Altered plasma triglyceride-rich lipoproteins and triglyceride secretion in feed-restricted pregnant ewes

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ABSTRACT: In late pregnancy, energy deficits in ewes can induce a metabolic imbalance, which often results in pregnancy toxaemia. This metabolic disorder is characterised by hypoglycaemia, hyperketonaemia, an increase in plasma concentrations of free fatty acids and by fatty infiltration in the liver. The purpose of the present study was to identify alterations in lipid and triglyceride-rich lipoprotein distribution and to evaluate triglyceride secretion in ewes during the third trimester of pregnancy, particularly in ewes with feed restriction. The study was performed on non-pregnant and on twin- and triplet-bearing pregnant Limousine × Romanov ewes fed a control or restricted diet. We show that in pregnant ewes, feed restriction resulted in strong lipomobilisation, as monitored by high plasma free fatty acid concentration, and in hypoglycaemia and hyperketonaemia. Plasma and very low density lipoprotein (VLDL) triglyceride concentrations were about four-fold higher in adequately-fed pregnant ewes than in non-pregnant ewes. Feed restriction in pregnant ewes resulted in VLDL triglyceride concentrations that were approximately two-thirds lower than in adequately-fed pregnant ewes. VLDL particles from pregnant ewes were found to be enriched in triglycerides, but to a lesser extent in feed-restricted pregnant ewes. Pregnant ewes that were fed an adequate diet exhibited greater triglyceride secretion rates (TGSR) than non-pregnant ewes. Feed restriction in pregnant ewes resulted in triglyceride secretion rates that were approximately 35% lower than those of pregnant ewes fed an adequate diet. Our results support the idea that exacerbated lipomobilisation in late pregnancy leads to profound lipid and lipoprotein metabolism disturbances.

Keywords: lipids; lipoproteins; triglycerides; pregnancy; sheep

In pregnant ewes, the increase in nutrient requirements necessary for support of rapid foetal growth over the third trimester of pregnancy can induce a metabolic imbalance, which often results in pregnancy toxemia. The relationship between nutrient supply and pregnancy toxemia is well known from early studies (Forbes and Singleton, 1964; Reid, 1968; Kronfeld, 1972). From a biochemical point of view, this metabolic disease is characterised by hypoglycaemia, hyperketonaemia and an increase in the plasma concentrations of free fatty acids. Also, the importance of fatty infiltration of the liver has long been recognised in this pathology (Forbes and Singleton, 1964). In fact, the drain on body fat reserves in response to energy deficit results in increased concentrations of free fatty acids, which in turn lead to fatty infiltration of the liver (Smith and Walsh, 1975; Remesy et al., 1986; Emery et al., 1992). The quantity of mobilised fatty acids from adipose tissue depends on the energy balance and has a direct relationship with the severity of the fatty liver (Reid et al., 1979). Even though the mechanisms and consequences of fat mobilisation have been studied extensively, there is paucity of information on lipid and lipoprotein metabolism in pregnancy in small ruminants. This knowledge is particularly important when considering the central role of triglyceride-rich lipoproteins in triglyceride secretion by the liver and fuel distribution within the body.

Ruminants present many nutritional, physiological and metabolic peculiarities in comparison to monogastric animals (Christie, 1981; Remesy et al., 1986). In particular, in sheep and other ruminants, the plasma lipid profile is characterised by low triglyceride and triglyceride-rich lipoprotein concentrations (Christie, 1981; Bauchart et al., 1986; Remesy et al., 1986; Butler et al., 1988). This is in agreement with species showing a weak participation of the dietary lipids to the lipemia and showing minor hepatic synthesis of fatty acids (Christie, 1981; Remesy et al., 1986; Emery et al., 1992). For this reason, fatty acids metabolised by the liver have to be imported via the blood (Emery et al., 1992). Therefore, limited triglyceride secretion by the liver in ruminants when free fatty acid uptake was high could be responsible for their particular sensitivity to developing liver steatosis (Bauchart et al., 1986; Mazur et al., 1992).

