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RUMINANT ADAPTATION TO NEGATIVE ENERGY BALANCE

Influences on the Etiology of Ketosis and Fatty Liver

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Ketosis and fatty liver are diseases associated with negative energy balance (NEB). Negative energy balance is essentially universal among dairy cows in the first few weeks of lactation. In spite of this, the large majority of cows do not develop ketosis or fatty liver. Most cows cope with NEB through an intricate mechanism of metabolic adaptation. Ketosis and fatty liver occur not because of NEB but because of the failure of these adaptive mechanisms. The objective of this article is to review the mechanisms of adaptation to NEB in ruminants and to point out places and reasons why those mechanisms might fail. References were taken as much as possible from studies of ruminant animals, but where necessary references to monogastric species are used. The objective is to give readers an understanding of the pathophysiologic basis of ketosis and fatty liver. Furthermore, it is the intent to point out two potentially important sites of maladaptation, each of which may lead to a different metabolic type of ketosis requiring different therapeutic and prophylactic strategies. It is hoped that this review will create a more meaningful appreciation of the more applied aspects of treatment, management, and prevention of ketosis and fatty liver discussed elsewhere in this issue.

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BODY FUEL ALLOCATION AND CONSERVATION DURING PERIODS OF NEGATIVE ENERGY BALANCE

The body fuels consist of carbohydrates, amino acids, and fats. Adaptation to NEB consists of physiologic shifts in the use and conservation of these fuels. In most herbivorous and omnivorous species, carbohydrates form the primary fuel source during periods of positive energy balance. Storage of carbohydrates in the body is limited, so when dietary carbohydrate intake does not meet the energy needs of the animal, carbohydrate must either be synthesized from other fuels, or an alternative fuel source must be used. Carbohydrates can be synthesized from proteins, but not from fats. Use of carbohydrates for fuel during periods of NEB results in the consumption of body protein, potentially depleting important structural and enzyme proteins. To protect against depletion of body proteins during carbohydrate synthesis, adaptive mechanisms exist to orchestrate the mobilization of reserve energy from fat. Concurrently, carbohydrate is conserved. Metabolic and endocrine controls are necessary to direct these changes in fuel use.

In ruminant animals, the conservation of carbohydrate is especially critical, because rumen digestion leads to the fermentative destruction of carbohydrate, resulting in limited carbohydrate absorption from the gut. This limited carbohydrate supply takes on special significance in animals that are concurrently lactating and in NEB, as is the case for most dairy cows in early lactation. Milk production requires large amounts of carbohydrate for the synthesis of lactose. Lactational carbohydrate demands are met in ruminants by the synthesis of glucose, referred to as *gluconeogenesis*. A major substrate for gluconeogenesis in ruminants is propionic acid, one of the volatile fatty acids arising from rumen fermentation. No other fatty acid, including long-chain fatty acids from body adipose tissue, is capable of supporting gluconeogenesis. Although propionic acid is efficiently converted to carbohydrate, there is still a net loss of dietary carbohydrate associated with rumen fermentation. This is because propionic acid accounts for no more than about a third of the total energy available from fermented carbohydrate, the rest being represented by acetic and butyric acids. These latter two acids cannot support gluconeogenesis. These unique aspects of ruminant metabolism make glucose synthesis and conservation of carbohydrate especially critical aspects of adaptation to NEB in lactating cows. An elaborate control system exists to regulate and facilitate these metabolic adaptations.

THE ROLE OF ORGANS AND TISSUES IN BODY FUEL STORAGE, MOBILIZATION, AND USE ALLOCATION DURING NEGATIVE ENERGY BALANCE

Many of the metabolic adaptations to NEB occur at an organ level. These adaptations involve mobilization of stored energy from energy

deposition sites, shifts in substrate use, and interconversions of metabolic fuels. Adipose and liver tissues are critical sites of metabolic adaptation, but skeletal muscle and the mammary gland are also very important.

Adipose tissue represents the body's reserve of stored energy. It consists of triglyceride-filled cells known as *adipocytes*. *Triglycerides* are molecules consisting of three long-chain fatty acids esterified to a glycerol molecule (Fig. 1). Within the adipocytes, triglycerides are continually being broken down and resynthesized. Breakdown of the triglycerides consists of cleavage of the ester bond, resulting in the release of nonesterified fatty acids (NEFAs). The process of breaking down adipose triglyceride is known as *lipolysis*. The establishment or reestablishment of the ester bond results in the synthesis or resynthesis of triglyceride. Synthesis of triglyceride is known as *lipogenesis*, although this term is also used to describe the synthesis of fatty acids.

