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The Role of Exogenous Insulin in the Complex of Hepatic Lipidosis and Ketosis Associated with Insulin Resistance Phenomenon in Postpartum Dairy Cattle

A. Hayirli*
Department of Animal Nutrition and Nutritional Disorders, School of Veterinary Medicine, Atatürk University, Erzurum 25700, Turkey
**Correspondence: E-mail: ahayirli_2000@yahoo.com; ahayirli@atauni.edu.tr*

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ABSTRACT

As a result of a marked decline in dry matter intake (DMI) prior to parturition and a slow rate of increase in DMI relative to milk production after parturition, dairy cattle experience a negative energy balance. Changes in nutritional and metabolic status during the periparturient period predispose dairy cattle to develop hepatic lipidosis and ketosis. The metabolic profile during early lactation includes low concentrations of serum insulin, plasma glucose, and liver glycogen and high concentrations of serum glucagon, adrenaline, growth hormone, plasma β-hydroxybutyrate and non-esterified fatty acids, and liver triglyceride. Moreover, during late gestation and early lactation, flow of nutrients to fetus and mammary tissues are accorded a high degree of metabolic priority. This priority coincides with lowered responsiveness and sensitivity of extrahepatic tissues to insulin, which presumably plays a key role in development of hepatic lipidosis and ketosis. Hepatic lipidosis and ketosis compromise production, immune function, and fertility. Cows with hepatic lipidosis and ketosis have low tissue responsiveness to insulin owing to ketoacidosis. Insulin has numerous roles in metabolism of carbohydrates, lipids and proteins. Insulin is an anabolic hormone and acts to preserve nutrients as well as being a potent feed intake regulator. In addition to the major replacement therapy to alleviate severity of negative energy balance, administration of insulin with concomitant delivery of dextrose increases efficiency of treatment for hepatic lipidosis and ketosis. However, data on use of insulin to prevent these lipid-related metabolic disorders are limited and it should be investigated.

Keywords: insulin resistance, insulin therapy, hepatic lipidosis, ketosis, dairy cows

Abbreviations: A, acidic; apoB, apolipoprotein B; B, basic; BCS, body condition score; BHBA, β-hydroxybutyrate; BW, body weight; CPT-I, carnitine palmitoyltransferase-I; C-peptide, connecting peptide; DMI, dry matter intake; FA, fatty acid; G1P, glucose-1-phosphate; G6P, glucose-6-phosphate; GTT, glucose tolerance test; GLUT, glucose transporter; GLY, glycogen; GH, growth hormone; hCG, human chorionic gonadotrophin; IRS, insulin receptor substrate; LPL, lipoprotein lipase; MW, molecular weight; NEFA, nonesterified fatty acid; NEL, net energy for lactation; NFC, non-fibre carbohydrates; PI3, phosphoinositol phosphate kinase; RRI, rapid-release insulin; RER, rough endoplasmic reticulum; SRI, slow-release insulin; TCA, tricarboxylic acid; TG, triglyceride; VFA, volatile fatty acids; VLDL, very low-density lipoprotein

INTRODUCTION

The periparturient period, from 3 weeks prior to parturition through the first 4 weeks of subsequent lactation, is characterized by metabolic and endocrine changes resulting from negative energy balance. In addition to shifting of metabolism towards catabolism, the flow of nutrients is primarily directed to the fetus and mammary gland during late prepartum and

early postpartum periods (Baird, 1981). This selectivity is associated with the insulin resistance phenomenon by extrahepatic tissues (Kronfeld, 1982; Sano *et al*., 1991, 1993), which may also be linked to the aetiology of hepatic lipidosis and ketosis (Holtenius, 1993; Steen *et al*., 1997). The aims of this review are to describe the relationship between the insulin resistance phenomenon and the aetiology of lipid-related peripartum hepatic lipidosis and ketosis and to discuss the feasibility of exogenous insulin use for their prevention and treatment.

INSULIN METABOLISM

Pancreas

The pancreas is located in the upper right quadrant of the abdominal cavity in close proximity to the duodenum. The pancreas is composed primarily of parenchyma with little stroma. The parenchymal tissue serves both endocrine and exocrine functions and plays an important role in regulations of nutrient metabolism during pre- and postprandial phases (Hsu and Crump, 1989). As an exocrine gland, the pancreas secretes enzymes (lipase and amylase) and pancreatic juice (water, sodium, chloride, and bicarbonate), which are required for breakdown of nutrients so that they can be absorbed across the epithelial cells of the small intestine (Johnson, 1997). As an endocrine gland, the pancreas secretes peptide hormones, such as insulin from β cells, glucagon from α cells, somatostatin from δ cells, and pancreatic polypeptide from F cells (Hsu and Crump, 1989). These endocrine cells constitute 1–3% of the total pancreatic mass and are located in the clusters of cells that are known as islets of Langerhans (Hadley, 1996). In humans, there are approximately one million islets. The proportions of β, α, δ, and F cells are about 60%, 30%, 8%, and 2%, respectively, of approximately 2500 cells in each islet (Hsu and Crump, 1989; Berne and Levy, 1993).

Biosynthesis and structure

Insulin biosynthesis is a complex event that is characterized by formation of preproinsulin and proinsulin (Hadley, 1996). The insulin gene on human chromosome 11 is a member of genes coding for a variety of growth factors. The transcription of mRNA is the initial step of insulin biosynthesis and takes place in the nucleus. This follows translation in the rough endoplasmic reticulum (RER) for the formation of preproinsulin on the cytosolic polysomes. Preproinsulin (insulin precursor) is composed of acidic (A) and basic (B) chains and a peptide chain connecting the A and B chains, which is known a connecting peptide (C-peptide). Proinsulin is formed after cleavage of arginine-arginine and lysine-arginine residues of C-peptide at positions 23 and 24 by trypsin-like and caboxypeptidase-like enzymes in the RER and formation of disulphide linkage between the A and B chains. After the proinsulin is transferred from the RER to the Golgi apparatus, C-peptide is removed in the secretory granules. Proinsulin is the definitive form of insulin and is stored in the cytosol. Although insulin produced by different species has similar chemical structure, the amino acid sequence of C-peptide is highly variable across species (Hadley, 1996).

Insulin consists of two peptide chains, A and B, which are linked by two disulphide bridges between position 7 of the A and B and between position 20 of the A and position 19 of the B

chains. The A and B chains are composed of 21 and 30 amino acids, respectively. There are only minor differences in chemical structures of insulin secreted by mammalians (human, cattle, sheep, pig, horse, rabbit, and dog). For instance, threonine located in position 30 of the B chain in human insulin ($C_{257}H_{383}N_{65}O_{77}S_6$, MW of 5807.7) is replaced with alanine in porcine ($C_{256}N_{381}N_{65}O_{76}S_6$, MW of 5777.6) and bovine insulin ($C_{254}H_{377}N_{65}O_{75}S_6$, MW of 5733.6). Also, threonine and isoleucine in positions 8 and 10 of the A chain in human insulin are replaced with alanine and valine in bovine insulin (Hsu and Crump, 1989).