Thus, the purpose of the present study was to evaluate how triglyceride rich-lipoprotein distribution and triglyceride secretion vary in ewes during pregnancy, particularly when pregnant ewes are submitted to feed restriction. These data will help improve our understanding of metabolic deviations leading to pregnancy toxemia and liver steatosis.

MATERIAL AND METHODS

Animals and diets

The experiment was performed on mature non-pregnant and pregnant Limousine × Romanov ewes aged 2–5 years. Animals were housed indoors during the experimental period. The Research Centre is approved for animal experimentation by the French Veterinary Service of Animal Health and Protection. Non-pregnant ewes (n = 20) weighed 55 ± 1 kg and received grass hay (crude protein 9.5% and cellulose 34% of dry matter) *ad libitum*. Twin- and triplet-bearing pregnant females that had been bred in the autumn were used in this experiment and received an additional 0.6 kg/day of concentrate (g/kg: corn 300, barley 300, oat 200, soy turtle 20) containing 20% (dry matter) crude protein. Three weeks before assumed parturition, ewes (weighed 69.2 ± 2 kg) were randomly divided into a control group (fed an adequate diet and a feed-restricted group (20 ewes per group). The feed-restricted group of animals was restricted to 0.75 kg/day of grass hay over a seven day period. INRAtion software (www.inration.educagri.fr) was used to calculate the nutritional value of feed (feed unit UF and protein value PDI). The calculated quantities of energy (UF) and protein (PDI) provided to the pregnant unrestricted ewes were 106% and 110% of recommended allowances, respectively, and 31% and 31% for restricted ewes. Experiments were performed at the end of this seven day period and animals then received the control diet until parturition. At parturition, there was no difference in the number of lambs in both groups (2.5 ± 0.2 and 2.2 ± 0.2 lambs/ewe, respectively).

Plasma and lipoprotein analyses

Blood was collected in heparinised tubes from the jugular vein of ewes in the morning just before feeding. Glucose (Trinder, 1969), β-hydroxybutyrate (Williamson and Mallenby, 1974), free fatty acids (Biolyon, Lyon, France), triglyceride (Biotrol, Paris, France), total cholesterol (BioMerieux, Lyon, France) and phospholipid (BioMerieux) concentrations were determined by enzymatic procedures.

Before lipoprotein separation, sodium azide (0.02%), merthiolate (0.005%), and EDTA (0.04%) were added to the plasma to prevent lipid oxidation and microbial contamination. Plasma lipoproteins were separated into various density fractions by sequential ultracentrifugation (Havel et al., 1955). Ultracentrifugation was performed at 15°C in a Beckman L-5 model ultracentrifuge (Beckman, Palo Alto, CA) with a type 50 titanium rotor. Very low density lipoproteins (VLDL) were isolated at plasma density > 1.006 g/ml by centrifugation at 100 000 g for 18 hours.

Measurement of triglyceride secretion rate

Triglyceride secretion rate (TGSR) was determined using Triton 1339 (Schultz and Esdale, 1971; Mamo et al., 1983). Briefly, Triton WR 1339 (Tyloxapol, Sigma, St. Louis, MO, USA) was injected into the jugular vein of experimental animals as a 15% solution (w/v) in 0.15M NaCl at a dose of 0.2 g/kg body weight. Ten non-pregnant ewes, seven adequately-fed pregnant ewes and four feed-restricted pregnant ewes were used in this study. Blood samples were taken before and every one hour after Triton injection for six hours. By preventing triglyceride egress from the plasma, Triton caused triglyceride plasma concentrations

to increase. The concentration of triglyceride in the plasma rose linearly during the six hours after Triton WR 1339 injection in all study animals (not shown). This increase in triglyceride concentration was used to calculate the secretion of triglycerides into the plasma. The results are expressed in nmole of triglyceride/ml plasma/min.

Statistics

Results are given as means ± SEM. A one-way analysis of variance (ANOVA) and Student-Neuman-Keuls post hoc test were used to analyse the data (InStat, Graphpad, La Jolla, CA, USA).

