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**METABOLIC AND PHYSIOLOGICAL ADAPTATIONS OF
LATE PREGNANT DAIRY COWS TO EXERCISE TRAINING
VOLUME I**

By

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ABSTRACT

METABOLIC AND PHYSIOLOGICAL ADAPTATIONS OF LATE PREGNANT DAIRY COWS TO EXERCISE TRAINING

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Conventional management practices during the prepartum period limit activity or exercise of cows in many dairy farms. This may reduce physical fitness, impairing their ability to respond to physiological and metabolic stressors of the periparturient period. The main focus of this research was to determine if prepartum exercise training of dairy cows causes physiological and metabolic changes in physical fitness, acid-base status, and energy metabolism that could facilitate adaptations transition to lactation. A system was designed to continuously collect heart rate data as an indicator of amount of exertion. Cows were exercised and evaluated with a variable speed mechanical walker with a circular lane and variable speed treadmill with adjustable slopes of the incline. The speed of walking and slope of the incline increased heart rates of cows more than the duration of walking and more co-mingling in a turnout lot or milking. This information was used to develop exercise training treatments along with acute exercise tests to assess physical fitness of dairy cows and adaptations of energy metabolism. After 60 d of exercise training, pregnant and non-pregnant cows that exercised had greater physical fitness than non-exercised cows in that the time to end of treadmill tests were greater, and heart rate and plasma lactate concentrations at given workloads (during treadmill tests) were reduced. The reduction of plasma lactate concentrations (less metabolic acidosis and (or) anaerobic metabolism or increased lactate utilization) at given workloads also influenced the maintenance of blood acid-base status during acute exercise. As indicators of extracellular buffering capacity, the partial pressures of CO₂

at given workloads were greater, whereas the concentrations of K^+ were reduced for exercised compared with non-exercised cows. However, exercise training treatments did not greatly affect other mineral element concentrations (Cl^- and ionized Ca^{2+}), or acid-base status during rest. Related to energy metabolism, prepartum body weight gains at similar dry matter intakes (DMI) were reduced for exercised compared with non-exercised cows, regardless of pregnancy status. Additionally, postpartum DMI during the first 4 wk of lactation was slightly greater for cows exercised prepartum compared with non-exercised cows, whereas milk yields were similar among treatment groups. Furthermore, exercise training also influenced the endocrine control of circulating concentrations of glucose and fatty acids, and the storage of substrates in muscle and liver tissues. During acute exercise tests, plasma lactate, glucose and glucagon concentrations were reduced for exercised cows compared with non-exercised cows. Prepartum liver glycogen concentrations increased, whereas postpartum liver glycogen concentrations were lower for pregnant, exercised cows compared with non-exercised cows. Exercise training increased prepartum plasma concentrations of non-esterified fatty acids (NEFA) and muscle and liver triglycerides, but decreased plasma β -hydroxy butyrate (BHBA) concentrations. During rest, a decline of plasma insulin for exercised cows may have increased lipolysis contributing to the increased plasma NEFA concentrations, whereas the decline of BHBA concentrations may indicate increased oxidation of fatty acids. Dairy cows profited from the physiological adaptations induced with prepartum exercise training. This may lead to reduced incidences of metabolic disorders and increase productivity of the periparturient dairy cow.

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LIST OF ABBREVIATIONS

1-h Exercise	1 h exercise treatment at 3 km/h
12min0.8%	initial time of 12 min at 0% slope followed by 0.8% increases of slope
12min1.6%	initial time of 12 min at 0% slope followed by 1.6% increases of slope
2-h Exercise	2 h exercise treatment at 3 km/h
6min0.8%	initial time of 6 min at 0% slope followed by 0.8% increases of slope
6min1.6%	initial time of 6 min at 0% slope followed by 1.6% increases of slope
BCS	body condition score
BE-B	base excess of blood
BE-ECF	base excess of extracellular fluid
BHBA	β -hydroxybutyrate
BPM	beats per min
BW	body weight
Cl⁻	chloride ion
CO₂	carbon dioxide
DIM	days in milk
DMI	dry matter intake
DR	drinking
E	exercise treatment
ECD	expected calving date
Ed	exercise day
ET	eating
GLUT4	glucose transport protein 4
H⁺	hydrogen ion
HCO₃⁻	bicarbonate ion
HGB	hemoglobin concentration
iCa²⁺	ionized calcium ion
INT	2 mM 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride
iP	inorganic phosphorous in the forms of PO ₄ ³⁻ , HPO ₄ ²⁻ , and H ₂ PO ₄ ⁻
K⁺	potassium ion
LY	lying
Mg²⁺	magnesium ion
Na⁺	sodium ion
NaF	sodium fluoride
NaF-KOxalate	sodium fluoride and potassium oxalate
NE	non-exercise treatment
NEFA	non-esterified fatty acids
No Exercise	no exercise treatment
NP	non-pregnant

List of Abbreviations, continued.

O₂	oxygen
P	pregnant
PC	pyruvate carboxylase
pCO₂	partial pressure of carbon dioxide
PEPCK	phosphoenolpyruvate carboxykinase
PS	pregnancy status effect
Rd	rest, non-exercise day
RT	ruminating
SDH	succinate dehydrogenase
SL	sleeping
ST	standing
T	treatment effect
TCO₂	total carbon dioxide
TG	triglycerides
TMR	total mixed ration
Trt	treatment effect

CHAPTER 1

INTRODUCTION

Conventional management practices during the prepartum period limit activity or exercise of cows in many dairy farms. This may reduce physical fitness, impairing their ability to respond to physiological and metabolic stressors of the periparturient period. In just a few days, cows that have had relatively small nutritional and metabolic demands must meet tremendous demands at parturition and onset of lactation. For cows to be productive and healthy in the face of physiological and metabolic stressors of the periparturient period, many physiological and metabolic adaptations occur during the periparturient period.

If the cow fails to adjust physiologically and metabolically during the periparturient period, a cascade of events can result causing health disorders, which can affect subsequent postpartum performance. Cows mobilize lipid stores as indicated by increased circulating concentrations of non-esterified fatty acids (NEFA) to support energy requirements during periparturient period (Bauman et al., 1988; Grummer, 1993). Greater circulating concentrations of NEFA are associated with greater incidences of dystocia, retained placenta, ketosis, displaced abomasum, and mastitis (Dyk, 1995). Uptake of NEFA by the liver increases proportionally to circulating concentrations (Bell, 1979). This can lead to accumulation of fat in the liver and the condition of "fatty liver," which has been linked to ketosis (Baird, 1982; Grummer, 1993; Veenhuizen et al., 1991). Furthermore, ketosis was associated strongly with displaced abomasum (Curtis et al., 1985); however, the exact cause and effect relationship has not been elucidated. Fatty liver, ketosis, and displaced abomasum during the periparturient period can have negative impact on milk yields (Drackley, 1999; Wallace

et al., 1996). The ability of cows to meet energy demands without excessive mobilization of lipid stores can reduce incidences of periparturient disorders. Maintenance of homeostasis of carbohydrate and lipid metabolism during the periparturient period is critical for productive, healthy dairy cows.

The use of prepartum exercise may be a management tool to stimulate physiological and metabolic processes prior to parturition to ease the transition from the non-lactating to lactating state. Cows may be able to adapt more quickly to metabolic and physiological demands of parturition and early lactation if fitness is improved with prepartum exercise. Furthermore, the ability to recover from the physical exertion of parturition may be related to prepartum activity and improved physical fitness. This may carryover to improved postpartum health during early lactation, greater milk yield, and more efficient reproductive performance.

A system to exercise dairy cows during the periparturient period was developed at Utah State University (Arave et al., 1978; Blake et al., 1982). At various speeds and durations of time (wk), the responses of pregnant dairy cows and heifers to prepartum exercise were variable (Anderson et al., 1979; Lamb et al., 1979; Lamb et al., 1981). Milk yield and dry matter intake (DMI) were not consistently greater for cows or heifers exercised prepartum compared with those that were not exercised. However, even with the small number of animals in these studies, udder edema was reduced and subsequent reproductive performance improved for exercised animals compared with non-exercised animals. These studies did not evaluate the interaction between exercise and pregnancy on physiological fitness responses or metabolism of carbohydrates and lipids. Prepartum exercise may stimulate adaptations of carbohydrate and lipid metabolism. These physiological adaptations of metabolism may improve the maintenance of homeostasis of carbohydrate and lipid metabolism during the periparturient period.

Overall goals of the current research were to examine the effects of exercise training on dairy cows and to characterize the responses of metabolism and milk production of periparturient dairy cows. It was hypothesized that exercised cows will have improved physical fitness and be more suited to maintain homeostasis of carbohydrate and lipid metabolism. This should result in improved transition to lactation. Specific objectives of the research reported herein are: 1) to develop a model to evaluate physical fitness of dairy cows as indicated by heart rate and acid-base homeostasis; 2) to develop a exercise training regimen for late pregnant dairy cows; 3) to assess prepartum effects of exercise training of late pregnant dairy cows on physical fitness and homeostasis of carbohydrate and lipid metabolism; 4) to evaluate potential interactions between prepartum exercise training and pregnancy on physical fitness and homeostasis of carbohydrate and lipid metabolism; and, 5) to evaluate postpartum responses of production and homeostasis of carbohydrate and lipid metabolism after a prepartum exercise training regimen.

CHAPTER 2

REVIEW OF LITERATURE

Introduction

The known physiological responses of the body during acute exercise at maximal or submaximal work capacities and physiological adaptations to chronic exercise are described in this review of literature. Physiological processes examined include energy expenditure, respiratory and cardiovascular changes, acid-base balance, carbohydrate and lipid metabolism, and regulation by the endocrine system. Additionally, changes in body tissue and muscle histology and biochemistry after periods of chronic exercise are described. Pregnancy and exercise can affect physiological processes. Limited research was available to elucidate the effects of prepartum exercise on physiological and metabolic responses of dairy cows. Where research results are not available from ruminants, results from nonruminant species are utilized to describe known responses of physiological systems.

Acute exercise, a single bout of activity, stimulates several of these physiological processes to meet the demands of tissues. Chronic exercise, repeated activity or training in a defined program or protocol, may cause adaptations of many physiological processes during the increased activity. Similarly, exercise training is a defined protocol of repeated exercise to cause a physiological response. Chronic exercise typically improves the physical and physiological fitness of an individual in that homeostasis of many physiological processes is improved during activity or exercise (Hodgson and Rose, 1994). For instance, less exertion is required to perform the exercise or activity. Additionally, the amount of acute or chronic exercise can be defined as a function of the maximal work that a body can complete. Usually, the maximal work capacity is

described in terms of the maximum oxygen (O_2) consumption or maximum heart rate of an individual during maximal activities (Derman and Noakes, 1994). In this thesis, submaximal exercise was usually defined as a percent of the maximal work capacity that an individual attempted.

Physical and Physiological Fitness

Physical fitness is defined as "the capacity to successfully meet the present and potential physical challenges of life" (Lamb, 1984). Physiology is the study of all functions of the body. Thus, physiological fitness can be defined as the capacity of all functions of the body to complete a task or challenge of life. Fitness can be evaluated by examining the ability of an animal through physiological processes to maintain homeostasis when a task or challenge is imposed. The physiological responses of an animal and how this occurs during a single bout of exercise may be altered by repeated chronic exercise (Astrand and Rodahl, 1986; Lamb, 1984). Therefore, physiological fitness of an animal may adapt and change with exercise training. Exercise training may cause adaptations in physiological processes such as the respiratory and circulatory systems, which deliver O_2 and nutrients to working tissues, and remove carbon dioxide (CO_2) and other waste products from working tissues. During exercise, changes in the acid-base status control the exchange, transport, delivery, and removal of O_2 and CO_2 throughout the body.

Furthermore, exercise increases the energy required by the contracting muscles. Exercise training may cause adaptations of the metabolism of carbohydrate and lipids to provide energy, and the endocrine system regulation of metabolism. During exercise, tissues have a greater utilization of O_2 and nutrients that were delivered by the circulation or stored in tissues (increased oxidative metabolism).

The exchange of O₂ and CO₂ by the respiratory system is dependent upon the ventilation volume of the lungs, frequency of respiration, and exchange of gases within the lung. The capacity of the circulatory system to carry the nutrients throughout the body is a function of the cardiac output (heart rate and stroke volume of the heart), carrying capacity of the blood (hemoglobin concentration), capillary network within tissues, and the exchange of substrates and end-products of metabolism between blood and cells. The following sections of this review describe the responses of these systems during acute and chronic exercise, considering the key aspects of each system critical to ruminants and nonruminants.

Physiological Responses to Acute Exercise

Energy expenditure during exercise. The Agricultural Research Council (ARC, 1980) defined energy requirements of cattle for horizontal walking as a function of body weight (**BW**), approximately 0.478 kcal/kg of BW per km. Walking up an incline has an additional energy requirement of 6.45 kcal/kg of BW per km of rise or vertical ascent (Lawrence and Stibbards, 1990; Ribeiro et al., 1977). For sheep, energy expenditure has been estimated at 0.621 kcal/kg of BW per km for horizontal walking and 6.69 kcal/kg of BW per km for vertical ascent (Clapperton, 1964). More recently, Di Marco and Aello (1998) and Mendez et al. (1996) using CO₂ dilution techniques in a study with Angus steers fed to maintenance requirements determined energy expenditure of horizontal walking to be 0.09 to 0.15 kcal/kg of BW per km. The values recommended by ARC (1980) for cattle were 0.478 kcal/kg of BW per km for horizontal walking and 6.69 kcal/kg of BW per km for vertical ascent.

The difference among these estimates could be attributed to different estimation methods. To determine energy expenditure, Ribeiro et al. (1977) used respiratory exchange of animals that were walking on a treadmill in chambers. However, Mendez

et al. (1996) used isotope dilution techniques with free ranging animals, which may cause fewer disturbances or adaptations of the animals compared with a treadmill in a respiration chamber. Utilizing measurements of energy expenditure for activity from Ribeiro et al. (1977) and Mendez et al. (1996), a 550-kg cow with a maintenance energy requirement of 9.08 Mcal/d (NRC, 2001) that was walking 1 km on flat terrain would have increased energy expenditure of 0.08 to 0.26 Mcal/d. The additional increment above the maintenance energy requirement is comparatively small for non-grazing animals. The increase in energy expenditure may be met with additional feed intake, increased metabolism, or partitioning of nutrients for activity from other processes.

Effects of exercise on feed and water intake. The different exercise activities did not affect DMI of diets with different composition. During a week of exercise (walking 10.6 km/d and climbing 0.48 km/d), ad libitum straw intake of crossbred lactating cattle was not increased (Matthewman et al., 1993b). Supplementing the straw with different carbohydrate and protein supplements during exercise also did not affected ad libitum straw intakes (Matthewman et al., 1993b). The voluntary DMI of non-lactating Holstein cows that were exercised compared with non-exercised cows was not different (Anderson et al., 1979; Lamb et al., 1979; Lamb et al., 1981). Voluntary water intakes of crossbred lactating cows increased with exercise (0 km/d vs. 2 km/d or 4 km/d), but feed consumption and milk yields were not affected (Benedetti et al., 1990). Similarly, draught force work for 36 d and diet with and without supplementation did not affect total DMI of pregnant and non-pregnant cows (Zerbini et al., 1996). In contrast to these results with year-long studies, draught cows pulling sledges had slightly greater DMI as a function of metabolic body size with or without additional concentrate supplement (Gemedda et al., 1995; Zerbini et al., 1995). However, cows fed a concentrate supplement had a greater increase in DMI than cows not fed a supplement. Additionally, apparent digestibility of dry matter was slightly greater for working cows,

and the supplement caused the greatest increase in apparent digestibility of the diet. Sheep that were exercised for 14 d at 3 km/h, on a 10° incline (17.6% slope) for 30 min, six times a day had reduced organic matter intakes and reduced apparent organic matter digestibility compared with non-exercised sheep (Henning, 1987). In all of these studies, diets were mostly roughage with a great potential for physical fill to limit intakes. Based on this limited evidence, ruminants do not appear to have increased DMI during periods of exercise, especially if physical fill already may be limiting intakes.

In summary, energy expenditure of cattle during walking was determined to be small relative to maintenance requirements of the animal (Mendez et al., 1996; Riberiro et al., 1977). Additionally, DMI to meet the increase in energy expenditure for walking did not appear to be affected by exercise. However, it is not known if increased energy expenditure will cause responses in carbohydrate or lipid metabolism of ruminants which could be beneficial to the dairy cow during the periparturient period. Dry matter intakes when physical fill was less has not been evaluated in response to exercise. Other physiological processes are involved to support the increased energy demand of muscles during exercise.

Respiration during acute exercise. During exercise, the respiratory system is stimulated to provide O₂ to working tissues and to remove CO₂. When 5- to 8-mo old Simmental calves were walked for 5 min at 3.6 km/h, 0° incline, respiration frequency and ventilation volume increased compared with rest (36 vs. 65 breaths per min and 16.5 vs. 47.4 L/min, respectively; Blum and Eichinger, 1988). Walking at the same rate up a 6° incline (10.5% slope), increased respiration frequency an additional 14 breaths per min and ventilation volume an additional 34.6 L/min. The O₂ uptake increased by 7 ml/min per kg of BW greater than rest (4.1 ml/min per kg of BW) after walking at 3.6 km/h, 0° incline. Increasing the incline to 6° (10.5% slope) increased O₂ uptake an additional 6.9 ml/min per kg of BW (Blum and Eichinger, 1988). Mature crossbred dairy

cows that were walked 0.5 km at 3.6 km/h, 0° incline had an increase of ventilation volume of 60 L/min and O₂ uptake of 4.6 L/min compared with resting values (Zerbini et al., 1992). Similarly, Arave et al. (1987) reported an increase in respiration rates of 2-yr old Holstein heifers after 30 min of exercise at 1.5 km/h, up a 9% slope (5.0° incline).

Crossbred beef heifers and steers working at various speeds (0 km/h to 14 km/h) had linear increases in ventilation volume and O₂ consumption, but respiration frequency was maximal after working at the slowest speed (2.88 km/h) compared with greater speeds (Jones et al., 1989; Kuhlmann et al., 1985b; Piguet et al., 1993; Piguet et al., 1994). Respiration frequency ranged from approximately 60 to 90 breaths per min across studies (Jones et al., 1989; Kuhlmann et al., 1985b; Piguet et al., 1993; Piguet et al., 1994). Oxygen consumption reached a plateau in cattle, sheep, and goats as they approached maximal exercise workloads (Kuhlmann et al., 1985b; Mundie et al., 1991; Piguet et al., 1994; Weber et al., 1993). Additionally, the new steady-state O₂ consumption was reached approximately 3 min after the start of exercise (Piguet et al., 1993). Steers (475 kg of BW) had a maximum O₂ consumption of 51 ml/min per kg of BW after exercising at a speed of 14 km/h (Jones et al., 1989) which was approximately 10-fold greater than resting O₂ consumption. Kuhlmann et al. (1985a) reported similar results of maximum O₂ consumption with 165-kg calves. Weber et al. (1993) measured O₂ consumption of trained pygmy goats of approximately 60 ml O₂/min per kg of BW. Maximal O₂ consumption of pregnant sheep was 57 ml/min per kg of BW and related linearly to incline and speed ($r^2 = 0.94$; Jones et al., 1992).

Increased respiration frequency, ventilation volume, and O₂ uptake during exercise potentially provided more O₂ for aerobic oxidation of working muscles and increased the removal of CO₂ from oxidation by working muscles. Furthermore, ventilation volume and O₂ consumption increased linearly with submaximal workloads. As workloads approached maximal exertion, the rate of increase of ventilation volume

and O₂ consumption decreased. The maximum O₂ consumption of ruminants was approximately 50 to 60 ml O₂/min per kg BW. Oxygen consumption was related linearly to the amount of exercise.

Cardiac output during acute exercise. Oxygen is carried to active tissues through the circulatory system as result of the cardiac output from the heart and redistribution of cardiac output. Cardiac output is a function of the stroke volume and frequency of the heart beat. In calves, heart beat frequency increased non-linearly with increases in workloads (Fosha-Dolezal and Fedde, 1988; Jones et al., 1989; Kuhlmann et al., 1985b; Pearson and Archibald, 1989; Piguet et al., 1993; Piguet et al., 1994). As heart rate approached maximum, the change in response of heart rates was reduced with increased speed of exercise (Fosha-Dolezal and Fedde, 1988). When Simmental calves were walked for 5 min at 3.6 km/h, heart beat frequency increased 49 beats per min (BPM) compared with rest (82 BPM). Walking at the same rate up a 6° incline (10.5% slope), heart rates increased an additional 65 BPM (Blum and Eichinger, 1988). Similarly, 2-yr old Holstein heifers had greater heart rates after 30 min of exercise at 1.5 km/h up a 9% slope (5.0° incline) compared with resting rates (Arave et al., 1987). During exercise for 30 min at 2.2 km/h up a 10% slope (5.7° incline), calves also had a greater heart rate compared with resting rates (Blum et al., 1979).

Maximum heart beat frequency was 205 BPM walking at a rate of 6.48 km/h for Hereford calves (Kuhlmann et al., 1985b); 216 BPM at 14 km/h for Red and White steers (Jones et al., 1989); and 230 BPM at 9.4 km/h for Hereford calves (Fosha-Dolezal and Fedde, 1988). Whereas these maximal rates were similar, the differences of workloads at which heart beat frequency were maximized may be a result of pre-measurement training or age of the animals in the studies. Furthermore, Jones et al. (1989) only evaluated three speeds whereas Kuhlmann et al. (1985a) compared responses at five speeds, and Fosha-Dolezal and Fedde (1988) evaluated 10 speeds.

The range of speeds to determine maximum heart rates also was different among the three studies.

Heart beat frequency had the greatest impact on increased cardiac output during exercise of steers in that the stroke volume remained unchanged (Jones et al., 1989; Kuhlmann et al., 1985b). Cardiac output increased linearly with increased workloads in horses and steers (Jones et al., 1989). Cardiac output was correlated highly with the increase in heart rates with increased walking speed, but not with stroke volume. Stroke volume (L per beat) is the difference between end diastolic volume (filling of left ventricle) and end systolic volume (emptying of left ventricle) of the heart. In humans, end systolic volume remained unchanged during mild, moderate, and peak exercise compared with rest; whereas, end diastolic volume increased similarly at all amounts of exercise over resting values (Poliner et al., 1980). Therefore, stroke volume increased during exercise compared with rest, but reached a plateau with greater workloads (Poliner et al., 1980). During exercise, stroke volume increased above resting values, but not linearly with increasing workloads. Therefore, stroke volume was not linearly correlated to cardiac output (Jones et al., 1989; Rowell, 1986). In sheep during exercise, stroke volume was not greater than resting values, but cardiac output increased as a function of changes in heart rates (Bell et al., 1983). In sheep, cardiac output was redistributed to muscle from the gut, kidney, spleen and central nervous system during exercise (Bell et al., 1983; Hales et al., 1984).

Mean blood pressure at the aorta of Hereford calves was greater when exercising at speeds greater than 6.48 km/h, whereas the blood pressure of the pulmonary artery remained unchanged (Kuhlmann et al., 1985b). Total peripheral resistance was less than resting resistance at all speeds tested. In sheep exercising for 30 min at 2.6 km/h up a 10° incline (17.6% slope), mean arterial blood pressure was not different from resting values, but total peripheral resistance was less than resting values (Bell et al.,

1983). Exercise possibly increased the O₂-carrying capacity of the blood because hematocrit percent and hemoglobin percent increased (Jones et al., 1989; Kuhlmann et al., 1985b). However, it is not clear if this is an artifact of extracellular water moving intracellularly to dilute increased solutes causing hemoconcentration of circulating blood or additional release of red blood cells from the spleen.

In summary, exercise increased heart beat frequency resulting in greater cardiac output. Maximum heart rates were greater than 200 BPM for calves and steers. During exercise, heart rates increased cardiac output providing more O₂ to exercising tissues. Additionally, changes in resistance of pulmonary and peripheral tissue may increase the gas exchange from the blood to air and blood to tissues. Finally, additional red blood cells during exercise also may contribute to the capacity of cardiovascular system to increase delivery of O₂ to exercising tissues.

Whole blood acid-base status. Responses of the respiratory and cardiovascular systems during exercise can be mediated through the endocrine system, end-products of metabolism, and the interaction of all systems of the body to maintain homeostasis during activity. As calves exercised, venous hydrogen ion (H⁺) concentrations and partial pressure of CO₂ (pCO₂) increased (Jones et al., 1989; Kuhlmann et al., 1985b), which in turn stimulated respiration. Venous peaks of pCO₂ were 60 torr and 81 torr, and H⁺ concentrations were approximately 50 nmol/L and 80 nmol/L for speeds at 7.9 and 14.8 km/h, respectively.

In non-pregnant and pregnant sheep, arterial pCO₂ and bicarbonate concentrations decreased whereas pH increased as exercise speed and incline increased (Palmer et al., 1984; Pethick et al., 1991). The increase of pCO₂ of venous blood and decrease in arterial blood may be a function of respiratory alkalosis with increased ventilation stimulated by exercise with increased venous CO₂ concentration.

Increased pCO_2 and H^+ concentrations in venous blood are reflective of the increased intracellular oxidation of substrate, yielding CO_2 and production of lactic acid as a result of incomplete oxidation of substrates (anaerobic metabolism). Furthermore, the effects of rumen-produced and absorbed CO_2 concentrations did not affect circulating CO_2 and ventilation rates during exercise (Kuhlmann et al., 1985a). At maximum activity, the accumulation of these end-products in the circulation overwhelms homeostasis of O_2 and CO_2 exchange in the pulmonary-cardiac systems and buffering of the extracellular matrix, thus preventing the current rate of exertion to continue. The ability to maintain homeostasis of O_2 and CO_2 exchange in the pulmonary-cardiac system and buffering of the extracellular matrix is an indicator of physical fitness of an animal.

Circulating mineral element concentrations. During submaximal exercise, the extracellular matrix osmolality, volume, and specific ion concentrations shift as the body transports metabolic acids and end-products away from contracting tissues. These changes of the extracellular matrix stimulate an increase in respiration and heart rates, and maintenance of blood pressure to continue to deliver O_2 and substrates to exercising tissues.

To maintain tonicity (osmolality) of the body, water intake, and excretion is altered. Extracellular volume is controlled by the osmotic solutes of sodium (Na^+), chloride (Cl^-), and potassium (K^+) ions with the amount of Na^+ in the extracellular fluid being the primary determinant of extracellular volume. Concentrations of other mineral elements in the extracellular fluid such as calcium (Ca^{2+}), magnesium (Mg^{2+}), and inorganic P (iP in the forms of PO_4^{3-} , HPO_4^{2-} , and H_2PO_4^-) are controlled by specific endocrine systems that are less responsive to short-term bouts of exercise (Genuth, 1993; Lawrence, 1994; Rose and Hodgson, 1994; Stanton and Koeppen, 1993).

As mentioned previously, H^+ concentrations of whole blood increase in response to exercise, while at the same time serum K^+ concentration increased. Hereford calves exercised at varying speeds exhibited a nonlinear increase in concentrations of serum K^+ with a peak of approximately 7.4 meq/L (Fosha-Dolezal and Fedde, 1988; Kuhlmann et al., 1985b). The increased K^+ concentrations may be because of the shift of water between intracellular and extracellular compartments, movement of K^+ from muscle cells in response to catecholamines, movement of K^+ from red blood cells, or increase in action potentials generated with incomplete uptake of K^+ during repolarization (Fosha-Dolezal and Fedde, 1988).

Hereford-Friesian lactating cows that were walked 10 km/d for 3 wk had reduced concentrations of Mg^{2+} and iP 1 h after walking compared with the same cows at rest (Matthewman et al., 1993a). Exercising calves for 0.5 h at 2.2 km/h on a 10% slope (5.7° incline) did not change plasma Ca^{2+} or Mg^{2+} concentrations (Blum et al., 1979). Kuhlmann et al. (1985a) demonstrated that serum iP, Ca^{2+} , and Na^+ concentrations were increased after exercising at speeds greater than 6.5 km/h. Draught oxen did not have changes in concentrations of Ca^{2+} , Na^+ or Cl^- during moderate or maximum work (Pearson and Archibald, 1989; Zanzinger and Becker, 1992). Parathyroid hormone was greater during exercise, but no change in circulating Ca^{2+} was detected (Blum et al., 1979).

At speeds greater than 6.5 km/h, calves were approaching maximum exertion, therefore the potential for fluid shifts from extracellular to intracellular were greater causing hemoconcentrations of solutes and greater hematocrit. Exercising Hereford calves had approximately a 9% shift of water from extracellular to intracellular compartments as indicated by changes in serum albumin concentrations before and after exercise (Kuhlmann et al., 1985b). Calves exercising at similar speeds to that study also had similar changes of Na^+ concentrations and hematocrit, but concentrations

of iP and Ca^{2+} were not measured (Fosha-Dolezal and Fedde, 1988). Lambs that exercised at 5.6, 7.2, or 8.8 km/h up a 9° incline had a 7% increase of total plasma protein concentration compared with resting values (Apple et al., 1994). Humans exercised at submaximal and maximal intensities for 1 h had hemoconcentration of circulating blood with 7.1 and 13.0% decrease in plasma volume, respectively (Novosadova, 1977). Therefore, changes in extracellular concentrations of Na^+ , iP, Mg^{2+} , and Ca^{2+} are likely a result of changes in water volume and (or) flux between intracellular and extracellular compartments during exercise.

During the periparturient period of the dairy cow, changes in extracellular matrix mineral element concentrations or in plasma volume also may occur to support lactogenesis and support of subsequent lactation. As parturition nears, plasma volume increased in humans (Pivarnik et al., 1990). During exercise, the osmolality of the extracellular matrix increased as mineral element concentrations of K^+ and Na^+ increased. However, it was not evident that movement of mineral elements or water between intracellular and extracellular compartments changed the osmolality of the extracellular matrix. During exercise, movement of water from the extracellular to intracellular matrixes was approximately 5 to 10% greater than resting values as indicated by changes of the concentrations of total plasma protein and albumin. However, the increase of K^+ concentrations during exercise was approximately 40% greater than resting values. Therefore, movement of water between cellular compartments alone cannot fully explain the increased osmolality of the extracellular matrix during exercise. No evidence was available to indicate if the increased osmolality during exercise can affect or stimulate changes in the mammary gland for lactogenesis and subsequent lactation of transition dairy cows.

Glucose metabolism. During intensive exercise of ruminants, circulating concentrations of glucose increased with increased hepatic output. Blood plasma

glucose concentrations of calves after exercise at 3.6 km/h for 5 min, 6° incline were greater than rest (4.85 vs. 4.72 mmol/L, Blum and Eichinger, 1988). Plasma glucose concentrations increased approximately 20 mg/dl (80 to 100 mg/dl) when Hereford calves were exercised at 7.9 km/h for 5 min (Kuhlmann et al., 1985a). At submaximal exercise, glucose concentrations remained unchanged compared with resting concentrations (Blum et al., 1979; Kuhlmann et al., 1985a). Draught animals working for extended time periods had less glucose concentrations compared with rest (Pearson and Archibald, 1989; Zerbini et al., 1995).

Similarly in sheep, arterial blood glucose concentrations increased during acute exercise (4.5 km/h, 0° for 4 h or 9° incline, 15.8% slope, for 2 h at either 7 km/h or 9 km/h); hepatic glucose release increased; and glucose uptake by muscle increased (Harman and Pethick, 1994; Pethick, 1991). Similar results of arterial glucose concentration, hepatic production of glucose, and muscle uptake of glucose occurred during exercise of sheep at 5 km/h, 7 km/h, and 9 km/h for 30 to 45 min at either 0° or 9° incline (Pethick et al., 1991). Speeds of 5.6 to 8.8 km/h used to exercise wethers caused greater plasma glucose concentrations (125 mg/dl) than resting values (89 mg/dl; Apple et al., 1994).

Nonruminants did not appear to have a hyperglycemic (greater than 150 mg/dl of plasma glucose) response to exercise even with increased hepatic output. In horses and humans, plasma glucose remained unchanged after 90 min of exercise (Geor et al., 2000; Lavoie et al., 1997). However, hepatic glucose output of horses was increased to a peak of 38.6 $\mu\text{mol/kg}$ per min to match the rate of disappearance of glucose (peak of 40.4 $\mu\text{mol/kg}$ per min; Geor et al., 2000). Hepatic output of glucose for exercising men was increased 2-fold compared with resting values (Bergeron et al., 1999; Lavoie et al., 1997).

After maximal exercise (7.9 km/h for 5 min), plasma glucose concentrations of calves returned to resting values after 1 h (Kuhlmann et al., 1985a). However, Hereford-Friesian lactating cows that walked 10 km/d for 3 wk had reduced concentrations of glucose 1 h after walking compared with rest (Matthewman et al., 1993a). Because these cows also were lactating, the reduced circulating concentrations of glucose may have been reflective of the mammary gland uptake of glucose for lactation.

Incomplete oxidation of glucose (anaerobic glycolysis) in muscle tissue during maximal exercise increased circulating concentrations of lactate in blood. Lactate concentrations increased approximately 70 mg/dl (10 to 80 mg/dl) when Hereford calves were exercised for 5 min at 7.9 km/h (Fosha-Dolezal and Fedde, 1988; Kuhlmann et al., 1985b). Murrah buffalo heifers walking 0 km vs. 3 km or 6 km had a 2-fold increase in blood plasma lactate concentrations (Singh and Singh, 1986). Moderate and maximum workloads in draught oxen increased lactate concentrations (Zanzinger and Becker, 1992; Zerbini et al., 1995). Concentrations of lactate declined to rest values 30 min after cessation of activity (Zanzinger and Becker, 1992; Zerbini et al., 1995). Additionally, plasma lactate concentrations increased non-linearly with increases in workloads in cattle and sheep. Lactate concentrations continued to increase at a decreasing rate at maximal workloads (Apple et al., 1994; Blum et al., 1979; Blum and Eichinger, 1988; Fosha-Dolezal and Fedde, 1988; Kuhlmann et al., 1985b; Piguet et al., 1994). In sheep, arterial lactate concentrations increased non-linearly with increased incline and speed of exercise in that concentrations increased at a decreasing rate as workloads increased (Gardner et al., 1998; Pethick et al., 1991). Similar results of increased lactate concentrations were seen in horses exercised for prolonged time (90 min) at a submaximal exercise (Geor et al., 2000).

Glycogen in hepatic and muscle tissues was used during prolonged submaximal exercise. Glycogen utilization in horses during 90 min of submaximal exercise was 164 mmol glucosyl units/kg dry tissue (Geor et al., 2000). In dogs, hepatic glycogen was depleted by 70% with exercise of 50% maximal O₂ consumption (Wasserman et al., 1993). In mice, swimming exercise for 3 h caused a 50% decrease in muscle glycogen compared with rest (Ryder et al., 1999). Hepatic glycogen of swimming rats was utilized to maintain circulating glucose concentrations (Borba-Murad et al., 1998). Furthermore, if hepatic glycogen was depleted with fasting, the capacity for hepatic gluconeogenesis increased (Borba-Murad et al., 1998). Research results for glycogen of hepatic and muscle tissues were not available for ruminants.

Hepatic gluconeogenesis is controlled primarily by phosphoenolpyruvate carboxykinase (PEPCK). During exercise, activity of hepatic PEPCK increased (Dohm et al., 1985). Additionally, Friedman (1994) demonstrated that hepatic PEPCK gene transcription in mice increased during exercise by activation from glucocorticoids.

In summary, exercise stimulated hepatic output of glucose in sheep causing increased circulating concentrations of glucose. Glucose is required by the mammary gland for lactose production. Homeostasis of glucose metabolism and providing required glucose for milk synthesis during the periparturient period of dairy cows may be stimulated by exercise.

Lipid metabolism. Circulating concentrations of NEFA increased slightly during acute exercise of ruminants with the largest increase occurring at the end of exercise and post-exercise. Hereford-Friesian lactating cows that walked 10 km/d for 3 wk had greater concentrations of NEFA (0.96 vs. 0.61 mmol/L) 1 h after walking (Matthewman et al., 1993a). Plasma NEFA concentrations of calves were greater (0.80 vs. 0.66 mmol/l) 5 min after exercise (3.6 km/h up a 6° incline, 10.5% slope, for 15 min; Blum et al., 1979; Blum and Eichinger, 1988). Cows that walked 9.6 or 12.8 km/d had greater

plasma NEFA concentrations immediately after exercise compared with non-exercised cows or cows that only walked for 6.4 km/d (D'Hour et al., 1994). Draught animals had greater NEFA concentrations at the end of work and post-work (Zanzinger and Becker, 1992; Zerbini et al., 1995). In sheep, concentrations and entry rates of NEFA into blood increased during exercise of 2 to 4 h (4.5 km/h, 9° incline, 15.8% slope; Pethick et al., 1987). In pygmy goats, glycerol concentrations increased during submaximal exercise with increases in workloads, and remained elevated for 20 to 60 min after exercise (Weber et al., 1993).

During exercise, incomplete oxidation of fatty acids may increase β -hydroxybutyrate (BHBA) concentrations or oxidation of BHBA may reduce circulating concentrations of BHBA. Hereford-Friesian lactating cows that walked 10 km/d for 3 wk had greater concentrations of BHBA 1 h after walking (Matthewman et al., 1993a). Draught animals had greater BHBA concentrations immediately after work (Zerbini et al., 1995). In sheep, concentrations of BHBA in blood were slightly greater than resting values after exercise of 2 h (4.5 km/h, 0° incline) or 4 h (4.5 km/h, 9° incline, 15.8% slope; Pethick, 1991).

Prolonged exercise (greater than 2 h) of ruminants caused significant increases in the blood concentrations of NEFA or BHBA. Increased circulating concentrations of NEFA have been linked to increased risk for ketosis, fatty liver, or displaced abomasum (Baird, 1982; Dyk, 1995). Prolonged exercise (greater than 2 h) also may increase the risk for ketosis, fatty liver and displaced abomasum.

Endocrine control of metabolism. Changes in carbohydrate and lipid metabolism during exercise likely are affected or controlled by insulin, glucagon, catecholamines and cortisol. Circulating glucose concentrations are controlled by insulin secretion and insulin receptor activity, which stimulates tissue uptake of glucose for glycolysis or lipogenesis. Additionally, glucagon will stimulate gluconeogenesis or

glycogenolysis to increase circulating concentrations of glucose. Catecholamines and glucocorticoids will stimulate lipolysis, glycogenolysis, and inhibit tissue responsiveness to insulin and insulin secretion.

Catecholamines and corticoids increase during exercise, potentially stimulating lipolysis and inhibiting insulin secretion or action. During a single bout of exercise of Simmental calves, plasma epinephrine and norepinephrine (catecholamines) concentrations were 20 and 40% greater than when at rest, respectively (Blum et al., 1979; Blum and Eichinger, 1988). Furthermore, during exercise of 3.6 km/h, an increase of 6° of the treadmill incline increased plasma epinephrine and norepinephrine of 40 and 60% than when at resting values (Blum and Eichinger, 1988). Holstein heifers exercised for 30 min up a 9% slope (5° incline) at 1.5 km/h, 1 h at 3.5 km/h, 2.75 h at 3.5 km/h, or 30 min at 5.5 km/h had greater blood serum glucocorticoid concentrations compared with concentrations in the same heifers at rest (Arave et al., 1987; Arave et al., 1978). Cortisol concentrations increased during exercise (20% increase) and were greatest 10 min post-exercise (65% increase) for calves exercised at speeds greater than 7.9 km/h compared with pre-exercise values (Kuhlmann et al., 1985b). Veal calves had greater cortisol concentrations during exercise compared with rest (Piguet et al., 1993).

Pregnant sheep exercised at 4.0 km/h for 45 min had increased blood plasma epinephrine, norepinephrine and dopamine concentrations compared with resting values (Palmer et al., 1984). Wethers exercised at speeds of 5.6 to 8.8 km/h for 10 min had greater venous adrenocorticotrophic hormone and cortisol concentrations than the same wethers at rest (Apple et al., 1994). Concentrations also remained greater during the 10 min following exercise (Apple et al., 1994). Plasma insulin concentrations of calves decreased during walking, but plasma glucagon concentrations were unchanged compared with resting values (Blum and Eichinger, 1988). Additionally, plasma insulin

concentrations of calves were greater approximately 15 min after exercise than resting values (Blum and Eichinger, 1988).

Compared with ruminants, glucose homeostasis of nonruminants was more tightly controlled by insulin and glucagon during exercise preventing a hyperglycemia (plasma glucose greater than 110 mg/dl) or hypoglycemia (plasma glucose less than 70 mg/dl) situation. Horses that were exercised for 90 min at 35% maximum O₂ consumption with a 2° incline (3.5% slope) had no change in circulating insulin, but an increase in glucagon, epinephrine, and norepinephrine concentrations compared with resting values (Geor et al., 2000). During submaximal exercise, non-ruminants (humans and dogs) had no change in plasma glucose concentrations, but had a decrease in plasma insulin concentrations (Lavoie et al., 1997; Wasserman et al., 1989a; Wasserman et al., 1989b; Wasserman et al., 1989c), and an increase in plasma glucagon (Bottger et al., 1972; Wasserman et al., 1989b) and catecholamine concentrations (Christensen and Galbo, 1983). Lavoie et al. (1997) demonstrated that these endocrine changes were responsible for the increase in hepatic output of glucose (2-fold increase) and gluconeogenesis (47% increase) during submaximal exercise of humans. Glucagon (20% increase) was necessary to stimulate hepatic output of glucose, and a decline in insulin concentration (26% decrease) was required to prevent hypoglycemia.

During the periparturient period of ruminants, glucose and lipid homeostasis change rapidly to support the increased nutrient demands of gestation, lactogenesis, and lactation. Similar changes in the endocrine hormones to maintain glucose homeostasis during a bout of exercise also may occur during parturition. During parturition, glucocorticoids and catecholamine concentrations are increased, which may stimulate gluconeogenic and glycogenolytic pathways. This can deplete the liver of glycogen making it unavailable to the mammary gland after parturition to support lactation. Acute exercise of ruminants can increase plasma glucose concentration,

whereas nonruminants maintain plasma glucose concentrations within the normal range during acute exercise. The inability of ruminants to maintain glucose homeostasis during acute exercise of ruminants also could occur at parturition and greatly affect the ability of the cow to adjust to the demands of early lactation.

Integration of acute responses to exercise. Acute exercise increased circulation of catecholamines, corticoids, and glucagon. The catecholamines, as well as shifts in acid-base status (greater $p\text{CO}_2$ and reduced pH in venous blood), stimulate increased respiration and cardiac output. Cardiac output was primarily a function of increased heart rates and to a lesser extent stroke volume. Some evidence exists indicating that hematocrit also is increased from spleen output of red blood cells which increases the O_2 carrying capacity of the blood. These increases allow for additional delivery of O_2 and other nutrients to exercising muscles.

Furthermore, hepatic output of glucose through gluconeogenesis and glycogenolysis pathways are stimulated by the increases of catecholamines, corticoids, and glucagon concentrations during exercise. During prolonged exercise, these hormones also stimulate adipose tissue to mobilize lipid stores. During exercise, muscle tissues consume more O_2 for aerobic oxidation of glucose and lipid for production of ATP to be utilized during muscle contractions. Eventually, the capacity of the tissue to oxidize substrates will be depleted thereby increasing anaerobic metabolism. Lactate will accumulate in blood accompanied by a decline in blood pH. As the animal approaches its maximum capacity to work or exercise, its O_2 consumption also will be maximal and physiological homeostasis can no longer be maintained. As anaerobic glycolysis with lactate accumulation causes metabolic acidosis, exercise cannot continue.

Similar processes also may occur during the transition from a non-lactating state to a lactating state, a pregnant state to a non-pregnant state, or during a prolonged

strenuous parturition of dairy cows. Referring to the definitions of physical and physiological fitness, it is apparent that a dairy cow that is more fit has a greater potential to transition through changes in physiology to support requirements during the periparturient period. If homeostasis was maintained at the more demanding state of lactation, the potential for periparturient disorders of dairy cows may be reduced.

Acute responses of ruminants compared with nonruminants to exercise.

Carbohydrate and lipid metabolism of ruminants differs greatly from that of nonruminant species such as rats and humans. Ruminants have less basal plasma glucose concentrations and a greater rate of hepatic gluconeogenesis. However, during a single bout of exercise ruminants and nonruminants respond similarly to exercise. Plasma concentrations of carbohydrates and lipids along with hormones regulating carbohydrate and lipid metabolism were altered by exercise in both ruminants and nonruminants. However, the magnitude of responses in carbohydrate and lipid metabolism were different. As described below, evidence indicates that nonruminants have tighter control of plasma glucose concentrations compared with ruminants.

Horses that were evaluated in the same study as steers had a greater aerobic capacity (2.6-fold greater mass-specific maximum O_2 consumption; Jones et al., 1989). Mass-specific maximum O_2 consumption is maximum O_2 consumption per kg of BW. Dogs had 2-fold greater mass-specific maximum O_2 consumption than goats during maximal exercise (McClelland et al., 1995; Roberts et al., 1996; Weber et al., 1996). Furthermore, on a muscle-mass basis, dogs had 1.6-fold greater maximum O_2 consumption than goats (Vock et al., 1996a; Vock et al., 1996b). Additionally, mass-specific cardiac output increases in horses and steers were due primarily to increased heart beat frequency, not due to changes in mass-specific stroke volume (Jones et al., 1989). Also, the horse had a larger stroke volume at rest and additional red blood cells from the spleen during exercise than did steers. Furthermore, the horse had a greater

lung volume with a greater pulmonary diffusion capacity than steers (Constantinopol et al., 1989). The most striking difference between nonruminants and ruminants was the increased aerobic capacity of nonruminants. The athletic species (dogs and horses) had a greater aerobic capacity than non-athletic species such as goats (Hoppeler, 1990; Hoppeler and Weibel, 1998).

Species with greater aerobic capacity such as humans, horses, or dogs utilize lipid and carbohydrate stores differently than species with less aerobic capacity such as goats, sheep, or cows. Dogs had a 2-fold greater absolute rate of fat and carbohydrate oxidation than goats (Roberts et al., 1996). At greater exercise intensities, the additional energy required is supplied by carbohydrate metabolism, primarily from intramuscular glycogen (Weber et al., 1996). In goats and dogs, the contributions of fat and carbohydrate to fuel oxidation as a percent of their maximum O₂ consumption was similar (Roberts et al., 1996).

In dogs, plasma concentrations of glucose (97.2 mg/dl) did not change during exercise intensities of 40, 60 and 85% of maximum O₂ consumption compared with rest or post-exercise (Weber et al., 1996). However, in goats, glucose concentrations increased from 79.2 mg/dl at rest to 169.2 mg/dl at 40 and 60% of maximum O₂ consumption and 207 mg/dl at 85% of maximum O₂ consumption (Weber et al., 1996). The rates of appearance in circulation were greater in dogs compared with goats. In contrast, the rates of degradation were less in goats compared with dogs. The contributions of circulating glucose to carbohydrate oxidation during exercise were approximately 23% for goats and 13% for dogs (Weber et al., 1996). The remaining portions were from intramuscular glycogen.

Dogs, when exercising at 40 or 60% of maximum O₂ consumption, have greater concentrations of plasma NEFA than goats exercising at a similar intensity (McClelland et al., 1995; Weber et al., 1996). Plasma NEFA increased 15% above resting values

after dogs exercised at 40% maximum O₂ consumption for 1 h, whereas plasma NEFA remained unchanged for goats exercising for the same duration and intensity (Weber et al., 1996). This provides additional substrate for oxidation in working muscle tissues of species with greater aerobic capacity (Weber et al., 1993). Furthermore, in horses, exercise caused 3- and 6-fold greater NEFA and glycerol concentrations in circulation compared with rest (Geor et al., 2000). During exercise of ruminants, reduced circulating NEFA during exercise may be a reflection of the maintenance of the balance between mobilization and oxidation of NEFA. In goats and dogs at exercise intensities of 40, 60 and 85% of maximum O₂ consumption, steady-state rates of fat oxidation were independent of exercise intensity and maximal at 40% of maximum O₂ consumption (Roberts et al., 1996; Weber et al., 1996). Fatty acids in circulation contributed more to total lipid oxidation during exercise for goats compared with dogs (Weber et al., 1996).

Distribution of muscle fiber types and enzymatic profiles also differs between ruminants and nonruminants (Karlstrom et al., 1994). The structural capacity (capillary erythrocyte volume and surface area) was greater in muscle of dogs compared with goats which contributed to increased delivery of O₂ (Vock et al., 1996b). Horse muscles have a greater capillary supply and contain more type IIA fibers along with a greater oxidative capacity than muscle of steers (Karlstrom et al., 1994).

Mitochondrial volume of muscle in a species with greater aerobic capacity such as horse or dog also was larger than for a sedentary steer or goat (Vock et al., 1996a). Intramuscular stores of lipid and glycogen were greater in dogs compared with goats (Friedman, 1994; Vock et al., 1996a; Weber et al., 1996; Weber et al., 1996; Weibel et al., 1996). The increased intramuscular stores of substrates allow the dog to maintain oxidative metabolism for longer periods at greater intensities compared with goats (Weibel et al., 1996). Citrate synthetase and acyl CoA-dehydrogenase activity were

greater and lactate dehydrogenase activity was less in muscle of horses compared with steers suggesting a greater oxidative capacity (Karlstrom et al., 1994).

The differences of oxidative capacity of muscle tissues between ruminants and nonruminants are critical when considering the limited information examining the effects of acute exercise and chronic exercise on metabolic and physiological adaptations of ruminants. Glucose homeostasis during exercise of ruminants was not as tightly controlled as in nonruminants. Ruminants have greater increases of plasma concentrations of glucose during exercise than nonruminants. Additionally, lipid catabolism was apparent for several species with greater oxidative capacity during exercise.

Physiological Adaptations in Response to Chronic Exercise

Chronic exercise. Chronic exercise or training is repeated activity (e.g., walking, running, or swimming) that causes adaptations in physiological processes and (or) systems. These adaptations increase the ability of subjects to perform the chronic exercise at a reduced rate of exertion. For example, homeostasis of acid-base balance or circulating glucose concentration may be maintained as the body changes to meet the repeated demands of activity that can improve subsequent performance.

Respiration. Chronic exercise increased maximal O₂ consumption and ability to exercise for long periods of time. It increases physical fitness. Physical fitness was improved for sheep that exercised for 12 wk, 2 to 3 times a week with a maximal incremental exercise conditioning program (increases in incline of treadmill from 0 to 12% slope and speed from 4.0 km/h to 5.6 km/h). Maximum O₂ consumption (ml/min per kg of BW) increased 27% and the length of time of exercise increased 40% compared with pre-conditioning values of each animal (Mundie et al., 1991). Chronic

exercise of men 6 to 7 wk increased maximal O₂ consumption (ml/min per kg of BW) by 6 and 10% of pre-conditioning values (MacDougall et al., 1998; Turner et al., 1997).

Changes in O₂ utilization with chronic exercise in horses were not a function of adaptations of the respiratory system. After 7 wk of exercise, racehorses had a 23% increase in maximal O₂ uptake with a 19% increase in treadmill speed, but there was no change in pulmonary ventilation at maximal O₂ uptake (Evans and Rose, 1988). Aerobic and anaerobic exercise of Thoroughbred horses did not affect tidal lung volume (the amount of air that moves into the lungs with each inspiration), respiratory frequency, or expired minute volume (Art and Lekeux, 1993). Therefore, chronic exercise appeared not to alter respiratory system of horses to increase maximal O₂ uptake, but rather adaptations of the cardiovascular system.

Chronic exercise increased maximal O₂ consumption capacity of ruminants and nonruminants. However, it does not appear that adaptations of the respiratory system were responsible for the increased O₂ consumption of horses. No results were available to determine if adaptations of the respiratory system contribute to the increased O₂ consumption of ruminants (sheep, goats, or cattle). Increases in maximal O₂ consumption of horses were attributed to increases in maximal stroke volume, cardiac output, and a decrease in arteriovenous O₂ difference (Evans and Rose, 1988).

Cardiac output. Chronic exercise reduced the heart rate at given workloads compared with pre-exercise values. In calves (170 kg initial BW to 210 kg final BW), chronic exercise increased the speed at which heart rate was maximized from 9.4 to 11.5 km/h (Fosha-Dolezal and Fedde, 1988). In horses that had chronic exercise for 4 mo (Aguera et al., 1995) or 10 wk (Rodiek et al., 1987), heart beat frequency was less during progressive stages of an exercise test compared with values prior to chronic exercise. Maximum heart rates in nonruminants or ruminants did not change after

chronic exercise (Aguera et al., 1995; Evans and Rose, 1988; Fosha-Dolezal and Fedde, 1988).

Whole blood acid-base status. When animals previously were exercised chronically, acid-base homeostasis was maintained during an exercise challenge or test. Dairy cows forced to exercise for 8 wk at 4 km/h for 2 h maintained homeostatic control of blood CO₂ content, bicarbonate, and base excess during the stress of exercise compared with pre-exercise values (Blake et al., 1982). After exercise training, the difference between post-exercise and resting concentrations of blood CO₂ content, bicarbonate, and base excess was less than the difference prior to training indicating that homeostatic control of blood CO₂ content, bicarbonate, and base excess was improved. After horses underwent exercise training for 4 mo (Aguera et al., 1995) or 5 wk (Rodiek et al., 1987), plasma venous lactate was less during an acute exercise test compared with values prior to exercise training. In humans, chronic exercise for 7 d reduced lactate concentrations following a bout of exercise compared with pre-training values following exercise (Putman et al., 1998).

Glucose metabolism. Glucose metabolism of ruminants after a period of chronic exercise training has not been examined. However, in humans, chronic exercise training altered carbohydrate and lipid metabolism during a bout of exercise. Chronic exercise training improved resistance to hypoglycemia during exercise (Coggan, 1997). In humans, reviewed by Coggan (1997) and Holloszy and Kohort (1996), plasma glucose utilization during exercise was reduced after a period of chronic exercise training. Tracer dilution methodology indicated that during acute exercise of similar intensities, the rate of appearance, rate of disappearance, and oxidation of glucose were reduced by 30% following 12-wk of moderate intensity training (Coggan et al., 1990). During high intensity exercise, training did not alter the rate of appearance of plasma glucose, but reduced the rate of disappearance (19% less than untrained

individuals; Coggan et al., 1995a; Coggan et al., 1995b). Therefore, plasma glucose was spared from oxidation or glycogen synthesis during exercise after a period of chronic training.

The primary source of carbohydrate that oxidized during exercise of humans was intramuscular glycogen. Chronic exercise training in humans (Bergstrom and Hultman, 1966) and in dogs (Reynolds et al., 1995) increased the accumulation of glycogen in exercised muscle tissues. Additionally, the rate of muscle glycogen degradation was reduced after chronic exercise compared with non-exercised individuals (Henriksson, 1977; Holloszy and Coyle, 1984).

Rats fasted to deplete liver glycogen maintained greater plasma glucose concentrations during an exercise bout following chronic exercise training (Donovan and Sumida, 1990). Additionally, the rate of appearance of glucose in rats increased during an exercise bout due to chronic exercise training (Donovan and Sumida, 1990). The uptake of glucose by skeletal muscle tissues during a bout of exercise was not suppressed following endurance exercise training (Leighton et al., 1989; Sumida and Donovan, 1994). Additionally, the rate of glycogen synthesis in rat muscle following a bout of exercise was greater compared with rest, but not different between sedentary and chronically exercised rats (Leighton et al., 1989). Insulin-stimulated glucose utilization (Leighton et al., 1989) and glucose uptake (Hokama et al., 1997) by rat muscle were greater for chronically exercised compared with sedentary rats.

A key enzyme of glucose metabolism in muscle, hexokinase, catalyzes phosphorylation of glucose to form glucose 6-phosphate, which then continues through pathways of glycolysis, glycogen synthesis, or pentose phosphate synthesis. As part of citric acid cycle, 2-oxoglutarate dehydrogenase (α -ketoglutarate dehydrogenase) catalyzes the reaction of α -ketoglutarate and NAD^+ to succinyl-CoA, CO_2 , and NADH. The maximal activities of hexokinase (glucose metabolism) and 2-oxoglutarate

dehydrogenase (citric acid cycle) were greater in trained rats compared with sedentary rats (Leighton et al., 1989). Chronic exercise increased muscle tissue enzymatic activity for glucose utilization and citric acid cycle compared with sedentary rats. Therefore, during exercise the capacity of muscle to utilize glucose was greater after chronic exercise.

Liver tissue from trained rats had greater gluconeogenic capacity compared with liver tissue from untrained rats (Sumida et al., 1993). In rats, endurance exercise training increased hepatic gluconeogenic capacity, however, the mechanism involved is not known. In rats, the hormonal control of glucose homeostasis during exercise also is not known. Insulin resistance, including glucose tolerance and metabolic action of insulin, was implicated in adaptations of glucose homeostasis to chronic exercise training (Donovan and Sumida, 1997).

Lipid metabolism. In humans, endurance exercise training enhances the capacity of skeletal muscle to oxidize fatty acids (Mole et al., 1971). Blood plasma fatty acid turnover and oxidation or depletion of intramuscular triglycerides provides energy to exercising muscle. At the same workload, plasma fatty acid concentrations were less, and plasma fatty acid oxidation was reduced after chronic exercise compared with pre-exercise training values (Martin, 1997). Chronic exercise increased the depletion of intramuscular triglycerides during exercise at the same absolute workload compared with pre-exercise training values (Martin, 1997). Additionally, chronic exercise increased the uptake of fatty acids and oxidation of fatty acids by the skeletal muscle during prolonged exercise compared with non-exercised skeletal muscle (Kiens, 1997; Turcotte et al., 1992). The extent to which NEFA uptake and oxidation occurred during exercise was dependent on intensity and length of time of exercise (Kiens, 1997). Total oxidation of fatty acids during exercise was 60% greater for chronically exercised than non-exercised men (Kiens, 1997). Chronic exercise caused adaptation in metabolism of fat

from plasma or intramuscular stores to provide energy for exercise and spare carbohydrate sources.

Concentrations of catecholamines increase in circulation during exercise and stimulate lipolysis from adipose tissue (Christensen and Galbo, 1983; Galbo, 1983). Chronic exercise reduced the plasma concentrations of catecholamines during a bout of exercise which may reduce the amount of circulating fatty acid available (Winder et al., 1979). Oxidation of intramuscular adipose by the exercising muscle increases after chronic exercise training compared with pre-exercise values (Martin, 1997) to meet the energy requirements of exercising tissues.

Endocrine control of metabolism. Glucose homeostasis is regulated by insulin, glucagon, catecholamines, and corticoids. Endurance exercise training may cause alteration in sensitivity of tissues to the hormones or in plasma concentrations of the hormones during exercise to maintain plasma glucose. After chronic exercise of 5 d per wk for 8 wk with a treadmill at 1.5 km/h, 9% slope incline for 30 min, Holstein heifers had less total blood corticoid concentrations during a bout of exercise compared with pre-exercise training values (Arave et al., 1987).

