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Metabolic effects associated with changes in the availability of glucose for lactating dairy cows

Amaral, Donna Marie, Ph.D.

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Metabolic effects associated with changes

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in the availability of glucose for lactating dairy cows

by

Donna Marie Amaral

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of the Requirements for the Degree of DOCTOR OF PHILOSOPHY

> Department: Animal Science Major: Nutritional Physiology

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INTRODUCTION

Classically, dairy nutritionists have balanced rations for lactating dairy cows so that they provide enough energy and protein to meet the needs of cows for maintenance and for the production of milk. Little attention is given to meeting the needs of cows for glucose. Nutritionists assume that the requirements for glucose will be met when the requirements for energy are met.

High-producing dairy cows require large amounts of glucose to support the synthesis of milk. For the synthesis of milk, the mammary gland uses 70% of the total amount of glucose available to lactating cows (Bickerstaffe et al., 1974). Young (1977) calculated that Beecher Arlinda Ellen required 7.4 kg of glucose daily to support her production of 89 kg of milk. With the mammary gland playing such a dominant role in the utilization of glucose, Brown (1969) concluded that "... we should perhaps consider the cow as an appendage on the udder rather than the reverse".

Because glucose plays a prominent role in the synthesis of milk, it is important to understand how changes in the availability of glucose affect the partitioning of energy-yielding substrates. Research on

this topic will aid dairy researchers in finding ways to maximize milk production and increase productive efficiency of cows without compromising their general health or reproductive performance. Results will be especially helpful during early lactation when cows are in negative energy balance and susceptible to ketosis and other metabolic disorders.

The present studies were undertaken to determine effects on energy metabolism when the availability of glucose for lactating dairy cows was either decreased (Manuscript 1) or increased (Manuscript 2). Subcutaneous injections of phlorizin were used to decrease the amount of glucose available to lactating cows, whereas, the availability of glucose was increased experimentally by infusing glucose into the jugular vein of lactating cows. Specific objectives of the two studies were:

- 1. To determine the amount of glucose excreted into the urine after administration of different doses of phlorizin to lactating cows in positive energy balance (Manuscript 1).
- 2. To determine how cows in negative energy balance adjust to a sudden decrease in availability of glucose caused by phlorizin (Manuscript 1).
- 3. To quantify the changes that additional glucose would cause in the metabolism of plasma

glucose, rumen propionate, and blood CO₂ when glucose is infused intravenously into peripheral blood of lactating cows at energy equilibrium (Manuscript 2).

4. To quantify the changes that additional glucose would cause in the concentrations of insulin and glucagon of peripheral blood when glucose is infused intravenously into peripheral blood of lactating cows at energy equilibrium (Manuscript 2).

REVIEW OF LITERATURE

The purpose of this review of literature is to familiarize the reader with the regulation and biological role of glucose for lactating dairy cows. Major topics to be discussed include: 1) requirement for glucose by various tissues, 2) sources of carbon for glucose, 3) hormonal regulation of the metabolism of glucose, and 4) effects of changing the availability of glucose on milk production and metabolism of glucose. When limited data are available from lactating dairy cows, supporting data from sheep and steers will be reported. The biochemical regulation of the metabolic pathways for glucose will not be covered. For such coverage, the reader is referred to a textbook of general biochemistry.

Requirement for Glucose by Various Tissues

The amount of glucose a cow uses, or the irreversible loss of glucose, can be estimated by measuring the dilution of isotopically-labeled glucose in the blood. Explanations of methods used to measure irreversible loss of glucose have been reviewed by Leng (1970), Young (1977), and Young et al. (1987) and will not be repeated here. Table 1 summarizes results from experiments where

the irreversible loss of glucose for lactating cows was measured. As shown in Table 1, the irreversible loss of glucose of lactating cows ranges from 1484 to 3048 g of glucose daily. Representative values obtained for steers, sheep, and lactating goats also are included for comparative purposes.

The daily amount of glucose used by ruminants depends on their physiological status (Bergman et al., 1974). With sheep, the irreversible loss of glucose increases for ewes pregnant with twins in comparison to nonpregnant, fed wethers (Table 1). The irreversible loss of glucose increases even more during lactation. Utilization of glucose also has been shown to be related to the amount of energy in the diet (Herbein et al., 1978) and to be independent of diet composition (Schmidt and Keith, 1983), energy density (Schmidt and Keith, 1983), and body size (Russell et al., 1986). Because of differences in methodology and energy intake, comparisons among data listed in Table 1 should be made with caution. <u>Tissues that require glucose for maintenance functions</u>

Some tissues have an absolute requirement for glucose. For the lactating cow, Elliot (1976) has calculated, from the data of Annison et al. (1974), that approximately 188 g of glucose is needed daily for maintenance of normal body functions in tissues such as

	Glucose	Method of	Milk	Glucose	
Energy	tracer	aivina	nroduction]064 TITC/GIDIN	
balance	used	tracerl	(ka/d)	(q/d)	Reference2
2424	abea	014001	(19/4)	(9/4)	Notet ende
Lactati	na cows				
+	U-14C	CI	14	1811	а
+ •	U-14C	PCI	30	2170	b
+	6- ³ H	SI	28	3048	C
+	U-14 C	PCI	29	2760	d
+	U-14C	CI	22	2048	e
-	6- ³ H	SI	27	2280	C
-	U-14 C	CI	34	2195	e
?				2560	` f
?	U-14 C	SI		1484	q
Steers					•
maint.	U-14C	PCI		452	h
maint.	U-14C	PCI		396	i
+	6- ³ H	SI		350-498	j
Nonpreq	nant, fed	<u>l sheep</u>			-
+	U-14C	CI		110	k
Twin-pre	eqnant, f	<u>fed sheep</u>			
+	U-14C	CI		180	k
Lactatir	ng sheep				
-	U-14C	PCI	3-4	283	1
Lactatir	ng goats				
+	U-14C	PCI	2-3	287	m

Table 1. Irreversible loss of glucose for lactating cows, steers, sheep, and goats

¹ CI, continuous infusion; PCI, primed-continuous infusion; SI, single injection.

2a= Bickerstaffe et al., 1974. b= Bauman et al., 1988. c= Bruckental et al., 1980. d= Clark et al., 1977. e= McDowell et al., 1977. f= Bennink et al., 1972. g= Kronfeld and Raggi, 1964. h= Armentano et al., 1983. i= Veenhuizen et al., 1988. j= Schmidt and Keith, 1983. k= Bergman et al., 1974. l= Bergman and Hogue, 1967. m= Buckley et al., 1982.

the brain, gastrointestinal tract, erythrocytes, liver, and muscle. Most available data relating to the specific requirements of these tissues have been obtained from fed, nonpregnant sheep and it is assumed that in lactating cows they represent the same fraction of maintenance.

<u>Cellular metabolism</u> Analogous to monogastric species, glucose serves three major functions in the cellular metabolism of ruminants. First, it serves as a source of carbon in the synthesis of other carbohydrates, amino acids, and mucopolysaccharides (Weekes, 1979). Second, metabolism of glucose by the pentose phosphate pathway provides a source of reducing equivalents, i.e., NADPH, necessary for biosynthetic reactions. Third, glucose also may be oxidized directly as a source of energy. However, glucose makes only a small contribution to respiratory CO_2 of ruminants. For lactating dairy cows in positive energy balance and producing 30 kg milk, 3.8 to 6.1% of respired CO_2 was derived from the oxidation of glucose with 12.3 to 17.4% of glucose being oxidized to CO₂ (Bauman et al., 1988; Clark et al., 1977).

In contrast to monogastrics, ruminants use fatty acids, not glucose, as the major source of energy. Annison et al. (1974) showed that 19 and 34% of total expired CO_2 on low- and high-fiber diets, respectively, was derived from the oxidation of acetate. Oxidation of

ketone bodies represents only 3 to 4% (Palmquist et al., 1969) and the oxidation of palmitate accounts for 3.5% of total CO₂ (Bauman et al., 1988).

Brain and central nervous system Brain and central nervous system tissues of ruminants have an absolute requirement for glucose when the ruminant is well-fed and during starvation. Lindsay and Setchell (1976) showed that the rate of utilization of glucose by the brain of sheep was .508 umol glucose/g brain tissue per min, or 12 g of glucose daily, in both conscious sheep and sheep that are hypoglycemic and hyperketonemic. This corresponds to 5 to 6% of glucose used by pregnant sheep. In contrast, the brain of man requires 110 to 145 g of glucose daily, or 80% of the total amount of glucose used within the body (Owen et al., 1967; Bergman, 1973). The major difference between these two species has been attributed to the difference in the sizes of the brain, with the brain of man weighing approximately 1400 g and that of sheep weighing only 90 g (Lindsay and Setchell, 1976).

<u>Erythrocytes</u> Red blood cells of adult ruminants contain little or no glucose and metabolize small amounts of glucose, which is equivalent to about 3% of the total glucose used. This is in direct contrast to man, whose

red blood cells use approximately 15% of the postabsorptive supply of glucose (Weekes, 1979).

Gastrointestinal tract (including spleen and Utilization of glucose by the pancreas) gastrointestinal tract accounts for 16% of the turnover of glucose in fed, nonpregnant and pregnant sheep and 22% of the glucose turnover of fasted sheep (Bergman, 1975). This major requirement for glucose may be related to the rapid turnover of mucosal epithelial cells. The total amount of glucose needed by these tissues would be expected to increase during early lactation of a dairy cow as feed intake increases (Fell and Weekes, 1975). Reynolds et al. (1988b) measured the net flux of glucose across the gastrointestinal tract of cows 4 or 8 wk postpartum and found that the gastrointestinal tract used 255 g of glucose daily or 8% of the glucose produced by the liver. These data represent the net amount of glucose used from the blood and do not account for the amount of glucose absorbed from the contents of the small intestine and used by mucosal tissue. Consequently, the data may underestimate the amount of glucose used by the gastrointestinal tract.

Some glucose used by the gastrointestinal tract may be recycled as lactate rather than being oxidized completely (Baird, 1977). Considering the large amount of

lactate originating from these tissues (1.9 mmol lactate/min) (Baird, 1981), recycling of carbon from glucose through lactate may account for about half of the glucose used by the gastrointestinal tract and for a large percentage of carbon recycled through the Cori cycle.

Liver The liver of ruminants continuously synthesizes glucose from gluconeogenic precursors. In fed, pregnant and nonpregnant sheep, the liver utilizes 15 g of glucose or 10% of the total glucose turnover (Bergman, 1975). Because the liver of ruminants contains no glucokinase (Ballard et al., 1969), the glucose that is extracted is stored as glycogen and little glucose is oxidized and used as an energy source.

Muscle Although considerable amounts of glucose are stored as glycogen in muscle, glucose is not oxidized to CO₂ by muscle (Weekes, 1979). Recycling of carbon from glucose through lactate, alanine, and glutamate accounts for 75% of the glucose taken up by the hind limb of fed sheep, with no net loss or utilization of the carbon in glucose. Only during exercise or exposure to cold is glucose used as an energy source by muscle (Weekes, 1979). <u>Tissues that require glucose for productive functions</u>

In addition to the amount of glucose required for maintenance of normal body functions, the reproductive tract and mammary gland have a specific requirement for

glucose. Of the total amount of glucose required by a lactating or pregnant ruminant, the pregnant uterus and mammary gland utilize a large percentage of glucose available.

Reproductive tract (nonpregnant cows) Little direct evidence exists that illustrates a relationship between fertility and the need for glucose by the uterus. McClure (1970) has suggested that hypoglycemia in pasturefed beef cows causes a decrease in fertility. Injections of 2-deoxy-D-glucose, a metabolic inhibitor of the metabolism of glucose, resulted in an inhibition of ovarian activity of well-fed, nonlactating beef heifers (McClure et al., 1978).

For lactating dairy cows, a negative relationship has been shown between energy balance during the first 20 d of lactation and the number of days to normal ovulation (r=-.60) (Butler et al., 1981). Butler et al. (1981) concluded that energy balance during the first 20 d of lactation was important in determining the onset of ovarian activity after parturition. Because the amount of energy a cow consumes directly determines the amount of glucose available (Herbein et al., 1978) and cows are in the greatest negative energy balance before milk production peaks, a limit in the amount of glucose

available to the reproductive tract may compromise reproductive performance.

Pregnancy (fetus and gravid uterus) Uptake of glucose by the fetus and the gravid uterus accounts for 43% (Prior and Christenson, 1978) to 70% (Setchell et al., 1972) of the total amount of glucose used by pregnant sheep. The amount of glucose used by the pregnant uterus increases both with progression of gestation and with an increasing number of fetuses. Ferrell and Ford (1980) showed that the amount of glucose taken up by the gravid uterus of Hereford cows increased from 7 to 174 g of glucose daily as the day of gestation increased from 31 to 258 d, and the amount of glucose taken up by the uterus increased exponentially during the last trimester of pregnancy. Thus, analogous to sheep, tissues associated with the pregnant uterus and the fetus use a large percentage of glucose available to the pregnant cow.

During pregnancy in sheep, the amount of glucose oxidized by other tissues in the body is decreased (Setchell et al., 1972) to preserve glucose. Thus, the fetus exerts a "pulling" effect on glucose metabolism in order to meet its metabolic needs.

<u>Synthesis of triglycerides by adipose tissue and</u> <u>mammary gland</u> Glucose is necessary for the synthesis of triglycerides by both adipose tissue and mammary gland.

First, glucose is necessary for the synthesis of α glycerol phosphate used during the esterification of fatty acids to form the triglyceride moiety. Free glycerol released during lipolysis can not be reutilized, because the activity of glycerol kinase is very low in both of these tissues (Vaughn, 1961). Glycerol released during lipolysis is used by the liver and kidney primarily for gluconeogenesis (Bergman et al., 1968). Second, glucose is required to furnish NADPH reducing equivalents through the pentose phosphate pathway.