Within adipocytes, both lipolysis and lipogenesis are continually occurring, creating a constant cycling of fatty acids from triglycerides to NEFAs and back again, as depicted in Figure 1. While present as NEFAs, fatty acids are available for transport out of the cell and into the blood for use as energy by other tissues. The rate of NEFA release is regulated by the relative rates of lipogenesis and lipolysis. Increased NEFA release can result from an increase in lipolysis or a decrease in lipogenesis. Both

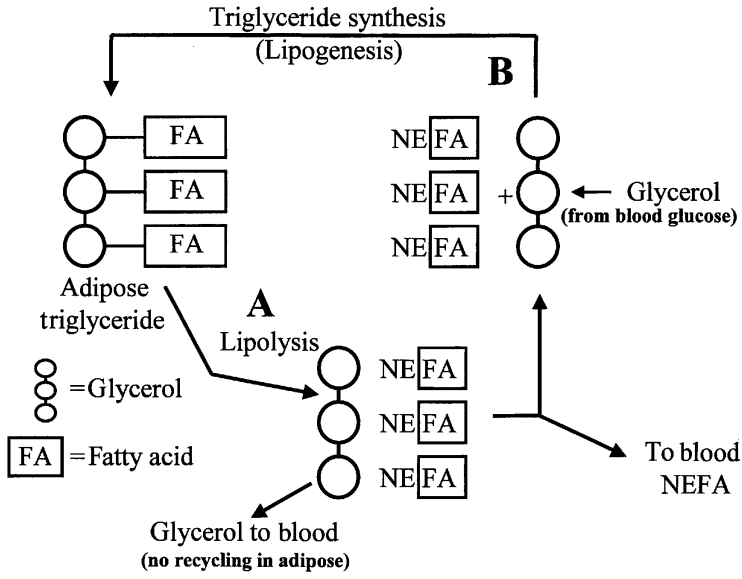

Figure 1. Dynamic recycling of fatty acids (FA) in adipose tissue. The relative rates of lipolysis *(arrow A)* and triglyceride synthesis *(arrow B)* determine the amount of nonesterified fatty acids (NEFAs) available for transport into blood. Fuel-mediated and endocrine effects, as explained in the text, control the activities of arrows A and B.

of these processes are subject to substrate and hormonal controls, as discussed in a further section of this article.

Skeletal muscle also plays an important role in adaptation to NEB and fuel homeostasis. This is partly because muscle is a major site of fuel use. Muscle metabolism can affect blood fuel concentration depending on which fuels are selected for use. During periods of NEB, muscles use fat-derived fuels, including NEFAs and ketone bodies,[10] although ruminants may be less efficient at NEFA use than other species.[53] By shifting fuel sources, glucose use is reduced, aiding in the maintenance of blood glucose concentrations. An additional activity of muscle in the adaptation to NEB is the provision of amino acids. Muscle is the major protein reservoir in the body. During NEB, skeletal muscle proteins are mobilized to support gluconeogenesis. A major aim of the adaptation process is to limit the use of muscle protein for this purpose.

The mammary gland and the fetal-placental unit each play an important role in influencing adaptation to NEB. These tissues cannot shift their fuel needs away from carbohydrates and proteins, in contrast to many other tissues. Glucose and amino acids form the major and obligatory fuel supply of the developing fetus.[8] Glucose is needed by the mammary gland for lactose synthesis, and amino acids are needed for synthesis of milk proteins. The fuel use of the mammary gland and placenta is not subject to insulin influence, a major means by which the body regulates fuel partitioning and allocation. Lactation and late-term gestation place exceptional stress on the body's mechanisms of adaption to NEB.

The liver serves as a linchpin in adaptation to NEB and the maintenance of body fuel supplies. It buffers fluctuations in fuel concentrations in the blood, maintaining a constant supply of fuels to tissues. To do this requires hepatic processing and interconversion of fuels. Hepatic metabolism modifies the distribution and allocation of all important body fuels, including glucose, amino acids, propionic acid, butyric acid, NEFAs, lactic acid, and ketone bodies.

