Journal of Dairy Research (2005) **72** 129–137. © *Proprietors of Journal of Dairy Research 2005*
doi:10.1017/S0022029905000774 *Printed in the United Kingdom*

Fatty liver in dairy cows *post partum* is associated with decreased concentration of plasma triacylglycerols and decreased activity of lipoprotein lipase in adipocytes

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Received 21 January 2004 and accepted for publication 6 July 2004

Cholesterol and phospholipid concentrations in serum lipoproteins, plasma activities of lecithin:cholesterol acyltransferase (LCAT) and phospholipid transfer protein (PLTP) and lipoprotein lipase (LPL) activity in adipose tissue biopsies were measured *ante* and *post partum* in dairy cows given either free or restricted access to feed during the dry period. After parturition, all cows were fed *ad libitum*. The purpose of this study was to try to understand the earlier observed marked drop *post partum* in plasma triacylglycerol (TAG) in terms of lipoprotein metabolism in cows developing fatty liver *post partum*. As would be expected, free access to feed during the dry period induced a rise of hepatic TAG concentrations *post partum* associated with a decrease in plasma TAG levels. Total and free cholesterol concentrations in the VLDL, IDL, LDL and HDL_2 fractions fell immediately after parturition. VLDL and IDL cholesterol concentrations remained at a constant, low level during the entire sampling period *post partum*, whereas the drop in LDL and HDL_2 cholesterol *post partum* was followed by a rebound rise. Plasma LCAT and PLTP activities decreased by on average 19% and 33%, respectively, after parturition and then rose to values seen before parturition, but there was no effect of feeding regimen during the dry period. Activities of LCAT and PLTP were significantly correlated with cholesterol and phospholipid concentrations in LDL and HDL_2. Plasma LCAT activity, as measured with exogenous substrate, and PLTP activity were both positively correlated with HDL_3 phospholipid levels. LPL activity in adipose tissue dropped after parturition, the drop being smaller after feeding *ad libitum* during the dry period. It is concluded that the drop in adipose tissue LPL activity *post partum* is at variance with the simultaneous fall in plasma TAG. Possibly, the decrease in adipose tissue LPL activity helps to channel fatty acids away from adipose tissue into the udder. The *post-partum* changes in lipid transfer proteins in the blood are in line with the changes observed in the levels of the lipoproteins.

Keywords: Fatty liver, lipid transfer proteins, adipocyte lipoprotein lipase.

High-producing dairy cows go into negative energy balance (NEB) for several weeks after parturition because the extra energy requirement for milk production is not met by energy intake (Harrison et al. 1990; McNamara 1991). As a consequence of NEB, cows mobilize fatty acids from adipose tissue causing an increase in the *post-partum* circulating concentrations of non-esterified fatty acids (NEFA) (Grummer, 1993; Waltner et al. 1993; Zurek et al. 1995; Katoh 2002). Liver has a great capacity to remove NEFA from the plasma. In the liver, the NEFA are used for energy production, the formation of ketone bodies, or are converted into triacylglycerols (TAG) and then secreted with very-low density lipoproteins (VLDL). Excessive influx of NEFA into the liver can lead to accumulation of TAG in this tissue (Mohamed et al. 2004). Unrestricted access to feed in the dry period enhances the release of NEFA *post partum* and increases the risk of developing fatty liver because the enhanced formation of TAG easily exceeds the low capacity for VLDL secretion (Fronk et al. 1980; Reid et al. 1986; Herdt 1988; Johannsen et al. 1988).

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TAG concentrations in blood drop sharply after parturition and are lower in cows developing fatty liver *post partum* (Van den Top et al. 1996). This observation is as yet unexplained (Drackley et al. 2001). The liver is a major player in the metabolism of VLDL, the main carrier of TAG. Therefore, the observed changes in hepatic metabolism in cows with fatty liver (Van den Top et al. 1996; Rukkwamsuk et al. 1999; Murondoti et al. 2004) may affect TAG concentrations. The drop in TAG concentrations in the blood *post partum* may indicate low hepatic synthesis and/or secretion of VLDL. Apolipoprotein B100 (apo B100), which is essential for the synthesis and secretion of VLDL from the liver (Boren et al. 1990; Young 1990) and in dairy cattle is exclusively synthesized in the liver (Gruffat et al. 1996), could be limiting in this respect. Impairment of the synthesis of apo B100 could result in decreased VLDL formation leading to enhanced TAG accumulation in the liver (Marcos et al. 1990) and decreased TAG concentration in the blood. Alternatively, the markedly decreased TAG concentrations in plasma *post partum* in cows with the fatty liver syndrome could result from increased activity of lipoprotein lipase (LPL). Plasma TAG concentrations and post-heparin plasma LPL activities are inversely related in horses (Geelen et al. 1999) and in man (Zampelas et al. 1994) which is explained by the fact that LPL catalyses the hydrolysis of TAG in VLDL. If LPL activity is indeed stimulated in cows with fatty liver then there will be adaptations in lipoprotein metabolism. In man, an increase in LPL activity causes VLDL levels to decrease and HDL_2 levels to increase as well as the interconversion of HDL_2 in HDL_3 (De Vries et al. 2003; Stein & Stein, 2003). LPL-catalysed VLDL catabolism is associated with transfer of surface material to HDL (Puppione, 1978). The activities of lecithin:cholesterol acyltransferase (LCAT), cholesteryl ester transfer protein (CETP) and phospholipid transfer protein (PLTP) are involved in HDL metabolism (Ha & Barter, 1982; Speijer et al. 1991; De Vries et al. 2003). It could be hypothesized that the marked drop *post partum* in plasma TAG concentration associated with fatty liver results from increased activity of LPL and that this in turn will increase HDL_2 and HDL_3 levels and affect the activities of LCAT, CETP and PLTP.

