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Ketone body metabolism during pregnancy in the rabbit.

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Summary. The aim of this study was to test the effect of lipid store mobilization on changes in ketone body metabolism in pregnant rabbits. Related blood parameters were studied in pregnant animals fed either *ad libitum* or submitted between days 21 of gestation and parturition first to 50 % food restriction for 4 days and then to a complete fast.

Ketogenesis from oleate, butyrate and endogenous substrates was measured on days 0, 8, 18 and 28 of gestation in isolated liver cells prepared from females fasted for 48 h.

In the does fed *ad libitum,* the concentration of non-esterified fatty acids (NEFA) was higher than in non-pregnant animals and then increased about 2-fold in the last week before term. Total ketone body concentrations increased slightly but significantly from day 27 until term.

In the same period, glycemia decreased significantly. No variations were observed in lactate, alanine and total amino acid concentrations. Food restriction on days 21 to 24 induced a quick rise in the plasma concentrations of NEFA, ketone bodies and glycerol. Further fasting resulted in the development of hyperketonemia which was more than 3 times that observed during prolonged fasting in non-pregnant rabbits. There was no further increase in plasma NEFA level after day 27 of gestation. Food restriction and fasting decreased only the plasma level of total amino acids but had no significant effect on plasma concentrations of lactate and alanine.

In isolated liver cells, a marked and significant increase in the rate of ketogenesis from oleate, butyrate and endogenous substrates was noted on day 28 of gestation in comparison with the preceding periods.

It is concluded that ketonemia was enhanced in late gestation, particularly with restricted feeding or in fasted animals ; this enhancement was partly related to the increase of plasma NEFA concentrations and partly to the enhancement of hepatic ketogenesis in the mothers. The fact that the rate of hepatic ketogenesis was increased equally with butyrate and oleate indicated that it could not be explained by a modification of acylcarnitine transferase activity as butyrate directly crosses the mitochondrial membrane without using this pathway.

Introduction.

No anabolism has been observed in pregnant rabbits (Lebas, 1975). Moreover, in the latter part of gestation food intake increases and the doe practically fasts during the day preceding delivery. During the last 7 days,

maternal body weight increases little in comparison with the increase of the uterine mass. This induces mobilization of maternal lipid stores, and fatty acids are taken up by the foetus (Elphick *et al.,* 1975 ; Elphick and Hull, 1977a,b ; Hudson and Hull, 1977 ; Stammers *et al.,* 1983). Fatty acids are also metabolized to ketone bodies in the maternal liver since pregnant females are more prone to hyperketonemia than non-pregnant ones. Ketone bodies are used by several maternal tissues in both fed and food-restricted animals, thus making more glucose available to the foetus. Ketone bodies can also be utilized by the foetus (see review by Robinson and Williamson, 1980). The only data available have shown that blood ketone bodies remain very low throughout pregnancy in rabbits (Gilbert *et al.,* 1984).

The aim of the present work was to study (1) variations in ketonemia and related plasma parameters in the second part of gestation in rabbits submitted to different feeding schedules (*ad libitum,* restricted, fast) and (2) variations in hepatic ketogenesis in isolated hepatocytes from rabbits at different stages of pregnancy.

Material and methods.

Animals and diets. — New Zealand does weighing 4 to 5 kg were used. After mating they were fed a U.A.R. commercial diet containing 16 % crude protein. 12.5 % crude fiber, 3 % fat. A group of 6 does was fed *ad libitum* until day 20 of pregnancy, then submitted to 50 % food restriction from days 21 to 24 and finally fasted from day 25 until delivery. A group of non-pregnant does, submitted to the same food restriction plan as the first group, was used as a control.

Surgery and blood sampling. — To allow the blood to be sampled without stress, a permanent intravascular catheter was introduced under Nembutal anesthesia into the femoral artery (Gilbert *et al.,* 1984) on day 12 of gestation. The animals recovered within 2 days. To avoid interference with fatty acid metabolism, the catheters were flushed every 3 days with a 0.9 % NaCl solution containing no heparin.