RESULTS

As shown in Table 1, pregnant ewes presented significantly lower concentrations of glucose and higher concentrations of free fatty acids and β-hydroxybutyrate in the plasma than non-pregnant ewes. Feed restriction in pregnant ewes resulted in a strong depression of plasma glucose and an increase in free fatty acid and β -hydroxybutyrate concentrations.

Plasma triglyceride concentrations were higher in adequately-fed pregnant ewes than in non-pregnant ewes (Table 1). The increase in plasma triglycerides in these animals was largely due to an increase in the amount of triglycerides carried

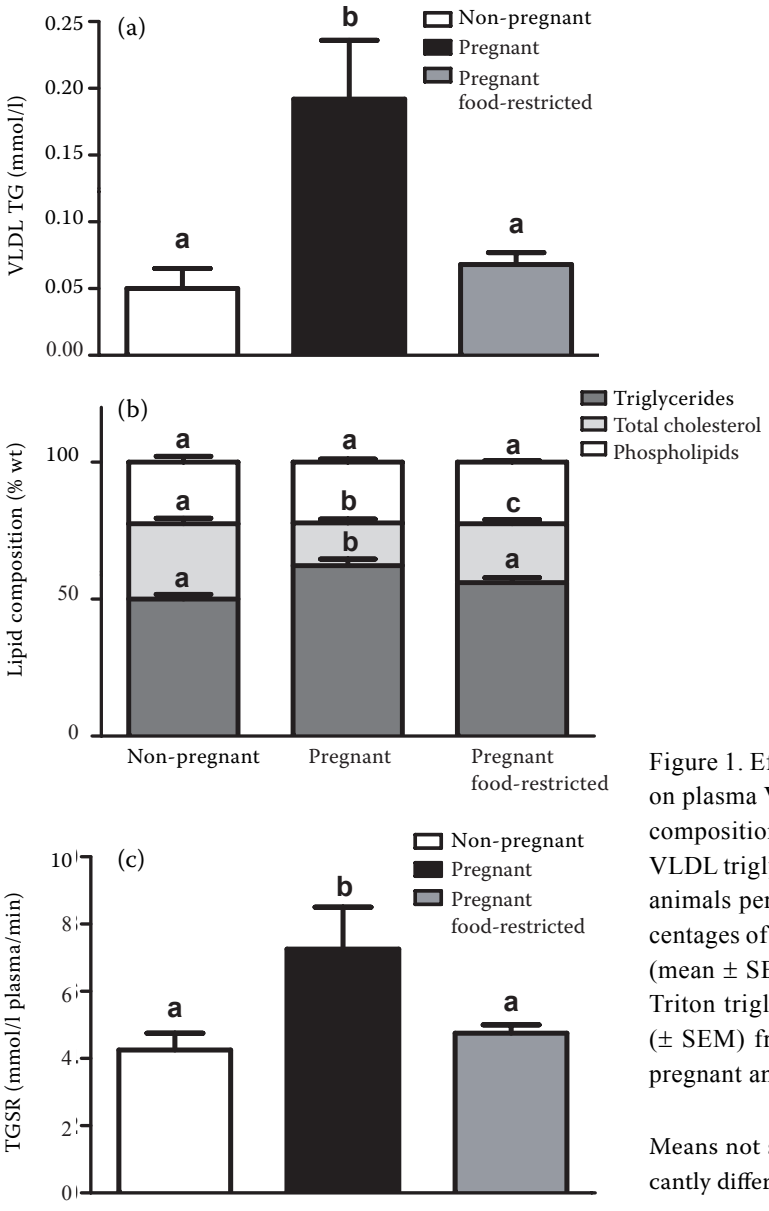

Figure 1. Effect of pregnancy and feed restriction in ewes on plasma VLDL triglyceride concentration, VLDL lipid composition, and post-Triton triglyceride secretion. (a) = VLDL triglyceride concentration (mean ± SEM of twenty animals per group); (b) = lipid composition (weight percentages of the total lipid concentration) of plasma VLDL (mean ± SEM of twenty animals per group); (c) = post-Triton triglyceride secretion rate (TGSR). Mean values (± SEM) from ten non-pregnant, seven adequately-fed pregnant and four feed-restricted pregnant ewes