The objective of the present study was to reveal the basis for the marked *post-partum* drop in plasma TAG in cows developing fatty liver *post partum*. Therefore, we determined the levels of VLDL and HDL and the activity of LPL in post-parturient dairy cows with concentrations of hepatic TAG similar to those observed in patients with fatty liver syndrome, i.e., exceeding 15% on a wet weight basis (Herdt et al. 1983). To detect any differences in lipoprotein metabolism secondary to a possible increase in LPL activity, we also determined the activities of LCAT, CETP and PLTP. It was anticipated that the results thus obtained would provide further clues as to mechanisms underlying the development of fatty liver and altered lipoprotein metabolism in cows overfed during the dry period.

Materials and Methods

Cows and diets

The experimental design was approved by the animal experiments committee of the Utrecht Faculty of Veterinary Medicine. We used 18 pregnant, multiparous Holstein Friesian × Friesian Holland cows aged 4·8±0·17 (mean± SEM) years and with 305-d milk production during the previous lactation period of 8888±457. Average body weight at 84 d before parturition was 646±12·5 kg. Body weight was recorded between 8·00 and 9·00 by weighing on a scale. Animals were housed in a stanchion barn with wheat straw as bedding. The feeding regimen of the experimental group during the dry period was designed to induce excess body fat at calving and fatty liver *post partum*. They were fed individually and had free access to tap water. All cows were fed grass silage and a mixed ration of corn silage and concentrates throughout (Van den Top et al. 1996). Cows were randomly allocated to two groups: 8 cows were assigned to the control group and 10 cows to the experimental group. During the dry period, control cows were fed at the energy level indicated by the National Research Council (2001) and the experimental cows were given free access to feed. From 1 week before the expected calving date, control cows were also given free access to feed in that period. Thus, after parturition all cows had free access to feed and also received daily, 1 kg of solvent extracted rapeseed meal per 10 kg dry matter. The diet has been described before (Van den Top et al. 1996). All cows were offered the same total mixed ration both before and after parturition. Feed intake was measured as the amount of feed offered minus the recorded refusals at 8·00. Milk production was recorded daily. Experimental treatments lasted for the entire dry period, which was about 80 d. The experiment finished at 12 weeks *post partum*.

Sampling procedure

Liver samples were collected at 0·5 week *ante partum* (4±0·7 d) and at 0·5, 1, 1·5, 2, 3, 5, 8 and 12 weeks *post partum*. Liver biopsies were obtained as described by Van den Top et al. (1996), snap frozen in liquid nitrogen and stored at −70 °C until analysis for TAG. Blood samples were collected at 4 weeks *ante partum* and at 0·5, 1, 5 and 12 weeks *post partum*. Blood samples were taken at 10·00 and liver samples at 12·00. Blood was collected from the tail vein in evacuated tubes containing K-EDTA (47 μmol per 10 ml blood) or no additive. Plasma samples were stored at −70 °C until analysed for concentrations of TAG, NEFA and β-hydroxybutyric acid (BHBA) and activities of LCAT, PLTP and CETP. Serum samples were stored at 4 °C for up to 3 d and analysed for lipoproteins. Subcutaneous adipose tissue samples were collected from the thoracic wall, caudal from the scapula at 4 weeks *ante partum* and at 1, 5 and 12 weeks *post partum*. After induction of local anaesthesia (2% Lidocaine-HCl) an incision was made.

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A sample of the adipose tissue was removed, immediately frozen in liquid nitrogen and stored at −70 °C until analysed for lipoprotein lipase activity.