The liver is a key regulator of blood glucose concentration and glucose supply to the tissues. The liver is essentially the only site of gluconeogenesis, although there is a small contribution by the kidney cortex. When the supply of glucose precursors, including propionic and amino acids, is high, glucose production can exceed metabolic demands, and excess glucose is stored in the liver as *glycogen*. During periods of high glucose demand, the liver can mobilize glucose from glycogen to support body needs. The amount of glycogen stored in the liver is relatively small compared to the metabolic demands of the cow, so glycogen reserves do not have a large effect on adaptation to NEB. Glycogen reserves are significant because they reflect the carbohydrate status of the cow.[3] Depletion of glycogen reserves, especially in association with increased liver triglyceride concentration, may increase the risk of clinical ketosis.[16]

The liver also plays a key role in the metabolism of fat and fat-derived fuels, especially during periods of NEB. Negative energy balance results in the release of large amounts of NEFA from adipose tissue.

These NEFAs circulate in the blood and are available as fuels to most body tissues; however, the liver removes a large portion of them.[9, 32, 61] This makes the liver a key modulator of blood NEFA concentration and a regulator of fuel distribution during NEB.

Within the liver, NEFAs can be metabolized to ketone bodies or reesterified for the production of triglycerides. The distribution of hepatic NEFAs into ketogenesis or triglyceride synthesis is a key factor in the adaption of cows to NEB. The major metabolic control point determining the conversion of NEFAs to ketone bodies appears to be the transport of NEFAs into mitochondria, a step necessary for the production of ketone bodies, as further discussed in the following section.

Nonesterified fatty acids that do not enter the mitochondria for oxidation or conversion to ketone bodies are reesterified to form triglycerides. During periods of intense lipid mobilization and high blood NEFA concentrations, large amounts of triglycerides can be synthesized in the liver.[23] Removal of these triglycerides from the liver requires the synthesis and secretion of very low-density lipoproteins, major carriers of triglycerides in blood. Once incorporated into very low-density lipoproteins and secreted into the blood, triglycerides can be used by various tissues for energy or transported to the mammary gland for synthesis of milk fat.

METABOLIC INTERACTIONS OF FUELS IN THE ADAPTATION TO NEGATIVE ENERGY BALANCE

Part of the "check-and-balance" system responsible for regulating the adaptation to NEB in cows comes from specific interactions among the fuels and fuel-using organs and tissues. These interactions are primarily based on the availability and supply of glucose, NEFAs, and ketone bodies.

The relationship of these three metabolites is circuitous, but let's begin the discussion with glucose. When blood glucose concentrations are ample, adipose lipogenesis is favored over lipolysis. This results in suppression of NEFA release from adipose tissue and low blood NEFA concentrations. Much of this effect is probably mediated by insulin, which is discussed below, but a portion of the effect seems to be a direct action of glucose on adipose tissue.[6, 12, 50, 66] The effect of glucose on adipose tissue is probably related to its role in glycerol synthesis. As adipose fatty acids are cycled between NEFA and triglycerides, a constant source of glycerol is needed for triglyceride synthesis. Glucose is the major precursor of adipose glycerol, and its presence increases glycerol availability and favors lipogenesis. As blood glucose concentrations diminish during NEB, NEFA mobilization from adipose tissue is stimulated because of the lack of a source of glycerol.

As blood NEFA concentrations increase in response to low blood glucose, the metabolic effect is to rebalance fuel availability by raising blood glucose concentration. The effect of NEFAs on blood glucose

occurs by several mechanisms. In peripheral (nonhepatic) tissues, NEFAs inhibit glucose use. This appears to result from direct suppression of glucose uptake by cells and from the provision of NEFA as an alternative fuel source.[10]