Every 3 days from day 15 of gestation until delivery, blood was sampled in the morning between 9 and 10 a.m. At that time of day, food intake is minimal in rabbits. Blood was collected on heparin for determination of glucose, lactate, ketone bodies, total amino acids and alanine, and on EDTA for non-esterified fatty acids (NEFA) and glycerol assays. The samples were kept on ice and then centrifuged at 3 000 × g for 10 min at 4 °C. The plasma was collected and stored at − 20 °C until assay.

Biochemical analysis. — All determinations were performed on plasma after perchloric precipitation. Ketone bodies, lactate, alanine and glycerol were assayed by standard enzymatic methods (Gutman and Wahlefeld, 1974 ; Mellanby and Williamson, 1974 ; Williamson and Mellanby, 1974 ; Williamson, 1974 ; Wieland, 1974), glucose by the glucose oxidase peroxidase method (Trinder, 1969), total

amino acids with the colorimetric method of Malangeau *et al.* (1963) and NEFA by enzymatic and colorimetric methods (Shimizu *et al.*, 1979 ; NEFA C test Wako, France).

Measurement of hepatic ketogenesis in isolated hepatocytes.

Isolation of hepatocytes. — Eighteen pregnant does of the New Zealand strain fed with a commercial diet were used for the preparation of isolated liver cells at three stages of pregnancy (days 8, 18 and 28) after a fast of 48 h. The isolated cells were prepared according to the method of Berry and Friend (1969) modified for adult rabbits by Jean-Blain and Martin (1980). Perfusion flow rate was 130 ml/min. The packed cells (0.4 ml, 10-15 mg of protein) were incubated in a final volume of 2 ml with Krebs-Ringer bicarbonate buffer containing 1 % of fatty acid-free bovine albumin.

Ketone body formation from 5 mM butyrate, 1 mM oleate in albumin + 0.1 mM carnitine and a mixture of butyrate and oleate was measured after 1 h of incubation according to the method of Williamson and Mellanby (1974). The results are given in µmoles of ketone bodies formed/mg protein/h. The protein content of the incubated cells was determined by the micro-kjeldhal method (N × 6.25).

Hepatocyte total lipid content was determined after Folch extraction (Folch *et al.*, 1957). Glycogen was measured with the amylo 1-4 1-6 glucosidase method (Keppler and Decker, 1974).

Statistical methods. — Differences between means were tested for significance by variance analysis or Student's t-test.

Results.

Changes in body weight, food intake and plasma metabolite concentrations in chronically catheterized does. — Changes in the mean body weight and food intake of the two groups of pregnant does are shown in figures 1 and 2. In does

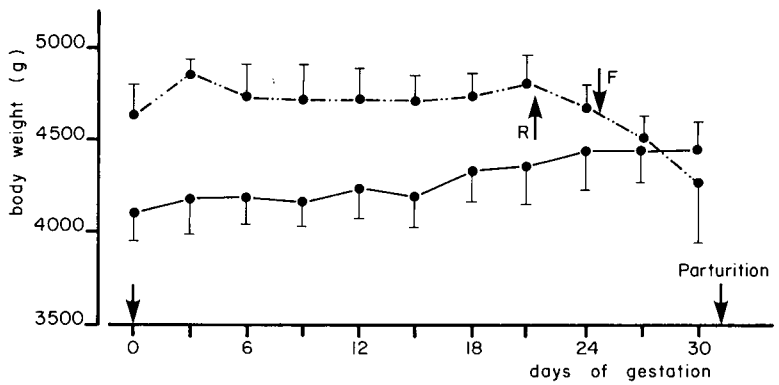

FIG. 1. — *Changes in rabbit body weight during gestation.* ● — — ● fed *ad libitum* ; ● — ... — ● restricted and fasted. R : start of 50 % food restriction ; F : start of fast (mean ± SEM ; n = 6).

fed *ad libitum* mean net gain of body weight throughout pregnancy was 341 g ; a marked weight loss was noted in the restricted group from day 21 until delivery. Mean body weight loss from days 1 to 31 of pregnancy was 317 g. Non-pregnant females submitted to the same feeding plan as the restricted group had a mean body weight loss of 263 g (fig. 1).