In contrast to monogastrics, glucose is not a source of carbon during de novo synthesis of fatty acids by adipose tissue (Vernon, 1981) and mammary gland (Bauman and Davis, 1974). This is in agreement with the very low activities of ATP-citrate lyase and NADP-malate dehydrogenase in both of these tissues (Ballard et al., 1969). Activities of these two enzymes and the amount of glucose incorporated into fatty acids in adipose tissue can be increased by infusing glucose or feeding a highconcentrate diet to sheep (Ballard et al., 1972). However, acetate still remains the primary source of carbon for fatty acids.

<u>Mammary gland</u> The requirement for glucose by the mammary gland of dairy cows increases dramatically with the onset of lactation. When feed intake was held

constant from -30 to +40 d postpartum, the irreversible loss of glucose increased from 1.48 to 2.56 kg/d (Bennink et al., 1972). Over the same period, the percentage of glucose oxidized to CO_2 decreased from 35 to 11%, thus illustrating the dominant role the mammary gland plays in controlling the metabolism of glucose.

Studies with lactating goats (Annison and Linzell, 1964), sheep (Bergman and Hogue, 1967), and cows (Bickerstaffe et al., 1974) have shown that the requirement for glucose by the mammary gland accounts for 60 to 85% of the glucose used by lactating ruminants. Further, the synthesis of lactose accounts for 50 to 85% of the glucose extracted by the mammary gland (Annison et al., 1974; Bickerstaffe et al., 1974). From values in the literature, Elliot (1976) calculated that at some point not far above a daily milk production of 30 kg, almost 100% of glucose available would be used by the mammary gland. No data are available in the literature on the amount of glucose used by the mammary gland of cows producing over 35 kg of milk and this point needs to be addressed in future research. The mammary gland also uses glucose to synthesize ATP, NADPH, and α -glycerol phosphate necessary for the synthesis of milk fat and protein. (See previous discussion under synthesis of triglycerides by adipose tissue and mammary gland.)

Precursors of Glucose

Absorption of glucose from the small intestine

Less than 10% of the glucose utilized by ruminants is absorbed from the lower gastrointestinal tract (Armstrong, 1965; Bensadoun et al., 1962). On a predominantly forage diet, essentially no glucose enters the portal blood from the lower gastrointestinal tract (Huntington, 1982; Janes et al., 1984). As the amount of concentrate in the diet increases, the amount of glucose present in portal blood also increases (Huntington et al., 1981; Janes et al., 1984; Wieghart et al., 1986). At the same time, the amount of glucose available to the ruminant increases. As a result, the percentage of glucose absorbed from the small intestine remains constant. Otchere et al. (1974) measured the amount of α -linked glucose polymers recovered in digesta from the duodenum of four steers fed low and high amounts of a high-concentrate diet. At both feed intakes, they estimated that the maximum contribution of α -linked glucose polymers recovered in the duodenum was 9% of the irreversible loss of glucose for those steers.

Gluconeogenesis, thus, must supply over 90% of the glucose utilized. In contrast to monogastrics, ruminants continuously rely on gluconeogenesis to provide a source of glucose that is required by various tissues.

Gluconeogenesis by the liver accounts for 85% of the total, whereas the kidneys account for 8 to 10% of the glucose used by fed sheep, which increases to 15% during fasting (Bergman, 1973).

Propionate

Two different approaches have been used to estimate the contribution of propionate to the synthesis of glucose. The first approach involves using isotope dilution techniques to calculate a transfer quotient. Transfer quotients reflect the fraction of glucose that is derived from the precursor, in this instance, propionate (Gurpide et al., 1963). Transfer quotients attempt to explain what is occurring in the whole animal and reflect the minimal contribution of propionate to the synthesis of glucose because crossover of carbon occurs in the tricarboxylic acid cycle (Wiltrout and Satter, 1972). The second approach involves calculating the net flux of a particular metabolite across the liver from the rate of blood flow to the liver and the concentration gradients across the liver for the particular metabolite. Such fluxes can be used to calculate net extraction of propionate by the liver, which represents the maximum contribution propionate can make to the synthesis of glucose (Bergman, 1975).

For well-fed, nonlactating ruminants, propionate, derived from rumen fermentation, is the major precursor of glucose (Weekes, 1979). In sheep, the amount of glucose derived from propionate, calculated by using isotope dilution techniques, has ranged from 19 to 60% depending on the type and amount of concentrate consumed (Bergman et al., 1966; Judson et al., 1968; Leng et al., 1967; Steel and Leng, 1973). Judson et al. (1968) varied the amount of starch fed to wethers and found that the percentage of glucose synthesized from propionate increased from 36 to 56% as the amount of starch fed was decreased. Recently, Veenhuizen et al. (1988) showed that 43% of glucose was derived from propionate, and this percentage increased to 67% when 600 g of sodium propionate was fed daily to steers.

Few studies have been done with lactating dairy cows to determine the contribution of propionate to gluconeogenesis during early lactation. By determining the trans-organ balances of propionate and glucose across the liver, Lomax and Baird (1983) and Reynolds et al. (1988a) determined that propionate accounted for, at most, 46 to 55% of hepatic glucose production. Wiltrout and Satter (1972), using isotope dilution techniques, determined that 45% of glucose was derived from propionate in lactating cows.

Wiltrout and Satter (1972) also discussed the difficulties of accurately determining the amount of glucose derived from propionate because of crossover of carbon, and subsequent isotope dilution, in the tricarboxylic acid (TCA) cycle. Radioactive carbon is lost from oxaloacetate as the labeled molecule of oxaloacetate "travels around" the TCA cycle, even though oxaloacetate eventually is converted to glucose. Therefore, Wiltrout and Satter (1972) concluded that the crossover of carbon in the TCA cycle results in an underestimation of the contribution of propionate to the synthesis of glucose. After correcting for crossover of carbon in the TCA cycle, Wiltrout and Satter (1972) estimated that 60% rather than 45% of glucose was derived from propionate. It is encouraging that both isotope dilution and trans-organ balance techniques for measuring the contribution of propionate to glucose synthesis give comparable results.

<u>Amino acids</u>

The contribution of amino acids to the synthesis of glucose is influenced by both the nutritional and physiological status of the ruminant (Bergman, 1973). In a review of carbohydrate metabolism, Weekes (1979) reported that 15 to 30% of the glucose synthesized by fed,

nonpregnant sheep came from amino acids and that this amount increased to 35% during a 3 to 6 day fast.

Bergman and Heitmann (1978) compared results from two different techniques to determine the contributions of amino acids to the synthesis of glucose in fed, nonpregnant sheep. Because of the problem of crossover of carbon in the TCA cycle, a minimal estimate of gluconeogenesis from amino acids was obtained by measuring the transfer of ¹⁴C from labeled amino acids into glucose. However, because the liver uses amino acids for protein synthesis and other metabolic processes, the maximum contribution was calculated from the net removal of amino acids from blood by the liver. Between 16 and 32% of glucose was derived from amino acids with alanine and the glutamine-glutamate couple being the largest contributors.

Few studies have been done with lactating cows to determine the contribution of amino acids to the synthesis of glucose. Lomax and Baird (1983) estimated that alanine, glycine, serine, and threonine accounted for no more than 8.6% of glucose synthesized by the liver. For early-lactation cows, Reynolds et al. (19882) calculated that the maximum contribution of amino acids to the synthesis of glucose was 16.5%, based on the flux of α amino nitrogen across the liver. No studies have been done to compare the contributions of amino acids to

gluconeogenesis in cows in negative and positive energy balance.

Lactate and pyruvate

Lactate, along with small quantities of pyruvate, can be used as a source of carbon for gluconeogenesis. For sheep and steers, estimates of the contribution of lactate to the synthesis of glucose range from .064 to 20% (Harmon et al., 1983; Lindsay, 1970; Prior, 1978; Weekes, 1979). The contribution of lactate to the synthesis of glucose depends on the physiological state of the ruminant (Weekes, 1979) and the availability of lactate (Harmon et al., 1983). Lindsay, as reported by Weekes (1979), calculated that lactate potentially could provide 15% of the glucose needed by fed, nonpregnant sheep, and this amount could increase to 40% after sheep were fasted for 3 to 6 d.

For lactating cows, the maximum contribution of lactate to the synthesis of glucose, as calculated from the flux of lactate and glucose across the liver, ranges from 16 to 23% (Baird, 1981; Lomax and Baird, 1983; Reynolds et al., 1988a). Van der Walt et al. (1983) calculated the minimal contribution of lactate to glucose flux to be 6.5%, and up to 80% of the lactate assimilated by the liver was used for purposes other than gluconeogenesis in ewes. As shown for sheep, a 6 day fast

of lactating cows increases the contribution of lactate to glucose synthesis from 16 to 74% (Lomax and Baird, 1983).

Lactate is formed during anaerobic metabolism of glucose in all cells, especially in muscle cells during exercise (Weekes, 1979). Much of the available lactate is absorbed from the rumen of ruminants fed highconcentrate diets, and small amounts can be produced by metabolism of propionate in the rumen epithelium (Weekes, 1979). In a review of lactate metabolism, Giesecke and Stangassinger (1980) indicated that up to 50% of blood lactate was derived from glucose or glycogen. The Cori cycle involves the conversion of glucose to lactate in peripheral cells; lactate then is transported to the liver and kidneys and converted back to glucose. Thus, no net synthesis of glucose occurs from such endogenous lactate.

It is possible to quantitate the contribution of the Cori cycle to the synthesis of glucose by using specifically labeled glucoses. The tritium on $6-[^{3}H]$ glucose is lost at the stage of pyruvate and, consequently, is not incorporated into glucose synthesized through gluconeogenesis. By comparing the irreversible losses of $6-[^{3}H]$ -glucose and $U-[^{14}C]$ -glucose, the amount of recycling through the Cori cycle can be estimated. Baird et al. (1983) used this approach and calculated that 7% of glucose came from the Cori cycle in nonlactating,

pregnant cows and this amount decreased to 2% for lactating cows. These values illustrate that the amount of glucose recycled through the Cori cycle is small and that most of the lactate is derived from dietary sources or from rumen fermentation.

<u>Glycerol</u>

Glycerol serves as a component of triglycerides in adipose tissue and is released upon their hydrolysis. In fed, nonpregnant sheep, 5% of glucose is synthesized from glycerol (Bergman et al., 1968), and a 3 to 5 day fast increases the percentage of glucose derived from glycerol to 23%. In lactating cows, glycerol would be an important source of carbon during early lactation when body stores are being mobilized to meet the energy demands of lactation. Lomax and Baird (1983) estimated that the maximum contribution of glycerol to the synthesis of glucose increased from .8 to 20% upon fasting lactating cows for 6 d.

<u>Glycogen stores in liver and muscle</u>

Glycogen stored in the liver provides a small reserve of glucose that can be used during starvation, stress, or exercise (Weekes, 1979). During the first month of lactation, cows deplete their stores of liver glycogen in order to support the need of the mammary gland for glucose. Assuming that liver contains 5% glycogen and

that essentially all of the glycogen is mobilized, 400 to 500 g of glucose can be obtained from the liver, a very small fraction of the glucose used by the mammary gland during the first month of lactation. Glucose derived from glycogen stored in muscle only can be used within the muscle and does not provide a source of glucose for the rest of the body (Weekes, 1979).

Hormonal Regulation of Glucose Metabolism

Insulin and glucagon are the major hormones involved in the regulation of glucose homeostasis in ruminants. The metabolic effects of these two hormones have been reviewed by Bassett (1975, 1978), Brockman (1978, 1986), McDowell (1983), Prior and Smith (1982), and Trenkle (1978, 1981). The discussion that follows centers around changes that occur in the concentrations and effects of these hormones when the availability of glucose is either increased or decreased.

<u>Insulin</u>

The predominant effect of insulin on metabolism is to increase the incorporation of nutrients into muscle and adipose tissue and, thus, stimulate protein and fat synthesis. Insulin has been shown to increase the uptake of glucose and amino acids by muscle and adipose tissue. In contrast, the uptake of glucose by the mammary gland (Laarveld et al., 1981) and uterus (Hay et al., 1984) seems to be dependent on the concentration gradient of glucose, and these tissues are not responsive to insulin. Insulin also has been shown to decrease gluconeogenesis and glycogenolysis in the liver.

Studies with sheep have shown a positive relationship between the concentration of insulin in plasma and the irreversible loss of glucose (Bassett et al., 1971; Leng, 1970) with no relationship between the concentrations of insulin and glucose in plasma (Bassett et al., 1971; Lomax et al., 1979). Bassett (1978) concluded from these data that insulin was a major regulator of the disposal of glucose, whereas concentration of glucose in plasma may not be an important determinant of the secretion of insulin.

In vitro experiments with adipose tissue from lactating dairy cows have shown that adipose tissue is insensitive to the effects of insulin and glucose during late pregnancy and the first month postpartum (Metz and van den Bergh, 1977; McNamara and Hillers, 1986). These results suggest that at one month postpartum, dairy cows use exogenous glucose differently than later in lactation. During early lactation when demands for energy can not be met from the feed consumed, such metabolic changes ensure
that the mammary gland has an adequate supply of acetate, nonesterified fatty acids, and glucose. As the availability of glucose is decreased, the concentration of glucose and insulin also decreases to adjust metabolism of the cows. When feed intake is decreased, the concentration of insulin in plasma decreases from 310 to 240 pg/ml in response to the perturbation (deBoer et al., 1985).