Assay procedures

Serum lipoproteins were isolated by density gradient ultracentrifugation (Van den Top et al. 1995) at the following densities (d, g/ml): VLDL, d<1·006, IDL, 1·006<d<1·019, LDL, 1·019<d<1·063, HDL_2, 1·063<d<1·125, HDL_3, 1·125<d<1·210. Lipoprotein total (Tietz, 1995) and free cholesterol (Stähler et al. 1977) and phospholipids (Takayama et al. 1977) were determined enzymically with commercial kits. Lipids were extracted from liver tissue by the method of Folch et al. (1957). Concentrations of TAG (Tietz, 1995), NEFA (Henry, 1974) and BHBA (McMurray et al. 1984) were measured enzymically with commercial kits. Activities in plasma of LCAT (LCATexo), PLTP and CETP were determined using exogenous substrates exactly as described by Meijer et al. (1993). LCAT activity with endogenous plasma lipoproteins as substrate (LCATendo) was also determined (Channon et al. 1990). LPL activity in adipose tissue biopsies was determined after extraction with acetone (Taskinen et al. 1980).

Statistical analyses

Owing to clinical complications there were missing values. *Ante-partum* data of seven control and ten experimental cows were used for statistical analyses; for the *post-partum* period, there were data from seven control and eight experimental cows (Van den Top et al. 1996). All statistical analyses were performed using a SYSTAT computer program (Wilkinson, 1990). To disclose diet effects, *post-partum* measurements were subjected to repeated measures; the model includes a constant and treatment as factors. *Pre-partum* measurements were evaluated with Student's *t* test. The level of statistical significance was pre-set at $P<0·05$ with the use of the SYSTAT computer program. Pearson-correlation coefficients and regression coefficients were calculated between serum lipids and activities of LCAT, PLTP or LPL. Student's *t* test for paired data was used to identify selected changes over time for the various variables.

Results

Performance

Daily dry matter intake in the period 10–14 weeks *ante partum* was 20·6±0·4 kg for the experimental group with free access to feed and 7 kg for the control group given a restricted amount of feed (Van den Top et al. 1996). During the 2 weeks just before calving, the intake of the experimental cows was 17·5±1·5 kg/d. Average dry matter intake in the experimental group was 15·5±2·9 kg/d during the first 0·5 week after parturition; in the control group it was 17·4±2·4 kg/d. In the period of 0–4 weeks after parturition, average daily dry matter intake was 22·0±0·8 kg in the experimental group and also 22·0±1·4 kg in the control group. Milk yields were not significantly different between treatments. Average milk yields in the first week of lactation were 30·6±2·0 kg/d and 30·7±2·5 kg/d for the control and experimental groups, respectively. For weeks 2–12 of lactation, milk yields were 37·0±1·1 kg/d for the experimental group and 38·1±1·5 kg/d for the control group. During the dry period, body weight gain in the control group was 112±15 kg and in the experimental group 152±16 kg, but the difference did not reach statistical significance. On the other hand, body weight loss after parturition, i.e., the difference in body weight measured immediately *post partum* and that at 4 weeks *post partum*, was significantly greater for experimental cows than for control cows (114±11 *v.* 79±9 kg).

TAG, NEFA and BHBA in plasma

Before parturition, neither plasma NEFA nor BHBA concentrations differed between the two groups (Van den Top et al. 1996). NEFA and BHBA concentrations rose after calving (NEFA: week 0·5 *v.* week −0·5, $P<0·01$; BHBA: week 0·5 *v.* week −0·5, $P<0·05$), the rise being greater and lasting longer in the experimental group than in the control group (Table 1). Mean plasma TAG concentrations were not different between the two groups before parturition. Following calving the concentrations dropped significantly in both groups (week 0·5 *v.* week −4, $P<0·01$) and remained lower throughout the experimental period. Again, plasma concentrations of TAG did not differ between the two groups (Table 2).

TAG in liver

At 0·5 week before parturition, liver TAG levels were similar in the two groups of cows (Table 1). Consistent with previous observations (Grummer et al. 1990; Harrison et al. 1990; McNamara 1991; Bertics et al. 1992), the mean concentrations in the two groups rose sharply at 0·5 week after parturition (week 0·5 *v.* week −0·5, $P<0·01$) and remained higher throughout the *post-partum* sampling period. The rise was markedly greater in the experimental cows than in the control cows (week 0·5, $P<0·05$).

Lipoprotein cholesterol

Total and free cholesterol concentrations in serum lipoproteins are presented in Table 2. At 4 weeks before parturition, total and free cholesterol in VLDL and IDL were significantly lower in the experimental group than in the control (week −4, $P<0·05$). Conversely, in the experimental group *ante partum* total cholesterol was higher in the HDL_2 and HDL_3 fractions (week −4, $P<0·05$). At 0·5 week after calving, total and free cholesterol concentrations in all lipoprotein fractions had fallen significantly in both

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Table 1. Concentrations of hepatic triacylglycerols (TAG), plasma non-esterified fatty acids (NEFA) and 3-hydroxybutyrate (BHBA), measured before and after parturition in control cows and experimental cows