In does fed *ad libitum* food intake decreased strongly from day 25 of gestation onwards. Wide individual variations in food intake were noted by that time, some does still eating 150 g per day on the day preceding delivery, while others ate only 25 g (fig. 2).

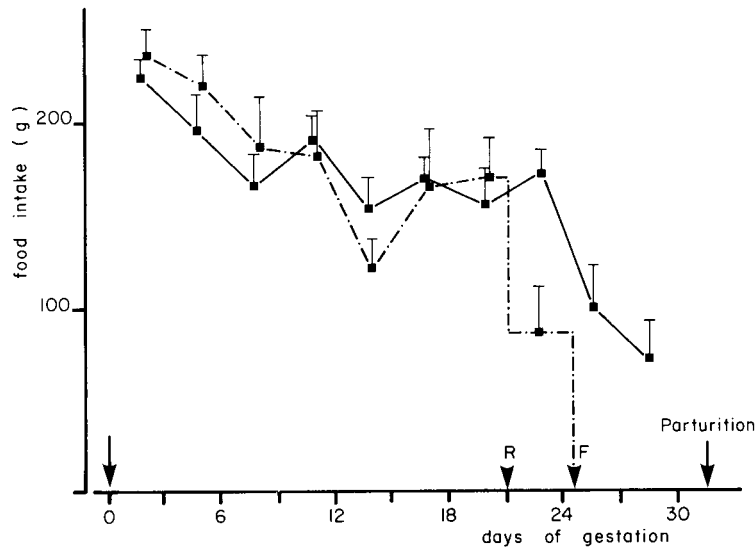

FIG. 2. — *Changes in rabbit food intake during gestation.* ■ — — ■ *fed ad libitum* ; ■ — . — . — ■ restricted and fasted. Each point represents the average food intake of 3 days. R : start of 50 % food restriction ; F : start of fast (mean ± SEM ; n = 6).

The level of plasma NEFA from days 15 to 21 of gestation was about 4-fold higher in pregnant than in non-pregnant animals (fig. 3a,c) ; these levels then increased until the end of gestation, reaching a concentration corresponding to the fasting value in the non-pregnant animals (1 mM). Food restriction on day 21 induced a more rapid elevation of the plasma NEFA concentration, but no further increase was observed after day 27 so that the concentration on day 30 was not significantly different from that in the group fed *ad libitum* (fig. 3a,b).

Plasma glycerol concentration increased almost 2-fold from day 18 to delivery in females submitted to food restriction and fasted (fig. 5). This concentration increased only 41 % in the former ; no significant change was noted in fed does.

The plasma concentration of ketone bodies in pregnant does fed *ad libitum* from days 15 to 24 of gestation was not significantly different from that observed in non-pregnant animals (fig. 3a, c) ; a slight but significant increase was noted on days 27 ($P < 0.05$) and 30 ($P < 0.01$) of pregnancy.