Signal transduction

Signal transduction is a very complex intracellular event for release of insulin and occurs in three stages: phosphorylation of insulin receptors, intracellular signalling of the secondary messengers, and translocation of glucose transporters (GLUT). The insulin receptor is a dimer of α and β subunits. The α subunit is located on surface of the cell and has the insulin-binding domain, whereas the β subunit is located inside the cell and has the tyrosine kinase domain (Kahn, 1994). Insulin binding to the α subunit causes autophosphorylation by ATP of the β subunit, which generates tyrosine kinase activity in the cell. Upon phosphorylation of the β subunit, the hormone–receptor complex is internalized. Internalization is the major stage where obesity or hyperinsulinaemia causes downregulation of insulin receptors through increasing degradation rates of insulin and its receptor (Berne and Levy, 1993; Hadley, 1996). Following insulin internalization, intracellular secondary messengers are propagated in a chain reaction through serine phosphorylation and dephosphorylation cascades. These messengers are specific for the final biological effects of insulin (Figure 1). For instance, phosphorylation of guanosine triphosphate (Ras complex) stimulates growth and gene expression; phosphorylation of insulin receptor substrate (IRS) stimulates glycogen synthesis; phosphorylation of phosphoinositol phosphate kinase (PI3) stimulates lipogenesis and protein synthesis, and translocation of GLUT 2 (Kahn, 1994). During the translocation of GLUT and internalization of insulin, potassium is required for the β cell membrane depolarization (Berne and Levy, 1993).

Glucose transporters are important parts of the intracellular signalling event. Differences in possession of different type of GLUT determine tissue responsiveness to insulin for uptake of glucose. For instance, GLUT 1 is a predominant type in brain, placenta, mammary gland and erythrocytes; GLUT 2 in the liver, kidney and pancreas; GLUT 3 in brain and placenta; GLUT 4 in adipose tissue and skeletal and heart muscle; and GLUT 5 in small intestine (DeFronzo *et al*., 1992). Among these, only GLUT 4 is found in insulin-sensitive tissues (muscle and adipose tissue) and requires insulin for uptake of glucose (Zhou *et al*., 1999).

Secretion

Insulin release is closely related to its synthesis. There are numerous factors that stimulate insulin secretion (Berne and Levy, 1993). These include nutrients (e.g. glucose, galactose, mannose, glyceraldehydes, arginine, lysine, leucine, alanine, long-chain fatty acids, potassium and calcium), gastrointestinal hormones (e.g. glucagon, pancreatic polypeptide, gastric inhibitory peptide, secretin and cholecystokinin), parasympathetic stimuli (e.g.

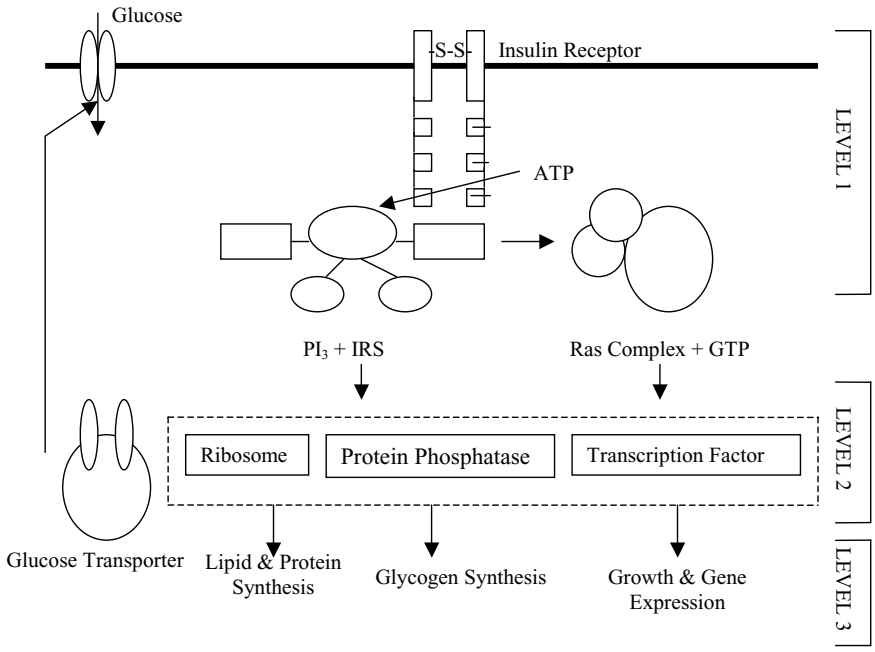

Figure 1. Overview of signal transduction

vagal activity, β-adrenergic activity and acetylcholine), and drugs (e.g. sulpha drugs). Factors that suppress insulin release include physiological conditions (e.g. fasting and exercise), gastrointestinal hormones (galanin, somatostatin, pancreastatin), sympathetic stimuli (α-adrenergic activity), and other specific compounds (e.g. IL-1 and PGF_2-α).

Owing to distinct differences in nutrient metabolism between ruminants and non-ruminants, the magnitude of insulin secretion in response to nutrients varies greatly (Brockman, 1984; Brockman and Laarveld, 1986). In ruminants, nutrients are subjected to microbial degradation and fermentation. Dietary carbohydrates and proteins are converted to volatile fatty acids (VFA) and some other biomolecules (e.g. ammonia and microbial protein) in the reticulorumen. Only a small amount of glucose passes through the reticulorumen and is absorbed from the intestine, while the majority of glucose is provided via gluconeogenesis in the liver (Young, 1976). Therefore, plasma glucose concentration in ruminants is lower than that in non-ruminants. Under normal physiological conditions, postprandial plasma glucose concentrations range from 80 to 120 mg/dl in non-ruminants and young ruminants, whereas they range from 40 to 60 mg/dl in adult ruminants (Hsu and Crump, 1989). In a comparative study, Horino and colleagues (1968) showed that fatty acids (FA) with 3- to 8-carbon chains increased insulin secretion in ruminants. Although there was no change in plasma glucose concentrations, serum insulin concentrations were at the maximum level when cows were infused with valerate and butyrate. Moreover, these FA were more potent for insulin secretion than glucose in ruminants, but they failed to stimulate insulin secretion in rabbits and pigs.

THE ROLE OF INSULIN IN METABOLISM

Insulin has numerous roles in metabolism of carbohydrates, lipids and proteins in adipose tissue, muscle and the liver. Insulin is an anabolic hormone and acts to preserve nutrients. In this review, discussion of the effect of insulin on protein metabolism will be excluded.

Carbohydrate metabolism

Insulin facilitates entry of glucose into cells by speeding up the basal rate of exocytosis and slowing down the basal rate of endocytosis of GLUT 2 in hepatocytes (Katzung, 1995). Insulin supports inward movement of glucose by stimulating the activity of glucokinase, which phosphorylates glucose to glucose 6-phosphate (G6P). The G6P is converted to glucose 1-phosphate (G1P) by phosphoglucomutase. The G1P is combined with uridine diphosphate to form uridine diphosphoglucose. Insulin then stimulates storage of glucose in the form of glycogen (GLY) by increasing the activity of glycogen synthase and by suppressing the activity of glycogen phosphorylase (glycogenesis). The latter enzyme breaks down glycogen to glucose units; this event (glycogenolysis) is mediated by glucagon and adrenaline. Insulin also suppresses glucose production by inhibiting activities of key enzymes (fructose-biphosphatase, pyruvate carboxylase and phosphoenolpyruvate carboxykinase) for gluconeogenesis (O'Brien and Granner, 1990). The liver is not an insulin-sensitive organ. Ortmeyer and Hansen (1998) compared responses of activity of glycogen synthase and glycogen phosphorylase to insulin between muscle and liver of insulin-resistant monkeys. Insulin failed to stimulate activity of glycogen synthase and inhibit activity of glycogen phosphorylase in muscle, but not in liver.

Except for uptake of glucose, the biochemistry of glycogen synthesis in liver and in muscle is essentially similar. The liver utilizes GLUT 2, whereas muscle utilizes GLUT 4. At the same time as glycogenesis, insulin stimulates glycolysis in the liver and muscle. The flow of glucose is directed to pyruvate and lactate by the actions of phosphofructokinase and pyruvate kinase. Insulin stimulates the activity of these glycolytic enzymes (Berne and Levy, 1993). In ruminants, insulin also stimulates glycogenesis. However, ruminants have little or no glucokinase activity in the liver (Brockman and Laarveld, 1986). Instead, in the ruminant liver, hexokinase plays a role in the activation of glucose for uptake (Brockman, 1984). Hexokinase is non-specific and has a substantially lower K_m value for glucose compared with glucokinase (Berne and Levy, 1993). Thus, the ruminant liver takes up only small amounts of glucose under normal physiological conditions.