Values are group means for 9 sampling times with pooled SEM

	Week from parturition									Pooled SEM	*P* value†
	−0·5	0·5	1	1·5	2	3	5	8	12		
					TAG, mg/g tissue						
Control	17·9	48·0	59·6	65·3	69·0	67·9	38·3	23·4	16·1	26·70	<0·001
Experimental	21·3	90·0	127·8	151·5	172·4	164·1	123·2	62·8	23·8		
					NEFA, mmol/l						
Control	0·28	0·72	0·82	0·59	0·62	0·55	0·51	0·39	0·25	0·089	0·001
Experimental	0·39	0·98	1·25	1·58	0·98	1·33	0·82	0·68	0·33		
					BHBA, mmol/l						
Control	0·48	0·83	1·13	0·82	0·60	0·76	0·63	0·46	0·39	0·462	0·090
Experimental	0·54	0·85	1·13	1·14	1·46	1·31	1·88	0·88	0·36		

† Based on repeated measurements analysis

Table 2. Serum concentrations (μmol/l) of triacylglycerols (TAG) and concentrations of total cholesterol (chol), free cholesterol (chol) and phospholipids (PL) in serum lipoproteins measured before and after parturition in control cows and experimental cows

Values are group means for 5 sampling times with pooled SEM

	Week from parturition											
	−4		0·5		1		5		12		Pooled SEM	*P* value†
	Control	Exp.	Control	Exp.	Control	Exp.	Control	Exp.	Control	Exp.		
Total chol												
VLDL	34·5	15·3	5·6	3·8	5·4	4·4	6·7	4·4	6·4	3·3	1·45	0·030
IDL	24·9	10·3	5·3	3·1	7·0	2·7	7·1	4·3	4·5	4·4	2·69	0·257
LDL	849	631	302	215	240	241	1011	1124	1543	1215	202·3	0·608
HDL_2	1216	1592	1063	1034	1243	1186	2209	1878	2540	2194	109·1	0·028
HDL_3	74	101	88	88	87	72	133	149	184	156	16·4	0·581
Free chol												
VLDL	30·5	12·8	2·1	3·9	2·2	7·0	5·6	4·2	4·8	4·5	5·10	
IDL	19·9	9·4	2·2	3·1	3·0	2·3	1·8	3·2	3·5	2·2	2·49	
LDL	215	143	80	50	66	63	255	284	366	327	40·7	
HDL_2	249	318	197	193	251	214	425	344	475	430	5·1	0·027
HDL_3	12·4	15·4	13·8	11·2	11·9	9·4	21·7	26·0	26·8	24·8	3·12	0·0274
PL												
VLDL	29·0	10·4	2·3	3·7	5·1	3·0	4·0	3·1	6·3	4·3	1·30	0·191
IDL	20·6	10·1	3·7	3·7	3·3	3·9	3·6	2·4	4·0	4·9	0·61	
LDL	271	236	145	110	104	119	473	446	666	571	101·5	0·716
HDL_2	608	802	596	585	712	632	1076	923	1197	1082	46·1	0·010
HDL_3	38	58	49	52	41	41	60	74	96	84	8·7	0·845
Total TAG in blood	196	146	51	43	59	47	68	51	71	64	6·5	0·038

† Based on repeated measurements analysis. (Where no *P* value is given, there were no more than 3 animals for which the data were complete)

groups (week 0·5 *v.* week −4, $P<0·05$) except in HDL_3, which remained rather stable around calving. Total and free cholesterol concentrations in VLDL and IDL remained lower throughout the sampling period. After parturition, total cholesterol in VLDL and IDL was consistently lower in the experimental group than in the controls. From 5 to 12 weeks after parturition, HDL_2 total cholesterol also was lower in the experimental group. From 1 to 12 weeks after calving, total and free cholesterol concentrations in the LDL, HDL_2 and HDL_3 fractions increased significantly (week 12 *v.* week 1, $P<0·05$).

Lipoprotein phospholipids

At 4 weeks before calving, phospholipid concentrations in VLDL and IDL were significantly lower (week −4,

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Table 3. Plasma activities of lecithin:cholesterol acyltransferase and phospholipid transfer protein and adipose tissue lipoprotein lipase, measured before and after parturition in control cows and experimental cows

Values are group means for 5 sampling times with pooled SEM

	Week from parturition					Pooled	
	−4	0·5	1	5	12	SEM	*P* value†
	Lecithin:cholesterol acyltransferase, nmol/h per ml (*exogenous substrate*)						
Control	40·6	28·6	32·4	42·7	42·6	5·81	0·556
Experimental	38·7	31·1	32·4	46·6	43·4		
	Lecithin:cholesterol acyltransferase, nmol/h per ml (*endogenous substrate*)						
Control	13·3	11·9	12·0	13·1	13·4	1·76	0·524
Experimental	11·9	10·0	10·5	13·1	12·4		
	Phospholipid transfer protein, nmol/h per ml						
Control	4651	2967	2538	3724	3909	387·3	0·470
Experimental	4271	3046	2875	3839	3768		
	Adipose lipoprotein lipase, mU/g wet weight						
Control	81·8	—	5·5	6·5	7·0	1·24	0·017
Experimental	103·6	—	8·0	9·7	9·9		