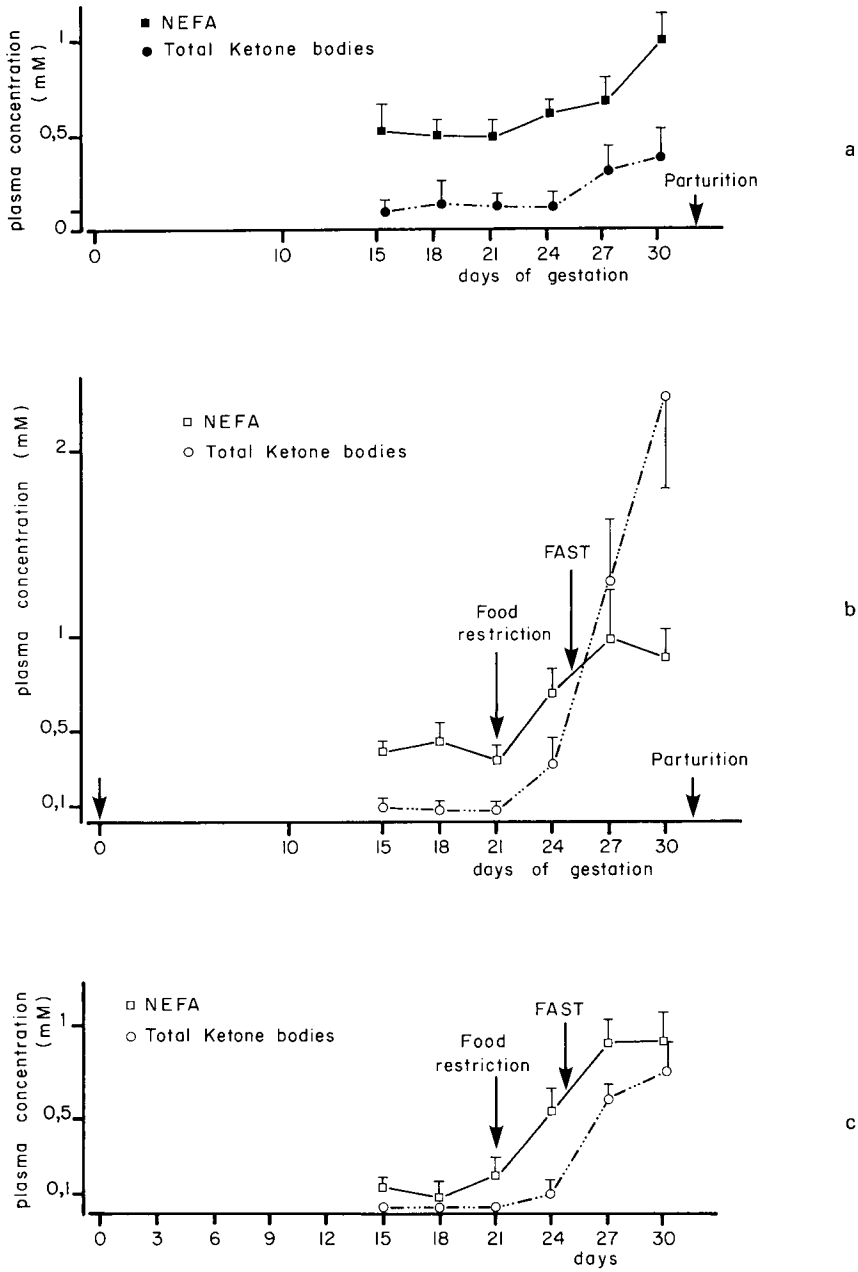

FIG. 3. — *Changes in arterial plasma concentration of NEFA and total ketone bodies.* a : pregnant rabbits fed *ad libitum* ; b : pregnant rabbits restricted and fasted ; c : non-pregnant rabbits submitted to a 4-day food restriction then to a 6-day fast. (mean ± SEM ; n = 6).

