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Roles for Insulin and Glucagon in the Development of Ruminant Ketosis — A Review

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SUMMARY

Ketonemia can be a physiological response to a reduction in dietary intake. It also may occur when energy demands exceed the energy intake. Normally, alimentary ketogenesis is the major source of ketone bodies in ruminants. During ketonemia there is increased hepatic ketone body production. During physiological ketosis, the mobilization of free fatty acids is inadequate to support a high rate of hepatic ketogenesis. However, during clinical ketosis, the hormonal status (low insulin, high glucagon/insulin ratio) in combination with hypoglycemia promotes excessive lipid mobilization and a greater hepatic removal of fatty acids and switches the liver to a higher rate of ketogenesis. The low insulin, furthermore, can impair maximal ketone body utilization, thus exacerbating the hyperketonemia.

RÉSUMÉ

Une revue des rôles de l'insuline et du glucagon dans le développement de la cétose des ruminants
L'acétonémie peut représenter une réaction physiologique à une ingestion moins grande d'aliments. Elle peut aussi survenir lorsque la demande d'énergie en excède l'ingestion. Normalement, la cétogénèse alimentaire représente la principale source des corps cétoniques, chez les ruminants. Au cours de l'acétonémie, la production de corps cétoniques par le foie subit un accroissement. Lors de la cétose physiologique, la mobilisation d'acides gras libres se révèle insuffisante pour supporter un taux élevé de cétogénèse hépatique. Cependant, au cours de la cétose clinique, le statut hormonal (peu d'insuline et rapport glucagon-insuline élevé), de concert avec l'hypoglycémie, favorise une mobilisation excessive des lipides et un plus grand retrait hépatique des acides gras; il oriente aussi le foie vers une cétogénèse plus élevée. Par ailleurs, la faible quantité d'insuline peut faire échec à l'utilisation maximale des corps cétoniques, exacerbant ainsi l'acétonémie.

INTRODUCTION

Ketonemia is a physiological response to a number of hormonal or nutritional disturbances. Among these are administration of thyroxine (22), glucagon (15) or growth hormone (7, 36) and starvation (3, 29) or a change in diet (48). Hyperketonemia or ketosis results from excessive ketone body (KB) production and reflects deranged metabolism. Both the cow and the ewe are susceptible to hyperketonemia. The predisposition peaks during lactation in the cow, and during multiple lamb pregnancies (47) in the ewe. This undoubtedly is partly due to the high requirements for glucose in the synthesis of lactose and in the developing feti, and to the reliance of ruminants on gluconeogenesis for their glucose needs. While hypoglycemia and lowered liver glycogen concentrations are characteristic of these conditions (9, 33), glucose production does not decrease in primary ketosis until feed intake is depressed (5, 33, 35). The hypoglycemia may, in part, be the effect of changes in glucose distribution (33).

The endocrine system, especially the pancreas, probably is intimately involved in the development of ruminant ketosis. This review considers the roles of the two major pancreatic hormones, glucagon and insulin, in the pathogenesis of ruminant ketosis. However, since key information in ruminant animals often is lacking references to more abundant studies in nonruminant animals are used. Extrapolation from findings in diabetes mellitus are taken to fill gaps in our understanding of the roles of pancreatic hormones in the pathogenesis of ruminant ketosis. This is done, not because of the similarity in pathogenesis of these conditions, but rather because the alloxan diabetic animal serves as a model to study glucagon and insulin.

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THE PROBLEM IN KETOSIS

Both bovine ketosis and ovine ketosis are diseases of production. The high-producing dairy cow and multiple-pregnant ewe have high glucose requirements for milk production and for growing feti.

A high-producing milk cow normally loses weight during lactation as energy output exceeds energy intake. Energy reserves must be utilized for production of milk constituents, especially milk fat. Lipolysis occurs in the animal's adipose tissue and creates elevated concentrations of free fatty acids (FFA) in the plasma. An increased supply of FFA to the liver favours increased production of β-hydroxybutyrate and acetoacetate or ketone bodies.

The ewe usually would require a minimum of two feti to develop ketosis (47). Undernutrition or stress resulting in decreased energy intake seems to be critical to the development of ovine ketosis. Pregnant ewes switched from a high energy diet to a lower energy diet in the latter part of pregnancy, when fetal growth is most rapid, exhibit lowered blood glucose concentrations and elevated FFA and KB levels (48). This is most evident in multiple lamb pregnancies, but also is true for single pregnant ewes to a lesser extent. The maternal hypoglycemia results from preservation of fetal well-being at the expense of maternal glucose homeostasis.