In humans, plasma insulin concentrations decreased during a bout of exercise for chronically exercised and non-exercised individuals (Coggan et al., 1995a; Coggan et al., 1995b). Additionally, chronic exercise reduced the magnitude of decline in plasma insulin concentrations during exercise as compared with untrained individuals (Coggan et al., 1995a; Coggan et al., 1995b). Plasma glucagon concentrations were maintained during an exercise bout and were not altered by chronic exercise (Coggan et al., 1995a; Coggan et al., 1995b). After chronic exercise, plasma epinephrine and norepinephrine concentrations declined during an exercise bout at the same absolute workload compared with the untrained state (Coggan et al., 1995a; Coggan et al., 1995b).

Body composition and muscle distribution. Chronic exercise training had minor effects on the body composition of ruminants. Growing lambs trained at 75% maximum O₂ consumption for 6, 12 or 18 wk had changes in body composition (Aalhus and Price, 1990b). Livers were slightly larger in exercised lambs compared with non-exercised lambs. However, exercised lambs also had increased diet intakes, which may have contributed to increased liver mass. Exercise treatment of lambs did not change the proportion of carcass that was fat, bone, and muscle as determined by dissection (Aalhus and Price, 1990b). Exercised lambs had a larger proportion of muscle in proximal pelvic limbs and abdominal walls with less muscle around the spinal column. After 18 wk of exercise, intramuscular fat in semimembranous, vastus lateralis, and gastrocnemius muscles were reduced. Subcutaneous fat was less in exercised wethers compared with non-exercised wethers (Pethick and Rowe, 1996). Load carrying exercise of 16 wk did not alter carcass composition, but altered weight distribution of muscle tissues of lambs compared with non-exercised lambs (Aalhus and Price, 1990a).

Muscle histological and compositional adaptations. Muscle fiber type and adaptations of oxidative capacity to chronic exercise were variable and dependent upon number of weeks of training and intensity of exercise. Growing lambs exercised for 6, 12, or 18 wk at 75% of maximum O₂ consumption had similar muscle size and fiber diameters, but an increase in slow-twitch fibers (Aalhus and Price, 1991). Additionally, the semitendinous muscle from exercised lambs had greater oxidative-glycolytic fibers. Adaptations to exercise of adult ruminant muscle were evaluated in one study comparing grazing vs. non-grazing cows. No differences in fiber composition, capillary density, or oxidative capacity were detected (Wiklund et al., 1998). After 7 wk of chronic exercise, the gluteal muscle of horses did not have different muscle fiber types or capillary numbers (Hodgson et al., 1985).

Glycogen and myofibrillar protein concentrations increased with chronic exercise training, whereas collagen concentrations decreased. Chronic exercise training increased the myofibrillar protein concentrations in muscle and reduced soluble collagen content of lambs (Aalhus et al., 1991). Wethers exercised daily for 1 h, 8 km/h for 5 wk had greater concentrations of glycogen in semimembranous, semitendinous, and longissimus dorsi muscles (Gardner et al., 1998; Pethick and Rowe, 1996). After 7 wk of chronic exercise, glycogen utilization within the gluteal muscle of horses was reduced (36% less than before exercise; Hodgson et al., 1985). Chronic exercise training of rats prevented net glycogen loss of muscle tissues during exercise (Azevedo et al., 1998). In humans, exercise training for 7 d increased the glycogen, coenzyme A, and phosphocreatine concentrations in muscle compared with pre-training values (Putman et al., 1998). Green et al. (1995) determined that 4 wk of exercise of men at 62% pre-training maximum O₂ consumption 5 to 6 times per wk reduced muscle lactate concentrations, phosphocreatine hydrolysis, and glycogen depletion. Two wk of wheel running of rats increased glycogen concentration in muscle compared with sedentary controls (Henriksen and Halseth, 1994).

In all species, glycogen concentration in muscle was increased in exercised individuals compared with sedentary individuals. A reduction of glycogen depletion and (or) repletion of glycogen may contribute to the greater glycogen content of exercised individuals with non-exercised.

Muscle enzyme adaptations. Enzymatic adaptations of ruminants after a period of chronic exercise have not been examined. Chronic exercise on a treadmill of rats for 12 wk caused increased mitochondrial protein and cytochrome oxidase activity of hindlimb muscles compared with sedentary controls (Martin, 1987). After 6 wk of endurance training, leg muscle mitochondrial volume was increased (Turner et al., 1997). After 7 wk of chronic exercise training, the potential oxidative capacity of the

gluteal muscle of horses was greater in that the concentrations of citrate synthetase and acyl-CoA dehydrogenase increased, whereas lactate dehydrogenase decreased (Hodgson et al., 1985). Exercise training of men caused greater maximal enzyme activity of hexokinase, phosphofructokinase, citrate synthase, succinate dehydrogenase, and malate dehydrogenase of vastus lateralis muscle (MacDougall et al., 1998). Increases in maximal citrate synthase activity occurred after only 6 d of exercise training in humans (Green et al., 1999), and succinate dehydrogenase activity increased by 31% after 4 wk of chronic exercise compared with pre-training values (Green et al., 1995).

Rats trained for 6 or 11 wk had greater maximal activities of hexokinase and 2-oxoglutarate dehydrogenase (Leighton et al., 1989). Exercise of rats for 6 wk increased oxidative capacity of muscle tissues as evident by increased citrate synthase and hexokinase activities (Kriketos et al., 1995). Activities of citrate synthase, ubiquinol-cytochrome-c oxidoreductase, and cytochrome oxidase increased after 3 wk of treadmill running of rats followed by elevations in activity after 6 and 12 wk of training (Murakami et al., 1994). However, mitochondrial DNA concentrations of muscle indicating mitochondrial replication did not increase until 12 wk of training of rats.

From these studies, glycolytic and oxidative enzyme activity of muscle was increased as early as 1 wk of chronic exercise, whereas increased mitochondrial volume of muscle after chronic exercise occurred after approximately 6 wk of chronic exercise. The increased capacity for oxidative metabolism enables trained muscle to better maintain energy and redox state during exercise than untrained muscle.

Chronic exercise increased insulin-stimulated glucose uptake by increasing glucose transport protein 4 (GLUT4) concentrations and activity of muscle (Ebeling et al., 1993). Two wk of wheel running of rats increased GLUT4 protein concentrations in rat muscle compared with sedentary controls (Henriksen and Halseth, 1994; Hokama et al., 1997). In rats, exercise stimulated adaptations of glucose transport after 1 and 2 d

of activity. Sixteen h after a single bout of swimming exercise by rats, GLUT4 mRNA increased 2-fold and GLUT4 protein expression increased by 50% compared with sedentary controls (Ren et al., 1994). Glucose transport activity stimulated by insulin, contractions, and hypoxia increased similarly as to changes in GLUT4 protein concentrations (Ren et al., 1994).

These changes in GLUT4 increased the ability to utilize circulating glucose to spare muscle glycogen during exercise. Contraction of muscle causes the translocation of GLUT4 to the plasma membrane to facilitate glucose transport. Additionally, post-exercise insulin-stimulated increased glucose transport intracellularly by translocation of GLUT4 to the plasma membrane. These early adaptations of glucose transport to chronic exercise contributes to the post-exercise increase in glycogen synthesis in the rat.

Liver enzyme adaptations. Hepatic gluconeogenesis and glycogenolysis are critical to maintain circulating concentrations of glucose during exercise (Coggan et al., 1995b). PEPCK, a key regulatory enzyme of gluconeogenesis with a half-life of approximately 30 min, catalyzes the formation of phosphoenolpyruvate from oxaloacetate during gluconeogenesis. Glucocorticoids and glucagon increase the transcription of hepatic PEPCK mRNA, whereas insulin decreases transcription. Little evidence was found of adaptations of this enzyme in response to chronic exercise. The adaptations of glucoregulatory hormones in response to chronic exercise most likely controlled the changes in hepatic gluconeogenesis rather than adaptations in PEPCK in humans or rats.

Rats, which were chronically and progressively exercised, had greater hepatic gluconeogenesis capacity compared with controls when alanine was the substrate (Burelle et al., 2000; Sumida and Donovan, 1995). Hepatocytes from trained rats had a greater rate of gluconeogenesis with alanine, lactate, and glycerol as substrates

compared with hepatocytes of sedentary rats (Burelle et al., 2000). Furthermore, intracellular alanine accumulation capacity increased by 50% compared with that of sedentary rats (Burelle et al., 2000). Burelle et al. (2000) attribute adaptations of hepatic gluconeogenesis as increased uptake of alanine by hepatocytes and deamination of alanine to pyruvate.

Physiological Responses during Pregnancy to Acute and Chronic Exercise

Maternal responses to acute exercise. During submaximal exercise of humans, pregnancy increased O_2 consumption at given workloads (McMurray et al., 1993). As pregnancy of humans progressed, the O_2 consumption at given workloads increased, potentially as a result of increased weight gain (Clapp et al., 1987; Pivarnik et al., 1990). Additionally, O_2 consumption during non-weight bearing exercise (cycle ergometry) was greater during the final trimester of pregnancy of humans than during early gestation (Carpenter et al., 1990; Pivarnik et al., 1990; Sady et al., 1989). Maximal O_2 consumption during pregnancy of women was not altered, but the maximal work capacity may be reduced (Lotgering et al., 1991; McMurray et al., 1991; Sady et al., 1989). The reduction of maximal work capacity may be due to the additional metabolic demand of increased maternal BW of women (Lotgering et al., 1991).

In women, pregnancy increased the pulmonary ventilation at a given workload to support the greater O_2 consumption (Kemp et al., 1997; Wolfer et al., 1989). Pivarnik et al. (1990) indicated that during pregnancy, the ventilation increased more than O_2 uptake during exercise of humans. The increase in pulmonary ventilation was attributed to increased lung tidal volume, rather than the frequency of respiration in that it did not change (Lotgering et al., 1991; McMurray et al., 1991). During exercise, the ventilation (ml/min) per O_2 uptake (ml/min) was greater for pregnancy than postpartum, possibly as a result of changes in lung tidal volumes.

Pregnancy increased resting cardiac output with increases in both stroke volume and heart rate. Submaximal acute exercise increased cardiac output during pregnancy of women compared with postpartum responses (Pivarnik et al., 1990; Sady et al., 1989). However, the relationship between cardiac output and O_2 consumption was not changed. During pregnancy, cardiac output increased at rest. This increase was maintained during exercise compared with reduced cardiac outputs of non-pregnant exercising women. Pregnancy did not cause an additional response of cardiac output to exercise (McMurray et al., 1993).

Pregnancy in women caused plasma volume to increase (McMurray et al., 1991; Pivarnik et al., 1990), which contributed to the increased stroke volume during rest and exercise. This is different from non-pregnant cattle in that exercise-induced changes in cardiac output were a function of increased heart rates without changes in stroke volume (Kuhlmann et al., 1985b). Furthermore, acute exercise of pregnant women caused a slight elevation of fetal heart beat frequency (Kemp et al., 1997; Lotgering et al., 1991).

In pregnant sheep, the intensity and duration of exercise reduced uterine blood flow. During exercise for 10 to 40 min at 70% maximal O_2 consumption, uterine blood flow decreased from 13 to 24% compared with resting values (Lotgering et al., 1983b). Additionally, exercise at 60, 70, 80, and 90% maximal O_2 consumption of sheep reduced uterine blood flows by 29, 45, 62, and 80% of pre-exercise values, respectively (Jones et al., 1992). Circulating norepinephrine concentrations altered uterine blood flow through vaso-constriction of utero-placental tissues of sheep (Bell et al., 1986; Palmer et al., 1984).

Acid-base status of pregnant compared with non-pregnant women following an acute maximal exercise test was different. At rest, plasma concentrations of hydrogen ion (H^+) and bicarbonate, and pCO_2 were less for pregnant compared with non-pregnant

women (Kemp et al., 1997). Immediately following a maximal exercise test, both groups responded similarly in that plasma venous H^+ concentrations increased whereas bicarbonate concentrations and pCO_2 decreased. These differences between the pregnant and non-pregnant groups at rest also were detected immediately after the exercise test. Differences in acid-base status associated with pregnancy have been attributed to adaptations induced by progesterone to increase respiratory sensitivity to venous pCO_2 (Bayliss and Millhorn, 1992).

Strenuous acute exercise during late gestation of ewes did not reduce glucose or O_2 uptake by conceptus. Pregnant ewes exercised during late gestation had greater arterial concentrations, entry rates, and uterine uptake of glucose compared with resting values (Leury et al., 1990a; Lotgering et al., 1983b). Pregnant ewes that were exercised for 1 h at 2.5 km/h on a 10° incline (17.6% slope) had greater maternal blood concentrations and entry rates of glucose. Uterine uptake of glucose was greater in exercised ewes, even though uterine blood flow was reduced (Chandler et al., 1985; Leury et al., 1990a). Uterine or umbilical O_2 uptake was not different during exercise of the ewe (Chandler et al., 1985; Lotgering et al., 1983a). Net uterine uptake of glucose was correlated with maternal arterial blood glucose concentrations. During exercise of ewes, fetal blood glucose concentrations increased, however fetal utilization of glucose did not change. Additionally, fetal insulin concentrations did not change during exercise (Leury et al., 1990b).

Circulating metabolites of pregnant women and pregnant ewes were different during submaximal acute exercise. Immediately after exercising at heart rates ranging from 135 to 150 BPM, pregnant women had less plasma glucose and insulin concentrations, whereas triglycerides and NEFA concentrations were greater compared with resting values (Bonen et al., 1995; McMurray et al., 1996). This may be reflective

of the differences between nonruminants and ruminants in aerobic capacity or fine control of glucose homeostasis in nonruminants as described previously.

Catecholamines and cortisol responses during acute exercise may be attenuated during pregnancy and exercise of women. Epinephrine and norepinephrine were not altered during submaximal exercise of pregnant women, whereas strenuous exercise increased circulating catecholamines (Bonen et al., 1995; McMurray et al., 1987). Submaximal and maximal exercise reduced or did not change plasma cortisol concentrations (Hackney et al., 1992; McMurray et al., 1996). McMurray et al. (1993) speculated that the blunted response of stress hormones during pregnancy and exercise may be to avoid a hyperglycemia response. It is crucial to recall the inherent differences in glucose homeostasis between ruminants and nonruminants when thinking of extrapolating these limited research results to dairy cows. Finally, there was no evidence that acute exercise caused significant negative effects on fetal well-being in humans or sheep (McMurray et al., 1993).

Maternal responses to chronic exercise training. Exercise during pregnancy caused increases in food intake and altered body composition. Rats that were exercised daily 3 wk prepartum for 2 h at 1.8 km/h had greater food intakes than sedentary rats and non-pregnant rats (Courant and Barr, 1990). Additionally, weight gain was greater for pregnant, exercised rats than for non-pregnant and sedentary rats. Other studies have not demonstrated increases in BW as a result of exercise treatment (Vanheest and Rodgers, 1997). No research results were available for ruminants.

Body composition was altered with exercise treatment. Exercise of pregnant rats caused a 2-fold decrease in body fat content, whereas water and ash percent were slightly greater, and percent protein was similar (Courant and Barr, 1990). The mean pup birth weight was similar between non-exercised and exercised rats. Crossbred beef cows and heifers forced to exercise 3.2 km daily for 90 d prepartum had 31% greater

feed intake to maintain the same BW gains compared with non-exercised controls (Bellows et al., 1994).

Maternal heart rates during pregnancy can be altered by exercise. Pregnancy increased resting heart rates compared with non-pregnant values (Clapp, 1985). Chronic exercise during pregnancy of sedentary women did not reduce this increase in resting heart rates (Erkkola, 1976). In contrast, if trained women continued to exercise during their pregnancy, they had reduced resting heart rates compared with sedentary controls (Pivarnik et al., 1993). After chronic exercise training, heart rates of pregnant women were less at given workloads compared with sedentary pregnant women indicating that they were more physically fit (Morton et al., 1985). Changes in cardiac stroke volume as a result of increased blood volume were difficult to determine because pregnancy and exercise increased blood volume (McMurray et al., 1993).

Physiological Adaptations of Dairy Cows during Periparturient Period

Several reviews describe physiological adaptations that dairy cows experience during the periparturient period (Bauman and Currie, 1980; Bell, 1995; Drackley, 1999; Drackley et al., 2001; Goff and Horst, 1997; Grant and Albright, 1995; Grummer, 1995; Ingvarsten and Andersen, 2000; Kelly et al., 1991; McNamara, 1991). Potential physiological adaptations during the transition from the non-lactating, pregnant state to the lactating, non-pregnant state that may respond to chronic exercise or exercise training are described in the following sections.

Energy expenditure. Prior to parturition, dairy cows and heifers have less voluntary DMI at parturition compared with other physiological states (reviewed by Ingvarsten and Andersen, 2000). The magnitude and duration of the decline in voluntary DMI around parturition can be variable and have a great influence on health and production of the dairy cow. Furthermore, the postpartum demand for energy or

peak milk production increases more than the rate of the increase of DMI. The physical constraints on voluntary DMI during the prepartum period may be a key regulator of the intake associated with the nadir of DMI at parturition (Ingvarsen and Andersen, 2000). However, many metabolic factors also are implicated. These include nutrients, metabolites, reproductive hormones, stress hormones, leptin, insulin, gut peptides, cytokines, and neuropeptides (Ingvarsen and Anderson, 2000).

Metabolizable energy requirements increase two- to three-fold during the 3 wk before and 3 wk after parturition of dairy cows compared with other physiological states. Bell (1995) calculated that during late pregnancy the maternal supplies of glucose, amino acids, and acetate were 1476, 998, and 2196 g/d, respectively. The uterine uptake to support fetal growth and uterine metabolism of these nutrients were 46, 72, and 12% of the maternal supply of glucose, amino acids, and acetate, respectively. Drackley et al. (2001) summarized that the demand of glucose by Holstein cows during 3 wk prepartum was 1000 to 1100 g/d and increased to approximately 2500 g/d at 3 wk postpartum. The large changes in nutrient demands during the periparturient period must be met by increasing the nutrient density of the diet and (or) adaptations in glucose and lipid metabolism of the body.

Glucose metabolism. As reviewed by Bell (1995), late-pregnant ruminants increase hepatic gluconeogenesis to meet increased demand for glucose at parturition. Gluconeogenesis from amino acids, lactate, propionate, and glycerol by the liver is required to meet the glucose demands during the periparturient period. Potential rate-limiting enzymes of hepatic gluconeogenesis are PEPCK and pyruvate carboxylase (PC). Propionate is metabolized to oxaloacetate followed by the action of PEPCK which converts oxaloacetate to phosphoenolpyruvate. Other gluconeogenic substrates such as alanine, lactate, and glycerol are converted to pyruvate followed by the action of PC to form oxaloacetate. PC mRNA was greatest on the day following parturition, whereas

PEPCK mRNA was greater on d 28 and 56 after parturition (Greenfield et al., 2000).

Drackley et al. (2001) suggested that PEPCK may be less restricting of the gluconeogenic capacity of the liver compared to PC during the periparturient period.

During the periparturient period, several endocrine changes are occurring to coordinate the homeorhetic adaptations necessary to support lactation (Bauman and Currie, 1980). Drackley et al. (2001) reviewed possible endocrine signals (insulin, glucagon, somatotropin, and cortisol) associated with or controlling the increase in hepatic gluconeogenesis. Concentrations of somatotropin increased at parturition (Simmons et al., 1994). Circulating glucose primarily is controlled by the secretion of insulin and glucagon from the pancreas. During the periparturient period, insulin that stimulates glucose uptake in peripheral tissues was greatest on d -28, -14, +1 compared with d +28 and +56 relative to parturition, whereas glucagon, which stimulates hepatic glucose output, was least on d -14 and +1 and greatest on d -28, +28, and +56 relative to parturition (Greenfield et al., 2000).

The low insulin-to-glucagon ratio, along with increased concentrations of somatotropin, would support increased hepatic gluconeogenesis. Along with changes in PC mRNA, Drackley et al. (2001) hypothesized that the conversion of alanine from body tissues or diet was a key to the increased gluconeogenic capacity of the liver at parturition. Alanine and glutamine, compared with all other amino acids, account for 40 to 60% of the gluconeogenic capacity of amino acids (Bergman and Heitmann, 1978). Uptake of alanine was greatest by the liver than other amino acids and available in greatest amounts in portal-drained viscera and from inter-organ amino acid exchange (Bergman, 1986; Wolff et al., 1972).

Furthermore, changes during the periparturient period of the insulin-to-glucagon ratio would stimulate hepatic glycogenolysis at parturition to increase supply of glucose. Liver glycogen concentrations were the least on d +1 compared with -28, -14, +28, and

+56 relative to parturition (Greenfield et al., 2000). Additionally, Rukkwamsuk et al. (1999) demonstrated in dairy cows that glucose 6-phosphatase activity (converts glucose 6-phosphate to glucose) was greater 0.5 wk postpartum compared with activity 1 wk prepartum. Concentrations of liver glycogen were greatest 1 wk prepartum compared with 0.5 to 3 wk postpartum (Rukkwamsuk et al., 1999).

Adaptations of the hepatic gluconeogenic and glycogenolytic capacity during the prepartum period of dairy cows may improve glucose supply during the postpartum period. Dairy cows with fatty liver had less gluconeogenic capacity compared with control cows (Hippen et al., 1999; Rukkwamsuk et al., 1999). Potentially, prepartum diets and (or) manipulations of the endocrine system could stimulate adaptations in liver carbohydrate and lipid metabolism during the periparturient period. It is not known if exercise of cows during the prepartum period could also cause similar adaptations, potentially increasing or sparing glucose for the mammary gland to support lactation.

Adjustments in endocrine control of carbohydrate and lipid metabolism during this time has been linked to incidences of metabolic disorders such as ketosis and fatty liver (Kaske et al., 2001). Physiological states altered the pancreatic insulin responses to glucose infusions, and peripheral tissue responses to insulin infusions (Kaske et al., 2001). Pancreatic insulin response to glucose infusion was greatest for non-lactating, nonpregnant cows compared with lactating cows and cows with fatty livers. The greatest response to the insulin infusion was for lactating cows compared with non-lactating, pregnant cows with a further reduction for cows with fatty liver (Kaske et al., 2001). This indicates that pancreatic and peripheral tissue responses to maintain glucose homeostasis were altered by the physiological state such as lactation and metabolic disorder such as fatty liver.

Lipid metabolism. During the periparturient period, increased energy demands and reduced DMI can increase lipid mobilization (Grummer, 1993). Furthermore,

plasma catecholamine concentrations at parturition are increased, which also may stimulate lipid mobilization (Grummer, 1993). Drackley (1999) reviewed the scientific literature and developed the following model of lipid metabolism among adipose, liver and mammary gland tissues.

Increased mobilization of lipid from adipose tissue possibly stimulated by epinephrine during the prepartum period resulted in increased uptake of NEFA by the liver. One fate of NEFA in the liver was re-esterification to triglycerides, which may accumulate in the liver. Accumulation of triglycerides in the liver of ruminants primarily occurs because of a low rate of synthesis and export of very low density lipoproteins. This can be detrimental to hepatic gluconeogenesis and has been linked to increased susceptibility to ketosis. Additionally, NEFA can be oxidized in peroxisomes by the liver to form acetyl-CoA or oxidized to ketone bodies by mitochondria. Although the dairy cow is susceptible to lipid accumulation during the periparturient period, factors controlling the flux between esterification and oxidation of NEFA by the liver have not been elucidated clearly. Acetate, insulin, and glucose decrease oxidation of fatty acids. Carnitine palmitoyltransferase-1 has the potential to be a key regulatory enzyme for uptake of NEFA by mitochondria for oxidation. However, little is known regarding its activity or expression during the periparturient period of dairy cows.

Circulating concentrations of NEFA from mobilization of lipids are controlled by the endocrine system. Hippen et al. (1999) demonstrated that exogenous glucagon during early lactation of dairy cows promoted the clearance of triglyceride from liver and prevented additional accumulation of triglycerides. No evidence was available to determine mechanisms of action of insulin and glucagon in controlling enzymes associated with lipid metabolism during the periparturient period. Potentially, chronic exercise of dairy cows prepartum may cause adaptations in lipid metabolism including enhanced oxidation of lipids by peripheral or hepatic tissues.

Effects of Prepartum Exercise on Dairy Cattle

Daily exercise of dairy cows may improve health during the periparturient period. During a 4-yr study in Sweden, lactating Red and White cows that were exercised 2 to 3 km/d during summer and 0.4 to 0.8 km/d during winter had fewer calving-related disorders, mastitis, and lameness (Gustafson, 1993). Veterinary treatments during the first 2 wk of lactation for cows that were exercised were the least compared with non-exercised cows. However, only 65 lactations were analyzed in this study, and milk production was not described. Pregnant crossbred beef cattle, that were forced to exercise (walking 3.2 km/d, 90 d prepartum), had a 14.8% increase in pregnancy rates in the subsequent breeding season compared with non-exercised controls (Bellows et al., 1994).

Additional research of exercise training by walking during the prepartum period with Holstein cattle had variable production responses postpartum. Exercise 5 d per wk for 7 wk prepartum by walking for 3.22 km at 3.54 km/h, 9.66 km at 3.54 km/h, or 3.22 km at 5.47 km/h did not affect pre- or postpartum feed intake, or subsequent milk yields of multiparous Holstein cows compared with non-exercised cows (Anderson et al., 1979). Eight weeks (5 d per wk) of prepartum exercise of Holstein heifers and cows for 1.6 km or 8.0 km at 4.0 km/h did not affect prepartum feed intake or milk yields for the entire lactation compared with sedentary cows and heifers (Lamb et al., 1981). Furthermore, pregnant Holstein heifers exercised for 4 wk prepartum (5 d per wk) for 1.6 km at 5.47 km/h did not have greater DMI or milk yield compared with non-exercised heifers (Lamb et al., 1979). Additional exercise of these heifers for 10 d postpartum resulted in reduced grain intake and milk yields compared with sedentary heifers (Lamb et al., 1979).

In contrast with these results, pregnant Holstein heifers that exercised 6 wk prepartum (5 d per week) for 1.6 km at 3.54 km/h had greater postpartum intakes of

digestible energy and greater milk yield through 50 DIM, and for the entire lactation than non-exercised heifers (Lamb et al., 1979). Pregnant Holstein heifers exercised for 4 wk prepartum (5 d per wk) for 1.6 km at 5.46 km/h had fewer days prepartum of udder edema, improved calving ease, and more rapid release of placental tissues compared with non-exercised heifers (Lamb et al., 1979).

Understanding conclusively whether or not exercise training by walking was beneficial to pregnant dairy cattle is not possible from previous research because of various deficiencies of protocol and data collection within experiments. Independent variables (e.g., age, time of initiation of exercise programs relative to expected calving date, and intensity and duration of exercise training) were not similar among various studies. Additionally in some studies, animals were offered free-choice hay with limited access to grain, so the nutrition program was not optimal to maximize nutrient intakes, to support subsequent milk yields or reproductive performance. Limited sample size (approximately 15 to 20 animals per treatment) in the studies makes it impossible to assess statistically whether or not reproductive performance was actually improved. Finally, assessment of altered physical fitness of dairy cows with the exercise programs outlined was inconclusive.

Measurements to assess fitness (e.g., changes in heart rate, lactate concentrations) were incomplete, therefore it is difficult to determine if cows' fitness was improved by the various exercise protocols. Additionally, the stage of gestation of cows can independently alter the response variables that should be used to assess fitness such as heart rates during a given workload. Furthermore, experiments were not designed to test the cows' actual physical or physiological fitness, or metabolic and physiological adaptations to exercise training (Blake et al., 1982). Based on the literature, it is not clear whether or not metabolic adaptations and physiological fitness,

or performance (reproduction or milk yields) was modified as a result of exercise training of prepartum dairy cows.

Statement of Problem

In many commercial management systems, prepartum dairy cows have reduced activity, which may impair fitness. Changes of management such as a exercise training may cause physiological adaptations that could optimize milk yield, health, and reproduction during the subsequent lactation. Calving problems and peripartum metabolic disorders account for large portions of the health problems in the lactation cycle.

The potential beneficial effects of greater physical fitness of pregnant cows during the periparturient period have not been fully examined. Possibly, cows with greater physical fitness would have less dystocia or quicker recovery after parturition. In humans, exercise training during first two trimesters reduced the odds for cesarean delivery in nulliparous women compared with sedentary women (Bungum et al., 2000). Women who exercised during pregnancy also had reduced pain perception and stress during labor (less cortisol and greater β -endorphin concentrations) than sedentary women during pregnancy (Varrassi et al., 1989). Pregnant crossbred beef cattle, that were forced to exercise (walking 3.2 km/d, 90 d prepartum), had a 14.8% increase in pregnancy rates in the subsequent breeding season compared with non-exercised controls (Bellows et al., 1994).

Greater NEFA and BHBA concentrations in plasma of dairy cows during the peripartum period have been implicated in the development of fatty liver and ketosis (Grummer, 1993). Insulin resistance of tissues during the peripartum period may contribute to the development of the metabolic disorders such as ketosis when dairy cows are in negative energy balance during early lactation (Bell, 1995). Exercise

training prepartum could minimize concentrations of these circulating metabolites and increase responsiveness to regulatory hormones such as insulin to reduce metabolic disorders. These adaptations could improve glucose homeostasis and increase fatty acid oxidation during the periparturient period.

It is not known if exercise training as a prepartum management practice will reduce metabolic disorders such as ketosis or hypocalcemia, or improve milk yield or reproduction. Furthermore, it is not known if prepartum exercise of dairy cows will improve physical or physiological fitness. Improved fitness may reduce the challenges or stresses that a cow experiences at parturition which would allow for a quicker transition to a healthy, lactating physiological state.

Objectives and Hypothesis

The main focus of this research is to determine if prepartum exercise training of dairy cows causes physiological and metabolic changes that could minimize the stress and adaptations associated with the transition to lactation and increased energy demands of lactation. The proposed research will determine if physical and physiological fitness of dairy cattle can be improved with exercise training; and, if adaptations of carbohydrate and lipid metabolism occur that could benefit peripartum metabolism, health, and lactational performance.

The hypothesis is that cows exercised prepartum will have improved homeostatic control of acid-base status and glucose and NEFA concentrations during the periparturient period. Cows that exercise will have increased physical fitness and increased capacity to perform a cardiovascular stress test. Additionally, pregnant cows that are exercised prepartum will have greater milk production and DMI during early lactation than non-exercised cows.

CHAPTER 3

HEART RATES OF DAIRY COWS DURING DAILY ACTIVITIES AND FORCED EXERCISE

ABSTRACT

Objective was to evaluate responses and among- and within-cow variances of heart rates of dairy cows during various activities and exercise at different durations, speeds, and slopes. In experiment 1, heart rates and activities of lactating, multiparous Holstein dairy cows ($n = 9$) were simultaneously recorded. Body position of standing increased heart rates compared with the position of lying with a total variance of 50.4. In experiments 2 and 3, eight multiparous Holstein dairy cows were assigned randomly to replicated 4 x 4 Latin squares with treatments arranged in a 2 x 2 factorial. In experiment 2, exercise treatments in a mechanical walker were speed of exercise (3.6 versus 4.0 km/h) and duration of exercise (1 h versus 2 h). Cows that walked at 4 km/h had greater increases of heart rates above resting values compared with cows that walked 3.6 km/h (35 versus 30 beats per minute; total variance equaled 108). In experiment 3, exercise treatments on a treadmill were 6 or 12 min at 0% slope followed by slope increases every 3 min of 0.8% or 1.6%. The increases of heart rates were greatest during the portion of exercise when slope increased. Overall, speed and slope of the walking surface had greater effects on heart rates than duration of exercise and activities of standing or lying. Heart rate as an indicator of exertion was related quadratically with estimated power required to complete exercise.

INTRODUCTION

Changes in heart rate may reflect the amount of exertion of cows during daily activities or forced exercise. The amount of exertion potentially can be influenced by the speed of exercise, slope of the walking surface, or the duration of exercise. Additionally, activities such as lying, standing, eating, ruminating, sleeping, or exercise may result in different heart rates, each with a different estimate of variance. These estimates of variance may differ among cows and within individual cows when repeated measurements are made. Previously, changes in heart rates of individual cows were used to evaluate responses of cows isolated from the herd or walked (Hopster and Blokhuis, 1994) and between agonistic encounters of cows (Lefcourt et al., 1999).

At various speeds and slopes of the walking surface, heart rates of calves and heifers increased compared with their resting values. When Simmental calves were walked at 3.6 km/h on a 0% slope, heart rates increased compared with rest (Blum and Eichinger, 1988). Heart rate increased additionally when calves walked at the same rate up a 10.5% slope. Similar responses were measured with 2-yr old Holstein heifers (Arave et al., 1987). In these studies, heart rates increased as workloads increased by greater slopes of walking surface or by speed of exercise. Effects of durations of exercise and workloads on heart rates of dairy cattle have not been characterized. The magnitude and variation of heart rates to these effects could be useful to quantify the amount of exertion. Estimations of cow (among-cow) and residual (within-cow) variances will be useful in designing future experiments and interpreting results.

The objective of our experiments was to compare heart rates of dairy cows during different daily activities and to forced exercise as altered by duration, speed, or slope of the walking surface. Additionally, the variances of heart rates among- and within-cows associated with activities and exercises were characterized.

MATERIALS AND METHODS

Animal care and procedures were approved and conducted under standards of the Michigan State University All-University Committee on Animal Use and Care, application number 05/98-081-00. Guidelines were compliant with the National Institutes of Health and the Guide for the Care and Use of Laboratory Animals. In this study, experiment 1 evaluated the effects of daily activities on heart rates (Table 3.1). Experiment 2 compared the effects of speed and duration of forced exercise in a mechanical walker on heart rates (Table 3.2). Experiment 3 compared the effects of different slopes of the walking surface of a treadmill on heart rates (Table 3.2).

Measurement of Heart Rate

An electromagnetic sensor measured the heart's electrical activity to determine beats. In experiment 1, heart rates were recorded every 15 sec with a wireless electrocardiogram monitor (Polar Vantage NV, Polar Electro, Port Washington, NY). In experiments 2 and 3, heart rates were recorded every 5 sec. A similar system was validated previously (Hopster and Blokhuis, 1994). Figure 3.1 illustrates the heart rate monitoring system. The hair of the chest floor and area behind the cow's elbow to the point of her shoulder was clipped to improve skin contact with electrodes. The electrode strap was attached around the girth with a 5-cm wide elastic strap on the left side of the cow. An outer, wider elastic strap (7.6-cm wide) and felt saddle girth were placed over the electrode to aid in maintaining skin contact while the cow walked. Electrocardiogram gel (Medi-Trace Conductivity Gel, Graphic Controls, Buffalo, NY) was placed on the electrode to skin contact points to insure conductivity of the signal. Care must be taken to avoid poor contact between the electrode and skin. Any electromagnetic disturbance,

static electricity, arrhythmia, or distance between receiver and transmitter can cause atypical readings.

While exercising in the mechanical walker (experiment 2), the cows' heart rates were transmitted to a small data logger about the size of a wrist watch that was attached to the outer elastic strap. Data were later downloaded with an interface to software on a portable computer (Polar Precision, Polar Electro, Port Washington, NY). During exercise on the treadmill (experiment 3), heart beat signals were transmitted directly to a computer, monitored in real-time, and recorded. Representative graphs of heart rate data from the software are shown when a cow was walking in the mechanical walker (Figure 3.2) and on the treadmill (Figure 3.3).

Data Editing

If the contact of the electrodes or the transmission of data was interrupted during a 5- or 15-sec interval, the heart rate was recorded as zero. The software allowed for manual editing of a single 5- or 15-sec reading. A span of 12 consecutive 5- or 15-sec readings was viewed to edit data. In experiments 1 and 2, no manual editing was done, and the values of zero were considered missing. In experiment 3, if more than six consecutive readings were atypical, the values were considered missing. Atypical measurements were defined as greater or less than 60 BPM from preceding and succeeding values. If the span of atypical measurements was less than six consecutive readings, the values were replaced with the average of the preceding and succeeding measurements.

For the statistical analyses of experiment 2 (mechanical walker exercise), the average, standard deviation, minimum and maximum heart rates were calculated using the software by selecting the time interval of interest. Missing data points were excluded

from these calculations. For the statistical analyses of experiment 3 (treadmill exercise), edited heart rate data (every 5 sec) were averaged for each minute.

Experiment 1: Effects of Daily Activities on Heart Rate

Nine lactating multiparous Holstein cows were evaluated for 7.5 h on 2 d, 1 wk apart. Beginning 4 wk prior to the start of the experiment, each cow was kept in the same tie-stall and fed a common TMR (corn silage-based diet with 1.61 Mcal of NE_L /kg and 18.1% CP, dry basis) ad libitum once daily. Heart rate monitors were placed on cows at approximately 0800 h prior to feeding. Every 15 min, the following activities or combinations of activities of cows while in tie-stalls were observed and recorded from 0900 h (at feeding) until 1330 h. Activities were eating (ET), ruminating (RT), drinking (DR), standing (ST), lying (LY), and sleeping (SL). Cows were defined as sleeping when recumbent with eyes closed and no other activity. To synchronize heart rate measurements and observed activities, the time of feeding was recorded on the data logger of the heart rate monitor for each cow. At approximately 1330 h, all cows were released into an outdoor cement-paved lot. They commingled with 33 other cows for approximately 1.5 to 2 h until time of milking. Cows then were moved as a group to the milking parlor holding pen, milked, and returned to their respective tie-stalls; time interval ranging from 15 to 60 min. The 15-sec heart rate measurements that were recorded prior and after (eight readings) the times of observation of each activity were used to determine the average heart rate for that event.

Experiment 2: Effects of Speed and Duration of Exercise on Heart Rate

Eight non-lactating, non-pregnant multiparous Holstein cows were assigned randomly in a replicated 4 x 4 Latin square design with treatments arranged in a 2 x 2 factorial. Exercise treatments were speed of exercise (3.6 or 4.0 km/h) and duration of

exercise (1 h or 2 h in a mechanical walker; Table 3.2). These speeds were evaluated to identify the maximum rate of walking at which cows could maintain a comfortable pace and gait for at least 2 h. One cow was replaced after completing two periods due to her refusal to walk in period 3. Data from this cow for periods 1 and 2 and data of a replacement cow of periods 3 and 4 were used in statistical analyses.

Exercise treatments were administered using an outdoor mechanical walker with a circular lane. The circular lane was 90-cm wide (Figure 3.4 A). The outside diameter of the circle was 10.8 m, and the circumference was 33.8 m. The circular lane was 36.5 m from the tie-stall area. The walking surface consisted of approximately 20 cm (depth) of rolled stone covered with geo-textile felt (Cow Carpet TM, S & R Textiles, Highpoint, NC). The felt was covered with about 15 cm of fine fill sand. The mechanical walker was a modified six-horse commercial walker (Hot to Trot Horsecwalkers, Chardon, OH). Partitions hanging from each arm of the walker were constructed of galvanized metal pipe and steel chain. Three 60-cm lengths of 2.5-cm metal pipe were bolted horizontally to three, 245-cm lengths of 0.5-cm steel chain. Chain lengths were then attached to a 90-cm length of polyvinylchloride pipe mounted to each arm of the walker. Plywood panels (0.6-cm thick, 56-cm wide, 70-cm long) were attached to the chain lengths (Figure 3.4 B). These panels separated individual cows and helped control walking speed. Partitions were flexible enough to pass over the cow if she refused to continue walking. Cows learned to use the mechanical walker system during three, 5- to 10-min training sessions completed about 3 wk before the actual experimentation began.

The experiment lasted 1 wk with a Latin square period every other day. Each cow was kept in the same tie-stall in a ventilated, closed-sided barn and fed a TMR (alfalfa silage-based diet with 1.47 Mcal of NE_L/kg and 15.6% CP, dry basis) ad libitum once daily. Heart rate monitors were placed on cows while they were standing in tie-stalls. Cows previously had been familiarized to the heart rate monitor straps. While cows

remained in tie-stalls, a resting heart rate was recorded for 5 min prior to moving to the mechanical walker. During exercise in the mechanical walker, cows assigned to the 2-h duration of exercise were stopped for 5 min after the first hour, so that cows assigned to the treatment of 1-h duration of exercise could be loaded into the mechanical walker. Both treatment groups then completed the second hour of exercising at the assigned speed (3.6 or 4.0 km/h).

Experiment 3: Effects of Slope of Walking Surface and Duration of Exercise on Heart Rate

To evaluate the effects of increasing slope (0 to 9.6%) of walking surface and duration of exercise on heart rate, cows were walked at a rate of 5 km/h on a treadmill (Classic Treadmill Conditioner, W. H. D. Lindsey, Kenilworth, Australia). Eight non-lactating, non-pregnant multiparous Holstein cows were assigned randomly in a replicated 4 x 4 Latin square design. Treatments in a 2 x 2 factorial arrangement were duration of exercise for an initial 6 or 12 min interval at 0% slope followed by increases of slope of 0.8 or 1.6% every 3 min. The four treatment groups were: 6 min initial exercise at 0% slope followed by 0.8% increases of slope (**6min0.8%**); 6 min initial exercise at 0% slope followed by 1.6% increases of slope (**6min1.6%**); 12 min initial exercise at 0% slope followed by 0.8% increases of slope (**12min0.8%**); and, 12 min initial exercise at 0% slope followed by 1.6% increases of slope (**12min1.6%**) (Table 3.2). Estimates of required power to exercise at workloads of each treatment as increased by duration of exercise and slope of walking surface are illustrated in Figure 3.5. Power estimates (cal/kg of BW per min) were calculated for each workload during the treadmill exercise as a summation of energy required for distance traveled at 0% slope (0.478 cal/kg of BW per km) and vertical rise at each slope (6.21 cal/kg of BW per km; Agricultural Research Council, 1980; Lawrence and Stibbards, 1990). Due to

mechanical limitations of the treadmill (maximum slope of 9.6%), maximum estimated power was 90 cal/kg of BW per min. Therefore, the potential durations of treadmill exercise were 42, 24, 48, and 30 min for treatment 6min0.8%, 6min1.6%, 12min0.8%, and 12min1.6%, respectively. The exercise continued for each individual cow until the cow refused to walk or until heart rate was greater than 180 BPM. The time at which the exercise actually ended was recorded for statistical analyses.

In preliminary observations, 5 km/h was determined to be the rate at which cows could maintain a comfortable walking gait. Cows were able to complete the slightly faster pace on the treadmill compared with the mechanical walker because of differences in walking surface. The lack of coordination of cows on this treadmill prevented evaluations of faster speeds.

Statistical Analyses

Data were analyzed by method of ANOVA using mixed model procedures (SAS, 1999). The appropriate covariance structure for the repeated measures was determined using the goodness of fit criteria of Aiaike's Information Criterion and Schwarz's Bayesian Criterion. The among- and within- animal variance components were estimated using residual maximum likelihood methods. These estimates were used to describe the number of observations per treatment group required to compare treatments of future experiments with t-test statistics and one-way ANOVA (SAS, 1999). For one-way ANOVA sample size estimations, the sums of squares were estimated from mixed model procedures using method option of type3. Results are presented as generalized least squares means with standard error of mean. Statistical significance was declared at $P < 0.05$; trends were noted at $0.05 < P < 0.15$.

Experiment 1. For each activity, heart rate data recorded every 15 sec were used to determine the average heart rate. The mixed model included the random effect

of cow, repeated measures effect of day, and fixed effects of activity and the two-way interaction of activity and day. In tie-stalls, the activities or combinations of activities for which heart rates were recorded included: ST; ST and ET; ST and RT; ST and DR; LY; LY and RT; and, LY and SL (Table 3.1). During commingling, activities were ST in the outdoor cement-paved lot, ST in the holding pen adjacent to the milking parlor, and ST during milking (Table 3.1). Preplanned orthogonal contrasts were used to test the effects of activities on heart rates (Table 3.1). The covariance structure of the repeated measures effect of day was compound symmetry.

Experiment 2. During the experiment with the mechanical walker, average heart rates in the tie-stall and during exercise, and minimum and maximum heart rates during exercise were determined. The mixed model included the random effect of cow and fixed effects of duration of exercise, speed of exercise, period, and all two-way interactions of period, duration, and speed of exercise. The averaged heart rates were weighted by their standard deviations in the mixed model analysis.

Experiment 3. The actual ending time of the treadmill exercise was determined as the minute at which the peak heart rate was recorded. The mixed model included the random effect of cow and the fixed effects of period, duration of exercise at 0% slope, increase of slope, and two-way interactions of duration of exercise at 0% slope and increase of slope. If heart rates reached more than 170 BPM, a treadmill test was declared a maximal workload challenge. Chi-square analysis was completed to determine if treatment affected the probability of completing a maximal workload session.

Heart rate data for every 5 sec were used to determine the average heart rate for each minute during the treadmill exercise. Some cows had longer sessions resulting in unequal observations among treatment groups (Figure 3.6). The frequency of observations per minute of exercise was three or less for min 26 to 33 of 6min0.8%, min

23 to 34 of 6min1.6%, min 38 to 43 of 12min0.8%, and min 28 to 29 of 12min1.6%. Due to variable length sessions and reduced frequency of observations, data were divided into two subsets. The first subset contained data from the initial 6 min of exercise of the session (Figure 3.7 A). The second subset were data from the starting time of slope increases through 14 min (Figure 3.7 B). The data from initial interval of min 6 to 12 at 0% slope for treatments of 12min0.8% and 12min1.6% were excluded from the analyses.

The mixed model included the random effect of cow, repeated measures effect of minute of exercise, and fixed effects of period, duration of exercise at 0% slope, increase of slope, and the two- and three-way interactions of duration of exercise at 0% slope, increase of slope, and minute. To account for any missing 5-sec heart rates during a given minute, the averaged heart rate data were weighted with the number of recorded heart rates within a minute in the mixed model analyses. The covariance structure of the repeated measures effect of minute of exercise was auto-regressive first order.

Heart rate during the final minute of exercise at a given power was assumed to be steady-state and reflective of the amount of exertion of the animal. Regression analysis was performed to evaluate the relationship between heart rate and the estimated amount of power required during the treadmill exercise.

RESULTS AND DISCUSSION

Experiment 1: Heart Rates during Daily Activities

Various activities of cows affected measured heart rates ($P < 0.001$; Table 3.1). Lying and LY associated with other activities decreased heart rates compared with ST and ST associated with other activities in tie-stalls or in commingling (Contrast A; Table

3.1; $P < 0.0001$). Heart rates were not different among cows standing in tie-stalls or during commingling (Contrast B; Table 3.1). Other activities of ET, DR, RT in tie-stalls did not increase heart rates of cows more than only standing in tie-stall (Contrast C, Table 3.1). Cows that were LY and SL tended to have lower heart rates compared with cows that were LY and LY and RT (Contrast D; Table 3.1; $P = 0.09$). When cows were lying, their average heart rates were 66 ± 1.2 BPM (pooled across other activities). When cows were standing, their average heart rates were 72 ± 1.2 BPM (pooled across tie-stall and commingling activities).

In the current study, the body position of standing increased heart rate 6 BPM compared with lying. Similarly, Lefcourt et al. (1999) found that heart rates decreased by 5 BPM when cows laid down. Also, releasing cows from the tie-stall and commingling them in a cement-paved lot and moving them to a holding pen for more than 1.5 h did not affect heart rates.

The estimated variances (s^2) among- and within-cows were 14.6 and 35.8, respectively. Therefore, the total estimated variance of heart rates of cows during routine activities in tie-stalls and commingling was 50.4. Using this estimate of variance and power of 90% for a two-tailed Type I error of 5% (t-statistic), a minimum of 12 observations per group would be required for future experiments to measure a potential mean difference of 10 BPM.

Experiment 2: Speed and Duration of Exercise Affected Heart Rates

The interaction of speed and duration of exercise was not significant for any dependent variable (Table 3.3). Assigned speed of exercise did not affect resting heart rates in tie-stalls prior to exercise (Table 3.3). Heart rates of cows that were assigned to 2 h duration of exercise tended to have slightly greater resting values than those assigned to 1 h durations ($P = 0.10$, Table 3.3). Duration and speed of exercise affected

the average heart rate during exercise (Table 3.3). Cows that walked for 2 h had greater average heart rates (93 ± 3.7 BPM) compared with cows that walked for 1 h (86 ± 3.7 BPM) pooled across speeds of exercise. Cows that walked at 4.0 km/h had greater average heart rates (92 ± 3.7 BPM) compared with cows that walked at 3.6 km/h (87 ± 3.7 BPM) pooled across durations of exercise. Speed and duration of exercise did not affect either maximum or minimum heart rates recorded (Table 3.3). Walking at 4.0 km/h, increased heart rate of cows by 35 ± 3.2 BPM above resting values compared with an increase of 30 ± 3.2 for cows walking at 3.6 km/h. Even though cows that walked for 2 h had greater average heart rates than cows that walked for 1 h (93 vs. 86 BPM), duration of exercise did not affect the increase in heart rates above resting values.

Additionally, average heart rates during the first hour of exercise were greater for cows walking 4.0 km/h compared with those walking at 3.6 km/h (93 vs. 87 ± 3.8 BPM; $P = 0.02$). Cows assigned to exercise for 2 h also had greater heart rates during the first hour than cows assigned to exercise for 1 h (94 vs. 86 ± 3.9 BPM; $P = 0.008$). The assigned duration of exercise did not affect the increase of average heart rates during the first hour over resting heart rates, whereas the speed of exercise did affect the magnitude of increase (34 vs. 28 ± 3.0 BPM for 4.0 vs. 3.6 km/h; $P = 0.003$).

The estimated among-, within-cow and total variances are described in Table 3.3. The maximum heart rates recorded had the largest total variance estimate compared with all other heart rate measurements. Total estimated variances were 231 for average heart rates during exercise and 108 for the increase during exercise above resting values. Using these estimates of variance and power of 90% for a two-tailed Type I error of 5% (t-statistic), a minimum of 50 and 24 observations per treatment group, respectively, would be required in future experiments to measure a potential mean difference of 10 BPM. Using one-way ANOVA, a comparison would require a minimum

of three observations per group for future experiments to measure a potential difference between treatments (assuming a power of 90% with Type I error of 5% and four treatments with sums of squares of 2800).

Mature oxen carrying loads at similar speeds to those in the current experiment had heart rates of approximately 80 BPM (Pearson and Archibald, 1989). In contrast, Hereford steers (approximately 4-mo old, 165-kg BW) had greater heart rates (148 BPM at 3.6 km/h) compared with heart rates (85 BPM) in the current study (Fosha-Dolezal and Fedde, 1988; Kuhlman et al., 1985). The differences of stride lengths between young steers and mature dairy cows may have resulted in observed differences in heart rates.

Previous research with calves and oxen demonstrated that duration of exercise of 15 to 30 min at speeds similar to those in our study did not affect the increase of heart rates above resting values (Pearson and Archibald, 1989; Piguet et al., 1994). In these studies, heart rates reached a steady-state after 3 to 5 min of exercise and were sustained for the entire duration of exercise. In the current experiment, extended duration of exercise did not cause an additional increase in heart rates above resting values. Overall, the speed of exercise had a greater effect on increasing steady state heart rates than did duration of exercise.

Experiment 3: Increased Slope of Walking Surface Affected Heart Rate

During treadmill exercise, the initial interval of time (6 or 12 min) at 0% slope and increase of slope (0.8 or 1.6%) affected the time to end of session ($P < 0.001$; Table 3.4). Successfully completing a treadmill session (time at which heart rates were greater than 170 BPM) was not affected by treatment (Chi-square, $P > 0.15$). When cows walked an initial interval of 12 min at 0% slope compared with 6 min at 0% slope, the time to end of the treadmill session was greater (31 vs. 25 ± 1.1 min, $P < 0.001$).

When the increase of slope was 0.8% compared with 1.6%, the time to end of treadmill session was greater (31 vs. 24 ± 1.1 min, $P < 0.001$). The interaction of initial interval of time at 0% slope by increase of slope did not affect the overall time to end of treadmill session ($P > 0.15$). The among- and within-cow variances were 0.6 and 17.7. Using a total estimated variance of 18.3 and power of 90% for a two-tailed Type I error of 5%, a minimum of 12 observations per treatment group would be required for future experiments to measure a potential mean difference of 6 min. Using one-way ANOVA, a comparison would require a minimum of two observations per group for future experiments to measure a potential difference between treatments (assuming a power of 90% with Type I error of 5% and four treatments with sums of squares of 670).

During the first 6 min of treadmill exercise, heart rate increased regardless of treatment from 68 to 106 BPM (Figure 3.7 A, $P < 0.0001$). The standard error during the first 6 min of ranged from 4.5 to 6.5 BPM. The interaction of increase of slope by minute of exercise affected the response of heart rates during the first 6 min of the treadmill exercise ($P < 0.004$; Figure 3.7 A). Nonetheless, the magnitude of the differences in heart rates among treatments during this portion of exercise was relatively small. Furthermore, heart rates of cows in all treatments increased with time during this portion of treadmill exercise, even though workload did not change. The among-cow variance was estimated to be 0, whereas the within-cow variances for each period of the Latin square were 2777, 1589, 2185, and 1332 for period 1 through 4, respectively.

With the increased slope (either 0.8 or 1.6%), heart rates increased from 119 to 149 BPM as time (minute) during exercise increased (Figure 3.7 B, $P < 0.0001$). The standard error after the start of slope increases ranged from 8.7 to 10.7 BPM. The initial interval of time at 0% slope, increases of slope, or the interaction did not affect the magnitude of increase in heart rates ($P > 0.15$). The among-cow variance estimate was

435, whereas the within-cow (residual) variances for each period of the Latin square were 2377, 3016, 4100, and 3802 for period 1 through 4, respectively.

The among- and within-cow variances estimated in this experiment with repeated measures within each Latin square period could be used to evaluate additional experimental designs and statistical analyses as described in Stroup (1999).

Hypothetical data sets with similar temporal relationships and mean differences could be developed to determine number of observations per treatment required in future experiments with designs such as randomized complete or incomplete block, Latin square, or cross-over.

With exercise in the mechanical walker (experiment 2), heart rate did not change with duration of exercise. Similarly, walking for an initial 6 or 12 min at 0% slope on the treadmill did not increase heart rates. During treadmill exercise, increases of 0.8 and 1.6% slope every 3 min increased workloads and heart rates. Additionally, heart rates of exercising calves and heifers increased as the slope of walking surface increased (Arave et al., 1987; Blum and Eichinger, 1988).

For the current experiment, the energy requirements for work were used to determine a power index associated with each workload of treadmill exercise as calculated by the Agricultural Research Council (1980). In the current experiment, heart rates as a function of the power index increased quadratically ($P < 0.001$, Figure 3.8). The regression equation was: $0.0033 \pm 0.0017 \times (\text{power index})^2 + 0.79 \pm 0.14 \times (\text{power index}) + 68.9 \pm 2.9$; $r^2 = 0.70$; $P < 0.001$. As an indicator of exertion, heart rate was related strongly to a given power index during treadmill exercise.

CONCLUSIONS

The daily activities, the duration and speed of exercise, and the slope of walking surface affected the average responses and variation of heart rates. Standing versus lying had the greatest effect on heart rate compared with other activities such as eating, ruminating, and drinking. Commingling cows while standing did not cause an additional increase of average heart rate compared with standing in tie-stalls. The total estimated variance of heart rates of cows during routine activities in a tie-stall management system was 50.4. Walking mature non-lactating, non-pregnant dairy cows at 3.6 or 4.0 km/h for any duration increased the average heart rate and variance compared to resting values. Furthermore, changes in workload by increasing the treadmill slope every 3 min during exercise caused an increase of heart rate and its associated variance. Changes in heart rate during walking activity reflect the amount of exertion during activity or exercise. The relationship of heart rates with an estimated power index was quadratic with a coefficient of variation of 70%. Therefore, we conclude that forced exercise of cows increased heart rates and its associated variance to a greater extent in a mechanical walker or on a treadmill than during other activities.

Table 3.1. Heart rates (beats per minute) of multiparous lactating Holstein cows during daily activities (experiment 1).¹

Activity number	Activity descriptions	Frequency of measurements ²	Heart rate ³	SEM
<u>Individual tie-stalls</u>				
1	Standing (ST)	32	72	1.7
2	Standing and eating (ST and ET)	124	72	1.4
3	Standing and drinking (ST and DR)	26	73	1.9
4	Standing and ruminating (ST and RT)	24	73	1.8
5	Lying (LY)	82	66	1.5
6	Lying and ruminating (LY and RT)	39	66	1.6
7	Lying and sleeping (LY and SL)	6	60	3.6
<u>Commingling⁴</u>				
8	Standing (ST) in cement-paved lot	89	73	1.4
9	Standing (ST) in holding pen of milking	46	69	1.6
10	Standing (ST) during milking	21	73	1.8
Pre-planned orthogonal contrasts ⁵			<u>P-value</u>	
Contrast A:	5, 6, 7 vs. 1, 2, 3, 4, 8, 9, 10 (LY vs. ST)		< 0.001	
Contrast B:	1, 2, 3, 4 vs. 8, 9, 10 (ST in tie-stall vs. ST commingled)		NS	
Contrast C:	1 vs. 2, 3, 4 (ST in tie-stall vs. ST plus other activities)		NS	
Contrast D:	7 vs. 5, 6 (LY and SL vs. LY and LY and RT)		0.09	
Variance estimates ⁶			<u>s²</u>	
Among-cows			14.6	
Within-cows			35.8	
Total			50.4	

¹ Values are least squares means and SEM.

² Equals the number of observations across cows and days of experiment.

³ Effect of activity, $P < 0.0001$.

⁴ Commingling = 42 cows mingled in one group.

⁵ Numerical representation of orthogonal contrasts of numbered activities listed above.

⁶ Compound symmetry was the covariance structure of repeated measures effect of day.

Table 3.2. Summary of treatment factors evaluated in experiments 2 and 3.

Experiment	Description and notation of treatments			
Experiment 2:				
Exercise (mechanical walker)				
Duration of exercise, h	1	1	2	2
Speed of exercise, km/h	3.6	4.0	3.6	4.0
Experiment 3:				
	Treatment abbreviations			
Exercise (treadmill) ¹	<u>6min0.8%</u>	<u>6min1.6%</u>	<u>12min0.8%</u>	<u>12min1.6%</u>
Time at 0% slope, min	6	6	12	12
Increases of slope, %	0.8	1.6	0.8	1.6

¹ The treatments were 6 or 12 min for an initial time interval of exercise at 0% slope followed by increases of slope of 0.8 or 1.6%.

Table 3.3. Heart rates (beats per minute) of cows prior to exercise and during exercise at two speeds for 1 or 2 h in the mechanical walker (experiment 2).¹

Dependent variable	<u>Duration of exercise²</u>		<u>Speed of exercise²</u>		<u>s²</u>			<u>P value³</u>		
	1 h	2 h	3.6 km/h	4.0 km/h	SEM	Among-cows	Within-cows	Total	D	S
	57	62	59	60	2.1	3	212	215	0.10	NS ⁴
Resting in tie-stall	57	62	59	60	2.1	3	212	215	0.10	NS ⁴
Average during exercise	86	93	87	92	3.7	109	122	231	0.005	0.01
Maximum during exercise	114	121	112	123	6.3	4	3489	3493	NS	NS
Minimum during exercise	67	66	68	65	4.0	77	656	733	NS	NS
Increase during exercise above resting in tie-stall	32	32	30	35	3.2	55	53	108	NS	0.05

¹ Values are least squares means.

² Pooled across each other.

