

Use of Glucagon to Prevent and Treat Fatty Liver in Transition Dairy Cows

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Summary and Implications

Because of the relationship of fatty liver to increase health problems and decreased productive and reproductive performance, scientists can improve the profitability of dairy farmers by developing nutritional and management technologies for preventing and treating fatty liver. Our research group has demonstrated that glucagon shows much promise for use in preventing and treating fatty liver in transition cows. Moreover, we have data to indicate that ultrasound technology can be used to estimate the incidence of fatty liver within a dairy herd. The development of a slow-release form of glucagon would seem necessary before adoption of our proposed glucagon technology is adopted by the dairy industry.

Introduction

Fatty liver is a major metabolic disease of animals and humans. The disorder occurs when the uptake and de novo synthesis of fatty acids exceeds the oxidation and secretion of fatty acids in the liver. The excess fatty acids accumulate in the liver as triacylglycerols (TAGs). This accumulation is of significance because it negatively affects critical metabolic functions of the liver. In dairy cattle, this condition is known as fat cow syndrome or fatty liver. Fatty liver occurs primarily in the first four weeks after calving and may lead to development of lactation ketosis. Although data are limiting, up to 50% of all cows have an accumulation of TAG in liver. What is the primary event that initiates this accumulation of TAG in liver? The primary cause is that the energy intake of postparturient (transition) dairy cows is insufficient to meet the demands for maintenance and for the increasing lactation. This energy deficit, and thus glucose shortage, causes a series of metabolic adaptations that result in markedly greater rates of mobilization of fatty acids from adipose tissue to liver and other organs, resulting in an increased probability of fatty liver development.

Severity of fatty liver can be defined on the basis of amount of TAG accumulation. My colleagues and I commonly categorize fatty liver into normal liver (< 2% TAG), mild (2-5% TAG), moderate (5-10% TAG), and severe (> 10% TAG) fatty liver. Total lipid content of liver would be about 2% greater because of the presence of phospholipids, cholesterol, and cholesteryl esters. Knowing the severity of fatty liver in cows at any given time can only be determined by collecting a sample of liver by biopsy and by performing a chemical analysis of that sample. Our research group has been studying a noninvasive procedure involving ultrasonography. To date, results for use of commonly available ultrasound instruments to estimate degree of fatty liver development seem promising.

The reason to be concerned about fatty liver development is that fatty liver has detrimental effects on health status, well-being, productivity, and reproductive performance of cows. Thus, fatty liver costs U.S. dairymen millions of dollars each year in lost income because of increased veterinary costs, longer calving intervals, decreased milk production, and decreased average lifetime. Our research group has studied the pathology and etiology of fatty liver and ketosis, metabolic diseases that often result from fatty liver, for over 20 years to provide a basic understanding of the disorders and to find possible treatments and preventatives. Our most recent research has led to the development of the technology of using the pancreatic hormone called glucagon as a treatment and a preventative.

Etiology of Fatty Liver

Although much remains to be learned about all of the causes (etiology) of fatty liver, the major metabolic pathways that are altered during fatty liver development are illustrated in Figure 1. As stated earlier, the energy or glucose deficit that frequently occurs during the early postparturient period causes a marked increase in the mobilization of fatty acids (NEFA; nonesterified fatty acids) from the stored TAG in the adipose tissue. The NEFA diffuse into the blood and provide energy to tissues throughout the body. Many NEFA diffuse into the liver where they provide energy via oxidation to CO₂ for liver function. If more NEFA arrive at the liver than needed for energy purposes, the excess may be oxidized incompletely

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and generate ketone bodies (β -hydroxybutyrate, acetoacetate, and acetone) for release to the blood. Ketone bodies are oxidized as a fuel by all muscles of the body. If ketone bodies accumulate in blood because of overproduction, symptoms of lactation ketosis begin to appear. Excess NEFA in liver also are converted to TAG for disposition. Excessive NEFA disposal by TAG synthesis is protective to the liver because excessive NEFA in liver cells disrupts normal liver function. Under normal conditions, TAG are secreted from the liver as very-low-density lipoproteins (VLDL). Inadequate secretion of VLDL, however, contributes to development of fatty liver. An additional metabolic adaptation during the postparturient energy deficit period is that amino acids (dietary and mobilized) that serve as glucose precursors may become limiting for VLDL synthesis, further contributing to fatty liver development.

In summary, the previously mentioned metabolic changes occur in response to a number of changes in secretion and concentrations of hormones and growth factors such as insulin, glucagon, and glucocorticoids that are caused primarily by the postparturient energy deficit. Theories have been proposed that a glucose deficiency is the initial metabolic change that causes the hormonal and metabolic changes that result in fatty liver development.