When a large bolus of glucose is given to lactating cows, dramatic increases in concentrations of glucose and insulin in plasma are seen immediately (Hove, 1978; Thompson et al., 1975; Sartin et al., 1985). In comparison to glucose-loading studies, glucose administered over a long period of time does not cause as dramatic a change in the concentration of insulin. Frobish and Davis (1977) infused glucose into the duodenum for 5 d at a rate equivalent to 1.5 times the amount of glucose secreted as lactose in milk. Concentration of insulin in plasma tended to increase from 9.5 to 13.4 uU/ml, a smaller increase than observed during the glucose-loading studies. Therefore, insulin may be involved only in the short-term regulation of the metabolism of glucose when additional glucose is provided. Lomax et al. (1979) infused glucose continuously into the jugular vein of three lactating dairy cows at 1089 g/d for

48 h. Concentration of insulin in arterial blood doubled and the concentration in portal vein tripled after 4.5 h of infusion but returned to preinfusion values at 24 h.

Propionate and butyrate at pharmacological concentrations are more potent stimulators of the secretion of insulin than is glucose (Brockman, 1986). With nonlactating ewes, Brockman (1982) showed that propionate infused into the portal vein at physiological concentrations doubles the concentration of insulin in plasma. Because Brockman did not obtain a dose response when different amounts of propionate or butyrate were infused into the portal vein, he concluded that these short-chain fatty acids were not the primary agents in the regulation of the secretion of insulin.

For the lactating dairy cow, 60% of insulin produced by the pancreas is removed as each volume of blood passes through the liver (Lomax et al., 1979). Concentrations of insulin in plasma or blood reflect the net balance between the clearance rate and pancreatic production rate of insulin. Trenkle (1981) suggested that increases in concentrations of insulin usually are the result of increases in the production of insulin by the pancreas. Also, the concentration of insulin at the receptor site, determined by rate of blood flow to the tissue, and the

number of receptors are important in determining the biological response elicited by insulin.

<u>Glucagon</u>

Pancreatic glucagon stimulates glycogenolysis and gluconeogenesis by the liver, thus increasing the availability of energy to the cells of the body. Glucagon increases the hepatic extraction of alanine, glutamine, serine, threonine, and lactate. The conversion 'of propionate to glucose, however, is not changed by glucagon (Brockman and Greer, 1980). The activity of pyruvate carboxylase, an enzyme that is not involved in the conversion of propionate into glucose, is increased by glucagon (Brockman and Manns, 1974).

At physiological concentrations, butyrate increases the secretion of glucagon from the pancreas. Propionate at physiological concentrations, however, does not stimulate increases in the concentration of glucagon in plasma (Brockman, 1982). Like insulin, physiological variations in the concentrations of butyrate, however, may not directly regulate the secretion of pancreatic glucagon.

When the availability of glucose is decreased, the secretion of glucagon should increase. Bloom et al. (1978) showed that injections of 2-deoxyglucose into calves increased the concentration of glucagon in blood.

In order for increases in the concentration of glucagon to have a beneficial effect, however, adequate substrate must be available for the increase in gluconeogenesis to occur. Increases in the concentration of pancreatic glucagon may not be beneficial to ruminants fasted or starved for longer than 48 h. Bassett (1972) showed a decrease in the concentration of pancreatic glucagon in wethers that had been fasted for 4 d.

With wethers and dry, nonpregnant ewes, infusion of glucose into the duodenum for 40 to 120 min decreases the concentration of glucagon in plasma (Bassett, 1972; Berzins and Manns, 1979). At the same time, the concentration of glucagon-like immunoreactivity increased (Berzins and Manns, 1979). Glucagon-like immunoreactivity, or gut-type glucagon, is immunologically similar to pancreatic glucagon, binds to glucagon receptors in the liver, and may inhibit the action of pancreatic glucagon (Berzins and Manns, 1979). These authors concluded that the inhibition of the secretion of pancreatic glucagon may have resulted from the localized effect of glucose on gastrointestinal cells or a direct inhibition of the release of glucagon from the pancreas caused by the sudden increase in availability of glucose. No studies have examined the effects of glucose infused into the duodenum for several days in lactating dairy cows

on the concentrations of either pancreatic or gut-like glucagon.

Molar ratio of insulin to glucagon

With their opposing effects on liver metabolism, the relationship between insulin and glucagon is of functional importance. Unger (1971) suggested that the molar ratio of the concentrations of insulin and glucagon in blood may be more important than their individual absolute concentrations. Although the ratio of insulin to glucagon in portal blood is the most important due to their effects on hepatic metabolism (Bassett, 1975), Unger has proposed that the ratio in peripheral plasma can be used as an index of the balance of the actions of these hormones on maintaining glucose homeostasis. In ruminants, the molar ratio of the concentrations of insulin and glucagon are lower than those obtained for nonruminants. However, the ratio of insulin to glucagon still may be important in regulating the rate of gluconeogenesis from absorbed metabolites, such as propionate and amino acids (Bassett, 1975).

Effects of Changing the Availability of Glucose on Milk Production and Metabolism of Glucose

Because large quantities of glucose are used by the mammary gland of high-producing dairy cows, understanding

the effects of either increasing or decreasing the availability of glucose is important to understand fundamentals of milk secretion. The availability of glucose can be increased by infusing glucose into the blood, abomasum, or duodenum or by increasing the intake of feed or specific gluconeogenic precursors, such as propionate and/or amino acids. On the other hand, the availability of glucose can be decreased experimentally by decreasing feed intake or by causing glucose to be excreted into the urine.

When the availability of glucose is changed by changing feed intake, the availability of all nutrients, not just glucose, is changed. Therefore, changes observed in glucose metabolism may be the result of changes in the amounts of other nutrients and not specifically effects associated with changes in the availability of glucose. For these reasons, the discussion that follows deals specifically with increasing the availability of glucose by infusing glucose into peripheral blood or gastrointestinal tract and with decreasing the availability of glucose by means that decrease the availability of glucose and not other dietary nutrients. <u>Increasing the availability of glucose</u>

Several researchers have investigated the effects of increased availability of glucose on milk production.

With an intravenous infusion of glucose, Linzell (1967) showed a 63% increase in the secretion of milk within 3 h in goats fasted for 24 h. Neither acetate alone nor acetate plus amino acids had any additional effect. Results obtained with lactating cows and goats are summarized in Table 2. Exogenous glucose has been infused into the abomasum, duodenum, or jugular vein of lactating cows under different energy balances. In addition, the amount of glucose infused daily has varied from 10 to over 100% of the amount of glucose used by a lactating cow. All studies, except one (Whitelaw et al., 1986), have shown either no changes or small increases in the production of milk when glucose was supplemented. Whitelaw et al. (1986) showed a 5% decrease in milk production when 800 g/d of glucose was infused into the abomasum for 7 d. With such a low amount of milk production, this decrease may not be biologically significant.

The concentration of glucose in plasma has been shown to increase with the long-term administration of glucose (Fisher and Elliot, 1966; Frobish and Davis, 1977; Rao et al., 1973; Whitelaw et al., 1986), however, the concentrations of ketone bodies and nonesterified fatty acids have been shown to decrease (Fisher and Elliot, 1966; Whitelaw et al., 1986). As discussed already, the

<u></u>	1	Amount of				Change in	
Energy	Infusion	glucose	Length of	Milk production		mīlk	
balance	sitel	infused	infusion	Control	+ Glucose	production	Reference ²
Lactati	ng cows	<u></u>		(kg	/d)		
	IA	300 g/d	12 d	14.4	14.1	ns	a
-	IA	800 g/d	7 d	13.0	12.3	- (5%)	b
0	IV	750 g/d	1 d	?	?	ns	С
+	IA	450 g/d	12 d	28.8	28.9	ns	đ
+	IA	300 g/d	6 d	15.5	16.4	+ (6%)	e
+	IA	300 g/d	6 d	23.3	23.9	ns	e
+	IA	2150 g/d	5 d	26.7	28.6	+ (7%)	f
+	IV	444 g/d	4 d	13.7	14.6	+ (6%)	q
+	IV	622 g/d	4 d	13.7	15.2	+ (118)	g
Lactati	ng goats						-
starved	IV	5.4 q/h	4 h	.46	.65	+ (41%)	h
fed	IV	5.4 g/h	4 h	1.59	1.59	ns	h
+	IA	45 g/d	14 d	1.59	1.62	ns	i
+	IA	40 g/d	14 d	1.26	1.34	ns	j

Table 2. Changes in milk production as affected by the infusion of glucose into lactating cows and goats

¹IA= into the abomasum; IV=into the jugular vein.

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<sup>2</sup>a=Orskov et al., 1977.
b=Whitelaw et al., 1986.
c=Rao et al., 1973.
d=Clark et al., 1977.
e=Vik-Mo et al., 1974.
f=Frobish and Davis, 1977.
g=Fisher and Elliot, 1966.
h=Chaiyabutr et al., 1983.
i=Ranawana and Kellaway, 1977.
j=Farhan and Thomas, 1977.
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concentration of insulin has been shown to increase slightly (Frobish and Davis, 1977; Whitelaw et al., 1986) or remain unchanged (Lomax et al., 1979) when additional glucose is infused into the abomasum or peripheral blood. Such changes in concentrations of key energy metabolites suggest that supplying additional glucose may spare other energy sources, such as fatty acids and acetate from oxidation, and may allow for additional storage of nutrients in adipose stores or as glycogen in the liver.

Studies with steers (Prior and Scott, 1980) and wethers (Pearce and Piperova, 1984 and 1985) lend support to the idea that some of the glucose infused is stored in adipose tissue. When glucose was infused into the duodenum of wethers for 11 to 14 d at 185 g of glucose daily, the specific activity of enzymes associated with lipogenesis in liver, mucosa from the small intestine, and perinephric adipose tissue increased along with the specific activities of phosphofructokinase and pyruvate kinase. Prior and Scott (1980) showed that the specific activities of acetyl CoA carboxylase, fatty acid synthetase, and NADP-malate dehydrogenase increased when steers were infused intravenously with 495 g of glucose daily for 14 d. Rao et al. (1973) showed that the specific activity of lipoprotein lipase in adipose tissue from lactating cows increased with the infusion of 750 g

of glucose for 24 h. Bartley et al. (1966) infused 1500 g of glucose daily into the duodenum of two lactating cows for 21 d and found that the concentration of glycogen in the liver increased from 1.7 to 2.6%. Bartley et al. (1966) concluded that most of the glucose infused was not stored as glycogen in the liver and at least part of the additional glucose was stored in adipose tissue.

Infusions of glucose into the jugular vein, abomasum, or duodenum have increased the irreversible loss of glucose in nonpregnant sheep (Judson and Leng, 1973) and lactating goats (Ranawana and Kellaway, 1977) and cows (Bartley and Black, 1966; Clark et al., 1977). In all of these studies except Ranawana and Kellaway (1977), decreases in the endogenous production of glucose were observed. Judson and Leng (1973) determined that the decrease in endogenous production of glucose was not caused by a decrease in gluconeogenesis from propionate and, most likely, was caused by a decrease in gluconeogenesis from amino acids.

The amount of glucose infused and the energy balance of ruminants may influence the response in the metabolism of glucose when the amount of glucose available is increased. When large amounts of glucose (1500 g/d) were infused into the duodenum of lactating cows producing under 8 kg of milk, the percentage of respiratory CO_2

derived from glucose increased from 10 to 20% (Bartley and Black, 1966). However, when 450 g of glucose was infused daily into lactating cows producing more milk (29 kg/d), the percentage of CO_2 derived from glucose did not change compared to the control treatment, with only 5% of CO_2 originated from glucose (Clark et al., 1977). Although cows in both of these studies were in positive energy balance, the degree of positive energy balance differed greatly between the two studies. Clark et al. (1977) fed their cows 4 Mcal above NRC recommendations for energy whereas the cows in the study by Bartley and Black (1966) were fed double their NRC recommendations for energy. <u>Decreasing the availability of glucose</u>

Previous research has provided indirect evidence that a decrease in the availability of glucose may influence milk production. By using perfused mammary glands of goats, Hardwick et al. (1961) showed that milk production ceased abruptly when the concentration of glucose fell below 20 mg/dl. Bergman (1973) suggested that the availability of glucose has a powerful influence on lactose synthesis and, thus, milk production. Kronfeld et al. (1968) showed that a linear relationship existed between milk production and the total amount of glucose used by lactating cows.

Only a few in vivo studies have examined possible direct relationships between glucose availability and secretion of milk. Linzell (1967) observed a decrease in the production of milk within 2 h when he injected insulin into fed goats, and this decrease in milk production could be reversed by infusions of glucose. Kronfeld et al. (1963) did an experiment with lactating cows similar to the experiment of Linzell (1967) and obtained similar results.

Injections of phlorizin, as well as injections of insulin, can be used to decrease the availability of glucose to a fed ruminant. Phlorizin prevents the reabsorption of glucose in the renal tubules and small intestine (Alvarado and Crane, 1962; Horsburgh et al., 1978) with the affinity of the glucose receptor for phlorizin being 1000 times greater than its affinity for glucose. The effects of phlorizin on specific tissues have been reviewed by Veenhuizen (1983) and will not be repeated here. Experimentally, phlorizin has been used to cause glucosuria in sheep (Goetsch and Pritchard, 1958), goats (Schultz et al., 1949), dairy heifers (Young et al., 1974), and steers (Lyle et al., 1984; Veenhuizen et al., 1988; Young et al., 1974). Steers given 2 g of phlorizin daily have excreted 213 to 269 g of glucose daily into urine (Lyle et al., 1984; Veenhuizen et al., 1988). No

studies have been done with lactating cows to determine the amount of glucose excreted into the urine with different dosages of phlorizin.

