Studies on the Pathogenesis of Fatty Liver in Cows

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Introduction

Increased fat mobilization in early lactation in cows is described by many authors.[1,8,9] Energy deficit in connection with the start of milk secretion is considered to be of importance as cause of the lipolysis. One consequence of increased mobilization of body fat can be development of a fatty liver.[7]

In an in vitro of lipid metabolism in bovine adipose tissue McNamara and Hillers[6] have shown that there is an adaptation in prepartum. There is a strong reduction in lipogenesis and esterification and increase in norepinephrine—and epinephrine—stimulated free fatty acid release. The lipoprotein lipase activity in adipose tissue decreases and increases in the mammary gland. This metabolic shift precedes the energy demand for lactation and is stimulated by prolactin and inhibited by progesterone. At two months postpartum there is a dramatic rebound in lipogenesis and esterification.

Besides the increased transport of fatty acids to the liver near partus, decreased removal of fat from the liver can be of importance in the development of fatty liver. The aim of the present investigation was to elucidate the pathogenesis of fatty liver in cows by analysing serum lipoproteins and plasma lipids in normal cows round partus on different prepartum feeding and in cows with fatty liver.

Materials and Methods

The study was done on 17 experimental cows at the department of clinical nutrition and the department of cattle and sheep diseases. All were of the Swedish Red and White Breed, multiparous and with an average milk yield of 7900 kg FCM/year. Besides these animals 20 cows with fatty liver were examined, almost all of them had been sent to the clinic for treatment of abomasal displacement. These cows had calved 3–5 weeks before the examination.

The experiment with different feeding before calving was done on 12 cows divided in three groups, one with low energy-low protein given only grass silage and hay, one high energy-low protein given hay and increasing amount of grain and one high energy-high protein given grass silage; hay and concentrates. The amount of metabolizable energy and digestible crude protein at calving appears

TABLE 1. Influence of feeding during the dry period on plasma lipids at calving.

Feeding	Low energy Low protein	High energy Low protein	High energy High protein
Number of cows	4	4	4
Metabolizable energy MJ	70	120	130
Digestible crude prot.g	840	950	1240
Protein/Energy	12	8	10
FFA mmol/l	0.88±0.29	0.74±0.12	0.49±0.14
Cholesterol mmol/1	2.31±0.05	1.66±0.18	2.38±0.03
Phospholipid mmol/1	1.13±0.10	0.93±0.19	1.42±0.11
FFA/cholesterol	0.38	0.46	0.20
Fatty liver score	1.2	3.0	0.5
Range	0-2	2-3.5	0-1

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from Table 1.

Blood samples were taken in the jugular vein at 1 p.m. every second week from four weeks before to six weeks after calving. A sample was also taken the day after calving. In the cows with fatty samples were taken the day after arrival at the clinic.

Liver biopsies according to the fine needle technique[3] were taken the day after calving and then 3-4 weeks later in the experimental cows and within one day after arrival to the clinic in the patients. The degree of fatty change was designated according to the earlier described key.[4] As fatty liver was designated livers that had such degree of fat infiltration that there also occurred degenerative nuclear changes, thus at least degree 3 according to the key.[4] As normal were designated livers which had not more than degree 2.

Samples for analysis of free fatty acids, cholesterol and phospholipids were taken with EDTA as anticoagulant and after centrifugation plasma was stored at -20°C until analysis. Serum for lipoprotein analysis was separated by centrifugation and then chilled to 5°C and sent in cold-storage bag to the laboratory.

Free fatty acids, cholesterol and phospholipids were determined by commercial enzymatic kits, NEFA C-test (Wako Industries Osaka, Japan) and for cholesterol and phospholipids (Boehringer Mannheim Diagnostica). The serum lipoproteins were quantified by Dr. L.A. Carlson, Stockholm as described.[2]

Results

The plasma lipids at calving in the cows on different feeding during the dry period are presented in Table 1. As can be seen in the table the cows on high energy-low protein differed from the animals in the other two groups. The cholesterol level was significant lower ($p<0.05$) than in both the other groups. Also the phospholipid level was lower than in the other cows. The difference was significant, ($p<0.05$) between this animal's and the high energy-high protein group but not significant between the low energy-low protein group. There was also higher degree of fatty change in the liver cells in the high energy-low protein fed cows.

The triglycerides in serum and in the different lipoprotein fractions are presented in Table 2. There was a significant ($p<0.01$) drop of the triglycerides in serum and in all lipoprotein fractions at calving. During the following four weeks there was a significant rise in serum ($p<0.01$) and in the LDL and HDL-fractions.

The cows with fatty liver had lower amounts of triglycerides in the serum and in the LDL and HDL fractions than the normal cows four weeks after calving. The amounts were almost exactly the same as in the normal cows at calving.

TABLE 2. Triglycerides in serum and in lipoproteins in normal cows and in cows with moderate fatty liver.

			Triglycerides mmol/l			
Cows	No	Time from Partus	serum	VLDL	LDL	HDL
	5	2 weeks a.p.	0.056±.16	0.20±.07	0.24±.10	0.10±.03
Normal	5	within 24 hours p.p.	0.12±.03	0.06±.03	0.03±.02	0.06±.01
	8	4 weeks p.p.	0.27±.04	0.06±.03	0.10±.03	0.13±.05
Fatty liver	6	3-5 weeks p.p.	0.12±.03	0.05±.03	0.02±.01	0.05±.03

Table 3 shows the amounts of cholesterol in serum and in the different lipoprotein fractions. There was a drop at calving and a rise thereafter in normal cows. The animals with fatty liver had lower level in serum and in the LDL and HDL-fractions. The difference in serum and in the HDL-fractions was significant, $p<0.05$. In all cows more than 80 percent of the cholesterol was in the HDL-fraction.