Our recent glucagon research resulted in us becoming the first to develop a cure or treatment for bovine fatty liver by using continuous 14-day intravenous infusions of glucagon. We also developed a preventative for fatty liver by using subcutaneous injections of glucagon every 8 hours for 14 days starting on day 2 postpartum (Nafikov et al., 2002). First, I will describe our experiments that demonstrated that glucagon is an effective treatment for bovine fatty liver. Then, I will describe the experiment that indicates glucagon is a preventative of fatty liver in dairy cows.

Treatment of fatty liver by glucagon

An experiment was designed to test the ability of glucagon to alleviate (or treat) fatty liver in early postparturient (transition) dairy cows. Twenty multiparous (two or more lactations) Holstein cows were fed about 5 kg of cracked corn daily in addition to their normal diet during the final 30 days before calving to increase susceptibility to fatty liver. From 14 to 42 days postpartum, all cows were fed 80% of their expected feed intake and were fed up to one liter of 1,3-butanediol to induce fatty liver and ketosis. To test glucagon as a treatment for fatty liver, either glucagon at 10 mg per day or carrier (control) was infused continuously via the jugular vein from 21 to 35 days postpartum. All cows had fatty liver at 14 days postpartum and had elevated concentrations of ketone bodies and lower glucose concentrations in plasma during the induction of fatty liver and ketosis. Glucagon increased the glucose concentration in blood by about 1.4 fold throughout the 14-day treatment period. The increased glucose in blood

occurred because of increased rates of mobilization of glucose from liver glycogen to the blood increased rates of glucose synthesis from 1) primarily propionate derived from the rumen and 2) from amino acids derived from the digestive tract, and 3) from body proteins. Insulin concentrations were abnormally low during the treatment period, and glucagon concentration was elevated as expected. The concentrations of β -hydroxybutyrate and NEFA in plasma were decreased by glucagon.

But, what happened to the concentration of lipid in the liver during the early postpartum period? At six days postpartum, liver TAG averaged 12.9% of liver weight (Figure 2).

Glucagon had decreased the TAG content of livers by 71% at 35 days postpartum or after 14 days of glucagon treatment. The concentration of TAG in the liver of control cows had not changed during the same 14-day period. Therefore, our results document that glucagon decreases the degree of fatty liver in early lactation cows, which also decreases the incidence of ketosis after alleviation of fatty liver. For commercialization of glucagon treatment for fatty liver, however, a slow-release preparation of glucagon would be much more desirable than the infusion procedure of our experiment.

Prevention of fatty liver by glucagon

Our research group hypothesized that, if glucagon would increase the rate of removal of TAG from liver (treatment), it also would lessen the increase in TAG during the early postparturient period. An experiment was designed whereby we administered glucagon at day 2 postpartum to determine whether glucagon lessened or prevented TAG accumulation. Twenty multiparous Holstein cows were selected during their dry period. They were fed supplemental cracked corn in addition to their normal diet for the last 30 days before calving to increase the probability that cows would develop pathological fatty liver during the early postpartal period. At parturition, they were assigned randomly to one of three treatment groups of eight cows in each. Each cow was injected subcutaneously with either 0.15 M sodium chloride solution (saline), 7.5 mg of glucagon, or 15 mg of glucagon daily for 14 days starting at day 2 postpartum. The daily dosage of glucagon was divided into three dosages that were injected subcutaneously between the third and fourth rib every 8 hours to maintain an elevated concentration of glucagon throughout the day for the 14-day treatment period.

Glucagon at both dosages increased blood glucose concentrations but did not alter NEFA concentrations in blood. The elevation in blood glucose was expected, but the lack of effect of glucagon at our chosen dosages on plasma NEFA was unexpected because of the well documented observation that glucagon promotes fatty acid mobilization from adipose tissues of nonruminant animals. In fact, NEFA concentrations tended to decrease because of the

glucagon administration to dairy cows. We observed that glucagon administered at day 2 postpartum prevented the accumulation of total lipids in liver of the transition dairy cows during the first 2 weeks after calving (Figure 3). Both dosages had similar effects. Currently, we are evaluating the interaction of feeding glycerol, a glucose precursor, with glucagon as a preventative of fatty liver in the transition dairy cow.