Glucose and NEFA availabilities play a large and interrelated role in influencing hepatic metabolism and systemic metabolism. To understand the relationship of glucose and NEFA in the liver, it is important to remember that metabolic reactions within hepatocytes are separated into cytosolic and mitochondrial events. The Krebs cycle, like ketogenesis, occurs only within mitochondria. During periods of adequate glucose availability, glucose entry into the Krebs cycle is uninhibited, resulting in more glucose flowing into the Krebs cycle than needed for energy generation. Energy surplus results in a slowing of Krebs cycle activity, resulting in an intramitochondrial accumulation of Krebs cycle intermediates, including citrate, the first intermediate in the cycle. Excess citrate is transported out of the mitochondria, where it is converted to *malonyl CoA,* the first dedicated metabolite in fatty acid synthesis. Malonyl CoA specifically inhibits the activity of the enzyme *carnitine palmityl transferase I* (CPT I). This enzyme is necessary for the transport of NEFA into the mitochondria for ketone body synthesis.[45] The CPT I control point for NEFA entry into mitochondria appears to be functional in cattle as well as other species.[1, 30] The citrate-malonyl CoA-CPT I relationship results in a reciprocal association between carbohydrate status and the rate of hepatic use of NEFAs for ketone body synthesis,[1, 14] as illustrated in Figure 2.

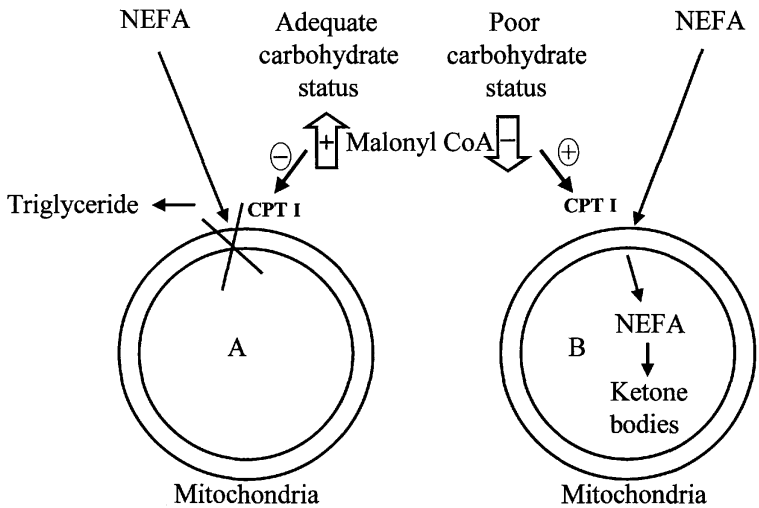

Figure 2. The influence of carbohydrate status on the metabolic fate of NEFAs in the liver. With adequate carbohydrate status, malonyl CoA concentration increases in the cytosol, resulting in the suppression of carnitine palmityl transferase I (CPT I) activity. Consequently, NEFA transport into the mitochondria is reduced. This leads to reduced ketogenesis and increased esterification of NEFAs to form triglycerides, resulting in fatty liver.

In contrast to periods of adequate glucose availability, in NEB, carbohydrate status is poor, especially in the face of high milk production. In such times, the supply of glucose and glucose precursors is limited and little glucose flows into the Krebs cycle, resulting in little or no citrate leaving the mitochondria for malonyl CoA production. Low malonyl CoA concentrations result in activation of CPT I, inducing rapid transport of NEFAs into the mitochondria. Mitochondrial metabolism of NEFAs stimulates the production of both glucose and ketone bodies.[15] These effects are mediated by the actions of acetyl CoA, an intermediate product in the metabolism of NEFAs. Acetyl CoA suppresses glucose use and stimulates gluconeogenesis, although acetyl CoA is not itself a precursor of glucose. Mitochondrial acetyl CoA is a precursor of the ketone bodies, and much of it is metabolized in the mitochondria to acetoacetate, an important ketone body. Acetoacetate leaves the mitochondria and enters the cytosol, where a portion of it is converted to the other major ketone body, beta-hydroxybutyrate, before leaving the liver and entering the general circulation.

In the systemic circulation, ketone bodies serve as an additional fuel source for muscle, further sparing glucose from use as an energy fuel[65] and promoting higher blood glucose concentrations. Furthermore, ketone bodies serve as a feedback regulator of NEFA release by suppressing adipose lipolysis.[21, 50, 52, 55]