† Based on repeated measurements analysis

$P<0·01$), and those in HDL_2 and HDL_3 were significantly higher (week −4, $P<0·01$) in the experimental group than in the control group (Table 2). At 0·5 week after calving, phospholipid concentrations in VLDL and IDL had dropped significantly in both groups (week 0·5 *v.* week −4, $P<0·05$). This lower level was maintained throughout the 12-week sampling period. Moreover, the phospholipid concentrations in LDL had dropped at 0·5 week *post partum* in the two groups of animals (week 0·5 *v.* week −4, $P<0·05$). Between 1 and 5 weeks after parturition there was a rebound rise in the phospholipid concentration of LDL in both groups (week 5 *v.* week −4, $P<0·05$). After calving, phospholipid concentrations in the HDL_2 fraction dropped in the experimental cows (week 0·5, $P<0·001$), but not in the control cows. Between 0·5 and 12 weeks *post partum*, phospholipid concentrations in the HDL_2 and HDL_3 fractions increased significantly in both groups (week 12 *v.* week −4, $P<0·01$) (Table 2).

Plasma LCAT, PLTL and CETP activities

The activity of plasma LCAT is shown in Table 3. Before parturition, plasma LCAT activity was not different between the two groups. When compared with values at 4 weeks before calving, the activity of LCAT (as measured with exogenous substrate) had dropped significantly by 0·5 week after calving (week 0·5 *v.* week −4, $P<0·05$). There was a rebound rise *post partum* in LCAT activity as measured with exogenous substrate, i.e., between 1 and 5 weeks *post partum* the LCAT activity rose significantly to values seen *ante partum*. Moreover, after calving there was no significant effect of *ante-partum* feeding regimen. With endogenous substrate there was a similar pattern for LCAT activity, but the differences were not statistically significant. Plasma CETP activities were not detectable (<3% of the activity in a human reference plasma sample). Plasma PLTP activity had decreased significantly at 0·5 and 1 week after calving (week 1 *v.* week −4, $P<0·01$) and then increased to a stable level. Activities of plasma PLTP before and after parturition were not different for the two groups of cows (Table 3). Activities of LCATexo (as measured with exogenous substrate), LCATendo (as measured with endogenous substrate) and PLTP were significantly correlated with LDLtotal cholesterol (LCATexo: $r=0·644$, $P<0·001$, $n=73$; LCATendo: $r=0·452$, $P<0·001$, $n=73$; PLPT: $r=0·415$, $P<0·001$, $n=73$) (Fig. 1A), LDLfree cholesterol (LCATexo: $r=0·637$, $P<0·001$, $n=76$; LCATendo: $r=0·419$, $P<0·001$, $n=76$; PLPT: $r=0·433$, $P<0·001$, $n=76$) (Fig. 1B) and phospholipid concentrations (LCATexo: $r=0·625$, $P<0·001$, $n=72$; LCATendo: $r=0·422$, $P<0·001$, $n=72$; PLPT: $r=0·415$, $P<0·001$, $n=72$) (Fig. 1C). LCAT and PLTP activities showed a positive, significant correlation (LCATendo: $r=0·274$, $P<0·014$, $n=79$; LCATexo: $r=0·303$, $P<0·007$, $n=79$) (Fig. 1D). Total cholesterol in HDL_2 ($r=0·535$, $P<0·001$, $n=73$) and in HDL_3 ($r=0·047$, $P<0·69$, $n=73$) both showed a positive correlation with LCATexo activity. Plasma LCATendo activity was significantly correlated with HDL_3 phospholipid levels ($r=0·326$, $P<0·005$, $n=73$). PLTP activity and HDL_3 phospholipids were significantly correlated ($r=0·261$, $P<0·026$, $n=73$).

Adipose tissue lipoprotein lipase

Before parturition, LPL activity did not differ between the two groups. It fell to very low values after calving (week 1 *v.* week −4, $P<0·01$) (Table 3). During the entire *post-partum* period under study, LPL activity remained at the low level. LPL activity *post partum* was significantly higher in the experimental group than in the control group, the difference on average being 45%. Adipose tissue LPL