With steers fed a maintanance diet, injections of 2 g of phlorizin daily for at least 7 d decreased the concentration of glucose (Lyle et al., 1984; Veenhuizen et al., 1988), the ratio of β -hydroxybutyrate to acetoacetate (Lyle et al., 1984), and the ratio of insulin to glucagon (Lyle et al., 1984) in plasma and whole blood. Concurrently, the concentration of nonesterified fatty acids increased in plasma. These results illustrate that changes in metabolism occur to accommodate a decrease in availability of glucose.

At the same time, injections of phlorizin into steers have caused increases in the irreversible loss of glucose (Lyle et al., 1984; Veenhuizen et al., 1988; Young et al., 1974). By determining the flux of carbon between plasma glucose, rumen propionate, blood CO_2 , and rumen CO_2 , Veenhuizen et al. (1988) determined that the decrease in the availability of glucose caused by injections of phlorizin resulted in an increase in the utilization of propionate for gluconeogenesis. No changes were detected in the combined contribution of amino acids, glycerol, and dietary lactate to gluconeogenesis. No studies have examined the effects of phlorizin on the metabolism of

glucose or the production of milk by healthy, lactating dairy cows.

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MANUSCRIPT 1: EFFECTS OF DECREASING THE AVAILABILITY OF GLUCOSE FOR HIGH-PRODUCING DAIRY COWS IN NEGATIVE ENERGY BALANCE

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ABSTRACT

Five Holstein cows, six weeks postpartum, were used to test how cows in negative energy balance would respond to a sudden decrease in availability of glucose, caused by phlorizin. Cows were fed equal amounts of feed twice daily to supply 100% of NRC recommendations for protein and 90% of NRC recommendations for NET. Cows were in negative energy balance throughout the experiment. Phlorizin, at 0, 2, and 4 g/d, was injected subcutaneously every 6 h for 48 h and caused excretion of glucose into urine of 0, 225, and 337 g/d. Milk production was not affected, but percentage of milk fat increased linearly (3.34, 3.56, and 3.70%) with increasing phlorizin. Concentrations of glucose (64.2, 62.6, and 59.4 mg/dl) and insulin (518, 432, and 329 pg/ml) in plasma decreased linearly, whereas B-hydroxybutyrate (6.11, 8.88, and 9.98 mg/dl) and nonesterified fatty acids (181, 220, and 271 ueg/l) increased linearly. The most dramatic changes were seen during the final 12 h of the 48-h injection interval. Results suggest that healthy, early-lactation cows in negative energy balance have the metabolic capacity to change substrates used for energy and milk synthesis and to compensate for short-term increased needs for glucose.

Key words: Dairy cows, glucose, phlorizin, energy balance, insulin, glucagon, nonesterified fatty acids, *B*-hydroxybutyrate

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INTRODUCTION

High-producing cows have a tremendous demand placed upon them to provide enough glucose to meet the requirements of the mammary gland and other organs and tissues of the body. Studies with lactating goats (Annison and Linzell, 1964), sheep (Bergman and Hogue, 1967), and cows (Bickerstaffe et al., 1974) have shown that the glucose requirement of the mammary gland accounts for 60 to 85% of the glucose used by lactating ruminants, with lactose synthesis accounting for 50 to 85% of the glucose extracted by the mammary gland (Annison et al., 1974; Bickerstaffe et al., 1974). From values in the literature, Elliot (1976) calculated that at some point not far above a daily milk production of 30 kg, almost 100% of the total glucose available would be used by the mammary gland. This suggests that glucose availability may limit the amount of milk a cow can produce.

Previous research has provided much indirect evidence that glucose availability influences milk production. By infusing glucose intravenously, Linzell (1967) showed a 63% increase in milk secretion within 3 h in goats fasted for 24 h. Neither acetate alone nor acetate and amino acids had any additional effect. By using perfused mammary glands of goats, Hardwick et al. (1961) showed

that milk production ceased abruptly when the concentration of glucose fell below 20 mg/dl. Few in vivo studies have been done to determine whether a direct relationship exists between glucose availability and secretion of milk. Kronfeld et al. (1968) indicated a linear relationship between milk production and amount of glucose taken up by the mammary gland.

Fhlorizin prevents the reabsorption of glucose in the renal tubules and small intestine (Horsburgh et al., 1978; Alvarado and Crane, 1962) with the affinity of the glucose receptor for phlorizin being 1000 times greater than its affinity for glucose. Experimentally, phlorizin has been used to cause glucosuria in sheep (Goetsch and Pritchard, 1958), goats (Schultz et al., 1949), dairy heifers, and steers (Lyle et al., 1984; Young et al., 1974); thus, phlorizin decreases the availability of glucose in ruminants. Work with steers given 2 g/d of phlorizin has caused 213 to 269 g of glucose to be excreted daily in urine (Lyle et al., 1984; Veenhuizen et al., 1988). No studies have been done with lactating cows to determine the amount of glucose excreted into the urine with different dosages of phlorizin.

Classically, researchers have either decreased feed intake or fasted lactating cows and studied how a decrease in energy intake affects metabolism. One problem with

this approach is that the availability of all nutrients is decreased, not just the availability of energy and glucose. By injecting phlorizin while keeping feed intake constant, only the availability of glucose is decreased and the effects of an immediate decrease in availability of glucose can be studied.

The purpose of the two trials reported herein was 1) to measure glucose excretion in urine of lactating cows in positive energy balance after administration of different doses of phlorizin and 2) to determine whether cows in negative energy balance adjust to a sudden decrease in availability of glucose caused by phlorizin. By studying how lactating cows respond to changes in glucose status, researchers can understand better what may limit and/or control milk production.

MATERIALS AND METHODS

Trial 1: Dose Response to Phlorizin of Midlactation Cows in Positive Energy Balance

Four multiparous cows (two Holstein and two Brown Swiss), 90 to 120 d postpartum, were used in a 4 X 4 Latin square design. Each period lasted 2 d with at least 5 d between periods to prevent carry-over effects. Phlorizin¹, which causes glucosuria, was injected subcutaneously in the scapular region twice daily on d 1 and 2 of each period. The doses of phlorizin (1 g phlorizin/4 ml propylene glycol) were 0, 2, 4, and 6 g/d. Cows were fed twice daily a total mixed diet (Table 1) ad libitum, and feed intake was measured daily throughout the trial. Milk production was measured daily and milk samples were collected for four milkings after the initial injection of phlorizin during each period and analyzed for fat, protein, and lactose content².

Foley catheters were inserted into the urethra 24 h before the first injection of phlorizin for each period. Total collections of urine were made over 2 to 4 h

¹Purchased from Sigma Chemical Co., St. Louis, MO.

²Analysis for components of milk courtesy of Mid-America Dairyman, Inc., Newton, IA. Milk was analyzed by using Milk O'Scan 104AB.

	Trial 1	Trial 2
Composition:		-% DM
Chopped alfalfa hay	25.1	27.6
Corn silage	25.1	30.4
Corn	35.6	18.2
Soybean meal	13.1	23.0
Dicalcium phosphate	.4	.3
Ground limestone	.2	
Trace mineralized salt ^a	.5	.5
Vitamins A, D, and E ^b	.02	.02
Analvsis:		
DM (%)	70.7	67.8
ADF (% DM)	19.9	21.6
NDF (% DM)	ndC	33.8
Crude protein (% DM)	16.9	18.4
Estimated digestible energy (Mcal/kg DM)	nd ^C	2.99

Table 1. Composition and analysis of total mixed diet fed to cows in trials 1 and 2

^aContains not more than 98.0% NaCl and not less than 92.0% NaCl, .35% Zn, .28% Mn, .175% Fe, .035% Cu, .007% Zn, and .007% Co.

^bEach kg contains 4,410,000 IU Vitamin A and D, 2205 IU Vitamin E, and 1654 mg Vitamin K (BP Feed Pak-Mate, Protein Blenders, Inc., Iowa City, IA).

^Cnd=not determined.

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intervals for 48 h after the first injection of phlorizin. Samples of urine were preserved by using toluene, frozen, and analyzed later for concentration of glucose by using glucose oxidase³. Blood samples for the determination of concentration of glucose in plasma were taken from the jugular vein by venipuncture before injection and feeding (pre-injection) and 4 h after phlorizin injections (postinjection). Values for the concentration of glucose in plasma represent samples taken after the second, third, and fourth injections of phlorizin.

Statistical analysis was by analysis of variance (SAS, 1982) in GLM, and differences between treatment means were determined by least significant difference. For all variables measured, except amount of glucose excreted into urine, the treatment by time interaction was not significant, therefore, only least-square means for the 48-h treatment period are reported. One cow bloated during period 4 and her data from period 4 have not been included.

³Diagnostic kit purchased from Worthington Diagnostic Systems, Inc., Freehold, NJ.

Trial 2: Effects of a Decrease in Availability of Glucose for Cows in Negative Energy Balance

Five Holstein cows, 6 wk postpartum, were used in a randomized complete block design with each cow representing a block. Each cow received all three treatments, which consisted of 0, 2, and 4 g/d of phlorizin⁴ injected subcutaneously in equal portions every 6 h. Each treatment lasted 48 h (2 d), with at least 5 d between treatments.

Cows were fed a total mixed diet (Table 1) twice daily. The diet was formulated to supply 100% of NRC recommendations for protein and 90% of NRC recommendations for NE_L (NRC, 1978). Cows consumed almost all of their ration within .5 h after being fed. Feed intake was recorded daily throughout the trial and representative feed samples were taken during each treatment period and analyzed for DM (by toluene extraction), ADF, NDF, and crude protein. During the last two periods, all feces were collected from three cows to estimate the digestible energy content of the diet. Milk production and composition⁵ were measured for four milkings after the initial injection of phlorizin.

⁴Purchased from Sigma Chemical Co., St. Louis, MO.

⁵Analysis for components of milk courtesy of Mid-America Dairyman, Inc., Newton, IA. Milk was analyzed by using Milk O'Scan 104AB.

Catheters were inserted into the urethra at least 24 h before the first injection of phlorizin for each period. Total collections of urine were made for 2 h intervals for the first and last 12 h after the initial injection of phlorizin and for 6 h intervals for the middle 24 h. Thus, all urine was collected for 48 h. Urine was preserved by adding concentrated HCl to the collection vessel; aliquots were frozen and analyzed later for concentration of glucose⁶.

Blood samples were collected from indwelling catheters in the jugular vein every hour for the first and last 12 h and every 6 h for the middle 24 h. Blood samples also were collected hourly for 12 h, for the interval 48 to 36 h before the first injection of phlorizin of each period. Plasma was prepared and analyzed for the concentration of glucose⁶ (GLU), nonesterified fatty acids⁷ (NEFA) (Eisemann et al., 1986), insulin (INS), and glucagon (GLG). Concentration of NEFA in plasma was determined every 2 h for the first and last 12 h and every 6 h for the middle 24 h. Plasma was deproteinized (Somogyi, 1945) and the resulting protein-

⁶Diagnostic kit purchased from Worthington Diagnostic Systems, Inc., Freehold, NJ.

⁷Diagnostic kit purchased from Wako Chemicals USA, Inc., Dallas, TX.

free filtrate was analyzed for concentration of Bhydroxybutyrate (BHBA) (Williamson and Mellanby, 1974).

Concentrations of INS (Elsasser et al., 1986) and GLG (Herbein et al., 1985) in plasma were determined by standard double-antibody radioimmunoassay procedures. Antiserum to bovine insulin⁸ bound 40% of porcine [¹²⁵-I] insulin⁹. Goat anti-guinea pig IgG¹⁰ was the second antibody. Both intraassay and interassay coefficients of variation were 8%. Samples kept for GLG analysis had been preserved with trasylol¹¹ (2500 KIU/ml plasma), an inhibitor of proteolysis. Antiserum against pancreatic glucagon¹⁰ bound 40% of [¹²⁵-I] glucagon⁹. Goat antirabbit IgG¹⁰ was the second antibody. Intraassay and interassay coefficients of variation were 6 and 4%, respectively.

Statistical analysis was by analysis of variance (SAS, 1982) with the model consisting of the three main effects: cows, treatments, and time. To test if treatments were significantly different, the cow by treatment interaction was the error term used. If the

⁸Purchased from ICN ImmunoBiologicals, Lisle, IL. ⁹Purchased from Cambridge Medical Technology, Billerica, MA.

¹⁰Purchased from BioTek Research, Inc., Lenexa, KS.
¹¹Purchased from Mobay Chemical Cooperation, FBA
Pharmaceuticals, New York, NY.

treatment effect was significant $(P\leq.10)$, treatment means were tested to see if the effect was linear.

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RESULTS AND DISCUSSION

Trial 1: Dose Response to Phlorizin of Midlactation Cows in Positive Energy Balance

The major emphasis was to measure the amount of glucose excreted into the urine for the three doses of phlorizin. No glucose was detected in the urine from cows on the 0 g dose, i.e., control (Table 2). The 207 g of glucose excreted daily into the urine from the 2 g dose was similar to that observed for steers fed to gain .3 to .5 kg/d and injected with 2 g of phlorizin (Lyle et al., 1984; Veenhuizen et al., 1988). Amounts of glucose excreted into the urine on the 4 and 6 g doses increased compared to the 2 g dose, but there was little additional benefit of 6 g over 4 g. For all treatments, cumulative excretion of glucose into the urine was linear over time (Figure 1), but closer examination of the data points shows a slight decline in the rate just before the time of the next injection.

