Veterinary Bioscience: Metabolism

LECTURE 8
BOVINE KETOSIS AND OTHER FATTY LIVER SYNDROMES IN DOMESTIC ANIMALS

INTENDED LEARNING OUTCOMES

At the end of this lecture, you should be able to:

- describe the normal biochemical processes that occur with fasting/starvation and the central role of the liver in these processes.
- explain how ruminants derive energy from complex carbohydrates.
- describe the metabolic changes associated with ketosis and fatty liver disease.

PHYSIOLOGY OF NEGATIVE ENERGY BALANCE

Fed state

The fed state represents the presence of abundant nutrients, including glucose and amino acids, in the bloodstream. Insulin is secreted by pancreatic β-cells in response to postprandial hyperglycaemia, the effect of which is to stimulate the storage of fuels and synthesis of proteins. Insulin stimulates glycogen synthesis in both the liver and skeletal muscle and suppresses gluconeogenesis by the liver; it also promotes the uptake of fatty acids into adipose depots and has a stimulating effect on protein synthesis.

Negative energy balance

Negative energy balance can refer to any situation in which energy expenditure exceeds energy intake. This can be due to insufficient food intake (e.g. fasting or starvation) or due to intense energy utilisation (e.g. disease states; pregnancy/lactation; physical exertion).

Early fasted state

Blood glucose levels decrease several hours after a meal, leading to a decrease in insulin secretion and an increase in glucagon secretion. Glucagon is secreted by pancreatic α-cells in response to low blood glucose concentrations, the effect of which is to mobilise glycogen stores (glycogenolysis) when there is no dietary intake of glucose. The main target of glucagon is the liver – glycogen stored in skeletal muscle is not available for release of glucose into the bloodstream. The entry of glucose into peripheral tissues also decreases due to reduced insulin concentrations.

The liver has a finite capacity to store glucagon, which will become depleted in around 24 hours. Beyond this, other metabolic processes are necessary to sustain basal energy requirements.

Prolonged fasting, starvation or intense energy demand

The *first priority of metabolism in starvation is to provide sufficient glucose to the brain* and other tissues (such as red blood cells) that are absolutely dependent on glucose for energy. Even under starvation conditions, blood glucose levels must be maintained above a certain threshold (2.2mmol/L) to prevent clinical signs of hypoglycaemia (e.g. depression, seizures, coma).

Both skeletal muscle and liver use fatty acids for energy when the blood glucose levels drop – glucagon stimulates lipolysis and inhibits lipogenesis. Thus, a constant blood glucose level is maintained by three major factors: (1) glycogenolysis and the release of glucose by the liver, (2) the release of fatty acids by adipose tissue, and (3) a shift in the predominant energy substrate used from glucose to fatty acids in tissues such as skeletal muscle and the liver.

The metabolic changes on the first day of starvation are like those after an overnight fast. Low blood glucose levels lead to decreased secretion of insulin and increased secretion of glucagon. The dominant metabolic processes are the mobilisation of triacylglycerols in adipose tissue and gluconeogenesis by the liver. The liver obtains energy for its own needs by oxidising fatty acids released from adipose tissue. The uptake of glucose by skeletal muscle and other tissues is markedly diminished because of the low insulin level, whereas fatty acids enter freely. Consequently, these tissues shift almost entirely from glucose to fatty acids for fuel. The β-oxidation of fatty acids halts the conversion of pyruvate into acetyl-CoA, reducing the rate of glycolysis and thereby preserving glucose for the brain.

Once glycogen stores in the liver have been exhausted, glucagon continues to promote gluconeogenesis (from glycerol, pyruvate/lactate, and glucogenic amino acids). However, precursors of glucose are not abundant. Glycerol released from adipose tissue during lipolysis provides some of the carbons for gluconeogenesis, with the remaining carbons mostly coming from the hydrolysis of muscle proteins. Proteins are not stored, so any breakdown will necessitate a loss of function. Therefore, the *second priority of metabolism in starvation is to preserve protein*, which is accomplished by shifting the fuel being used from glucose to fatty acids and ketone bodies.

Ketone bodies (acetone, acetoacetate, β-hydroxybutyrate) are an important source of energy during severe or prolonged negative energy balance. Their synthesis from acetyl-CoA increases markedly because the TCA cycle is unable to oxidise all the acetyl units generated by the degradation of fatty acids. Gluconeogenesis depletes the supply of oxaloacetate, which is essential for the entry of acetyl-CoA into the TCA cycle. Consequently, the liver produces large quantities of ketone bodies, which are released into the blood as an alternative energy source for many tissues. After several days, the brain begins to consume appreciable amounts of ketone bodies and less glucose.

How long an animal can sustain these processes during a period of prolonged negative energy balance will depend on the size of the triacylglycerol depot. Once triacylglycerol stores are depleted, the only source of fuel that remains is proteins. Protein degradation accelerates, and death will inevitably result from a loss of heart, liver and/or kidney function, if hypoglycaemia and coma do not occur earlier.

The refed state

When refed following a period of fasting or starvation, the liver does not initially absorb glucose from the blood, but rather leaves it for the peripheral tissues. The liver remains in a gluconeogenic mode, with newly synthesised glucose being used to replenish the liver's glycogen stores. As blood glucose levels continue to rise, the liver completes the replenishment of glycogen stores and begins to process the remaining excess glucose for fatty acid synthesis. Otherwise, absorbed fat and amino acids are processed exactly as it is processed in the normal fed state.