The overall adaptive response is illustrated in Figure 3. In review,

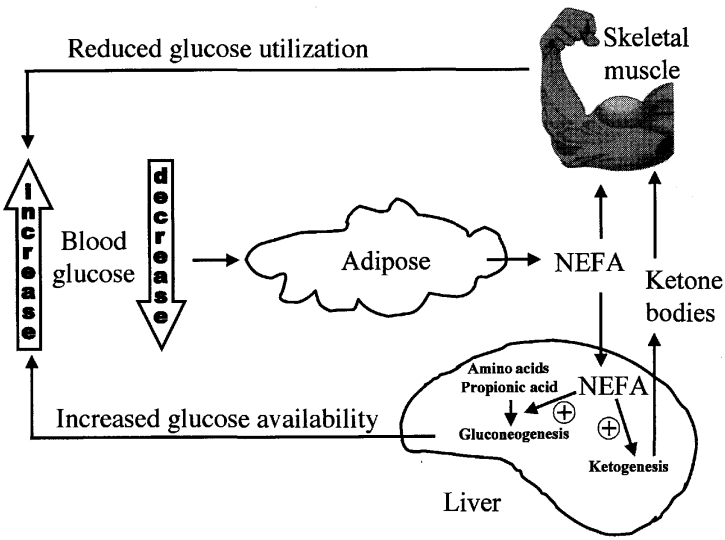

Figure 3. Fuel- or metabolite-mediated feedback in the control of adipose mobilization. The overall effect of this scheme is that a decrease in blood glucose results in reduced glucose use and stimulation of gluconeogenesis, resulting in stabilization of blood glucose at a new set point. Fuel-mediated feedback control of metabolism is not sensitive enough to function without the influence of hormones.

substrate-mediated events initiated by a decline in blood glucose concentration result in mobilization of NEFAs, which in turn results in several actions that stabilize or increase blood glucose. As blood glucose concentrations stabilize, NEFA mobilization is moderated and a new steady state is achieved, with NEFA mobilization rates adjusted to values adequate to maintain blood glucose concentration.

ROLE OF HORMONES AND NEUROREGULATORY SUBSTANCES IN THE ADAPTATION TO NEGATIVE ENERGY BALANCE

Endocrine mediators are essential in the adaptation to NEB because fuel-mediated adaptation is not sensitive enough to function independently; however, endocrine actions primarily serve to control rates of fuel metabolism. The general pattern of metabolic adaptation to NEB, as described above and in Figure 3, is not altered by endocrine influences but is regulated by them.

Insulin is the key metabolic hormone. Its concentration in the blood is influenced primarily by the availability of glucose and glucose precursors such as propionic acid. Insulin increases glucose use by muscle tissue and decreases hepatic gluconeogenesis, resulting in declining blood glucose concentrations. When blood concentrations of glucose and propionic acid fall, as in NEB, so does the blood concentration of insulin.

In addition to its role in glucose and carbohydrate metabolism, insulin has important effects on lipid metabolism. In adipose tissue, insulin stimulates lipogenesis and inhibits lipolysis,[18, 58] resulting in marked suppression of NEFA mobilization. In the liver, insulin results in reduced activity of CPT I, reducing NEFA transport into mitochondria and suppressing ketogenesis.[33, 34] Furthermore, insulin enhances the esterification of NEFAs, resulting in increased triglyceride synthesis.[14, 67]

Insulin's actions in lipid metabolism are powerful and occur at low blood concentrations. This appears to allow insulin to function as a liporegulatory hormone and a glucoregulatory hormone. The insulin liporegulatory circuit is completed by NEFAs[43, 44] and ketone body stimulation of insulin secretion.[5, 11, 26, 51] The liporegulatory circuit of insulin secretion is illustrated in Figure 4.

Glucagon is the primary counter-regulatory hormone to insulin, and it is also important in adaptation to NEB. There is evidence that glucagon stimulates lipolysis in some species, but its primary site of action in ruminants appears to be the liver.[13] The hepatic effect of glucagon is to stimulate gluconeogenesis. In addition, glucagon promotes activation of CPT I, stimulating NEFA entry into the mitochondria for ketone body synthesis. The ratio of glucose to insulin seems to be an important determining factor in hepatic metabolism, with increases in this ratio promoting gluconeogenesis and ketogenesis.[29, 30]

Perhaps the second most important regulatory substances, after insulin, in the control of adipose tissue metabolism are epinephrine and norepinephrine, the catecholamines. These are potent stimulators of