Daily DM intake and milk production and composition were not changed by phlorizin injections (Table 2). Assuming that 70% of the glucose extracted by the mammary gland is used for the synthesis of lactose (Annison et al., 1974), one can calculate that the amount of glucose excreted into the urine corresponds to 3 kg of milk for

Table 2. Amount of glucose excreted into the urine, daily feed intake, milk production and composition, and concentration of glucose in plasma for lactating cows in positive energy balance injected with phlorizin (Trial 1)

	<u>Treatment¹(g phlorizin/d)</u>					
	0	2	4	6	Sem	
Amount glucose excrete into urine (g/d) ²	d _O a	207b	379 ^C	454 ^C	24	
DM intake (kg)	21.8	20.3	22.2	22.1	1.1	
Milk production (kg) 4% fat-corrected milk	27.0	26.4	25.9	27.0	.9	
(kg)	27.1	25.7	25.4	27.3	1.0	
Milk composition:						
Fat (%)	3.99	3.83	4.01	4.05	.20	
Fat production (kg)	1.07	1.00	1.00	1.09	.04	
Protein (%) Protein production	3.39	3.38	3.40	3.38	.08	
(kq)	.92	.88	.86	.90	.04	
Lactose (%)	5.06	5.10	5.12	5.10	.03	
Lactose production (kg)	1.38	1.36	1.32	1.40	. 04	
Plasma glucose (mg/dl)	_	_	•			
Pre-injection ³	58.8 ^a	59.3 ^a	62.6 ^D	59.7 ^a	.7	
Post-injection	58.8	58.3	57.8	59.6	1.1	

¹Means within a row with different superscripts differ, P<.05.

²Significant treatment effect, P<.01.

³Significant treatment effect, P<.08.



Figure 1. Cumulative excretion of glucose into the urine for cows in positive energy balance (Trial 1). (Phlorizin was injected every 12 h for 48 h. No glucose was detected in the urine on the 0 g/d dose, which is not shown.)

the 2 g dose, 5 kg of milk for the 4 g dose, and 6 kg of milk for the 6 g dose. Therefore, phlorizin can be used to decrease the availability of glucose in lactating cows without affecting feed intake.

The pre-injection concentration of GLU in plasma increased with the 4 g dose compared to the other 3 treatments (Table 2). The physiological reason for this increase is unknown, but it may relate to the absence of data from the cow that bloated during this treatment. No changes were seen in the concentration of GLU postinjection.

Trial 2: Effects of a Decrease in Availability of Glucose for Cows in Negative Energy Balance

In contrast to trial 1, the doses of phlorizin were injected every 6 h, rather than every 12 h, to cause a more constant excretion of glucose into the urine (Figure 2). Injection of 2 g of phlorizin daily resulted in 225 g glucose/d being excreted into the urine (Table 3). A slight decrease (337 versus 379 g/d) in the amount of glucose excreted into the urine was seen for the 4 g dose in cows in negative energy balance (Trial 2) compared to cows in positive energy balance (Trial 1). These differences may reflect differences in the excretion patterns of glucose into the urine and/or differences in



Figure 2. Cumulative excretion of glucose into the urine for cows in negative energy balance (Trial 2). (Phlorizin was injected every 6 h for 48 h. No glucose was detected in the urine on the 0 g/d dose, which is not shown.)

	Treatment (g phlorizin/d)				Treatment effect	Linearity of treatment
Measurement	0	2	4	SEM	(P>F)	(P>F)
Amount glucose excreted into urine (g/d)	0	225	337	19	.01	.01
DM intake (kg)	17.4	17.6	17.9	.1	ns	
Milk production (kg) 4% fat-corrected milk	30.2	29.8	29.6	• 5	ns	
production (kg)	27.3	27.9	28.0	.6	ns	
Milk composition:						
Fat (%)	3.34	3.56	3.70	.07	.10	.05
Fat production (kg)	1.01	1.06	1.09	.03	ns	
Protein (%)	2.82	2.84	2.87	.01	ns	
Protein production (kg)	.85	.84	.84	.01	ns	
Lactose (%)	4.96	5.01	4.95	.01	ns	
Lactose production (kg	1.50	1.48	1.45	.02	ns	

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Table 3.	Daily excretion of glucose	into the urine,	feed intake,	and milk
	production and composition	after phlorizin	was given to	lactating cows
	in negative energy balance	(Trial ²)	-	-

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the gluconeogenic potential of the diets used in the trials. For Trial 2, the excretion of glucose into the urine was more constant when phlorizin was injected every 6 h (Figure 2) compared to trial 1 where phlorizin was injected every 12 h (Figure 1). Young et al. (1974) fed heifers equal amounts of DM from either grain or hay-based diets and found that the grain-based diet resulted in two times more glucose being excreted into the urine.

Daily DM intake and responses of milk production and composition also are shown in Table 3. Dry matter intake was held constant intentionally across the three treatments for each cow. At the beginning of the experiment, dry matter intake was set at 100% of NRC recommendations for protein and at 90% of NRC recommendations for NE_L (NRC, 1978), thus cows were held in negative energy balance by dietary adjustment. Milk production was not decreased by the injection of phlorizin; thus, cows in negative energy balance were able to adjust for the glucose excreted into the urine. Percentage of fat in milk increased linearly with increasing dose of phlorizin, which suggests that body stores were mobilized to account for the decrease in glucose availability. No changes were seen in the percentages of protein or lactose in milk. The amount of glucose excreted into the urine on the 2 and 4 g doses of

phlorizin represents 15 and 22% of the amount of glucose used to synthesize milk lactose on the 0 g dose. <u>Changes in concentration of metabolites and hormones in</u> <u>relation to time after feeding</u>

Figures 3 and 4 show changes in the concentrations of GLU, BHBA, NEFA, INS, and GLG in plasma for a 12-h interval in relation to time after feeding. Blood samples were taken from 48 to 36 h before the initial injection of phlorizin during all three periods and, thus, represent values from cows that are 6 to 10 weeks postpartum and held in negative energy balance by dietary restriction. Diurnal variations clearly are shown in response to changes associated with time after feeding. Concentration of GLU in plasma decreased for the first 3 h after feeding (Figure 3), then increased, which may possibly coincide with the influx of propionate into the liver and its conversion to glucose through gluconeogenesis.

Concentration of BHBA increased for the first 4 h after feeding and was related negatively to the decreases seen in concentration of NEFA in plasma during this time. Concentration of NEFA in plasma then peaked 10 h after feeding when BHBA was at the lowest concentration. Similar responses in relation to feeding have been observed for both lactating cows (Blum et al., 1985) and ewes (Thye et al., 1970). The initial increase in BHBA

Figure 3. Changes in the concentrations of glucose, ß-hydroxybutyrate, and nonesterified fatty acids in plasma in relation to time after feeding (Trial 2). (Cows were fed at 0 and 12 h. Each point represents the mean of 15 samples, 3 per cow, and all samples were taken before any injections of phlorizin were given for that period.) (• - - •, plasma glucose; + --- +, ß-hydroxybutyrate; X-- --X, nonesterified fatty acids)


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Figure 4. Changes in the concentrations of insulin and pancreatic glucagon in plasma in relation to time after feeding (Trial 2). (Cows were fed at 0 and 12 h. Each point represents the mean of 15 samples, 3 per cow, and all samples were taken before any injections of phlorizin were given for that period.) (0----0, insulin; 0----0, glucagon) may be the result of an increase in the ruminal production of butyrate, which is converted to BHBA in the rumen wall and liver. Part of the increase in BHBA also may be from the oxidation of NEFA by the liver and in turn, BHBA may inhibit release of additional NEFA from adipose tissue. Menahan et al. (1966) observed a significant decrease in concentration of NEFA during an intravenous infusion of sodium *B*-hydroxybutyrate in fasted, nonlactating goats.

Concentration of INS decreased after feeding and was lowest 7 to 11 h after feeding (Figure 4). There is no evidence suggesting any cyclic release of INS beyond that associated with feeding. Vasilatos and Wangsness (1981) fed lactating cows ad libitum at 30 and 90 d postpartum and observed 4.8 and 8.5 spikes, respectively, in the concentration of INS over 24-h periods. In our study, blood samples were taken only hourly, and pulses of INS release can not be assessed accurately. Concentration of GLG did not change significantly except for the first hour after feeding.

<u>Changes in concentration of metabolites and hormones with</u> <u>phlorizin injections</u>

Concentration of GLU in plasma decreases linearly with phlorizin (Table 4), with the decrease primarily occurring within the first 12 h after the initial injection of phlorizin (Table 5). By plotting

Table 4.	Concentrations of metabolites and hormones in plasma averaged over the
	48 h infusion period of phlorizin for cows in negative energy balance (Trial 2)

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Metabolite or	Treatme	Treatment (g phlorizin/d)				Linearity	
hormone in plasma	0	2	4	SEM	(P>F)	(P>F)	
Glucose (mg/dl) B-hydroxybutyrate	64.2	62.6	59.4	.4	.01	.01	
(mg/dl)	6.11	8.88	9.98	.26	.01	.01	
Nonesterified fatty	acids						
(ueg/l)	181	220	271	7	.05	.02	
Insulin (pg/ml)	518	432	329	23	.05	.02	
Glucagon (pg/ml)	182	182	193	6	ns		
Insulin:glucagon (molar ratio)	1.89	1.55	1.07	.09	.05	.01	

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Metabolite T or hormone initi	ime after al injection	Treatm	ent (a	phlor	izin/d)	Treatment effects	Linearity between treatments
in plasma	(h)	0	2	4	SEM	(P>F)	(P>F)
Glucose (mg/dl)	1-12	64.4	62.5	60.7	.7	.02	.01
	36-48	64.8	62.4	58.1	1.4	.03	.02
B-hydroxybutyrate							
(mg/dl)	1-12	6.10	7.92	7.98	.06	.10	.10
	36-48	6.57	10.34	13.37	1.03	.01	.01
Nonesterified fatt	y acids						
(ueg/l)	- 1-12	191	228	250	22	.23	.11
	36-48	178	221	287	17	.01	.01
Insulin (pq/ml)	1-12	461	381	340	41	.17	.10
	36-48	524	450	351	42	.06	.02
Glucagon (pg/ml)	1-12	200	176	193	19	ns	ns
	36-48	172	185	199	7	.07	.02
Insulin: glucagon							
(molar ratio)	1-12	1.64	1.37	7 1.01	.15	.10	.05
•	36-48	1.94	1.59	1.16	.18	.05	.02

Table 5. Concentrations of metabolites and hormones in plasma by time after the initial injection of phlorizin in cows in negative energy balance (Trial 2)

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concentration of GLU in plasma versus time after the initial injection of phlorizin (Figure 5-A), the largest decreases in concentration for the 2 g dose are seen when the concentration of glucose is the lowest, i.e. at 2 to 3 h post-feeding. On the 4 g dose, concentration of glucose tends to remain lower for 36 to 48 h after the initial injection of phlorizin.

Concentrations of BHBA and NEFA increase linearly with increasing amount of phlorizin for the entire 48-h injection period (Table 4). Increases in the concentrations of BHBA and NEFA, however, become more dramatic during the last 12-h of injection period (Table 5 and Figure 5-B and 5-C).

Mean concentration of INS in plasma decreases linearly with increasing dose of phlorizin when averaged over the entire 48-h phlorizin-injection interval (Table 4). No changes were seen in the overall mean of the concentration of pancreatic GLG in peripheral plasma (Table 4). The concentration of GLG shows a slight linear increase over the last 12 h (Table 5 and Figure 5-E) which may suggest an increase in gluconeogenesis. Molar ratio of INS to GLG decreased linearly with phlorizin (Table 4) with the most dramatic changes occurring during the last 12 h (Table 5). For steers given 2 g phlorizin for at





Figure 5. Changes in the concentration of glucose, B-hydroxybutyrate, nonesterified fatty acids, insulin, and pancreatic glucagon in plasma for the three phlorizin treatments (Trial 2). (Phlorizin was injected every 6 h (denoted by the hash marks on X-axis). Cows were fed every 12 h.) (•---•, 0 g/d; •---••, 2g/d; A ----A, 4 g/d.)

least 12 days, conversion of propionate to glucose was increased by 39% (Veenhuizen et al., 1988).

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GENERAL DISCUSSION

The two areas to be discussed are partitioning of substrates and estimated "glucose balance".

Partitioning of Substrates

Short-term decreases in the availability of glucose did not affect milk production adversely for our lactating cows in negative energy balance. The cows apparently compensated for the imposed glucose drain by changing the proportions of substrates used for energy, which probably represent mobilization of some body stores.

One possible explanation follows. In trial 2, milk production did not change significantly after injecting phlorizin and, therefore, the glucose requirement of the mammary gland remained constant. After the first injection of phlorizin, the availability of glucose to various tissues decreased suddenly and remained lower throughout the 48-h phlorizin injection. Such changes are indicated by the initial decrease in the concentration of glucose. The lack of a further decrease in the concentration of glucose coupled with no decrease in milk production suggests that cows adjust to an immediate decrease in glucose availability by partitioning

differently the substrates used to provide glucose needed for milk synthesis.

During the first few hours after the initial injection of phlorizin, liver glycogen could supply most of the additional glucose needed. If the liver represents 1.5% of body weight and contains 3% glycogen on a wet weight basis, approximately 300 g of glucose can be obtained from liver glycogen. After reserves of liver glycogen are depleted, fat and protein could be mobilized from adipose and muscle to spare glucose. The fact that concentrations of β -hydroxybutyrate and nonesterified fatty acids increased in blood in response to phlorizin supports this idea. In addition, more carbon from gluconeogenic precursors may be converted to glucose. Thus, early-lactation cows are able to adjust their metabolism to cope with a short-term decrease in glucose availability.

Estimated "Glucose Balance"

An estimated "glucose balance" can be calculated by subtracting the amount of glucose required by the mammary gland and other tissues from the theoretical amount of glucose derived from the diet. The gluconeogenic potential of the diet can be estimated by using the

regression equation derived by Herbein et al. (1978), which relates digestible energy intake to glucose turnover. The concept for calculating the amount of glucose needed by a lactating dairy cow was presented by Elliot (1976). The requirements of glucose for milk production and maintenance can be calculated by the equation:

where milk contains 5% lactose, and 70% of the glucose taken up by the mammary gland is used for the synthesis of lactose (Elliot, 1976). The estimate of the glucose required for maintainance (188 g of glucose) is based on work by Annison et al. (1974) and is a small fraction of the total glucose needed by the mammary gland.