³ D = effect of duration of exercise; S = effect of exercise speed. Interaction of S x D was not significant for any dependent variable ($P > 0.15$).

⁴ NS, $P > 0.15$.

Table 3.4. Time to end of treadmill exercise session as affected by treatment (experiment 3).¹

Treatment	min ²
6 min at 0% slope followed by 0.8% slope increases (6min0.8%)	27 ± 1.5
6 min at 0% slope followed by 1.6% slope increases (6min1.6%)	22 ± 1.6
12 min at 0% slope followed by 0.8% slope increases (12min0.8%)	35 ± 1.6
12 min at 0% slope followed by 1.6% slope increases (12min1.6%)	27 ± 1.5
<u>Variance estimates</u>	<u>s²</u>
Among-cows	0.6
Within-cows	17.7
Total	18.3

¹ Values are least squares means and SEM.

² Significant effects of initial interval of time at 0% slope and increase of slope, $P < 0.001$. The interaction of time at 0% slope by increase of slope was not significant ($P > 0.15$).

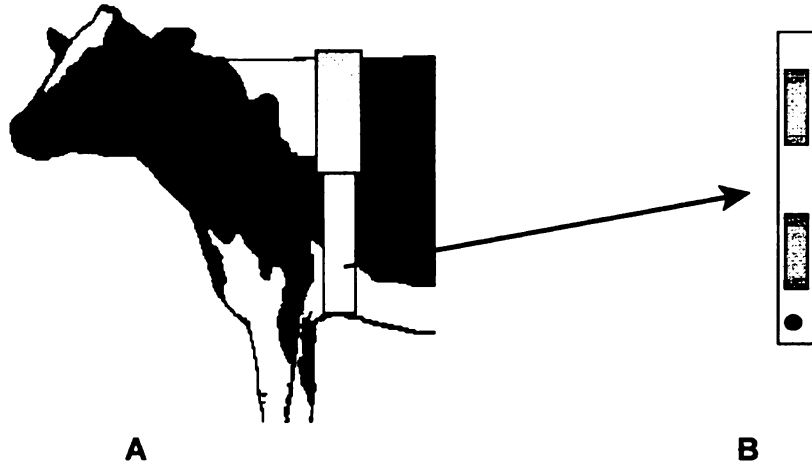


Figure 3.1. Illustration of heart rate monitoring system. *Panel A:* Outer elastic strap (white) and felt saddle girth (gray) and cut away of the location of the heart rate electrode strap on the cow. *Panel B:* Short electrode strap that must maintain skin contact. The gray rectangles represent the electrode contact sites, and the circles represent the sites for connecting to elastic straps.

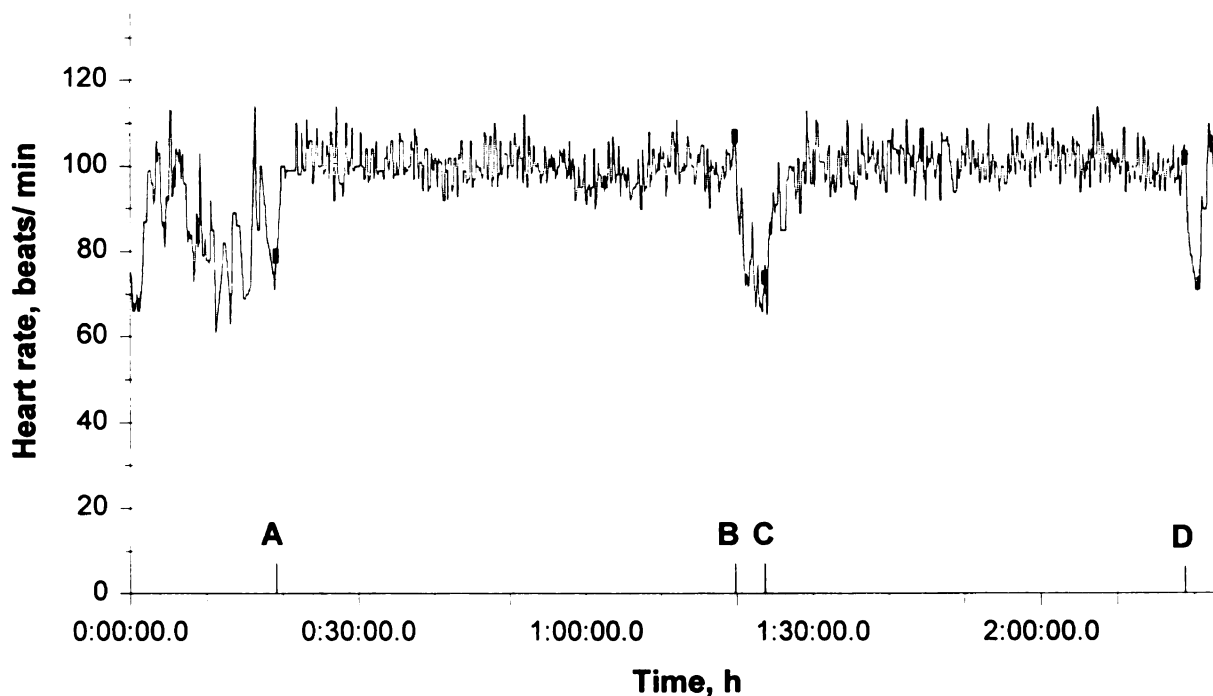


Figure 3.2. Example of representative data from one cow during exercise in the mechanical walker. Letters in the graph represent the following events: beginning of the first hour of exercise, **A**; exercise stopped to load additional cows into mechanical walker, **B**; beginning of second hour of exercise, **C**; and, cessation of exercise, **D**.

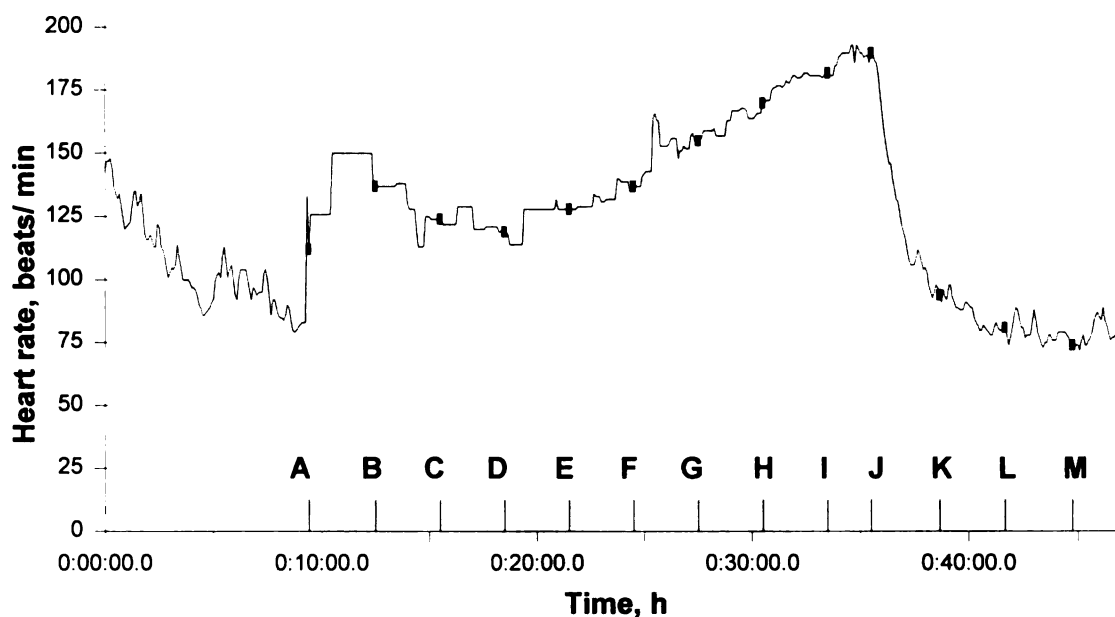


Figure 3.3. Example of representative data from one cow during a treadmill exercise session. The letters on the graph indicate the beginning of the exercise, **A**; each increase of slope every 3 min, **B** to **I**; the end of the exercise at which peak heart rate was recorded, **J**; and, post-exercise, **K** to **M**.

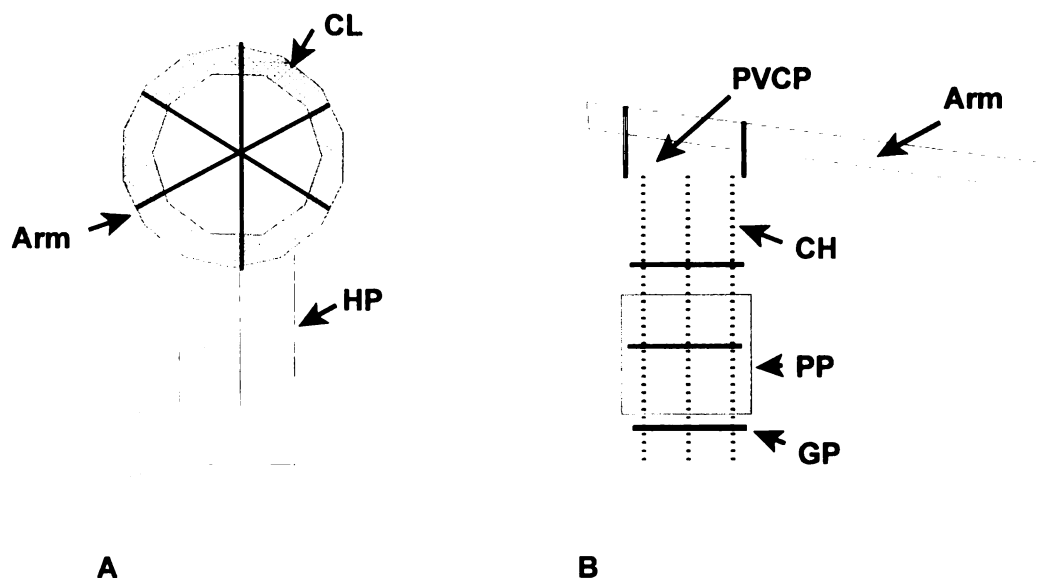


Figure 3.4. Illustration of circular mechanical walker. *Panel A:* Overhead view of holding pen (HP) and circular lane (CL, 10.8-m outside diameter, 33.8-m circumference, 90-cm width of lane) in gray with arms of walker extending from the center point. *Panel B:* View of flexible partition hanging from the walker arm to control spacing and forward motion of cows. Light gray section represents polyvinylchloride pipe (PVCP) with chain lengths (CH, black dotted lines) attached. Dark gray section represents plywood panel (PP) with galvanized metal pipe (GP, solid dark horizontal lines) attached to the chains.

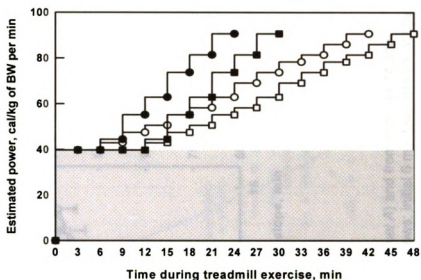


Figure 3.5. Estimated power of each treatment for a cow at each workload during treadmill exercise at 5.0 km/h. Gray area (about 40 cal/kg of BW per min) represents the power of each treatment associated with horizontal forward movement (workloads at 0% slope). Treatments are as follows: initial 6 min time interval at 0% slope followed by 0.8% increases of slope (○); initial 6 min time interval at 0% slope followed by 1.6% increases of slope (●); initial 12 min time interval at 0% slope followed by 0.8% increases of slope (□); and, initial 12 min time interval at 0% slope followed by 1.6% increases of slope (■). Increases of slope occurred every 3 min.

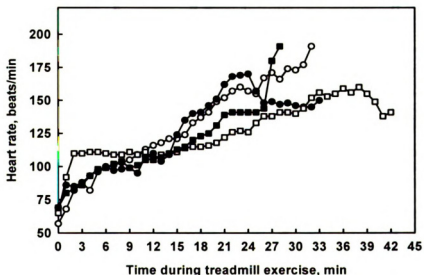


Figure 3.6. Heart rates of cows during all minutes of treadmill exercise. Treatments are as follows: initial 6 min time interval at 0% slope followed by 0.8% increases of slope (○); initial 6 min time interval at 0% slope followed by 1.6% increases of slope (●); initial 12 min time interval at 0% slope followed by 0.8% increases of slope (□); and, initial 12 min time interval at 0% slope followed by 1.6% increases of slope (■). The frequency of observations per minute of exercise was three or less for min 26 to 33 of 6min0.8%, min 23 to 34 of 6min1.6%, min 38 to 43 of 12min0.8%, and min 28 to 29 of 12min1.6%.

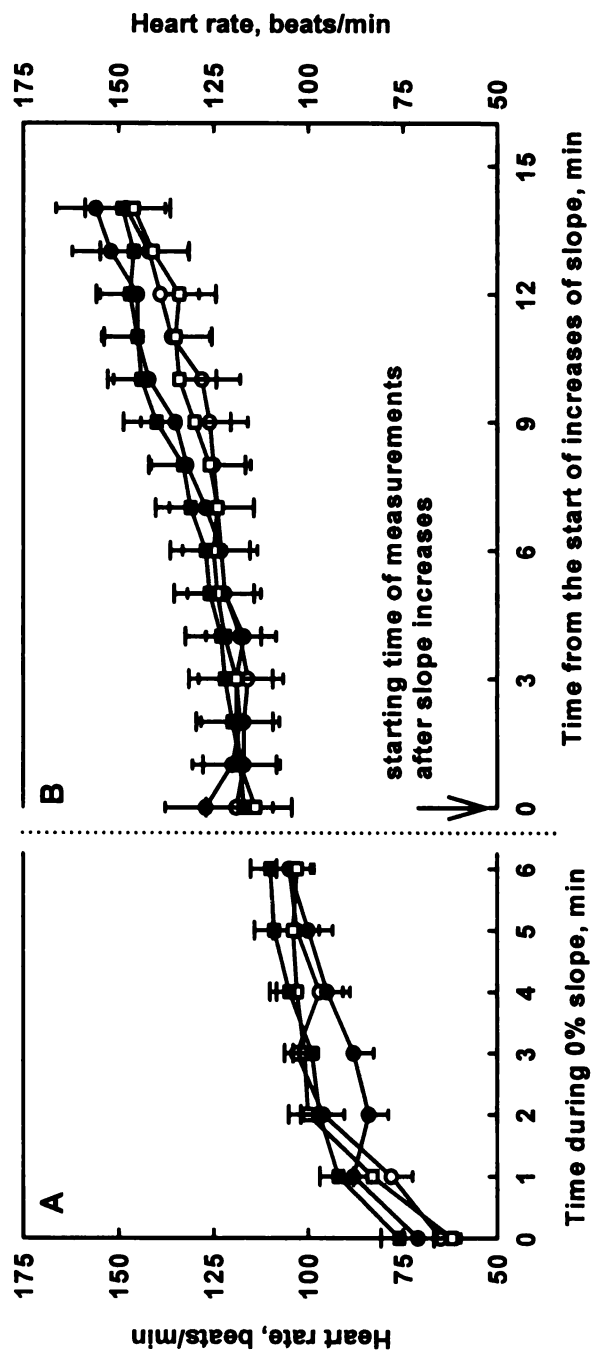


Figure 3.7. Heart rates of cows during the initial 0 to 6 min of treadmill exercise at 0% slope (*Panel A*) and from min 0 or 14 after the start of increases of slope (*Panel B*) (least squares means and SEM). Treatments are as follows: initial 6 min time interval at 0% slope followed by 0.8% increases of slope (□); initial 6 min time interval at 0% slope followed by 1.6% increases of slope (●); initial 12 min time interval at 0% slope followed by 0.8% increases of slope (○); and, initial 12 min time interval at 0% slope followed by 1.6% increases of slope (■). *Panel A:* Minute during exercise and interaction of treatment of slope increases by minute affected heart rate ($P < 0.01$). *Panel B:* Minute of exercise affected the magnitude of increase in heart rate ($P < 0.001$).

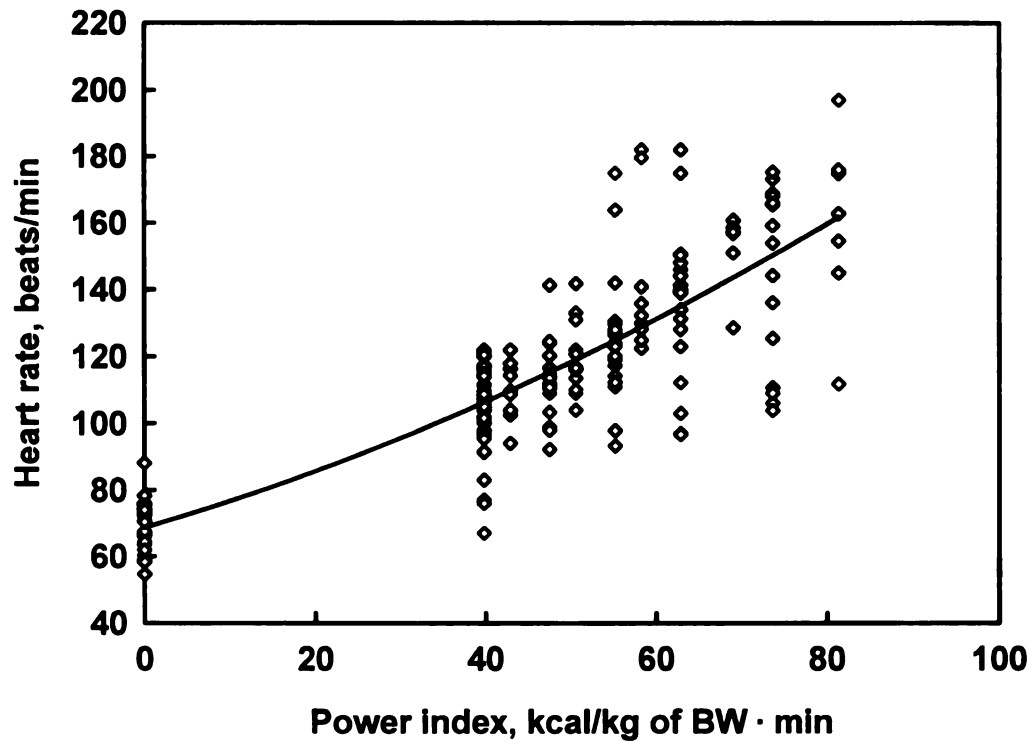


Figure 3.8. Heart rate during treadmill exercise versus power index for individual cow observations (◇). The line represents the quadratic response of heart rates to the power indices during treadmill exercise. The equation of the regression line was $0.0033 \pm 0.0017 \times (\text{power index})^2 + 0.79 \pm 0.14 \times (\text{power index}) + 68.9 \pm 2.9$; $r^2 = 0.70$; $P < 0.001$.

CHAPTER 4

A SYSTEM TO ASSESS FITNESS OF DAIRY COWS DURING EXERCISE TRAINING

ABSTRACT

Objectives of this study were to develop a system to assess physical fitness during a treadmill test and to affect potential physiological indicators of fitness of dairy cows. Nineteen non-lactating, non-pregnant multiparous Holstein dairy cows were blocked by parity and BCS and assigned randomly to one of three exercise training treatments: No Exercise; 1-h Exercise; or, 2-h Exercise. Cows in 1-h and 2-h Exercise walked at 3 km/h every other day for 60 d in a mechanical walker. Physical fitness was evaluated during treadmill exercise tests on d 15, 30, 45, and 60 of the experiment. Treadmill tests consisted of walking (5 km/h) with 1.6% increases in slope of the walking surface every 3 min until cows refused to walk or heart rates reached 180 beats per minute. Time to end of the treadmill test was greater for cows in exercise training treatments compared with cows that did not exercise. After 60 d of exercise training treatment for either 1 or 2 h, cows had reduced heart rates and lower plasma L-lactate concentrations at given workloads of the treadmill test compared with non-exercised cows. Exercise training did not affect blood acid-base status or mineral elements concentrations during treadmill tests. Using this system to characterize heart rates and plasma lactate changes, fitness of dairy cows can be quantified comparing exercise training treatments or different management approaches.

INTRODUCTION

Management systems that limit exercise may reduce physical fitness of dairy cows. Physical fitness may be important to the cows' ability to respond to physiological and metabolic demands associated with the periparturient period. Characterizing and identifying useful indicators of physical fitness is the first step in elucidating the possible benefits of exercise training. Physical fitness is defined as "the capacity to successfully meet the present and potential physical challenges of life" (Lamb, 1984). Thus, physiological fitness can be defined as the capacity of all functions of the body to complete a task, workload, or challenge of life. Fitness may be evaluated by examining the ability of an animal to maintain homeostasis or steady-state during defined work performed during a given period (workload). After repeated exercise in a defined regimen (exercise training), physical and physiological fitness may be improved. In horses exercised for 5 wk, plasma venous lactate concentrations were less during an exercise test compared with values before training (Rodiek et al., 1987). Physical fitness assessments of dairy cows with an exercise test have not been described after extended confinement or exercise training.

Objectives of this study were to develop a system to assess physical fitness of dairy cows during a treadmill test and to affect and assess physiological indicators of fitness after exercise training. The hypothesis was that exercise training improves physical fitness of dairy cows. Potential physiological indicators of fitness are reduced heart rate and plasma lactate concentrations and maintenance of acid-base status during given workloads of a treadmill test. Estimation of cow (among-cow) and residual (within-cow) variances of these indicators will be useful to design future experiments.

MATERIALS AND METHODS

Animal care and procedures were approved and conducted under standards of the Michigan State University All-University Committee on Animal Use and Care, application number 05/98-081-00. Guidelines were in compliance with National Institutes of Health and the Guide for the Care and Use of Laboratory Animals.

Cows and Exercise training Treatments

The exercise system consisted of a mechanical walker to facilitate exercise training of cows and a treadmill to assess physical fitness and physiological responses. Nineteen non-lactating, non-pregnant multiparous Holstein dairy cows were blocked by parity and BCS and assigned randomly to exercise training treatments: control (**No Exercise**); 1 h exercise at 3 km/h (**1-h Exercise**); or, 2-h exercise at 3 km/h (**2-h Exercise**). Non-lactating, non-pregnant cows were utilized to develop and characterize the system to avoid any influences of gestation and(or) lactation on physiological responses. Exercise training was completed every other day for 60 d in the mechanical walker previously described (Chapter 3).

Cows had been dried-off at least 3 wk before the start of the experiment. They also were trained to use the mechanical walker and treadmill with 2 to 3 short duration (5 min) practice sessions, 2 to 3 wk before experimentation began. All cows walked approximately 36.5 m from the tie-stall alley to the holding pens (8.5 m by 3 m) adjacent to the mechanical walker. Cows assigned to No Exercise were placed in holding pens adjacent to the walker, while cows in the exercise treatments walked. After cows in the 1-h Exercise treatment completed their exercise, they were moved into the holding pens while cows in the 2-h Exercise treatment walked for an additional hour. This ensured

that all cows were exposed to the same environmental conditions and were denied access to feed and water for the same length of time.

Except when receiving treatments, all cows were kept in tie-stalls in a ventilated pole barn with full side walls and fed ad libitum once daily a TMR containing 62% alfalfa haylage, 15% corn silage, 18% spelt hull, 3.8% soybean meal, plus mineral and vitamins (1.42 Mcal of NE_L/kg, 15.6% CP, 38.5% ADF, and 51.7% NDF, dry basis). Daily DMI and water intake of individual cows were recorded. Body weights and BCS (Wildman et al., 1982) were measured every 15 d. On d 0 and 60 of the experiment, backfat thickness above the 12th and 13th ribs was determined with ultrasound using a linear scan 7.5-MHz probe (Aloka 500 Ultrasound, Corometrics Medical Systems, Inc., Wallingford, CT) (VandeHaar et al., 1999).

Treadmill Tests

As a bioassay to assess physical fitness, treadmill tests were performed by all cows on d 15, 30, 45, and 60 of the experiment. The test consisted of walking (5 km/h) on a treadmill (Classic Treadmill Conditioner, W. H. D. Lindsey, Kenilworth, Australia). Slope of the treadmill walking surface was increased 1.6% every 3 min to increase workloads. Heart rates were recorded every 5 sec with a wireless electrocardiogram monitor (Polar Advantage, Polar Electro, Port Washington, NY) as previously described (Chapter 3). Heart rates were transmitted to a computer, monitored in real time, and recorded (Polar Precision software, Polar Electro, Port Washington, NY). The test continued until cows refused to walk or until heart rates were greater than 180 BPM for two consecutive 3-min intervals. The heart rate of a cow that refused to walk may not have reached 180 BPM. If heart rates reached at least 165 BPM, a treadmill test was declared a maximal workload challenge. The time to end of the exercise portion of each test was recorded. Actual elapsed times ranged from 9 to 30 min depending on cow

and day of experiment. The treadmill was stopped and the walking platform was lowered to 0% slope for an additional 12-min period (the post-exercise portion of the test) during which time experimental measurements continued.

An indwelling catheter was inserted into the jugular vein 1 d prior to the treadmill test. Blood (10-ml syringe) was sampled during the final min of each 3-min interval of the exercise and post-exercise portions of the test. Blood was transferred to evacuated glass test tubes containing Na-heparin or sodium fluoride/potassium oxalate (**NaF-KOxalate**) (Vacutainer; Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ). Blood with Na-heparin was analyzed for pH, partial pressure of carbon dioxide (**pCO₂**), hematocrit (**HCT**), and concentrations of potassium (**K⁺**), ionized calcium (**iCa²⁺**), and chloride (**Cl⁻**) (Stat 5 Profiler, Nova Biochemical, Princeton, NJ). From these measurements, the concentrations of bicarbonate (**HCO₃⁻**), total carbon dioxide (**TCO₂**), hemoglobin (**HGB**), base excess of extracellular fluid (**BE-ECF**), and base excess of blood (**BE-B**) were computed (Stat 5 Profiler, Nova Biochemical, Princeton, NJ).

Blood with NaF-KOxalate was centrifuged within 15 min of collection and plasma was harvested. Plasma was stored at -20°C until analysis for L-lactate concentrations using a lactate oxidase kit modified for use in a 96-well plate reader (Sigma Diagnostics, St. Louis, MO). Intra- and inter-assay coefficients of variation of the assay were 3.9% and 4.0%, respectively.

Statistical Analysis

Data were analyzed by methods of ANOVA using mixed model procedures of SAS (1999). The appropriate covariance structure for the repeated measures was determined using the goodness of fit criteria of Aiaike's Information Criterion and Schwarz's Bayesian Criterion. Unless otherwise described, results are presented as generalized least squares means with standard error of mean. The among- and within-

animal variance components were estimated using residual maximum likelihood methods of mixed models. Statistical significance was declared at $P < 0.05$; trends were noted at $0.05 < P < 0.15$.

Variation of plasma L-lactate concentrations was not homogeneous across time within day of experiment during all portions of the treadmill test. Therefore, data were logarithmic transformed prior to statistical analysis. The back-transformed least squares means are presented. The 67% confidence intervals of back-transformed least squares means were calculated and plotted.

The time to end of the treadmill test was analyzed with mixed models. The mixed model included random effect of cow, repeated measures effect of day of test and fixed effects of treatment and two-way interaction of day by treatment. The following orthogonal contrasts were performed: No Exercise vs. 1-h Exercise and 2-h Exercise, and 1-h Exercise vs. 2-h Exercise. Chi-square analysis was performed to determine if treatment affected the probability of completing maximal workloads of the treadmill exercise test.

During the exercise portion of the test, the frequency of observations per cow for heart rate, blood, and plasma variables was different among tests due to the differences in time to end tests. The frequency of observations per minute of tests was eight or less at min 22 to 25 of No Exercise; at min 22 to 27 of 1-h Exercise; and, at min 22 to 30 of 2-h Exercise (Figure 4.1). Because of the reduced frequency of observations beyond 20 min, only the data from min 0 to 20 of the exercise portion of the test were included in the statistical analyses. The measurement at the final minute of the exercise portion of each test was included as min 0 measurements of the post-exercise portion of the test for each dependent variable.

Heart rates, blood, and plasma variables from min 0 to 20 during the exercise portion test and from min 0 to 12 min during post-exercise portion were analyzed

separately. Heart rate data for every 5 sec were used to calculate the average for each minute during the exercise portion of the test. Because of the rapid decline of heart rates once exercise ceased, 5-sec heart rate data were averaged for every 15 sec for the post-exercise portion. To account for missing heart rate measurements during a given minute or 15-sec interval, the average heart rate was weighted by the number of observed heart rates within a time interval in the mixed model analyses. The mixed model included the random effect of cow nested within treatment, repeated measures of day of experiment and minute of test, and the fixed effect of treatment. All two- and three-way interactions of treatment, day of experiment, and minute of test were included in the model. The covariance structures were unstructured for day of experiment and auto-regressive first order for minute of test.

All other data (DMI, water intake, BW, BCS, and backfat thickness) were analyzed with a mixed model including the random effect of cow nested within treatment, the repeated measures of day of experiment, and fixed effects of treatment and two-way interaction of treatment by day of experiment. The covariance structure was auto-regressive first order for day of experiment. Changes in BW, BCS, and backfat thickness from d 0 to 60 were analyzed with the mixed model including the random effect of cow nested within treatment and the fixed effect of treatment. Orthogonal contrasts were as follows: No Exercise vs. 1-h Exercise and 2-h Exercise, and 1-h Exercise vs. 2-h Exercise.

RESULTS AND DISCUSSION

Effect of Exercise training on Physical Fitness and Acid-Base Status

Time to end of treadmill tests. Exercise treatment did not affect successfully completing maximal workloads of treadmill tests (heart rates greater than 165 BPM)

(Chi-square analysis, $P > 0.15$). Exercise treatment tended to affect the time to end of treadmill tests ($P = 0.11$). Pooled across day of experiment, time to end of test was greater for cows that exercised compared with cows that did not exercise (Table 4.1; $P < 0.05$). The interaction of treatment by day of experiment did not affect the length of time of treadmill tests ($P > 0.15$). Sheep trained at greater workloads than the cows of the current study also had a 40% increase in the length of time of exercise test compared with values prior to training (Mundie et al., 1991). The ability to continue the treadmill test for greater lengths of time was one indicator that physical fitness was improved for dairy cows after exercise training.

The estimates of among- and within-cow variances of time to the end of test were 0.23 and 14.95, respectively. Using a total estimated variance of 15.13 and power of 90% for a two-tailed Type I error of 5% (t-statistic), a minimum of 14 observations per treatment would be required in future experiments to measure a potential mean difference of 5 min.

Heart rates during the exercise portion of treadmill tests. Cardiac output is a function of stroke volume and frequency of strokes. During exercise as heart rates increase, cardiac output increases. The capacity of a subject to maintain exercise is dependent on the heart rate and increased cardiac output.

Regardless of treatment or day of experiment, heart rates during the exercise portion of the treadmill test increased as minute of test progressed ($P < 0.0001$). During the exercise portion of the test, the magnitude of increase in heart rates as a function of minute of test and day of experiment was different among exercise training treatments ($P < 0.05$; Figure 4.2). Pooled among cows that exercised (1 and 2 h), the magnitude of increase of heart rates during the treadmill tests was less on d 45 and 60 of the experiment compared with cows that did not exercise ($P < 0.05$; Figure 4.3). The among-cow variance was 175, and the within-cow variances for each day of the

experiment were 3067, 3882, 4198, and 3063 for d 15, 30, 45, and 60, respectively. At similar workloads of the treadmill test, cows that exercised for 60 d for 1 or 2 h had lower heart rates compared with cows that did not exercise.

Horses that trained for 10 wk had reduced heart rates by the third stage of a 5-stage treadmill test compared with untrained horses (Thomas et al., 1983). In horses that had chronic exercise for 10 wk, heart beat frequency was less during progressive stages of an exercise test compared with values prior to chronic exercise (Rodiek et al., 1987). After chronic exercise, the heart rates of calves at similar speeds of exercise were lower compared with pre-training values (Fosha-Dolezal and Fedde, 1988). Similarly in the current study, heart rates were reduced during the treadmill test of cows that underwent 60 d of exercise training. The reduction of heart rates during exercise indicated that these cows were more physically fit. They were able to meet the workload challenge with less effort from the heart. Previously, over a wide range of workloads, heart rates of dairy cows were related quadratically to the estimated power or exertion required to complete exercise (Chapter 3). With the treadmill test in the current study, the physical fitness of cows was assessed by the change in magnitude of increase of heart rates. Exercise training resulted in lower increases of heart rates during treadmill tests at d 45 and 60.

Heart rates during post-exercise portions of treadmill tests. Regardless of exercise training treatment, heart rates declined rapidly during the post-exercise portion of the treadmill test ($P < 0.0001$; Figures 4.2 and 4.3). Interactions of treatment by day of experiment and treatment by minute of test affected the decline of heart rates ($P < 0.05$; Figure 4.2). Pooled across exercise treatment (1-h Exercise and 2-h Exercise), the interaction of treatment by day of experiment also affected heart rates ($P < 0.05$; Figure 4.3).

The dramatic decline in heart rates of cows within 3 min post-exercise was similar among treatments and similar to the decrease measured in horses (Ehrlein et al., 1973). The time required for heart rates to return to resting values can be affected by the workloads and duration of work, environmental temperature and humidity, and working capacity of the horse (Fregin and Thomas, 1983). In the current system with dairy cows, the pattern of decline in heart rate post-exercise was not greatly different among treatments. Heart rates returned within 6 min after cessation of exercise to values similar to those at the beginning of the exercise portion of the test. The decline in heart rates post-exercise was very rapid with small variability. Therefore, as part of this system, they are not a definitive assessment of physical fitness.

Plasma lactate concentrations during treadmill tests. During prolonged exercise, anaerobic metabolism of glucose by muscle tissue can cause an increase in blood L-lactate concentrations. An increase in anaerobic glycolysis (reduced oxidative capacity) during exercise was reflected by greater plasma lactate concentrations as work effort increased (Perrson, 1983). Therefore, reduced plasma lactate concentrations during exercise may be one indicator of the greater oxidative capacity of muscle. Physical fitness could be assessed by the maintenance of oxidative metabolism as indicated by plasma lactate concentrations during exercise.

During the exercise portion of the treadmill test, plasma L-lactate concentrations increased as minute increased on d 15, 30, 45 and 60 of the experiment ($P < 0.0001$; Figures 4.4 and 4.5). The interactions of treatment by minute of test and day of experiment by minute of test affected the plasma L-lactate concentrations ($P < 0.05$; Figure 4.4). Pooled across exercise training treatments (1 h and 2 h), treatment and interaction of treatment by minute of test affected plasma lactate concentrations ($P < 0.05$; Figure 4.5). The pattern of increase of L-lactate concentrations of cows that exercised (1 h vs. 2 h) was similar across minutes of the tests on d 30, 45 and 60

(Figure 4.4). However, as day of experiment progressed to d 60, the L-lactate concentrations of cows on No Exercise increased more within a test than for cows that exercised. By d 60, cows that exercised had reduced plasma lactate concentrations during the treadmill test compared with non-exercised cows. The increases of plasma lactate concentrations during exercise were clearly less for cows that exercised compared with cows that did not exercise (Figure 4.5). Similarly, in horses that were trained for 5 wk, plasma venous lactate concentrations were less during an exercise test compared with values prior to training (Rodiek et al., 1987).

During the post-exercise portion of the treadmill tests, plasma L-lactate concentrations declined as minute of test increased ($P < 0.0001$; Figures 4.4 and 4.5). The day of experiment by minute of test interaction affected the pattern of decline in L-lactate concentrations ($P < 0.05$), and tended to be influenced by the interaction of treatment by minute of test ($P = 0.06$). When exercise treatments were pooled (1 and 2 h of exercise), the day of experiment and minute of test tended to alter plasma lactate concentrations ($P = 0.11$; Figure 4.5). By d 60, cows that exercised (1 and 2 h) had lower plasma lactate concentrations than cows that did not exercise (Figure 4.5). Lower plasma lactate concentrations during the treadmill test indicated that cows in the exercise training treatments likely were experiencing less anaerobic glycolysis.

For the natural logarithmic transformed plasma lactate concentrations, the among-cow variance was 0.0086 and the within-cow variances for each test of the experiment were 0.0358, 0.0252, 0.0187, and 0.0271 for d 15, 30, 45, and 60, respectively. Based on a total estimated variance of 0.0444 and power of 90% for a two-tailed Type I error of 5% (t-statistic), a minimum of 16 observations per treatment would be required to measure a potential mean difference of 0.25 mmol/L (natural logarithm value). In the current system, the accumulation of lactate in plasma (or the

lack of accumulation) was another indicator that can be used to assess physical fitness of dairy cows.

Acid-base status during treadmill tests. Exercise training treatment did not affect acid-base status during the treadmill test. During the exercise portion of the treadmill test, changes of blood pH were very small and still within the normal physiological range for dairy cows ($P < 0.05$; Figure 4.6). Post-exercise, blood pH declined slightly within the 3 min and was slightly less than at min 0 of exercise portions of tests (Figure 4.6). For all treatments during exercise portions of treadmill tests, concentrations of TCO_2 and HCO_3^- , and pCO_2 of blood increased slightly at min 3 and then declined as minute of test progressed. During post-exercise, TCO_2 , HCO_3^- , and pCO_2 concentrations were less than measured at min 0 of exercise portion of the tests (Figure 4.6).

The decline in venous plasma pH, pCO_2 , and HCO_3^- concentrations with acute exercise also was described for horses during an exercise test (Taylor et al., 1995). Additionally, Judson et al. (1983) described a decline in plasma HCO_3^- concentrations after submaximal and maximal exercise of horses with concentrations returning to pre-exercise values within 15 min post-exercise. For the cows in our system, sampling for a greater duration of time post-exercise apparently was required for acid-base measurements to return to pre-exercise test values.

Responses of venous pCO_2 during exercise were different among studies. For mature steers (Jones et al., 1989) and calves (Kuhlmann et al., 1985), venous pCO_2 increased as speeds of work increased (increased workloads). In both of these studies, the duration of exercise at various speeds was 5 to 6 min. Similar to the current study, blood pCO_2 of sheep decreased after exercising for 25 min compared with resting values (Pethick et al., 1991). The difference in response of pCO_2 between these studies and the current study likely was due solely to the differences of duration of exercise.

In the current study, the relatively small blood acid-base measurement changes may reflect the net effects of the simultaneous occurrences of metabolic acidosis and respiratory alkalosis to sustain physiological pH and acid-base homeostasis.

Respiratory rates were not measured in the current study. However, Arave et al. (1987) reported an increase above basal of 40 to 70 breaths per minute of 2-yr old Holstein heifers after 30 min of exercise at 1.5 km/h up a 9% slope. Crossbred beef heifers and steers working at 2.88 km/h had increased respiration frequency over resting values (approximately 25 to 60 breaths/min; Kuhlmann et al., 1985; Piguet et al., 1994). During exercise in the current study, the increased plasma lactate concentrations may indicate metabolic acidosis, whereas the changes of $p\text{CO}_2$ may reflect respiratory alkalosis.

Calculations of BE-ECF and BE-B attempt to separate the metabolic and respiratory influences on acid-base status (Fencl and Leith, 1993). Base excess of extracellular fluid and BE-B were calculated as the amount of base needed to adjust blood pH to 7.4 at a standardized $p\text{CO}_2$ of 40 mm Hg. These calculations are a function of HCO_3^- concentrations which were determined from the $p\text{CO}_2$ and pH of the blood. As would be expected, minute of exercise and post-exercise portions of treadmill tests affected BE-ECF and BE-B in a similar manner as $p\text{CO}_2$, and TCO_2 , HCO_3^- concentrations (Figure 4.7). During exercise, concentrations of BE-ECF and BE-B increased initially within 3 min of test and then declined as time progressed. During post-exercise, these concentrations were less than initial concentrations at min 0 (beginning) of exercise test.

These calculated concentrations of base excess are questionable indicators of acid-base status when $p\text{CO}_2$ varies greatly from 40 mm Hg, as is the case in the current study (Fencl and Leith, 1993). Therefore, it is unlikely that the effects of metabolic acidosis and respiratory alkalosis using these calculations can be separated in the current study without additional assessments of the respiratory system.

In our system, changes of acid-base status (pH, pCO₂, HCO₃⁻, TCO₂, BE-ECF, and BE-B) were not considered useful indicators of physical fitness because of lack of differences among treatment groups. Additionally, the inability to separate simultaneous changes in metabolic and respiratory influences on acid-base status pose a difficulty to assessing changes in fitness.

Hematocrit and hemoglobin during treadmill tests. Hematocrit and HGB contents are potential indicators of the oxygen-carrying capacity of the blood. Exercise training treatments did not affect HCT and HGB concentrations. Hematocrit and HGB contents of blood increased slightly as time during treadmill exercise increased ($P < 0.05$, Figure 4.8). During post-exercise, HCT and HGB declined as time increased. During the post-exercise portion of the test, HCT returned to values prior to exercise.

In calves, HCT was greater as speeds of exercise increased (Kuhlmann et al., 1985). Following exercise of dairy cows and steers, packed cell volumes were greater than resting values (Blake et al., 1982; Blum et al., 1979). The changes of HCT and HGB contents and mineral element concentrations (reported subsequently) during exercise may be due to the movement of water from plasma to the extracellular fluid or intracellular or interstitial fluid compartments of muscle.

Mineral element concentrations during treadmill tests. Minute of exercise and post-exercise portions of treadmill tests affected K⁺, Cl⁻, and iCa²⁺ concentrations ($P < 0.05$, Figure 4.9), whereas exercise training treatment did not. Estimated among- and within-cow variances are reported in Table 4.2 which can be utilized to develop future experiments.

Concentrations of blood K⁺ increased about 25% as time during exercise progressed and then declined to initial concentrations during the post-exercise phase of the treadmill test. Similarly, serum concentrations of K⁺ increased as exercise speed increased in calves (Fosha-Dolezal and Fedde, 1988; Kuhlmann et al., 1985). In

horses, plasma K^+ concentrations increased after maximal exercise compared with resting values (Judson et al., 1983; Taylor et al., 1995). Potassium is released from contracting cells during exercise. Previously, Fosha-Dolezal and Fedde (1988) demonstrated that training of calves reduced the magnitude of increases of serum K^+ concentrations during exercise at a given speed compared with pre-training values. In dogs, training or conditioning increases the intracellular concentration of K^+ and Na^+-K^+ -ATPase activity which may be involved with reduced concentrations of circulating K^+ during exercise (Knochel et al., 1985). However, in the current study, the main effect of exercise training treatments did not alter the blood K^+ concentration during the treadmill test.

Changes of Cl^- and iCa^{2+} concentrations were small during the entire treadmill test. During post-exercise, concentrations of iCa^{2+} returned to initial values prior to exercise. However, during the post-exercise portion of the test, Cl^- concentrations increased slightly. The relatively small changes of iCa^{2+} and Cl^- concentrations are potentially reflective of changes in plasma volume with hemo-concentration due to the movement of water from plasma to extracellular, intracellular, or interstitial fluid compartments of muscle.

Blood venous acid-base status and concentrations of gases and mineral elements were not affected by exercise training treatment on any day of the experiment during either portion of the treadmill test. Blood venous acid-base status, blood gases, and associated variables did not return to initial values within 12 min post-exercise indicating sampling for a longer time post-exercise should be done in future experiments. In our system, changes of acid-base status and concentrations of gases and mineral elements in blood were not considered reliable indicators of physical fitness.

Effect of Exercise training on Water and Dry Matter Intake

Exercise training treatments or the interaction of treatment by day of experiment did not affect daily water and DM intakes (Table 4.3). In contrast, crossbred lactating dairy cows that were exercised had greater water intakes compared with non-exercised cows (Benedetti et al., 1990). In that study, exercise treatment did not affect feed consumption and milk yields. In other studies, exercise training did not affect DMI. Previous research with exercising cows used diets of greater forage content (Matthewman et al., 1993), or a TMR was not fed (Anderson et al., 1979; Lamb et al., 1981; Lamb et al., 1979). The diets in all of these studies were mostly roughage which had the potential to limit intakes due to increased physical fill or access to feed may have been limited. The current study along with previous research demonstrated that exercise did not greatly stimulate DM consumption when the diets were mostly forage. It is not known if DMI will increase with greater workloads and energy requirements, or if a diet of greater energy density is fed.

Initial BW and BCS of cows were not different among treatments (Table 4.4). Average initial BW and BCS pooled across all treatments were 752 kg and 3.6, respectively. Treatment or the interaction of treatment by day of experiment did not alter BW, BCS, and backfat thickness ($P > 0.15$). Body weight increased from d 0 to 60 of experiment, regardless of treatment. Cows that exercised (1 or 2 h) gained less BW than cows that did not exercise ($P < 0.03$). Treatment did not affect the change of BCS or the change of backfat thickness from d 0 to 60 (Table 4.4).

CONCLUSIONS

A system was developed for dairy cows to assess physical fitness during a treadmill test and to affect potential physiological indicators of fitness with a mechanical

walker. Cows assigned to exercise training for 1 or 2 h every other day for 60 d were more physically fit than cows that did not exercise as indicated by increased length of time of treadmill tests, reduced heart rates, and lower plasma lactate concentrations at given workloads of exercise during the treadmill test. The reductions of heart rate and plasma lactate concentrations were apparent after 45 d of exercise training with the greatest magnitude of difference on d 60 between exercise and non-exercised cows. However, acid-base status variables and mineral element concentrations were not affected by improved physical fitness in our study. The among- and within-cow variances estimated for measurements made in this study are a basis for the design of future experiments. Changes of heart rates and plasma lactate concentrations during a treadmill test quantified the physical fitness of dairy cows in order to compare potential responses of exercise training treatments or different management approaches.

Table 4.1. Time to end of treadmill tests.¹

	Exercise training treatments				<i>P</i> -value ²	
	No Exercise	1-h Exercise	2-h Exercise	SEM	No Exercise vs. 1-h and 2-h Exercise	1-h vs. 2-h Exercise
Time to end of test, min	18.9	20.4	21.5	0.83	0.05	NS ³

¹ Values are least squares means.² Orthogonal contrasts.³ NS, *P* > 0.15.**Table 4.2.** Estimated among and within-variances for blood acid-base variables and mineral element concentrations during the exercise portion of the treadmill test.¹

Dependent variables ²	Among-cow variance	Within-cow variances for each test			
		d 15	d 30	d 45	d 60
pH	9.7 x 10 ⁻⁵	3.2 x 10 ⁻⁴	2.8 x 10 ⁻⁴	2.4 x 10 ⁻⁴	2.9 x 10 ⁻⁴
pCO ₂ , (mm Hg) ²	2.4	12.1	9.6	6.3	9.7
HCO ₃ ⁻ , (mmol/L) ²	0.48	5.1	5.7	3.5	5.6
TCO ₂ , (mmol/L) ²	0.55	5.6	6.1	3.7	6.0
BE-ECF, (mmol/L) ²	0.37	5.2	6.4	4.0	5.9
BE-B, (mmol/L) ²	0.22	3.9	5.0	3.1	4.4
HCT, (%) ²	0.61	4.5	2.7	3.7	3.5
HGB, (g/dl) ²	0.07	0.48	0.30	0.28	0.23
K ⁺ , (mmol/L) ²	0.03	0.45	0.06	0.04	0.06
Cl ⁻ , (mmol/L) ²	1.7	4.1	2.0	2.4	2.3
iCa ²⁺ , (mmol/L) ²	4.6 x 10 ⁻⁴	1.7 x 10 ⁻³	1.2 x 10 ⁻³	2.0 x 10 ⁻³	1.4 x 10 ⁻³

¹ Covariance structures of repeated measure effects of day of experiment and minute during exercise portions of tests were unstructured and auto-regressive first order.² pCO₂ = partial pressure of carbon dioxide, HCO₃⁻ = bicarbonate ion, TCO₂ = total carbon dioxide, BE-ECF = base excess of extracellular fluid, BE-B = base excess of blood, HCT = hematocrit, HGB = hemoglobin concentration, K⁺ = potassium ion, Cl⁻ = chloride ion, iCa²⁺ = ionized calcium ion.

Table 4.3. Water and DM intakes during the experiment.¹

	Exercise training treatments			SEM	P-value ²	
	No Exercise	1-h Exercise	2-h Exercise		Trt	Trt x D
Water intakes, L/d	38	41	37	4.0	NS ³	NS
DMI, kg/d	13	15	13	0.8	NS	NS
DMI, kg/100 kg BW	1.7	1.9	1.8	0.11	NS	NS

¹ Values are least squares means.

² Trt = main effect of exercise treatment; D = day of experiment; and, Trt x D = interaction of exercise treatment by day of experiment.

³ NS, $P > 0.15$

Table 4.4. Initial, final, and change in BW, body condition score (BCS), and backfat thickness during experiment.¹

	Exercise training treatments				P-value ²	
	No Exercise	1-h Exercise	2-h Exercise	SEM	No Exercise vs. 1-h and 2-h Exercise	1-h Exercise vs. 2-h Exercise
BW, kg						
Initial	737	777	740	22	NS ³	NS
Final	789	808	753	22	NS	0.14
Change	52	32	13	11	0.03	NS
BCS ⁴						
Initial	3.5	3.6	3.6	0.15	NS	NS
Final	3.6	3.5	3.4	0.15	NS	NS
Change	0.06	-0.13	-0.19	0.10	0.10	NS
Backfat thickness, cm						
Initial	0.35	0.41	0.38	0.06	NS	NS
Final	0.38	0.36	0.37	0.06	NS	NS
Change	0.03	-0.05	-0.01	0.04	NS	NS

¹ Values are least squares means.

² Orthogonal contrasts.

³ NS, $P > 0.15$.

⁴ BCS on scale of 1 to 5 with 1 being thinnest (Wildman et al., 1982).

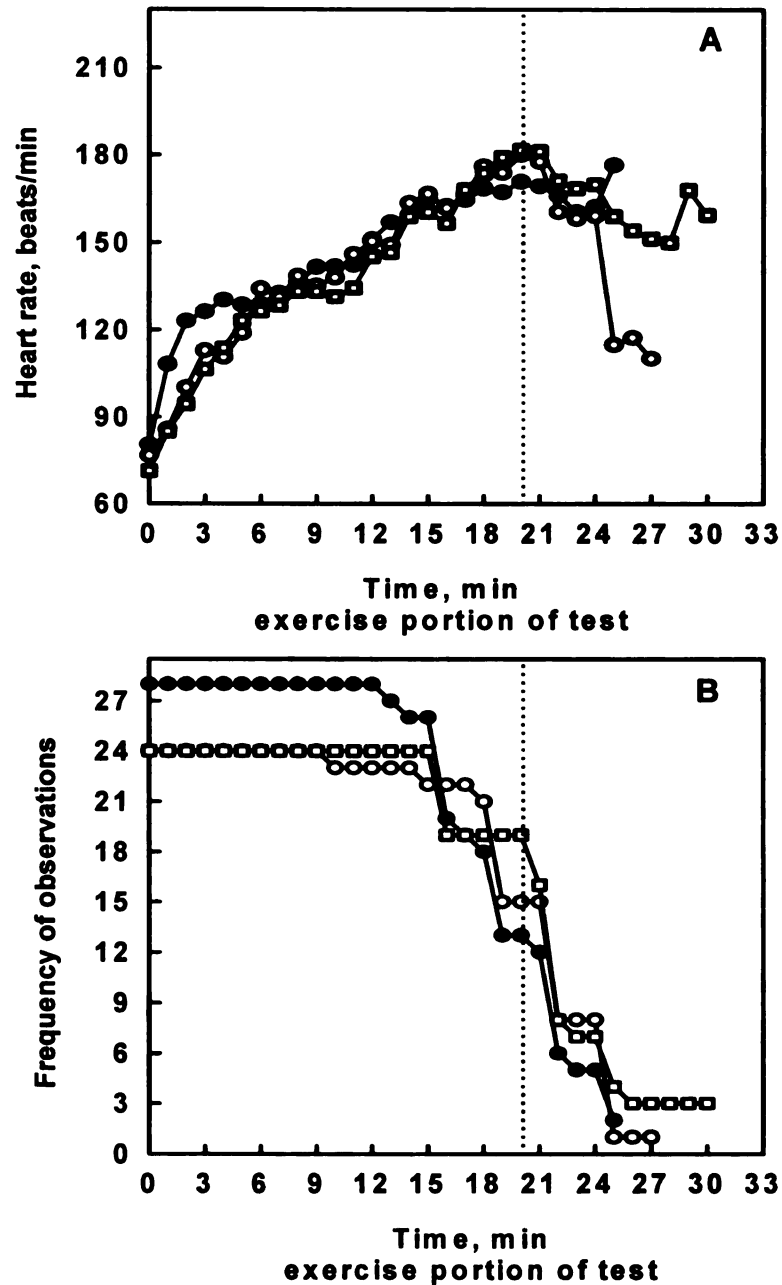


Figure 4.1. Mean heart rates and frequency of experiment observations of cows during exercise portions of treadmill tests pooled across d 15, 30, 45 and 60 of the experiment. Treatments were No Exercise (●), 1-h Exercise (○), and 2-h Exercise (□). The final minute of test varied among treatment groups. The dotted lines represents min 20 of treadmill test. Heart rate data during minute of test greater than 20 min were excluded from statistical analyses (*Panel A*). The frequency of experimental observations per minute of tests was eight or less at min 22 to 25 of No Exercise; at min 22 to 27 of 1-h Exercise; and, at min 22 to 30 of 2-h Exercise (*Panel B*).

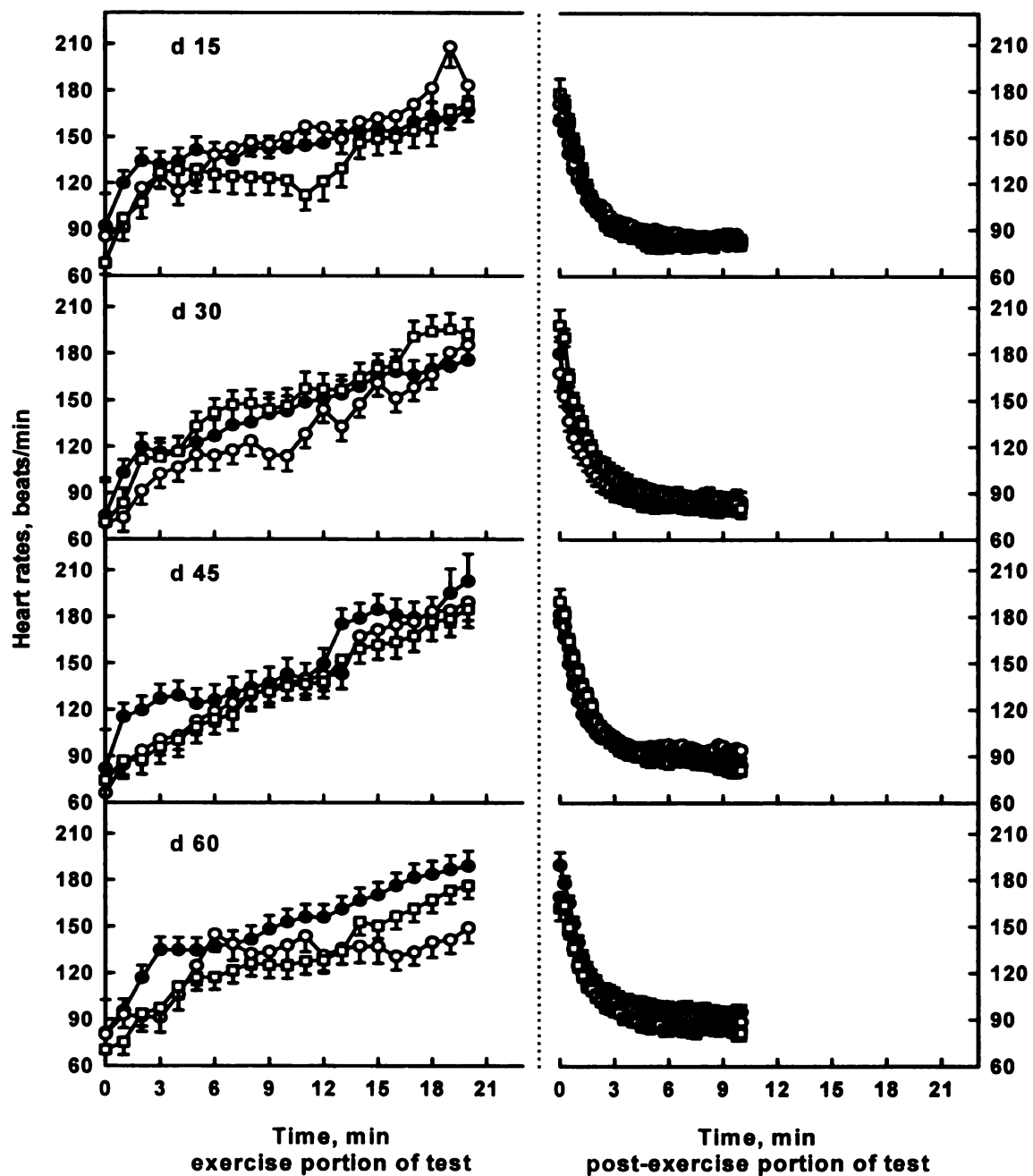


Figure 4.2. Heart rates of cows during exercise and post-exercise portions of treadmill tests on d 15, 30, 45 and 60 of the experiment (least squares means and SEM). Treatments were No Exercise (●), 1-h Exercise (○), and 2-h Exercise (□). Interaction of treatment by day of experiment by minute of test affected the magnitude of increases in heart rates during the exercise portion of the test ($P < 0.05$). Interactions of treatment by 15-sec interval (time) of test and treatment by day of experiment affected the decline of heart rates during the post-exercise portion of the test ($P < 0.05$).