Means not sharing the same superscript letter are significantly different at a significance level of $P < 0.05$

Table 1. Effect of pregnancy and feed restriction during late pregnancy on plasma parameters in ewes

Ewes	Non-pregnant	Pregnant	Pregnant feed-restricted
Glucose (mmol/l)	3.33 ± 0.04[a]	2.80 ± 0.09[b]	2.16 ± 0.05[c]
Free fatty acids (mmol/l)	0.14 ± 0.01[a]	0.68 ± 0.08[b]	1.22 ± 0.09[c]
β-hydroxybutyrate (mmol/l)	0.38 ± 0.02[a]	0.66 ± 0.05[b]	0.97 ± 0.10[c]
Triglycerides (mmol/l)	0.18 ± 0.01[a]	0.40 ± 0.05[b]	0.21 ± 0.01[a]
Total cholesterol (mmol/l)	1.28 ± 0.07[a]	1.41 ± 0.05[a]	1.90 ± 0.04[b]
Phospholipids (mmol/l)	0.99 ± 0.05[a]	1.30 ± 0.03[b]	1.21 ± 0.04[b]

Mean ± SEM of 20 animals per group. Means not sharing the same superscript letter are significantly different at a significance level of $P < 0.05$

by VLDL (Figure 1a). compared to adequately-fed pregnant ewes, adequately-fed pregnant ewes (Table 1). Feed-restricted pregnant ewes presented lower plasma triglyceride (Table 1) and VLDL-triglyceride concentrations (Figure 1b). Plasma cholesterol concentrations were not affected by pregnancy but were highest in feed-restricted pregnant ewes (Table 1).

Differences in plasma triglyceride-rich lipoprotein–VLDL composition among experimental groups were also found, as shown in Figure 1b. In fact, VLDL particles are more enriched in triglycerides in pregnant ewes than in non-pregnant ewes. This enrichment was less pronounced in feed-restricted pregnant ewes than in adequately-fed pregnant ewes (Figure 1b). As shown in Figure 1c, pregnant ewes that were fed an adequate diet exhibited a greater TGSR than non-pregnant ewes. Feed restriction in pregnant ewes resulted in a lower TGSR in comparison with pregnant ewes fed an adequate diet.

DISCUSSION

In the present work, we show that at the end of pregnancy, adequately-fed ewes present lower glycaemia, higher free fatty acid plasma concentrations and higher plasma levels of ketone bodies (β-hydroxybutyrate) than non-pregnant ewes. This finding is in agreement with previous reports (Wastney et al., 1983; Remesy et al., 1986; Emery et al., 1992). The observed metabolic response during pregnancy results from a higher lipid mobilisation in response to high energy needs (Christie, 1981). This pattern is well known and depends on the gestation period, the number of foetuses and the nutritional conditions (Patterson et al., 1964; Remesy and Demigne, 1976; West, 1996; Schlumbohm and Harmeyer, 2008). This metabolic situation could be particularly harmful for late pregnancy multiparous ewes and can lead to the pregnancy toxemia (Remesy et al., 1986; Schlumbohm and Harmeyer, 2008). In our study, we reproduced this metabolic stress response in pregnant ewes by feed restriction. Exacerbated hypoglycaemia, lipid mobilisation (monitored by an increase in plasma free fatty acid concentration) and higher level of plasma β-hydroxybutyrate were observed.

Our study focused on identifying how these metabolic conditions affect lipid (specifically triglyceride) metabolism. Available data on the lipoprotein distribution and composition in sheep are scarce. According to previous studies on sheep (Nelson, 1973; Bouchat et al., 1980) and other ruminants (Raphael et al., 1973; Stead and Welch, 1975; Puppione 1978; Mazur and Rayssiguier, 1988; Marcos et al., 1990; Schweigert, 1990), the level and composition of lipoproteins of these animals are quite different from monogastric species; in particular, the ruminant plasma is characterised by very low concentrations of triglycerides. Thus, the fraction of VLDL is a minor component of plasma lipoproteins which reflects the lipid metabolism characteristics in the ruminant liver, and VLDL-triglyceride secretion by the ruminant liver is considered to be very low (Mamo et al., 1983; Bauchart et al., 1986; Kleppe et al., 1988; Pullen et al., 1990; Emmison et al., 1991; Mazur et al., 1992).