For trial 2, the diet could supply an estimated 2,335 g of glucose per day, and the requirements of the mammary gland and other tissues can be estimated at 2,345 g/d. Thus, these cows would be deficient only 10 g of glucose during the control treatment (0 g/d) and are close to "glucose equilibrium" when they were fed 94% of their recommended digestible energy. For the 2 and 4 g phlorizin treatments, cows should be in a negative "glucose balance" of 235 and 347 g glucose per day, but they compensated for the negative "glucose balance" without decreasing milk production, possibly by mobilizing body stores.

A decreased availability of 347 g of glucose daily for 48 h did not decrease the amount of milk earlylactation cows produced. These results may not apply to long-term decreases in availability of glucose. If the availability of glucose was decreased more drastically or was maintained for a longer period of time, milk production and/or overall health and reproductive performance might be compromised.

There is indirect evidence that glucose balance may affect the milk production response of cows given somatotropin. When somatotropin was administered daily to cows at 20 to 29 d postpartum, milk production increased by 2.2 kg/d, however, increases of 4.1 kg/d were observed at 60 to 69 d postpartum (Richard et al., 1985). Calculation of glucose balance shows that cows receiving somatotropin at 20 to 29 d postpartum were in a 324 g/d glucose deficit compared to a 73 g/d deficit at 60 to 69 d postpartum. Possibly milk production would have increased to the same extent at 20 to 29 d postpartum if those cows were given intravenous glucose.

Our calculations represent an estimation of the requirement for glucose in lactating cows and should be used only as a tool to estimate the glucose status of cows

and to estimate whether a treatment could be potentially benefical or cost effective. Peel et al. (1982) showed no increases in milk production when glucose and casein were infused into the abomasum of cows injected with somatotropin. Close examination of their data show that milk production would have to be increased by 13 kg before their cows would have had no surplus glucose, therefore, no increases in milk production would be expected from the supplementation of glucose alone. The glucose balance of an early-lactation cow may have to be below the 350 g/d glucose deficit calculated in the present research before a response in milk production is obtained.

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MANUSCRIPT 2: METABOLISM OF PROPIONATE, GLUCOSE, AND BLOOD CO₂ AS AFFECTED BY EXOGENOUS GLUCOSE IN DAIRY COWS AT ENERGY EQUILIBRIUM

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ABSTRACT

Four Holstein cows, fed to meet their digestible energy requirements, were used to quantify changes that occur in the metabolism of propionate, glucose, and CO2 when glucose was infused continuously into the peripheral blood supply at 0, 342, or 737 g/d for at least 5 d. Neither production nor composition of milk was changed by the glucose infusions. Isotope dilution techniques were used to calculate irreversible loss of rumen propionate, plasma glucose, and blood CO_2 and to determine a unique solution for the flux of carbon in a three-pool system composed of rumen propionate, plasma glucose, and blood CO₂. Irreversible losses of rumen propionate and blood CO2 were not changed. The glucose treatments increased glucose irreversible loss over the control in proportion to the amount of glucose infused, but did not change endogenous glucose production. For the control, 52% of plasma glucose was derived from rumen propionate, with an additional 26% coming from other gluconeogenic substances. During glucose treatments, the flux of carbon into plasma glucose from sources other than rumen propionate or blood CO_2 increased in proportion to the amount of glucose infused, with the flux of carbon from rumen propionate remaining constant. The rate of carbon leaving the plasma

glucose pool, other than as CO_2 , increased with infusion of glucose, and the oxidation of glucose tended to increase for the highest glucose treatment.

Key words: Dairy cows, in vivo kinetics, glucose, propionate, carbon dioxide, insulin, glucagon

INTRODUCTION

High-producing dairy cows have a tremendous metabolic challenge to provide enough glucose to support milk production. Young (1977) calculated that Beecher Arlinda Ellen required 7.4 kg of glucose daily to meet the requirements of the mammary gland and other tissues to produce 89 kg of milk. In ruminants, less than 10% of the glucose required is absorbed from the lower gastrointestinal tract (Armstrong, 1965; Otchere et al., 1974). Therefore, gluconeogenesis must supply more than 90% of the glucose required. In steers, sheep, and lactating cows, propionate has been estimated to provide 27 to 59% of the carbon in glucose (Herbein et al., 1978), amino acids provide 2 to 30% (Weekes, 1979), and lactate and glycerol provide most of the remainder (Weekes, 1979).

Bauman et al. (1988) recently showed a 12% increase in the amount of glucose used in the body of cows receiving bovine somatotropin. Less glucose was oxidized to CO_2 , but the decrease was not large enough to account for the increase in glucose irreversible loss. Because the irreversible losses of glucose and nonesterified fatty acids were the only kinetic measurement made, Bauman et al. (1988) could not account for the origin of the additional glucose.

By infusing separate carbon-14 tracers on different days, it is possible to calculate rates of carbon flux between several pools, such as rumen propionate, plasma glucose, and blood CO_2 , and to simultaneously measure the interconversion of these metabolites by using stochastic analysis. This technique has been used to study the interconversion of rumen propionate, plasma glucose, and CO_2 in steers (Veenhuizen et al., 1988) and sheep (Wilson et al., 1983). Veenhuizen et al. (1988) found that the resulting solution accurately predicted the changes imposed on metabolism when either propionate was added to the diet or glucose was excreted into the urine. Stochastic analysis is different from predictive modeling, which has been used to study glucose metabolism in cows (Kronfeld et al., 1971) and sheep (Wastney et al., 1983).

The purpose of the present study was to infuse glucose intravenously into lactating cows at energy equilibrium and then quantify the changes that additional glucose causes in the metabolism of rumen propionate, plasma glucose, and blood CO_2 . Several research groups have measured these metabolites separately, but our study is the first to measure the interconversion of these metabolites simultaneously in lactating cows.

MATERIALS AND METHODS

Experimental Design and Management of Cows

Three multiparous Holstein cows, 60 to 90 d postpartum, were used in a 3 X 3 Latin square design. A fourth cow was added with the treatment sequence such that each treatment was preceded and followed by the other two treatments. All four cows received each of the three treatments. Treatments were 1) a control diet (control), 2) control diet plus a continuous, intravenous infusion of 342 (\pm 6) g glucose/d¹ (low-glucose treatment), and 3) control diet plus a continuous, intravenous infusion of 737 (\pm 24) g glucose/d² (high-glucose treatment). Each period was 11 d, and at least 3 d were allowed between each period.

Before the start of the experiment, each cow was fitted with a rumen fistula. Bilateral jugular catheters were inserted a day before the start of each period and remained patent until the end of each period. Infusions of nonradioactive glucose were begun on d 1 and continued for all 11 d for both the low- and high-glucose

^{130%} dextrose was purchased from Travenol Laboratories, Inc., Deerfield, IL.

²60% dextrose was purchased from Travenol Laboratories, Inc., Deerfield, IL.

treatments. Glucose solutions were infused at about 1.0 ml/min by using a peristaltic $pump^3$. The infusion of tracer began 90 h later (on d 5) to ensure metabolic and hormonal steady state and to allow sufficient time for enzymatic changes to occur.

Cows were fed a total mixed ration every 2 h to meet NRC recommendations for digestible energy (NRC, 1978). Previous results indicate that steers fed every 2 h are in metabolic steady state (Armentano et al., 1984). Composition and chemical analysis of the diet are listed in Table 1. Diet DM was determined by toluene extraction. Digestible energy intake was estimated by collecting fecal grab samples four times daily the last 8 d of each period and by using acid detergent insoluble ash as an internal marker (Porter and Sniffen, 1986).

Cows were milked at 0730 and 1900 h daily. Milk production was recorded throughout the experiment and will be reported for d 3 through 11 of each period. Milk samples were collected at both milkings on d 3 through 11 of each period, composited every three d, and analyzed for fat (Babcock) and protein (Kjeldahl) content.

³Rainin Rabbit, Model HP-4, Woburn, MA.

mixed diet	
Ingredient:	\$DM
Alfalfa hay (chopped) Corn silage Soybean meal ^a Monosodium phosphate Trace mineralized salt ^b	42.9 42.9 13.1 0.5 0.5
Chemical analysis:	
Dry matter (%) Crude protein (% DM) ADF (% DM) NDF (% DM) Digestible energy (Mcal/kg DM)	58.7 18.1 28.4 45.0 2.94

Table 1. Composition and chemical analysis of total

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^aExpeller processed soybean meal, Soy-plus, West Central Cooperative, Ralston, IA.

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^bContained not more than 98.0% NaCl and not less than 92.0% NaCl, .35% Zn, .28% Mn, .175% Fe, .035% Cu, .007% Zn, and .007% Co.

Protocol for Infusion of Tracers

To control rumen volume and turnover rate during all tracer infusions, cows were denied free access to water 14 h before the start of each tracer infusion. Water was, in turn, infused directly and continuously into the rumen at the rate of daily consumption.

For each period, three separate primed-continuous infusions of radioactive tracer were completed. Tracer infusions consisted of $[U-1^4C]$ -propionate infused intraruminally and $[U-1^4C]$ -glucose and NaH¹⁴CO₃ infused intravenously into the right jugular vein. Amounts of each tracer infused are summarized in Table 2. For the intraruminal infusions of $[U-1^4C]$ -propionate, appropriate amounts of tracer were mixed with water in 4-liter bottles as needed and replaced the intraruminal infusion of water. This protocol limited the time that microbial degradation of propionate could occur. During both low- and highglucose treatments, $[U-1^4C]$ -glucose and NaH¹⁴CO₃ were mixed with the nonradioactive glucose. Physiologically buffered saline⁴ was used as the carrier for $[U-1^4C]$ glucose and NaH¹⁴CO₃ during the control treatment. When

⁴Purchased from Travenol Laboratories, Inc., Deerfield, IL.

Tracer	Priming dose	Infusion rate	
	uCi	uCi/min	
[U- ¹⁴ C]-glucose ^a	42	2.7	
[U-14C]-propionate ^b	250	1.0	
NaH14CO3b	55	7.5	

Table	2.	Isotopic	tracers	used	for	primed-continuous
		infusions	5			

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^aAmersham, Arlington Heights, IL.

^bNew England Nuclear, Boston, MA.

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NaH¹⁴CO₃ was infused, pH of the infusate was adjusted to 8.2 to 8.5 by using nonradioactive NaHCO₃⁵.

Tracer infusions were done on d 5, 8, and 11 of each period and were randomized within each period. Three days were allowed between infusions to ensure that radioactivity returned to background amounts. Thus, three infusions of tracer were completed per period for a total of nine separate infusions per cow.

Protocol for Sample Collection and Analysis

Each primed-continuous infusion of tracer was for 10 h, and rumen fluid and blood were sampled before all infusions to assess background radioactivity. Samples for determining specific radioactivity (SRA) of rumen propionate, plasma glucose, and blood CO_2 were collected every 30 min between 4 and 10 h of tracer infusion. Samples for determining the concentration of glucose, β hydroxybutyrate, insulin, and glucagon in plasma were collected every hour between 4 to 10 h of tracer infusion.

Rumen fluid was sampled via the rumen cannula with a stainless-steel probe equipped with a strainer at the distal end. One milliliter of saturated mercuric chloride

⁵Purchased from Vitarine Co, Inc., New York, NY and Tech America Group, Inc., Elwood, KS.

was added immediately to the sample to inhibit microbial action. The sample was centrifuged, and the supernatant frozen for subsequent analysis. Propionate SRA was calculated after isolation of propionate by high-pressure liquid chromatography (Veenhuizen et al., 1988) and counted by using liquid scintillation spectrometry.

Blood was sampled via a catheter in the left jugular vein. A subsample was analyzed immediately for CO_2 SRA (Russell and Young, 1982) and packed cell volume. Whole blood was deproteinized immediately by using cold .6 N perchloric acid and centrifugation (Tyopponen and Kauppinen, 1980), and the supernatant was frozen. Before enzymatic analysis of *B*-hydroxybutyrate (Williamson and Mellanby, 1974), the supernatant was neutralized by using 2 M K₂CO₃ to precipitate perchlorate ions.

Plasma, prepared by centrifugation, was frozen. Later, it was assayed for glucose SRA (Mills et al., 1981) and concentration of glucose by glucose oxidase⁶. Trasylol⁷ (2500 KIU/ml plasma), a proteolytic inhibitor, was added to subsamples of plasma kept frozen for analysis of glucagon. Concentrations of insulin (Elsasser et al., 1986) and glucagon (Herbein et al., 1985) in plasma were

⁶Glucose (Trinder) 500 kit was purchased from Sigma Chemical Company, St. Louis, MO.

⁷Purchased from Mobay Chemical Cooperation, FBA Pharmaceuticals, New York, NY.

determined by standard double antibody radioimmunoassay procedures. Antiserum to bovine insulin⁹ bound 40% of porcine $[^{125}I]$ -insulin⁹. Goat anti-guinea pig IgG¹⁰ was the second antibody. Intraassay and interassay coefficients of variation were 7 and 8%, respectively. Antiserum against pancreatic glucagon¹⁰ bound 40% of $[^{125}I]$ -glucagon⁹. Goat anti-rabbit IgG¹⁰ was the second antibody. Intraassay and interassay coefficients of variation were 5 and 7%, respectively.

Analysis of Data

Data for SRA (DPM/mmol C) for the three metabolites studied were plotted against time. A representative set of precursor and product curves is shown in Figure 1. Although data were available from 4 to 10 h, plateau SRA was determined by averaging data between 6 and 10 h of infusion, and 92% of the product and precursor curves during this time span had slopes that were not different from zero when tested by linear regression (SAS, 1982). Coefficients of variation, averaged for product and

⁸Purchased from ICN Immunobiologicals, Lisle, IL. ⁹Purchased from Cambridge Medical Technology, Billerica, MA.

