c}	
$ApoB_{100}$ mRNA#		SP	1.167^{a}			1.115^{b}		1.052^{b}	0.141
			**			**		**	
		SU	0.897^{A}			0.480^{B}		0.526^{B}	
$ApoB_{100}$ LP	µg/µg TP¥	SP	0.548^{a}			0.054^{c}		0.310^{b}	0.077
			**					*	
		SU	0.220^{a}			0.103^{b}		0.240^{a}	
$ApoB_{100}$ PP	µg/ml	SP	235.4^{a}	200.4^{a}	91.3^{c}	55.4^{d}	102.1^{c}	160.0^{b}	21.1
		SU	216.3^{a}	149.2^{b}	64.0^{d}	47.4^{d}	120.5^{c}	121.7^{c}	
DL	a.u.	SP	0.00^{B}			$2,17^{A}$		2.16^{A}	0.53
			*			*			
		SU	1.00^{c}			3.50^{a}		2.50^{b}	

*A, B, C=P<0.01 and a, b, c, d=P<0.05 within season, *P<0.05 and **P<0.01 within time;*
#normalized to bovine RPS9 expression; ¥Total Proteins.

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