Plasma lipids in normal cows and in cows with fatty liver are presented in Table 4. The latter differed very much from the normal ones. They had higher FFA-levels, lower cholesterol and phospholipids and higher FFA/cholesterol quotient.

Discussion

The increased lipolysis round partus is hormonally regulated and not primary an expression for energy deficit. The liver is an important site for removal of free fatty acids (FFA) from circulating blood plasma.[1] After esterification to triglycerides they are transported from the liver

TABLE 3. Cholesterol in serum and in lipoproteins in normal cows and in cows with moderate fatty liver.

Cows	No	Time from partus	Cholesterol mmol/l			
			serum	VLDL	LDL	HDL
Normal	5	2 weeks a.p.	2.39±.15	0.06±.04	0.41±.24 17%	1.96±.14 82%
	5	within 24 hours p.p.	2.04±.41	0.01±.01	0.13±.10 5%	1.95±.33 95%
	8	4 weeks p.p.	3.55±.78	0.01±.01	0.53±.41 14%	3.08±.87 86%
Fatty	6	3-5 weeks p.p.	2.32±.83	0.03±.03	0.28±.13 12%	1.95±.77 84%

in very low density lipoproteins (VLDL). In increased lipolysis due to energy deficit the fatty acids are oxidized to ketone bodies in the liver cells and then removed.

The strong reduction of triglycerides in serum and in the lipoprotein fractions at calving (Table 2) is connected with the start of milk secretion. The triglycerides have a rapid turn over rate and the concentration in serum is therefore relatively small. Four weeks after calving the level had raised in the normal cows which indicates increased lipoprotein production in the liver. But in the cows with fatty liver the level was low, probably depending on reduced lipoprotein production in the liver.

Even if the feed contains almost no cholesterol, this lipid is the dominating in serum and more than 80 percent of it is in the HDL-fraction (Table 3). The cholesterol must emanate from synthesis in the liver from acetate. It is known if there is a secretion of HDL from the liver in cattle. Probably the large amount of cholesterol in HDL represents an important fat metabolism and after removal of triglycerides the main part of cholesterol is on way back to the liver for excretion in the bile. However, cholesterol is needed for a lot of functions and Koper et al.[5] have shown by in vitro studies with cells from bovine adrenal cortex that both LDL and HDL from bovine blood stimulated steroidogenesis but HDL was approximately 3-fold more active. These authors suggest that in cattle is HDL a major source of cholesterol for steroidogenesis.

The cows with fatty liver had lower amount of cholesterol in serum and in the HDL-fraction than the normal cows four weeks post partum. This like the low triglyceride level indicates reduced lipoprotein synthesis in the liver. Also the plasma lipid levels presented in Table 4 shows that there are big differences between normal cows and cows with fatty liver in this respect. The latter had lower cholesterol and phospholipid and higher FFA and FFA/cholesterol quotient.

As FFA is a metabolite that not has passed the liver and cholesterol probably is synthesized in the liver and removed by VLDL, the quotient FFA/cholesterol can be used as an expression for fat retention in the liver.

The feeding prepartum seems to have influence on plasma lipids and fat infiltration in the liver cells at partus.

TABLE 4. Plasma lipids in normal cows 4 weeks after calving and in cows with fatty liver 3-5 weeks after calving.

Cows	No	FFA mmol/l	Cholesterol mmol/l	Phospholipids mmol/l	FFA/Cholesterol
Normal	10	0.27±.16	4.10±.97	2.25±.63	0.07
Fatty liver	20	1.26±.43	2.16±.97	1.15±.43	0.58

Of the three combinations of feed it was high energy-low protein that differed from the other in this respect. The cows in this group had lower plasma cholesterol and phospholipids and higher tendency to develop fatty liver.

During the period around partus when there is a hormonally induced lipolysis increased uptake of fatty acids in the liver, it is important that the metabolism and their removal functions. When the energy supply is high there are conditions for their esterification to the triglycerides. If some factor for synthesis of lipoproteins is missing the triglycerides are accumulated in the liver cells. As high energy-low protein feeding seems to dispose for such accumulation, one can suggest that lack of protein or some component in the protein is of importance in the development of fatty liver when there is increased lipolysis.

Summary

Plasma lipids and serum lipoproteins were studied in normal cows round calving and in cows with fatty liver. A study of plasma lipids in cows on different prepartum feeding was also done. The types of feeding were low energy-low protein, high energy-low protein and high energy-high protein.

Cows with fatty liver had lower triglyceride and cholesterol amounts in serum and in the LDL- and HDL-fractions than normal cows at the same time after calving. They also had lower amounts of phospholipids and higher level of FFA and higher FFA/cholesterol quotient. Of the different types of feeding high energy-low protein resulted in decreased plasma cholesterol and phospholipids and increased FFA and higher FFA/cholesterol quotient and increased fat infiltration in the liver cells. The results indicate that decreased transport of fat from the liver in VLDL during lipolysis is a cause of fatty liver. They also indicate that the feeding prepartum is of importance for the fat metabolism and increased fat storage in the liver.

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