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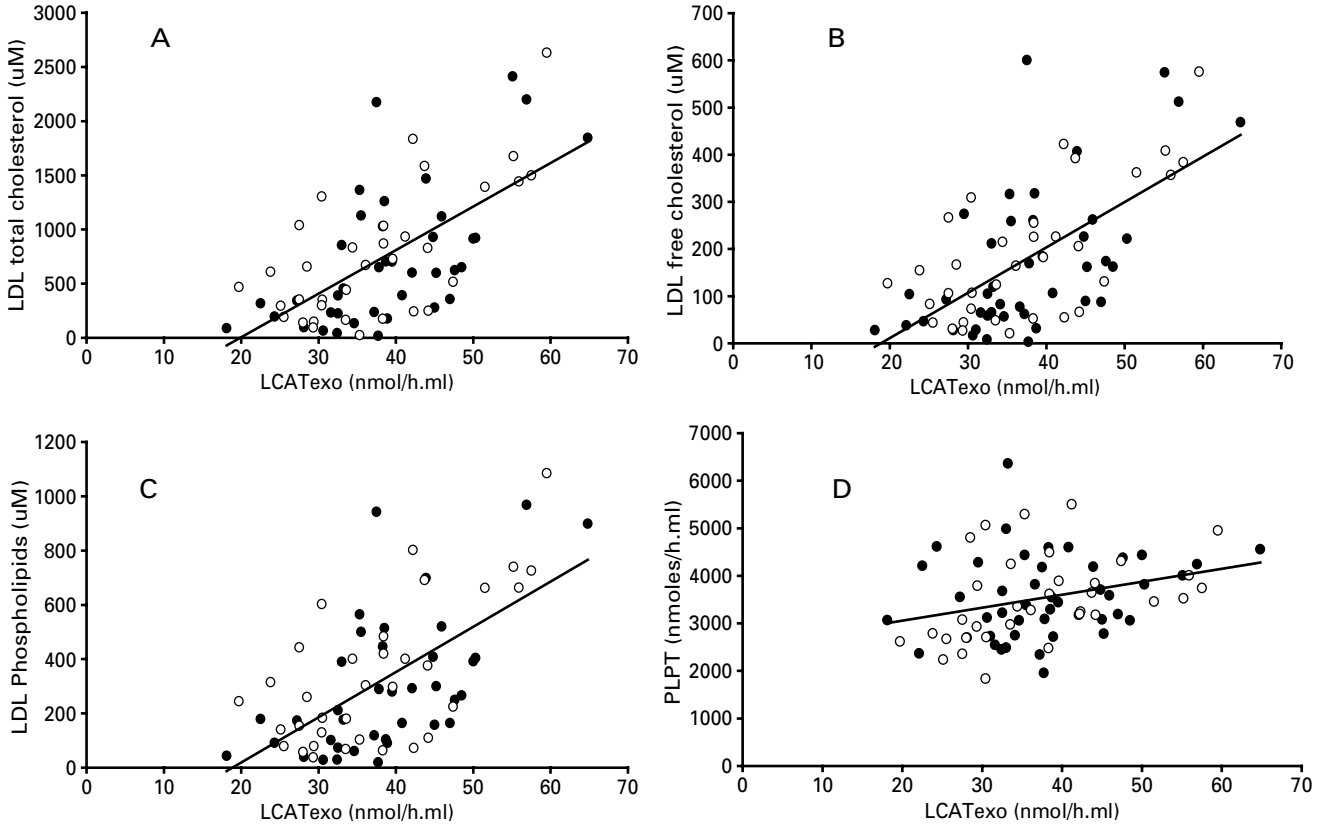

Fig. 1. Relationship between *ante-partum* and *post-partum* individual plasma activities of lecithin:cholesterol acyltransferase (LCAT), as measured with exogenous substrate, and low density lipoprotein (LDL) total cholesterol (A), LDL free cholesterol (B), LDL phospholipids (C) concentrations and phospholipid transfer protein (PLTP) activity. The linear correlation coefficients and regression formulas were: panel A, r=0·644, P<0·001, y=40·2x−796·9 (n=73); panel B, r=0·637, P<0·001, y=9·6x−181·6 (n=6); panel C, r=0·625, P<0·001, y=16·7x−314·4 (n=72); panel D, r=0·303, P<0·007, y=27·3x+2513·1 (n=79). Dependence of the values was not taken into account. Symbols: ○, control diet; ●, experimental diet.

activity and VLDL total (r=0·566, P<0·001, n=53) and free cholesterol (r=0·560, P<0·001, n=39) or VLDL phospholipids (r=0·464, P<0·001, n=50) were all positively correlated. LPL was negatively correlated with HDL_2 total cholesterol (r=−0·300, P<0·031, n=52) and phospholipids (r=−0·340, P<0·015, n=51).

Discussion

As would be expected (Fronk et al. 1980; Reid et al. 1986; Johannsen et al. 1988; Van den Top et al. 1995; Rukkwamsuk et al. 1998; Murondoti et al. 2004), the cows with unrestricted instead of restricted access to feed in the dry period accumulated markedly greater amounts of TAG in the liver after parturition. Clearly, fatty liver development *post partum* can be counteracted by restricted feeding during the dry period. However, the composition of the ration fed during the periparturient period influences hepatic fatty acid metabolism and thereby could control TAG accumulation. Supplemental fat may increase hepatic fatty acid oxidation relative to fatty acid synthesis (Grum et al. 1996) while oxidation may be negatively correlated with TAG in liver (Grum et al. 2002). Both fatty acid oxidation and esterification are stimulated and depressed, respectively, by glucagon (Geelen et al. 1980) while subcutaneous injection with this hormone decreases hepatic TAG concentrations of dairy cows early in lactation (Bobe et al. 2003). The degree of fatty liver in the overfed cows was of the same order of magnitude as that seen in practice in patients with *post-partum* fatty liver syndrome (Herdt et al. 1983). TAG concentrations in blood dropped sharply after parturition and were lower in cows that developed fatty liver *post partum* (Table 2 and Van den Top et al. 1996). The extra drop *post partum* in TAG concentrations in the blood of cows with the fatty liver syndrome could relate to low hepatic synthesis of VLDL (Murondoti et al. 2004). Alternatively, the decrease in TAG concentrations could result from an increased activity of LPL which in turn would enhance HDL_2 and HDL_3 as well as the activities of LCAT, CETP and PLTP.