RUMINANT METABOLISM

How ruminants derive energy from food

Large amounts of volatile fatty acids (VFAs) are produced through microbial fermentation in the rumen. They are of critical importance as they provide 70-80% of a ruminant's energy supply. The acetate (acetic acid), propionate (propionic acid) and butyrate (butyric acid) formed in the rumen are rapidly absorbed across the ruminal epithelium, through a process of diffusion down a concentration gradient. Ruminal veins then carry absorbed VFAs to the liver via the portal vein. Acetate and propionate pass through the epithelium largely unchanged, but almost all butyrate is metabolised in the ruminal epithelium to β-hydroxybutyrate, a type of ketone body.

The three major VFAs absorbed from the rumen have somewhat distinctive metabolic fates:

1. **Acetate (2-C; ≈70% VFAs):** There is minimal uptake of acetate by the liver, which then circulates throughout the body to be oxidised in many tissues (via acetyl-CoA) for energy production. Acetate is also the main precursor for *de novo* fatty acid synthesis, for storage in adipose tissue or secretion via the mammary gland.
2. **Propionate (3-C; ≈20% VFAs):** Almost all propionate is taken up by the liver, where it serves as the principal substrate for gluconeogenesis. This is critical, as almost no glucose reaches the intestines in ruminants. Glucose production occurs via the methylmalonyl pathway, which requires several cofactors such as biotin (vitamin B7), magnesium, cobalamin (vitamin B12) and cobalt, which can explain why deficiency of these vitamins/minerals can lead to illthrift in ruminants.
3. **Butyrate (4-C; ≈10% VFAs):** Almost all butyrate is metabolised in the ruminal epithelium to β-hydroxybutyrate, a type of ketone body, which then circulates throughout the body to be oxidised in many tissues for energy production (via acetyl-CoA).

Negative energy balance in ruminants

Next, we will discuss examples of disease syndromes associated with negative energy balance in ruminants. Note that at DVM1 level we are most interested in the pathophysiology of disease. We will mention some of the clinical aspects such as clinical examination findings, diagnostics and treatment, but the focus should remain on *principles* rather than a detailed understanding of clinical medicine in practice.

Ketosis in dairy cows

Ketosis is a relatively common disease of high producing dairy cows, which typically occurs in early lactation (4-6 weeks postpartum), characterised by partial anorexia and depression. Ketosis results from negative energy balance associated with high glucose demand and intense fatty acid mobilisation, with a mismatch often occurring between peak lactation and peak feed intake. During periods of intense gluconeogenesis, a large portion of circulating fatty acids will be directed to ketone body synthesis in the liver. Clinical subclassifications of bovine ketosis (type I or type II) can be made but are beyond the scope of this lecture.

The clinicopathological characterisation of ketosis is based on high concentrations of serum fatty acids (NEFA) and ketone bodies (β-hydroxybutyrate most commonly measured; can also be measured in urine and milk) and low blood glucose concentrations. Cows with ketosis are generally found to be dull and inappetent and may be dehydrated with a poor milk yield. The principles of treatment are aimed at restoring normoglycaemia and reducing serum ketone body concentrations, to reverse the negative energy balance. This can include the administration of glucose (via intravenous bolus/infusion) and provision of gluconeogenic substrates, such as propylene glycol administered orally, which will be metabolised to pyruvate. Prevention is aimed at ensuring appropriate body condition and optimising nutrition of cows in late-pregnancy and early-lactation to ensure that energy supply will meet demand.

[See case example presented in lecture slides for illustration of the above concepts in practice.]

OTHER FATTY LIVER SYNDROMES

Pregnancy toxaemia

Pregnancy toxaemia (pregnancy ketosis; 'twin lamb disease'; fatty liver disease) is a manifestation of ketosis primarily associated with inadequate nutrition during late gestation, when there is intense energy demand from the growing foetus(es). Energy requirements of ewes in late pregnancy can increase by 23% with a single lamb, 36% for twins or 42% with triplets. Pregnancy toxaemia (a severe clinical manifestation of ketosis) is associated with either low energy density of feed and/or decreased rumen capacity due to the enlarged uterus.

In late gestation, the liver increases gluconeogenesis to preferentially deliver glucose to the developing foetus(es) via the placental circulation. Mobilisation of fatty acids from adipose depots will be occurring to assure adequate energy delivery. However, when negative energy balance occurs, the rapid mobilisation of fatty acids can overwhelm the liver's capacity and result in hepatic lipidosis, with subsequent impairment of function. [See Lecture 7 for further detail related to the pathology of fatty liver disease.] Ewes or cows with twin pregnancies, and especially if overconditioned, appear to have more difficulty producing glucose and clearing ketone bodies, which increases their risk of pregnancy toxaemia. Affected animals will therefore have low blood glucose concentrations and high serum concentrations of ketone bodies and fatty acids (NEFA).

The clinical signs of pregnancy toxaemia can include depression and inappetence, weakness and recumbency, and sudden death. The principles of treatment are as described for bovine ketosis (glucose, propylene glycol) with an additional consideration of inducing premature parturition to relieve the intense energy demand. Treatment of advanced cases of pregnancy toxaemia, especially once recumbent, is frequently unrewarding, so prevention through appropriate management of body condition and ensuring optimal nutrition is vital.

Hepatic lipidosis in horses

Horses have a poorly developed pathway of hepatic ketogenesis, so they do not develop overt ketosis like ruminants, which makes for an interesting contrast. Instead, the fatty acids mobilised from adipose depots during negative energy balance (e.g. starvation, systemic illness, pregnancy/lactation) are directed to VLDL synthesis. This process can become rapidly overwhelmed and result in hyperlipaemia, in which the serum can take on a grossly lipaemic (opalescent) appearance. Ponies are especially predisposed to hyperlipaemia and subsequent hepatic lipidosis. Clinical signs include depression and inappetence, weakness and recumbency. Principles of treatment include the administration of glucose solution (no propylene glycol since they are not ruminants) and provision of high-energy feed to reverse the negative energy balance.