In adipose tissue, insulin facilitates entry of glucose into cells through GLUT 4 in the same mechanism as explained earlier for muscle tissue (Katzung, 1995). Glucose is then oxidized to form α-glycerophosphate, which is used for esterification of free FA during lipogenesis.

Lipid metabolism

In adipose tissue and muscle, insulin enhances triglyceride (TG) synthesis by providing FA substrate through stimulation of lipoprotein lipase (LPL). Additionally, insulin suppresses

lipolysis by lowering the level of cAMP and inhibiting the activity of protein kinase A and hormone sensitive-lipase (Berne and Levy, 1993; Brockman, 1978, 1979). Acetate is the major precursor for lipogenesis in adipose tissue of ruminants, whereas glucose is the major precursor for lipogenesis in adipose tissue of non-ruminants. Although precursors for lipogenesis are different (Prior and Scott, 1980), the stimulatory role of insulin on lipogenesis in adipose tissue of ruminants and non-ruminants is the same as in hepatic lipogenesis (Brockman and Laarveld, 1986).

In the liver, insulin stimulates lipogenesis and inhibits ketogenesis (Brockman, 1978, 1979). The free FA taken up by the liver is re-esterified with glycerophosphate, derived either from insulin-stimulated glycolysis or from glycerol formed by glycerophosphate kinase. Insulin also stimulates lipogenesis from glucose in non-ruminants. Mitochondrial acetyl-CoA generated by pyruvate dehydrogenase during glycolysis is transferred to the cytoplasm and then converted to malonyl-CoA by acetyl-CoA carboxylase. This is the rate limiting-step for hepatic lipogenesis and is stimulated by insulin.

The antiketogenic effects of insulin in the liver of ruminants and non-ruminants are the same (Brockman and Laarveld, 1986). Insulin decreases liver non-esterified fatty acid (NEFA) uptake through stimulating lipogenesis and inhibiting lipolysis in adipose tissue, enhancing peripheral tissue ketone utilization, and altering enzyme activities and availability of substrates involved in ketogenesis in the liver (Brockman, 1979). Insulin decreases the activity of carnitine palmitoyltransferase-I (CPT-I) and increases affinity of CPT-I for malonyl-CoA (Grantham and Zammit, 1988). In the liver, CPT-I is a primary regulator of translocation of long chain FA from the cytoplasm into the mitochondria, where they partition for esterification and oxidation. Moreover, insulin's inhibitory effect on ketogenesis is also related to its stimulatory actions on the activity of acetyl-CoA carboxylase and the formation of malonyl-CoA, which inhibits the activity of CPT-I and directs the flux of acetyl-CoA to lipogenesis (Zammit, 1981, 1990, 1996).

Ruminants use a negligible amount of glucose for lipogenesis (Hood *et al*., 1972). The $NADPH_2$ and α-glycerophosphate generated during the direct oxidation of glucose via hexose monophosphate shunt (Smith, 1983) are indispensable for FA synthesis from lactate and acetate in ruminants (Prior and Scott, 1980). The effect of insulin on carboxylation of acetyl-CoA directly derived from acetate is the same as on that derived from glucose in non-ruminants (Brockman and Laarveld, 1986). Unlike in non-ruminants, the ruminant liver is not a primary organ for lipogenesis. The gastrointestinal tract and liver contribute only 8% of whole-body lipogenesis in ruminants (Ingle *et al*., 1972). Additionally, NEFA mobilized from adipose tissue is the primary source of hepatic lipogenesis in ruminants (Emery *et al*., 1992).

INSULIN RESISTANCE PHENOMENON

Insulin resistance describes the state in which a physiological level of insulin produces a less than normal biological response (Kahn, 1978). Berson and Yalow (1970) described insulin resistance as a condition in which a greater amount of insulin is required to produce a normal response, implying that insulin resistance can be altered by provision of exogenous insulin. This may be true when insulin resistance is due to defects located at the pre-receptor level.

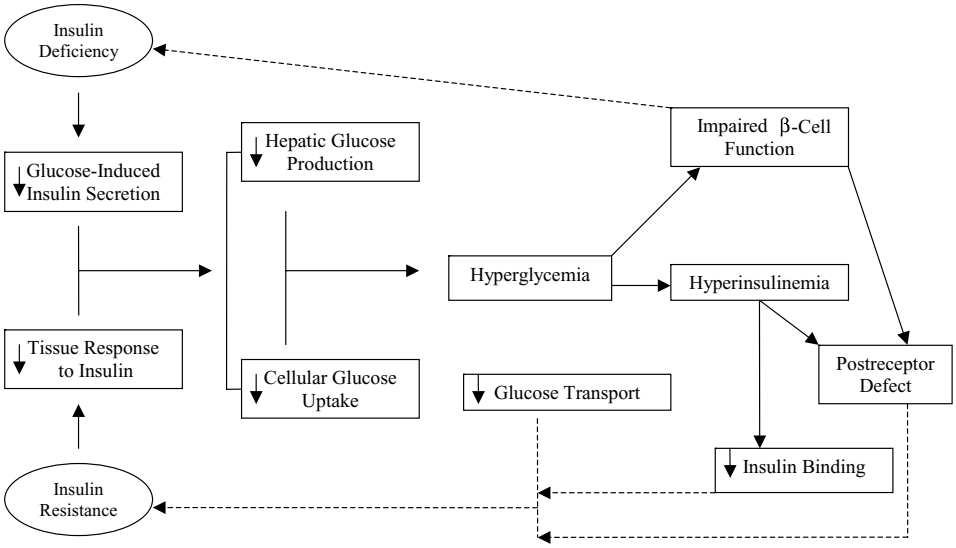

Figure 2. Factors involved in the insulin resistance phenemenon

However, provision of exogenous insulin may not alter insulin resistance when defects are located at the receptor and post-receptor levels. Insulin resistance is a generic term that can be evaluated by insulin responsiveness (insulin response to glucose), insulin sensitivity (tissue responsiveness to insulin), or both (Kahn, 1978; Sano *et al*., 1991). The molecular mechanism of insulin resistance may be localized (1) prior to the interaction of insulin with the receptors (pre-receptor level), which includes decreased insulin production, increased insulin degradation, or both; (2) in alteration of the interaction of insulin with its receptor (at receptor level), which include decreased number of receptors and decreased binding affinity; and (3) in defects associated with alteration of the intracellular steps in insulin action (post-receptor defect), which includes impaired intracellular signalling and translocation of GLUT (Figure 2). In general, defects at the pre-receptor level cause hypoinsulinaemia; defects at the receptor level cause reduced insulin responsiveness; and defects at the post-receptor level cause reduced insulin sensitivity (Kahn, 1978).