A 50 % food restriction (fig. 3b) from days 21 to 24 of pregnancy induced a significant increases in total ketone bodies ($P < 0.001$). The most striking observation was the considerable enhancement of ketonemia induced by further fasting (2.3 mM). In comparison, the same food restriction plan and fasting applied to non-pregnant rabbits increased ketonemia to only 0.6 mM (fig. 3c). Glycemia decreased significantly ($P < 0.001$) (fig. 4) at the end of gestation in does fed *ad libitum*. On the other hand, no significant changes occurred in plasma lactate (fig. 4), alanine and total amino acid concentrations (table 1). Food restriction and fasting had no effect on plasma glucose, lactate and alanine

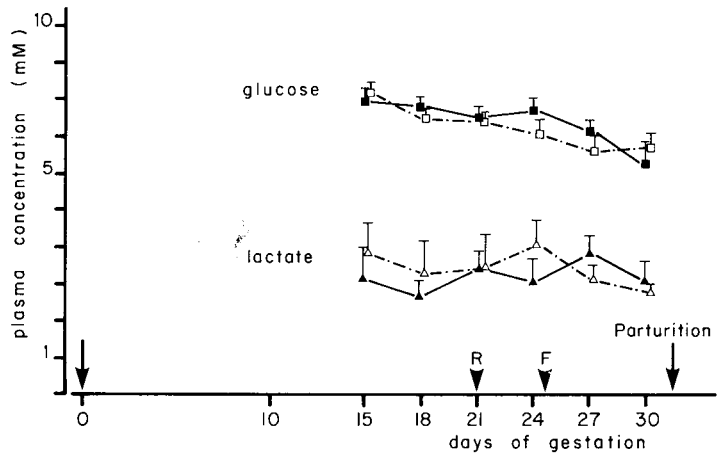

FIG. 4. — *Changes in arterial plasma concentrations of glucose and lactate during gestation in rabbits.*
■ ▲ —— fed *ad libitum* ; □ △ — . — restricted and fasted. R : start of 50 % food restriction ; F : start of fast.
(mean ± SEM ; n = 6).

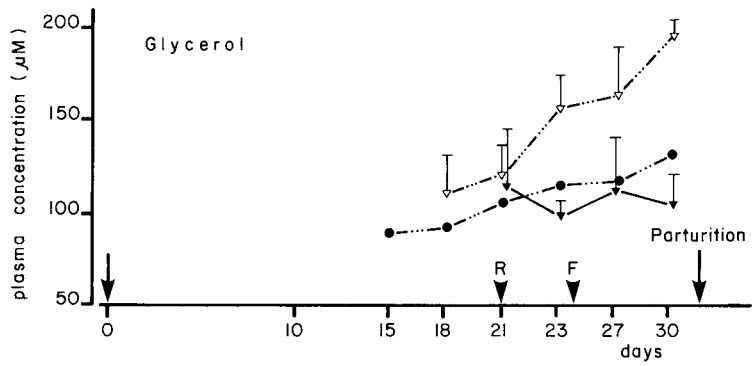

FIG. 5. — *Changes in arterial plasma glycerol concentration.*
▼— —▼ pregnant rabbits fed *ad libitum* ; ▽— ... —▽ pregnant rabbits restricted and fasted ; ● — ... ● non-pregnant rabbits restricted and fasted.
(mean ± SEM ; n = 6).

TABLE 1

Arterial plasma concentrations of alanine and total amino acids during the second half of pregnancy in rabbits. Effect of food restriction and fasting.

Day of gestation	18	24		30	
Feeding mode	*ad libitum*	*ad libitum*	restricted	*ad libitum*	restricted
Alanine mM	0.274 ± 0.024[a]	0.273 ± 0.06[a]	0.261 ± 0.09[a]	0.280 ± 0.031[a]	0.226 ± 0.033[a]
Total amino acids mM	5.88 ± 0.67[a]	6.61 ± 0.64[A]	4.34 ± 0.10	7.02 ± 0.42[A]	5.22 ± 0.073[B]

(means ± SEM)
Means with different superscripts are significantly different.
A,B $P < 0.01$; a, b, $P < 0,05$.

concentrations. Only plasma total amino acid concentration was significantly decreased (table 1).

Ketogenesis in isolated liver cells. — The rates of ketogenesis in isolated liver cells prepared from rabbits on days 0, 8, 18 and 28 of gestation are shown in table 2.