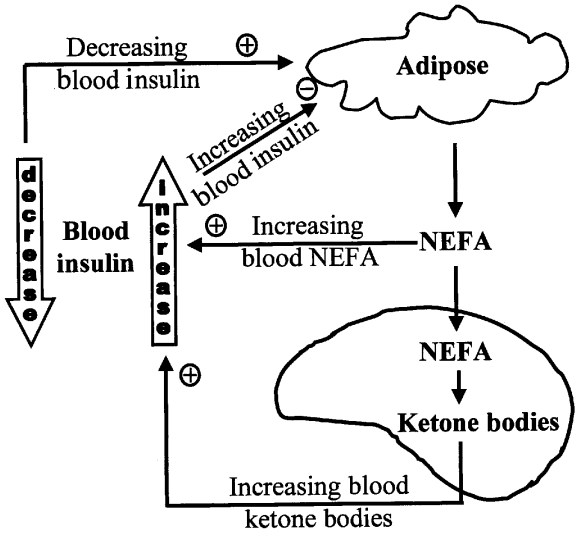

Figure 4. Feedback mechanisms in insulin-mediated control of adipose mobilization. Both NEFA and ketone bodies stimulate insulin secretion, which creates a negative feedback on NEFA release.

lipolysis. Norepinephrine is secreted by sympathetic nerve endings in adipose tissue, resulting in marked release of NEFAs into the circulation. Control of sympathetic nervous activity is by centers in the brain, allowing control of adipose lipolysis by brain centers sensitive to energy balance.[7] Epinephrine from adrenal medullary secretion is also a potent stimulator of adipose lipolysis, indicating that stress can stimulate NEFA secretion.

Growth hormone is an additional fuel regulatory hormone with an important role in the adaptation to NEB. Growth hormone reduces lipogenesis, favoring NEFA mobilization. Growth hormone secretion is stimulated by hypoglycemia, and growth hormone concentrations are naturally high in early lactation in dairy cows compared with cows in later lactation. Other hormones, including leptin, cortisol, and the thyroid hormones, influence metabolism and may affect adaptation to NEB, but their roles do not seem as critical to this process as those of insulin, the catecholamines, and growth hormone.

Our discussion to this point outlines the major mechanisms involved in the adaptation to NEB. The overall effect of these is to shift the body's fuel supply away from glucose and toward the use of lipid energy sources. Lactation and late gestation present special challenges, because these metabolic functions have an obligatory requirement for glucose that cannot be met by other fuels. Nevertheless, many cows are capable of meeting this challenge and are able to sustain high milk yields without developing metabolic disease, even in the presence of substantial NEB.

WHEN ADAPTATION TO NEGATIVE ENERGY BALANCE FAILS

Ketosis and fatty liver represent a failure in the adaptation to NEB. The adaptation system is complex, and failures may occur at various points involving the adipose tissue, liver, or other sites.

Maladaptive Responses of Adipose Tissue

Adaptive responses of adipose tissue may function inappropriately because of alterations in adipose sensitivity. Adipose sensitivity is usually defined as an increase in the lipolytic response to a given stimulus. In in vivo experiments, adipose sensitivity is usually measured by the plasma NEFA response after a measured dose of catecholamine. As adipose sensitivity increases, so does the NEFA response. When adipose tissue is highly sensitive, antilipolytic feedback responses may be blunted, releasing adipose tissue from metabolic and endocrine controls and allowing the rate of NEFA mobilization to go unchecked.

Adipose sensitivity is affected by various factors, some of which are influenced by husbandry and some of which are not. High genetic merit in dairy cows is associated with increased adipose sensitivity.[47, 48, 59] No attempt should be made to select against adipose sensitivity, because such an attempt would result in reduced intensity of selection for milk production. Advancing gestation is another factor leading to increasing adipose sensitivity.[50] Little can be done to prevent this, but it is important to recognize the role this might play in peripartum adaptation to NEB. Other factors influencing adipose sensitivity are more amenable to managerial control.

Adipose mass, or the general fatness of the cow, may be a critical factor affecting adipose sensitivity. In humans, obesity is associated with increased plasma NEFAs, implying increased adipose sensitivity.[20] This increased sensitivity may be partially caused by an expanded adipose tissue mass, with similar rates of lipolysis per adipocyte[28]; however, effects at the adipocyte level are also possible, as discussed below. It is likely that obesity has a similar effect on NEFA mobilization in cattle as it does in humans, although experimental evidence of this, unconfounded by factors such as feed intake, is difficult to locate.