¹⁰Purchased from BioTek Research, Inc., Lenexa, KS.



Figure 1. Representative specific radioactivity (SRA) curves for one cow (#66) for the low-glucose treatment. (The isotopic tracer infused is listed at the top of each section. The metabolite with the highest SRA is the precursor, labeled for an individual infusion, and the other two are the products.)

precursor pools, were 22% for rumen propionate, 10% for blood CO_2 , and 12% for plasma glucose. Similar coefficients of variation have been observed for both lactating cows (Wiltrout and Satter, 1972) and growing steers (Veenhuizen et al., 1988).

Plateau SRA and infusion rates of tracers were used to calculate irreversible loss of rumen propionate, plasma glucose, and blood CO_2 (White et al., 1969). Irreversible loss refers to the amount of a metabolite that disappears from the pool labeled with tracer and does not return during the sampling period. Transfer quotients were calculated to determine both the fraction of product derived from precursor and the fraction of precursor converted to product (Gurpide et al., 1963).

Rates of carbon flux between rumen propionate, plasma glucose, and blood CO_2 pools were calculated through the use of stochastic analysis of an open system (Gurpide et al., 1963). This technique has been used to study in steers the interconversions of ruminal VFA (Armentano and Young, 1983) and rumen propionate, plasma glucose, and rumen and blood CO_2 (Veenhuizen et al., 1988). The kinetic solution was calculated separately for each cow for each treatment. Rates of carbon flux were calculated by using a Fortran computer program (Russell et al., 1985) that uses a series of simultaneous equations to generate a

solution. In this three-pool system, six individual rates of carbon flux between the three pools and an overall rate of carbon flux into or out of each pool were calculated. Rates of carbon flux into and out of pools were calculated by computer by using mass balance, thus resulting in 12 individual rates of carbon flux between the three pools (Veenhuizen et al., 1988).

Data were analyzed as a randomized block with the model consisting of two main effects, cows and treatments. For the concentrations of glucose, β -hydroxybutyrate, insulin, and glucagon in plasma for which multiple measurements were taken, no differences were seen within or between tracer infusions within a period. Consequently, all values within a period were averaged for the 6- to 10-h interval after the start of the tracer infusion. In the tables, significance level for the overall treatment effect has been reported when the P value is less than .20. Differences between treatments were tested by using Fisher's protected least-significance difference (SAS, 1982).

RESULTS AND DISCUSSION

Feed Intake and Milk Production

Daily feed intake and milk production and composition are shown in Table 3 for the three treatments. Dry matter and digestible energy intake did not change significantly among treatments. Therefore, feed consumed had approximately the same gluconeogenic potential for the three treatments. Average body weight for cows was 555 kg. Cows for all treatments were at energy equilibrium (+.3 Mcal/d).

Daily milk production did not change with the continuous infusion of glucose into the blood (Table 3). Others who have infused glucose into the blood or duodenum have shown either no changes or small increases in milk production for lactating cows either in positive energy balance or at energy equilibrium (Clark et al., 1977; Frobish and Davis, 1977; Fisher and Elliot, 1966). No changes were seen in percentage of milk fat or protein. Protein production increased slightly for the high-glucose treatment.

	т	reatmentl		Treatment	
Measurement	Control	Low glucose	High glucose	SEM	effect (P>F)
Feed intake: Dry matter (kg/d)	18.5	18.8	19.7	- 6	ng
Digestible energy	10.5		13.1		119
(Mcal/d)	54.4	55.4	57.8	1.7	ns
Milk production (kg/d)	26.5	28.0	28.4	1.1	ns
Milk composition:					
Fat (%)	3.56	3.46	3.43	.05	ns
Fat production (kg/d)	.95	.97	.98	.03	ns
Protein (%)	2.88	2.94	2.99	.07	ns
Protein production (kg/d)	.76 ^a	.82 ^{ab}	.84 ^b	.02	.09

Table 3. Daily feed intake and milk production for cows infused intravenously with glucose

¹Treatment means within a row with different superscripts differ, P<.05.
Concentration of Metabolites and Hormones

Concentrations of metabolites and hormones associated with energy metabolism were averaged for the last 5 h of each tracer infusion and pooled for each treatment; means are reported in Table 4. Packed cell volume did not change across the three treatments (26.6, 27.2, and 26.8% for control, low-glucose, and high-glucose treatments, respectively). Thus, cows were able to adapt to the changes in glucose loads within 5 d from the start of infusion of nonradioactive glucose. Concentration of glucose in plasma did not change with the infusion of glucose into peripheral blood, whereas the concentration of B-hydroxybutyrate was less with the low- and highglucose treatments compared with the control treatment. These results suggest that B-hydroxybutyrate either was cleared more rapidly or ketogenesis decreased when glucose availability increased in relation to energy requirements of the cows.

Concentration of insulin in plasma tended to increase on the low- and high-glucose treatments in comparison with control (Table 4). Concentration of glucagon in plasma was not changed, whereas the molar ratio of insulin to glucagon tended to increase with lowand high-glucose treatments. Lomax et al. (1979) infused

	Tre	atmentl		Treatment		
Metabolite or hormone	Control	Low glucose	High glucose	SEM	effect (P>F)	
Plasma glucose (mg/dl)	63.8	64.4	65.3	.4	ns	
Blood B-hydroxybutyrate (mg/dl)	6.01 ^a	5.16 ^b	4.37 ^b	.11	.01	
Plasma insulin (pg/ml)	499	608	634	21	.12	
Plasma glucagon (pg/ml)	153	157	149	1	.17	
Molar ratio insulin:glucagon	2.01	2.37	2.63	.08	.11	

Table 4. Average concentrations of metabolites or hormones in plasma or wholeblood over the last 5 h of tracer infusions

¹Treatment means within a row with different superscripts differ, P<.05.

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glucose continuously into the jugular vein at 1089 g/d for 48 h in three lactating dairy cows that were 2 to 3 mo postpartum and were fed 93% of their requirements. Concentration of insulin in arterial blood doubled and insulin concentration in the portal vein tripled after 4.5 h of infusion but returned to preinfusion values at 24 h. Although their cows were fed twice daily and blood samples were taken over a 1 h interval, homeostatic equilibrium with respect to whole-body glucose metabolism was reached within the first 24 h of glucose infusion. In our experiment, no conclusions can be drawn concerning the clearance rate of insulin or the production of pancreatic insulin, which could have increased at the same time, keeping the concentrations of insulin and glucose constant.

Kinetic Measurements

Irreversible losses of rumen propionate and blood CO_2 did not change because of treatments (Table 5). Because digestible energy intake was held constant during the experiment, irreversible loss of propionate would be expected to remain constant. Irreversible loss of blood CO_2 was five times greater than that observed in growing

	Tre	atment ¹			Treatment effect (P>F)	
Irreversible loss	Control	Low glucose	High glucose	SEM		
Rumen propionate:		<u> </u>	<u></u>			
(mol C/d)	90.0	82.1	92.0	8.1	ns	
(g/d)	2190	1997	2239	197	ns	
Blood C0 ₂ :						
(mol C/d)	341.3	318.2	358.0	17.8	ns	
Plasma glucose:						
(mol C/d)	57.0 ^a	72.6 ^{ab}	87.1 ^b	5.3	.02	
(g/d)	1711 ^a	2177 ^{ab}	2614 ^b	159	.02	
Exogenous glucose supplied (g/d)	0	342	737			
Estimated endogenous glucose production (g/d) 1711	1835	1877	159	ns	

Table 5. Irreversible loss of rumen propionate, plasma glucose, and blood CO_2

¹Treatment means within a row with different superscripts differ, P<.05.

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steers (Veenhuizen et al., 1988), thus illustrating the increase in metabolic activity in a lactating cow.

For the control treatment, irreversible loss of glucose averaged 1711 g glucose/d (Table 5), an amount lower than that observed by other workers with lactating cows (Bauman et al., 1988; Bruckental et al., 1980; Clark et al., 1977). The regression equation derived by Herbein et al. (1978), which relates digestible energy intake and propionate production rate to glucose irreversible loss, does not predict accurately the glucose irreversible loss observed in our study. Bauman et al. (1971) showed a decrease in propionate production rates in relation to digestible energy intake on high-forage diets in comparison with high-grain diets. In our study, the diet consisted predominantly of forage, and cows were at energy equilibrium, which may account for part of the decrease observed in irreversible loss of glucose. If we assume that milk contains 4.9% lactose, then lactose accounts for 1299 g glucose/d or 76% of glucose irreversible loss on the control treatment, which is similar to observations by other researchers (Bickerstaffe et al., 1974; Bauman et al., 1988).

Irreversible loss of plasma glucose increased with the infusion of exogenous glucose (Table 5), increasing significantly on high-glucose in comparison with control

treatment. For low- and high-glucose treatments, the amount of glucose infused represents 20 and 43% of the irreversible loss of glucose for the control. In cows fed a predominantly forage diet, as in this study, essentially no glucose enters the portal blood from the lower gastrointestinal tract (Huntington, 1982). For the control, therefore, glucose irreversible loss represents the production of glucose by liver and kidney. For lowand high-glucose treatments, glucose irreversible loss represents the rate of endogenous glucose production plus the rate at which exogenous glucose was infused. The continuous, intravenous infusions of glucose did not change the estimated endogenous glucose production (Table 5). These results are in contrast to those obtained by Clark et al. (1977) and Bartley and Black (1966), who used cows in positive energy balance and showed a decrease in endogenous glucose production rates when glucose was infused either into the duodenum or blood.

Interconversion of Metabolites

By infusing separate, uniformly labeled carbon-14 tracers on different days, the interconversions of rumen propionate, plasma glucose, and blood CO_2 were calculated as transfer quotients (Table 6) and as a direct flux of

		Treatment ¹	Treatment		
Transfer quotient	Control	Low glucose	High glucose	SEM	effect (P>F)
Rumen propionate:					
from plasma glucose	.4	.5	.4	.3	ns
from blood CO ₂	7.3	7.6	7.9	0.9	ns
to plasma glucose	36.2	38.1	35.4	3.4	ns
to blood CO ₂	77.8 ^a	65.7 ^b	77.8 ^a	3.2	.05
Plasma glucose:					
from rumen propionate	56.6 ^a	41.6 ^{ab}	37.7 ^b	3.9	.03
from blood CO ₂	25.2	18.2	18.2	2.4	.14
to rumen propionate	.6	.5	.4	.4	ns
to blood CO ₂	24.6	22.1	27.9	3.7	ns
Blood CO ₂ :					
from rumen propionate	20.6 ^{ab}	17.0 ^b	21.5 ^a	.9	.04
from plasma glucose	4.1 ^b	5.2 ^{ab}	6.8 ^a	.6	.05
to rumen propionate	1.9	2.0	2.0	.2	ns
to plasma glucose	4.3	4.3	4.4	.6	ns

Table 6.	Transfer	quotients	for	the	interconversions	of	rumen	propionate,	plasma
	glucose,	and blood	C02					_	_

¹Treatment means within a row with different superscripts differ P<.05.

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carbon between the three metabolites (Figure 2). Transfer quotients include both direct and indirect carbon flux. In contrast, the flux of carbon calculated by using stochastic analysis represents only direct transfer of carbon and is expressed as a rate. No differences between the values calculated by these two methods were seen, thus suggesting little indirect transfer of carbon between these three metabolites. By multiplying the irreversible loss of a metabolite by its respective transfer quotient, the flux of carbon between two metabolites can be calculated. This allows comparisons to be made with transfer quotients reported in the literature for these three metabolites.

Several observations are consistent across the three treatments (Figure 2) with respect to carbon flux into and out of the rumen propionate pool. Carbon flux from plasma glucose to rumen propionate was zero. Only 7% of the carbon entering the rumen propionate pool came from blood CO_2 , presumably from blood CO_2 diffusing across the rumen epithelium or from salivary bicarbonate. Flux of carbon from rumen propionate to outside the three-pool system is essentially zero, considering the large standard deviation associated with this pool and the small flux of carbon. For control treatment, 32% of the carbon in rumen propionate pool (30.1 mol C/d) is converted to glucose

Figure 2. Results of the three-pool system for the control, low-glucose, and high-glucose treatments. (Values associated with each arrow represent the mean for the four cows and units of mol C/d. The upper values represent the control; middle values, the low-glucose treatment (11.4 mol C/d); and the bottom values the high-glucose treatment (24.6 mol C/d). Pooled SEM are listed in parentheses. Different subscripts on a particular arrow for different treatments are different, P<.05.)



through gluconeogenesis. The remaining 67% or 63.1 mol C/d is oxidized to CO_2 .

For the control treatment, 52% of the carbon in plasma glucose (30.1 mol C/d) was derived from rumen propionate (Figure 2), which agrees closely with the calculated transfer quotient (56.7%) in Table 6. An additional 15.1 mol C/d or 26% comes from sources outside the three-pool system, such as amino acids, rumen and dietary lactate, and glycerol. Because a primedcontinuous infusion of $[U-1^4C]$ -tracer was used in this study, the amount of carbon recycled through Cori and alanine cycles can not be calculated because lactate and alanine released from muscle recycle back to glucose within the 10-h tracer infusion. The remaining 22%, or 12.4 mol C/d, enters the plasma glucose pool from blood CO_2 . This value is greater than can be explained by metabolic pathways that are known to incorporate CO2 into glucose. Veenhuizen et al. (1988) reported that the percentage of plasma glucose derived from blood CO2 ranged from 17 to 31% in steers fed to gain .3 kg/d. Similar observations have been seen in steers (Armentano and Young, 1983; Russell and Young, 1980) and may involve futile cycling of carbon between glucose and CO_2 .