The euglycaemic clamp technique and glucose tolerance test (GTT) are commonly used to evaluate insulin resistance or glucose intolerance. During the euglycaemic clamp, the amount of insulin required to achieve the maximum response indicates insulin responsiveness, whereas the amount of insulin required to reach the half-maximal response indicates insulin sensitivity (Kahn, 1978). During insulin infusion, the system is clamped when euglycaemia is maintained by concomitant glucose infusion. Glucose infusion rate is equal to metabolic rate of glucose utilization (Sano *et al*., 1991, 1993). The GTT is a more practical and simple test than the euglycaemic clamp technique for determining glucose intolerance. In the GTT, basal and peak concentrations, plasma disappearance rate, half-life, time to reach basal concentration, area under the curve for plasma glucose, and ratio of plasma glucose to serum insulin are parameters for evaluation of glucose tolerance (Hayirli *et al*.,

2001). However, information obtained from GTT is not as easily interpreted as that generated from euglycaemic clamps. For instance, during the GTT, it is not known whether increased plasma glucose disappearance rate is due to increased glucose utilization or decreased glucose production. In this case, the molar ratio of serum insulin to glucose or the ratio of their disappearance rates is a better indictor of insulin resistance than plasma glucose disappearance rate alone (Subiyatno *et al*., 1996).

Insulin resistance is a multi-factorial phenomenon that is characterized by metabolic acidosis, hyperglycaemia, glucose intolerance, glucosuria, ketonaemia, ketonuria, diuresis, hypovolaemia, dehydration, polydipsia, central nervous system depression, and shock in humans (McCance and Huether, 1994). These physiopathological events are also seen in ruminant animals with induced or spontaneous hepatic lipidosis and ketosis (DeBoer *et al*., 1985; Veenhuizen *et al*., 1991; Drackley *et al*., 1992). Factors that cause insulin resistance in humans are also related to those involved in development of ruminant hepatic lipidosis and ketosis. These factors include advancing pregnancy, obesity, hyperinsulinaemia, fat feeding, hyperlipidaemia, malnutrition, and other hormones.

Gestation

Insulin resistance is commonly observed during late gestation and early lactation (Prior and Christenson, 1978; Debrass *et al*., 1989; Faulkner and Pollock, 1990; Petterson *et al*., 1994). Glucose utilization by peripheral tissues is lower for pregnant animals than for non-pregnant and lactating animals (Hay *et al*., 1988; Nieuwenhuizen *et al*., 1998). During late pregnancy, fetal glucose uptake is approximately 42–50% of glucose production in ewes (Prior and Christenson, 1978). Uptake of glucose and numbers of GLUT 4 in heart muscle and white and brown adipose tissues were lower in pregnant rats compared to non-pregnant rats (Nieuwenhuizen *et al*., 1998). In a study involving ewes, Schlumbohm and colleagues (1997) showed that insulin-mediated uptake of glucose by muscle and adipose tissues and insulin-mediated inhibition of lipolysis were decreased during late pregnancy compared to during nonpregnancy and lactation. Moreover, reduced insulin response is substrate-selective. Hjolliund and colleagues (1986) reported that insulin reduced basal adipose tissue lipogenesis, maximal responses of lipogenesis, and glucose oxidation in pregnant women. Guesnet and colleagues (1991) ascertained that reduced sensitivity of peripheral tissues to insulin in pregnant ruminants was specific to glucose utilization, not to lipid utilization. This suggests that insulin administration may reduce lipolysis without causing hypoglycaemia and thereby prevent cows from hepatic lipidosis around parturition.

During late gestation, increased serum concentrations of the hormones estradiol, progesterone and prolactin affect the sensitivity of peripheral tissues to insulin. Ryan and Enns (1988) investigated the effects of these hormones on insulin action in isolated cells from adipose tissue of pregnant, non-pregnant, and virgin rats. Addition of hCG to culture did not change the numbers of GLUT 4; addition of estradiol to culture medium increased maximum insulin binding; addition of progesterone and cortisol decreased glucose transport and maximum insulin binding; and addition of prolactin and placental lactogen decreased glucose transport without changing maximum insulin binding. It appears that oestrogen

enhances the action of insulin during non-pregnancy and lactation and progesterone suppresses insulin actions during late pregnancy.

In summary, transfer of glucose from dam to fetus is an insulin-independent process, whereas transfer of glucose from blood to muscle or adipose tissue and vice versa is an insulin-dependent process. Reduced insulin sensitivity by peripheral tissues during late gestation assures adequate transfer of glucose from dam to fetus. If insulin-stimulated glucose utilization by insulin-sensitive tissues is not limited, the fetus might not survive due to hypoglycaemia.

Obesity

A number of studies reveal that obesity is associated with increased likelihood of metabolic disturbances. Hyperinsulinaemia (McCann *et al*., 1986) and insulin resistance (Mahler, 1981; McCann and Reimers, 1985; Bergman *et al*., 1998) are common metabolic signs of obesity in non-ruminants and ruminants. Unlike obese non-ruminant species, obese ruminants have poor appetites (Garnsworthy and Topps 1982; Treacher *et al*., 1986; Hayirli *et al*., 2002b). Therefore, obesity in non-ruminants is associated with hyperglycaemia and hyperinsulinaemia, whereas obesity in ruminants is associated with hypoglycaemia and hypoinsulinaemia.

Hyperinsulinaemia

In general, hyperinsulinaemia downregulates insulin actions at the receptor and post-receptor levels (Berne and Levy, 1993). In hepatic insulin resistance, hyperinsulinaemia is associated with inability of insulin to suppress hepatic glucose production. In peripheral tissue insulin resistance, hyperinsulinaemia is associated with impairment of glucose uptake and oxidation by muscle and adipocytes and inability to suppress FA release from adipose tissue (Sparks and Sparks, 1995).

Fat feeding

Increased plasma NEFA concentration is a common observation during high-fat feeding due to incomplete oxidation of fat. Feeding of fat (ketogenic diet) causes reduced insulin sensitivity, which is characterized by reduced glucose disposal rate in skeletal muscle and decreased suppression of hepatic glucose production (Oakes *et al*., 1997). Ruth and Kor (1992) reported that reduction of dietary fat concentration from 40% to 30% improved glucose tolerance in rats *in vivo*. Ruth (1992) conducted a similar study to clarify the mechanism by which a high-fat diet reduces glucose tolerance and found that increasing dietary fat concentration from 21% to 60% resulted in a decrease in number of receptors and insulin binding as well as decreased glucose oxidation and lipogenesis. Watari and colleagues (1988) demonstrated that high-fat feeding resulted in a 65% reduction of maximal 2-deoxyglucose uptakes by rat adipocytes and a 50% reduction in insulin binding and kinase activity. Laville and colleagues (1995) measured NEFA and total lipid oxidation in lipid-induced insulin resistance in humans by using indirect calorimetry. Following infusions

of labelled glucose and palmitate, NEFA concentration, turnover rate and oxidation were reduced by insulin in the control group. In contrast, NEFA concentration was not altered in the group that received intravenous lipid solution.

In summary, ketogenic diets elevate NEFA and cause insulin resistance for the following reasons: (1) acute hyperinsulinaemia fails to quench elevated lipid availability in muscle and liver; (2) elevated lipid oxidation opposes insulin stimulation of glucose oxidation and suppression of gluconeogenesis in muscle; (3) decreased cell membrane fluidity and numbers of GLUT 4; (4) decreased hepatic glucokinase activity in the liver and muscle; and (5) impaired insulin signal transduction at the receptor and post-receptor levels (Sebokova *et al*., 1995).