In both the lactating cow and the pregnant ewe the body compensates for inadequate energy intake by adipose tissue lipolysis and tissue catabolism. However, in ketosis the body seems to overcompensate for inadequate energy intake by excessive lipolysis and a concomitant excessive production of ketone bodies.

ORIGIN OF KETONE BODIES

Major sites (Figure 1) of ketogenesis in the ruminant are liver (29, 44) and ruminal epithelium (1, 23, 45, 49). In normally fed sheep, alimentary ketogenesis accounts for nearly all the KB production, while hepatic ketogenesis may account for over one-third of KB production in twin-pregnant ewes (29). These KB are derived primarily from butyrate absorbed into the blood, and when the animal stops eating, alimentary ketogenesis ceases (3, 29). Hepatic ketogenesis, at least during starvation-induced ketosis in sheep, accounts for the bulk of the KB production (10, 29, 49). Similar observations have been found in fasted dairy cows. Baird (3) reported a net removal of ketones by the portal-drained viscera and a twofold increase in net hepatic production of ketones after 48 h of food deprivation. The substrate for hepatic ketogenesis during food-deprivation is undoubtedly FFA (40, 41). The hepatic uptake of FFA definitely is increased during starvation and starvation-induced ketosis (29).

In addition to producing KB from acetyl-CoA derived by oxidation of FFA, the liver can produce

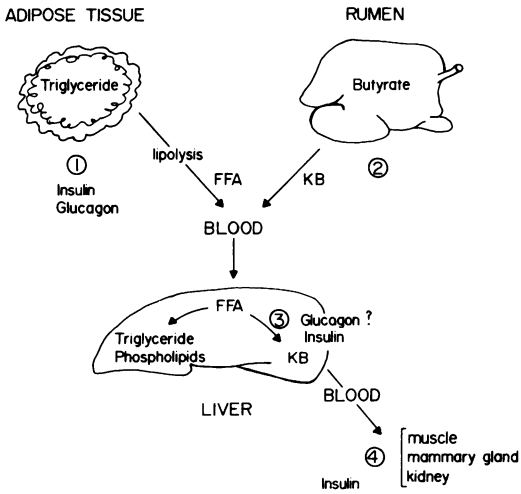

FIGURE 1. Summary of ketone body metabolism in ruminants. Possible sites of regulation are numbered. Site (1) — Lipolysis/lipogenesis. Site (2) — Dietary regulation. Site (3) — Hepatic factors. Site (4) — Utilization of ketone bodies.

acetate (19). Blood acetate concentrations may be elevated in bovine ketosis (35). No increase in hepatic acetate output occurs during starvation in cattle and sheep (3, 11), but during diabetes mellitus in sheep hepatic acetate production is increased (32). Similar to KB production, endogenous acetate production depends to a large extent on blood FFA levels (2). It has been speculated that the balance between hepatic production of acetate and KB may be determined by the availability of carnitine (A.M. Snoswell cited by 3) during the metabolism of FFA.

UTILIZATION OF KETONE BODIES

Ketone bodies are utilized by many tissues except the brain (38). Skeletal muscle (28), kidney (30) and lactating mammary gland (34, 52) all show a net removal of KB. Skeletal muscle utilizes more KB during starvation (28) and even more during ketosis. This implies that overproduction, not underutilization, is the initial cause of ketonemia. However, in the later stages of ketosis, when blood ketone concentrations are sufficiently elevated, KB metabolic clearance rates are down and the activities of KB catabolizing enzyme are decreased (53). Thus, underutilization may become a factor.

HORMONAL INFLUENCES ON KETOGENESIS

Insulin has long been associated with the ketoacidosis of diabetes mellitus. It is known to influence a) the mobilization of FFA from adipose tissue (Figure 1, site 1) and b) utilization of KB by peripheral tissues (Figure 1, site 4). The lipogenic effect of insulin is well-recognized (14). A fall in plasma insulin concentration triggers lipolysis in adipose tissue and elevates plasma FFA concentration. However, lipid mobilization is not a major

ketogenic stimulus without a contribution from the liver (40). Insulin inhibits ketogenesis when FFA levels remain high (12). During starvation and/or ketosis, hepatic removal of FFA is increased (29) and a greater proportion of FFA is converted to KB by the liver than normally.

Insulin also appears to be important in regulating the utilization of KB. The uptakes of β-hydroxybutyrate and acetate by the sheep hindlimb are impaired during alloxan diabetes and are restored by insulin (27). This is consistent with studies in dogs (4) showing that insulin increases the rate of removal of KB from blood and that during insulin deficiency maximal utilization of KB is impaired.