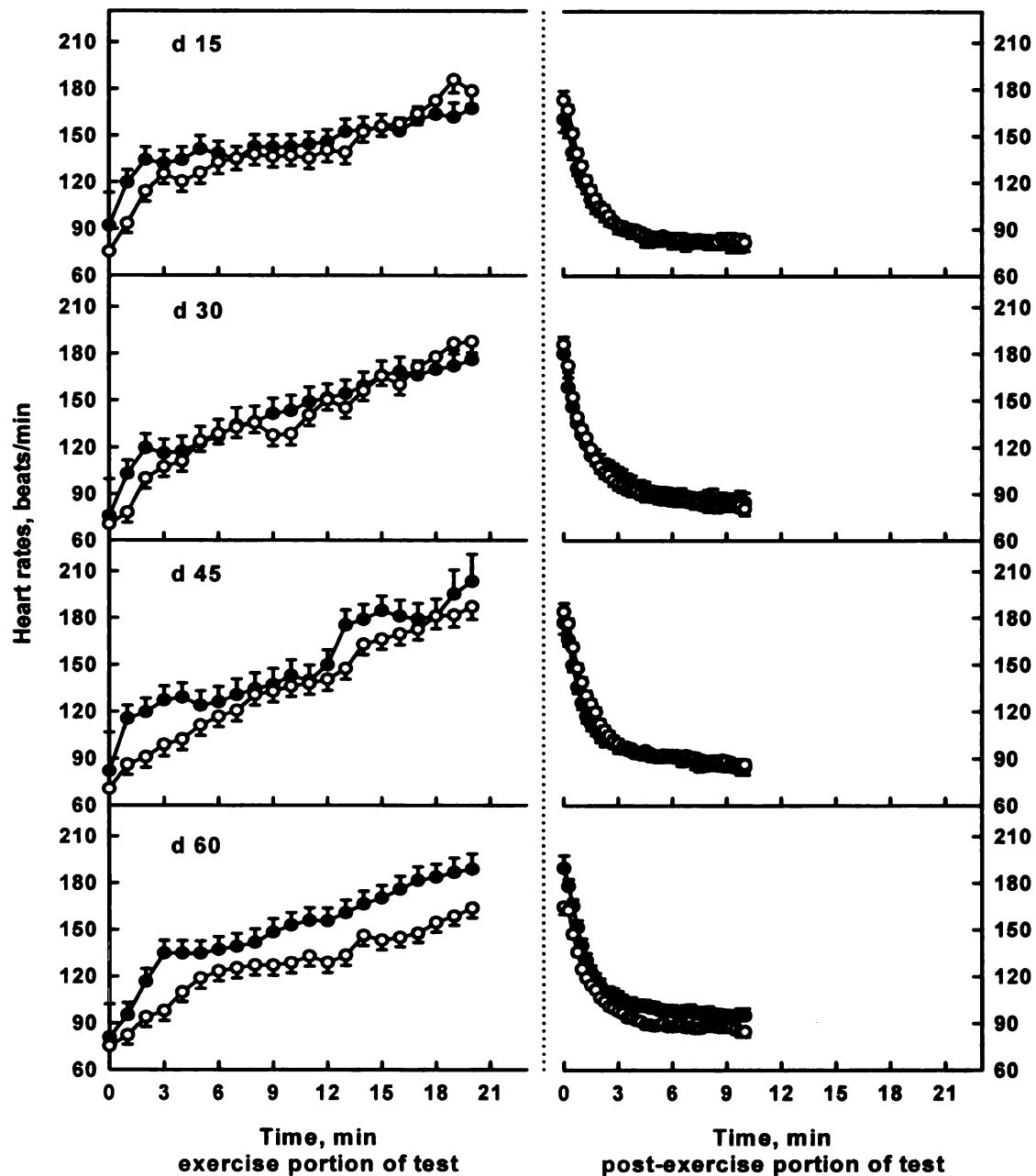


Figure 4.3. Heart rates of cows during exercise and post-exercise portions of treadmill tests on d 15, 30, 45 and 60 of the experiment (least squares means and SEM). Treatments were pooled exercise (1-h Exercise and 2-h Exercise, ○) and No Exercise (●). Interactions of treatment by day of experiment, treatment by minute of test, and treatment by minute of test affected the magnitude of increases in heart rates during the exercise portions of the test ($P < 0.05$). Interaction of treatment by day of experiment affected the decline of heart rates during post-exercise of the test ($P < 0.05$).

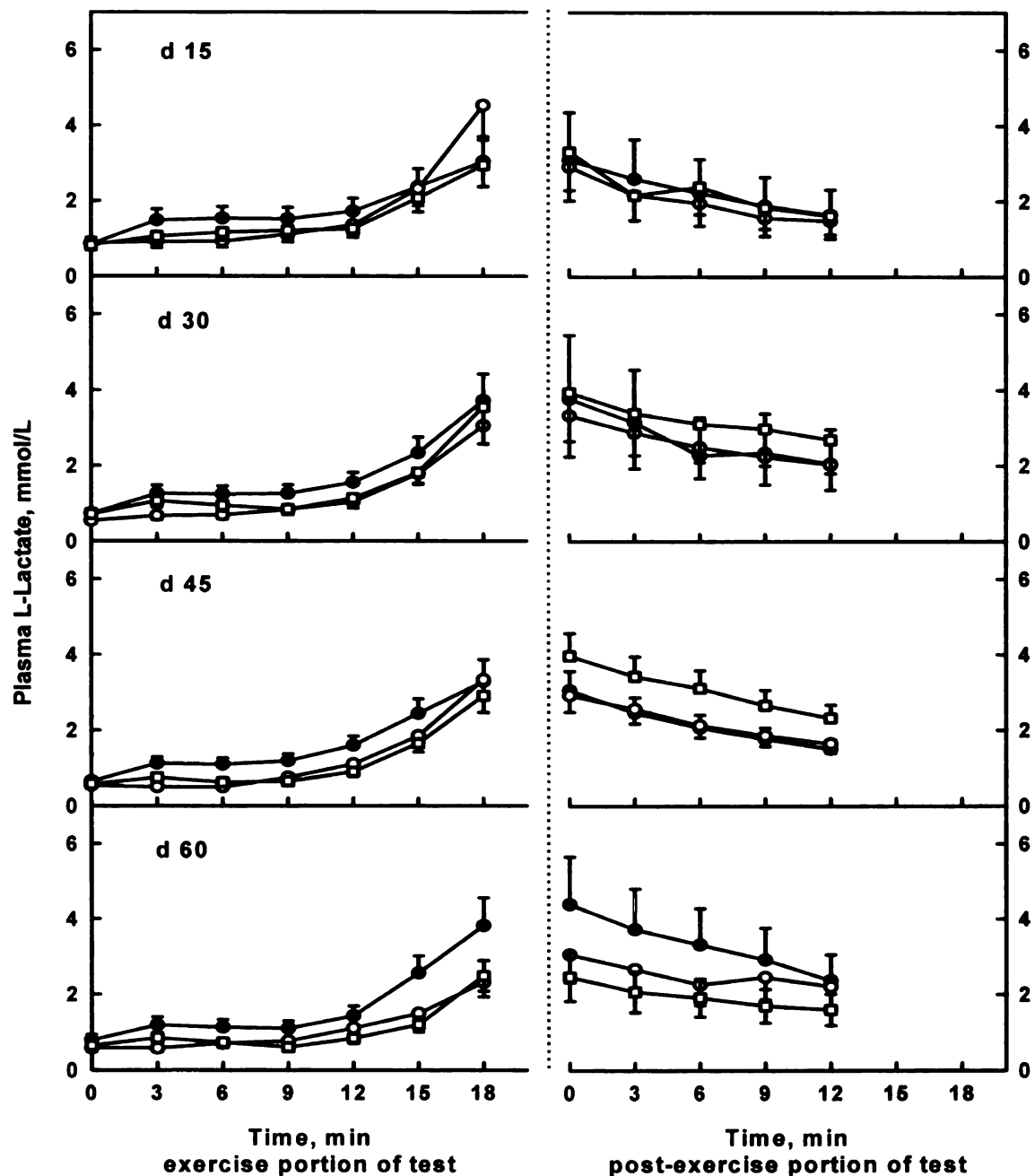


Figure 4.4. Plasma L-lactate concentrations during exercise and post-exercise portions of treadmill tests on d 15, 30, 45 and 60 of the experiment (back-transformed least squares means and 67% confidence intervals). Treatments were No Exercise (●), 1-h Exercise (○), and 2-h Exercise (□). Interactions of treatment by minute of test and day of experiment by minute of test affected the magnitude of increases in plasma lactate concentrations during the exercise portion of the test ($P < 0.05$). During post-exercise portions of the test, interactions of treatment by minute of test tended to affect concentrations ($P = 0.06$) and day of experiment by minute of test affected the decline of plasma lactate concentrations ($P < 0.05$).

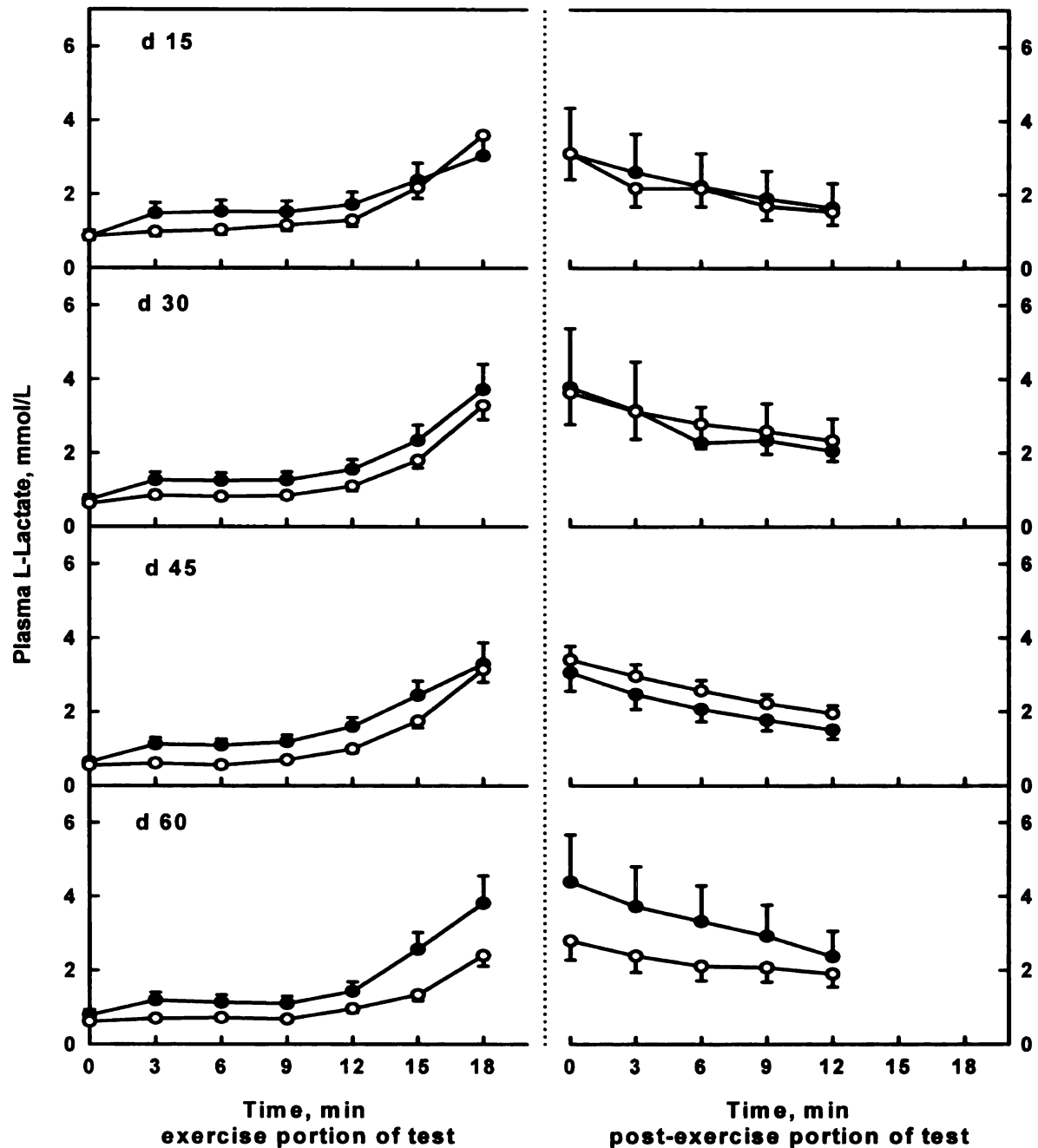


Figure 4.5. Plasma L-lactate concentrations during exercise and post-exercise portions of treadmill tests on d 15, 30, 45 and 60 of the experiment (back-transformed least squares means and 67% confidence intervals). Treatments were pooled across exercise (1-h Exercise and 2-h Exercise, ○) vs. No Exercise (●). Main effects of treatment, day of experiment, and minute of test, and the interaction of treatment by minute of test affected the magnitude of increases of plasma lactate concentrations during the exercise portion of the test ($P < 0.05$). Interaction of treatment by day of experiment by minute of test tended to affect the decline of plasma lactate concentrations during post-exercise portions of the test ($P = 0.11$).

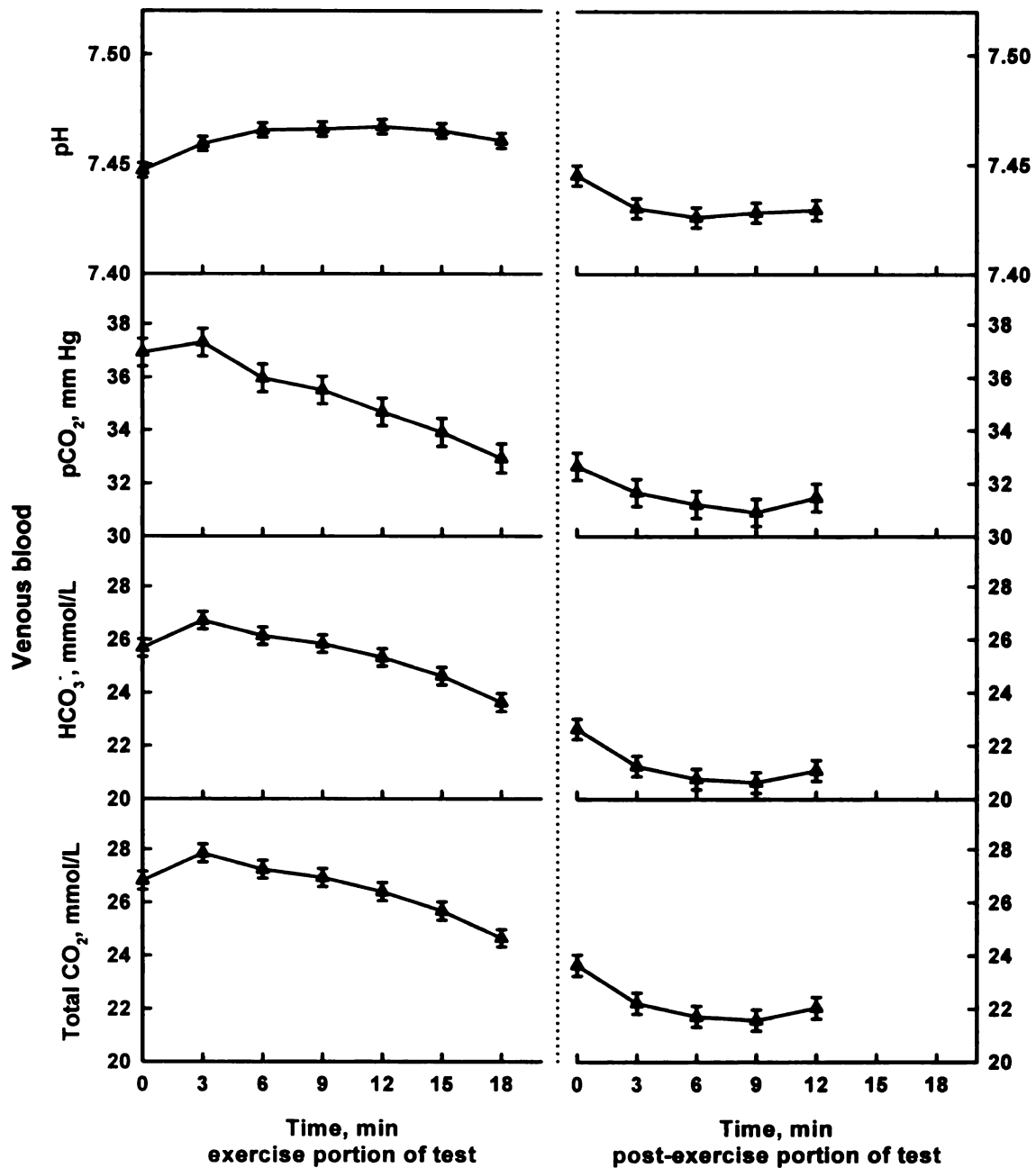


Figure 4.6. Blood pH, pCO₂, HCO₃⁻, and TCO₂ concentrations during exercise and post-exercise portions of treadmill tests pooled across treatment groups and day of experiment (least squares means and SEM). Minute of test affected responses of all variables during exercise and post-exercise portions of the test ($P < 0.05$).

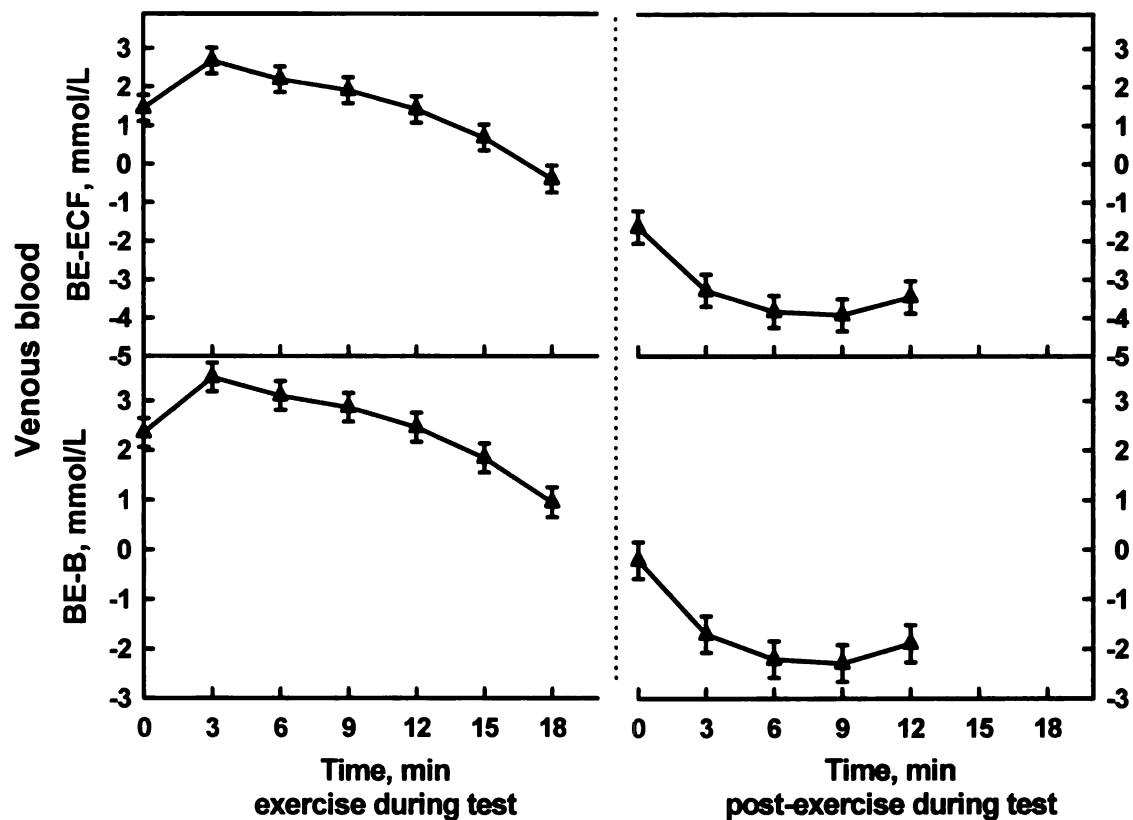


Figure 4.7. Base excess of extracellular fluid (BE-ECF) and of blood (BE-B) concentrations during exercise and post-exercise portions of treadmill tests pooled across treatment groups and day of experiment (least squares means and SEM). Minute of test affected concentrations during exercise and post-exercise portions of the test ($P < 0.05$).

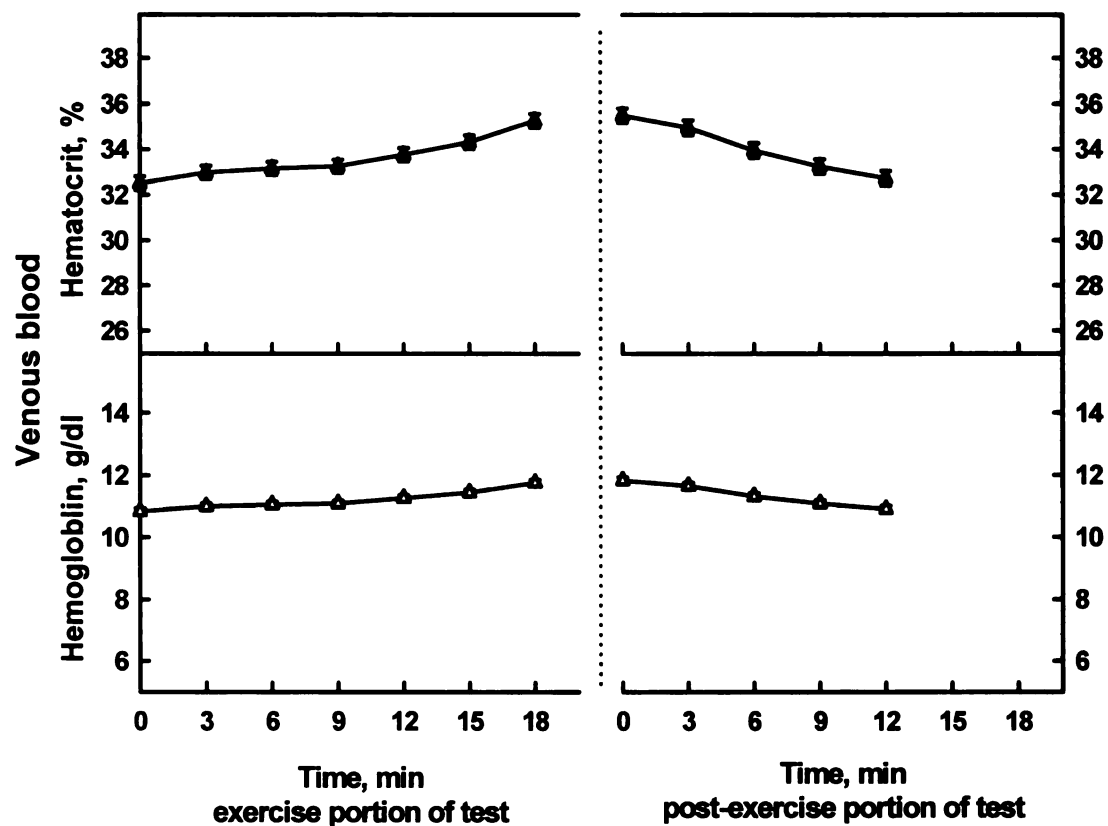


Figure 4.8. Blood hematocrit and hemoglobin concentrations during exercise and post-exercise portions of treadmill tests pooled across treatment groups and day of experiment (least squares means and SEM). Minute of test affected responses during exercise and post-exercise portions of the test ($P < 0.05$).

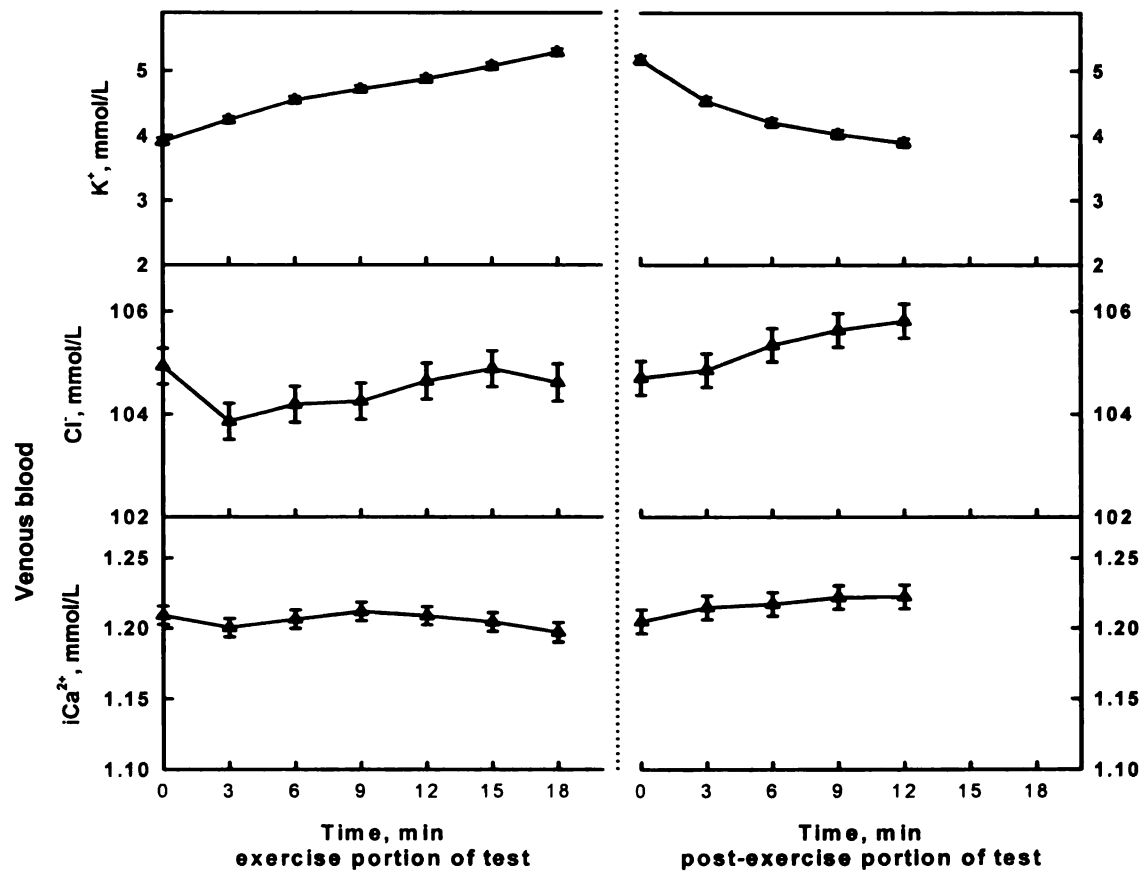


Figure 4.9. Blood K^+ , Cl^- , and ionized Ca^{2+} (iCa^{2+}) concentrations during exercise and post-exercise portions of treadmill tests pooled across treatment groups and day of experiment (least squares means and SEM). Minute of test affected concentrations during exercise and post-exercise portions of the test ($P < 0.05$).

CHAPTER 5

EXERCISE TRAINING OF PREGNANT DAIRY COWS AFFECTS PHYSICAL FITNESS AND ACID-BASE HOMEOSTASIS

ABSTRACT

Objectives of this experiment were to determine if prepartum exercise training improves physical fitness of pregnant cows and alters acid-base homeostasis during exercise test on a treadmill. Pregnant (n = 26) and non-pregnant (n = 26), non-lactating multiparous Holstein cows were assigned to a factorial arrangement of treatments: exercise training (**E**) or no exercise (**NE**), and pregnant (**P**) or non-pregnant (**NP**). Exercise training was walking (1.25 to 1.5 h at 3.25 km/h) every other day in an outdoor mechanical walker for 70 d. All cows completed treadmill exercise tests on d 0, 30, and 60 of the experiment (d 70, 40, and 10 before expected parturition of pregnant cows). On d 0, physical fitness was similar among all cows based on durations of treadmill tests, heart rates, and acid-base measurements at given workloads. After 60 d of exercise training, the physical fitness of E cows was improved compared with NE cows (pooled across pregnancy status) as indicated by longer duration of treadmill exercise tests. Heart rates and plasma lactate concentrations at given workloads were lower for E cows than NE cows indicating greater fitness. Additionally, E cows more effectively maintained acid-base homeostasis during the treadmill test compared with NE cows. During the treadmill test, blood pH and K^+ concentrations were lower for E cows compared with NE cows, whereas pCO_2 and HCO_3^- concentrations were greater. Physiology associated with pregnancy status did not alter the ability to improve physical

fitness by exercise training. Overall, prepartum exercise training of pregnant cows for 60 d increased physical fitness.

INTRODUCTION

Current management systems that limit exercise of dairy cows may reduce their physical fitness during the periparturient period. Poor physical fitness may affect deleteriously periparturient health and performance. Fitness is characterized as the maintenance of homeostasis of physiological processes during an exercise challenge (Lamb, 1984). To improve physical fitness, repeated exercise training may cause adaptations of physiological processes to meet the increased demands of exercise. This in turn may be beneficial to dairy cows during the periparturient period.

In a previous experiment (Chapter 4), exercise training of non-lactating, non-pregnant dairy cows increased physical fitness. Heart rates and plasma lactate concentrations of exercised cows compared with non-exercised cows were lower at given workloads of a treadmill test. However, pregnancy in addition to exercise training may affect measurements of physical fitness and acid-base status differently. After chronic exercise training, heart rates of pregnant women were less at given workloads compared with sedentary pregnant women indicating that they were more physically fit (Morton et al., 1985). No results were found to describe the response of heart rates of pregnant ruminants after chronic exercise training.

Metabolic acidosis is the addition of strong acid or loss of base (bicarbonate) in the extracellular fluid. During exercise, the addition of plasma lactate increases the acid load (metabolic acidosis) of the extracellular fluid. Without compensatory mechanisms of respiration, renal excretion, and extracellular and intracellular buffering, an acid-base disturbance (decreased blood pH) will result. Chronic exercise training of pregnant dairy

cows may reduce metabolic acidosis (reduction in lactate concentrations) during an exercise challenge.

Objectives of this experiment were to determine: 1) if exercise training improves physical fitness of pregnant cows; 2) if pregnancy status and exercise training alters acid-base status during treadmill exercise tests; and, 3) if pregnancy status alters physiological responses to exercise. The hypotheses were that exercise training improves the physical fitness of both pregnant and non-pregnant cows, cause physiological adaptations to maintain acid-base homeostasis, and, that pregnancy status independent of exercise training affects measures of physical fitness and acid-base homeostasis.

MATERIAL AND METHODS

Animal care and experimental procedures were approved and conducted under standards of the Michigan State University All-University Committee on Animal Use and Care, application number 05/98-081-00. Guidelines were compliant with National Institutes of Health and the Guide for the Care and Use of Laboratory Animals.

Cows and Treatments

Twenty-six pairs of non-pregnant and pregnant multiparous Holstein cows were blocked by parity and expected calving date (ECD) of the pregnant animal. Pairs of cows were assigned randomly to exercise treatments. Treatments in a factorial arrangement were exercise training (E) or no exercise (NE), and pregnant (P) or non-pregnant (NP).

Cows had been dried-off at least 1 wk before the start of the experiment. They also were trained to use the mechanical walker and treadmill (described subsequently)

with two to three short duration (5 min) practice sessions, 1 to 2 wk before the experiment. The start of experiment for a block was approximately 70 d before ECD of the pregnant cows of each pair. Exercise training was completed every other day until the parturition of the pregnant cow of the pair.

Exercise training was accomplished in an outdoor mechanical walker with a circular lane that was 90-cm wide and 33.8-m long approximately 36.5 m from the alley in the tie-stall barn where cows were housed (Chapter 3). Cows assigned to NE remained in tie-stalls blocked from feed and water while cows assigned to E were exercised. From d 2 through 28 of the experiment (about 68 to 42 d before ECD), E cows walked for 1.25 h at 3.25 km/h and from d 32 to parturition for 1.5 h at 3.25 km/h. On the day of their treadmill test (d 0, 30 and 60), cows were not exercised in the mechanical walker.

All cows were fed a TMR ad libitum, once daily. From d 0 to 34 of experiment, the diet consisted 47% alfalfa haylage, 33% corn silage, 10% spelt hulls, 9% soybean meal, plus mineral and vitamins (1.44 Mcal of NE_L /kg, 15.9% CP, 31.5% ADF, and 45.8% NDF, dry basis with a dietary cation-anion difference, **DCAD** $[(Na + K) - (Cl + S)]$ meq/100 g of dietary DM) of 20.2). From d 35 to parturition, the diet consisted 24% alfalfa haylage, 32% corn silage, 18% ground corn, 14% soybean meal, plus mineral and vitamins (1.65 Mcal of NE_L /kg, 17.7% CP, 22.4% ADF, and 33.3% NDF, dry basis with a DCAD of -15.7 meq/100 g of dietary DM).

Treadmill Exercise Test

To assess physical fitness, all cows completed treadmill exercise tests on d 0, 30, and 60 of the experiment (about d 70, 40, and 10 before ECD). Exercise tests consisted of increased workloads as cows walked on the treadmill (Classic Treadmill Conditioner, W. H. D. Lindsey, Kenilworth, Australia). During the test, heart rates were recorded

every 5 sec with a wireless electrocardiogram monitor (Polar Advantage, Polar Electro, Port Washington, NY) as previously described (Chapter 3). Resting heart rates were recorded while cows stood in tie-stalls and during the minute prior to the start of treadmill test (min 0) while standing on the treadmill platform. From min 0 to 3, cows walked at 4 km/h followed by an increase at min 3 to 5 km/h for the remainder of the test. Beginning at min 6, slope of the walking surface was increased by 0.8% every 3 min. The test continued until the cow refused to continue walking or until heart rate was greater than 180 beats per minute (**BPM**). The time to end of test and the greatest heart rate at the end of test were recorded. At the end of the test, the walking surface was returned to 0% slope. Cows remained standing on the treadmill platform from 0 to 18 min after the exercise portion of the test before returning to their individual tie-stalls.

An indwelling catheters was inserted into a jugular vein 1 d prior to the treadmill test. Blood was sampled from the catheter into 10-ml syringes containing 20 IU of Na-heparin per ml of blood at min 0 and prior to each increase in workload during the test (every 3 min). Blood sampling continued post-exercise every 3 min from min 0 through 18 (standing on treadmill platform) and every 15 min from min 33 through 78 (in tie-stalls). Blood (3 ml) from the Na-heparin syringe was transferred to a tube with sodium fluoride (**NaF**, 2.5 mg/ml of blood) and centrifuged ($2000 \times g$) within 15 min of collection. Plasma was harvested and stored at -20°C until analysis for L-lactate. L-lactate concentrations were determined using the Sport Lactate Analyzer 1500 (YSI, Yellow Springs, OH). Intra- and inter-assay coefficients of variation were 1.6 and 4.3%, respectively. The remaining blood in syringes was analyzed for pH, partial pressure carbon dioxide (**pCO₂**), hematocrit (**HCT**), and concentrations of potassium (**K⁺**), ionized calcium (**iCa²⁺**), and chloride (**Cl⁻**) using a blood gas analyzer (Stat 5 Profiler, Nova Biochemical, Princeton, NJ). From these measurements, concentrations of bicarbonate (**HCO₃⁻**), total carbon dioxide (**TCO₂**), and hemoglobin (**HGB**) were calculated.

Statistical Analysis

Data were analyzed by method of ANOVA using mixed model procedures of SAS (1999). The appropriate covariance structure for the repeated measures (day of the experiment and minute of test) was determined using the goodness of fit criteria of Aiaike's Information Criterion and Schwarz's Bayesian Criterion. Plasma L-lactate concentrations had unequal variances across minute of treadmill test and were transformed using the natural logarithm before analyses. Unless otherwise described, results are presented as generalized least squares means with standard error of mean. Statistical significance was declared at $P < 0.05$ with tendencies at $0.05 < P < 0.10$.

Heart rates in the tie-stall, at min 0 of the treadmill test, and at the end of test, and the time to end of the treadmill test were analyzed using the following mixed model. A treadmill test in which the heart rates of an individual cow were never greater than 150 BPM were excluded from analyses (12 of 152 tests were excluded). The model included the random effect of cow nested within exercise treatment (T) and pregnancy status (PS), repeated measures effect of day of experiment, and fixed effects of T, PS, and block, and all two- and three-way interactions of T, PS, and day of experiment. Covariance structure of repeated measures effect of day of experiment was autoregressive first order. Additionally, means for time to end of treadmill test data were compared by the following orthogonal contrasts: T effect for d 0 vs. 30 and 60; T effect for d 30 vs. 60; PS effect for d 0 vs. 30 and 60; PS effect for d 30 vs. 60; interaction of T by PS effect for d 0 vs. 30 and 60; and, interaction of T by PS effect for d 30 vs. 60.

The means and frequency of observations per treatment group for heart rates for each minute of tests pooled across day of experiment are shown in Figure 5.1. The frequency of observations were different among treatment groups due to the differences in the time to end of the exercise portion of tests (Panel B, Figure 5.1). The frequency of experimental observations at each minute of tests was eight or less at min 25 to 28

for NP, NE; at min 22 to 28 for P, NE; at min 29 to 39 for NP, E; and, at min 25 to 29 for P, E. Because of the reduced frequency of observations beyond 18 min of tests, only the data from min 0 to 18 were used in analyses, so that all treatments and tests were represented with a similar number of observations. Additionally, treadmill tests in which the cow's heart rate never was greater than 150 BPM or the time to end of the test was less than 16 min were excluded from analyses of dependent variables (heart rates, blood, and plasma variables) measured during tests; 32 of 152 tests were excluded. Of these 32 excluded tests pooled across day of the experiment, the distribution among treatment groups were 4, 16, 4, and 8 for NP, NE; P, NE; NP, E; and, P, E, respectively.

Heart rate data for every 5 sec were used to calculate the average for each minute during the exercise portion of the treadmill test. During the post-exercise portion (0 to 12 min), 5-sec heart rate data were averaged for every 15 sec because of the rapid decline of heart rates. To account for missing heart rate measurements during a given minute or 15-sec interval, the average heart rate was weighted by the number of observed heart rates within a time interval in the mixed model analyses. Due to the large amount of data and long computation time to solve the model, the post-exercise heart rate data were analyzed as four separate data sets: 0 to 3 min; 3 to 6 min; 6 to 9 min; and, 9 to 12 min.

Model for the dependent variable heart rate during the treadmill test included the random effect of cow nested within T and PS, the fixed effects of T, PS, and block, and repeated measures of day of experiment and minute of test. All two-, three-, and four-way interactions of T, PS, day of experiment, and minute of test were included in the model. The covariance structures of the data were unstructured for day of experiment and auto-regressive first order for minute of test.

Dependent variables of blood and plasma concentrations measured during the treadmill test were analyzed separately as three data sets: 1) exercise portion of test

during min 0 to 18; 2) post-exercise during min 0 to 18; and, 3) post-exercise during min 33 to 78. Models included the random effect of cow nested within T and PS and the fixed effects of T, PS, and block, and repeated measures of day of experiment and minute of test. All two-, three-, and four-way interactions of T, PS, day of experiment, and minute of test were included in the model. The covariance structures of the data were unstructured for day of experiment and auto-regressive first order for minute of test.

RESULTS AND DISCUSSION

The target time for the start of the experiment (d 0 of treadmill test) was 70 d prior to ECD; it actually averaged 67 ± 6 d with a range of 80 to 56 d. Days on experiment for cows that exercised were 69 ± 0.77 , whereas cows that did not exercise were on experiment for 65 ± 0.77 d (effect of T, $P = 0.005$). Four cows (two in P and two in NP) did not complete the final treadmill test because parturition occurred earlier than predicted for the pregnant cow of each pair.

Time to End of Treadmill Test

The day of the experiment, T, and PS affected the time to end of the treadmill tests (Figure 5.2). Pooled across PS, cows in E had longer time to end of treadmill tests on d 30 and 60 compared with d 0 of experiment; whereas, cows in NE pooled across PS had shorter time to end of treadmill tests for d 30 and 60 compared with d 0 (contrast of T effect at d 0 vs. 30 and 60, interaction of T by day of experiment, $P < 0.01$, Panel B; Figure 5.2). Regardless of T, the time to end of tests of P cows was shorter at d 60 compared with d 0 and 30; whereas, the time to end of tests for NP cows

were longer at d 60 compared with d 0 and 30 (contrast of PS effect at d 0 vs. 30 and 60, interaction of PS by day of experiment, $P < 0.01$, Panel C; Figure 5.2).

Exercise training increased the time to end of the treadmill test as day of experiment increased, whereas pregnancy status reduced the time to end of the treadmill test near parturition. Non-pregnant sheep that exercised also had a 40% increase in the length of time of exercise (Mundie et al., 1991). As described previously in non-pregnant dairy cows and sheep, exercise improved their ability to exercise for an extended time during the treadmill test (Mundie et al., 1991; Chapter 4). Additionally, pregnancy status greatly reduced the cow's ability to complete the test closest to parturition. Additional weight associated with the conceptus and(or) other physiological factors associated with pregnancy affected the performance of late pregnant cows during treadmill tests. Regardless of pregnancy status, the extend time to end of exercise portion of the treadmill test of cows in E indicated that they were more physically fit than cows in NE.

Heart Rates during Treadmill Tests

In horses and steers, cardiac output increased linearly with increased workloads (Jones et al., 1989). During exercise, cardiac output was correlated strongly with an increase in heart rates, but not with stroke volume. In the current experiment, heart rates were used to quantify the effort of a cow's heart when subjected to varying workloads.

Heart rates were not different among cows in E or NE treatments while standing in tie-stalls. Prior to the test either in tie-stalls or at min 0 on treadmill, resting heart rates of P cows were greater on d 30 and 60 compared with d 0, whereas the heart rates of NP cows were only greater on d 60 compared with d 0 and 30 of the experiment (interaction of PS by day of experiment, $P < 0.05$, Table 5.1). In humans, pregnancy

also increased resting heart rates compared with non-pregnant values (Clapp, 1985). Additionally, chronic exercise training during pregnancy of women who had not trained previously did not reduce the change of resting heart rates (Erkkola, 1976). This increase of resting heart rates associated with pregnancy may be associated with the increases of metabolic activity, BW, or vascularization of reproductive organs. Cows that exercised tended to have slightly reduced heart rates at min 0 on the treadmill compared with non-exercised cows (pooled across pregnancy status and day of experiment, 83.2 ± 1.29 vs. 86.8 ± 1.31 , $P = 0.06$). This difference may be attributed to different behavioral responses related to repeated handling of exercised cows or anticipation of treadmill exercise.

Treatments of NE and E, or P and NP did not affect the greatest heart rates recorded by the end of treadmill tests. However, the greatest heart rates recorded during a test pooled across all cows tended to be influenced by the day of test (Table 5.1). In other studies, the maximum heart rates of nonruminants or ruminants did not increase after chronic exercise (Aguera et al., 1995; Fosha-Dolezal and Fedde, 1988). The repeated testing among all treatment groups in the current experiment may have affected measurements of greatest heart rates.

During the exercise portion of the treadmill test, the four-way interaction of T by PS by day of experiment by minute of test affected heart rates (Table 5.2; Figure 5.3). On d 0 of experiment, heart rates increased similarly for all groups during exercise as the test progressed (Figure 5.3). As day of experiment increased, cows in NP, NE and P, NE had greater increases of heart rates compared with cows in NP, E and P, E. In horses exercised for 5, 10, or 16 wk, heart rates were lower during exercise testing compared with untrained horses (Thomas et al., 1983) or pre-exercise training values (Aguera et al., 1995; Rodiek et al., 1987). As in a previous experiment (Chapter 4), heart rates were lower during the treadmill test after 60 d of exercise training treatment.

During the post-exercise portion of the treadmill test on all days of the experiment, heart rates declined rapidly among all treatment groups during the first 3 min (effect of minute of test, $P < 0.001$, Table 5.2) and continued to decline during min 3 to 6 (Figure 5.3). After min 6 post-exercise, the differences of heart rates among all treatment groups and tests were small. As most evident on d 60 of experiment, post-exercise decline of heart rates was not as great for P cows compared with NP cows (interaction of PS by day of experiment, $P < 0.05$). During min 3 to 6 on d 60, heart rates were slightly less for E cows compared with NE cows (interaction of T by day of experiment, $P = 0.032$).

The dramatic decline in heart rates of cows within 3 min post-exercise was similar among treatments and similar to the decrease measured in horses (Ehrlein et al., 1973). The time required for heart rates to return to resting values can be affected by the workloads and duration of work, environmental temperature and humidity, and working capacity of the horse (Fregin and Thomas, 1983). In the current system with dairy cows, the pattern of decline in heart rates post-exercise was very similar among treatments. Heart rates returned within 6 min after cessation of exercise to values similar to those at the beginning of the exercise portion of the test. On d 60 of the experiment, the differences of heart rates during post-exercise between P and NP cows were similar to differences of resting heart rates measured prior to starting the treadmill test. As in humans (Erkkola, 1976), chronic exercise during pregnancy in the current experiment did not reduce the increase in resting heart rate above non-pregnant values.

The greater time to end of tests and reduction of heart rates during the exercise portion of tests after 30 d of chronic exercise indicated that exercised cows were more physically fit than NE cows. They completed given workloads with less effort from the heart. Also, the slightly greater heart rates of P cows compared with NP cows could be

related to differences in BW associated with the conceptus and(or) the physiology associated with pregnancy.

Acid-Base Status during the Treadmill Test

All cows were able to compensate for the increased plasma lactate (acid load of metabolic acidosis) during the treadmill test in that blood acid-base status was within normal physiological ranges. Acid-base homeostasis was controlled tightly throughout this experiment apparently by compensatory mechanisms of respiration, renal excretion, and extracellular and intracellular buffering. Few studies are available to compare with the results of the current experiment. As described previously (Chapter 4), acute exercise of non-pregnant, non-lactating dairy cows altered acid-base status as indicated by changes in plasma concentrations of L-lactate and blood pH, $p\text{CO}_2$, and concentrations of HCO_3^- , and TCO_2 . In the present experiment, acid-base responses to exercise training and pregnancy were within normal physiological ranges as evaluated using the treadmill test.

Plasma L-lactate. During the exercise portion of the treadmill test, plasma lactate concentrations increased similarly in all treatment groups as minute of test progressed on d 0 of the experiment (Figure 5.4). Cows in NE had greater plasma lactate concentrations than cows in E on d 30 and 60 of experiment (interaction of T by day of experiment, $P < 0.001$). During the exercise portion of the test, pooled across day of experiment, cows in NP, NE and P, NE had greater concentrations of lactate than those in P, E and NP, E (interaction of T by PS by minute of test; $P = 0.017$; Figure 5.4). Additionally, cows in P, E had slightly greater concentrations of lactate than those in NP, E.

On d 0 of the experiment during the exercise portion of the treadmill test, plasma lactate concentrations increased from approximately 10 to 40 mg/dl. Concentrations

increased approximately 70 mg/dl (10 to 80 mg/dl) when Hereford calves were exercised for 5 min at 7.9 km/h (Fosha-Dolezal and Fedde, 1988; Kuhlmann et al., 1985). On d 60 of the current experiment during the exercise portion of the test, the increase of plasma lactate concentrations were lower for cows in E compared with those in NE (approximately 15 vs. 30 mg/dl, respectively). In horses exercise-trained, plasma venous lactate was lower during an exercise test compared with pre-exercise values (Aguera et al., 1995; Rodiek et al., 1987).

During min 0 to 18 post-exercise, all cows had similar decreases in plasma lactate concentrations as time post-exercise increased on d 0 of experiment ($P < 0.001$; Figure 5.4). Cows in E had less plasma lactate during min 0 to 18 post-exercise as day of experiment progressed compared with those in NE (interaction of T by day of experiment, $P = 0.045$; Figure 5.4). Additionally, the reduction of plasma lactate as time progressed post-exercise was greater for NP than P cows (interaction of PS by minute of test, $P < 0.001$; Figure 5.4). During min 33 to 78 post-exercise, P cows had greater plasma lactate concentrations than NP cows ($P < 0.001$). Lactate concentrations of all cows returned to pre-exercise values by the end of the post-exercise period. Similarly, concentrations of lactate declined to resting values 30 min after cessation of activity of draught animals (Zanzinger and Becker, 1992).

Plasma lactate concentrations among all treatment groups increased during the exercise portion of treadmill tests on d 0, 30, and 60. Increased plasma lactate during exercise indicates an increase of anaerobic metabolism, and subsequent metabolic acidosis. After 60 d of exercise training, plasma lactate concentrations were less for E than NE cows. The post-exercise concentrations also were lower for E cows compared with NE cows. Therefore, E cows were experiencing less anaerobic metabolism and metabolic acidosis than NE cows.

Blood pH and H⁺ concentrations. As measured during the treadmill tests, exercise training and pregnancy status affected the occurrence and magnitude of metabolic acidosis as indicated by changes of plasma lactate concentrations. During the exercise portion of the treadmill test, changes of blood pH (H⁺ concentrations) were very small and still within the normal physiological range for dairy cows (Figures 5.5 and 5.6). Therefore, all dairy cows were able to compensate for the increased metabolic acidosis to prevent a disturbance of blood acid-base. The interaction of T by day by experiment by minute of test, and interaction of PS by day of experiment by minute of test affected blood pH and H⁺ concentrations during the exercise portion of the treadmill ($P < 0.03$; Figures 5.5 and 5.6). The four-way interaction of T by PS by day of experiment by minute of test also tended to affect blood pH and H⁺ concentrations ($P = 0.067$).

During exercise on any day pooled across minute of test, cows in E had greater blood pH (less H⁺ concentrations) than cows in NE (Figures 5.5 and 5.6). On d 0, blood pH did not consistently change from min 0 as the test progressed for all groups. On d 30, E cows had greater blood pH (less H⁺ concentrations) than NE cows. On d 60, pooled across T, PS and minute of test, all cows had lower pH (greater H⁺ concentrations) than previous days. On d 60 of the experiment, cows in NP, E and NP, NE had similar blood pH (H⁺ concentrations), whereas cows in P, E had greater pH (less H⁺ concentrations) compared with those in P, NE.

Post-exercise min 0 to 18, the four-way interaction of T by PS by day of experiment by minute of test tended to affect blood pH (H⁺ concentrations) ($P = 0.061$; Figures 5.5 and 5.6). Blood pH was lowest for all groups on d 60 compared with other days. Exercised cows had greater pH (reduced H⁺ concentrations) than NE cows on all days of experiment and as time post-exercise increased. During post-exercise min 33

to 78, no T effects were detected. However, the interaction of PS by minute of test affected blood pH (H^+ concentrations) ($P = 0.038$).

Exercised cows had lower plasma lactate concentrations on d 60 which may indicate reduction of metabolic acidosis compared with other treatment groups. Among all cows, post-exercise values of blood pH were slightly lower than pre-exercise values. Cows may have slightly over-compensated for metabolic acidosis experienced during the exercise portion of the test. After the cessation of activity, the respiratory and renal systems may have influenced acid-base status during post-exercise period. Respiration rates were not measured in the current experiment. Respiration rates of calves after 15 min of exercise at 4.0 km/h remained greater than pre-exercise values for 15 min post-exercise (Piguet et al., 1993). Additionally, respiration rates of dairy cows after 18 min of exercise at 5.4 km/h remained greater than pre-exercise values for 1 h post-exercise (Blake et al., 1982). In the previous experiment with non-pregnant cows (Chapter 4), exercise training treatment did not affect on acid-base profiles of blood. However, the plasma lactate concentrations of the previous experiment were not as great as in the current experiment.

Blood pCO_2 and concentrations of HCO_3^- and TCO_2 . During the treadmill test, blood pCO_2 and HCO_3^- and TCO_2 concentrations responded in a similar fashion as the treadmill test progressed (Figures 5.7, 5.8, and 5.9). Calculated BE-B and BE-ECF concentrations responded in a similar fashion as pCO_2 to the effects of T, PS, day of experiment, and minute of test (Appendix O).

On d 0, 30 and 60 during the exercise portion, blood pCO_2 and concentrations of HCO_3^- and TCO_2 declined as time of test progressed ($P < 0.001$; Figures 5.7, 5.8, and 5.9). During post-exercise, blood pCO_2 and HCO_3^- and TCO_2 concentrations declined until min 6 followed by increases during the remaining time of post-exercise ($P < 0.001$; Figures 5.7, 5.8, and 5.9). Additionally, when all cows were being fed a negative DCAD

on d 60 of experiment, blood $p\text{CO}_2$ and concentrations of HCO_3^- and TCO_2 were reduced compared with d 0 and 30 when positive DCAD was fed ($P < 0.001$; Figures 5.7, 5.8, and 5.9). Increased respiration rates may have influenced the changes of blood $p\text{CO}_2$ and HCO_3^- and TCO_2 concentrations. Though not measured in the current study, respiration rates increased from 25 to 70 breaths per min during exercise of cattle (Arave et al., 1987; Kuhlmann et al., 1985; Piguet et al., 1993).

During the exercise portion of treadmill tests, blood $p\text{CO}_2$ and HCO_3^- and TCO_2 concentrations were less for P cows compared with NP cows regardless of day of experiment or minute of test ($P < 0.05$; Figures 5.7, 5.8, and 5.9). On d 60 of test, blood $p\text{CO}_2$ were slightly greater for E cows than NE cows (interaction of T by day of experiment, $P = 0.046$; Figure 5.7). During the exercise portion of the test regardless of day of experiment and PS, concentrations of HCO_3^- and TCO_2 were greater for E cows (25.3 ± 0.31 mmol/L) compared with NE cows (24.0 ± 0.034 mmol/L, $P < 0.007$). By 60 d of exercise training, E cows had slightly greater blood $p\text{CO}_2$ and concentrations of HCO_3^- and TCO_2 than NE cows. Similarly, dairy cows forced to exercise for 8 wk at 4 km/h for 2 h maintained homeostatic control of blood CO_2 , bicarbonate, and base excess during exercise compared with pre-exercise values (Blake et al., 1982).

During the post-exercise portion of the treadmill tests, blood $p\text{CO}_2$ and HCO_3^- and TCO_2 concentrations were less for P cows compared with NP cows as day of experiment and minute of test progressed (interaction of PS by day of experiment by minute of test, $P < 0.03$; Figures 5.7, 5.8, and 5.9). From min 33 to 78 post-exercise regardless of day of experiment and minute of test, blood $p\text{CO}_2$ tended to be less for cows in P, NE (37.5 ± 0.64 mmol/L) compared with those in NP, NE (41.8 ± 0.47 mmol/L); NP, E (41.7 ± 0.46 mmol/L); and, P, E (39.4 ± 0.49 mmol/L, $P = 0.057$).

In a previous experiment (Chapter 4), adaptations in acid-base status after exercise training were not detected. Plasma lactate concentrations and training

workloads were greater in the current study compared with previous experiment. Pregnant cows had slightly greater blood pH and lower blood $p\text{CO}_2$ and concentrations of HCO_3^- and TCO_2 compared with NP cows. No similar information was found in the literature to compare with these results. Pregnant cows likely have greater plasma volume than non-pregnant cows which may have diluted the acid-base measurements to explain the differences in blood pH and $p\text{CO}_2$ and concentrations of HCO_3^- and TCO_2 between the P and NP cows. Even though the absolute concentrations were different between P and NP cows, the relative changes of acid-base status during the treadmill tests were similar for both groups. Feeding a negative DCAD on d 60 of experiment decreased blood pH and $p\text{CO}_2$ and concentrations of HCO_3^- and TCO_2 at min 0 of the test compared with d 0 and 30 of the experiment when a positive DCAD was fed. However, the relative changes of acid-base status during the treadmill tests were similar whether cows were fed a positive (d 0 and 30 tests) or negative DCAD (d 60 test). The ability of P cows to maintain acid-base homeostasis during metabolic acidosis (greater plasma lactate concentrations) while exercising was not impaired or compromised by a negative DCAD.

Mineral Element Concentrations During Treadmill Test

During exercise, blood K^+ concentrations increased by approximately 25% as minute of test progressed on all days of the experiment ($P < 0.001$; Figure 5.10). Hereford calves that were exercised exhibited an increase in concentrations of serum K^+ with a peak of approximately 7.4 meq/L (Fosha-Dolezal and Fedde, 1988; Kuhlmann et al., 1985). The increase K^+ concentrations may result from the release of K^+ from muscle cells or incomplete uptake of K^+ during repolarization of muscle. A decrease in plasma volume during the treadmill test may be not great enough to fully explain the increases in measured K^+ concentrations.

On d 0, blood K^+ concentrations were similar among all treatment groups as minute of test increased. On d 30 and d 60, cows in NP, NE had greater K^+ concentrations compared with other groups of cows; and, on d 60, cows in P, NE had greater K^+ concentrations than those in P, E (interaction of T by PS by day of experiment, $P = 0.023$; Figure 5.10). During post-exercise min 0 to 18, all groups of cows responded similarly in that K^+ concentrations declined as time post-exercise increased ($P < 0.001$; Figure 5.10). In contrast, exercise training treatment of non-pregnant, non-lactating dairy cows did not generally affect the blood concentrations of K^+ during a treadmill test (Chapter 4). Previously, Fosha-Dolezal and Fedde (1988) demonstrated that training of calves reduced the increases of serum K^+ concentrations during exercise at a given speed compared with pre-training values.

During exercise or post-exercise portions of the treadmill test, T did not alter blood Cl^- and iCa^{2+} concentrations (Figures 5.11 and 5.12). Pregnant cows had greater Cl^- concentrations than NP cows with the largest increase occurring on d 60 of the experiment when cows were fed a negative DCAD (108 vs. 104 mmol/L; interaction of PS by day of experiment; $P < 0.025$). In other research, exercise or work activity of calves and draught oxen did not change plasma Ca^{2+} or Cl^- concentrations (Blum et al., 1979; Zanzinger and Becker, 1992).

The relatively small changes of iCa^{2+} and Cl^- concentrations may reflect changes in plasma volume due to the movement of water from plasma to extracellular, intracellular, or interstitial fluid compartments of muscle. Exercising Hereford calves and lambs had approximately a 7 to 9% shift of water from blood to muscle compartments during exercise (Apple et al., 1994; Kuhlmann et al., 1985). Additionally, PS affected the concentrations of Cl^- in that P cows had greater values throughout the test compared with NP cows. Pregnant cows have more plasma volume than non-pregnant

cows which may explain some of the differences in concentrations of Cl^- between the P and NP cows.

Hematocrit During Treadmill Test

During the exercise and post-exercise portions of the treadmill test, E did not affect blood HCT and HGB concentrations. On d 60 during exercise portion of treadmill test, the increases of HCT and HGB concentrations were less for P cows compared with NP cows and values on d 0 and 30 of the experiment (interaction of PS by day of experiment by minute of test, $P = 0.035$; Figure 5.13). Min 0 to 18 post-exercise, HCT and HGB concentrations declined as minute of test progressed with the greatest change for NP cows on d 60 compared with values on d 0 and 30 and for P cows (interactions of PS by day of experiment, $P < 0.001$; interaction of PS by minute of test, $P = 0.042$; Figure 5.13). Differences of HCT and HGB concentrations were not detected min 33 to 78 post-exercise.

Hematocrit increased as time during exercise increased at a faster rate for NP cows compared with P cows on d 60 of the experiment. The transient changes in HCT and HGB as the treadmill test progressed are suggestive of potential fluid shifts and changes of plasma volume. Humans exercising at submaximal and maximal intensities had hemoconcentration of circulating blood with 7.1 and 13.0% decreases in plasma volume, respectively (Novosadova, 1977). In the current study, pregnancy status affected concentrations of blood cells (HCT and HGB), potentially due to differences of plasma volume between P and NP cows.

CONCLUSIONS

The physical fitness was improved for NP and P cows that were exercised. The ability to continue the treadmill test for greater lengths of time, and the reduction of heart rates and plasma lactate during given workloads were indicative of improved physical fitness after 60 d of exercise training. Additionally, PS and days of gestation greatly affected heart rates during the treadmill test and rest, and the time to end of treadmill test near parturition (d 60 treadmill test). The reduced plasma lactate concentrations during exercise after 60 d of exercise training likely contributed to the improved acid-base homeostasis of exercised cows compared with non-exercised cows. Cows that exercised had greater $p\text{CO}_2$ and HCO_3^- concentrations during the treadmill test on d 60 than non-exercised cows. Exercise training did not affect greatly the concentrations of Cl^- or $i\text{Ca}^{2+}$, but did alter K^+ concentrations during the treadmill test on d 60. Pregnant cows had similar responses of acid-base status as NP cows. However, P cows had lower HCT and HGB during exercise than NP cows, potentially due to differences of plasma volume. Therefore, we conclude that regardless of pregnancy status of dairy cows, exercise training improved the physical fitness and acid-base status during the treadmill exercise tests.