The decrease in VLDL-TAG *post partum* is probably due to increased VLDL catabolism by the udder (Moore & Christie, 1981) in combination with unaltered VLDL

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secretion by the liver (Herdt et al. 1988). Thus, the liver takes up large amounts of NEFA after parturition while the export capacity remains unaltered. In cows with unrestricted instead of restricted access to feed during the dry period the activity of 3-hydroxy-acyl-CoA dehydrogenase was reduced *post partum* (Murondoti et al. 2004), indicating a decrease in fatty acid oxidation. It follows that most incoming fatty acids are esterified and eventually accumulate as TAG. Because liver TAG concentrations did not increase further after 2 weeks *post partum*, an equilibrium must have been attained between NEFA input and VLDL output. Because of the increased milk production, VLDL turnover rises after parturition (Raphael et al. 1973). VLDL lipid concentration remained more or less constant after 0·5 week *post partum*, but an increased VLDL turnover would be mirrored by increasing concentrations of HDL lipids because VLDL catabolism is associated with transfer of surface material to HDL (Puppione, 1978). Indeed, in our study, concentrations of HDL lipids gradually increased after 0·5 week *post partum*, which is consistent with increased VLDL turnover after parturition.

Plasma LCAT and PLTP activity levels, as measured with exogenous substrate, showed a decrease after parturition followed by an increase to values seen before parturition, which was also reported by Poso et al. (2000). In contrast to our cows, LCAT activities reported for cows with spontaneous fatty liver (Nakagawa et al. 1997) and ketotic cows (Nakagawa & Katoh, 1998) were markedly lower when compared with healthy cows (Uchida et al. 1995; Nakagawa-Ueta & Katoh, 2000). However, those low values in affected cows were much higher than the values in our cows fed restrictedly during the dry period. Moreover, in our experiment, the decrease in LCAT activity in the cows developing fatty liver were smaller than those in ethionine-induced fatty liver (Uchida et al. 1995). The reasons for these differences are not known, but may relate to differences in experimental protocol, diet and methodology for determining LCAT activities.

As shown in Fig. 1, activities of LCAT and PLTP were correlated with LDL total cholesterol. LCAT may directly produce cholesteryl esters in LDL (Knipping et al. 1986) yielding lighter LDL particles (Fig. 2) whereas cholesteryl esters produced by LCAT in HDL_2 or HDL_3 cannot be transferred to LDL because the cows were found to lack CETP activity in plasma. Thus, the correlation between LCAT activity and LDL cholesterol, which essentially represents cholesteryl esters, could reflect an enzyme-product relationship. The positive correlation between PLTP activity and LDL phospholipids (Fig. 1) could imply that PLTP is involved in the transfer of phospholipids to LDL (Fig. 2).

The strong, significant positive relationship between LCAT activity and HDL_2 total cholesterol agrees with the notion that HDL_2 is produced in the LCAT reaction from HDL_3 (Eisenberg, 1984) (Fig. 2). LCAT may also produce HDL_3 (Fig. 2), but the weak correlation between these two variables indicates that, apart from the conversion of

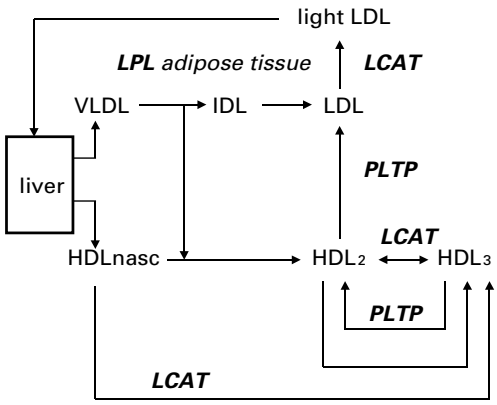

Fig. 2. Possible pathways for the conversion of serum lipoproteins in the cow as based in part on Eisenberg (1984), Knipping et al. (1986) and Jauhiainen et al. (1993). In cows, serum CETP (present study) and hepatic LPL (Emery et al. 1992) are essentially absent. LDL,low density lipoprotein; VLDL, very-low density lipoprotein; LPL, lipoprotein lipase; IDL, intermediate-density lipoprotein; PL, phospholipid; CH, cholesterol; CE, cholesteryl ester; LCAT, lecithin : cholesterol acyltransferase; HDL, high-density liprotein; nasc, nascent; PLTP, phospholipid transfer protein.

nascent HDL into HDL_3 in plasma (Fig. 2), the rate of HDL_3 metabolism (e.g., removal from plasma and conversion into HDL_2) also are determinants of the concentration of HDL_3 cholesterol.