There is mounting evidence that glucagon plays a role in regulating hepatic ketogenesis (Figure 1, site 3). *In vivo* studies in sheep suggest that high concentrations of glucagon may be lipolytic (6, 15, 16), although perhaps only marginally so, and ketogenic (15, 17). *In vitro* studies (8) have failed to demonstrate a lipolytic effect of glucagon in ruminant adipose tissue. Studies with normal sheep and alloxan diabetic sheep treated with insulin (15) reveal that: a) high concentrations of glucagon enhance hepatic ketogenesis, b) low levels of insulin can block the ketogenic effect of high concentrations of glucagon and c) insulin has a much more potent effect on adipose tissue lipogenesis/lipolysis and on hepatic ketogenesis than glucagon. These findings in sheep also are consistent with those in man (21, 37, 42, 50) and dogs (31), meaning that glucagon does play somewhat of a role in the development of hepatic ketogenesis. Glucagon appears to affect the liver itself, perhaps by activating the carnitine acyltransferase reaction, the first step in the oxidation of fatty acids (41, 42). Hepatic carnitine levels correlate with the rate of hepatic ketogenesis (43).

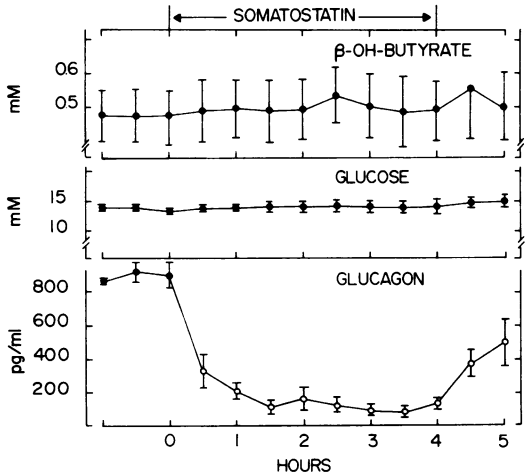

FIGURE 2. Effect of somatostatin infusion (200 μg/hr) on glucagon, glucose and β-hydroxybutyrate production in alloxan diabetic sheep. Values are means ± SE of three sheep (Unpublished observations, R.P. Brockman). Open symbols represent significant differences from preinfusion values ($P<0.01$).

TABLE I
SUMMARY OF THE EFFECTS OF INSULIN AND GLUCAGON ON ADIPOSE TISSUE AND ON LIVER METABOLISM IN RUMINANTS

	Adipose Tissue	Liver	
	Lipolysis	Ketogenesis	Glucogenesis
Insulin	↓	↓↓	↓
Glucagon	↑	↑	↑↑

If glucagon can promote ketogenesis, then removal of glucagon should reduce ketogenesis. This was examined in diabetic sheep. In an unpublished study (R.P. Brockman) from this laboratory (Figure 2) where somatostatin was infused for four hours into ketotic diabetic sheep to inhibit glucagon secretion no change in blood KB concentration was observed during low glucagon concentrations. Perhaps, in the absence of insulin and without a reduction in FFA supplied to the liver, low glucagon is not an adequate stimulus to turn off hepatic ketogenesis.

The effects of insulin and glucagon on liver and adipose tissue metabolisms are summarized in Table I. It would appear that insulin is the primary modifier of lipid and ketone metabolism while glucagon has secondary effects when insulin concentrations are low.

HORMONES AND RUMINANT KETOSIS

Only limited data are available on the insulin and glucagon status of preketotic and/or ketotic cows and ewes. During early lactation plasma insulin concentrations are lower than at any other time during bovine lactation (18, 26, 51). Furthermore, the lowest concentrations of insulin in plasma seen during early lactation occur in cows with the highest blood concentrations of KB and lowest glycemia (24, 26). Not only are insulin concentrations low, but insulin responsiveness also is impaired (26). These observations do not support the contention that hyperinsulinemia due to high concentrations of KB precipitates the hypoglycemia (33). They are consistent with a high rate of gluconeogenesis that is inadequate to meet the increased requirements for glucose at these times. The situation with glucagon is not clear. However, one report indicates that immunoreactive glucagon in plasma may be increased at the onset of lactation (39).