Ketogenesis from endogenous substrates was the same in non-pregnant and pregnant does on days 8 and 18 of gestation, but was strongly enhanced on day 28. The same pattern was observed when oleate and carnitine were added. A significant increase was observed with butyrate on day 18. In all cases, the mixture of butyrate and oleate gave a higher rate of ketogenesis than each individual substrate.

Hepatocyte glycogen content after 48 hours of fasting was equal to 26 ± 18 mg/g dry weight. Non-significant differences were noted at different stages of gestation. On the other hand, total lipid content increased significantly ($P < 0.05$) on day 28 (363 ± 96 mg/g dry weight) in comparison with the two preceding stages (252 ± 82 mg/g dry weight). On day 28 of pregnancy, the liver often had a lactescent aspect during perfusion.

TABLE 2

Ketogenesis in isolated liver cells of 48-hour fasted, non-pregnant and pregnant rabbits at 8, 18 and 28 days of pregnancy.

Substrate(s) added into the incubation medium	Day of gestation			
	0	8	18	28
None (endogenous substrates)	32 ± 4[A]	36 ± 3[A]	35 ± 4[A]	87 ± 9[B]
Oleate 1 mM + carnitine 0,1 mM	125 ± 10[A]	116 ± 3.6[A]	118 ± 2[A]	193 ± 10[B]
Butyrate 5 mM	132 ± 6[A]	126 ± 7.2[A]	151 ± 9[ab]	197 ± 14[B]
Oleate 1 mM + Butyrate 5 mM + carnitine 0,1 mM		166 ± 11.6[A]	196 ± 14[a]	253 ± 14[B]

Duration of incubation : 1 h ; Number of animals : 18 (6 for each period).
Total ketone bodies formed in µmoles/g protein/hour (means ± SEM).
Means with different superscripts are significantly different A,B $P < 0.001$; a, b $P < 0.05$.

Discussion.

Changes in the body weight and food intake of the rabbits were similar to those observed by Lebas (1975), although total weight gain in those fed *ad libitum* was a little lower, probably because of stress due to surgery and manipulation.

With the exception of lactate, the changes in blood metabolites in rabbits fed *ad libitum* were analogous to those reported by Acebal *et al.* (1973) and Gilbert *et al.* (1984). We found no increase in plasma lactate concentration before delivery. As compared with the results of Gilbert *et al.* (1984), a lower plasma NEFA concentration occurred in the non-pregnant state, probably because the catheters we used were flushed with an NaCl solution containing no heparin. We had observed that the use of heparinized catheters could artificially increase the plasma NEFA level.

Rabbits in late pregnancy have a fast-like metabolism which results in a decrease of glycemia. However, we did not find significant modifications in the gluconeogenic substrates (lactate, alanine and glycerol) during this period. In contrast with humans (see review by Robinson and Williamson (1980) and ewes (Lemons *et al.*, 1984), no hypoalanineamia was observed, even during fasting.

No data are available in rabbits concerning the effect of food restriction or fasting during pregnancy on plasma metabolites levels, despite the fact that the effect of food restriction on further lactation has been studied (Lebas, 1975). The most interesting fact in the present study is the important increase of ketogenesis compared with the same feeding states in non-pregnant animals (Jean-Blain and Durix, 1985). Fasting did not induce a decrease in glycemia in late pregnancy. As plasma NEFA concentration was not increased further by food restriction, the number of fatty acids directly available to the foetus was probably not enhanced. Moreover, maternal circulating triglycerides, which are normally transferred to the foetus owing to the high activity of placental lipase in rabbits (Elphick and Hull, 1977b), reach a minimal value in the last week of pregnancy (Kriesten and Murawski, 1981).

The present study shows that ketonemia in rabbits submitted to food restriction or a complete fast is enhanced in late pregnancy compared with non-pregnant animals, despite the fact that the increase in plasma NEFA concentration is almost the same and that the formation of butyrate and other ketogenic substrates in the alimentary tract decreases (Bonnafous and Raynaud, 1979).