Table 5.1. Heart rates (beats per minute) prior to treadmill test in tie-stall and at min 0 on treadmill; and, at end of the exercise portion of the test.¹

Independent variables	Day of experiment				<i>P</i> -value ²		
	0	30	60	SEM			
<u>Tie-stall</u>					PS	D	PS x D
NP ³	75.4	77.4	81.8	1.40	NS	< 0.001	0.036
P ³	75.8	79.7	89.4	1.45			
<u>Min 0 of test</u>					PS	D	PS x D
NP ³	80.9	79.1	84.6	1.85	< 0.001	< 0.001	0.022
P ³	82.1	87.2	95.9	1.89			
					T	D	T x D
NE ⁴	81.4	85.5	93.4	1.89	0.06	< 0.001	NS
E ⁴	81.6	80.8	87.2	1.85			
<u>End of test</u>					PS	D	PS x D
Pooled ⁵	175.5	179.9	182.9	2.43	NS	0.099	NS

¹ Values are least squares means.

² T = exercise treatment; PS = main effect of pregnancy status; D = main effect of day of experiment; T x D = interaction of exercise treatment by day of experiment; PS x D = interaction of pregnancy status by day of experiment; and NS = $P > 0.10$.

³ Least squares means pooled across exercise treatment. T x PS interaction ($P > 0.10$). NP = non-pregnant cows; and, P = pregnant cows.

⁴ Least squares means pooled across pregnancy status. T x PS interaction ($P > 0.10$). NE = non-exercised cows; and, E = exercised cows.

⁵ Least squares means pooled across exercise treatment and pregnancy status. T x PS interaction ($P > 0.10$).

Table 5.2. Probability values from ANOVA (mixed model) for heart rates during exercise and post-exercise portions of treadmill test.

Source of variation	Heart rates, beats per minute				
	Exercise	Post-exercise			
	0 to 18	0 to 3 min	3 to 6 min	6 to 9	9 to 12
Exercise treatment, T	0.0249	NS ¹	NS	NS	NS
Pregnancy status, PS	NS	NS	0.0138	0.0109	0.0129
T x PS	NS	NS	NS	NS	NS
Day of experiment, D	0.0007	0.0166	< 0.0001	0.0002	< 0.0001
T x D	0.0409	NS	0.0318	NS	0.0948
PS x D	NS	NS	0.0033	0.0139	0.0019
T x PS x D	NS	NS	NS	0.0840	0.0485
Minute of test, M	< 0.0001	< 0.0001	< 0.0001	0.0038	NS
T x M	< 0.0001	NS	NS	NS	NS
PS x M	NS	0.0288	NS	0.0597	NS
T x PS x M	NS	NS	NS	NS	NS
D x M	NS	NS	NS	NS	NS
T x D x M	NS	NS	NS	NS	NS
PS x D x M	0.0166	NS	0.0689	NS	NS
T x PS x D x M	0.0458	NS	NS	NS	NS
Block ²	NS	NS	NS	NS	NS
<u>Covariance</u>		<u>Estimate</u>			
Cow (T x PS) ³	104.68	102.07	61.8725	49.7799	47.0044
d 0 ⁴	3293.98	1049.06	225.08	157.34	124.56
d 30	2281.18	297.29	99.9695	150.54	173.83
d 60	1623.72	256.33	122.39	142.35	129.04
Minute of	0.7889	0.9258	0.9022	0.9049	0.8868
Pooled SEM ⁷	7.31	6.04	4.12	3.92	3.88

¹ NS = $P > 0.10$.

² Cows were blocked by expected calving date of the pregnant animal and parity. Thirteen blocks (4 cows each) were used in the experiment.

³ Among cow variance; Cow (T x PS) = cow nested within exercise treatment and pregnancy status.

⁴ Residual variance for each day of experiment; unstructured (UN) was the covariance structure of the repeated measures of day of experiment.

⁵ Correlations of minute of test, auto-regressive first order (AR(1)) was the covariance structure of the repeated measures effect of minute of test.

⁶ Because the data set was unbalanced, the largest standard errors are presented.

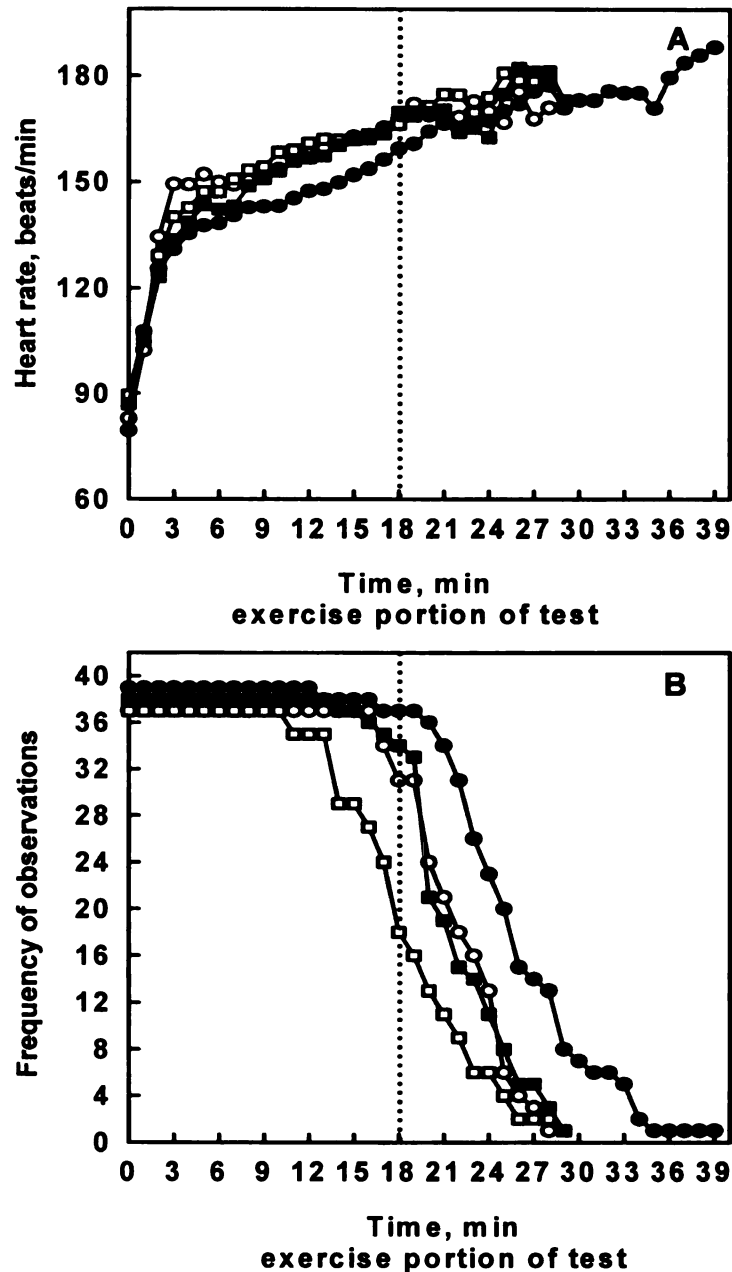


Figure 5.1. Mean heart rates and frequency of experiment observations of cows during the exercise portions of treadmill tests pooled across d 0, 30, and 60 of the experiment. Treatments were non-pregnant, non-exercise (○); pregnant, non-exercise (□); non-pregnant, exercise (●); and, pregnant, exercise (■). The time to end of tests varied among treatment groups. The vertical dotted line represents min 18 of the treadmill test. Heart rate data during minute of test greater than 18 min were excluded from statistical analyses of the exercise portion of the test (*Panel A*). The frequency of experimental observations per minute of tests was eight or less: at min 25 to 28 for non-pregnant, non-exercise; at min 22 to 28 for pregnant, non-exercise; at min 29 to 39 for non-pregnant, exercise; and, at min 25 to 29 for pregnant, exercise (*Panel B*).

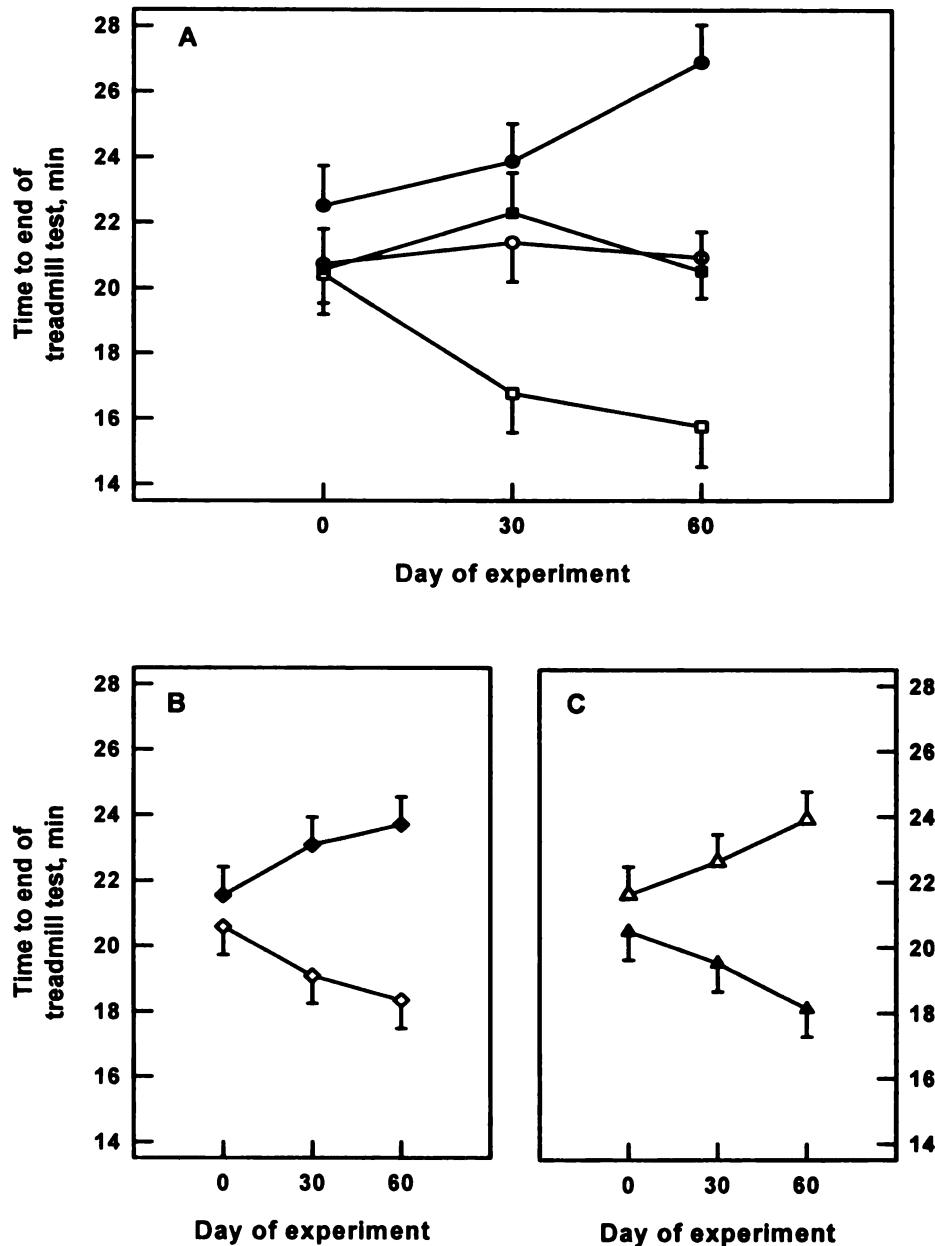


Figure 5.2. Time to end of treadmill test on d 0, 30, and 60 of experiment. *Panel A:* treatments were non-pregnant, non-exercise (○); pregnant, non-exercise (□); non-pregnant, exercise (●); and, pregnant, exercise (■). Interaction of treatment by pregnancy status by day of experiment was not significant ($P > 0.10$). *Panel B:* pooled across pregnancy status, treatments were exercised (◆) and non-exercised (◇). Interaction of treatment by day of experiment affected the time to end of treadmill test ($P < 0.05$). *Panel C:* pooled across exercise treatment, treatments were pregnant (▲) and non-pregnant (Δ). Interaction of pregnancy status by day of experiment affected the time to end of treadmill test ($P < 0.05$).

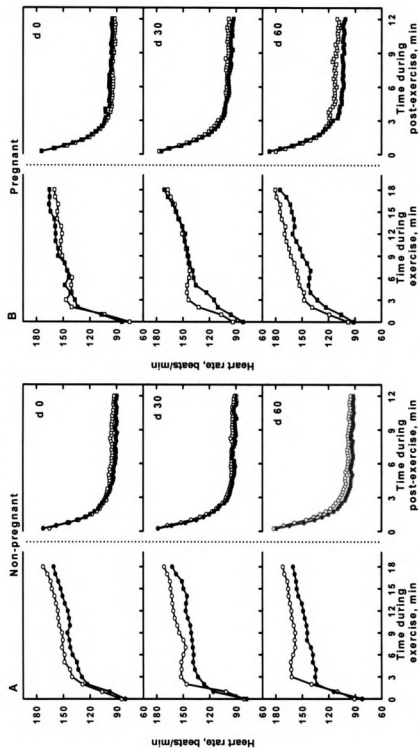


Figure 5.3. Heart rates during the exercise and post-exercise portions of treadmill test on d 0, 30, and 60 of the experiment. Treatments were non-pregnant, non-exercise (○); non-pregnant, exercise (●); pregnant, non-exercise (□); and, pregnant, exercise (■). Panel A shows results for non-pregnant cows, and Panel B shows results for pregnant cows. During exercise, heart rates were affected by the interaction of exercise treatment by pregnancy status by day of experiment by minute of test (pooled SEM = 7.31, $P < 0.05$). During 0 to 3 min in post-exercise, pregnancy by minute post-exercise interaction was significant (pooled SEM = 6.04, $P < 0.05$). During 3 to 6 min in post-exercise, the interaction of pregnancy status by day of experiment by minute tended to be significant (pooled SEM = 4.12, $P = 0.07$); and, treatment by day of experiment interaction was significant ($P < 0.05$). During 6 to 9 min in post-exercise, the interaction of pregnancy status by day of experiment tended to be significant (pooled SEM = 3.92, $P = 0.08$); and, treatment by minute post-exercise interaction tended to be significant ($P = 0.06$). During 9 to 12 min in post-exercise, the interaction of exercise treatment by pregnancy status by day of experiment was significant (pooled SEM = 3.88, $P < 0.05$).

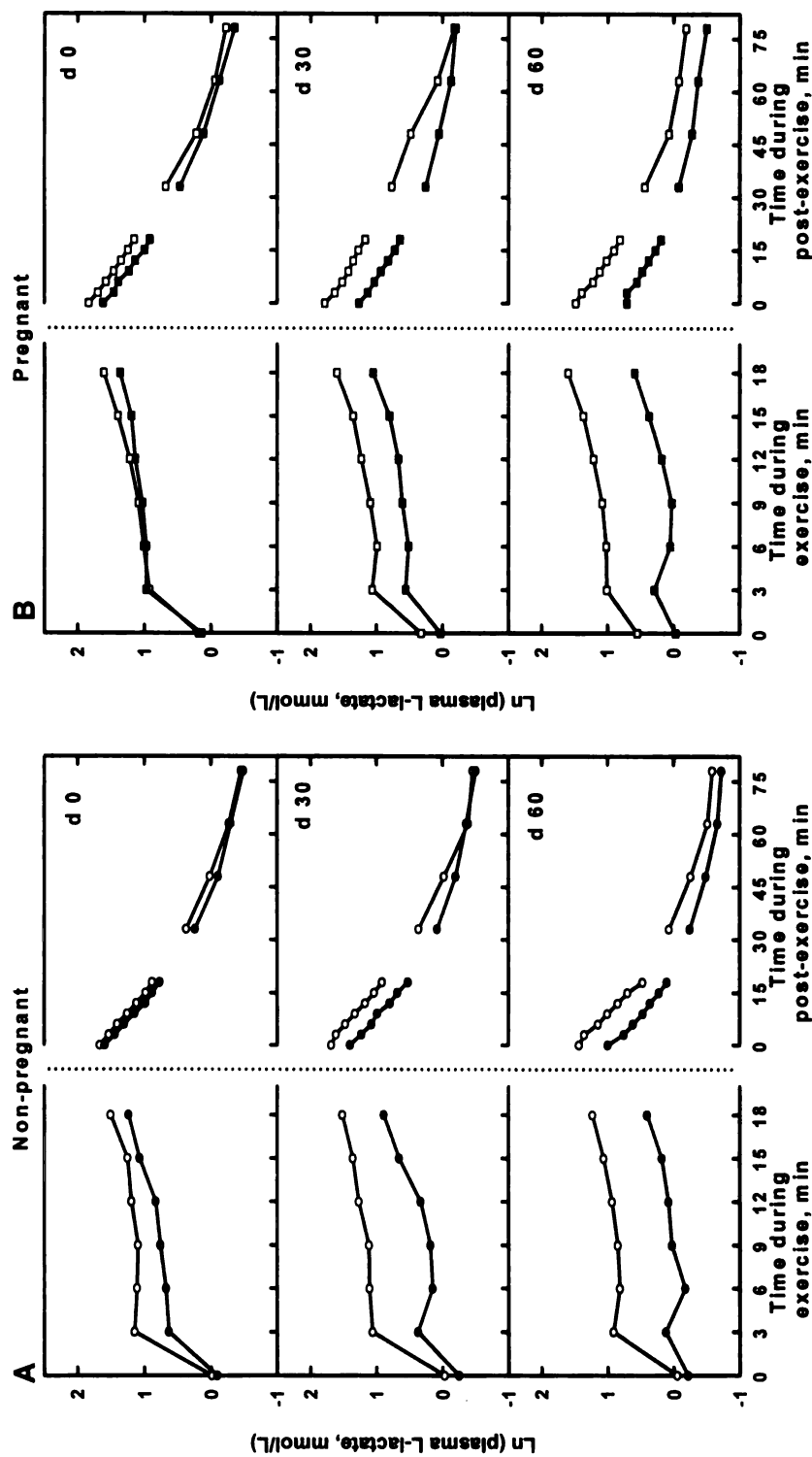


Figure 5.4. Natural logarithmically transformed plasma L-lactate concentrations during the exercise and post-exercise portions of treadmill test on d 0, 30, and 60 of experiment. Treatments were non-pregnant, non-exercise (○); non-pregnant, exercise (●); pregnant, non-exercise (□); and, pregnant, exercise (■). *Panel A* shows results for non-pregnant cows, and *Panel B* shows results for pregnant cows. During exercise, 0 to 18 min post-exercise, and 33 to 78 min post-exercise pooled SEM were 0.233, 0.204, and 0.157, respectively. During 0 to 18 min of exercise, the interaction of exercise treatment by pregnancy status by minute of test and the interaction of exercise treatment by day of experiment affected plasma lactate concentrations ($P < 0.02$). During 0 to 18 min post-exercise, plasma lactate was affected by interaction of exercise treatment by day of experiment and the interaction of pregnancy status by minute of test ($P < 0.05$). During 33 to 78 min post-exercise, the interaction of exercise treatment by minute was significant ($P = 0.007$). Also, the pregnancy status was significant between 33 to 78 min post-exercise ($P < 0.001$).

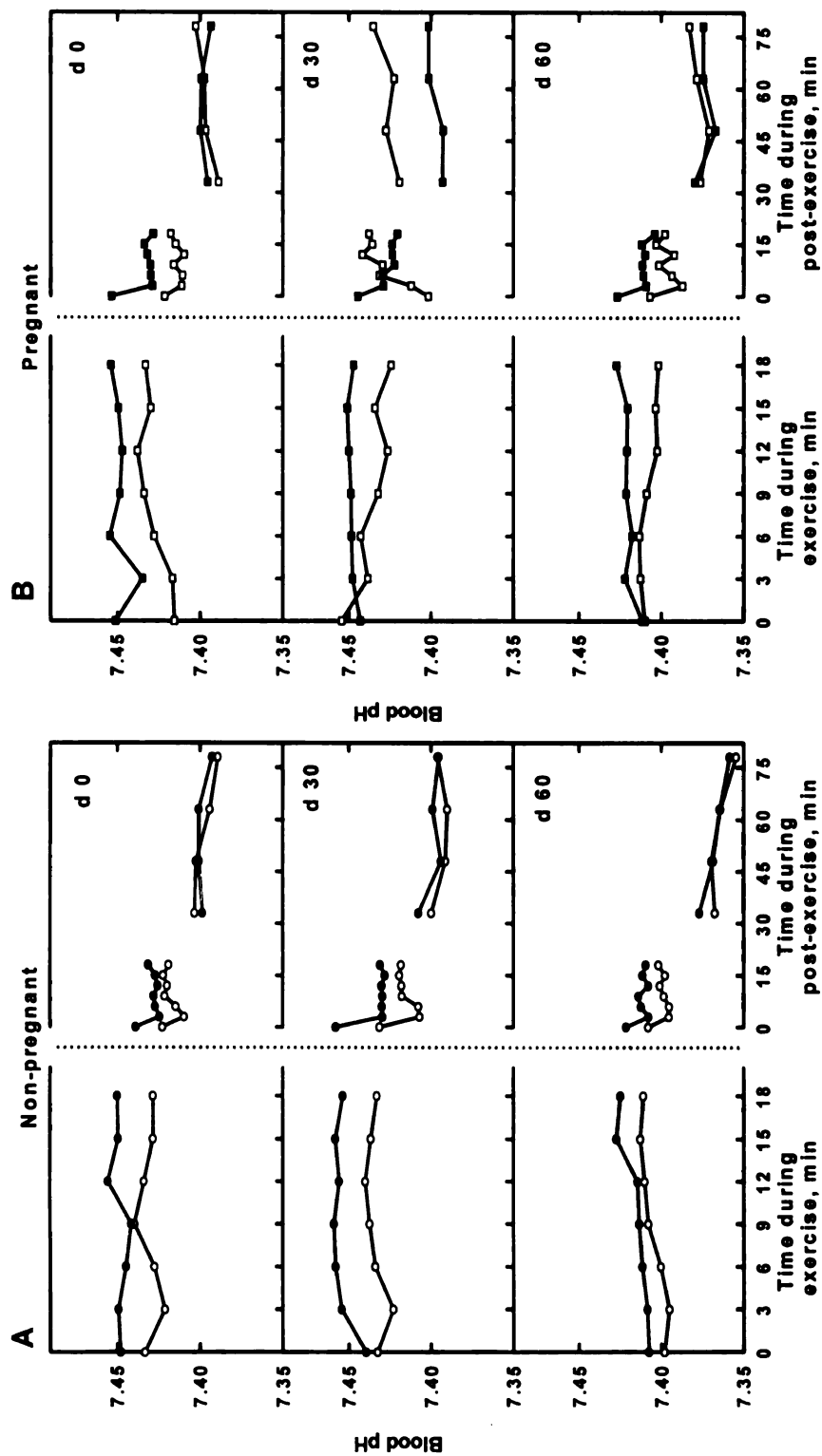


Figure 5.5. Blood pH during the exercise and post-exercise portions of treadmill test on d 0, 30, and 60 of experiment. Treatments were non-pregnant, non-exercise (○); non-pregnant, exercise (●); pregnant, non-exercise (□); and, pregnant, exercise (■). *Panel A* shows results for non-pregnant cows, and *Panel B* shows results for pregnant cows. During exercise, blood pH was affected by the interaction of exercise treatment by pregnancy status by day of experiment (pooled SEM = 0.0116, $P = 0.053$). During 0 to 18 min post-exercise, the interaction of exercise treatment by pregnancy status by day of experiment by minute of test (pooled SEM = 0.00957, $P = 0.088$) and interaction of pregnancy status by day of experiment by minute of test ($P < 0.001$) were significant. During 33 to 78 min post-exercise, the interaction of pregnancy status by minute of test was significant (pooled SEM = 0.0116, $P = 0.030$).

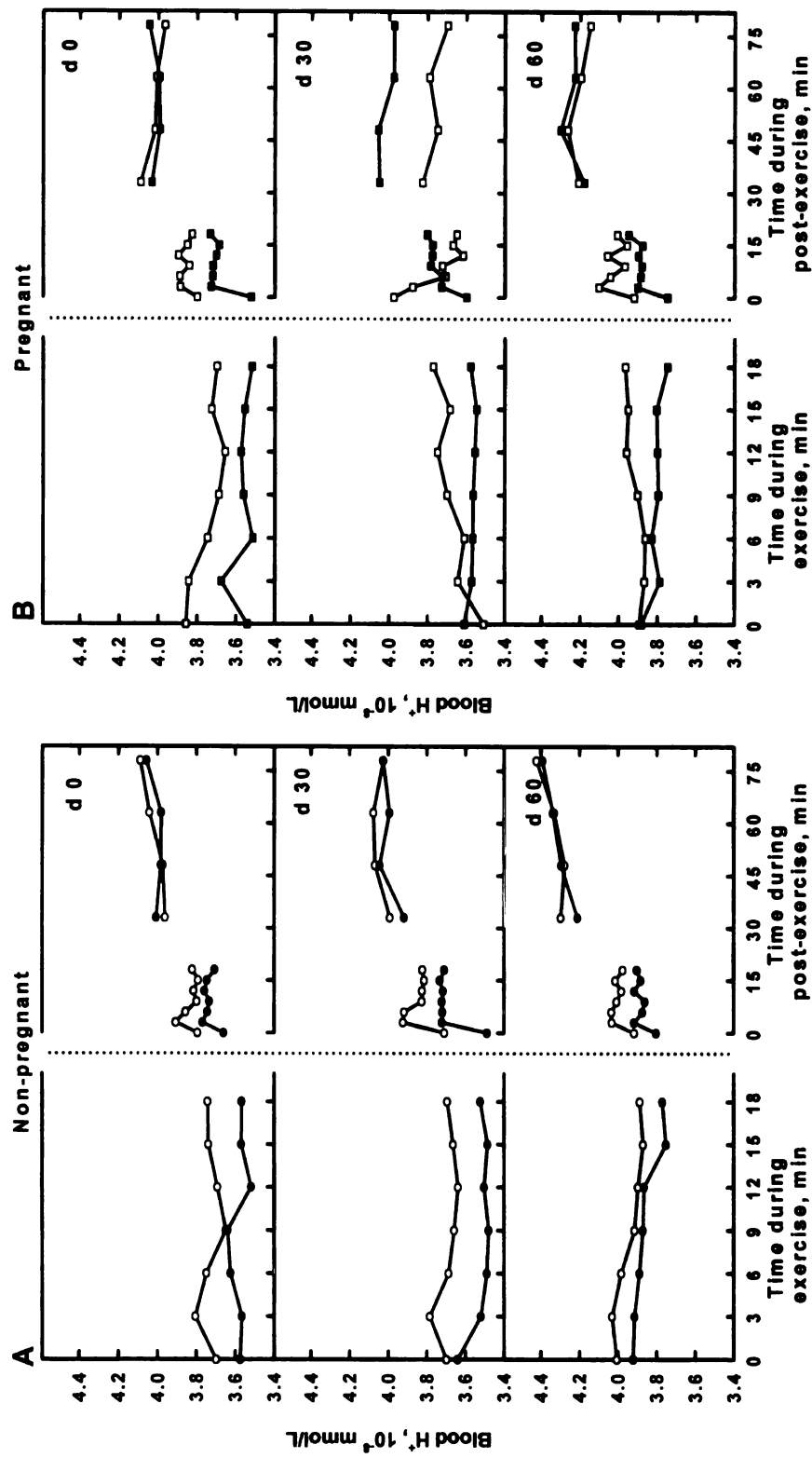


Figure 5.6. Concentrations of H^+ during the exercise and post-exercise portions of treadmill test on d 0, 30, and 60 of experiment. Treatments were non-pregnant, non-exercise (○); non-pregnant, exercise (●); pregnant, non-exercise (□); and, pregnant, exercise (■). *Panel A* shows results for non-pregnant cows, and *Panel B* shows results for pregnant cows. During exercise, H^+ concentrations were affected by the interaction of exercise treatment by pregnancy status by day of experiment by minute of test (pooled SEM = 0.104, $P = 0.067$). During 0 to 18 min post-exercise, the interaction of exercise treatment by pregnancy status by day of experiment by minute of test (pooled SEM = 0.087, $P = 0.061$) and interaction of pregnancy status by day of experiment by minute of test ($P < 0.001$) were significant. During 33 to 78 min post-exercise, the interaction of pregnancy status by minute of test was significant (pooled SEM = 0.083, $P = 0.038$).

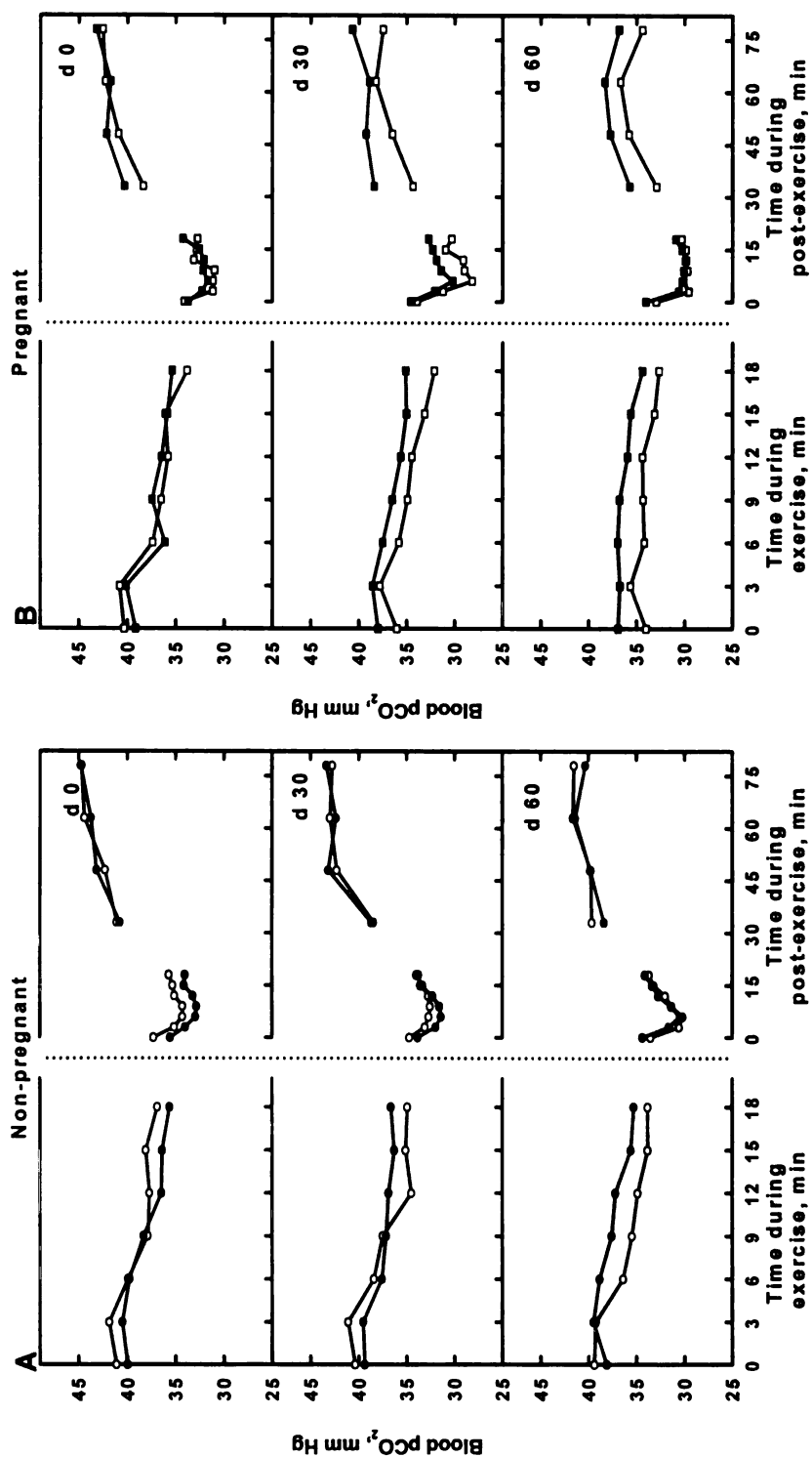


Figure 5.7. Blood partial pressure of carbon dioxide (pCO_2) during the exercise and post-exercise portions of treadmill test on d 0, 30, and 60 of experiment. Treatments were non-pregnant, non-exercise ($\square$); non-pregnant, exercise ($\circ$); pregnant, non-exercise ($\blacksquare$); and, pregnant, exercise ($\bullet$). *Panel A* shows results for non-pregnant cows, and *Panel B* shows results for pregnant cows. During exercise, blood pCO_2 was affected by minute of test ($P < 0.001$), pregnancy status ($P = 0.015$), and the interaction of exercise treatment by day of experiment ($P = 0.046$, pooled SEM = 1.597). During 0 to 18 min post-exercise, minute of test ($P < 0.001$), pregnancy status ($P = 0.003$), and the interaction of pregnancy status by day of experiment by minute of test (pooled SEM = 1.204, $P = 0.030$) were significant. During 33 to 78 min post-exercise, minute of test ($P < 0.001$), pregnancy status ($P < 0.001$), the interaction of pregnancy status by day of experiment ($P = 0.084$), and the interaction of exercise treatment by pregnancy status ($P = 0.057$) tended to be significant (pooled SEM = 1.645).

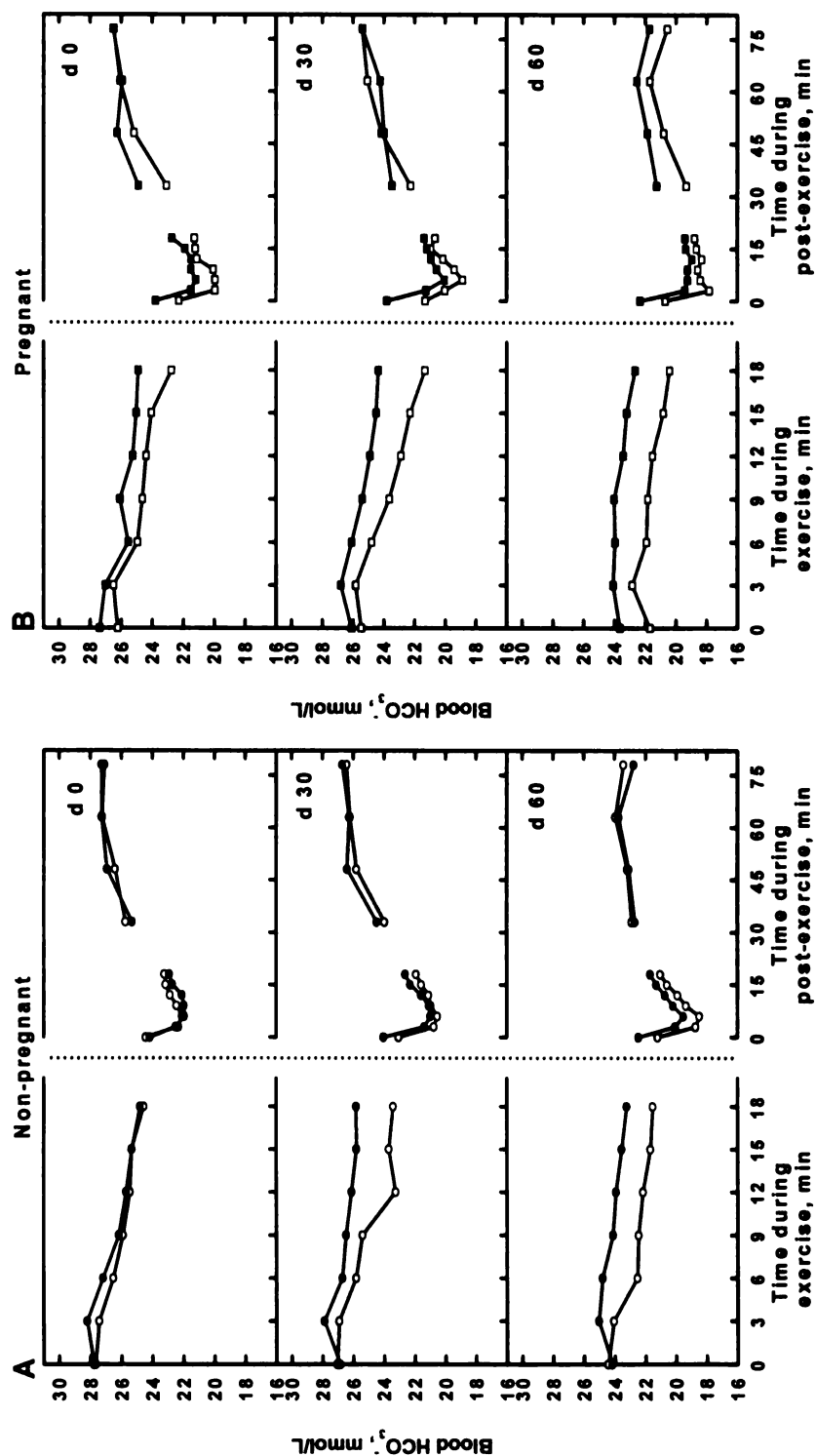


Figure 5.8. Blood HCO_3^- concentrations during the exercise and post-exercise portions of treadmill test on d 0, 30, and 60 of experiment. Treatments were non-pregnant, non-exercise (○); non-pregnant, exercise (●); pregnant, non-exercise (□); and, pregnant, exercise (■). *Panel A* shows results for non-pregnant cows, and *Panel B* shows results for pregnant cows. During exercise, blood HCO_3^- concentrations were affected by minute of test ($P < 0.001$), pregnancy status ($P = 0.039$), and exercise treatment ($P = 0.007$, pooled SEM = 1.099). During 0 to 18 min post-exercise, minute of test ($P < 0.001$), pregnancy status ($P = 0.013$), and the interaction of pregnancy status by day of experiment by minute of test (pooled SEM = 0.843, $P = 0.037$) were significant. During 33 to 78 min post-exercise, minute of test ($P < 0.001$) and pregnancy status ($P = 0.001$) was significant (pooled SEM = 0.852).

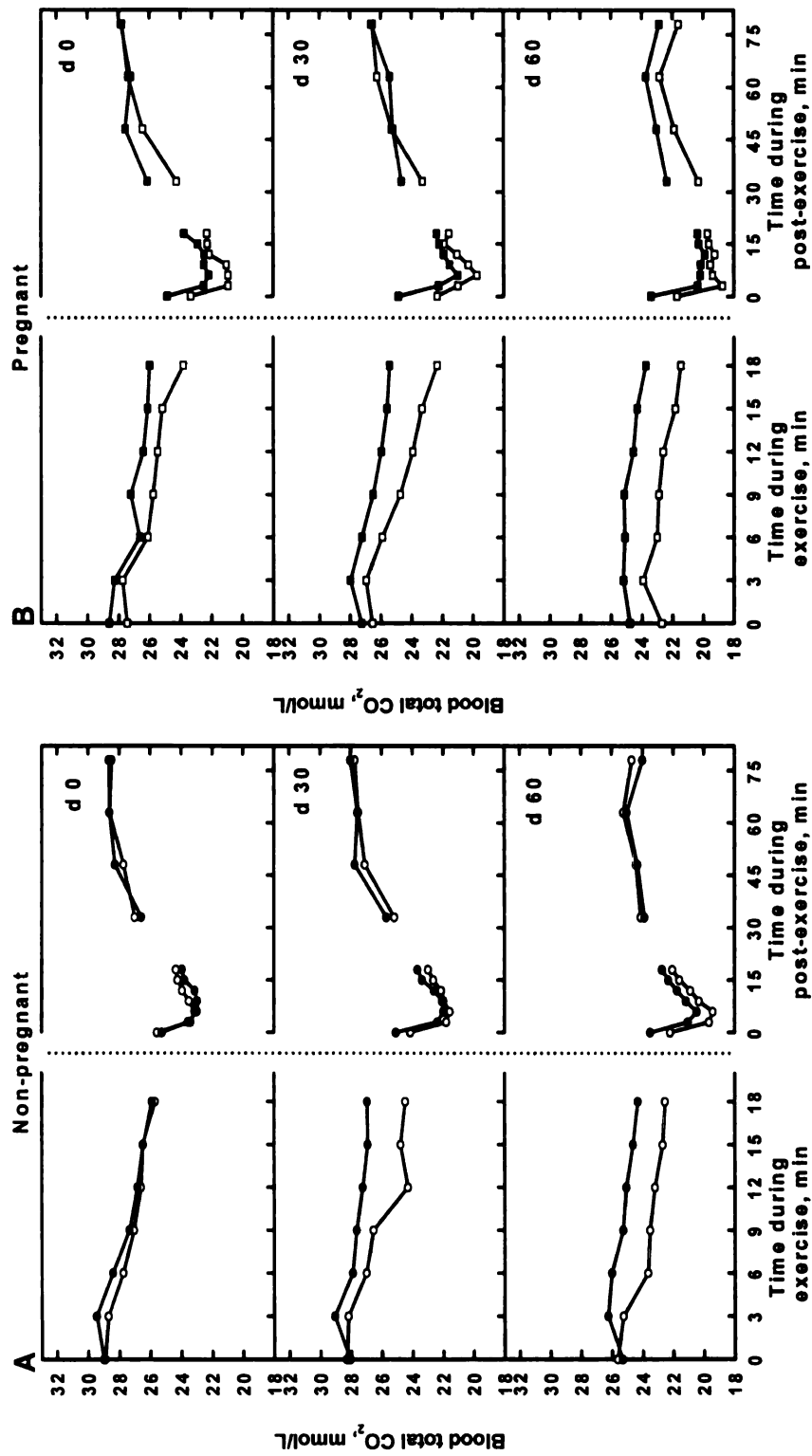


Figure 5.9. Blood total carbon dioxide (TCO_2) concentrations during the exercise and post-exercise portions of treadmill test on d 0, 30, and 60 of experiment. Treatments were non-pregnant, non-exercise ($\circ$); non-pregnant, exercise ($\square$); pregnant, non-exercise ($\bullet$); and, pregnant, exercise ($\blacksquare$). *Panel A* shows results for non-pregnant cows, and *Panel B* shows results for pregnant cows. During exercise, blood TCO_2 concentrations were affected by minute of test ($P < 0.001$), pregnancy status ($P = 0.037$), and exercise treatment ($P = 0.007$, pooled SEM = 1.140). During 0 to 18 min post-exercise, minute of test ($P < 0.001$), pregnancy status ($P = 0.011$), and the interaction of pregnancy status by day of experiment by minute of test (pooled SEM = 0.874, $P = 0.040$) were significant. During 33 to 78 min post-exercise, minute of test ($P < 0.001$) and pregnancy status ($P < 0.001$) was significant (pooled SEM = 0.972).

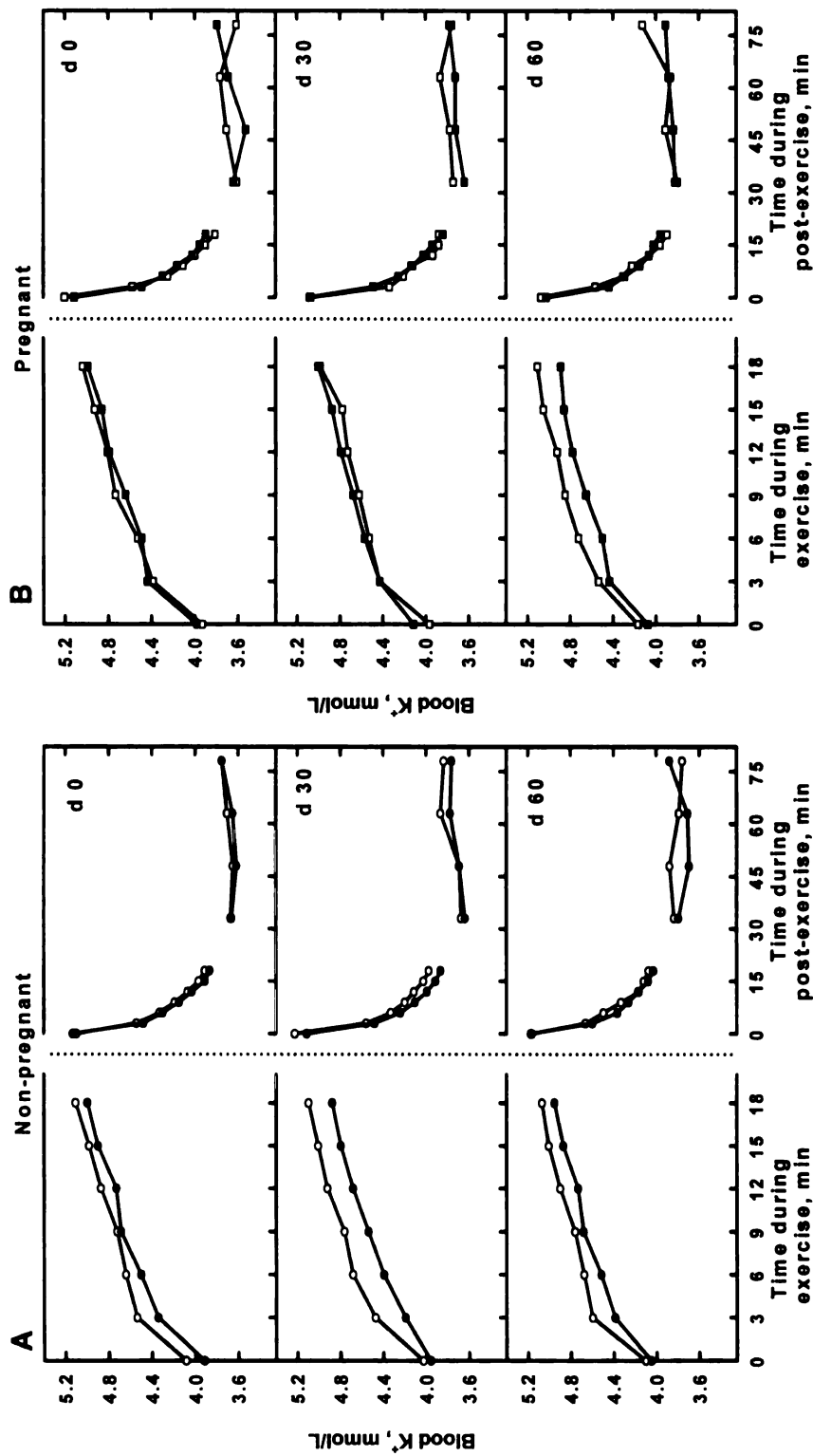


Figure 5.10. Blood K^+ concentrations during the exercise and post-exercise portions of treadmill test on d 0, 30, and 60 of experiment. Treatments were non-pregnant, non-exercise ($\circ$); non-pregnant, exercise ($\bullet$); pregnant, non-exercise ($\square$); and, pregnant, exercise ($\blacksquare$). *Panel A* shows results for non-pregnant cows, and *Panel B* shows results for pregnant cows. During exercise, blood K^+ concentrations were affected by minute of test ($P < 0.001$), and the interaction of treatment by pregnancy status by day of experiment ($P = 0.023$, pooled SEM = 0.0911). During 0 to 18 min post-exercise, minute of test ($P < 0.001$, pooled SEM = 0.0896) was significant. During 33 to 78 min post-exercise, minute of test ($P = 0.004$) and interaction of exercise treatment by pregnancy status by day of experiment by minute of test ($P = 0.022$) were significant (pooled SEM = 0.115).

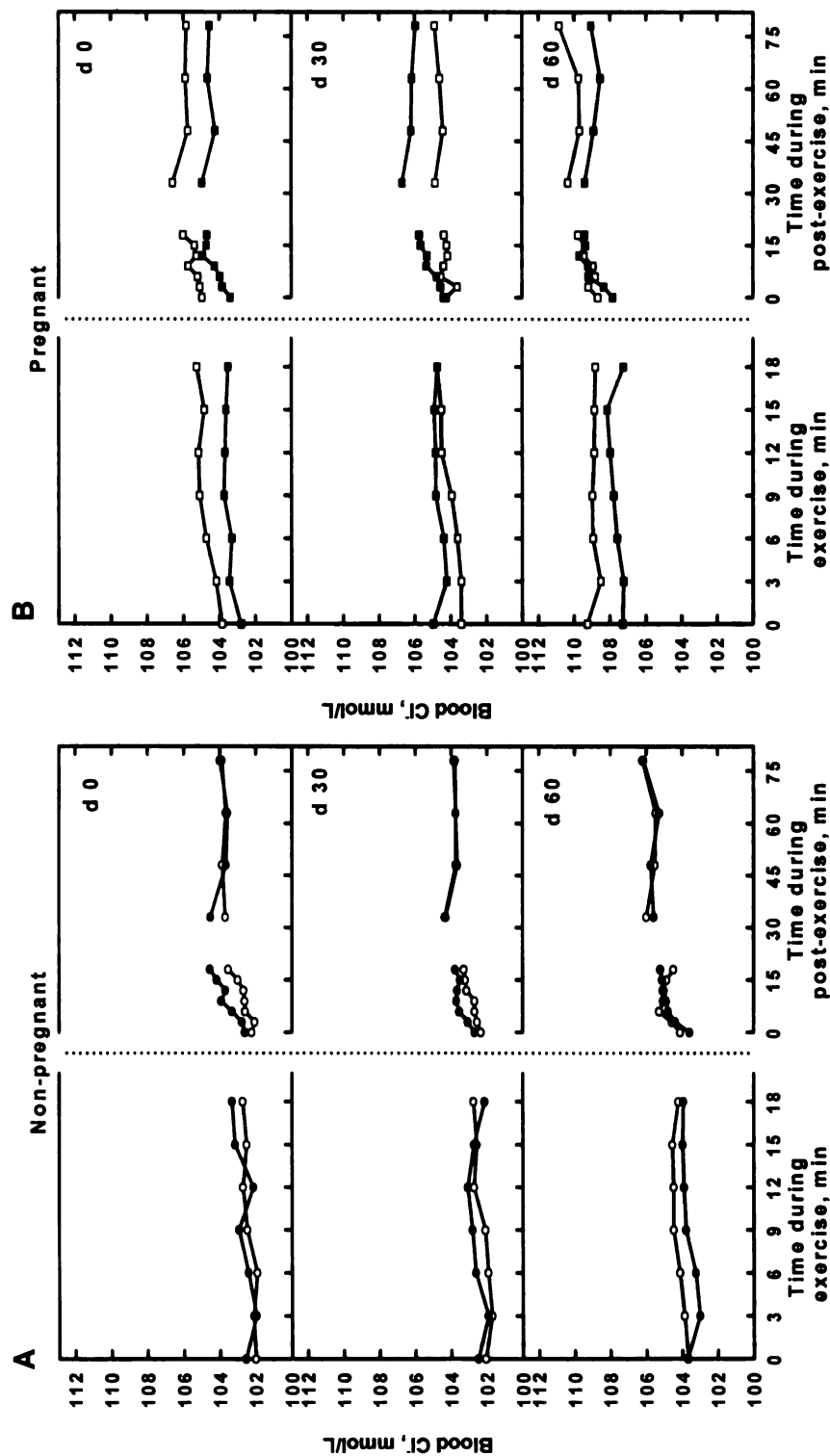


Figure 5.11. Blood Cl^- concentrations during the exercise and post-exercise portions of treadmill test on d 0, 30, and 60 of experiment. Treatments were non-pregnant, non-exercise (○); non-pregnant, exercise (●); pregnant, non-exercise (□); and, pregnant, exercise (■). *Panel A* shows results for non-pregnant cows, and *Panel B* shows results for pregnant cows. During exercise, blood Cl^- concentrations were affected by minute of test ($P < 0.001$), and the interaction of pregnancy status by day of experiment ($P = 0.005$, pooled $\text{SEM} = 1.061$). During 0 to 18 min post-exercise, minute of test ($P < 0.001$) and the interaction of pregnancy status by day of experiment ($P = 0.002$, pooled $\text{SEM} = 1.108$) were significant. During 33 to 78 minute post-exercise, minute of test ($P < 0.001$) and interaction of pregnancy status by day of experiment ($P = 0.025$) were significant (pooled $\text{SEM} = 1.014$).

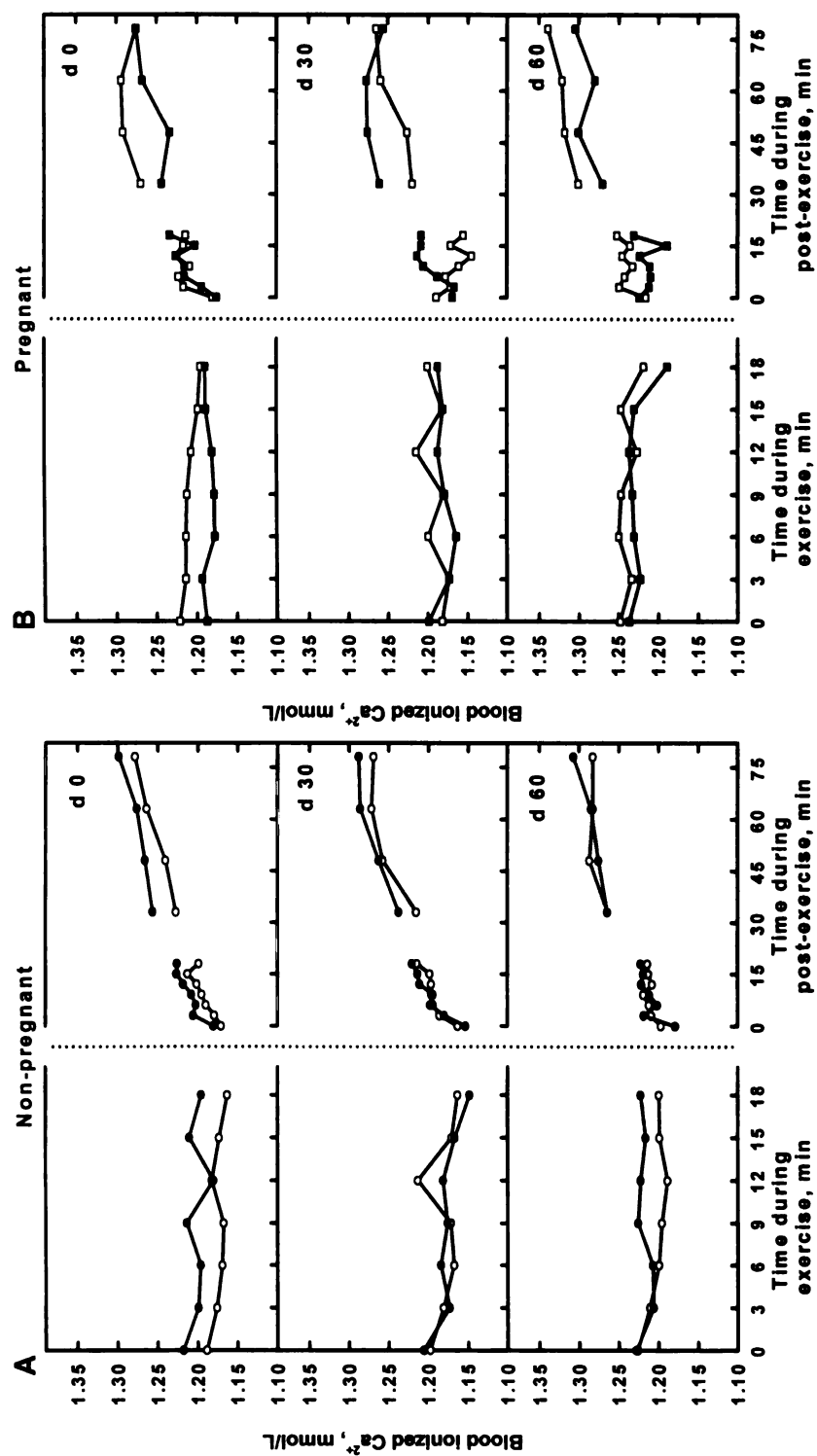


Figure 5.12. Blood ionized Ca^{2+} (iCa^{2+}) concentrations during the exercise and post-exercise portions of treadmill test on d 0, 30, and 60 of experiment. Treatments were non-pregnant, non-exercise (○); non-pregnant, exercise (●); pregnant, non-exercise (□); and, pregnant, exercise (■). *Panel A* shows results for non-pregnant cows, and *Panel B* shows results for pregnant cows. During exercise, blood iCa^{2+} concentrations were affected by minute of test ($P = 0.003$, pooled SEM = 0.0286). During 0 to 18 min post-exercise, minute of test ($P < 0.001$, pooled SEM = 0.0268) was significant. During 33 to 78 min post-exercise, minute of test ($P < 0.001$) and interaction of exercise treatment by pregnancy status by day of experiment by minute of test ($P = 0.10$) tended to be significant (pooled SEM = 0.0227).

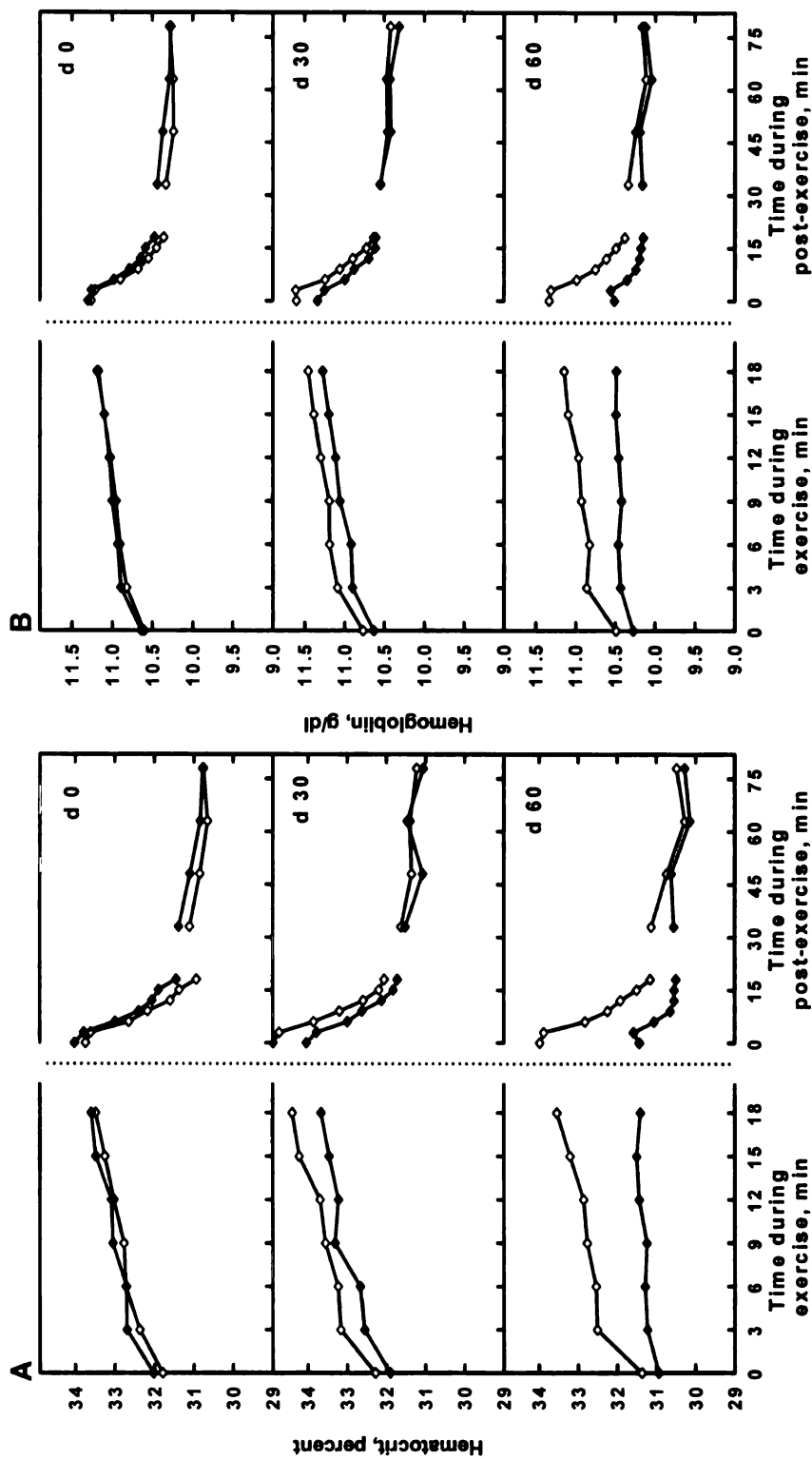


Figure 5.13. Blood hematocrit (HCT) and hemoglobin (HGB) concentrations during the exercise and post-exercise portions of treadmill test on d 0, 30, and 60 of experiment. Pooled across exercise treatments, symbols represent non-pregnant (◇) and pregnant (◆). *Panel A* shows results for HCT concentrations and *Panel B* shows results for HGB concentrations. During exercise, blood HCT and HGB concentrations were affected by minute of test ($P < 0.001$) and the interaction of pregnancy status by day of experiment by minute of test ($P = 0.035$, pooled SEM = 0.831 and 0.256, respectively). During 0 to 18 min post-exercise, minute of test ($P < 0.001$), interaction of pregnancy status by day of experiment ($P < 0.001$), and interaction of pregnancy status by minute of test ($P = 0.042$, pooled SEM = 0.735 and 0.249, respectively) were significant. During 33 to 78 min post-exercise, minute of test was significant ($P < 0.001$, pooled SEM = 0.656 and 0.226, respectively).