Similarly, in pregnant ewes, plasma insulin concentrations are lower near term (13). This is particularly evident in ewes carrying more than one fetus. The concentrations of KB, acetate and FFA are increased at this time (20, 48). Plasma glucagon concentrations have not been reported during pregnancy. Unpublished observations (R.P. Brockman) from this laboratory indicate that pregnant ewes on a roughage diet have higher plasma glucagon concentrations than ewes fed a

TABLE II

EFFECT OF INSULIN ON REMOVAL OF GLUCOSE FROM BLOOD BY THE MAMMARY GLAND IN THE LACTATING DAIRY COW[a]

	Control	Insulin-Glucose	Glucose
Glucose			
Art. Conc. (mM)	3.70 ± 0.22	3.70 ± 0.14	4.05 ± 0.17
V-A Conc. (mM)	0.75 ± 0.08	0.85 ± 0.14	0.80 ± 0.08
Insulin			
Plasma Conc. (μU/ml)	35 ± 1	95 ± 8	45 ± 7

[a]These data are unpublished observations by B. Laarveld and R.P. Brockman. They are means ± SE of seven experiments. The individual values from each experiment are the means of four sampling times in each two-hour period. Each experiment on a cow consisted of a two-hour control period, a two-hour period during which insulin and glucose were infused simultaneously into the jugular vein at rates of 0.62 mg/h and 0.40 mol/h, respectively, and a two-hour glucose infusion (0.40 mol/h) period. Glucose was infused with insulin to avoid effects by insulin-induced hypoglycemia. V-A conc. (venous-arterial blood glucose concentration differences) were derived from mammary venous and carotid arterial blood samples taken simultaneously.

roughage-grain diet. This suggests that undernourished pregnant ewes may have elevated glucagon levels.

Insulin can enhance the uptake of glucose, acetate and KB (27, 54) by most extrahepatic tissues, particularly muscle and adipose tissues. However, the uptake of glucose by the ruminant mammary gland (25, Table II) and pregnant uterus (46) does not appear to be influenced by insulin. The low insulin concentrations in lactating goats and cows, and in pregnant ewes might decrease glucose utilization by extramammary and extrauterine tissues, sparing it for milk production and fetal growth. Furthermore, low insulin would also a) promote a maximal rate of gluconeogenesis, b) a high rate of lipolysis in adipose tissue and c) hepatic ketogenesis. During physiological ketosis, the mobilization of FFA seems inadequate to support a high rate of hepatic ketogenesis. However, when insulin concentrations are sufficiently low the rate of lipolysis is adequate to provide high levels of FFA. The liver is switched to a high rate of ketogenesis and utilization of KB is reduced; clinical ketosis develops.

It is important to emphasize that while hormonal imbalances may be central to the development of ruminant ketosis, they are secondary to the inability of dietary intake to provide sufficient substrates to meet the demands put on by lactation and/or pregnancy. They represent adjustments by the cow or ewe to mobilize energy stores and/or spare metabolites for use by the mammary gland and/or uterine contents. For the cow, the solution can be simple. Milk production may decrease and lower the demands for gluconeogenesis and lipolysis thus, the disease is self-limiting. For the ewe, the situation is more complex. She cannot stop the fetal demands and without surgical removal of the lamb(s) prematurely, the prognosis is grave.

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LETTER TO THE EDITOR

Myocarditis in Young Dogs Associated with a Parvovirus-like Agent

DEAR SIR:

During the months of September to November 1978, we encountered a previously unrecognized fatal disease of young pups in the 4 to 6 week age range. A total of 25 previously healthy pups among 31 in six litters from Saskatchewan, Alberta and the Northwest Territories died suddenly, usually without a history or clinical evidence of disease. Slight depression occurred for less than a day in a few cases, and several became distressed and were heard to cry out immediately before being found dead.

At necropsy, the major gross lesion found in all cases was pulmonary edema, which, in several instances, lead to a suspicion of interstitial pneumonia, but on histological examination, the pulmonary change could be attributed to edema resulting from cardiac failure.

The outstanding microscopic lesion involved the ventricular myocardium, in which widespread, focally-intense, nonsuppurative inflammation was consistently found, although the degree of mononuclear inflammatory infiltration was variable. Intranuclear inclusion bodies of variable density were recognizable within cardiac myofibers in each of the eleven pups necropsied, but in several they were infrequent and not present in every section. However, in the most severely affected cases, several inclusions were usually evident in each high magnification microscopic field. The most dense inclusion bodies were purple on hematoxylin and eosin stained sections, and were surrounded by a narrow, clear space within the nuclear envelope, but other inclusions were less densely stained and completely filled the nuclei. Inclusion bodies were not found in other tissues of affected pups.

On ultrastructural examination, inclusion bodies contained large numbers of small regular particles which closely resembled parvoviruses, having both empty capsids and complete virions and being of similar size (approximately 20 nm).

No bacterial pathogens were cultured, and no cytopathic agents were isolated in canine kidney cell cultures from various tissues, including myocardium. Microscopic lesions, as described in parvoviral enteritis, were not evident in any of these cases.

Parvoviruses have not been recorded as a cause of myocarditis in dogs, so the recent recognition of these cases in pups, at a time when canine parvoviral enteritis has also been recognized in Canada (1), is of particular interest. We are presently attempting to isolate and identify the parvovirus-like agent observed in this disease.

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