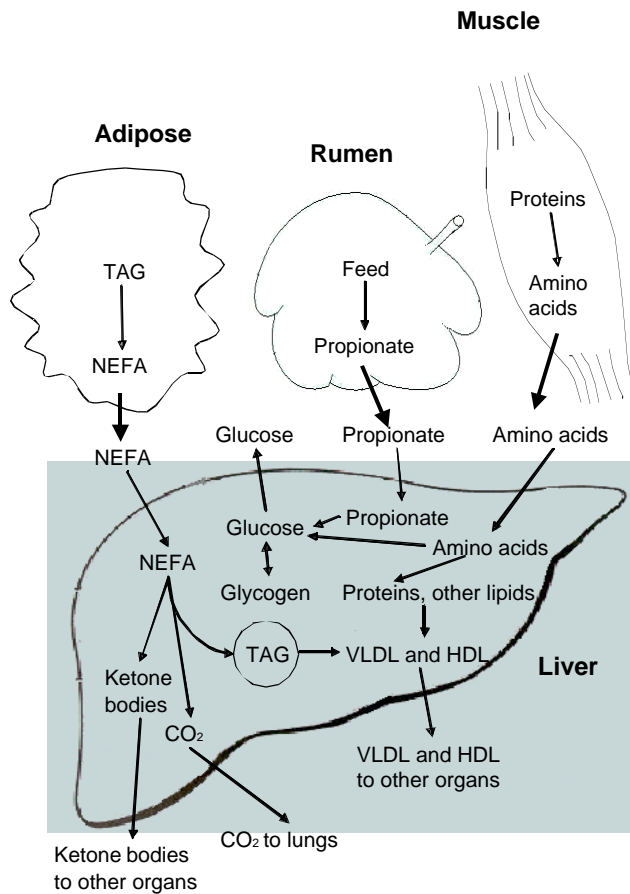


Figure 1. Etiology of fatty liver. TAG = triacylglycerol, NEFA = nonesterified fatty acid, VLDL = very-low-density lipoproteins, and HDL = high-density lipoproteins.

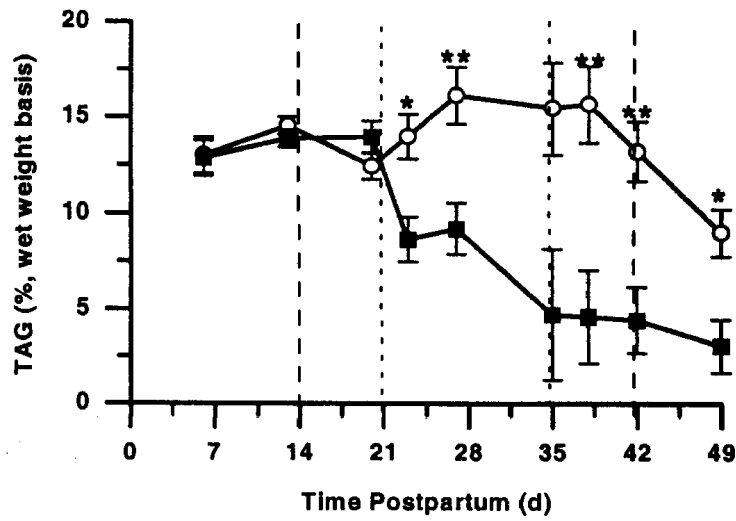


Figure 2. Treatment of fatty liver by glucagon. Data for control cows are indicated by open (o) circles; those for glucagon-treated cows are indicated by closed (●) circles. Ketosis induction period began on day 14 and concluded on day 42. Glucagon infusion occurred from day 21 to day 35.

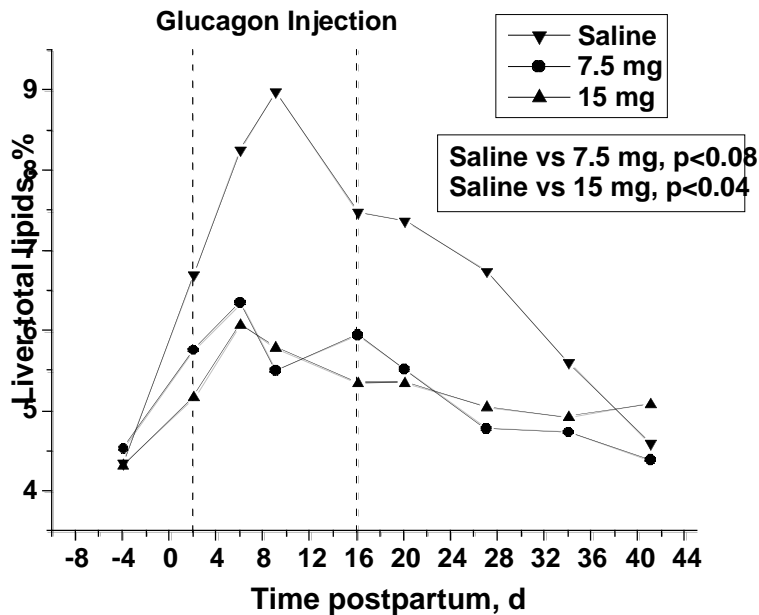


Figure 3. Prevention of fatty liver by glucagon. Glucagon (7.5 and 15 mg daily) was injected subcutaneously three times per day from day 2 to day 16.