CHAPTER 6

EXERCISE TRAINING OF PREGNANT DAIRY COWS AFFECTS GLUCOSE AND LIPID METABOLISM

ABSTRACT

Objective of this experiment was to determine if prepartum exercise training affects glucose and lipid metabolism of pregnant and non-pregnant cows during exercise tests on a treadmill. Pregnant (n = 26) and non-pregnant (n = 26), non-lactating multiparous Holstein cows were assigned to a factorial arrangement of treatments of exercise training (**E**) or no exercise (**NE**), and pregnant (**P**) or non-pregnant (**NP**). Exercise training was walking (1.25 to 1.5 h, 3.25 km/h) every other day in an outdoor mechanical walker for 70 d. All cows completed treadmill tests on d 0, 30, and 60 of experiment (d 70, 40, and 10 before expected parturition). After 60 d of exercise training, plasma glucose, lactate, and BHBA were greater for NE cows than E cows during the treadmill exercise tests. Exercise treatment did not affect plasma NEFA concentrations during the treadmill tests. Cows in P or E treatments had reduced concentrations of insulin and glucagon during the treadmill test on d 60 compared with NP or NE cows. On d 60 during acute exercise, P cows had lower plasma glucose and greater lactate, NEFA and BHBA concentrations than NP cows. Prepartum exercise training supported the maintenance of glucose and NEFA concentrations during exercise tests of similar workloads. Concentrations of insulin and glucagon required to maintain glucose homeostasis were reduced for cows undergoing exercise training. Therefore, we conclude that exercise training of dairy cows stimulated adaptations of glucose metabolism and its endocrine modulation to maintain glucose homeostasis.

INTRODUCTION

Prepartum exercise of dairy cows may be a management tool to stimulate physiological and metabolic processes prior to parturition to support homeostasis and ease the transition from the non-lactating to lactating state. During the periparturient period, glucose and lipid homeostasis changes rapidly to support the increased demands of gestation, lactogenesis and lactation (Bauman and Currie, 1980; Collier et al., 1984). Around parturition, changes in concentrations of insulin, somatotropin, glucocorticoid and catecholamine may stimulate lipolytic, gluconeogenic, and glycogenolytic pathways (Bauman and Currie, 1980; Collier et al., 1984; Drackley, 1999; Drackley et al., 2001). Excessive catabolic activities (excess mobilization of adipose tissue and depletion of liver glycogen) during the periparturient period to support lactogenesis and lactation can be detrimental. Exercise training of dairy cows may induce adaptations of glucose and lipid metabolism and affect endocrine control of metabolism. Prepartum exercise may improve the maintenance of homeostasis of carbohydrate and lipid metabolism during the periparturient period which is essential for productive, healthy dairy cows.

In humans and rats, prior exercise training altered carbohydrate and lipid metabolism during a bout of exercise. Plasma glucose utilization during exercise was reduced after a period of exercise training (Coggan, 1997; Holloszy and Kohort, 1996). The rate of appearance, rate of disappearance, and oxidation of glucose was reduced by 30% during prolonged exercise following 12 wk of moderate intensity training (Coggan et al., 1990). Liver tissue from trained rats had greater gluconeogenic capacity compared with liver tissue from untrained rats (Sumida et al., 1993).

In humans, endurance exercise training enhances the capacity of skeletal muscle to oxidize fatty acids (Mole et al., 1971). At the same workload, plasma fatty acid

concentrations were reduced after exercise training compared with values prior to training (Martin, 1997). Additionally, chronic exercise increased the uptake and oxidation of fatty acids by skeletal muscle during prolonged exercise compared with non-exercised skeletal muscle (Kiens, 1997; Turcotte et al., 1992). Total oxidation of fatty acids during exercise was 60% greater for exercised-trained men compared with non-exercised men (Kiens, 1997).

The objective of this experiment was to determine if prepartum exercise training alters circulating metabolites and hormones during treadmill exercise tests and if the additional dynamics of pregnancy alter physiological responses to exercise training. The hypothesis was that exercise training improves the glucose and lipid homeostasis of cows during exercise.

MATERIAL AND METHODS

Animal care and experimental procedures were approved and conducted under standards of the Michigan State University All-University Committee on Animal Use and Care, application number 05/98-081-00. Guidelines were in compliance with National Institutes of Health and the Guide for the Care and Use of Laboratory Animals. More detailed descriptions of the experimental methods are presented in Chapter 5.

Cows and Treatments

Twenty-six pairs of non-pregnant and pregnant multiparous Holstein cows were blocked by parity and expected calving date (ECD) of the pregnant animal as described previously (Chapter 5). Pairs of cows were assigned randomly to exercise treatments. Treatments in a factorial arrangement were exercise training (E) or no exercise (NE),

and pregnant (P) or non-pregnant (NP). Exercise training of the pair was conducted every other day until parturition of the pregnant cow.

Exercise training began approximately 70 d before ECD of pregnant cows and was accomplished in an outdoor mechanical walker with a circular lane that was 90-cm wide and 33.8-m long approximately 36.5 m from alley in the tie-stall barn where cows were housed (Chapter 3). Cows assigned to NE remained in tie-stalls blocked from feed and water while cows assigned to E were walked. From d 2 to 28 of experiment, E cows walked for 1.25 h at 3.25 km/h and from d 32 to parturition for 1.5 h at 3.25 km/h. To assess changes in glucose and lipid metabolism, all cows completed treadmill exercise tests on d 0, 30, and 60 of experiment (d 70, 40, and 10 before ECD). On the day of their treadmill test (d 0, 30 and 60), cows were not exercised in the mechanical walker.

Treadmill Exercise Test

The treadmill exercise test consisted of increases of workload every 3 min while cows walked on the treadmill (Classic Treadmill Conditioner, W. H. D. Lindsey, Kenilworth, Australia) as described previously (Chapter 5). An indwelling catheter was inserted into jugular vein 1 d prior to the treadmill test. Blood was sampled from the catheter into 10-ml syringes containing 20 IU of Na-heparin per ml of blood at min 0 and prior to each increase in workload during the test (every 3 min). Blood sampling continued post-exercise every 3 min from min 0 through 18 (standing on treadmill platform) and every 15 min from min 33 through 78 (in tie-stalls). Blood from the syringe containing Na-heparin was transferred to two glass test tubes with one containing aprotinin (500 KIU/ml of blood) and EDTA (1 mg/ml of blood), and the other NaF (2.5 mg/ml of blood).

Blood tubes with NaF or aprotinin and EDTA were centrifuged (2000 x *g*) within 15 min of collection, and plasma was harvested and stored at -20°C until analysis. Plasma from NaF tubes was analyzed for L-lactate, glucose, BHBA, and NEFA concentrations. Plasma from aprotinin and EDTA tubes was analyzed for insulin and glucagon concentrations.

Metabolite and Hormone Assays

Glucose concentrations were determined colorimetrically with a glucose oxidase assay modified for microtiter plates (510-A, Sigma Diagnostics, St. Louis, MO). Intra- and inter-assay coefficients of variation were 1.7 and 4.4%, respectively. L-lactate concentrations were determined using the Sport Lactate Analyzer 1500 (YSI, Yellow Springs, OH). Intra- and inter-assay coefficients of variation were 1.6 and 4.3%, respectively.

Concentrations of NEFA, BHBA, insulin, and glucagon were determined on a subset of samples collected during the exercise portion of the test (min 0, 6, 12, and 18) and during the post-exercise portion min 0, 3, 9, 15, 48, and 78. Concentrations of NEFA were determined with an enzymatic, colorimetric assay (NEFA-C assay, Wako Chemical, USA, Richmond, VA) modified for microtiter plates. Intra- and inter-assay coefficients of variation were 1.9 and 5.4%, respectively. Concentrations of BHBA were determined with an enzymatic assay (310-A, Sigma Diagnostics, St. Louis, MO) modified for microtiter plates. Intra- and inter-assay coefficients of variation were 2.3 and 5.8%, respectively. Insulin concentrations were determined using a Coat-A-Count kit (TKINX, Diagnostics Product Corp, Los Angeles, CA). Intra- and inter-assay coefficients of variation were 5.9 and 10.4%, respectively. Glucagon concentrations were determined by a radioimmunoassay (GL-32K, Linco Research, Inc., St. Charles, MO). Intra- and inter-assay coefficients of variation were 2.6 and 8.3%, respectively.

Statistical Analysis

Data were analyzed by method of ANOVA using mixed model procedures of SAS (1999). The appropriate covariance structure for the repeated measures (day of the experiment and minute of test) was determined using the goodness of fit criteria of Aiaike's Information Criterion and Schwarz's Bayesian Criterion. Plasma L-lactate, NEFA, BHBA, insulin, and glucagon concentrations had unequal variances across minute of treadmill test and were transformed using the natural logarithm before analyses. To simplify presentation and interpretation of results, generalized least squares means of natural logarithmically transformed plasma L-lactate, NEFA, BHBA, insulin, and glucagon concentrations are shown as back-transformed least squares means. Other results (plasma glucose) are presented as generalized least squares means with the standard error of mean. Statistical significance was declared at $P < 0.05$; trends were noted at $0.05 < P < 0.10$.

The frequency of observations per cow for blood and plasma variables was different among individual tests due to the differences in the time to end of the exercise portion of tests as described previously (Chapter 5). The frequency of experimental observations per minute of tests was eight or less at min 25 to 28 for NP, NE; at min 22 to 28 for P, NE; at min 29 to 39 for NP, E; and, at min 25 to 29 for P, E. Because of the reduced frequency of observations beyond 18 min of the tests, only the data from min 0 to 18 were used in analyses, so that all treatments and tests were represented with a similar number of observations. Additionally, treadmill tests in which the cow's heart rate never was greater than 150 BPM or the time to end of the test was not greater than 16 min were excluded from analyses of dependent variables; 32 of 152 tests were excluded (Chapter 5).

Dependent variables measured during the treadmill tests were analyzed as three data sets: 1) exercise portion of test during min 0 through 18; 2) post-exercise during

min 0 through 18; and, 3) post-exercise during min 33 through 78. Models included the random effect of cow nested within treatment (T) and pregnancy status (PS), the fixed effects of T, PS, and block, and the repeated measures of day of experiment and minute of test. All two-, three-, and four-way interactions of T, PS, day of experiment and minute of test were included in the model. The covariance structures of the data were unstructured for day of experiment and auto-regressive first order for minute of test.

RESULTS AND DISCUSSION

Treadmill tests were done to characterize the acute responses of plasma metabolite and hormone concentrations during exercise and to examine possible adaptations of these responses after 60 d of exercise training. Acute responses of metabolite and hormone concentrations to a bout of exercise have been described in sheep (Bell et al., 1982; Brockman, 1979a; Harman and Pethick, 1994; Judson et al., 1976; Pethick et al., 1987). However, effects of exercise training and(or) gestation have not been evaluated in dairy cows. These factors may cause additional adaptations or alterations of energy metabolism.

Carbohydrate Metabolism

Plasma glucose. Regardless of T, PS, or day of the experiment, plasma glucose concentrations increased as time during the exercise portion of the treadmill test progressed ($P < 0.001$; Figure 6.1). During post-exercise min 0 to 18, plasma glucose concentrations were greatest at min 3 and then declined as time increased ($P < 0.001$; Figure 6.1). During min 33 to 78 post-exercise pooled across T, PS and day of the

experiment, plasma glucose concentrations continued to decline and were approximately equivalent to values at the start of the test ($P < 0.001$; Figure 6.1).

Sheep that exercised for 45 min at 5.5 km/h up a 8% slope had increased plasma glucose concentrations compared with resting values (approximately 85 vs. 65 mg/dl; Brockman and Halvorson, 1981). Additionally, plasma glucose concentrations remained greater than resting values at 15 and 45 min post-exercise. Overall for these studies and the current experiment, plasma glucose concentrations during acute exercise of ruminants was 20 to 30% greater than resting values. In the current study, glucose concentrations were greatest 3 min after cessation of exercise, possibly because peripheral tissue uptake and oxidation of glucose were reduced while hepatic output was maintained. It cannot be ascertained directly if changes in the uptake of glucose by peripheral tissues or output of glucose by hepatic gluconeogenesis, increased glycogenolysis, or reduced uptake of glucose may have contributed to increased circulating concentrations during and after exercise.

During the exercise portion of test, pooled across PS, day of experiment, and minute of test, cows in E tended to have reduced concentrations of plasma glucose compared with those in NE ($P = 0.086$; Table 6.1). Pregnant cows had reduced concentrations of plasma glucose compared with NP cows during exercise portion of test pooled across days of experiment ($P < 0.01$; Table 6.1). On d 0 of experiment during the exercise portion of the test, plasma glucose concentrations at any given minute were similar among treatment groups (Figure 6.1). On d 30 and 60 of experiment, cows in treatment combinations of P, NE; NP, NE; and NP, E had similar concentrations of plasma glucose, whereas cows in P, E had reduced plasma glucose as time increased compared with all other groups (Figure 6.1).

During the post-exercise portion of the test on d 0 of experiment, plasma glucose concentrations at any given minute were similar among treatment groups. During post-

exercise min 0 to 18, T or PS did not affect plasma glucose concentrations on any day of experiment. As time increased during min 0 to 18 post-exercise, plasma glucose concentrations were greatest at min 3 compared with all other minutes regardless of day of experiment, T, and PS ($P < 0.001$; Figure 6.1). On d 30 and 60 as time increased post-exercise during min 33 to 78, cows in P, E had lower plasma glucose concentrations compared with those in NP, NE; NP, E; and P, NE (effects of day, minute of test, and interaction of T by PS, $P < 0.01$; Figure 6.1). Post-exercise min 33 to 78, plasma glucose concentrations were less for cows in P, E compared with all other groups ($P < 0.015$, Table 6.1).

Plasma lactate. Pooled across T, PS, and day of the experiment, plasma lactate concentrations increased as time during exercise portion of the treadmill test progressed, as an indicator of greater anaerobic metabolism by exercising muscle tissue ($P < 0.001$; Figure 6.2). During min 0 to 18 post-exercise, plasma lactate concentrations were greatest at min 0 then declined as time increased ($P < 0.001$; Figure 6.2). Post-exercise min 33 to 78, plasma lactate concentrations continued to decline and were less than values measured at the start of treadmill test ($P < 0.001$; Figure 6.2).

After exercising for 45 min at 5 km/h up a 5% slope, plasma lactate concentrations of sheep peaked at 2.75 mmol/L from 0.5 mmol/L at rest and remained elevated for 45 min post-exercise (Brockman, 1979b). In sheep and cattle, exercise increased circulating concentrations of lactate based on this study and the current experiment. However, it is not known if increased anaerobic metabolism or reduced hepatic uptake of lactate has a greater impact on observed concentrations. Post-exercise, the reduced entry rate of lactate into the blood and continued hepatic uptake of lactate may have caused the steady decline of plasma lactate concentrations.

On d 0 of experiment, plasma lactate concentrations changed similarly among treatment groups during the exercise and post-exercise portions of the test as time increased (Figure 6.2). During the exercise portion of the test on d 30 and 60 of experiment, plasma lactate concentrations were greater for cows in NP, NE and P, NE as time of test increased compared with those in P, E and NP, E (interaction of T by day of experiment, $P < 0.001$; Figure 6.2). During the exercise portion of the test, the interaction of T by PS by minute of test also was significant ($P = 0.017$; Figure 6.2). Pooled across day of experiment, cows in NP, NE and P, NE had greater concentrations of lactate than cows in P, E and NP, E as time of test increased.

During min 0 to 18 post-exercise on d 30 and 60, cows that were exercised had reduced plasma lactate compared with non-exercised cows (interaction of T by day of experiment, $P = 0.045$; Figure 6.2). During min 33 to 78 post-exercise pooled across T, day of experiment, and minute of test, P cows had greater plasma lactate concentrations than NP cows (1.0 vs. 0.8 mmol/L; $P < 0.001$; Figure 6.2). Pooled across PS and day of experiment, the plasma lactate concentrations of E cows were less than for NE cows as minute post-exercise (min 33 to 78) increased (interaction of T by minute post-exercise, $P = 0.007$; Figure 6.2).

Based on the findings of the current study, exercise training and day of gestation altered carbohydrate metabolism of dairy cows as reflected by differences in circulating glucose and lactate concentrations. Glucose and lactate concentrations were less for E cows than NE cows at similar workloads. The reduced concentrations of E cows may reflect differences of rate of appearance, disappearance, or oxidation of glucose or lactate. In humans, using tracer dilution methodology, the rates of appearance and disappearance, and oxidation of glucose were reduced by 30% following 12 wk of moderate intensity training (Coggan et al., 1990). After horses underwent exercise training, plasma venous lactate was less during an acute exercise test compared with

values prior to exercise training (Aguera et al., 1995; Rodiek et al., 1987). It is not possible to ascertain whether training induced adaptations of circulating concentrations of glucose or lactate were due to changes of substrate uptake, output, or oxidation of peripheral and hepatic tissues.

Lipid Metabolism

Plasma NEFA. Pooled across T, PS, and day of experiment, plasma NEFA concentrations increased slightly as time during the exercise portion of the treadmill test increased compared with values at min 0 ($P < 0.001$; Figure 6.3). Post-exercise min 0 to 15, plasma NEFA concentrations were greatest at min 6 and then declined with time ($P < 0.001$; Figure 6.3). During min 48 to 78 post-exercise, plasma NEFA concentrations were less than values measured at the start of treadmill test ($P = 0.052$; Figure 6.3).

In the current study, concentrations of NEFA as indicators of lipolysis were not affected greatly during exercise. However, glycerol concentrations, another indicator of lipolysis, were greater for sheep compared with resting values after exercise of 5 km/h for 45 to 75 min up a 10% incline (0.125 vs. 0.025 meq/L; Brockman, 1979b). Arterial plasma NEFA concentrations were greater than resting values in sheep after exercising for 60 min at 5 km/h up a 27% slope (1.145 vs. 0.227 mmol/L; Jarrett et al., 1976). Possibly, differences in the duration and intensity of exercise may explain the differences in responses between these studies.

On d 0 of the experiment, plasma NEFA concentrations changed similarly among treatment groups during the exercise and post-exercise portions of the test with time (Figure 6.3). Additionally, exercise training did not affect concentrations of NEFA during the exercise portion of test on d 30 and 60. During the exercise portion of the test, P

cows tended to have greater concentrations of NEFA compared with NP cows as time of test increased (interaction of PS by minute of test, $P = 0.10$; Figure 6.3).

During min 0 to 15 post-exercise on d 30 and d 60, E cows tended to have greater NEFA concentrations than NE cows as time increased (interaction of T by day of experiment by minute post-exercise, $P = 0.058$; Figure 6.3). Additionally, on d 60, P cows had greater NEFA concentrations than NP cows as time increased (interaction of PS by day of experiment by minute post-exercise, $P = 0.004$; Figure 6.3). Post-exercise min 33 to 78, NEFA concentrations were greatest on d 60 for P cows compared with other days and NP cows (interaction of PS by day of experiment, $P = 0.003$; Figure 6.3).

Plasma BHBA. Pooled across T, PS, and day of the experiment, plasma BHBA concentrations as time increased during the exercise portion of test were slightly less than values at min 0 ($P < 0.001$; Figure 6.4). Post-exercise min 0 to 15, plasma BHBA concentrations were greatest at min 15 ($P < 0.001$; Figure 6.4). During min 48 to 78 post-exercise, plasma BHBA concentrations did not change and were similar to values measured at the start of treadmill test. Similarly, arterial BHBA concentrations were not different for sheep exercised for 60 min at 5 km/h up a 27% slope (Jarrett et al., 1976).

During the exercise portion, there was a tendency for the interaction of T by day of experiment by minute of test to affect BHBA concentrations ($P = 0.066$; Figure 6.4). On d 0, all groups had similar BHBA concentrations during the exercise and post-exercise portions of the test regardless of minute of test. On d 30 and d 60 during exercise pooled across pregnancy status, E cows had reduced BHBA concentrations (18% reduction from min 0 to 18) compared with NE cows (10% reduction from min 0 to 18) as time of test increased (Figure 6.4). Pooled across exercise treatment, day of experiment, and minute of test, pregnancy status did not affect the BHBA concentrations during any portion of the treadmill test.

On d 0, 30 and 60 during 0 to 15 min post-exercise, changes of BHBA concentrations among E and NE cows were different as time progressed (interaction of T by day of experiment by minute of test, $P = 0.005$; Figure 6.4). During min 48 to 78 post-exercise, cows in NP, NE and NP, E tended to have similar BHBA concentrations and those in P, E tended to have greater concentrations than cows in P, NE (interaction of T by PS, $P = 0.087$; Figure 6.4).

As a result of exercise training, NEFA and BHBA concentrations were affected during exercise and post-exercise portions of tests. At similar workloads during the exercise portion of tests, concentrations of NEFA were not different for E cows compared with NE cows, whereas BHBA concentrations were less for E cows compared with NE cows. Rates of lipolysis and uptake of NEFA and BHBA by peripheral tissues, ketogenesis, or increased oxidation of lipid may have contributed to these differences between treatment groups .

Previously, in humans, plasma fatty acid concentrations were less, and plasma fatty acid oxidation was reduced after exercise training compared with values prior to training (Martin, 1997). During prolonged exercise, exercise training increased the uptake of fatty acids and oxidation of fatty acids by skeletal muscle compared with non-exercised skeletal muscle (Kiens, 1997; Turcotte et al., 1992).

Glucoregulatory Hormones

Plasma insulin. During the exercise portion of the treadmill test pooled across T, PS, and day of the experiment, plasma insulin concentrations declined as time of test increased ($P < 0.001$; Figure 6.5). During, post-exercise min 0 to 15 min, plasma insulin concentrations were greatest at min 9 and remained greater than values at the start of the test ($P < 0.001$; Figure 6.5). From min 48 to 78 post-exercise, plasma insulin concentrations were similar to values measured at the start of treadmill test. Similar to

the current experiment, sheep that were exercised 45 min at 5.5 km/h up a 8% slope had less plasma insulin compared with resting values (Brockman, 1987). In sheep, plasma insulin concentrations also were greater 15 min post-exercise compared with 45 min post-exercise and resting values (Brockman and Halvorson, 1981).

On d 0 of experiment, plasma insulin concentrations were altered similarly as time during exercise and post-exercise increased among all treatment groups. On d 30 and d 60 pooled across PS, plasma insulin concentrations were less for E cows (45% reduction from min 0 to 18) compared with NE cows (33% reduction from min 0 to 18) as time during exercise increased (interaction of T by day of experiment by minute of test, $P = 0.031$; Figure 6.5). Additionally on d 30 and 60, P cows had lower concentrations of insulin compared with NP cows regardless of minute of test (interaction of PS by day of experiment, $P = 0.007$; Figure 6.5). Post-exercise min 0 to 15, insulin concentrations were greatest at 9 min for all groups. On d 30 and 60 during min 0 to 15 and min 48 to 78 post-exercise, P cows had lower concentrations of insulin compared with NP cows (interaction of PS by day of experiment, $P = 0.010$; Figure 6.5).

Plasma glucagon. Pooled across T, PS, and day of the experiment, plasma glucagon concentrations increased as time during the exercise portion of the treadmill test increased ($P < 0.001$; Figure 6.6). Post-exercise min 0 to 15, plasma glucagon concentrations were greatest at min 0 then declined as time increased and returned to values similar to those at the beginning of the test by 15 min post-exercise ($P < 0.001$; Figure 6.6). Post-exercise 48 to 78 min, plasma glucagon concentrations were similar to values measured at the start of treadmill test. Similarly, sheep that were exercised 45 min at 5.5 km/h up a 8% slope had greater plasma glucagon compared with resting values (Brockman, 1987; Brockman and Halvorson, 1981). Plasma glucagon returned to pre-exercise values at min 15 and 45 post-exercise (Brockman, 1987; Brockman and Halvorson, 1981).

The responses of plasma glucagon concentrations during exercise and post-exercise for cows in NP, NE and NP, E did not change from d 0 compared with d 30 and 60 of experiment. The cows in NP, NE had numerically greater concentrations compared with those in NP, E on d 0, 30, and 60 of experiment. On d 0 during the exercise and post-exercise portions of the test, cows in P, NE and P, E had very similar concentrations of plasma glucagon at all minutes of tests. On d 60, P cows had lower plasma glucagon concentrations than NP cows across minutes of the test compared with d 0 and 30 (interaction of PS by day of experiment, $P < 0.001$; Figure 6.6). Also, pooled across day of experiment and PS, E cows had lower plasma glucagon than NE cows as time of test increased (interaction of T by minute of test, $P = 0.04$; Figure 6.6). On d 60 post-exercise (min 0 to 15 and min 48 to 78), P cows had less plasma glucagon compared with NP cows (interaction of PS by day of experiment, $P < 0.001$; Figure 6.6). Pooled across minute of test and day of experiment during post-exercise min 48 to 78, glucagon concentrations were greatest for cows in NP, NE (215 pg/ml) whereas those in P, NE (109 pg/ml) had the least concentrations compared with cows in NP, E (167 pg/ml) and P, E (125 g/ml; interaction of T by PS, $P = 0.027$).

The increase of glucagon during exercise would stimulate hepatic output of glucose via gluconeogenesis or glycogenolysis pathways. Insulin would increase tissue uptake of glucose at the same time that glucagon stimulates hepatic glucose output. Furthermore, muscle contractions can increase tissue uptake of glucose independent of insulin (Goodyear, 2000). Additionally, after cessation of exercise, control of glucose uptake was dependent on insulin. Possibly, at this point, insulin was increased to remove glucose from the circulation to avoid a hyperglycemic condition and to increase hepatic and muscle glycogen synthesis.

Furthermore, α - and β -adrenergic receptors affect glucose metabolism during exercise of sheep (Brockman and Halvorson, 1981). During exercise, inhibition of β -

adrenergic receptors did not affect concentrations of glucagon and insulin, and the subsequent hepatic glucose output compared with non-exercised controls (Brockman and Halvorson, 1981). However, the uptake of glucose by peripheral tissues during exercise was slightly greater compared with control sheep (Brockman and Halvorson, 1981). Inhibition of α -adrenergic receptors did reduce plasma glucose concentrations and increase concentrations of insulin during exercise of sheep compared with controls (Brockman and Halvorson, 1981). Therefore, during exercise, the α -adrenergic responses inhibits insulin secretion without affecting glucagon secretion resulting in greater plasma glucose concentrations (Brockman and Halvorson, 1981) as found in the current study.

Results similar to the current experiment were obtained in sheep trained for 3 wk. During exercise, plasma glucose, lactate, and glucagon concentrations were reduced, whereas insulin concentrations remained unchanged compared with values prior to training (Brockman, 1979a). In contrast to the current experiment, no measurements with sheep that were not trained were included in this study. The effects of repeated exercise testing or day of testing were not evaluated, which may influence the catecholamines concentrations and their actions through α -adrenergic receptors.

Exercise training of pregnant dairy cows did not affect plasma insulin during exercise, but corresponded to a dampened rise in plasma glucagon. In humans, plasma insulin concentrations decreased during a bout of exercise for trained and untrained individuals (Coggan et al., 1995a; Coggan et al., 1995b). In contrast to results of the current experiment, exercise training reduced the decline in plasma insulin concentrations during exercise as compared with untrained individuals (Coggan et al., 1995a; Coggan et al., 1995b). Additionally in humans, plasma glucagon concentrations were maintained during an exercise bout and were not altered by exercise training (Coggan et al., 1995a; Coggan et al., 1995b). These differences possibly may be

attributable to the differences in glucose metabolism between ruminants and non-ruminants.

The reduction of glucagon concentrations of cows in the current experiment would indicate less gluconeogenesis and glycogenolysis of hepatic tissues during exercise in response to exercise training. Potential adaptations in response to exercise training include increased muscle or hepatic glycogen, increased oxidative capacity for fatty acids or glucose, or utilization of intramuscular fat which may spare plasma glucose.

CONCLUSIONS

Circulating concentrations of glucose, lactate, glucagon, and BHBA were different for NP and P cows that were exercised. Furthermore, changes of plasma concentrations of glucose, NEFA, BHBA, and glucagon were different for cows in P, E compared with cows in NP, E. Pregnancy status clearly influenced responses of dairy cows to exercise training. Exercise training improved the ability of P and NP dairy cows to maintain plasma glucose concentrations (improved glucose homeostasis) and reduced plasma lactate (less anaerobic metabolism) during and after acute treadmill exercise. However, exercise training did not greatly affect circulating concentrations of NEFA and BHBA (lipid metabolism) of P and NP cows. Most dramatically, glucagon concentrations were reduced for cows in P, E compared with all other groups during exercise. Additionally, PS greatly affected glucoregulatory hormones and circulating metabolites. For P cows, concentrations of insulin and glucagon were reduced, whereas concentrations of NEFA were greater compared with NP cows. The concentrations of insulin and glucagon required to maintain glucose homeostasis were reduced for cows in E. Therefore, we conclude that exercise training of dairy cows stimulates adaptations of glucose homeostasis and its endocrine control during

exercise. The modification of glucoregulatory hormone patterns after exercise training may benefit dairy cows in the maintenance of homeostasis of glucose metabolism during the periparturient period.

Table 6.1. Plasma concentrations of glucose (mg/dl) during the exercise and post-exercise portions of treadmill exercise test.¹

Item	Treatment		Pregnancy		Treatment and Pregnancy					P-value ²		
	NE ³	E ³	NP ⁴	P ⁴	NP, NE ⁵	NP, E ⁵	P, NE ⁵	P, E ⁵	T	PS	T x PS	
Exercise												
mean	67.8	64.9	68.6	64.1	68.8	68.3	66.8	61.4	0.086	0.009		NS
SEM	1.23	1.08	1.07	1.23	1.53	1.50	1.92	1.56				
Post-exercise (0 to 18 min)												
mean	82.0	80.6	84.2	78.4	82.0	86.3	81.9	74.8	NS	NS		NS
SEM	2.81	2.56	2.55	2.82	3.63	3.58	4.31	3.70				
Post-exercise (33 to 78 min)												
mean	69.6	66.6	70.0	66.3	69.6	70.4	69.6	62.9	0.047	0.014		0.015
SEM	1.12	0.99	0.98	1.12	1.40	1.37	1.75	1.42				

¹ Values are least squares means.

² Probability values of main effects of exercise treatment (T), pregnancy status (PS), and the interaction of T by PS (T x PS), and NS = non-significant, $P > 0.10$.

³ Pooled least squares mean regardless of pregnancy status, day of experiment, or minute of test. NE = non-exercised cows; E = exercised cows.

⁴ Pooled least squares mean regardless of exercise treatment, day of experiment, or minute of test. NP = non-pregnant cows; P = pregnant cows.

⁵ Pooled least squares mean regardless of day of experiment or minute of test. NP, NE = non-pregnant, non-exercised cows; NP, E = non-pregnant, exercised cows; P, NE = pregnant, non-exercised cows; P, E = pregnant, exercised cows.

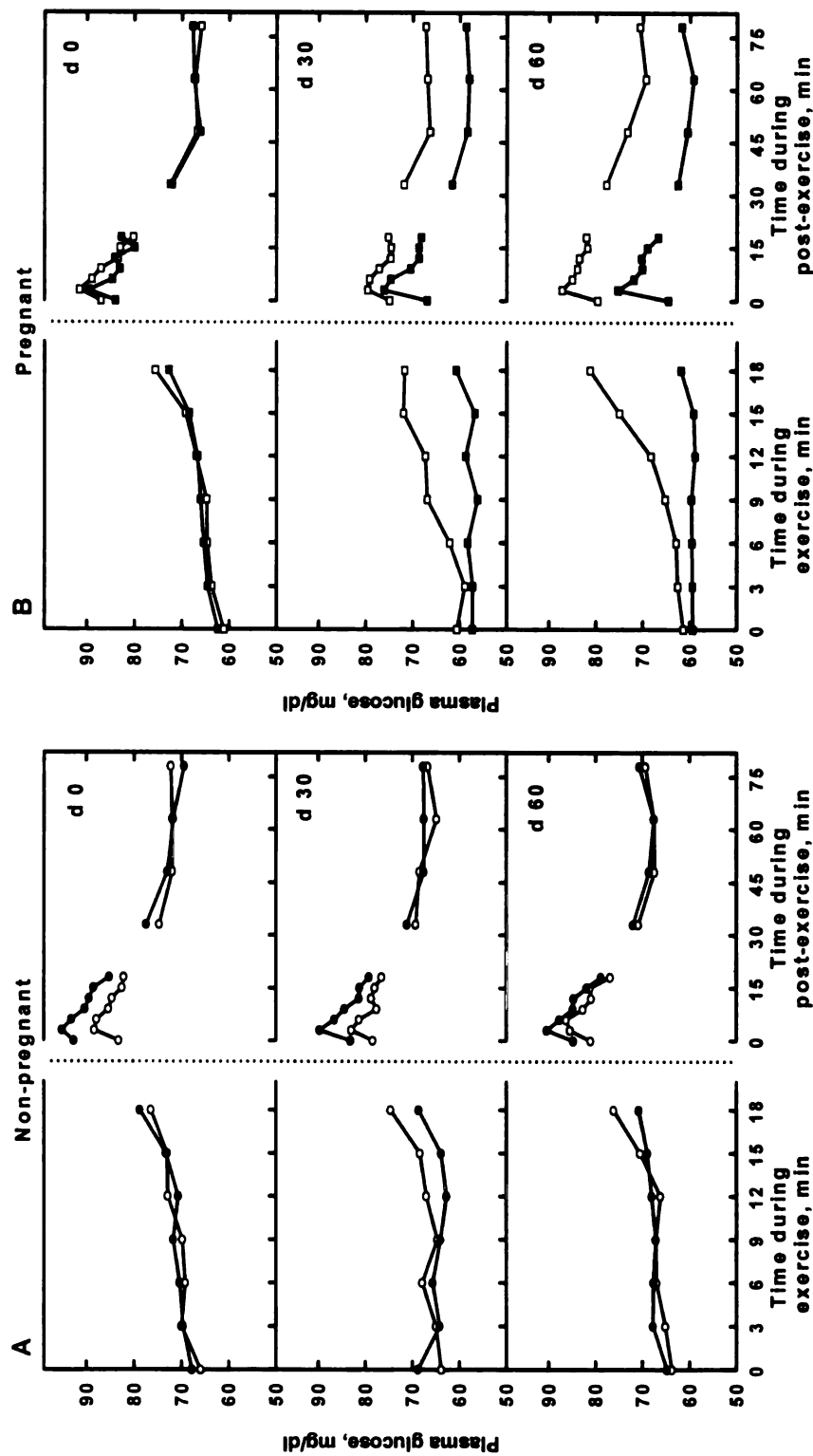


Figure 6.1. Plasma glucose concentrations during the exercise and post-exercise portions of treadmill test on d 0, 30, and 60 of the experiment. Treatments were non-pregnant, non-exercise (○); non-pregnant, exercise (◻); pregnant, non-exercise (●); and, pregnant, exercise (■). *Panel A* shows results for non-pregnant cows, and *Panel B* shows results for pregnant cows. During exercise, min 0 to 18 post-exercise, and min 33 to 78 post-exercise, plasma glucose was affected by minute of test and day of experiment (pooled SEM = 4.75, 6.48, and 3.60, respectively, $P < 0.01$). During exercise, pregnancy status affected concentrations of glucose ($P < 0.01$). During min 0 to 18 post-exercise, pregnancy status and exercise treatment did not affect plasma glucose concentrations. During min 33 to 78 post-exercise, the interaction of exercise treatment by minute was significant ($P = 0.007$). Also, the interaction of exercise treatment by pregnancy status was significant at this time ($P = 0.015$).

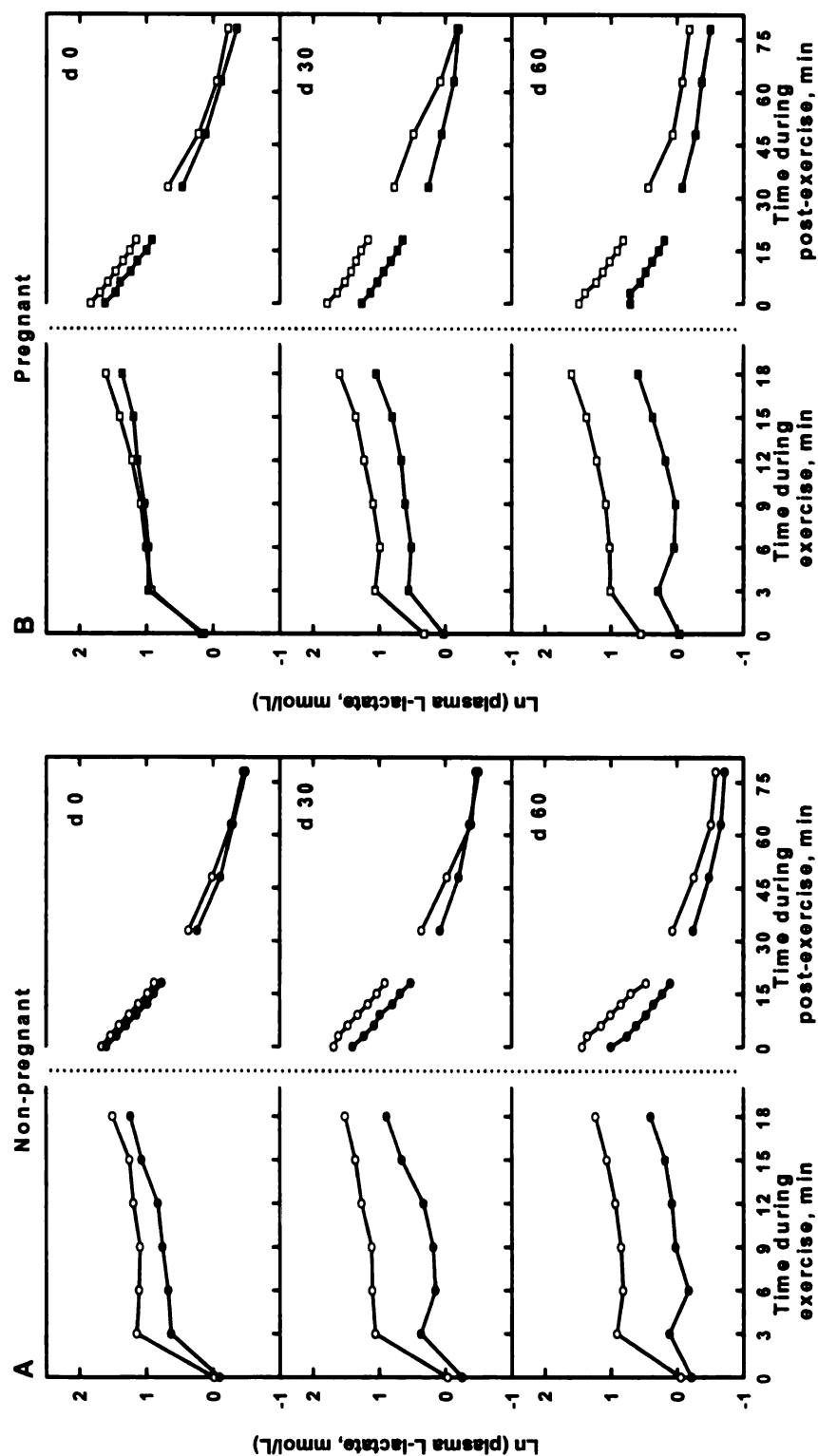


Figure 6.2. Natural logarithmically transformed plasma L-lactate concentrations during the exercise and post-exercise portions of treadmill test on d 0, 30, and 60 of the experiment. Treatments were non-pregnant, non-exercise (□); non-pregnant, exercise (●); pregnant, non-exercise (□); and, pregnant, exercise (■). *Panel A* shows results for non-pregnant cows, and *Panel B* shows results for pregnant cows. During exercise, min 0 to 18 post-exercise, and 33 to 78 min post-exercise pooled SEM were 0.233, 0.204, and 0.157, respectively. During min 0 to 18 of exercise, the interaction of exercise treatment by pregnancy status by minute of test and the interaction of exercise treatment by day of experiment affected plasma lactate concentrations ($P < 0.02$). During min 0 to 18 post-exercise, plasma lactate was affected by interaction of exercise treatment by day of experiment and the interaction of pregnancy status by minute of test ($P < 0.05$). During min 33 to 78 post-exercise, the interaction of exercise treatment by minute was significant ($P = 0.007$). Also, the pregnancy status was significant at this time ($P < 0.001$).

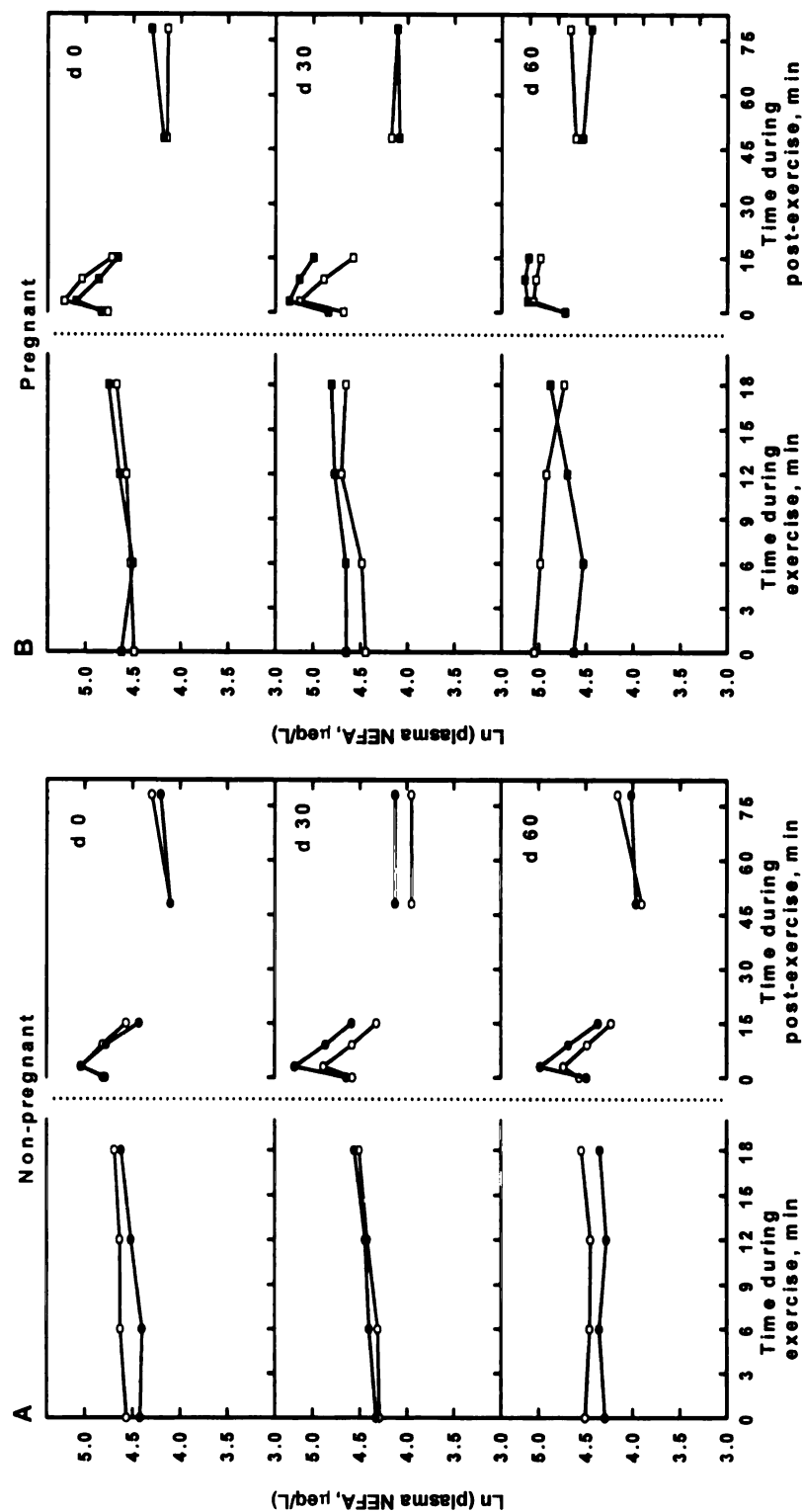


Figure 6.3. Natural logarithmically transformed plasma NEFA concentrations during the exercise and post-exercise portions of treadmill test on d 0, 30, and 60 of the experiment. Treatments were non-pregnant, non-exercise (○); non-pregnant, exercise (□); pregnant, non-exercise (●); and, pregnant, exercise (■). *Panel A* shows results for non-pregnant cows, and *Panel B* shows results for pregnant cows. During exercise, min 0 to 15 post-exercise, and min 48 to 78 post-exercise pooled SEM were 0.217, 0.167, and 0.184, respectively. During min 0 to 18 of exercise, the interaction of pregnancy status by minute of test ($P = 0.10$) and the main effect of pregnancy status ($P < 0.001$) affected plasma NEFA concentrations. During min 0 to 15 post-exercise, plasma NEFA was affected by interaction of exercise treatment by pregnancy status by minute of test ($P = 0.054$), the interaction of pregnancy status by day of experiment by minute of test ($P = 0.058$). During min 48 to 78 post-exercise, the interaction of pregnancy status by day of experiment was significant ($P = 0.003$). Also, the pregnancy status by minute of test interaction tended to be significant at this time ($P = 0.066$).

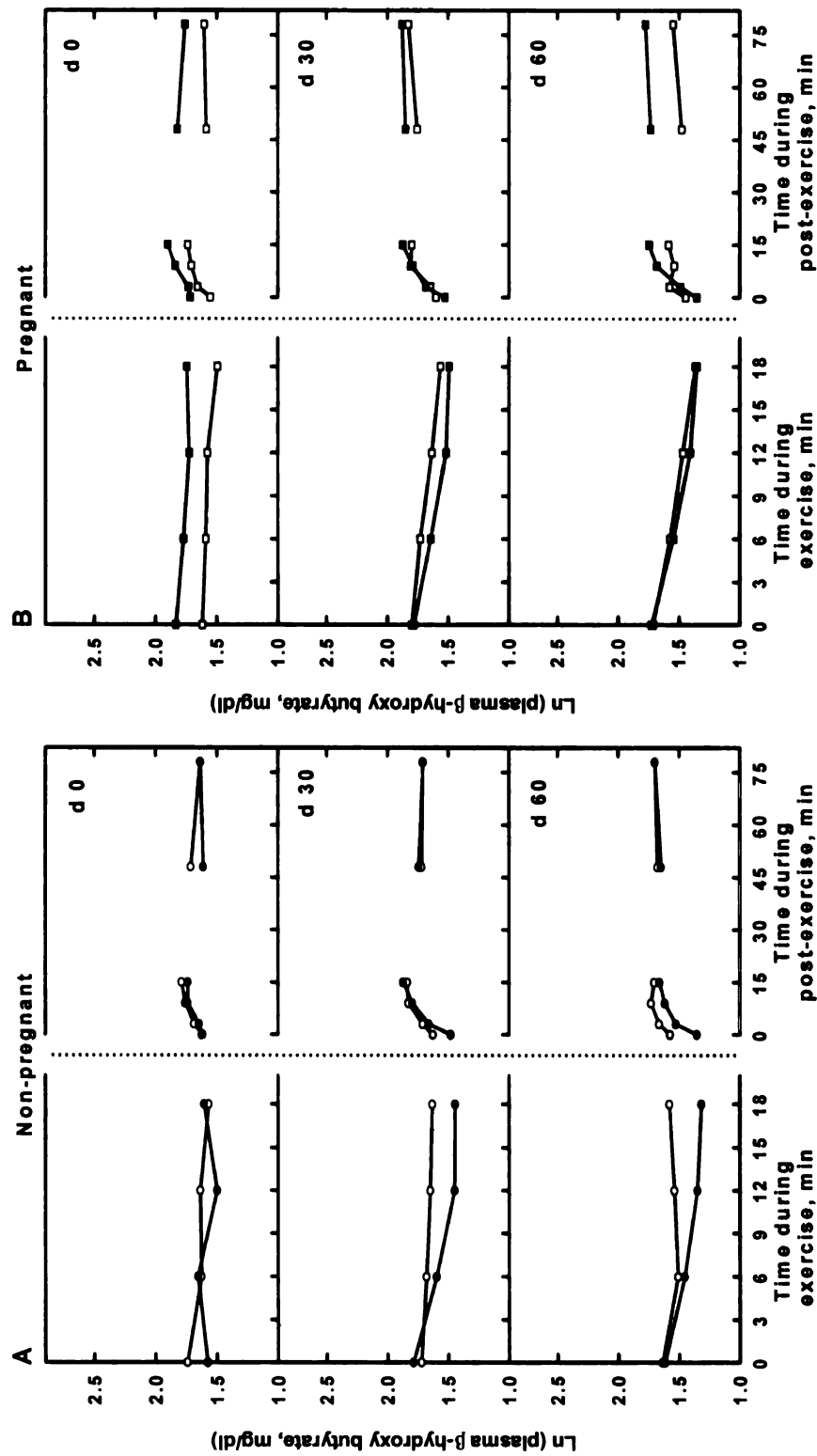


Figure 6.4. Natural logarithmically transformed plasma BHBA concentrations during the exercise and post-exercise portions of treadmill test on d 0, 30, and 60 of the experiment. Treatments were non-pregnant, non-exercise (○); non-pregnant, exercise (●); pregnant, non-exercise (□); and, pregnant, exercise (■). Panel A shows results for non-pregnant cows, and Panel B shows results for pregnant cows. During exercise, min 0 to 15 post-exercise, and min 48 to 78 post-exercise pooled SEM were 0.114, 0.095, and 0.114, respectively. During min 0 to 18 min of exercise, the interaction of exercise treatment by minute of test ($P = 0.029$) and the interaction of exercise treatment by day of experiment by minute of test ($P = 0.066$) affected plasma BHBA concentrations. During min 0 to 15 post-exercise, plasma BHBA was affected by interaction of exercise treatment by day of experiment by minute of test ($P = 0.005$). During min 48 to 78 post-exercise, the interaction of exercise treatment by pregnancy status tended to be significant ($P = 0.087$).

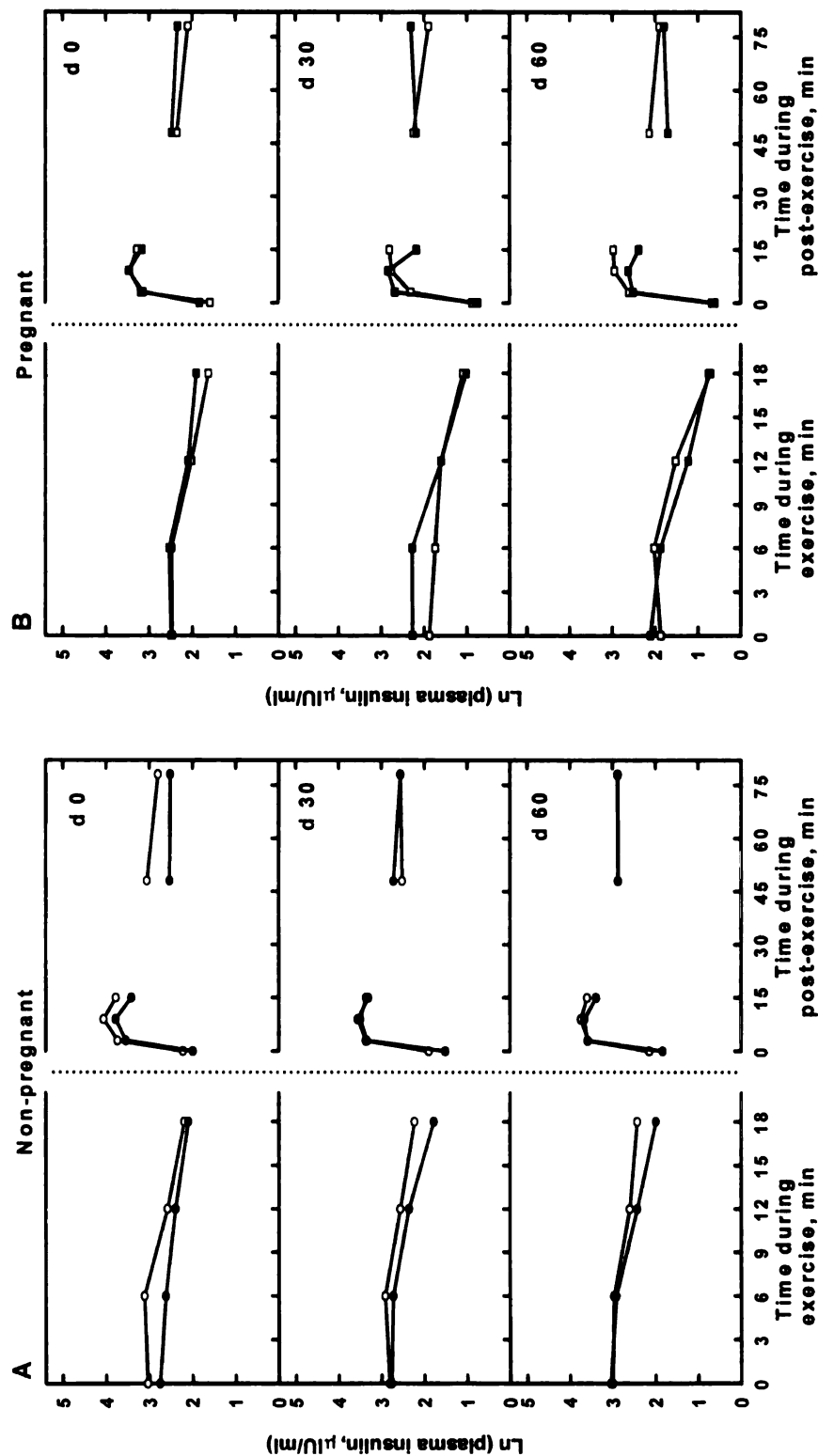


Figure 6.5. Natural logarithmically transformed plasma insulin concentrations during the exercise and post-exercise portions of treadmill test on d 0, 30, and 60 of the experiment. Treatments were non-pregnant, non-exercise (○); non-pregnant, exercise (●); pregnant, non-exercise (□); and, pregnant, exercise (■). *Panel A* shows results for non-pregnant cows, and *Panel B* shows results for pregnant cows. During exercise, min 0 to 15 post-exercise, and min 48 to 78 post-exercise pooled SEM were 0.321, 0.345, and 0.258, respectively. During min 0 to 15 of exercise, the interaction of exercise treatment by day of experiment by minute of test ($P = 0.031$) affected plasma insulin concentrations. During min 0 to 15 post-exercise, plasma insulin was affected by interaction of pregnancy status by day of experiment ($P = 0.010$) and minute post-exercise ($P < 0.001$). During min 48 to 78 post-exercise, the interaction of pregnancy status by day of experiment was significant ($P = 0.010$).