Recent research from the Netherlands demonstrates the potential effects of obesity in cattle on adipose sensitivity. In the Dutch experiments, experimental cows received high-energy diets ad libitum throughout the dry period, and control cows were fed an energy-restricted diet. Cows on the high-energy diet had adipose tissue that was less responsive in vitro to the antilipolytic effects of glucose and ketone bodies compared with restricted-fed controls.[55, 56] This response would reduce the effects of fuel-induced, autoregulatory negative feedback signals on lipolysis, allowing increased NEFA mobilization in obese animals.

Insulin resistance may be another means by which obesity increases

adipose sensitivity in cattle. Obesity results in insulin resistance in ruminants[40–42] and other species. Resistance to insulin is usually measured in reference to the rate of infusion insulin necessary to lower blood glucose concentration. In humans, insulin resistance results in reduced suppression of lipolysis,[27, 36, 57] meaning that the antilipolytic effect of insulin is blunted in insulin-resistant, obese individuals. Limited evidence suggests this is true in cattle as well.[31] If this is generally true in cattle, it means that obese cows have less effective insulin feedback regulation in the adaptive response to NEB and greater adipose sensitivity and NEFA mobilization compared to leaner cows.

Adipocyte cell size may be another important underlying factor in increased adipose sensitivity of obese animals. The size of individual adipocytes increases with adipose mass in ruminants[2, 46, 59] and other species. Large adipocytes are more responsive to lipolytic stimuli than small adipocytes,[64] further evidence that obesity may lead to overly sensitive adipose tissue in cows.

From the above discussion, it appears that a number of potential mechanisms may lead to increased adipose sensitivity in obese cows, resulting in an interruption of homeostatic mechanisms regulating adipose mobilization. This can result in inappropriately high blood NEFA concentrations that overwhelm the capacity of the liver for fuel processing and interconversion.

Maladaptive Responses in the Liver

The hepatic response to NEB is to produce glucose and ketone bodies and take up large amounts of NEFAs from the blood for mobilization as other forms of energy. Maladaptive problems can arise at several points in these metabolic processes.

Failure to Meet Gluconeogenic Demands

The relationship involving glucose use, the gluconeogenic capacity of the cow, and the incidence of ketosis has recently been cast in new light. Swedish researchers have suggested two classifications for ketosis, which they identify as type I and type II.[25] *Type I ketosis* occurs when the demand for glucose outstrips the capacity of the liver for gluconeogenesis. It is proposed that gluconeogenic pathways are maximally stimulated in type I ketosis cases, but the supply of glucose precursors is insufficient to permit maximal glucose production. This hypothesis is supported by the observation that attempts to further stimulate gluconeogenesis in these ketotic animals by supplying exogenous glucagon fail to alleviate the hypoglycemia and clinical signs of ketosis.[24] This suggests that gluconeogenesis is already stimulated maximally and that glucose production is limited only by substrate supply. These observations are consistent with earlier observations using tracer kinetic studies of gluconeogenesis in cows with naturally occurring ketosis. These tracer studies

indicated that the overall rate of glucose formation was not decreased in ketosis.[37]

In type I ketosis, blood glucose and insulin concentrations are very low, resulting in a low insulin/glucagon ratio with high activity of CPT I. Rapid entry of NEFAs into hepatic mitochondria is stimulated by these conditions. This results in high rates of ketogenesis and high blood ketone body concentrations. The extensive use of NEFAs for ketone body synthesis means that little NEFA is converted to triglycerides, resulting in little fat accumulation in the liver, and type I ketosis is characterized by high blood ketone body concentrations and the absence of severe fatty liver. In most cases, type I ketosis occurs 3 to 6 weeks postpartum, near the time of peak lactation. Type I ketosis fits the classical description of "spontaneous ketosis."[4]

The term *type I* is used to describe this variety of ketosis because blood insulin concentrations are very low, making the insulin status similar to insulin-dependent diabetes mellitus, which is frequently called *type I diabetes mellitus*. Individuals with uncompensated type I diabetes mellitus have very high blood ketone body concentrations.