Hyperlipidaemia

Free FA competes with glucose for utilization by insulin-sensitive tissues (Boden, 1977; Koopmans *et al*., 1996) and has toxic effects on peripheral tissues (Spector and Fletcher, 1978). Noshiro and colleagues (1997) tested insulin action on insulin-sensitive lean rats and insulin-resistant obese Zucker rats. In both groups, elevated serum NEFA and triglyceride concentrations resulted in 30% reduction in ED_{30} following the oral GTT. The adverse effect of NEFA concentration was more pronounced in obese rats than in lean rats. Van Epps-Fung and colleagues (1997) also demonstrated the adverse effect of elevated NEFA concentration on adipose tissue insulin sensitivity. There was a 50% reduction in insulin-induced glucose transport in cell culture in which 3T3-L1 cells (adipose tissue cell line) were exposed to 1 mmol/L of palmitate, myristate and stearate overnight; 0.3 mmol/L palmitate produced insulin resistance. This amount of palmitate is within the physiological range of obese humans. Inhibition of insulin action was observed after 4 h that was specific to GLUT 4 translocation (Van Epps-Fung *et al*., 1997). The adverse effects of elevated NEFA concentration on peripheral tissue insulin sensitivity can be diminished after supplementation with Acipimox (a nicotinic acid analogue) while maintaining euglycaemia (Walker *et al*., 1991, 1993; Farrer *et al*., 1992). Hennes and colleagues (1990) investigated the effects of increasing NEFA concentration on insulin receptor binding and insulin internalization processes (signalling, degradation, and dissociation) in hepatocytes. Cells from fasted rats with depleted liver glycogen were incubated with 10 mmol/L lactate, 1 mmol/L pyruvate, 3% albumin and 0, 0.05, 0.2, 0.5, 1 and 2 mmol/L palmitate. Increasing palmitate concentration was associated with a reduction in numbers of insulin receptors and in insulin binding, and the lowest numbers of receptors and insulin binding and greatest receptor-mediated insulin degradation were observed in cultures incubated with 2 mmol/L palmitate. Moreover, it was shown that elevated NEFA reduced permeability of adipocytes and limited the transport rate of insulin across endothelium, and consequently lowered glucose uptake (Bergman and Mittelman, 1998).

In summary, elevated NEFA concentration causes inhibition of insulin-stimulated glucose uptake by peripheral tissues, decreases the number of GLUT 4, and disturbs intracellular insulin signalling pathways in the liver and peripheral tissues. Defects in intracellular signalling pathways include abnormality of GLUT 4 translocation, decreased IRS-1

phosphorylation (Zierath *et al*., 1998; Le Marchand-Brustel *et al*., 1999), increased risks for receptor downregulation, and decreased coupling efficiency between occupied receptors and stimulated glucose transport (Garvey *et al*., 1986).

Malnutrition

Malnutrition causes imbalance in glucose homeostasis and glucose intolerance (Okitolonda *et al*., 1988). Prolonged malnutrition results in a decrease in islet numbers and a reduction in islet size, which may cause lower insulin secretion (Tse *et al*., 1998). Reis and colleagues (1997) reported that basal serum insulin and plasma glucose concentrations and glucose clearance rate were lower in malnourished rats than in well-nourished rats during the oral GTT. Moreover, insulin secretory response to glucose addition to cell media containing pancreas islets isolated from malnourished rats was lower compared to those isolated from well-nourished rats. The lack of difference in insulin-mediated receptor phosphorylation between treatment groups may suggest that adaptation to hypoinsulinaemia affects insulin sensitivity. Forhead and Dobson (1997) also demonstrated that feed restriction resulted in hypoinsulinaemia, which was accompanied by hyperlipidaemia, in donkeys.

In summary, malnutrition and/or feed restriction reduces the glucoregulatory actions of insulin. Consequently, hypoinsulinaemia suppresses adipose tissue LPL activity and therefore increases plasma NEFA concentrations. Similarly, in dairy cattle, decreased plasma insulin concentration may reflect regression of the pancreas as a result of depression in DMI prior to parturition and low DMI during the first two months of lactation. However, the magnitude and duration of malnutrition required to develop lipid-related metabolic disorders in dairy cows are largely unknown. Cows subjected to 30% feed restriction and those with about 30% reduction in DMI as they approach parturition are known to develop postpartum hepatic lipidosis (Vazquez-Anon *et al*., 1994; Hayirli and Grummer, 2004) and ketosis (Veenhuizen *et al*., 1991).

Other hormones

During late gestation, increased serum concentrations of growth hormone (GH) (Smith *et al*., 1997) and thyroxine (Fickova *et al*., 1997) affect nutrient partitioning and responses of peripheral tissues to insulin. Nieuwenhuizen and colleagues (1997) reported that subcutaneous injection of insulin during pregnancy decreased lipolysis, decreased the serum concentration of GH, and did not influence concentrations of estradiol and progesterone in rats. Smith and colleagues (1997) injected GH (1 mg/kg body weight, s.c.) for 3 days into rats to evaluate the effects of GH on insulin action. During the GTT, the concentration of fasting plasma glucose did not change, but the ratio of insulin to glucose decreased due to GH injection. There was also a 48% elevation in insulin β-subunit autophosphorylation and numbers of GLUT 4. Similar adverse effects on insulin action were reported after thyroxine injection (50 μg/100 g body weight) (Fickova *et al*., 1997).

SIGNIFICANCE OF THE PERIPARTUM PERIOD

Metabolic and energetic status

Parturition is associated with a complex series of synchronized metabolic and endocrine adjustments (homeorhesis) that are initiated during the final three weeks of gestation and continue through the first four weeks of subsequent lactation (Bauman and Currie, 1980). Physiological changes occurring during the periparturient period have an impact on the nutritional (Van Saun, 1991; Grummer, 1995), metabolic (Bell, 1995, 1996; Nocek, 1995; Drackley, 1999) and immune (Goff and Horst, 1997; Mallard *et al*., 1998) status of dairy cattle.

Development of negative energy balance prior to parturition and its continuation through early lactation are due to significant DMI depression (Bertics *et al*., 1992; Hayirli *et al*., 2002b) and the lag time between peaks of intake and milk yield, respectively (Baird, 1981; Grummer, 1995). Dairy cattle lose body weight BW and body condition score (BCS) to compensate for the energy deficit (Coppock, 1985). Negative energy balance may compromise the ability of dairy cattle to adapt to physiological changes (Van Saun, 1991; Bell, 1995; Grummer, 1995). Therefore, lactogenesis is accompanied by alterations of metabolism, which include increased lipolysis and decreased lipogenesis in adipose tissue, decreased glycogenesis and increased gluconeogenesis and glycogenolysis in the liver, decreased use of glucose and increased use of lipid as energy sources by body tissues, and increased mobilization of protein reserves from muscle tissue (Bauman and Currie, 1980; Collier *et al*., 1984; Reynolds *et al*., 2003). These changes predispose dairy cows to hepatic lipidosis and ketosis. For instance, Young (1976) accentuated the challenge in supplying glucose for milk synthesis. Based on hypothetical estimation, realistically for a cow producing 35 kg of milk per day with 4.9% lactose, about 2.9 kg glucose is used for lactose secretion in the mammary gland, of which 2.7 kg is provided via gluconeogenesis. To quantify homeorhetic changes as cows approach parturition, Reynolds and colleagues (2003) reported that lower arterial insulin and acetate, higher blood NEFA, and increased removal rate of glycerol were related to the magnitude of DMI depression. Moreover, because of dependence on gluconeogenesis, net liver removal of propionate, lactate, alanine, and glycerol accounted for 69%, 20%, 8%, and 4%, respectively, of glucose released from the liver during the final two weeks of gestation. That is, the metabolic profile during early lactation includes low concentrations of serum insulin, plasma glucose and liver glycogen and high concentrations of serum glucagon, adrenaline and GH, plasma β-hydroxybutyrate (BHBA) and NEFA, and liver triglycerides (Herbein *et al*., 1985; Vazquez-Anon *et al*., 1994). This metabolic pattern is also reported in cases of induced or spontaneous hepatic lipidosis and ketosis (DeBoer *et al*., 1985; Veenhuizen *et al*., 1991; Drackley *et al*., 1992).