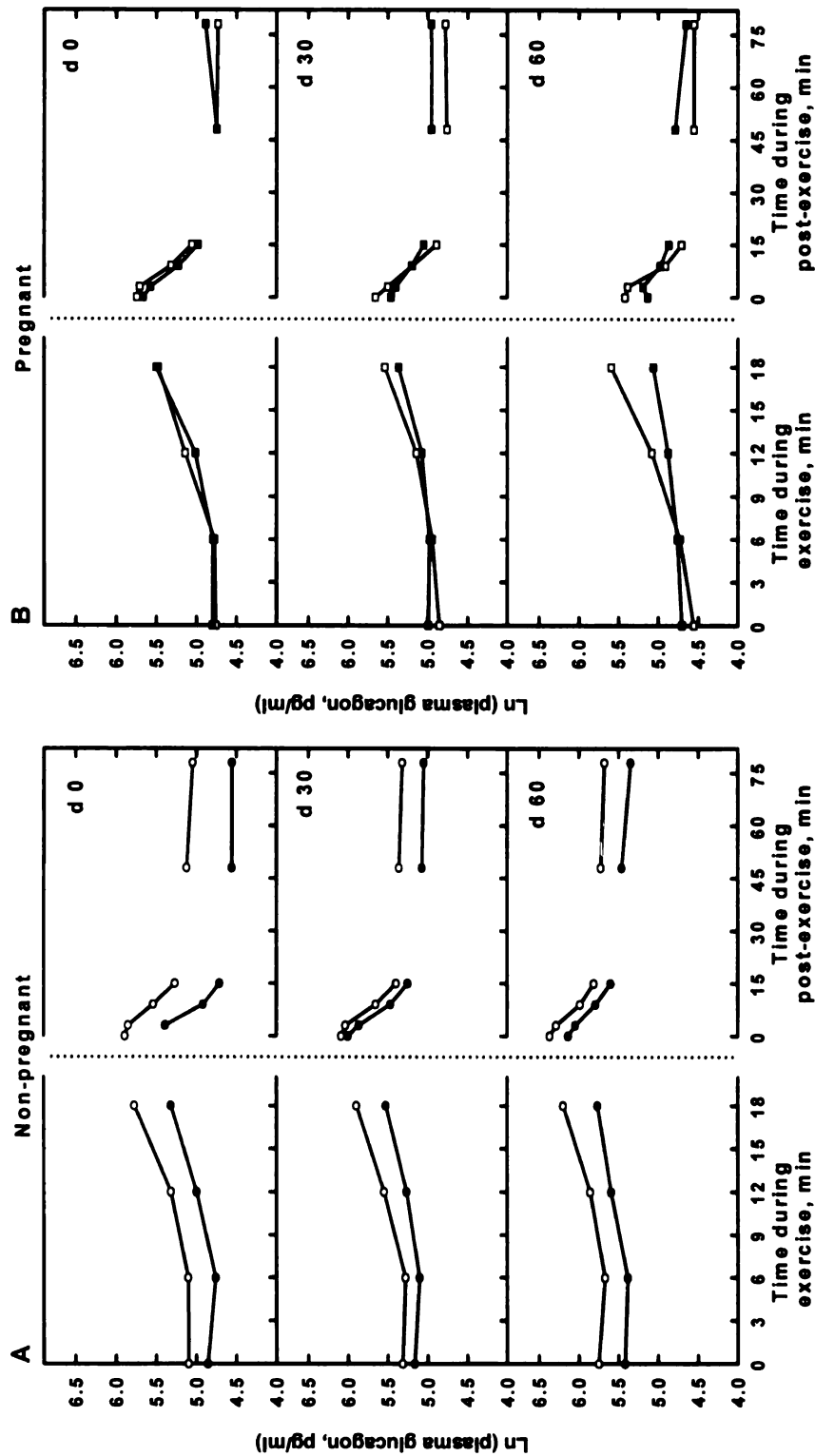


Figure 6.6. Natural logarithmically transformed plasma glucagon concentrations during the exercise and post-exercise portions of treadmill test on d 0, 30, and 60 of the experiment. Treatments were non-pregnant, non-exercise (○); non-pregnant, exercise (●); pregnant, non-exercise (□); and, pregnant, exercise (■). *Panel A* shows results for non-pregnant cows, and *Panel B* shows results for pregnant cows. During exercise, min 0 to 15 post-exercise, and min 48 to 78 post-exercise pooled SEM were 0.184, 0.164, and 0.141, respectively. During min 0 to 18 of exercise, the interaction of exercise treatment by minute of test ($P = 0.040$) and interaction of pregnancy status by day of experiment ($P < 0.001$) affected plasma glucagon concentrations. During min 0 to 15 post-exercise, plasma glucagon was affected by the interaction of pregnancy status by day of experiment ($P < 0.001$). During minute 48 to 78 post-exercise, the interaction of pregnancy status by day of experiment was significant ($P < 0.001$).

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**METABOLIC AND PHYSIOLOGICAL ADAPTATIONS OF
LATE PREGNANT DAIRY COWS TO EXERCISE TRAINING**

VOLUME II

By

Jill Anna Davidson

A DISSERTATION

Submitted to
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Professor David K. Beede

CHAPTER 7

EFFECTS OF EXERCISE TRAINING OF DAIRY COWS ON ENERGY BALANCE

ABSTRACT

The objective of this experiment was to determine if prepartum exercise training altered energy balance of pregnant and non-pregnant cows (BW, DMI, or water intake). Pregnant (n = 26) and non-pregnant (n = 26), non-lactating multiparous Holstein cows were assigned in a factorial arrangement of treatments: exercise training (E) or no exercise (NE), and pregnant (P) or non-pregnant (NP). Exercise training was walking (1.25 to 1.5 h at 3.25 km/h) every other day in an outdoor mechanical walker for 70 d. Exercised cows had reduced BW gains compared with NE cows, but E did not affect BCS or DM and water intake. Pregnant cows weighed more and consumed less DM and water than NP cows. Near parturition, DM intake decreased for P cows, regardless of exercise treatment. Exercise treatment did not greatly affect muscle glycogen or triglycerides, whereas liver glycogen was increased. Pregnant cows had greater liver triglyceride concentrations near parturition. During the prepartum period, pregnancy status of cows had a greater effect on energy balance and energy requirements than exercise training. All cows were in positive energy balance during the experiment. We conclude that exercise training of pregnant cows does not increase the risk of negative energy balance during the prepartum period.

INTRODUCTION

Exercise training, defined as repeated activity or training in a specific program, may cause adaptations of physiological processes to meet the demands of increased activity. Exercise training of non-pregnant and pregnant dairy cows increased physical fitness of cows as determined during a treadmill test (Chapters 4 and 5). However, it is not known energy utilized for walking would affect energy balance of prepartum dairy cows. Previously during the prepartum period, dairy cows and heifers that exercised for 8 km/d had lower BW gains than non-exercised cows (Lamb et al., 1981). In other studies, BW gains of prepartum dairy cows and heifers were not affected by exercise (Anderson et al., 1979; Lamb et al., 1979). In all three of these studies, cows and heifers were given limited access to feed managers of 6 h/d and provided ad libitum alfalfa hay and corn silage with limited concentrate supplement of 4.5 kg/d. Therefore, it is not clear if prepartum exercise in current management systems would affect energy balance in a similar manner as these studies.

The objective of this experiment was to determine if prepartum exercise training altered energy balance of pregnant and non-pregnant cows (BW, DMI, or water intake); and, if additional dynamics associated with pregnancy would alter energy balance. The hypotheses were that exercise training would improve the energy status of pregnant cows during prepartum period.

MATERIALS AND METHODS

Animal care and experimental procedures were approved and conducted under standards of the Michigan State University All-University Committee on Animal Use and

Care, application number 05/98-081-00. Guidelines were compliant with National Institutes of Health and the Guide for the Care and Use of Laboratory Animals.

Cows and Exercise Training Treatments

Twenty-six pairs of non-pregnant and pregnant multiparous Holstein cows were blocked by parity and expected calving date (ECD) of the pregnant animal. Pairs of cows were assigned randomly to exercise treatments. Treatments in a factorial arrangement were exercise training (E) or no exercise (NE), and pregnant (P) or non-pregnant (NP).

Cows had been dried-off at least 1 wk before the start of the experiment. They also were trained to use the mechanical walker with 2 to 3 short duration (5 min) practice sessions, 1 to 2 wk before the experiment. The start of experiment for a block of cows was approximately 70 d before ECD of the pregnant cows. Exercise training was completed every other day until the parturition of the pregnant cow of the pair.

Exercise training was accomplished in an outdoor mechanical walker with a circular lane that was 90-cm wide and 33.8-m long approximately 36.5 m from the alley in the tie-stall barn where cows were housed (Chapter 3). Cows assigned to NE remained in tie-stalls blocked from feed and water while cows assigned to E were walked. From d 2 through d 28 of experiment (about 68 to 42 d before ECD), E cows walked for 1.25 h at 3.25 km/h and from d 32 to parturition for 1.5 h at 3.25 km/h. On the day of their treadmill test (d 0, 30 and 60), cows were not exercised in the mechanical walker.

All cows were fed a TMR ad libitum, once daily at 1030 (Table 7.1). From wk 1 to 5 of experiment, the diet had a dietary cation anion difference (DCAD, $[(Na + K) - (Cl + S)]$ meq/100 g of dietary DM) of 20.2 meq/100 g of dietary DM and contained 1.44 NE_L Mcal/kg, 15.9% CP, dry basis. From wk 5 to parturition, the diet had a DCAD of -15.7

meq/100 g of dietary DM and contained 1.65 NE_L Mcal/kg, 17.7% CP, dry basis. Daily DMI and water intake of individual cows were recorded. Body weights were measured on d -1, 29, and 59 of the experiment. Weekly BCS were recorded independently by three technicians on a 1 to 5 point scale (Wildman et al., 1982).

Daily prepartum net energy balance (Mcal/d) was calculated using equations from NRC (2001) to estimate net energy of intake, net energy requirement of maintenance, net energy requirement of gain, net energy requirement of gestation, and net energy requirement of walking activity (Table 7.2). For both periods of wk 1 to 5 and wk 5 to parturition, the average daily net energy expenditure for each cow was estimated from NRC (2001) equations and the average BW, daily DM intake, distance walked, and days of gestation for each cow. To estimate the net energy of gain, net energy value (Mcal/kg of BW) for each cow's BW was estimated from the average BCS of all cows on wk 1, 4, and 8. Additionally, the activity equation of NRC (2001) was modified to exclude the grazing component in that these cows were not permitted to graze.

Liver and Muscle Tissue

Approximately 1 h after feeding on d -2, 3, 33, and 63 of experiment, liver and semitendinous muscle samples were collected by biopsy (see Appendix J). Biopsy sites were prepared aseptically and anaesthetized by local infiltration of lidocaine hydrochloride. Liver tissue sample (approximately 100 mg) was taken from a single entry site on the right side between the 10th and 11th rib at the intersecting diagonal line between the elbow joint and the point of the ilium. Multiple liver samples (5 to 6) were taken with 14 gauge 15-cm long biopsy needle (VET-core, Cook Veterinary Products, Bloomington, IN). Muscle tissue was taken from the center of semitendinous muscle of one hindlimb with a needle (5.0-mm outside diameter and 120-mm long, U. H. C. Skeletal Muscle Biopsy Needle, Popper & Sons, New Hyde Park, NY). Muscle biopsies

were performed on the same hindlimb on d -2 and 33 and the other hindlimb on d 3 and 63. Tissues were collected on filter paper and transferred to cryo-genic tubes and placed in liquid N₂. Samples were stored at -80° C until analyses for protein, triglyceride, and glycogen concentrations. Tissues were homogenized (Polytron, Kinematica GMBH, Luzern, Switzerland) 20 µl/mg of wet muscle and 40 µl/ml of wet liver with 20 mM Na- phosphate buffer, pH 7.4 and 0.5 mM EDTA (see Appendix L). Protein concentrations were determined in microtiter plates using Bradford reagent (Sigma Chemical, St. Louis, MO). Intra- and inter-assay coefficients of variation were 3.3 and 4.0%, respectively. Aliquots of homogenate were stored at -20° C until analyses of glycogen and triglycerides (TG).

Glycogen concentrations were determined using an enzymatic colorimetric microtiter plate assay (Andersson et al., 2000; Suzuki et al., 2001, see Appendix M). Tissue homogenates (200 µl) were incubated with 400 µl of 2 M potassium hydroxide for 30 min for liver samples and 60 min for muscle samples in a 70° C water bath. After samples were cooled to room temperature, 200 µl of 1 M Na- sulfate was added followed by 1 ml of cold-95% ethanol. Solution was centrifuged for 5 min, 2000 *g*. Tubes were inverted and shaken to remove supernatant. The precipitate containing glycogen was dried overnight at room temperature. To liver glycogen precipitates, 400 µl of 0.375% amyloglucosidase (w/v) in 0.3 M Na- acetate buffer, pH 4.8 was added. To muscle glycogen precipitates, 400 µl of 0.75% amyloglucosidase (w/v) in 0.3 M Na- acetate buffer, pH 4.8 was added. After mixing, samples were incubated for 1 h at 55° C. Glucosyl units were determined using a glucose oxidase colorimetric kit (Sigma Diagnostics, St. Louis, MO). Intra- and inter-assay coefficients of variation were 4.8 and 7.1%, respectively.

Triglyceride concentrations were determined with an enzymatic colorimetric microtiter plate assay (Hara and Radin, 1978; Moore et al., 2000, see Appendix N).

Tissue homogenates and standards (triolein) of 500 µl were extracted with the addition of 2 ml of hexane : isopropyl alcohol (3:2) followed by mixing for 5 min. To separate organic phases, 800 µl of 8% Na- sulfate (w/v) were added to tubes. The upper layer was transferred to 12 x 75 mm borosilicate glass tubes. The interface was washed two times with 200 µl of 7:2 hexane : isopropyl alcohol and added to the glass tubes. To the samples, 600 µl of 2% Triton-x100 in 7:2 hexane : isopropyl alcohol was added followed by drying at 45° C in a water bath under a steady stream of N₂. Samples and triolein standards were then re-constituted with 500 µl of de-ionized water to be assayed for triglycerides (No. 343, Sigma Diagnostics, St. Louis, MO). Intra- and inter-assay coefficients of variation were 2.7 and 5.2%, respectively. Extraction recovery of known amounts of triolein was approximately 85%.

Statistical Analysis

Data were analyzed by method of ANOVA using mixed model procedures of SAS (1999). The appropriate covariance structure for the repeated measures was determined using the goodness of fit criteria of Aiaike's Information Criterion and Schwarz's Bayesian Criterion. Dependent variables that had unequal variances across day or week of experiment were transformed using the natural logarithm before analyses. Unless otherwise described, results are presented as generalized least squares means with standard error of mean. Statistical significance was declared at $P < 0.05$ and was between $P > 0.05$ and $P < 0.10$ was denoted a tendency.

Dry matter and water intakes and BW were analyzed with a mixed model including the random effect of cow nested within treatment (T) and pregnancy status (PS), the repeated measures effect of day of experiment, fixed effects of T, PS, and block. All two- and three-way interactions of T, PS, and day of experiment were included in the model. Covariance structure of repeated measures effect of day of

experiment was auto-regressive first order. Because cows were fed two diets and to allow for an adjustment period to the diet change, intake data were analyzed as two data sets: d 0 to 30 and d 49 to parturition. The adjustment period of d 31 to 48 of the experiment was excluded from analyses. Additionally, the initial BW and changes in BW were analyzed with a model including the random effect of cow nested within T and PS, the fixed effects of T, PS, and block, and the interaction of T and PS.

Weekly average BCS were analyzed with a mixed model including the random effect of cow nested within exercise training treatment (T) and pregnancy status (PS), the repeated measures effect of week of experiment, and fixed effects of T, PS, and block. All two- and three-way interactions of T, PS, and week of experiment were included in the model. The covariance structure of repeated measures effect of week of experiment was auto-regressive first order. Additionally, the initial BCS and changes in BCS were analyzed with a model including the random effect of cow nested within T and PS, the fixed effects of T, PS, and block, and the interaction of T and PS.

Daily energy balance variables (energy intake and estimated requirements of maintenance, gestation, BW gains, and activity) were analyzed with a model including the random effect of cow nested within T and PS and the fixed effects of T and PS, and the interaction of T and PS. The energy balance variables for each period (wk 1 to 5 and wk 5 to parturition) were analyzed separately.

The dependent variables from the tissue samples were analyzed with a mixed model including the random effect of cow nested within T and PS, the repeated measures effect of day of experiment, fixed effects of T, PS, and block. All two- and three-way interactions of T, PS, and day of experiment were included in the model. Additionally, appropriate tissue data from d -3 of experiment were included in the model as a covariate for that dependent variable. The covariance structure of the repeated measures effect of day of experiment was auto-regressive first order.

RESULTS AND DISCUSSION

Body Weight

On d -1 of experiment, T assignment or interaction of T by PS did not affect BW. On d -1 of experiment, cows that were pregnant weighed 725 kg, whereas cows that were not pregnant weighed 677 kg ($P < 0.001$). Body weight increased in all treatment groups from d -1 to 59 of experiment (effect of day of experiment, $P < 0.001$; Table 7.2). Exercise treatment and PS affected the increases in BW from d -1 to 59 (Table 7.2). Pregnant cows or non-exercised cows had greater BW on d 59 compared with cows that exercised or were not pregnant.

Regardless of PS, the increase in BW from d -1 to 29 was less for cows that exercised (32 kg) compared with non-exercised cows (45 kg) ($P = 0.037$). From d 29 to 59, cows that were not pregnant and did not exercise tended to have greater increases of BW compared with all other treatment groups (interaction of T by PS, $P = 0.055$; Table 7.2). Similarly, from d -1 to 59, NP, NE cows tended to have the largest increase in BW compared with all other treatment groups (interaction of T by PS, $P = 0.081$; Table 7.2). Exercising cows that were either P or NP reduced the increase in BW from d -1 to 59 of the experiment compared with NE cows (effect of T, $P = 0.019$).

Pregnant cows had greater BW than NP cows at the start of the experiment (about 70 d before ECD) and throughout the entire experimental period. Pooled across PS, the increases in BW from d -1 to d 59 of the experiment were less for exercised cows compared with non-exercised cows which was similar to a previous study (Chapter 4). The greater caloric density of the diets and DMI of the current experiment probably contributed to the greater gains measured in this experiment compared with the previous study. Furthermore, beef cattle that were forced to exercise had a 31%

increase in feed requirement and reduced BW gains compared with restricted activity cows (Bellows et al., 1994).

Body Condition Score

On wk 1 of experiment, average BCS were 3.6, 3.5, 3.6, and 3.6 ± 0.17 for NP, E; NP, NE; P, E; and P, NE cows; respectively. Average BCS pooled across T and PS increased from 3.6 to 4.0 ± 0.054 from wk 1 to 10 of experiment ($P < 0.001$; Figure 7.1). Pregnancy status and T did not affect weekly BCS (Table 7.3). However, from wk 1 to 4, cows that exercised tended to have slightly greater increase in BCS than non-exercised cows ($P = 0.097$, Table 7.3). From wk 4 to 8, cows that exercised had a decrease in BCS compared with cows that did not exercise ($P < 0.05$, Table 7.3). From wk 1 to 8, T or PS did not affect the overall change of BCS (Table 7.3). The inability to detect differences of body composition using BCS in the current study was not surprising with the number of cows per treatment group. The changes from initial to final BW were 77, 43, 63, and 58 ± 8 kg for NP, E; NP, NE; P, E; and P, NE; respectively, which would be approximately a change of 1 BCS for the entire duration of the experiment.

In sheep, exercise training had only slight effects on body composition. Exercise treatment of Suffolk lambs did not change the proportion of carcass that was fat, bone, and muscle as determined by dissection (Aalhus and Price, 1990). Exercised lambs had a larger proportion of muscle in the proximal pelvic limb and abdominal wall with less muscle around the spinal column than non-exercised lambs. After 7 wk of exercise, subcutaneous fat and carcass weight were less in exercised Merino wethers compared with non-exercised wethers (Pethick and Rowe, 1996). Differences in detected responses between these studies may be due to breed differences, maturity of lambs, and initial body weight at the start of experiments. It is not clear if the reduction of BW

gain of dairy cows in this study was influenced by a re-distribution of body tissues or changes in body composition after exercise training.

Muscle and Liver Tissue

Muscle and liver tissue collected by biopsy may provide some insight to changes in body composition after exercise training. Exercise treatment and PS did not affect muscle protein concentrations or glycogen concentrations (Table 7.4). In contrast to our findings, exercise training increased the myofibrillar protein concentrations in muscle and reduced soluble collagen content of lambs (Aalhus et al., 1991). Wethers exercised daily for 1 h, 8 km/h for 5 wk had greater concentrations of glycogen in semimembranous, semitendinous, and longissimum dorsi muscles (Gardner et al., 1998; Pethick and Rowe, 1996).

The P, E and NP, NE cows had the greatest increase of muscle triglyceride concentrations compared with P, NE and NP, E cows as day of experiment increased (interaction of T by PS by day of the experiment, $P < 0.01$; Figure 7.2). Cows that were P, NE and NP, E had on slightly changes in muscle triglycerides as day of experiment increased. After 18 wk of exercise, the intramuscular fat in semimembranous, vastus lateralis, and gastrocnemius muscles of lambs was reduced (Aalhus and Price, 1990). Subcutaneous fat was less in exercised wethers compared with non-exercised wethers (Pethick and Rowe, 1996). The differences between growing and mature animals and (or) pregnancy status may have affected the measurements of muscle tissue composition between studies.

Exercise treatment did not affect liver protein concentrations or triglyceride concentrations (Table 7.5). Pregnant cows had greater accumulation of triglycerides in liver than NP cows (interaction of PS by day of experiment, $P < 0.01$; Figure 7.3).

Accumulation of liver triglycerides of dairy cows during late gestation has been previously described (Grummer, 1993).

The NP, E cows had greater liver glycogen concentrations than all other groups (interaction of T by PS, $P = 0.03$, Table 7.5). Interaction of T by day of experiment affected glycogen concentrations ($P = 0.02$; Figure 7.4). Cows that exercised had greater liver glycogen on d 63 than cows that did not exercise. The increased liver glycogen in exercised cows may be due to increased gluconeogenesis capacity. In other studies, rats, that were progressively exercise trained, had greater hepatic gluconeogenesis capacity compared with controls (Burelle et al., 2000; Sumida and Donovan, 1995). Hepatocytes from trained rats had greater rate of gluconeogenesis with substrates of alanine, lactate, and glycerol compared with hepatocytes of sedentary rats (Burelle et al., 2000).

Dry Matter Intake

During d 0 to 30 of the experiment, DMI (kg/d and as kg/100 kg BW) was less for P cows compared with NP cows ($P < 0.05$; Figure 7.5, Panels A and B). Also, cows that were exercised tended to eat less than NE cows (14.8 kg/d vs. 15.8 ± 0.38 kg/d, $P = 0.07$). However, T did not affect DMI expressed as kg/100 kg BW (2.2 vs. 2.1 ± 0.05 , NE vs. E, respectively).

From d 49 to parturition, P cows had reduced DM intake (kg/d and kg/100 kg BW) compared with NP cows with a reduction of intake near parturition (interaction of PS by day of experiment, $P < 0.001$; Figure 7.5, Panels C and D). Furthermore pooled across day of the experiment, E cows ate less DM than NE cows (13.9 vs. 15.5 ± 0.43 kg/d, $P = 0.014$). However, when DMI was adjusted for BW, exercise treatment did not affect DMI (kg/100 kg BW).

Previous research evaluating the effects of exercise of cows on DMI utilized diets of poor forage quality (Matthewman et al., 1993b), or a TMR was not fed (Anderson et al., 1979; Lamb et al., 1981; Lamb et al., 1979). As in the current experiment, exercise training did not affect DMI in these experiments. In the current experiment regardless of PS, exercise training did not affect DMI, even though diets of different energy density were fed as TMR (Chapter 4). In contrast, sheep that were exercised for 14 d at 3 km/h up a 17.6% slope for 30 min, six times a day had reduced organic matter intake and reduced apparent organic matter digestibility compared with non-exercised sheep (Henning, 1987). Limiting access to feed during exercise may have affected these results.

Additionally, P cows ate less DM (kg/d or kg/100 kg BW) than NP cows during the entire experimental period. During the final week of gestation, pregnant cows had a 30% reduction in DMI (kg/d) as described previously (Bertics et al., 1992). In the current experiment, exercise training did not affect the reduction of DMI of pregnant cows near parturition.

Water Intake

Exercise treatment during d 0 to 30 of experiment or d 49 to parturition did not affect water intakes (L/d; L/100 kg BW; or L/kg DMI). During d 0 to 30 and d 49 to parturition, P cows drank less water (L/d and L/100 kg BW) than NP cows ($P < 0.05$; Figure 7.6, Panels A, B, D and E). Water intakes as a function of kg DMI were approximately 1 L for all groups during d 0 to 30 of the experiment (Figure 7.6, Panel C). As parturition approached, water intakes as a function of DMI (L/kg DMI) were greater for P cows compared with NP cows (interaction of PS by day of experiment, $P < 0.001$; Figure 7.6, Panel F). Compared with NP cows, water intake of P cows near parturition was maintained, whereas DMI was reduced.

Exercise training treatments or interaction of T by day of experiment in the current experiment and previous study (Chapter 4) did not affect water intake. This differs from results with exercising crossbred lactating cows that had greater water intakes compared with non-exercised cows (Benedetti et al., 1990). In that study, exercise treatment did not affect feed consumption or milk yield. The difference between these studies may be due to different physiological states of cows (non-lactating vs. lactating), environments, or DM content of the diets.

Pregnant cows had less water intake (L/d and L/100 kg of BW) than non-pregnant cows. During d 0 to 30 of experiment, water intake as a function of kg of DMI was approximately 1 L/kg DMI for P and NP cows. Interestingly, as DMI was reduced during the last week of gestation for P cows, water intake as a function of kg of DMI increased to greater than 2 L/kg DMI. No other research was found to compare with these results.

Energy Balance

During wk 1 to 5 of the experiment, daily energy expenditure (Mcal/d) was greater for P cows compared with NP cows, whereas daily energy intake was less (effect of PS, $P < 0.05$; Table 7.6). The energy expenditure associated with gestation (average 3.38 Mcal/d) accounted for a large portion of the increase in total energy expenditure of P cows compared with NP cows (Figure 7.7, Panel A). Pregnant cows had slightly greater energy expenditure associated with maintenance compared with NP cows ($P < 0.001$).

Exercising cows resulted in less energy expenditure associated with gain compared with NE cows ($P = 0.028$). The reduction of energy associated with gain for E cows could be due to the additional energy required for walking (1 Mcal/d; Figure 7.7, Panel A). The overall energy balance (intake minus gestation, maintenance, and walking energy, Mcal/d) and unaccounted energy (intake minus total energy expenditure, Mcal/d) were less for P cows compared with NP cows ($P < 0.001$; Table

7.6). Energy balance also was reduced for exercised cows compared with non-exercised cows ($P < 0.02$; Table 7.6).

During wk 5 to parturition, daily energy expenditure (Mcal/d) was greatest for P, E cows and least for NP, NE cows compared with other groups of cows (interaction of T by PS, $P = 0.017$; Table 7.6; Figure 7.7, Panel C). Pregnant cows had slightly greater energy expenditure associated with maintenance than NP cows ($P < 0.001$). Cows that were NP, NE had the greatest energy associated with gain, whereas NP, E cows had the least compared with other groups (interaction of T by PS, $P = 0.035$; Table 7.6). Energy intake was greatest for NP cows compared with P cows, and less for E cows compared with NE cows (effects of T and PS, $P < 0.05$; Table 7.6). Exercise treatment and PS affected the overall energy balance ($P < 0.05$; Table 7.6; Figure 7.7, Panel D). Cows that were exercised and pregnant had reduced energy available for gain than NP, NE cows.

As measured in the current study, beef cows forced to exercise required 31% more feed compared to non-exercised cows and were unable to maintain similar BW gains as non-exercised cows (Bellows et al., 1994). In the current study, all cows were in positive energy balance and increased BW gain as day of experiment increased. However, the gain of BW was less for E cows than NE cows. No additional research was found to compare with the results of the current experiments.

Estimates of energy balance (calculated as energy intakes minus energy expenditure for maintenance, gestation, and walking) ranged from 5 to 18 Mcal/d for the entire experiment (Table 7.6). However, the estimates of energy associated with gain (calculated from change in BW and average BCS) ranged from 2 to 9 Mcal/d. Based on the differences of energy intake and maintenance, gestation, and walking, it would have seemed that all cows would have had greater weight gains than what was measured. Particularly, NP cows would have had much greater weight gains than P cows. The

unaccounted energy (daily energy intake - daily energy expenditure) ranged from -2 to 14 Mcal/d (Table 7.6). In this study, potential errors associated with determination of energy balance may have included measurement of BW due to the effects of gut fill; estimation of energy of body reserves from an average BCS; determination of TDN of diet in that neutral detergent and acid detergent insoluble CP were estimated from NRC (2001); and(or) estimated NE_L at actual intake (discounted for DMI over maintenance intake for each cow).

CONCLUSIONS

Exercise training treatment reduced the BW gains during the experiment, but did not alter water or DM intake. The reduction of DMI typically measured near parturition occurred for exercise trained, pregnant cows as well as non-exercised, pregnant cows. Pregnancy status did not affect BW gains during the experiment. However, pregnant cows had lower DM and water intakes throughout the experiment compared with NP cows. Furthermore, water intakes were maintained for pregnant cows near parturition, whereas DMI declined. Pregnancy status or exercise training did not affect BCS. After 60 d of exercise training, liver glycogen concentrations were greater. However, pregnant cows regardless of exercise training accumulated triglycerides in liver tissue as day of experiment increased.

During the prepartum period, pregnancy status of cows had a greater effect on energy balance and energy requirements than exercise training. All cows were in positive energy balance and gained BW; however, exercised cows had slightly lower gains and energy balance than NE cows. Furthermore, the differences associated with BW gains among the treatment groups were equivalent to energy required for exercise. Thus, exercise training treatment in this experiment slightly reduced the partitioning of

energy to BW gain for NP and P cows. However, pregnant cows still had an accumulation of triglycerides in liver samples. We conclude that exercise training of pregnant cows does not increase the risk of negative energy balance during the prepartum period.

Table 7.1. Formulated ingredient and analyzed chemical composition of diet fed d 0 to 34 and d 35 to parturition of experiment.

Item	d 0 to 34 of experiment	d 35 of experiment to parturition
Ingredient	———— % of DM ————	
Alfalfa silage	46.9	23.6
Corn silage	33.1	32.0
Spelt hulls	9.9	0
Soybean meal	9.4	0
Supplement ¹	0	14.3
Alfalfa hay	0	7.0
Vitamin-mineral mix	0.7 ²	23.2 ³
Chemical composition		
DM	43.9	50.0
CP	15.9	17.7
ADF	31.5	22.4
NDF	45.8	33.3
EE	3.2	4.1
Ash	7.9	8.1
Ca	0.90	1.17
P	0.34	0.38
Mg	0.24	0.29
K	1.59	1.35
Na	0.13	0.04
Cl	0.51	1.25
S	0.19	0.27
DCAD ⁴ , meq/100 g of dietary DM	20.2	-15.7
TDN for maintenance ⁵	61.3	69.5
DE for maintenance ⁵ , Mcal/kg	2.77	3.14
NE _L ⁶ , Mcal/kg	1.44	1.65

¹ Supplement = SoyChlor 16-7™, West Central ® Soy, Ralston, IA.

² Contained 16.66% Ca, 0.58% P, 0.16% Mg, 0.12% K, 15.0% Na, 0.49% S, 19.70% Cl, 111 ppm of Se, 1233 KIU of vitamin A/kg, 616.2 KIU of vitamin D/kg, and 12.0 KIU of vitamin E/kg.

³ Contained 76.9% of DM as ground corn. Contained 2.70% Ca, 0.37% P, 0.33% Mg, 0.27% K, 0.09% Na, 0.49% S, 0.20% Cl, 2.2 ppm of Se, 73 KIU of vitamin A/kg, 10.8 KIU of vitamin D/kg, and 0.370 KIU of vitamin E/kg.

⁴ DCAD = [(Na + K) - (Cl + S)] meq/100 g of dietary DM.

⁵ Energy equations and estimates of acid-detergent insoluble CP and neutral-detergent insoluble from NRC (2001).

⁶ Based on equations from NRC (2001). Calculated for each cow's average intake over maintenance and then averaged.

Table 7.2. Body weight (BW) and change in BW during experiment.¹

	Treatments					<i>P</i> -value ²		
	NP, NE ³	NP, E ³	P, NE ³	P, E ³	SEM	T	PS	T x PS
<u>BW, kg</u>								
Day of Experiment ⁴						0.067	< 0.001	NS ⁵
-1	684	669	731	718	13.9			
29	728	696	777	757	13.9			
59	762	713	794	776	13.9			
<u>Change in BW, kg</u>								
Day of Experiment								
-1 to 29	44	27	45	38	5.6	0.037	NS	NS
29 to 59	33	17	18	19	4.6	0.104	NS	0.055
-1 to 59	77	43	63	58	8.0	0.019	NS	0.081

¹ Values are least squares means.² T = effect of exercise treatment; PS = effect of pregnancy status; T x PS = interaction of exercise treatment by pregnancy status.³ NP = non-pregnant cows; P = pregnant cows; E = exercised cows; NE = non-exercised cows.⁴ Effect of day, $P < 0.001$, no significant interactions with day.⁵ NS, $P > 0.10$.

Table 7.3. Body condition score (BCS) and change in BCS.¹

	NP, NE ³	NP, E ³	P, NE ³	P, E ³	SEM	<i>P</i> -value ²		
						T	PS	T x PS
<u>Initial BCS</u>	3.5	3.6	3.6	3.6	0.17	NS ⁴	NS	NS
<u>BCS⁵</u>								
Pooled across week	3.9	3.8	3.8	4.0	0.08	NS	NS	NS
<u>Change of BCS</u>								
Wk of Experiment								
1 to 4	0.22	0.37	0.21	0.50	0.13	0.097	NS	NS
4 to 8	0.25	-0.05	0.16	-0.12	0.09	0.003	NS	NS
1 to 8	0.46	0.42	0.31	0.39	0.16	NS	NS	NS

¹ Values are least squares means.

² T = effect of exercise treatment; PS = effect of pregnancy status; T x PS = the interaction of exercise treatment by pregnancy status.

³ NP = non-pregnant cows; P = pregnant cows; E = exercised cows; NE = non-exercised cows.

⁴ NS = non-significant, $P > 0.10$.

⁵ Effect of week, $P < .0001$, no significant interactions with week.

Table 7.4. Muscle concentrations of protein, glycogen (glucosyl units), and natural logarithmically transformed triglycerides (TG).¹

	Treatments				SEM	<i>P</i> -value ²			
	NP, NE ³	NP, E ³	P, NE ³	P, E ³		T	PS	T x PS	D
Protein, mg per g wet tissue	122	122	122	116	3.6	NS	NS	NS	NS
Glycogen (glucosyl units), mg per g wet tissue	1.1	1.1	1.1	1.1	0.04	NS	NS	NS	NS
Glycogen (glucosyl units), µg per mg protein	9.2	9.4	9.1	9.7	0.4	NS	NS	NS	NS
Ln(TG, mg per g wet tissue)	1.24	0.94	1.32	1.48	0.16	NS	0.08	NS	0.005
Ln(TG, µg per mg protein)	3.36	3.07	3.43	3.64	0.18	NS	0.09	NS	0.002

¹ Values are least squares means.

² T = effect of exercise treatment; PS = effect of pregnancy status; T x PS = the interaction of exercise treatment by pregnancy status; D = effect of day of experiment; NS = non-significant, *P* > 0.10.

³ NP = non-pregnant cows; P = pregnant cows; E = exercised cows; NE = non-exercised cows.

Table 7.5. Liver concentrations of protein, glycogen (glucosyl units), and natural logarithmically transformed triglycerides (TG).¹

	Treatments				SEM	<i>P</i> -value ²			
	NP, NE ³	NP, E ³	P, NE ³	P, E ³		T	PS	T x PS	D
Protein, mg per g wet tissue	189	186	183	187	2.7	NS	NS	NS	NS
Glycogen (glucosyl units), mg per g wet tissue	44.7	50.4	45.7	45.1	2.0	NS	NS	0.11	NS
Glycogen (glucosyl units), µg per mg protein	230	274	252	247	11	0.10	NS	0.03	NS
Ln(TG, mg per g wet tissue)	0.19	0.12	0.59	0.70	0.11	NS	0.01	NS	0.001
Ln(TG, µg per mg protein)	1.9	1.8	2.3	2.4	0.12	NS	0.01	NS	0.001

¹ Values are least squares means.

² T = effect of exercise treatment; PS = effect of pregnancy status; T x PS = the interaction of exercise treatment by pregnancy status; D = effect of day of experiment; NS = non-significant, *P* > 0.10.

³ NP = non-pregnant cows; P = pregnant cows; E = exercised cows; NE = non-exercised cows.

Table 7.6. Average prepartum energy expenditure and balance.¹

	Energy, Mcal/d				SEM	P - value ²		
	NP, NE ³	NP, E ³	P, NE ³	P, E ³		T	PS	T x PS
<u>wk 1 to 5⁴</u>								
Daily energy intake ⁵	24	22	21	21	0.8	NS	0.038	NS
Maintenance ⁶	11	11	12	11	0.2	NS	< 0.001	NS
Gain ⁷	8	7	9	8	0.52	0.028	0.095	NS
Gestation ⁸	0	0	3	4	0.10	NS	<0.001	NS
Walking ⁹	0	1	0	1	0.01	<	NS	NS
Daily energy expenditure ¹⁰	19	18	23	23	0.6	NS	< 0.001	NS
Energy balance ¹¹	13	10	7	5	0.8	0.016	< 0.001	NS
Unaccounted energy ¹²	5	4	-2	-2	0.6	NS	< 0.001	NS
<u>wk 5 to parturition⁴</u>								
Daily energy intake	30	26	23	22	0.9	0.023	< 0.001	0.099
Maintenance	11	11	12	12	0.2	NS	< 0.001	NS
Gain	4	2	3	3	0.4	0.062	NS	0.035
Gestation	0	0	4	4	0.1	NS	< 0.001	NS
Walking	0	1	0	1	0.02	<	NS	NS
Daily energy expenditure	15	14	18	20	0.5	NS	< 0.001	0.017
Energy balance	18	14	7	6	0.9	0.002	< 0.001	NS
Unaccounted energy	14	12	5	3	0.7	0.004	< 0.001	NS

¹ Values are least squares means.

² T = effect of exercise treatment; PS = effect of pregnancy status; T x PS = the interaction of exercise treatment by pregnancy status; NS = non-significant, $P > 0.10$.

³ Pooled least squares mean for the following groups: NP, NE = non-pregnant, non-exercised cows; NP, E = non-pregnant, exercised cows; P, NE = pregnant, non-exercised cows; P, E = pregnant, exercised cows.

⁴ Weeks of experiment.

⁵ Daily energy intake, Mcal/d = average DMI * NE_L of diet as a function of each cow's level of intake over maintenance.

⁶ Maintenance, Mcal/d = $0.08 \times BW^{0.75}$

⁷ Gain, Mcal/d = $(0.64/0.6) \times \{[(BW \text{ final} \times BCS \text{ final} \times 0.51826 + 0.65176 \times BW \text{ final})] - [BW \text{ initial} \times BCS \text{ initial} \times 0.51826 + 0.65176 \times BW \text{ initial}]\}$. Simplified equation derived from equations of NRC (2001).

⁸ Gestation, Mcal/d = $(0.00318 \times \text{day of gestation} - 0.0352) \times (\text{calf weight}/45)/0.218$.

⁹ Walking, Mcal/d = $(BW \times 0.478 \times \text{distance, km})/1000$.

¹⁰ Daily energy expenditure, Mcal/d = maintenance + gain + gestation + walking.

¹¹ Energy balance, Mcal/d = daily energy intake - (maintenance + gestation + walking).

¹² Unaccounted energy, Mcal/d = daily energy intake - daily energy expenditure.

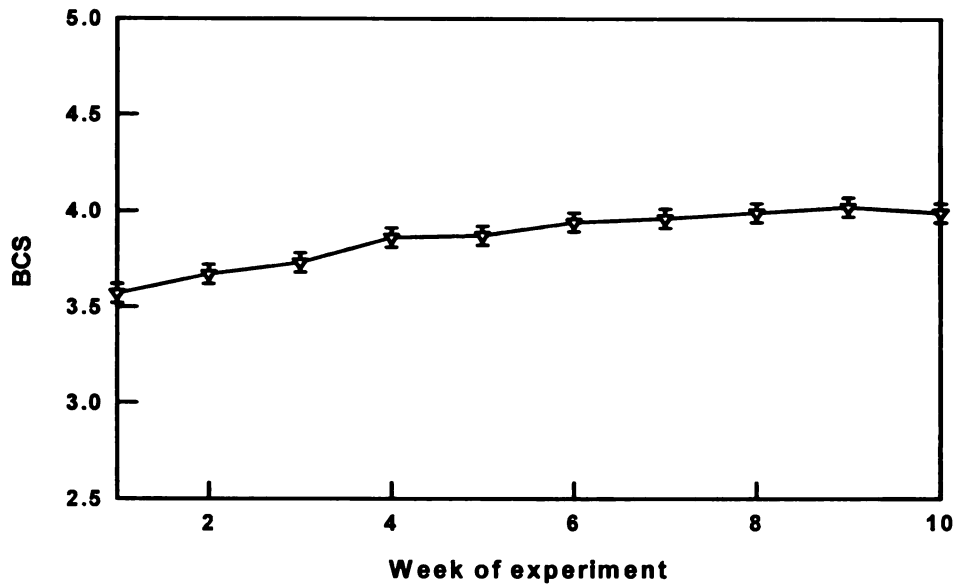


Figure 7.1. Weekly average body condition scores (BCS, least squares means and SEM). Exercise training treatment and pregnancy status did not affect BCS, therefore results are pooled across these effects. Week of experiment affected BCS, ($P < 0.001$).

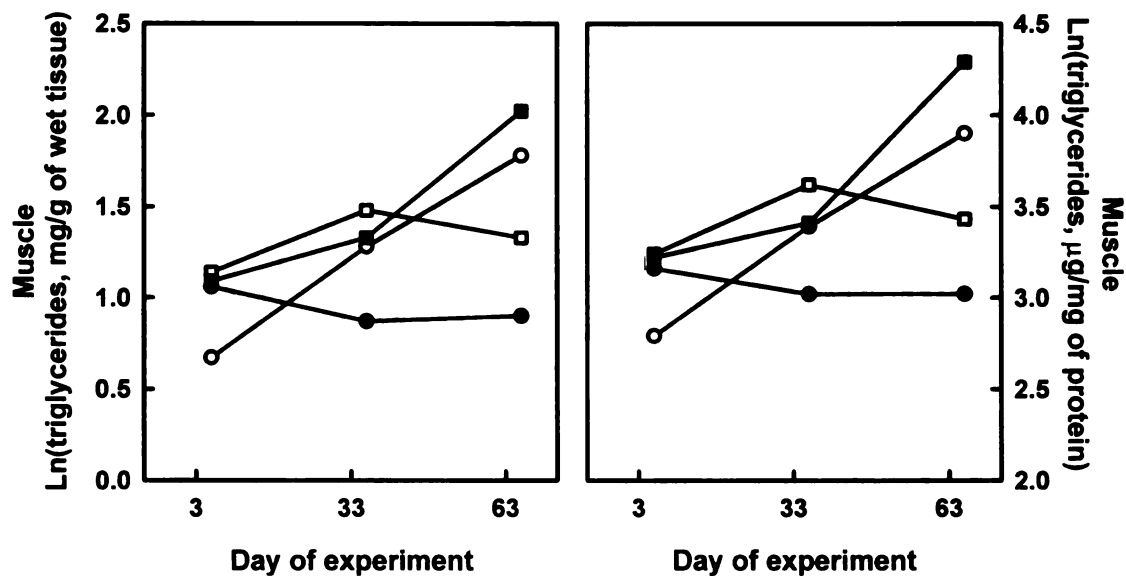


Figure 7.2. Natural logarithmically transformed triglyceride concentrations of muscle tissue on d 3, 33, and 63 of the experiment. Treatments were non-pregnant, non-exercise (○); non-pregnant, exercise (●); pregnant, non-exercise (□); and, pregnant, exercise (■). Interaction of exercise treatment by pregnancy status by day of experiment affected concentrations ($P < 0.01$).

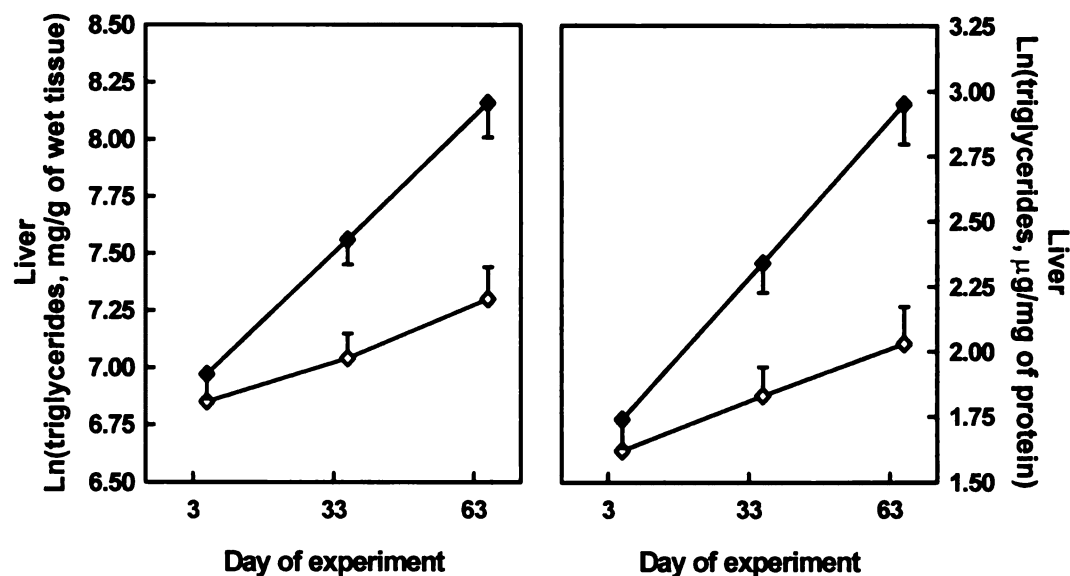


Figure 7.3. Natural logarithmically transformed triglyceride concentrations of liver tissue on d 3, 33, and 63 of the experiment. Results were pooled across exercise treatment: non-pregnant (◇) and pregnant (◆). Interaction of pregnancy status by day of the experiment affected triglyceride concentrations ($P < 0.01$).

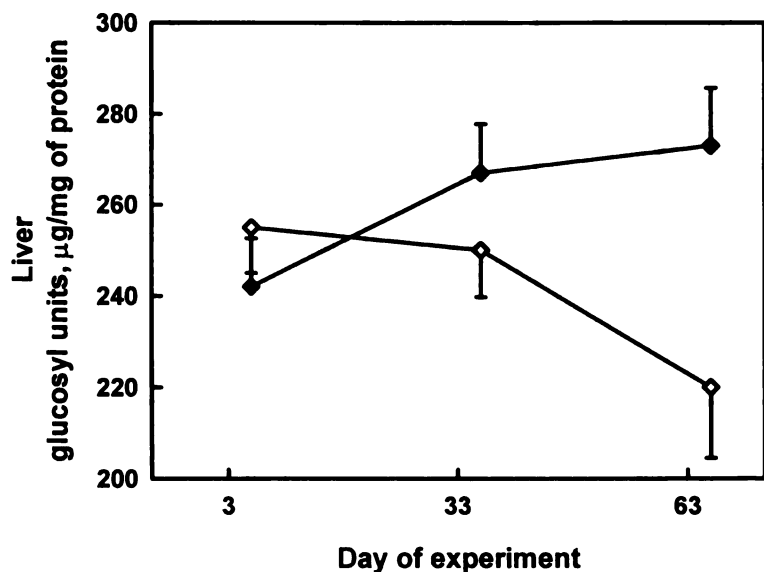


Figure 7.4. Glycogen concentrations of liver tissue on d 3, 33, and 63 of the experiment. Results were pooled across pregnancy status: non-exercise (◇) and exercise (◆). Interaction of exercise treatment by day of the experiment affected glycogen concentrations ($P = 0.02$).

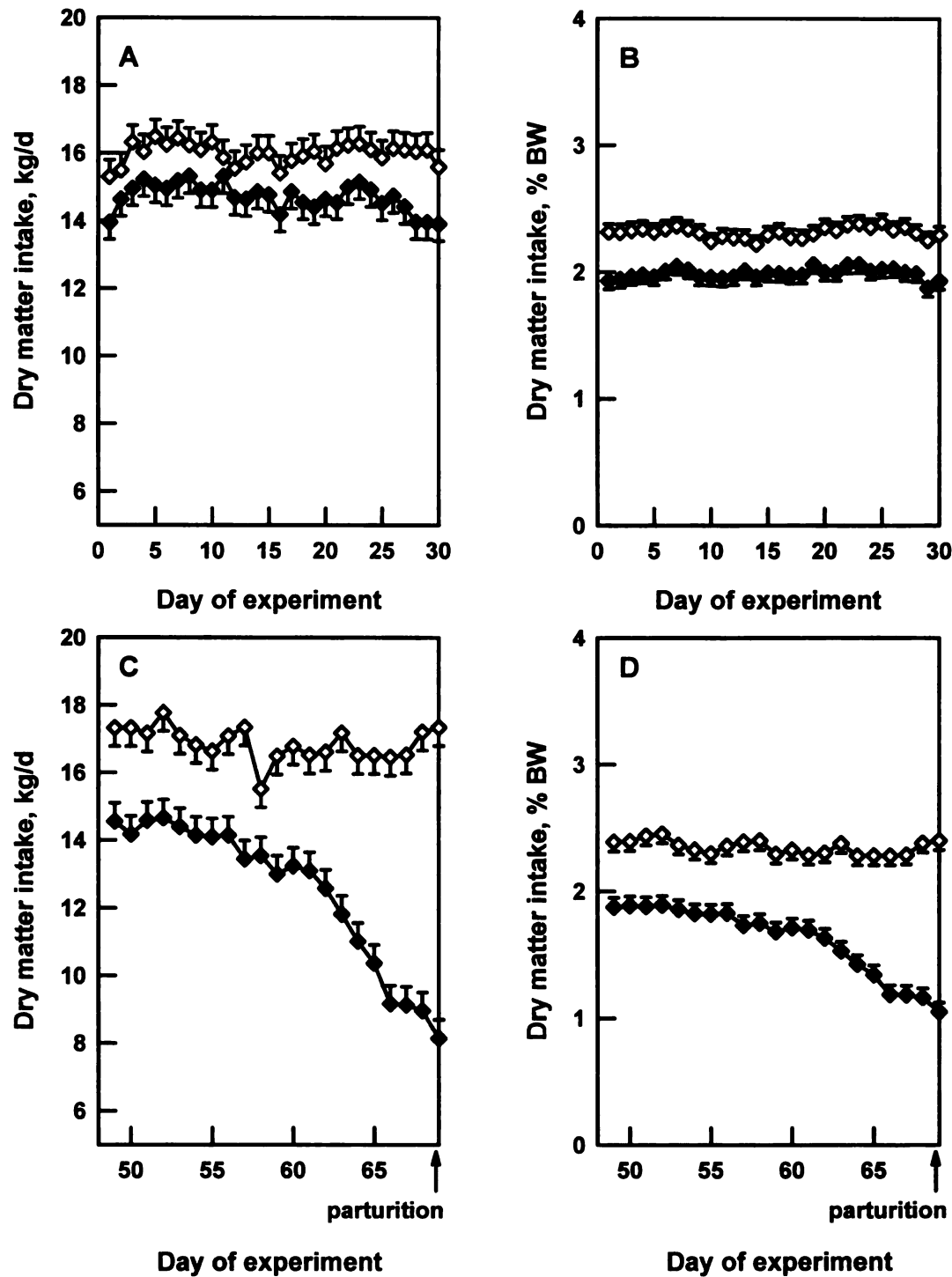


Figure 7.5. DM intake (least squares means and SEM, kg/d and kg/100 kg BW) for each day of experiment (effect of day, $P < 0.05$). *Panel A and B:* during d 0 to 30 of experiment, pregnancy status affected DM intake; pooled across exercise treatment, non-pregnant ($\diamond$) and pregnant ($\blacklozenge$) ($P < 0.05$). *Panel C and D:* during day 49 to parturition, interaction of pregnancy status by day of experiment affected DM intake; pooled across exercise treatment, non-pregnant ($\diamond$) and pregnant ($\blacklozenge$) ($P < 0.001$).

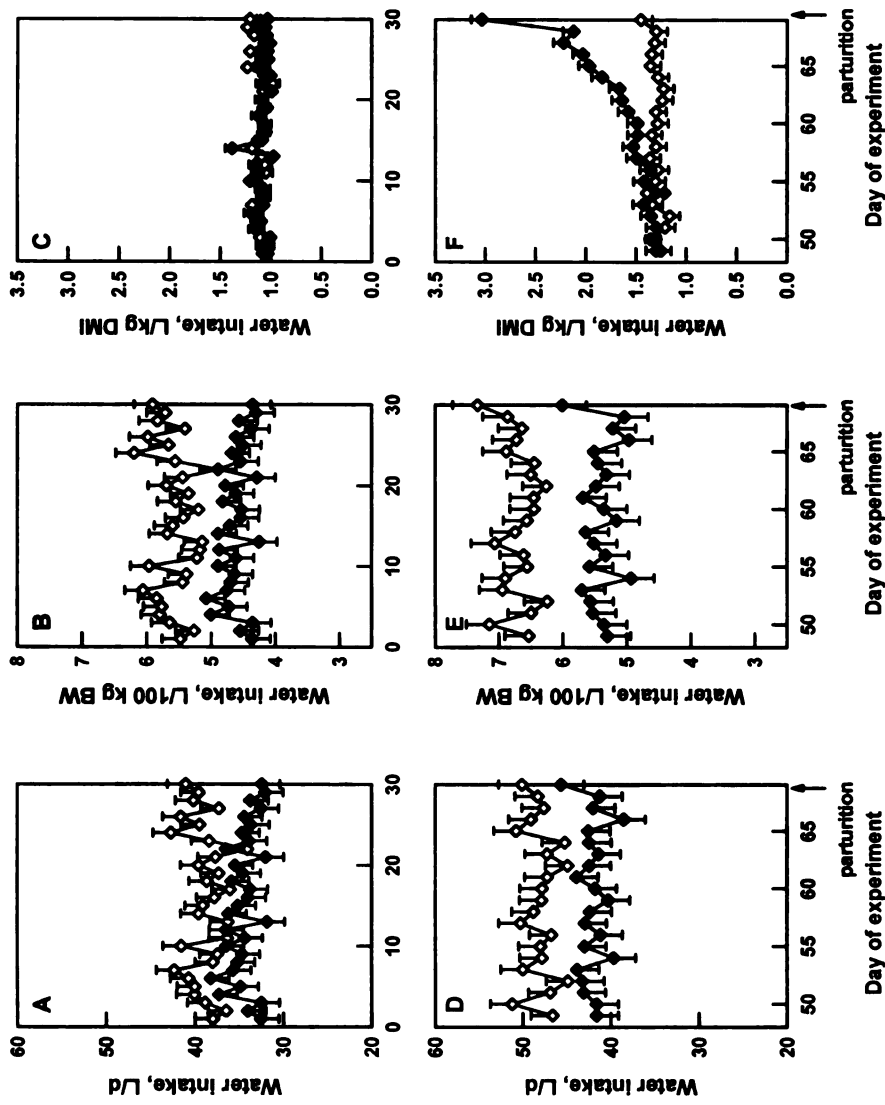


Figure 7.6. Water intake (least squares means and SEM; L/d; L/100 kg of BW; and L/kg of DMI) for each day of experiment. *Panel A and B:* during d 0 to 30 of experiment, pregnancy status and day of experiment affected water intake; pooled across exercise treatment, non-pregnant ($\diamond$) and pregnant ($\blacklozenge$) ($P < 0.05$). *Panel C:* on d 0 to 30 of experiment, exercise treatment, pregnancy status, and day of experiment did not affect water intake (L/kg of DMI). *Panel D and E:* during d 49 to parturition, pregnancy status affected water intake; pooled across exercise treatment, non-pregnant ($\diamond$) and pregnant ($\blacklozenge$) ($P < 0.05$). *Panel F:* during d 49 to parturition, pregnancy status and day of experiment affected water intake (L/kg of DMI); pooled across exercise treatment, non-pregnant ($\diamond$) and pregnant ($\blacklozenge$) ($P < 0.001$).

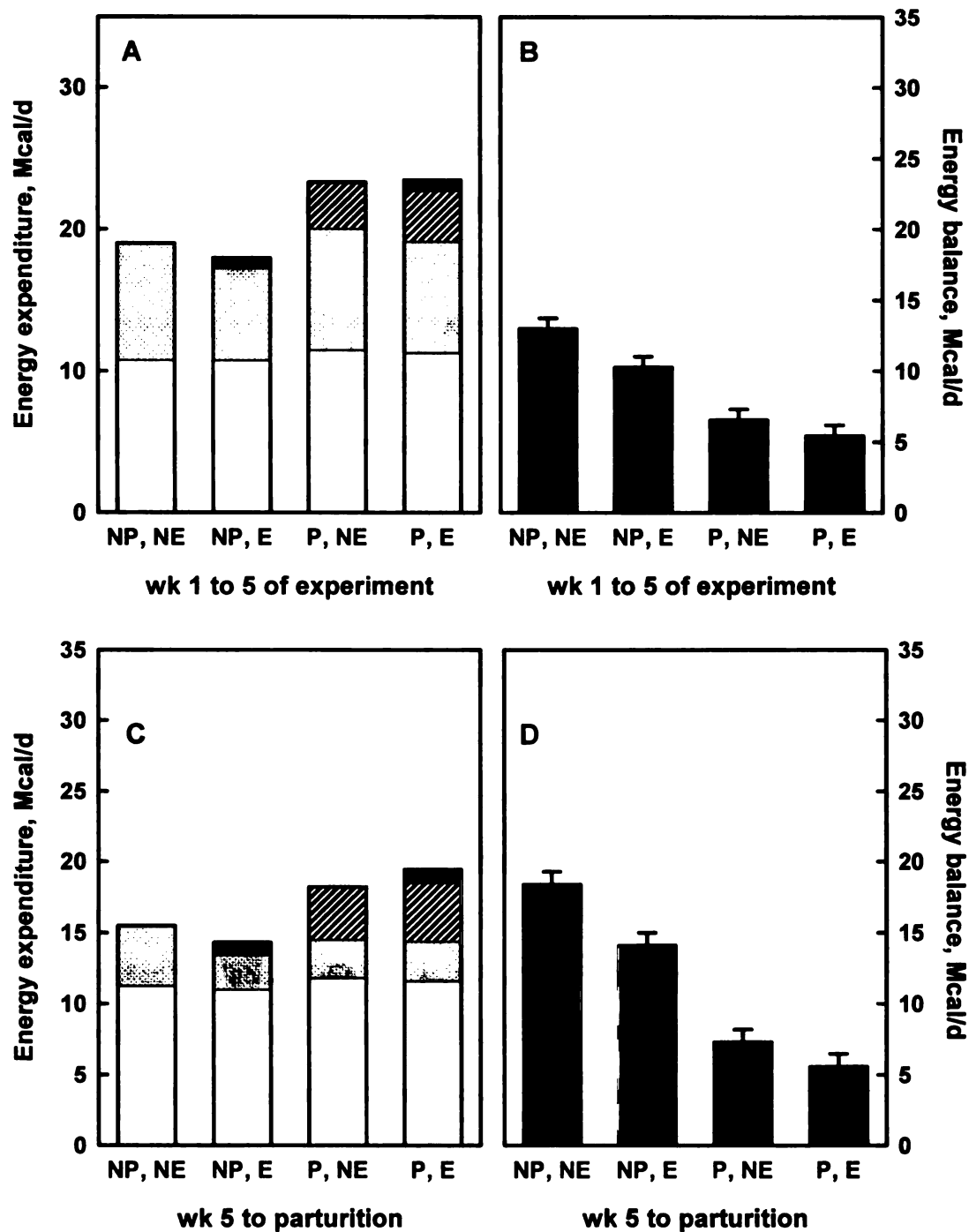


Figure 7.7. Energy expenditure (Mcal/d) and energy balance (ntake minus energy of gestation, maintenance, and walking) for wk 1 to 5 of experiment and wk 5 to parturition. *Panel A and C:* Total energy expenditure was affected by pregnancy status, ($P < 0.001$). Bars represent the total energy expenditure as a function of the expenditures: energy of maintenance (white); energy of gain (gray); energy of walking (black); and energy of gestation (slashed). *Panel B and D:* Energy balance (intake minus energy of gestation, maintenance, and walking) was affected by pregnancy status during wk 1 to 5 ($P < 0.05$). During wk 5 to parturition, exercise treatment and pregnancy status affected energy balance, ($P < 0.05$).