Fatty Liver and Type II Ketosis

According to the scheme of the Swedish researchers Holtenius and Holtenius,[25] *type II ketosis* occurs when large amounts of NEFAs are delivered to the liver, but gluconeogenesis and ketogenesis are not maximally stimulated. Under these circumstances, mitochondrial uptake of NEFA is not as active as in type I ketosis. Nonesterified fatty acids not transported into the mitochondria for ketone body synthesis are esterified in the cytosol, forming triglyceride. Transport of triglycerides from the liver to other tissues requires the synthesis and secretion of very low-density lipoprotein particles; however, the inherent capacity of ruminant liver for synthesis and secretion of very low-density lipoprotein is low.[35, 54] This inherently low rate may be even further suppressed in early lactation,[38, 39] and the capacity of cows for mobilization of triglyceride from their liver is easily overwhelmed when blood NEFA concentrations are high and ketone body synthesis rates are relatively moderate. When this occurs, fatty liver develops.

The effects of fatty liver on the adaptation to NEB are unclear. Some evidence suggests that fatty liver interferes with hepatic gluconeogenic capacity,[14, 63] although conflicting evidence exists.[17, 60] If indeed fatty liver suppresses gluconeogenesis, then fatty liver presents a special challenge to the cow's ability to adapt to NEB. This is because fat mobilization, which normally enhances gluconeogenesis (see Fig. 3), would in the case of fatty liver result in suppressed gluconeogenesis. The ensuing hypoglycemia would then be expected to lead to further adipose mobilization, creating a destructive spiral of events leading to fatty liver of increasing severity. More research is necessary to determine the effect of fatty liver on gluconeogenesis in vivo.

The term *type II ketosis* derives its name from type II diabetes

mellitus. Holtenius and Holtenius[25] propose this terminology because they contend that type II ketosis is associated with hyperglycemia, hyperinsulinemia, and insulin resistance, similar to the metabolic conditions in type II diabetes mellitus. This appears to have created some resistance in the veterinary research community to the acceptance of their classification scheme, because very few cows with ketosis are hyperglycemic and hyperinsulinemic at the time that ketosis is diagnosed; however, I think this should not detract from the possibility that different metabolic lesions in the scheme of adaptation to NEB may lead to distinctly different types of bovine ketosis. The period of hyperinsulinemia associated with type II ketosis may occur prior to the development of clinical signs, as suggested by the data of Rukkwamsuk et al.[55, 56] In their experiments, prepartum insulin concentrations were increased ($P = 0.15$) in cows fed high-energy diets throughout the dry period compared to cows on lower-energy diets. Blood insulin concentrations were similar after calving, but serum NEFA and liver triglyceride concentrations were greater in the group fed high-energy diets during the dry period. These observations are complemented by those of Meirhaeghe et al,[49] in which ketotic cows may demonstrate insulin resistance even in the face of low serum insulin concentrations. The period of hyperglycemia and hyperinsulinemia that is predicted for type II ketosis might precede the development of clinical signs. This period of hyperinsulinemia could then lead to the insulin resistance, increased adipose sensitivity, and relatively high rates of hepatic NEFA esterification (triglyceride synthesis) characteristic of type II ketosis and fatty liver.

There are important differences in the clinical presentation and management of these two forms of ketosis. Fatty liver and type II ketosis are usually seen clinically shortly after parturition, well before peak milk production. The blood ketone body concentrations are usually lower and the blood glucose concentrations higher in type II ketosis than in type I ketosis. The clinical response to treatment is usually less dramatic and less favorable in type II ketosis than in type I.[22] The occurrence of type II ketosis close to calving probably reflects its association with adipose sensitivity, which begins to increase with approaching parturition. The important role of adipose sensitivity and mobilization in the cause of fatty liver and type II ketosis is illustrated by the data of Gerloff et al[19] and of Vazquez-Anon et al.[62] These studies show that fat accumulation in the liver begins to occur in the last weeks of gestation, as adipose sensitivity increases. Factors influencing adipose sensitivity and energy balance in the late dry period are important concerns in the prevention of fatty liver and type II ketosis, as discussed in detail in the article by Gerloff elsewhere in this issue.

In conclusion, adaptation to NEB involves metabolic interrelationships between the energy storage site in the adipose tissue, the fuel processing site in the liver, and the energy use sites in skeletal muscle, mammary gland, placenta, fetus, and other tissues of the body. Normally, these interrelationships function to allow the cow to sustain a period of NEB in early lactation; however, problems can occur at several steps in

the adaptation process, leading to ketosis and fatty liver. Therapeutic and prophylactic intervention should be targeted at specific sites of maladaptation, which are different in different forms of ketosis.

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