The significant correlation of plasma LCATendo activity and HDL_3 phospholipids could simply point to a physical association between LCAT and HDL_3. Alternatively, LCAT activity might be subject to feed-forward control in that HDL_3 phospholipids serve as acyl donor for the LCAT reaction. The physiological function of plasma PLTP is still uncertain, but a possible function (Tall, 1986) is that of transferring phospholipid-rich surface fragments to HDL_3 and thus providing substrate for LCAT. The correlation of PLTP activity and HDL_3 phospholipid levels substantiates the putative role of PLTP. Another putative function of PLTP is in the interconversion of HDL subfractions (Jauhiainen et al. 1993). The positive correlation between PLTP and LCAT activities (Fig. 1) could indicate that these plasma proteins act in concert, but would also corroborate a physical association of the two enzymes with HDL. The mechanism(s) responsible for the reduction of LCAT activity in *post-partum* cows is probably multiple. One explanation may be reduction in hepatic synthesis or secretion of LCAT. Decreased concentrations of substrates for LCAT (free cholesterol and phosphatidylcholine) or of apo A-I (activator of LCAT) by reduced secretion of HDL (Table 2) may also be involved.

Adipose tissue LPL hydrolyses core TAG of VLDL while surface fragments produced are transferred to HDL_2 (Eisenberg, 1984), which could result in HDL_2 levels and LPL activities being positively correlated and VLDL levels and LPL activities being negatively correlated. However, this idea is not supported by the present results. So, here

https://doi.org/10.1017/S0022029905000774 Published online by Cambridge University Press

multiple factors, such as clearance etc, may also be involved. LPL activity dropped markedly after parturition, in accordance with findings from other studies (Shirley et al. 1973; McNamara, 1991). At the same time, there was a pronounced fall of serum TAG and of VLDL cholesterol and phospholipids. The apparently minor role of adipose tissue LPL in determining VLDL levels can be explained by an increase in mammary LPL activity which occurs around parturition (Vernon, 1981). The drop *post partum* in adipose tissue LPL then helps to shuttle more VLDL-TAG towards the udder. A rise in mammary LPL may also explain the increase in HDL cholesterol and phospholipids *post partum*.

Feeding *ad libitum* instead of at a restricted level during the dry period influenced the levels of lipoprotein cholesterol and phospholipids both before and after parturition. *Ante-partum* levels of phospholipids, total and free cholesterol were lower in VLDL and IDL and higher in HDL_2 and HDL_3 in the experimental group than in the control. *Post-partum* VLDL and HDL_2 total cholesterol and HDL_2 phospholipids were all lower in the experimental group that was fed *ad libitum* before parturition. This group difference was not associated with group differences in LCAT or PLTP activities. However, the experimental group had higher *post-partum* adipose tissue LPL activities which agrees with its lower VLDL cholesterol levels, but not with its lower HDL_2 cholesterol and phospholipid concentrations. The results should be interpreted with caution because any group differences in LPL activities in tissues other than adipose tissue cannot be excluded. Moreover, it is not known which part of the measured LPL activity is either functional, endothelial LPL or non-functional, intracellular LPL.

Compared with control cows, experimental cows showed a greater *post-partum* increase in the amount of hepatic TAG. A plausible explanation for this phenomenon would be the greater NEB *post partum* in the experimental cows, due to a depression of appetite (Lodge et al. 1975; Hayirli et al. 2002), which causes a greater influx of fatty acids into the liver (Van den Top et al. 1996). However, in the present study, feed intake during the first 0·5 week after parturition by the overfed cows was only 11% lower than in the control cows while milk production was not increased. Thus, there was no major difference in NEB between experimental and control cows, but plasma NEFA concentrations *post partum* were much higher in the former cows. The high NEFA concentrations may relate to the greater adipose tissue mass in the experimental cows (Murondoti et al. 2004) and would cause a high influx of fatty acids into the liver. Apart from the *post-partum* development of fatty liver, changes in lipoprotein metabolism in experimental cows occur which cannot yet be interpreted unambiguously. In any event, the periparturient dairy cow appears to be an interesting animal model for studies on the regulation of lipid metabolism.

It is concluded that the *post-partum* drop in adipose LPL activity is at variance with the simultaneous fall in plasma TAG. Possibly, the decrease in adipose tissue LPL activity helps to channel fatty acids away from adipose tissue into the udder. The *post-partum* changes in lipid transfer proteins in the blood are in line with the changes observed in the levels of the lipoproteins.

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