The hepatic origin of ketone bodies has been confirmed by our investigations of isolated liver cells. The rate of ketogenesis in the liver of the mother is increased to the same extent with butyrate and oleate of endogenous substrates. This indicates that a change in carnitine-acyltransferase activity is not the prime cause of this modification, for butyrate crosses the mitochondrial membrane without using the acylcarnitine shuttle.

The theory of a foetal origin for the ketone bodies formed can also be discarded, as it has been shown that liver ketogenesis is very low in foetal rabbits (El Manoubi, Ferré and Girard, 1981).

Comparisons with other species show marked differences. In rats, the rate of ketogenesis in isolated liver is increased from oleate but not from butyrate (Whitelaw and Williamson 1977), as is palmitoylcarnitinetransferase activity (Saggerson and Carpenter, 1982). In contrast, there are no modifications in the rate of ketogenesis in the liver homogenates of pregnant twin ewes, despite the considerable enhancement of fasting ketonemia in late gestation in this species (Varnam *et al.*, 1978).

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Résumé. *Métabolisme des corps cétoniques pendant la gestation chez la lapine.*

Afin d'étudier chez la lapine gestante les modifications métaboliques liées à la mobilisation des réserves lipidiques et, en particulier, celles qui ont trait au métabolisme des corps cétoniques, on a, dans un premier temps, mesuré les variations des paramètres sanguins concernés par ces modifications sur des lapines gestantes nourries *ad libitum* ou soumises préalablement à une restriction alimentaire de 50 % par rapport au régime *ad libitum* du 21e au 24e jour de gestation, puis à un jeûne complet jusqu'à la mise bas.

Dans un deuxième temps, on a préparé des hépatocytes isolés à partir de lapines à jeun depuis 48 h, à différents stades de la gestation (0, 8, 18, 28e jour de gestation). On a mesuré sur ces hépatocytes la production de corps cétoniques à partir d'oléate, de butyrate et des substrats endogènes.

Chez les lapines nourries *ad libitum,* le taux plasmatique des acides gras non estérifiés est plus élevé que chez les non gestantes et double au cours de la dernière semaine de gestation. La cétonémie augmente très légèrement mais significativement du 27e jour de gestation jusqu'à la mise bas. Dans le même temps, la glycémie diminue d'une façon significative. Les taux plasmatiques de lactate, d'alanine et d'acides aminés totaux ne varient pas. La restriction alimentaire du 21e au 24e jour de gestation provoque une élévation rapide des taux plasmatiques d'acides gras non estérifiés (AGNE), de corps cétoniques et de glycérol. Le jeûne ultérieur induit une hypercétonémie 3 fois plus grande que celle qui est observée chez l'animal non gestant au cours d'un jeûne prolongé. Néanmoins, les AGNE n'augmentent pas davantage après le 27e jour de gestation. La restriction alimentaire et le jeûne qui suit ne diminuent que le taux des acides aminés totaux. Aucune modification n'est observée pour le lactate et l'alanine.

Dans les hépatocytes isolés, la cétogenèse à partir de l'oléate, du butyrate et des substrats endogènes est fortement augmentée au 28e jour de gestation par rapport aux stades précédents.

Il est permis de conclure que l'augmentation de la cétonémie observée en fin de gestation, importante en cas de restriction alimentaire ou de jeûne total, est due en partie à l'augmentation des taux plasmatiques d'AGNE et en partie à la capacité cétogénique accrue du foie des lapines. Le fait que la cétogénèse soit augmentée aussi bien avec le butyrate qu'avec l'oléate indique qu'il ne s'agit pas d'une modification de l'activité des acylcarnitine transférases puisque le butyrate est capable de traverser directement la membrane mitochondriale sans utiliser le système de transport des acylcarnitines.

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