For the control treatment, 18% of the carbon flux into the blood CO_2 pool was derived from the oxidation of

rumen propionate, 4% from the oxidation of glucose, and the remaining 78% from sources outside the three-pool system, such as the oxidation of acetate, ketone bodies, nonesterified fatty acids, and amino acids. Annison et al. (1974) found that acetate contibuted 34% of the total CO₂ in cows fed a high-roughage diet. Oxidation of ketone bodies represents only 3 to 4% of the total CO₂ (Palmquist et al., 1969). The remaining 40% in our study possibly came from the oxidation of amino acids and nonesterified fatty acids.

Carbon that exits the three-pool system from plasma glucose can be partitioned between the synthesis of body stores and components of milk; i.e., lactose, citrate, nonessential amino acids, and glycerol phosphate. For control treatment, 76% of the carbon flux (43.5 mol of C/d) leaves the plasma glucose pool, and the remaining 24% of carbon is oxidized to CO_2 . For midlactation cows, Clark et al. (1977) observed that 7.8 to 13.4% of glucose was oxidized to CO_2 , and Bauman et al. (1988) observed that 12.3 and 17.4% of glucose oxidized to CO_2 in cows receiving bovine somatotropin or excipient for 9 d. The differences in diet and energy balance may account for the differences seen between our study and those reported in the literature.

The two glucose treatments did not change the flux of carbon between rumen propionate and plasma glucose. Oxidation of rumen propionate to blood CO_2 decreased on the low-glucose treatment, but the high-glucose treatment was not different from the control treatment. On lowglucose treatment, the flux of carbon into and out of the plasma glucose pool increased in proportion to the amount of glucose infused '(11.4 mol C/d). Oxidation of plasma glucose to CO_2 was not changed. For high-glucose treatment, the flux of carbon into and out of the threepool system was similar to low-glucose treatment except that the oxidation of plasma glucose tended to increase (+7.9 mol C/d).

GENERAL DISCUSSION

One advantage of the stochastic approach to in vivo kinetics is that the flux of carbon and the relationships between several metabolites can be studied simultaneously. In our study, the relationships between rumen propionate, plasma glucose, and blood CO₂ were investigated in lactating cows at energy equilibrium fed a high-forage diet. For the control treatment, propionate provided more than 53% of the carbon in plasma glucose, with another 25% coming from other gluconeogenic precursors & i.e., amino acids, rumen and dietary lactate, and glycerol. The remaining 22% comes from blood CO2 from the crossover of carbon in metabolic pathways. These contributions did not change when glucose was infused into peripheral blood supply, which suggests that enzymatic and hormonal changes occurred to allow the extra glucose to be used. Our results are in contrast to those obtained for lactating cows in positive energy balance (Bartley and Black, 1966; Clark et al., 1977) and suggest that cows in negative energy balance or at energy equilibrium use supplemental nutrients differently than do cows in positive energy balance.

Carbon that leaves the three-pool system from plasma glucose can be partitioned between the synthesis of body

stores and components of milk, with lactose accounting for most of the glucose carbon used. Glucose carbon that is used to synthesize ATP and NADPH in the mammary gland is accounted for in the oxidation of plasma glucose to blood CO_2 . By assuming that milk contains 4.9% lactose and that the synthesis of milk is constant over a 12-h milking interval, the amount of glucose carbon used to synthesize milk lactose can be estimated. For control treatment, 43.3 mol C/d was used to synthesize milk lactose, with 43.5 mol C/d leaving the system as carbon other than blood CO_2 , which left a surplus of .2 mol C/d. Thus, the threepool system accounted for the glucose carbon secreted as lactose.

For the low- and high-glucose treatments, stochastic analysis suggests that 11.0 and 17.5 mol C/d were deposited in body tissues after the requirements for milk lactose were met. For low-glucose treatment, the exit of the additional glucose carbon from the three-pool system corresponded to the amount of glucose infused (11.4 mol C/d), whereas the increase in oxidation of plasma glucose and the amount calculated to be deposited in body stores accounted for the amount of glucose carbon infused (24.6 mol C/d) for the high-glucose treatment. Increases in specific activities of enzymes associated with lipogenesis have been shown for steers (Prior and Scott, 1980) and

sheep (Pearce and Piperova, 1985) that were infused with glucose into the blood or duodenum for 11 to 14 d, which supports the increase in body stores shown by our threepool system.

Although it seems that any extra glucose above that required for milk production was stored as glycogen or glycerol in different body stores, concentration of insulin in peripheral blood did not increase to the same extent as that seen during short-term glucose loading studies (Hove, 1978). Brockman (1986) suggested that ruminants are relatively insensitive to glucose as a stimulator of insulin release, with propionate being more potent. When propionate production remains constant, glucose may exert a direct effect on metabolism in cows in energy equilibrium, thus directing glucose deposition into body stores after homeostatic equilibrium is reached. In our study, glucose was infused continuously into the peripheral blood supply, and the results do not apply to cows in positive energy balance or to glucose infused into the duodenum.

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GENERAL SUMMARY

The major objective of the research reported in this dissertation was to understand how changes in the availability of glucose may influence the production of milk and overall energy metabolism of lactating dairy cows. Basic research in this area is important if the dairy industry wishes to find ways of increasing milk production and productive efficiency of cows and, in turn, increase the profitability of dairying. Two separate experiments were conducted in which the availability of glucose was either decreased or increased for lactating dairy cows.

Decreasing the Availability of Glucose

In the first experiment (Manuscript 1), the availability of glucose was decreased, and changes in milk production and concentrations of key energy metabolites were measured in five Holstein cows in early lactation and in negative energy balance. Phlorizin was injected subcutaneously at 0, 2, or 4 g/d every 6 h for 48 h and resulted in the excretion of 0, 225, or 337 g of glucose daily into the urine. Assuming that 70% of the glucose extracted by the mammary gland is used for the synthesis

of lactose, the amount of glucose excreted into the urine would correspond to 3 kg of milk for the 2 g dose and 5 kg of milk for the 4 g dose. Milk production was not decreased by the loss of glucose via urine; thus, cows fed 94% of their recommendations for net energy of lactation (NE_L) are able to adjust for glucose excreted into urine. Percentage of fat in milk increased linearly with increasing dose of phlorizin, which suggests that body stores were mobilized to account for the decrease in availability of glucose.

Blood samples were collected hourly from the jugular vein for the first and last 12 h of the 48-h phlorizin injection interval. Irrespective of treatment, diurnal variations relative to time of feeding were seen for concentrations of glucose, insulin, β -hydroxybutyrate, and nonesterified fatty acids. Concentrations of glucose and insulin decreased linearly with increasing dose of phlorizin over the 48-h injection period, whereas concentrations of β -hydroxybutyrate and nonesterified fatty acids increased linearly. These changes indicate that body stores can be mobilized to allow cows to compensate for short-term decreases in the availability of glucose.

Results from this study suggest that healthy, early-

metabolic capacity to change substrates used for energy and for milk synthesis in order to compensate for shortterm decreases in the availability of glucose. Thus, after milk production has peaked within a particular lactation, cows can adjust to day-to-day variations in the amount of energy consumed without compromising milk production. However, milk production might have decreased if the availability of glucose had been decreased within the first three weeks postpartum when cows are in a greater energy deficit or if the availability of glucose had been decreased for a longer period of time.

Increasing the Availability of Glucose

In the second experiment (Manuscript 2), four cows were used to quantify changes that occur in energy metabolism when glucose was infused continuously into the peripheral blood at 0, 342, or 737 g/d for at least 5 days. Cows were fed a predominantly forage diet to met their digestible energy requirements. Neither production nor composition of milk was changed by the infusion of glucose.

Concentration of glucose in plasma was not changed by the continuous infusion of glucose into the jugular vein, whereas the concentration of *B*-hydroxybutyrate decreased

for the glucose treatments over the control. Concentrations of insulin in peripheral plasma tended to increase slightly for the glucose treatments over the control treatment. No changes were seen in the concentration of glucagon in peripheral plasma. Changes observed in the concentrations of insulin are smaller than those observed during glucose-loading studies (Hove, 1978; Thompson et al., 1975; Sartin et al., 1985) and may suggest that insulin is involved in the short-term regulation of glucose metabolism and may not be as important in the long-term regulation of the glucose economy in ruminants.

Isotope dilution techniques were used to calculate the irreversible losses of rumen propionate, plasma glucose, and blood CO_2 . Irreversible loss refers to the amount of a metabolite that disappears from the pool labeled with tracer and does not return during the sampling period. Irreversible loss of propionate and blood CO_2 did not change with the infusion of glucose into peripheral blood. The glucose treatments increased glucose irreversible loss over the control in proportion to the amount of glucose infused, but did not change endogenous glucose production. These results are in contrast to those obtained by Clark et al. (1977) and Bartley and Black (1966), who used cows in positive energy

balance and showed a decrease in endogenous glucose production rates when glucose was infused either into the abomasum or into blood. Differences between the present study and studies of Clark et al. (1977) and Bartley and Black (1966) may be related to energy balance, stage of lactation, and/or the diet fed. In the present study, cows were at energy equilibrium and, therefore, were not given the opportunity to replenish body stores depleted during early lactation. In addition, our cows were fed a predominately forage diet, which favors a higher acetate to propionate ratio in the rumen, in comparison to a typical concentrate/forage diet. The influence of energy balance and/or type of diet are areas that should be addressed with research in the future.

By infusing separate, uniformly labeled carbon-14 tracers on different days, the interconversions of rumen propionate, plasma glucose, and blood CO_2 were calculated. For the control treatment, 52% of the carbon in plasma glucose was derived from rumen propionate. An additional 26% comes from sources outside the three-pool system, such as amino acids, rumen and dietary lactate, and glycerol. The remaining 22% enters the plasma glucose pool from blood CO_2 through reincorporation of carbon in the tricarboxylic cycle and gluconeogenic pathways, and,

also, may involve futile cycling of carbon between glucose and CO_2 .

During the glucose treatments, the flux of carbon into plasma glucose from sources other than rumen propionate or blood CO_2 increased in proportion to the amount of glucose infused, with the flux of carbon from rumen propionate remaining constant. The rate of carbon leaving the plasma glucose pool, other than as CO_2 , increased with the infusion of glucose. Oxidation of plasma glucose to CO_2 was not changed for the low-glucose treatment (342 g/d), but tended to increase for the highglucose treatment (737 g/d).

The effect of exogenous glucose on endogenous glucose production and glucose metabolism may depend on the site of glucose infusion. In the present study, glucose was infused into the peripheral blood supply and, thus, peripheral tissues could remove a substantially greater proportion of the infused glucose before it reached the liver and pancreas. On the other hand, if glucose had been infused into the duodenum, it would come in contact with the liver and pancreas quickly and may have a larger impact on endogenous glucose production and, thus, overall glucose metabolism. Preliminary results with steers (M. H. Cooley, Nutritional Physiology Group, Department of Animal Science, Iowa State University, personal

communication) suggests that glucose infused into the duodenum decreases endogenous glucose production to a greater extent than when an equal amount of glucose is infused into peripheral blood.

Areas for Future Research

Understanding glucose metabolism in lactating, dairy cows during the first 30 days postpartum is an area of research with many unanswered questions. During this period, cows are in the greatest negative energy and protein balances and they are more susceptible to metabolic diseases associated with carbohydrate insufficiency, i.e., fatty liver and ketosis. Changes in the availability of glucose during this period may cause more dramatic perturbations in energy metabolism and influence milk production more than changes observed in cows after the first month postpartum. Evaluating how precarious the glucose economy is during the first month postpartum will become even more important should repartitioning agents, i.e., somatotropin, be approved for use in lactating dairy cows.

For research to be geared toward meeting the nutritional and metabolic needs of lactating cows in the future, researchers must use the very best cows, not mediocre cows producing the breed average or below. The classical studies by Annison et al. (1974) and Bickerstaffe et al. (1974) on the uptake of glucose and other nutrients by the mammary gland were done with Jersey and Friesian cows producing 12 to 26 kg of milk daily. How relevant these data are to todays highproducing Holsteins that produce over 50 kg of milk at peak lactation is not clear. In order to understand the glucose needs of todays and tomorrows high-producing cows during early lactation, researchers should be utilizing cows producing in excess of 40 kg of 4% fat-corrected milk.

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APPENDIX

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Flux ²	Treatment ¹			Treatment		
	Control	Low glucose	High glucose		effect (P>F)	
				SEM		
		(mo]	les C/d)			
F ₀₁	-2.0	6.9	-4.0	4.2	ns	
F ₁₀	84.7	76.8	86.5	7.8	ns	
F ₀₂	43.5	56.7	63.9	5.8	.11	
F ₂₀	15.1 ^a	33.8 ^b	44.5 ^b	5.2	.02	
F ₀₃	330.2	307.4	346.8	17.8	ns	
F ₃₀	272.0	260.6	275.8	13.0	ns	
F ₁₂	0	0	0	.3	ns	
F ₂₁	30.1	28.2	30.0	. 2.4	ns	
F ₁₃	6.6	6.3	7.2	.9	ns	
F ₃₁	63.1 ^a	47.8 ^b	67.7 ^a	5.3	.08	
F ₂₃	14.0	16.4	24.3	2.4	ns	
F32	12.4	11.2	13.8	1.9	.05	

Table Al.	Solution to the three-pool system with the intravenous infusion
	of glucose in lactating dairy cows (Manuscript 2)

¹Represents direct flux of carbon to pool measured from pool carbon originated (Pool 0, outside model; Pool 1, rumen propionate; Pool 2, plasma glucose; Pool 3, blood CO_2).

²Treatment means within a row with different superscripts differ, P<.05.

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