The greater extent of DMI depression during the transition period of cows compared to heifers (Hayirli *et al*., 2002b) suggests a greater decrease in energy balance, which may relate to their greater predisposition to postpartum metabolic disorders (Curtis *et al*., 1985). Also, due to the refusing of food intake (Garnsworthy and Topps, 1982; Hayirli *et al*., 2002b), obese cows (BCS $>$ 4) greater than four experience severe and rapid fat mobilization from adipose tissue, which increases the predisposition to hepatic lipidosis and ketosis (Curtis *et al*., 1985; Reid *et al*., 1986; Grummer, 1993). Minimizing DMI depression or increasing

nutrient density of the diet during the transition period would be an essential strategy to alleviate the severity of negative energy balance, maintain body reserves, increase nutrients available for rapid fetal growth, ease metabolic transition from pregnancy to lactation, and acclimate rumen microorganisms to lactation diets (Van Saun, 1991; Grummer, 1995; Nocek, 1995). Increasing dietary concentration of non-fibre carbohydrates (NFC) (1.58–1.63 Mcal NEL/kg DM or 38–44% NFC) increases energy density of the diet by providing greater amounts of glucogenic precursors (Flipot *et al*., 1988; Minor *et al*., 1998; Dann *et al*., 1999; Rabelo *et al*., 2003) and maximizes DMI and energy intake by decreasing gut fill (Forbes, 1996). This feeding regimen stimulates papilla length (Driksen *et al*., 1985) and the microbial flora (Forbes, 1996). Increased growth of papillae enlarges the surface area and VFA absorptive capacity of the rumen epithelium (Driksen *et al*., 1985). In turn, this could prevent accumulation of VFA and normalize pH of the reticuloruminol fluid.

Lipid-related postpartum metabolic disorders

Hepatic lipidosis and ketosis, common lipid-related metabolic disorders, occur as a result of inability to keep pace with homeorhetic changes during the periparturient period (Herdt *et al*., 1983, 1988; DeBoer *et al*., 1985; Veenhuizen *et al*., 1991; Drackley *et al*., 1992; Grummer, 1993; Vazquez-Anon *et al*., 1994). The aetiologies of hepatic lipidosis and ketosis are similar (DeBoer *et al*., 1985; Veenhuizen *et al*., 1991; Drackley *et al*., 1992; Rukkwamsuk *et al*., 1999a) and liver function is impaired in both cases (Strang *et al*., 1998; Zhu *et al*., 2000). Numerous nutritional strategies have been used to alleviate the severity of hepatic lipidosis and ketosis (Minor *et al*., 1998; Rukkwamsuk *et al*., 1998, 1999b; Bremmer *et al*., 2000; Hayirli *et al*., 2001; Rabelo *et al*., 2003).

Hepatic lipidosis refers to accumulation of lipids in hepatocytes (Pearson and Maas, 1990). Triglyceride is the major type of lipid that accumulates in the liver of normal (Collin and Reid, 1980) and obese (Fronk *et al*., 1980) dairy cattle. Many cows experience hepatic lipidosis and ketosis of varying degrees of severity during the periparturient period (Grummer, 1993). The incidence of hepatic lipidosis is about 66% in Holsteins and 33% in Guernseys (Reid, 1980) and the severity of hepatic lipidosis is related to the degree of mobilization of adipose tissue fat reserves (Roberts *et al*., 1981). Hepatic lipidosis may compromise production (Gerloff *et al*., 1986a), immune function (Ropstad *et al*., 1989; Kaneene *et al*., 1997), and fertility (Reid *et al*., 1979a,b) and increases the likelihood of ketosis (Drackley *et al*., 1992).

Elevated plasma NEFA and liver TG concentrations during the pre-fresh transition period are associated with a gradual decline in DMI prior to parturition and a slow rate of increase in DMI in early postpartum (Bertics *et al*., 1992; Vazquez-Anon *et al*., 1994; Grummer, 1995). In association with greater DMI, lower mitochondrial glycerol-phosphate acyltransferase activity (Van Den Top *et al*., 1996; Rukkwamsuk *et al*., 1998, 1999b) diverts FA from esterification into β-oxidation in order to protect the hepatocytes against further accumulation of TG (Bruss, 1993). Because liver contributes a small fraction of total body fat synthesis (Ingle *et al*., 1972), FA mobilized from adipose tissue is the primary sources of hepatic TG in ruminants (Emery *et al*., 1992; Bruss, 1993; Grummer, 1993). Non-esterified fatty acids are either directly transported to mammary gland (Annison *et al*., 1967) or are taken up by

the liver in relation to blood flow and their concentration in plasma (Heimberg and Wilcox, 1972). In the liver, FA is re-esterified to TG that can be stored or exported as VLDL, or FA is oxidized either completely to carbon dioxide in tricarboxylic acid (TCA) cycle or incompletely to ketone bodies. When the export of TG as VLDL from the liver cannot keep pace with increased NEFA uptake and TG synthesis by the liver, hepatic lipidosis becomes significant (Grummer, 1993).

Major factors causing hepatic lipidosis include increased supply of long-chain FA, impairment of TG incorporation into VLDL, and defects in VLDL transport (Gruffat *et al*., 1996). Thus, improving TG export ability of the liver and limiting fat mobilization from adipose tissue are essential strategies for alleviation of hepatic lipidosis (Grummer, 1993). For unknown reasons, the export of TG from the liver is slower in ruminants than in non-ruminants (Kleppe *et al*., 1988; Gruffat *et al*., 1996). Decreased plasma apolipoprotein B 100 (apoB), the major lipoprotein incorporated into VLDL by the liver without change in its mRNA expression level (Gruffat *et al*., 1997), is accompanied by increased liver TG during the periparturient period (Gibbons, 1990; Bauchart, 1993). The effects of insulin on synthesis and degradation of apoB and on export of TG as VLDL from hepatocytes are controversial. Some studies conducted using hepatocytes from rats (Beynen *et al*., 1981; Sparks and Sparks, 1995; Chirieac *et al*., 2000) suggest that insulin suppresses hepatic VLDL secretion and apoB synthesis, whereas other studies (Satoh *et al*., 1987; Bjornsson *et al*., 1992) suggest that insulin enhances TG secretion rate and inhibits apoB degradation. It was also shown that nutritional status did not alter the activity or mass of microsomal triglyceride transfer protein (Bremmer *et al.,* 2000), which is required for assembly and secretion of apoB-containing lipoproteins (Lin *et al*., 1995). Supplementations of choline (Hartwell *et al*., 2000; Piepenbrink and Overton, 2003), inositol (Gerloff *et al*., 1986b) and methionine (Bertics and Grummer, 1999), which was intended to improve TG export by enhancing lipoprotein synthesis, failed to alleviate hepatic lipidosis in dairy cattle. Therefore, until factors impeding hepatic VLDL-TG export are identified, limiting fat mobilization from adipose tissue will play a key role in prevention of hepatic lipidosis because decreased DMI and negative energy are associated with hypoglycaemia and hypoinsulinaemia. Moreover, liver TG concentration is negatively correlated with plasma glucose and serum insulin concentrations and positively correlated with plasma NEFA and BHBA concentrations (Studer *et al*., 1993). As explained earlier, improving DMI and energy balance by increasing the proportion of fermentable carbohydrates in the diet of transition cows provides more glucogenic precursors and is associated with increased plasma glucose concentration, decreased concentrations of plasma NEFA and BHBA, increased liver glycogen concentration, and decreased liver TG concentration (Minor *et al*., 1998; Rabelo *et al*., 2003). Studer and colleagues (1993) reported similar changes in transition dairy cows supplemented with propylene glycol. The antilipolytic effect of niacin supplementation during the periparturient period on alleviation of hepatic lipidosis is controversial. Minor and colleagues (1998) found a tendency for decreased liver TG, whereas Skaar and colleagues (1989) found no effect on hepatic lipidosis. Although there were decreases in plasma NEFA concentration and plasma glucose to serum insulin ratio, no effect on hepatic lipidosis was reported in cows supplemented with chromium (Yang *et al*., 1996; Besong *et al*., 2001; Hayirli *et al*., 2001; Pechova *et al*., 2002), which was intended to increase insulin action on extrahepatic tissues to inhibit lipolysis.