The most prominent observation in our study was the increase in plasma triglyceride concentrations in ewes during late pregnancy (on an adequate diet) in comparison with non-pregnant females. This difference was accompanied by an increase

in the plasma level of triglyceride-rich lipoprotein fraction (VLDL) and by their enrichment with triglycerides. We also have shown that triglyceride secretion was higher in pregnant and adequately fed ewes in comparison with non-pregnant ewes. Unfortunately, it was not possible to obtain liver samples in our study to assess the lipid content of the liver. However, it is generally accepted that in ruminants, lipid mobilisation leads to triglyceride accumulation in the liver and that the severity of triglyceride accumulation depends on the extent of lipomobilisation (Emery et al., 1992; Mazur et al., 1992). Studies of fasted cows have suggested that an increased hepatic uptake of free fatty acids associated with an inadequate hepatic secretion of triglycerides may constitute a major mechanism for lipid accumulation in the liver (Reid et al., 1979). As previously discussed, ruminants present some unique characteristics that limit triglyceride-rich lipoprotein secretion; ruminants are thus more susceptible to liver steatosis during fat mobilisation than are monogastric animals (Emery et al., 1992). The observed increase in the proportion of triglycerides of VLDL in adequate fed pregnant ewes also supports an increased triglyceride secretion by increasing triglyceride load of these lipoproteins. During pregnancy it has been observed in various species a decrease in lipoprotein lipase activity which also can lead to hypertriglyceridemia (Knopp et al., 1986). Thus, both reduced triglyceride secretion and catabolism are altered during pregnancy.

Our study has firmly demonstrated that feed-restricted pregnant ewes do not show increased plasma triglyceride and triglyceride-rich lipoprotein concentrations, even if they present a higher fat mobilisation than adequately-fed ewes. The VLDL particles of feed-restricted ewes are also not as enriched in triglycerides as adequately-fed pregnant ewes. The reasons for the differences in the lipid and lipoprotein patterns observed between feed-restricted and adequately-fed ewes could be due to the increased catabolism of free fatty acids in feed-restricted ewes, which can also result in increased plasma β-hydroxybutyrate concentrations due to energy restriction. The study of triglyceride secretion (in ruminants principally of the hepatic origin) shows a low secretion rate in feed-restricted pregnant ewes in comparison with pregnant adequately-fed ewes. This difference in secretion rates could be responsible for the fatty livers observed in pregnant ewes (Patterson et al., 1964; Butler et al., 1988) and early lactating cows (Bauchart et al., 1986; Mazur et al., 1992) during severe energy deficits. Also, changes in the lipoprotein profile have been reported in periparturient cows that spontaneously developed fatty livers, as well as in fasted cows (Herdt et al., 1983). The degree of these changes is also dependant on the severity of the fatty liver (Rayssiguier et al., 1988; Mazur et al., 1992). Some studies have shown that a high accumulation of liver lipids in periparturient cows is accompanied by a low level of plasma triglycerides and triglyceride-rich lipoproteins (Mazur et al., 1988), while low apolipoprotein B levels have also been observed in cows with fatty livers (Marcos et al., 1990; Oikawa et al., 1997). This strongly suggests that triglyceride secretion in ruminants is limited during energy deficit and enhanced lipomobilisation.

In conclusion, we have found that exacerbated lipomobilisation during late pregnancy in ewes is accompanied by alterations in lipid and lipoprotein profiles as well as by a reduced triglyceride secretion. These alterations suggest marked disturbances to the triglyceride-rich lipoprotein metabolism in this physiopathological condition which could contribute to exacerbated fat accumulation in the liver.

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