Ketosis occurs after hepatic lipidosis in high-producing dairy cows (Reid, 1980) and is characterized by hypophagia, decreased milk production, increased BW and BCS loss, lethargy, hyperexcitability, hypoglycaemia, hypoinsulinaemia, hyperketonaemia, hyperlipidaemia, and depleted hepatic glycogen (Veenhuizen *et al*., 1991; Drackley *et al*., 1992). The incidence of clinical ketosis varies from 3.5% to 15% in the USA; the estimated treatment cost is about $150 per case of clinical ketosis and annual treatment costs vary from $60 to 70 million nationwide (Littledike *et al*., 1981). Ketosis generally occurs 21–28 days after parturition and increases the risk for peripartum diseases such as displaced abomasum (53.5-fold), retained fetal membrane (16.4-fold), metritis (15.3-fold), and milk fever (23.6-fold) (Curtis *et al*., 1985; Correa *et al*., 1990, 1993). Moreover, elevated plasma ketones impair immune function through suppressing mitogenic response of lymphocytes (Targowski *et al*., 1985; Franklin *et al*., 1991; Sato *et al*., 1994).

Earlier, it was accepted that an imbalance of gluconeogenic to lipogenic nutrients in the diet resulted in ketosis (Kronfeld, 1982; Fleming, 1990). In this scenario, the demand for glucose for milk synthesis exceeds the supply, which may be the consequence of insufficient availability of gluconeogenic precursors of insufficient rate of gluconeogenesis. This decreases the amount of glucose carbon entering the TCA cycle. The demand for oxaloacetate for gluconeogenesis depletes the supply available for acetyl-CoA. Consequently, oxidation is not complete and acetyl-CoA is diverted towards ketone production. However, this theory has been deemed untenable for numerous reasons as outlined by Drackley and colleagues (2001). During negative energy balance, hepatic FA metabolism shifts to incomplete oxidation to ketone bodies instead of complete oxidation to carbon dioxide and water (Schultz, 1971). In clinical cases of ketosis, the liver contributes up to 60% of total ketones (Brockman, 1979). According to recent studies, hepatic ketogenesis is regulated by increased NEFA supply (due to extensive lipolysis in adipose tissue) to the liver (Brockman, 1978, 1979) and intramitochondrial activities of CPT-I (Grantham and Zammit, 1988) and 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase (Hegardt, 1999). CPT-I promotes entry of fatty acids to mitochondria and HMG-CoA synthase converts acetyl-CoA to ketones. The ratio of TG to glycogen is a useful predictor of a cow's likelihood of developing ketosis and impairment of liver function (Drackley *et al*., 1992).

Nutritional factors (e.g., feeding more energy as fermentable carbohydrates) that alleviate severity of hepatic lipidosis also affect ketogenesis (Hayirli and Grummer, 2004). The effects of this feeding regimen on the development of ketosis are mediated through insulin's potential antiketogenic effects (Schultz, 1971; Schalm and Schultz, 1976). Brockman (1979) summarized direct and indirect antiketogenic effects of insulin, which include decreasing liver NEFA uptake through stimulating lipogenesis and inhibiting lipolysis in adipose tissue; enhancing peripheral tissue ketone utilization; and altering enzyme activities and availability of substrates that are involved in ketogenesis in the liver. The impact of insulin on enhancing utilization of BHBA and acetoacetate by extrahepatic tissues was demonstrated in normal and alloxan-induced diabetic sheep (Jarret *et al*., 1976). Insulin decreases the activity of CPT-I and increases the affinity of CPT-I for malonyl-CoA (Grantham and Zammit, 1988). Moreover, insulin's inhibitory effect on ketogenesis is also related to its stimulatory effect on the activity of acetyl-CoA carboxylase and the formation of malonyl-CoA, which inhibits the activity of CPT-I (Zammit, 1981, 1990, 1996).

Relationship of the insulin resistance phenomenon to development of hepatic lipidosis and ketosis

Insulin sensitivity decreases during late pregnancy (Prior and Christenson, 1978; Debrass *et al*., 1989; Faulkner and Pollock, 1990; Sano *et al*., 1991, 1993; Petterson *et al*., 1994; Nieuwenhuizen *et al*., 1997, 1998). Metabolic changes during the periparturient period are controlled through homeorhesis. As defined by Bauman and Currie (1980), homeorhesis refers to a control mechanism that involves coordination of the metabolism of body tissues necessary to support the physiological state. During late gestation and early lactation the flow of nutrients to the fetus and mammary tissues is accorded a high degree of metabolic priority (Baird, 1981). This priority coincides with lowered responsiveness and sensitivity of extrahepatic tissues to insulin (Kronfeld, 1982; Sano *et al*., 1991, 1993; Nieuwenhuizen *et al*., 1997, 1998), which presumably plays a key role in the development of hepatic lipidosis and ketosis (Holtenius, 1993; Steen *et al*., 1997). In a recent study by Holtenius and colleagues (2003), it was reported that plasma glucose disappearance rate, and thereby the effectiveness and concentration of insulin, was related to feeding regimen offered during the prefresh transition period of cows fed to consume 6, 9 and 14.5 kg DM.

Cows with ketosis have low tissue responsiveness to insulin (Sakai *et al*., 1993) and ketoacidosis is one of the reasons for insulin resistance (Van Putten *et al*., 1985; Holtenius, 1993; Steen *et al*., 1997). Sakai and colleagues (1993) conducted GTT in healthy cows and cows with ketosis. Elevation of plasma glucose and serum insulin concentrations in healthy cows was greater than cows with ketosis during the intravenous GTT. Moreover, ketotic cows treated with daily intravenous glucose recovered later than those treated with intravenous glucose and subcutaneous insulin injection for 4 days. Van Putten and colleagues (1985) conducted a study to elucidate the mechanism by which ketoacidosis causes insulin resistance *in vitro*. Exposure of 3T3-L1 adipocytes to low pH and ketoacids for 48 h resulted in decrease in pH from 7.4 to 6.9 and a 50% reduction in insulin binding. Addition of BHBA to the medium increased insulin sensitivity (ED_{50} = 10 mmol/L). A similar study was conducted by Yki-Jarvinen and Koivisto (1984) in 14 patients with type I diabetes. The rate of glucose clearance in the patients with ketoacidosis was 35% lower than that in healthy people. Following three months of insulin therapy, the rate of plasma glucose disappearance rate was not different between groups. The ratio of glucose to insulin increased 2-fold in the patients with ketoacidosis, without changing basal glucose production, suggesting that decreasing plasma ketone body concentrations enhance insulin sensitivity (Holtenius, 1993; Steen *et al*., 1997).

USE OF EXOGENOUS INSULIN

Bovine and porcine insulin have been used for therapy of diabetes mellitus until the last decade. Differences in the structures of insulin produced by different mammalian species are minor and often restricted to positions 8, 9, and 10 of the A chain and to position 30 of the B chain, where intrachain disulphide bonds form (Hsu and Crump, 1989). These differences do not greatly alter the biological potency of interspecies insulin used in replacement therapy for diabetes mellitus (Hadley, 1996). The use of insulin produced by other species,

particularly bovine insulin, has been complicated by immunogenic reactions (hypersensitivity, anaphylactic shock, and formation of lipdystrophic plaque and antibody) in some human subjects (Reynolds, 1989). Two major companies (Eli Lilly Co. and Novo Nordisk Inc.) halted production of bovine insulin in 1994. Today, only enzymatically modified porcine insulin and recombinant human insulin are available. The duration and promptness of insulin actions vary depending on the method of delivery and type of insulin (Reynolds, 1989). According to the pharmacokinetics and compatibility, insulin is categorized as rapid-release (crystalline or regular), intermediate-release (Isophane and Lente), and slow-release insulin (protamine zinc insulin, UltraLente). In humans, serum concentrations of insulin from subcutaneous delivery of rapid, intermediate and slow-release insulin peak from 0.5 to 1.5, 4 to 12, and 10 to 24 h following injection and the effects of insulin last for 7, 24 and 36 h, respectively. Moreover, intramuscular injection hastens the action of insulin due to the enriched vascular network around muscle tissue compared to adipose tissue (Reynolds, 1989).

Effects on feed intake

Insulin is a potent regulator of feed intake (Grovum, 1995) and nutrient partitioning (Laarveld *et al*., 1981; Kronfeld, 1982) in ruminants. Moreover, insulin increases VFA absorption indirectly by stimulating growth of papillae (Sakata *et al*., 1980), which may prevent accumulation of VFA and stabilize ruminal pH, and consequently allow greater feed intake. The effects of insulin on feed intake of ruminants are inconsistent. Injection of goats with slow-release insulin (SRI) for a day (100 and 160 IU, s.c.) and for 14 days (80 IU s.c.) caused hyperinsulinaemia and hypoglycaemia without altering feed intake (Baile and Mayer, 1968). Deetz and Wangsness (1981) reported that five injections of rapid-release insulin (RRI) (6 IU/kg BW, i.v.) during 24 h decreased feed intake by 8.5% compared with injections of saline (i.v.) in sheep. In another study, Deetz and Wangsness (1980) reported that injection of a very high dose of RRI (2000 IU/kg BW, i.v.) caused severe hypoglycaemia without affecting feed intake, whereas injection of a moderate dose of RRI (6 IU/kg BW, i.v.) decreased feed intake without causing hypoglycaemia. DMI increased quadratically in early postpartum cows (day 5) injected with a single dose of SRI (0, 10.14, 0.29 and 0.43 IU humilin/kg BW, i.m.) (Hayirli *et al*., 2002a). Deetz and colleagues (1980) showed that increasing doses of RRI (0, 2, 4 and 6 IU/kg BW) via intraportal delivery decreased feed intake linearly. In contrast, increasing doses of RRI via intrajugular delivery increased feed intake quadratically; feed intake was the maximum in sheep injected with 4 IU/kg BW. These studies suggest that type, dose and means of delivery may interact directly with other metabolites to regulate feed intake. Moreover, it appears that ruminants do not overcome hypoglycaemia by increasing feed intake.

Effects on milk production

Milk yield responses to insulin are also inconsistent in the literature. Hayirli and colleagues (2002a) reported a quadratic increase in milk yield of early lactating cows (day 5) injected

with a single dose of SRI (0, 10.14, 0.29 and 0.43 IU humilin/kg BW, i.m.). In other studies, injections of cows with several doses of RRI (0.2–0.6 IU/kg, i.m.; Kronfeld *et al*., 1963) (0.35 IU/kg BW, i.m.; Schmidt, 1966) decreased both milk and lactose yields. In these studies, milk yield was restored following infusion of dextrose, suggesting that depression of milk yield is due to hypoglycaemia, not a direct effect of insulin *per se*. Insulin-partitions nutrients towards insulin-responsive tissues (i.e., adipose and muscle tissues) for storage. Thus, exogenous insulin may lead to decreased availability of glucose for mammary tissue (Kronfeld, 1982) because insulin does not regulate uptake of glucose through facilitative GLUT 1, which predominates in the mammary gland (Laarveld *et al*., 1981; Zhao *et al*., 1993).

Effects on metabolic profile

Administration of an excessive dose of insulin causes severe hypoglycaemia. Hypoglycaemic shock was characterized by anorexia, unconsciousness, tonic extensor spasms, coldness in the ears, excessive foamy salivation, and disturbed behaviour such as pica and seizure. These symptoms are similar to those observed during the nervous stage of ketosis (Kronfeld, 1982). Because of this, it is often misinterpreted that insulin causes ketosis. Hypokalaemia is another adverse effect of administration of excessive insulin. Hypokalaemia is characterized by an inversion of the T wave and a prolongation of the Q-T interval on the electrocardiogram (Goth, 1968). Potassium is used for transport of glucose and insulin and plays a role in regulation of heart rhythm (Berne and Levy, 1993).

During the periparturient period, it is well established that plasma metabolites and hormones reflect catabolic activities due to negative energy balance. Little is known about the potential of altering insulin sensitivity or insulin administration during the periparturient period for the prevention of hepatic lipidosis and ketosis. In addition to major replacement therapy to alleviate severity of negative energy balance, subcutaneous injection of SRI (100–200 IU of protamine zinc insulin, s.c.), with concomitant infusion of dextrose, is suggested for treatment of hepatic lipidosis (Pearson and Maas, 1990) and ketosis (Fleming, 1990). Sakai and colleagues (1993) showed that injection of SRI (200 IU, s.c.) with concomitant infusion of dextrose compared with infusion of dextrose alone for 4 days enhanced the effectiveness of treatment for ketosis and shortened the recovery period. As reported by Sakai and colleagues (1993), injections of insulin alone (40–50 IU, i.v.) (Sato *et al*., 1986) and as adjunct to infusion of dextrose (120–160 IU, i.m.) (Tayoda *et al*., 1987) for 3 days were effective treatments for hyperlipoproteinaemia and hepatic lipidosis. To determine a dose of SRI that decreases hepatic TG concentration without causing hypoglycaemia and hypophagia, Hayirli and colleagues (2002a) injected cows on the fifth day of lactation with several doses of humilin (0, 10.14, 0.29 and 0.43 IU/kg BW). One cow and two cows injected with 0.43 SRI IU/kg BW suffered from hypoglycaemic shock (<20 mg/dl). Serum glucagon concentration increased linearly, plasma glucose concentration decreased linearly, and plasma NEFA and BHBA concentrations decreased quadratically with increasing exogenous insulin dose. These parameters were returned to basal level within 24 h, but insulin effect on these parameters continued during the second day relative to injection. Serum insulin concentration was maximum 12 h after injection. Moreover, increasing

exogenous insulin dose quadratically decreased hepatic TG concentration and TG:GLY ratio and quadratically increased hepatic GLY concentration.

SUMMARY

Hepatic lipidosis and ketosis, common lipid-related metabolic disorders, occur as a result of inability to keep pace with homeorhetic changes during the periparturient period. To increase efficiency of prevention and/or treatment strategies, TG export ability of the liver should be improved and fat mobilization from adipose tissue should be limited. The effect of insulin on the former is controversial. Surging insulin secretion as a result of provision of glucogenic precursors in the diet or direct administration of insulin changes metabolic status from catabolism to anabolism. Insulin is an anabolic hormone and acts to preserve nutrients and plays a role in feed intake regulation. Insulin decreases liver NEFA uptake through stimulating lipogenesis and inhibiting lipolysis in adipose tissue; enhances utilization of ketones by extrahepatic tissues; and alters enzyme activities and availability of substrates that are involved in ketogenesis in the liver. The occurrence of lowered responsiveness and sensitivity of extrahepatic tissues to insulin as a result of negative energy balance should be alleviated by enhancing nutritional status, which increases the efficiency of exogenous insulin for treating hepatic lipidosis to maintain health and improve performance.

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