CHAPTER 8

EFFECTS OF EXERCISE TRAINING OF DAIRY COWS ON GLUCOSE, LIPID, AND ACID-BASE STATUS

ABSTRACT

Objective of this experiment was to determine if prepartum exercise training and pregnancy status affected weekly glucose, lipid, and acid-base status as parturition became imminent. Pregnant (n = 26) and non-pregnant (n = 26), non-lactating multiparous Holstein cows were assigned in a factorial arrangement of treatments: exercise training (E) or no exercise (NE), and pregnant (P) or non-pregnant (NP). Exercise training was walking (1.25 to 1.5 h at 3.25 km/h) every other day in an outdoor mechanical walker for 70 d. Blood was collected on two consecutive days during wk 2, 3, 4, 6, 7, 8, and 10 of the experiment. Exercise training did not affect weekly concentrations of plasma glucose and lactate. Cows that exercised had greater plasma non-esterified fatty acid (NEFA), whereas plasma β -hydroxybutyrate (BHBA), insulin and glucagon concentrations were lower compared with NE cows. During glucose tolerance tests, E cows tended to have reduced increase of plasma insulin than NE cows. Exercise training did not affect acid-base status. Pregnant cows had greater plasma and whole blood volumes on d 61 of experiment than NP cows. Pregnant cows had slightly lower partial pressure of carbon dioxide and bicarbonate than NP cows. Exercise training did not affect mineral element concentrations; however, pregnant cows had slightly greater Cl^- and iCa^{2+} concentrations than NP cows. Cows that were exercised had decreased insulin resistance and increased glucagon to maintain plasma

glucose concentrations. We conclude that exercise training caused physiological adaptations to glucoregulatory hormones during prepartum period.

INTRODUCTION

Exercise training, defined as repeated activity or training in a specific program, may cause adaptations of physiological processes to meet the demands of increased activity. Exercise training of nonpregnant and pregnant dairy cows increased physical fitness of cows as determined during a treadmill exercise test (Chapters 4 and 5). However, it is not known if additional adaptations while dairy cows are at rest or during late gestation are influenced by exercise training.

During the periparturient period of ruminants, glucose and lipid homeostasis change rapidly to support the increased nutrient demands of gestation, lactogenesis, and lactation (Bauman and Currie, 1980; Collier et al., 1984). Around parturition, changes of concentrations of insulin, somatotropin, glucocorticoid and catecholamine may stimulate lipolytic, gluconeogenic, and glycogenolytic pathways (Bauman and Currie, 1980; Collier et al., 1984; Drackley, 1999; Drackley et al., 2001). An excessive amount of catabolic activities during the periparturient period (excess mobilization of adipose tissue and depletion of liver glycogen) to support lactogenesis and lactation can be detrimental. Exercise training-induced adaptations of glucose and lipid metabolism and the endocrine control of metabolism prior to parturition may affect the ability of the cow to adjust to the demands of early lactation.

Exercise training may cause adaptations of glucose and lipid metabolism that are beneficial during the transition period of dairy cows. Chronic exercise training in humans (Bergstrom and Hultman, 1966) and in dogs (Reynolds et al., 1995) increased the accumulation of glycogen in exercised muscle tissues. Exercise training increases

muscle uptake of glucose which may affect the insulin-stimulated uptake of glucose to maintain glucose homeostasis (Goodyear and Kahn, 1998). Exercise training can cause adaptations in metabolism of fat from plasma or intramuscular stores to provide energy for exercise and spare carbohydrate sources. In humans, endurance exercise training enhanced the capacity of skeletal muscle to oxidize fatty acids (Mole et al., 1971).

When animals were trained previously, acid-base homeostasis was maintained during an exercise challenge. After exercise training of dairy cows, the differences between post-exercise and resting concentrations of blood CO₂ content, bicarbonate, and base excess were reduced compared with the difference prior to training (Blake et al., 1982). At rest, plasma concentrations of hydrogen ion (H⁺) and bicarbonate (HCO₃⁻), and partial pressure of carbon dioxide (pCO₂) were less for pregnant compared with non-pregnant women (Kemp et al., 1997). However, it was not clear if these same adaptations of acid-base status also extend to a resting cow or if late gestation alters these adaptations.

The objective of this experiment was to determine: if prepartum exercise training affected glucose, lipid, and acid-base status of pregnant and non-pregnant cows at rest and immediately after exercise training. Additionally, the additional dynamics of pregnancy also may affect these physiological responses to exercise training. The hypotheses were that exercise training would improve the carbohydrate and lipid status of pregnant cows.

MATERIALS AND METHODS

Animal care and experimental procedures were approved and conducted under standards of the Michigan State University All-University Committee on Animal Use and

Care, application number 05/98-081-00. Guidelines were compliant with National Institutes of Health and the Guide for the Care and Use of Laboratory Animals.

Cows and Exercise Training Treatments

Twenty-six pairs of non-pregnant and pregnant multiparous Holstein cows were blocked by parity and expected calving date (ECD) of the pregnant animal. Pairs of cows were assigned randomly to exercise treatments. Treatments in a factorial arrangement were exercise training (E) or no exercise (NE), and pregnant (P) or non-pregnant (NP).

Cows had been dried-off at least 1 wk before the start of the experiment. They also were trained to use the mechanical walker with 2 to 3 short duration (5 min) practice sessions, 1 to 2 wk before the experiment. The start of experiment for a block of cows was approximately 70 d before ECD of the pregnant cows. Exercise training was completed every other day until the parturition of the pregnant cow of the pair.

Exercise training was accomplished in an outdoor mechanical walker with a circular lane that was 90-cm wide and 33.8-m long approximately 36.5 m from the alley in the tie-stall barn where cows were housed (Chapter 3). Cows assigned to NE remained in tie-stalls blocked from feed and water while cows assigned to E were walked. From d 2 through d 28 of experiment (about 68 to 42 d before ECD), E cows walked for 1.25 h at 3.25 km/h and from d 32 to parturition for 1.5 h at 3.25 km/h. On the day of their treadmill test (d 0, 30 and 60), cows were not exercised in the mechanical walker.

All cows were fed a TMR ad libitum, once daily. From wk 1 to 4, the diet consisted 47% alfalfa haylage, 33% corn silage, 10% spelt hulls, 9% soybean meal, plus mineral and vitamins (1.44 Mcal of NE_L/kg, 15.9% CP, 31.5% ADF, and 45.8% NDF, dry basis with dietary cation-anion difference, DCAD $[(\text{Na} + \text{K}) - (\text{Cl} + \text{S})]$ meq/100 g of

dietary DM) of 20.2 meq/100 g of dietary DM). From wk 6 to parturition, the diet consisted 24% alfalfa haylage, 32% corn silage, 18% ground corn, 14% soybean meal supplement, plus mineral and vitamins (1.65 Mcal of NE_L/kg, 17.7% CP, 22.4% ADF, and 33.3% NDF, dry basis with DCAD of -15.7 meq/100 g of dietary DM).

Coccygeal Blood Measurements

Coccygeal blood samples before feeding were collected on two consecutive days during wk 2, 3, 4, 6, 7, 8, and 10 of the experiment. During wk 1, 5, and 9, cows completed a treadmill exercise test as described in Chapters 5 and 6; therefore, coccygeal blood samples were not collected during these weeks of the experiment. For all cows, samples were collected on a day of no exercise (rest) day (**Rd**) and a day of exercise day (**Ed**) within 15 min after E cows returned to tie-stall following exercise session. Blood was collected in two air-evacuated glass test tubes; one contained Na-heparin and the other contained sodium fluoride/potassium oxalate (**NaF-K Oxalate**; Vacutainer; Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ). Within 15 min, blood (approximately 3 ml) was transferred from Na-heparin tube to a tube with aprotinin (500 KIU/ml of blood) and EDTA (1 mg/ml of blood).

Tubes containing whole blood with NaF-K Oxalate or Na-heparin with aprotinin and EDTA were centrifuged within 15 min after collection. Plasma was harvested and stored at -20°C until analysis. Plasma from tubes with NaF-K Oxalate was analyzed for glucose, lactate, non-esterified fatty acids (**NEFA**), and β -hydroxy butyrate (**BHBA**). Plasma samples collected in tubes with Na-heparin including aprotinin and EDTA were analyzed for glucagon and insulin concentrations. Concentrations of glucose, L-lactate, NEFA, BHBA, insulin, and glucagon were determined as described in Chapter 6. Blood from Na-heparin only tubes was analyzed for pH, partial pressure of carbon dioxide (**pCO₂**), hematocrit (**HCT**), and concentrations of potassium (**K⁺**), ionized calcium

($i\text{Ca}^{2+}$), and chloride (Cl^- ; Stat 5 Profiler, Nova Biochemical, Princeton, NJ, see Appendix A). From these measurements, the following variables were calculated: bicarbonate (HCO_3^-), total carbon dioxide (TCO_2), hemoglobin (HGB), base excess of blood (BE-B), and base excess of extracellular fluid (BE-ECF).

Glucose Tolerance

Indwelling catheters (see Appendix I) were inserted on the left and right jugular veins of each cow on d -1, 29, and 59 of experiment. Approximately 1 h after feeding on d 1, 31, and 61 of experiment, glucose tolerance tests were performed on all cows. Jugular blood was collected with a syringe containing Na-heparin from one indwelling catheter at min 0, 5, 10, 15, 20, 25, 30, 35, and 40. In the other indwelling catheter, cows were infused at min 10 with a volume of 50% dextrose solution to deliver 150 mg of glucose/kg of BW. Whole blood was transferred to glass test tubes containing aprotinin (500 KIU/ml of blood) and EDTA (1 mg/ml of blood) or NaF (2.5 mg/ml of blood). As described previously, plasma was analyzed for glucose, NEFA, insulin, and glucagon concentrations (Chapter 6).

Whole Blood Volume

On d 1, 31, and 61 of experiment, approximately 2.5 h after feeding and 1 h after infusion of a glucose bolus for the glucose tolerance test, whole blood volume was measured for all cows based on methods described by Delaquis et al., (1995); Hamilton (1958); and, Holmes and Weiskopf (1987) (see Appendix K). A saline solution with 10 mg/ml Evans blue (Sigma-Aldrich, St. Louis, MO) was filtered through a 0.22 micron sterile filter. Jugular blood was collected with a syringe containing Na-heparin from one indwelling catheter at min 0, 5, 10, 12.5, 15, 20, 30, 40, 50, 70, and 90. In the other indwelling catheter, 50 ml of saline solution containing Evans Blue was infused at min

10. Blood (4 ml) was transferred to glass test tubes and centrifuged. Plasma was harvested and stored at -20°C until analysis for plasma concentrations of Evans Blue. Blood collected at min 0, 5, and 10 was analyzed for HCT (Stat 5 Profiler, Nova Biochemical, Princeton, NJ).

Plasma concentrations of Evans Blue were determined using a colorimetric assay (Hamilton, 1958; Holmes and Weiskopf, 1987). The intra- and inter-assay coefficients of variation were 1.9 and 4.0%, respectively. Background absorbency readings were determined with plasma from min 0 for each cow on each day and used to correct absorbency readings of samples after infusion. Plasma samples and standards (10 to 125 µg/ml of sterile-filtered Evans Blue in saline) were assayed (100 µl/well) in a 96-well flat bottom microtiter plate. The absorbency of the Evans Blue in standards and plasma were measured at 625 nm and 740 nm. To correct for hemoglobin and(or) turbidity, the plasma concentration of dye was calculated using the equation of (absorbance₆₂₅ - 1.794 x absorbance₇₄₀; Hamilton, 1958).

Least squares linear regression of the natural logarithmic transformed plasma dye concentrations versus time of sampling were calculated for each cow on each day. Using these equations, the plasma dye concentration at time 0 was determined. The amount of dye infused was determined as the difference of weight of syringe and solution before and after infusion. Thus, with the known amount of dye infused, the plasma volume at time 0 could be calculated (dye concentration at min 0, µg/ml / µg dye infused). Blood cell volume was calculated as the function of (HCT x plasma volume)/(1 - HCT). Summation of plasma volume and blood cell volume equaled total whole blood volume.

Statistical Analysis

Data were analyzed using method of ANOVA by mixed model procedures of SAS (1999). The appropriate covariance structure for the repeated measures was determined using the goodness of fit criteria of Aikai's Information Criterion and Schwarz's Bayesian Criterion. Plasma L-lactate, NEFA, BHBA, insulin, and glucagon concentrations had unequal variances across week of experiment and were transformed using the natural logarithm before analyses. Unless otherwise described, results are presented as generalized least squares means with standard error of mean. Statistical significance was declared at $P < 0.05$ and between $P > 0.05$ and $P < 0.10$ denoted as a tendency.

Weekly blood glucose, lipid, and acid-base status were analyzed with a mixed model including the random effect of cow nested within T and PS, the repeated measures effect of week of experiment, fixed effects of T, PS, and block. All two- and three-way interactions of T, PS, and week of experiment were included in the model. Spatial power provided the best fit for the covariance structure of the repeated measures effect of week of experiment. Data were analyzed separately (four data sets) according to daily activity (Rd or Ed) and diet being consumed (positive DCAD during wk 2 to 4 of experiment and negative DCAD during wk 6 to 10 of experiment).

During the glucose tolerance test, plasma concentrations (glucose, NEFA, insulin, glucagon, insulin : glucose, and insulin: glucagon) were analyzed with a mixed model. Model included the random effect of cow nested within T and PS, the fixed effects of T, PS, and block, and repeated measures of day of experiment and minute of test. All two-, three-, and four-way interactions of T, PS, day of experiment, and minute of test were included in the model. The covariance structures of the data were unstructured for day of experiment and auto-regressive first order for minute of test. Additionally, the basal, peak, and increase of insulin concentrations during the glucose tolerance test were

analyzed with a mixed model including the random effect of cow nested within T and PS, the repeated measures effect of day of experiment, fixed effects of T, PS, and block. All two- and three-way interactions of T, PS, and day of experiment were included in the model. The covariance structure of the repeated measures effect of day of experiment was auto-regressive first order.

Volumes of blood cells, plasma, and whole blood were analyzed with a mixed model including the random effect of cow nested within T and PS, the repeated measures effect of day of experiment, fixed effects of T, PS, and block. All two- and three-way interactions of T, PS, and day of experiment were included in the model. The covariance structure of repeated measures effect of day of experiment was auto-regressive first order.

RESULTS AND DISCUSSION

Weekly changes in the circulating metabolites, hormones, and acid-base status may be in response to exercise training or gestation as parturition became imminent. Glucose, lipid, and acid-base status of ruminants after exercise training has not previously been assessed or characterized.

Glucose and Lipid Status

Plasma glucose. The differences of plasma glucose concentrations between groups were small and within the normal physiological range (Table 8.1). Concentrations ranged from 62 to 66 ± 1.3 mg/dl. Pregnancy status and exercise training treatment did not affect concentrations during wk 2 to 4 and 6 to 10 on either Rd or Ed of experiment. During wk 2 to 4 on Rd, P cows had greater plasma glucose concentrations compared with NP cows on wk 4, whereas on wk 2 and 3 concentrations

were similar between P and NP cows (interaction of PS by week, $P = 0.04$; Figure 8.1). After exercising (Ed) during wk 2 to 4 and 6 to 10, E cows had greater plasma glucose concentrations compared with NE cows on wk 4 and 10, whereas during other weeks E cows had less or equal concentrations than NE cow (interaction of T by week, $P < 0.01$; Figure 8.2). However, the numerical differences were small and only detected for wk 4 and 10. It seems unlikely that exercise training (wk 4 and 10) or pregnancy (wk 4) stimulated an adaptation of plasma glucose concentrations.

An increase of plasma glucose concentrations after acute exercise of calves or sheep compared to resting values has been previously reported (Blum and Eichinger, 1988; Brockman, 1987; Harman and Pethick, 1994; Kuhlmann et al., 1985b; Leury et al., 1990a; Pethick, 1991). No additional research after chronic exercise was found for ruminants to verify that differences detected on wk 4 or 10 may be related to training adaptations.

Plasma lactate. The overall range of untransformed plasma lactate concentrations (0.7 to 1.3 mmol/L) was very small pooled across all weeks and days of experiment and treatment groups. During wk 2 to 4 on Ed, NP, E cows had greater plasma lactate concentrations compared with NP, NE cows, whereas P, E and P, NE cows had similar concentrations of lactate ($P = 0.018$, Table 8.1). Following exercise, E cows tended to have greater concentrations of lactate than NE cows at 2 wk, but not during wk 3 and 4 (interaction of T by week, $P = 0.060$; Figure 8.3). Exercised cows may have experienced adaptations of oxidative capacity which would eliminated the increase of plasma lactate that was measured in wk 2 after exercise training. In humans, exercise training for 7 d reduced lactate concentration following a bout of exercise compared with pre-training values (Putman et al., 1998).

Incomplete oxidation of glucose or anaerobic metabolism in muscle tissue during acute exercise increase circulating concentrations of lactate. The average

concentrations of lactate were small for all groups of cows compared with other studies. Lactate concentrations increased approximately 7.8 mmol/L (1.1 to 8.9 mmol/L) when Hereford calves were exercised for 5 min at 7.9 km/h (Fosha-Dolezal and Fedde, 1988; Kuhlmann et al., 1985b). Moderate and maximum workloads in draught oxen increased lactate concentrations (Zanzinger and Becker, 1992; Zerbini et al., 1995). With respect to pregnancy, no net exchange of lactate by utero-placental tissue of cows was measured (Ferrell, 1991). Therefore, it seems unlikely that utilization of lactate by the fetus of P cows accounted for the response to exercise and pregnancy status detected.

Plasma NEFA. The overall range of untransformed plasma NEFA concentrations were 28 to 923 μ eq/L among cows on either day or week of the experiment. Both exercise treatment and pregnancy status increased circulating concentrations of NEFA (Table 8.2). During wk 2 to 4 on Rd, E cows had greater plasma NEFA concentrations than NE cows on wk 4, whereas on wk 2 and 3 concentrations were similar among groups (interaction of T by week, $P = 0.05$; Figure 8.4). During wk 6 to 10 on Rd, plasma NEFA concentrations in P cows continued to increase for each week, whereas concentrations in NP cows did not change (interaction of PS by week, $P = 0.005$; Figure 8.4). After exercise during wk 2 to 4 and 6 to 10, cows that were pregnant and exercised had the greatest increase of NEFA concentrations as week increased compared with all other groups (interaction of T by PS by week, $P < 0.02$; Figure 8.5). The increase was greatest at wk 10. Plasma NEFA concentrations did not change for NE, NP cows compared with other groups.

In other studies with ruminants, circulating concentrations of NEFA increased slightly during acute exercise with the largest increase occurring at the end of exercise and post-exercise. Hereford-Friesian lactating cows that walked 10 km/d for 3 wk had greater concentrations of NEFA 1 h after walking compared with non-walking periods (0.96 vs. 0.61 mmol/L; Matthewman et al., 1993a). Plasma NEFA concentrations of

calves were greater 5 min after exercise compared with resting values (0.80 vs. 0.66 mmol/l; Blum et al., 1979; Blum and Eichinger, 1988). In sheep, blood concentrations of NEFA increased during exercise of 2 to 4 h (4.5 km/h up a 15.8% slope; Pethick et al., 1987). In pygmy goats, glycerol concentrations increased during submaximal exercise with increases in workload, and remained elevated for 20 to 60 min after exercise (Weber et al., 1993).

Lipid status as indicated by increased circulating concentrations of NEFA was influenced by E treatment and PS. As parturition neared and DMI declined for P cows (Chapter 7), P cows had greater concentrations of NEFA compared with NP cows (122 vs. 60 $\mu\text{eq/L}$). These values were lower than previous reports by Hayirli et al. (2001) and Stephenson et al. (1997), but similar to results of Mashek and Beede (2000).

Plasma BHBA. During the experiment, concentrations of BHBA ranged from 1.8 to 6.9 mg/dl among all cows. Differences among treatment groups were extremely small and within the normal physiological range (Table 8.2). During wk 2 to 4 on Rd, P cows tended to have greater concentrations of BHBA than NP cows ($P = 0.06$), whereas E cows had less plasma BHBA than NE cows after exercise ($P = 0.006$). During wk 6 to 10 on Rd, NP, E cows had the lowest circulating concentrations of BHBA compared with all other groups. Additionally, during wk 6 to 10 on Ed, P cows had greater plasma BHBA than NP cows. After exercise, E cows had reduced concentrations of BHBA during wk 6, 7, and 8 compared with NE cows, whereas on wk 10 concentrations were similar (interaction of T by week, $P = 0.01$; Figure 8.6).

During exercise, incomplete oxidation of fatty acids may increase BHBA concentrations or oxidation of BHBA may reduce circulating concentrations of BHBA. Hereford-Friesian lactating cows that walked 10 km/d for 3 wk had greater concentrations of BHBA 1 h after walking (Matthewman et al., 1993a). In sheep, concentrations of BHBA in blood were slightly greater than resting values after exercise

(Pethick, 1991). The concentrations measured in these studies were approximately 1.5 to 2-fold greater than values in the current experiment. These values were lower than reports of Hayirli et al. (2001), but similar to results of Mashek and Beede (2000) and Stephenson et al. (1997).

Plasma insulin and glucagon. Plasma insulin concentrations were less for P cows compared with NP cows during all weeks of experiment on Ed and Rd ($P < 0.001$, Table 8.3). Similarly, in beef cows, P cows had approximately 25% less plasma insulin than NP cows (Sano et al., 1991). As parturition neared (wk 8 and 10), plasma insulin of P cows declined compared with NP cows (interaction PS by week, $P = 0.016$; Figure 8.7).

During wk 2 to 4 and wk 6 to 10 on either a rest or exercise day, plasma insulin concentrations were lower for E cows compared with NE cows ($P < 0.05$, Table 8.3). Plasma insulin concentrations of calves decreased during walking compared with resting values (Blum and Eichinger, 1988). It was evident that the concentrations of insulin required to maintain glucose concentrations for NP and P cows were reduced after exercise training.

Plasma glucagon concentrations were less for P cows compared with NP cows during wk 2 to 4 and wk 6 to 10 of experiment on Rd and Ed ($P < 0.008$, Table 8.3). The greatest difference between plasma glucagon of P and NP cows occurred on wk 10, near parturition (interaction of PS by week, $P < 0.001$; Figure 8.8). Additionally, concentrations of glucagon were less for E cows compared with NE cows during wk 2 to 4 and 6 to 10 on Ed ($P < 0.05$, Table 8.3). However in another experiment with less duration of exercise, plasma glucagon concentrations of calves were unchanged during walking compared with resting values (Blum and Eichinger, 1988).

Exercised cows had greater plasma NEFA and less BHBA concentrations than NE cows. At the same time, E cows also had reduced insulin concentrations and

slightly less plasma glucagon compared with NE cows; whereas, plasma glucose concentrations were not different among treatment groups. Furthermore, pregnant cows had greater BHBA and NEFA concentrations with no differences in concentrations of glucose and lactate compared with NP cows. Additionally, insulin and glucagon concentrations were reduced for P cows compared with NP cows. Pregnancy status had a greater effect on reducing plasma insulin and glucagon concentrations than exercise training treatment. The reduction of plasma insulin may have contributed to the increased concentrations of NEFA of P and E cows compared with NE and NP cows.

Glucose Tolerance

On day 1, 31, and 61 of experiment, an intravenous glucose infusion (150 mg/100 kg of BW) increased plasma glucose concentration from 60.5 ± 0.94 before infusion to a peak of 145.9 ± 0.94 mg/dl 5 min after infusion ($P < 0.001$; Figure 8.9). After 1 h, glucose concentrations were back to pre-infusion values (data not shown). Exercise treatment or day of experiment did not affect plasma glucose concentrations before or after infusion. However, pooled across minute relative to infusion and day of experiment, P cows tended to have slightly less plasma glucose concentrations compared with NP cows (92.1 vs. 94.4 ± 0.92 mg/dl, $P = 0.090$).

During hyperinsulinemic euglycemic clamp of beef cows, glucose infusions were greater for P cows compared with NP cows in order to maintain similar concentrations of circulating glucose (Sano et al., 1991). Net umbilical uptake of glucose was correlated with maternal arterial blood glucose concentrations (Leury et al., 1990a). Glucose transport across placental tissues is dependent on the maternal-fetal plasma glucose concentration gradient (Bell, 1995) and unresponsive to insulin (Hay et al., 1984). The removal of glucose from the maternal circulation by the conceptus regardless of

endocrine responses may contribute to the differences of plasma glucose concentrations between P and NP cows.

Exercise treatment did not affect natural logarithm of NEFA concentrations which peaked at 10 to 15 min after glucose infusion. As parturition neared on d 61, P cows (112.3 $\mu\text{eq/L}$) had greater NEFA concentrations compared with NP cows (73.7 $\mu\text{eq/L}$, interaction PS by day of experiment, $P = 0.005$). Additionally, the increase of NEFA concentrations were greater for NP cows (11.3 $\mu\text{eq/L}$) compared with P cows (6.1 $\mu\text{eq/L}$, interaction of PS by minute relative to infusion, $P = 0.007$; Figure 8.9). In other research, prepartum NEFA concentrations of dairy cows after intravenous infusion of glucose decreased as minute after infusion increased (Hayirli et al., 2001). In that study, cows were fasted prior to glucose tolerance tests, and plasma NEFA concentrations were greater than values of the current study. The use of heparin as an anticoagulant in the current study may have also affected measurement of NEFA concentrations.

The four-way interaction of T by PS by day of experiment by minute relative to glucose infusion affected insulin concentrations ($P < 0.001$; Figure 8.9). Average basal insulin concentrations prior to infusion were lower for P cows, but greater for NP cows as day of experiment progressed (interaction of T by day of experiment, $P < 0.001$, Table 8.4). Peak concentrations of insulin occurred at either a min 10 or 15 after glucose infusion among all treatment groups. After glucose infusion, insulin concentrations returned to pre-infusion values within 1 h (data not shown). Regardless of exercise treatment, pregnant cows had lower peak concentrations of insulin compared with NP cows after infusion ($P < 0.001$, Table 8.4). In beef cows, during hyperglycemic clamp, P cows compared with NP cows had lower plasma insulin concentrations and required more glucose infusion to maintain similar concentrations of circulating glucose (Sano et al., 1991). As day of experiment increased, peak insulin

concentrations tended to be greater for NP cows, but remained unchanged for P cows (interaction of PS by day, $P = 0.10$, Table 8.4). The increase over the average basal concentrations of insulin were greater for NP cows compared with P cows (Table 8.4). Additionally, as day of experiment increased, the increase over basal concentrations of insulin tended to be greater for NE cows than for E cows (interaction of T by day, $P = 0.09$, Table 8.4). Exercise treatment affected insulin responsiveness to the glucose bolus and (or) tissue sensitivity to insulin as described in other species (Goodyear, 2000).

During the glucose tolerance test, E cows tended to have lower plasma glucagon concentrations than NE cows ($P = 0.08$; Figure 8.9). On d 61 of experiment, P cows had lower concentrations of plasma glucagon compared with NP cows (interaction of PS by day of experiment by minute relative to infusion, $P = 0.078$; Figure 8.9). The decline in plasma glucagon after glucose infusion was greater for NP cows (40% decline) compared with P cows (30% decline). Similar results were reported for dairy cows after intravenous glucose infusion (Hayrili et al., 2001).

The interaction of T by PS by day of experiment by minute relative to glucose infusion affected the molar ratios of insulin : glucose and insulin : glucagon ($P < 0.05$; Figure 8.10). Prior to glucose infusion on d 61, the basal insulin : glucose ratios were lower for P, NE cows compared with P, E cows; and, both were lower than ratios for NP, NE and NP, E cows. During late gestation, the insulin : glucose ratios were reduced for P cows compared with NP cows. Similarly on d 61, the basal ratios of insulin : glucagon were lower for P, NE cows compared with all other treatment groups (Figure 8.10). Ratios after glucose infusion were similar among treatment groups. The differences of ratios among treatment groups were primarily due to the reduced basal insulin concentrations of P, NE cows compared with all other groups. However, if the effects of

T or PS were due to improved insulin responsiveness to the glucose bolus and(or) tissue sensitivity to insulin.

Whole Blood Volume

Volumes of blood cells, plasma, and whole blood increased as day of experiment progressed (effect of day, $P < 0.001$; Figure 8.11). As parturition approached, volume of blood cells, plasma, and whole blood increased more for P cows compared with NP cows (interaction of PS by day, $P < 0.05$; Figure 8.5). Exercise treatment did not affect plasma and whole blood volumes. However, blood cell volumes of E cows were slightly less than NE cows ($P = 0.040$, Table 8.5). The NP, E cows tended to have the smallest blood cell volumes compared with all other groups; whereas, P, NE and P, E cows tended to have similar blood cell volumes (interaction of T by PS, $P = 0.069$, Table 8.5).

Hematocrit and HGB concentrations of blood collected weekly during the experiment on Rd and Ed were not affected by T or PS (HCT: mean = 30.6%; HBC: mean = 10.1 g/dl). However, immediately after exercise training during wk 6 to 10 of experiment, HCT of P cows tended to be reduced compared with HCT of NP cows (29.5 vs. 30.2 ± 0.26 , respectively, $P = 0.082$). In our previous experiment (Chapter 4) and that of Blake et al. (1982), exercise training treatment did not affect HCT or packed cell volume. The difference between measured HCT and the calculated blood cell volume may be due to the differences of time of day of sampling or determinations were after glucose tolerance test.

In humans, pregnancy and exercise training both increased whole blood volume (McMurray et al., 1993). In the current experiment, whole blood and plasma volumes on d 61 were greater for P cows compared with NP cows (6.1 vs. 5.6 kg/100 kg BW and 4.3 vs. 3.9 kg/100 kg BW for whole blood and plasma volumes, respectively). Similarly, plasma volumes (as a percent of BW) for non-lactating cows were described as less

than for lactating cows (Reynolds, 1953). Woodford et al. (1984) reported plasma volumes during 21 d prepartum to 42 d postpartum as 5.6 and 6.9 kg/100 kg BW, respectively. In contrast, plasma volumes of non-lactating, pregnant cows (Delaquis and Block, 1995a) compared with three stages of lactation (Delaquis and Block, 1995b) were especially greater 58.7 vs. 30.7, 35.5, and 39.6 L/cow or 8.1 vs. 6.3, 14.6, and 6.6 kg/100 kg BW, respectively. In the current experiment, P cows had greater whole blood and plasma volumes without changes in blood cell volume compared with NP cows. It is not known how this may affect the ability of pregnant cows to maintain acid-base homeostasis during the periparturient period.

Acid-Base Status

Acid-base homeostasis was controlled tightly and within normal physiological ranges throughout this experiment. Even though the changes of acid-base status were small, significant differences between treatment groups were detected. As described previously (Chapter 4), acute exercise increases plasma lactate concentrations which increases the H^+ concentrations. Through compensatory mechanisms of respiration and extracellular and intracellular buffer, the acid-base status was maintained as indicated by concentrations of H^+ , pH, pCO_2 , and concentrations of HCO_3^- , TCO_2 , BE-B, and BE-ECF.

The resting concentrations of plasma lactate were not affected by PS or T. During the early stages of training, plasma lactate concentrations after exercise training were greater for NP, E cows compared with NP, NE cows; whereas for P, E and P, NE cows, plasma lactate concentrations were not different (Figure 8.3). Murrah buffalo heifers walking 0 km vs. 3 km or 6 km had a 2-fold increase in lactic acid concentrations (Singh and Singh, 1986). Therefore, cows may have experienced relatively small increases of the acid-load during exercise training. The time in which samples were

collected relative to the cessation of activity (5 to 15 min) also may have prevented the detection of differences in plasma acid-base status related to exercise training.

On Rd for wk 2 to 4, blood concentrations of H^+ and pH were similar for NP, E; NP, NE; and P, NE cows, whereas P, E cows had decreased concentrations of H^+ (greater pH) compared with all other treatment groups (Table 8.6). Immediately following exercise training (Ed) during wk 2 to 4, H^+ concentrations and pH were not different among treatment groups. During wk 6 to 10, when cows were fed a negative DCAD diet (-15.7 meq/100 g of DM) concentrations of H^+ were greater (lower pH) than wk 2 to 4 for all groups (Table 8.6). On Rd, concentrations of H^+ tended to be less (greater pH) for E cows compared with NE cows for wk 7, 8, and 10 (interaction T by week, $P < 0.070$; Figure 8.12). On Ed, concentrations of H^+ were less (greater pH) for E cows compared with NE cows for wk 7, 8, and 10 (interaction T by week, $P < 0.040$; Figure 8.12). Additionally, during wk 6 to 10 on Ed, P cows tended to have less H^+ concentrations (greater pH) compared with NP cows ($P < 0.090$, Table 8.6).

The pCO_2 and concentrations of HCO_3^- and TCO_2 responded in a similar fashion among all treatment groups. On Rd and Ed during wk 2 to 4, P cows had reduced pCO_2 and concentrations of HCO_3^- and TCO_2 than NP cows ($P < 0.02$, Table 8.7). Additionally, during wk 2 to 4 on Ed, E cows had reduced pCO_2 and HCO_3^- and TCO_2 concentrations in blood compared with NE cows ($P < 0.05$, Table 8.7). During wk 6 to 10 on Rd, E cows generally had lower pCO_2 and HCO_3^- and TCO_2 concentrations compared with NE cows (the interaction of T by week of experiment, $P = 0.016$; Figure 8.13 A, C, and D; Table 8.7). During wk 6 to 10 on either day (Rd or Ed), P cows had less pCO_2 and HCO_3^- and TCO_2 concentrations compared with NP cows ($P < 0.02$, Table 8.7). Pregnant cows tended to have lower pCO_2 compared with NP cows ($P = 0.061$, Figure 8.13 B). Pregnancy status tended to affect concentrations of TCO_2 , in that P cows had slightly lower concentrations than NP cows ($P = 0.91$, Table 8.7).

Calculations of BE-ECF and BE-B attempt to separate the metabolic and respiratory influences on acid-base status (Fencl and Leith, 1993). Base excess of extracellular fluid and BE-B were calculated as the amount of base needed to adjust blood pH to 7.4 at a standardized $p\text{CO}_2$ of 40 mm Hg. These calculations are a function of HCO_3^- concentrations which were determined from the $p\text{CO}_2$ and pH of the blood. Calculated BE-B and BE-ECF concentrations responded in a similar fashion to each other on Rd and Ed during wk 2 to 4 and wk 6 to 10 of experiment. The BE-B and BE-ECF were greater for NP cows compared with P cows on Rd and Ed during wk 2 to 4, and during wk 6 to 10 on Rd, but not on Ed ($P < 0.05$, Table 8.8). Additionally, during wk 2 to 4 on Ed, NE cows had greater BE-B and BE-ECF concentrations compared with E cows ($P < 0.001$, Table 8.8).

At wk 5 of the experiment, all cows were fed a diet with a DCAD of -15.2 meq/100 g of DM. This increased the concentrations of H^+ (lower pH) of blood and reduced $p\text{CO}_2$ and concentrations of HCO_3^- , TCO_2 , BE-B, and BE-ECF, similarly for all cows regardless of exercise treatment, pregnancy status, and day of sampling (resting or exercised) compared with wk 2 to 4 of the experiment. Following exercise treatment, no differences in concentrations of H^+ were measured during wk 2 to 4, whereas during wk 6 to 10 concentrations of H^+ were lower (greater pH) for E cows than NE cows. During wk 2 to 4, $p\text{CO}_2$, and concentrations of HCO_3^- , TCO_2 , BE-B, and BE-ECF were less for E cows than NE cows after exercise treatment, whereas during wk 6 to 10 on Ed only $p\text{CO}_2$ was reduced for E cows compared with NE cows.

The small changes of weekly plasma lactate concentrations support the concept that cows did not experience uncompensated metabolic acidosis after exercise training. However, the differences of concentrations of H^+ (lower pH), $p\text{CO}_2$, and concentrations of HCO_3^- , TCO_2 , BE-B, and BE-ECF of exercised cows compared with NE cows may be reflective of increased ventilation after the exercise training. Increased ventilation in

response to exercise would reduce extracellular $p\text{CO}_2$ as reported for the current experiment.

Although respiration frequency and ventilation volume were not measured in the current experiment, previous research has shown that exercising cattle increases the ventilatory responses. Respiration frequency and ventilation volume increased compared with rest (36 breaths per min and 16.5 L/min, respectively) when 5- to 8-month old Simmental calves were walked for 5 min at 3.6 km/h (65 breaths per min and 47.4 L/min, respectively). Walking at the same rate up a 10.5% slope, increased respiration frequency an additional 14 breaths per min and ventilation volume an additional 34.6 L/min. Similarly, Arave et al. (1987) reported an increase in respiration rate of 2-yr old Holstein heifers after 30 min of exercise at 1.5 km/h, up a 9% slope. No research was found to determine if adaptations of the respiratory system can occur in ruminants after exercise training or if these adaptations may correlate with differences in the acid-base status of blood. However, heat stress of dairy cows, which increased respiration rates compared with cows in a thermoneutral environment (40.2 vs. 86.0 cycles/min), also decreased arterial $p\text{CO}_2$ and HCO_3^- concentrations (Schneider et al., 1988). Therefore, it seems plausible that the changes in $p\text{CO}_2$ and concentrations of HCO_3^- , TCO_2 , BE-B, and BE-ECF measured in the current experiment may be in response to increased respiration rate and (or) volume after exercise.

Pregnancy status did not affect concentrations of H^+ (pH) during the entire experiment (wk 2 to 4 and 6 to 10) regardless of day of measurement (Rd and Ed). However, blood $p\text{CO}_2$ and concentrations of HCO_3^- , TCO_2 , BE-B, and BE-ECF were less for P cows compared with NP cows regardless of the DCAD of the diet. Potentially, differences in plasma and blood volumes or ventilation volume or rate between pregnant and nonpregnant cows may have contributed to these findings.

Mineral Element Concentrations

During wk 2 to 4 on Rd and Ed, K^+ concentrations were slightly greater for NP cows compared with P cows ($P < 0.06$, Table 8.9). During wk 6 to 10, exercise treatment and pregnancy status did not affect concentrations of K^+ during Rd. However, on Ed during wk 6 to 10, E cows had slightly less blood K^+ concentrations than NE cows ($P = 0.03$, Table 8.9). In contrast, serum concentrations of K^+ increased as exercise speed increased in calves (Fosha-Dolezal and Fedde, 1988; Kuhlmann et al., 1985b). In horses, plasma K^+ concentrations increased after maximal exercise compared with resting values (Judson et al., 1983; Taylor et al., 1995). The increased K^+ concentrations may be due to a shift of plasma volume to intracellular, interstitial, and extracellular compartments of muscle, release of K^+ from muscle cells in response to catecholamines, or incomplete uptake of K^+ during repolarization of muscle (Fosha-Dolezal and Fedde, 1988). Previously, Fosha-Dolezal and Fedde (1988) demonstrated that conditioning of calves reduced the magnitude of increase of serum K^+ concentration during exercise at a given speed compared with pre-conditioned values. In our experiment, exercise treatment only slightly reduced blood K^+ concentrations.

Exercise treatment did not affect concentrations of Cl^- during Rd or Ed for any week of experiment. However, concentrations of Cl^- were greater for P cows compared with NP cows during wk 2 to 4 on both Rd and Ed ($P < 0.001$, Table 8.9). During wk 6 to 10 on both Rd and Ed, P cows had greater concentrations of Cl^- than NP cows, with the greatest difference occurring during wk 10 (interaction of PS by week of experiment, $P < 0.08$; Figure 8.14 A B).

On Rd and Ed, during wk 2 to 4, the NP, NE cows had the least concentration of iCa^{2+} , whereas P, NE cows had the greatest compared with other treatment groups (interaction of T by PS, $P < 0.06$, Table 8.9). In contrast, on Ed during wk 2 to 4, NP, E and P, NE cows had similar iCa^{2+} concentrations, and were greater than concentrations

of NP, NE and P, E cows. During wk 6 to 10 on Rd, the NP, E and P, NE cows had greater concentrations of iCa^{2+} than the concentrations of NP, NE and P, E cows (interaction of T by PS, $P = 0.03$, Table 8.9). However, on Ed, the NP, NE and NP, E cows tended to have greater concentrations of iCa^{2+} than P, NE and P, E cows (interaction of T by PS, $P = 0.08$, Table 8.9). On Ed, exercise treatment reduced the concentrations of iCa^{2+} : 1.249 vs. 1.213 mmol/L, NE vs. E, respectively. Additionally, on Rd during wk 6 to 10, E cows had greater concentrations of iCa^{2+} at wk 6, 7, and 8, but not wk 10 when compared with NE cows ($P < 0.05$, Figure 8.15 A). During wk 6 to 10 on Ed, E cows had less concentrations of iCa^{2+} than NE cows with the greatest magnitude of difference during wk 10 ($P < 0.05$, Figure 8.15 B). Although PS and T affected concentrations of iCa^{2+} , the differences were slight and well within acceptable ranges for prepartum dairy cows. The physiological significance of differences of mineral element concentrations among treatment groups were minor and may be reflective of variation of in plasma and blood volume among cows.

CONCLUSIONS

Prepartum exercise training and pregnancy status affected weekly glucose, lipid, and acid-base status as parturition became imminent. Exercise training treatment did not affect greatly circulating concentrations of metabolites, but reduced insulin and glucagon concentrations to maintain similar glucose concentrations. This adaptation of glucoregulatory hormones was similar to physiological responses of pregnancy. Non-exercised cows required more insulin and glucagon to maintain plasma glucose compared with exercise cows. During the glucose tolerance test, the increases over basal insulin concentrations were reduced for exercised cows compared with non-exercised cows, which indicates that the responsiveness of insulin to glucose or the

tissue sensitivity to insulin was improved. Additionally, cows that were pregnant had less plasma glucose, more NEFA and BHBA concentrations than NP cows. Pregnant cows required less insulin and glucagon to maintain similar concentrations of plasma glucose. The fetal uptake of glucose independent of maternal glucoregulatory control probably contributed to this response related to pregnancy status. Acid-base status and mineral element concentrations were not greatly affected by pregnancy status or exercise training. Pregnancy status increased plasma and whole blood volumes from d 1 to d 61 of experiment. Pregnant cows had greater plasma volumes than non-pregnant cows, which may explain the differences in concentrations of H^+ , pCO_2 , and concentrations of HCO_3^- , TCO_2 , BE-B, BE-ECF, K^+ , Cl^- , and iCa^{2+} between the P and NP cows. It was not clear if the changes of plasma volume would affect the ability of the pregnant cow to maintain acid-base homeostasis during activity such as parturition. We conclude that pregnancy status and exercise training of dairy cows affected the endocrine control of glucose homeostasis and NEFA concentrations during the prepartum period. The implications are that exercised cows may have a greater control of glucose supply during the periparturient period to support subsequent cow health and lactation.

Table 8.1. Plasma concentrations of plasma glucose and L-lactate (natural logarithmically transformed) on a rest day (**Rd**) and immediately after exercise training (**Ed**) during wk 2 to 4 and 6 to 10 of experiment.¹

	Treatment		Pregnancy		Treatment and Pregnancy						P-value ²	
	NE ³	E ³	NP ⁴	P ⁴	NP, NE ⁵	NP, E ⁵	P, NE ⁵	P, E ⁵	SEM	T	PS	T x PS
<u>Glucose, mg/dl</u>												
wk 2 to 4 ⁶												
Rd	63.9	65.1	64.1	64.8	63.2	65.0	64.5	65.1	1.31	NS	NS	NS
Ed	64.8	65.0	64.1	65.7	63.6	64.7	66.1	65.2	1.28	NS	NS	NS
wk 6 to 10 ⁶												
Rd	64.3	64.1	63.3	65.0	64.4	62.2	64.2	65.9	1.18	NS	NS	NS
Ed	64.9	64.6	64.1	65.4	64.2	65.5	63.9	65.3	1.40	NS	NS	NS
<u>Ln(lactate, mmol/L)</u>												
wk 2 to 4 ⁶												
Rd	-0.14	-0.19	-0.22	-0.11	-0.21	-0.23	-0.06	-0.15	0.13	NS	NS	NS
Ed	-0.07	0.13	0.06	0.005	-0.17	0.28	0.03	-0.02	0.11	0.047	NS	0.018
wk 6 to 10 ⁶												
Rd	-0.34	-0.24	-0.26	-0.32	-0.31	-0.20	-0.36	-0.28	0.11	NS	NS	NS
Ed	-0.22	-0.27	-0.17	-0.32	-0.18	-0.16	-0.26	-0.38	0.11	NS	NS	NS

¹ Values are least squares means.

² Probability values of main effects of exercise treatment (T), pregnancy status (PS), and the interaction of T by PS (T x PS), and NS = non-significant, $P > 0.10$.

³ Pooled least squares mean regardless of pregnancy status, or week of experiment. NE = non-exercised cows; E = exercised cows.

⁴ Pooled least squares mean regardless of exercise treatment, or week of experiment. NP = non-pregnant cows; P = pregnant cows.

⁵ Pooled least squares mean regardless of week of experiment. NP, NE = non-pregnant, non-exercised cows; NP, E = non-pregnant, exercised cows; P, NE = pregnant, non-exercised cows; P, E = pregnant, exercised cows.

⁶ Wk = week of experiment. Wk 2 to 4, cows were fed a diet with a DCAD of 20.2 meq/100 g of DM. Wk 6 to 10, cows were fed a diet with a DCAD of -15.2 meq/100 g of DM.

Table 8.2. Plasma concentrations (natural logarithmically transformed) of non-esterified fatty acids (NEFA) and β -hydroxy butyrate (BHBA) on a rest day (Rd) and immediately after exercise training (Ed) during wk 2 to 4 and 6 to 10 of experiment.¹

	Treatment		Pregnancy		Treatment and Pregnancy						P-value ²		
	NE ³	E ³	NP ⁴	P ⁴	NP, NE ⁵	NP, E ⁵	P, NE ⁵	P, E ⁵	SEM	T	PS	T x PS	
<u>Ln(NEFA, μeq/L)</u>													
wk 2 to 4 ⁶													
Rd	3.97	4.05	3.97	4.05	3.94	4.01	3.99	4.10	0.04	0.035	0.072	NS	
Ed	4.06	4.66	4.30	4.42	4.02	4.57	4.09	4.75	0.10	< 0.001	NS	NS	
wk 6 to 10 ⁶													
Rd	4.10	4.19	4.00	4.28	3.98	4.03	4.21	4.35	0.07	NS	< 0.001	NS	
Ed	4.06	4.78	4.17	4.66	3.94	4.41	4.19	5.14	0.10	< 0.001	< 0.001	0.026	
<u>Ln(BHBA, mg/dl)</u>													
wk 2 to 4 ⁶													
Rd	1.35	1.35	1.29	1.41	1.27	1.32	1.43	1.38	0.06	NS	0.061	NS	
Ed	1.37	1.18	1.23	1.32	1.30	1.15	1.44	1.20	0.06	0.0056	NS	NS	
wk 6 to 10 ⁶													
Rd	1.38	1.27	1.30	1.36	1.41	1.18	1.36	1.36	0.05	0.036	NS	0.026	
Ed	1.28	1.11	1.13	1.27	1.26	1.00	1.31	1.23	0.05	0.005	0.017	NS	

¹ Values are least squares means.

² Probability values of main effects of exercise treatment (T), pregnancy status (PS), and the interaction of T by PS (T x PS), and NS = non-significant, $P > 0.10$.

³ Pooled least squares mean regardless of pregnancy status, or week of experiment. NE = non-exercised cows; E = exercised cows.

⁴ Pooled least squares mean regardless of exercise treatment, or week of experiment. NP = non-pregnant cows; P = pregnant cows.

⁵ Pooled least squares mean regardless of week of experiment. NP, NE = non-pregnant, non-exercised cows; NP, E = non-pregnant, exercised cows; P, NE = pregnant, non-exercised cows; P, E = pregnant, exercised cows.

⁶ Wk = week of experiment. Wk 2 to 4, cows were fed a diet with a DCAD of 20.2 meq/100 g of DM. Wk 6 to 10, cows were fed a diet with a DCAD of -15.2 meq/100 g of DM.

Table 8.3. Plasma concentrations (natural logarithmically transformed) of insulin and glucagon on a rest day (Rd) and immediately after exercise training (Ed) during wk 2 to 4 and 6 to 10 of experiment.¹

	Treatment		Pregnancy				Treatment and Pregnancy					P-value ²		
	NE ³	E ³	NP ⁴	E ³	NP ⁴	P ⁴	NP, NE ⁵	NP, E ⁵	P, NE ⁵	P, E ⁵	SEM	T	PS	T x PS
<u>Ln(insulin, µIU/ml)</u>														
wk 2 to 4 ⁶														
Rd	2.29	2.05	2.47	1.87	2.56	1.72	2.38	2.01	1.72	1.72	0.09	0.013	< 0.001	NS
Ed	2.25	1.97	2.55	1.67	2.64	1.67	2.46	1.86	1.49	1.49	0.11	0.017	< 0.001	NS
wk 6 to 10 ⁶														
Rd	2.55	2.27	2.88	1.94	3.01	1.79	2.74	2.08	1.79	1.79	0.13	0.045	< 0.001	NS
Ed	2.52	2.05	2.77	1.81	2.99	1.56	2.55	2.05	1.56	1.56	0.13	0.001	< 0.001	NS
<u>Ln(glucagon, pg/ml)</u>														
wk 2 to 4 ⁶														
Rd	4.96	4.84	4.99	4.81	5.05	4.81	4.93	4.87	4.75	4.75	0.06	0.082	0.008	NS
Ed	4.96	4.81	4.99	4.79	5.08	4.79	4.89	4.84	4.74	4.74	0.07	0.033	0.005	< 0.001
wk 6 to 10 ⁶														
Rd	5.14	5.06	5.40	4.80	5.49	4.80	5.31	4.79	4.82	4.82	0.08	NS	< 0.001	NS
Ed	5.18	5.00	5.34	4.83	5.49	4.83	5.20	4.88	4.79	4.79	0.07	0.0144	< 0.001	NS

¹ Values are least squares means.

² Probability values of main effects of exercise treatment (T), pregnancy status (PS), and the interaction of T by PS (T x PS), NS = non-significant, $P > 0.10$.

³ Pooled least squares mean regardless of pregnancy status or week of experiment. NE = non-exercised cows; E = exercised cows.

⁴ Pooled least squares mean regardless of exercise treatment or week of experiment. NP = non-pregnant cows; P = pregnant cows.

⁵ Pooled least squares mean regardless of week of experiment. NP, NE = non-pregnant, non-exercised cows; NP, E = non-pregnant, exercised cows; P, NE = pregnant, non-exercised cows; P, E = pregnant, exercised cows.

⁶ Wk = week of experiment. Wk 2 to 4, cows were fed a diet with a DCAD of 20.2 meq/100 g of DM. Wk 6 to 10, cows were fed a diet with a DCAD of -15.2 meq/100 g of DM.

Table 8.4. Plasma basal insulin, peak insulin, and increase over basal concentrations of insulin after an intravenous infusion of glucose.¹

	Treatment and Pregnancy					<i>P</i> -value ²		
	NP, NE ³	NP, E ³	P, NE ³	P, E ³	SEM	T	PS	T x PS
<u>Average basal insulin concentrations, μU/ml</u>								
d 1	21.2	16.2	10.6	11.6	2.31	NS	< 0.001	NS
d 31	21.8	22.4	11.2	10.2	2.26			
d 61	26.5	25.3	7.7	10.1	2.31			
<u>Peak insulin concentrations, μU/ml</u>								
d 1	101.7	91.7	72.0	82.8	8.83	NS	< 0.001	NS
d 31	118.7	103.9	85.3	77.5	8.52			
d 61	131.6	111.7	71.3	80.8	9.59			
<u>Increase over basal insulin concentrations, μU/ml⁴</u>								
d 1	80.6	75.5	61.4	71.1	7.30	NS	0.003	NS
d 31	96.8	81.5	74.2	67.3	7.12			
d 61	104.8	86.5	75.5	70.6	7.30			

¹ Values are least squares means.

² T = exercise treatment; PS = main effect of pregnancy status; T x PS = interaction of treatment by pregnancy status; NS = non-significant, $P > 0.10$.

³ NP, NE = non-pregnant, non-exercised cows; NP, E = non-pregnant, exercised cows; P, NE = pregnant, non-exercised cows; P, E = pregnant, exercised cows.

⁴ Interaction of T by day of experiment, $P = 0.09$.

Table 8.5. Blood cells, plasma, and whole blood volumes of cows.¹

	<u>Treatment</u>		<u>Pregnancy</u>		<u>Treatment and Pregnancy</u>					<u>P-value²</u>	
	NE ³	E ³	NP ⁴	P ⁴	NP, NE ⁵	NP, E ⁵	P, NE ⁵	P, E ⁵	T	PS	T x PS
<u>Blood cell volume, L</u>											
	13.2	12.5	12.2	13.5	12.8	11.6	13.5	13.4	0.04	< 0.01	0.07
SEM	0.22	0.22	0.22	0.22	0.31	0.31	0.31	0.31			
<u>Plasma volume, L</u>											
	30.3	29.6	28.2	31.6	29.0	27.5	31.6	31.6	NS	< 0.01	NS
SEM	0.52	0.52	0.52	0.52	0.74	0.74	0.74	0.73			
<u>Whole blood volume, L</u>											
	43.5	42.1	40.5	45.1	41.8	39.1	45.2	45.0	NS	< 0.01	NS
SEM	0.71	0.70	0.70	0.70	0.99	1.00	1.00	0.99			

¹ Values are least squares means.

² Probability values of main effects of exercise treatment (T), pregnancy status (PS), the interaction of T by PS (T x PS), and NS, P > 0.10.

³ Pooled least squares mean regardless of pregnancy status, or day of experiment. NE = non-exercised cows; E = exercised cows.

⁴ Pooled least squares mean regardless of exercise treatment, or day of experiment. NP = non-pregnant cows; P = pregnant cows.

⁵ Pooled least squares mean regardless of day of experiment. NP, NE = non-pregnant, non-exercised cows; NP, E = non-pregnant, exercised cows; P, NE = pregnant, non-exercised cows; P, E = pregnant, exercised cows.

Table 8.6. Blood concentrations of H⁺ and pH on a rest day (Rd) and immediately after exercise training (Ed) during weeks 2 to 4 and 6 to 10 of the experiment.¹

	Treatment		Pregnancy		Treatment and Pregnancy					P-value ²		
	NE ³	E ³	NP ⁴	P ⁴	NP, NE ⁵	NP, E ⁵	P, NE ⁵	P, E ⁵	SEM	T	PS	T x PS
<u>H⁺, mmol/L</u>												
wk 2 to 4 ⁶												
Rd	3.70	3.57	3.63	3.64	3.65	3.61	3.74	3.53	0.041	0.003	NS	0.035
Ed	3.70	3.64	3.70	3.64	3.73	3.67	3.67	3.61	0.063	NS	NS	NS
wk 6 to 10 ⁶												
Rd	3.94	3.80	3.92	3.82	4.03	3.81	3.86	3.78	0.072	0.053	NS	NS
Ed	4.02	3.67	3.92	3.78	4.08	3.75	3.96	3.60	0.074	< 0.001	0.086	NS
<u>pH</u>												
wk 2 to 4 ⁶												
Rd	7.434	7.450	7.442	7.441	7.440	7.445	7.428	7.454	0.0050	0.004	NS	0.036
Ed	7.434	7.441	7.434	7.441	7.431	7.438	7.437	7.444	0.0076	NS	NS	NS
wk 6 to 10 ⁶												
Rd	7.406	7.424	7.410	7.419	7.398	7.422	7.414	7.424	0.0081	0.043	NS	NS
Ed	7.397	7.438	7.410	7.425	7.391	7.430	7.404	7.447	0.0080	< 0.001	0.092	NS

¹ Values are least squares means.

² Probability values of main effects of exercise treatment (T), pregnancy status (PS), and the interaction of T by PS (T x PS), and NS, P > 0.10.

³ Pooled least squares mean regardless of pregnancy status, or week of experiment. NE = non-exercised cows; E = exercised cows.

⁴ Pooled least squares mean regardless of exercise treatment, or week of experiment. NP = non-pregnant cows; P = pregnant cows.

⁵ Pooled least squares mean regardless of week of experiment. NP, NE = non-pregnant, non-exercised cows; NP, E = non-pregnant, exercised cows; P, NE = pregnant, non-exercised cows; P, E = pregnant, exercised cows.

⁶ Wk = week of experiment. Wk 2 to 4, cows were fed a diet with a DCAD of 20.2 meq/100 g of DM. Wk 6 to 10, cows were fed a diet with a DCAD of -15.2 meq/100 g of DM.

Table 8.7. Blood partial pressure of CO₂ (pCO₂), concentrations of bicarbonate (HCO₃⁻), and total CO₂ (TCO₂) on a rest day (Rd) and immediately after exercise training (Ed) during weeks 2 to 4 and 6 to 10 of the experiment.¹

	Treatment		Pregnancy				Treatment and Pregnancy					P-value ²		
	NE ³	E ³	NP ⁴	NP ⁴	E ⁴	P ⁴	NP ⁵	NP, E ⁵	P, NE ⁵	P, E ⁵	SEM	T	PS	T x PS
<u>pCO₂, mm Hg</u>														
wk 2 to 4 ⁶														
Rd	42.12	41.15	42.71	40.56	42.60	42.82	41.63	42.82	41.63	39.48	0.82	NS	0.012	NS
Ed	42.12	39.81	42.43	39.49	43.88	40.99	40.36	40.99	40.36	38.63	1.13	0.048	0.013	NS
wk 6 to 10 ⁶														
Rd	40.71	39.03	41.64	38.09	42.64	40.64	38.78	40.64	38.78	37.41	1.27	NS	0.006	NS
Ed	41.16	37.55	40.51	38.21	41.81	39.21	40.51	39.21	40.51	35.90	0.98	< 0.001	0.015	NS
<u>HCO₃⁻, mmol/L</u>														
wk 2 to 4 ⁶														
Rd	28.28	28.49	29.06	27.72	28.82	29.29	27.75	29.29	27.75	27.68	0.36	NS	< 0.001	NS
Ed	28.10	27.00	28.35	26.75	29.11	27.60	27.09	27.60	27.09	26.41	0.43	0.015	< 0.001	NS
wk 6 to 10 ⁶														
Rd	25.64	25.31	26.24	24.71	26.26	26.23	25.02	26.23	25.02	24.39	0.46	NS	< 0.001	NS
Ed	25.38	25.31	25.70	24.99	25.48	25.91	25.27	25.91	25.27	24.71	0.46	NS	NS	NS

Table 8.7. (continued).

	<u>Treatment</u>		<u>Pregnancy</u>		<u>Treatment and Pregnancy</u>					<u>P-value²</u>	
	NE ³	E ³	NP ⁴	P ⁴	NP,	NP, E ⁵	P, NE ⁵	P, E ⁵	SEM	T	T x PS
<u>TCO₂, mmol/L</u>											
wk 2 to 4 ⁶											
Rd	29.56	29.75	30.35	28.96	30.10	30.60	29.02	28.90	0.38	NS	< 0.001 NS
Ed	29.40	28.23	29.66	27.96	30.46	28.86	28.33	27.60	0.46	0.016	< 0.001 NS
wk 6 to 10 ⁵											
Rd	26.88	26.51	27.51	25.88	27.55	27.47	26.22	25.54	0.49	NS	< 0.001 NS
Ed	26.64	26.46	26.94	26.16	26.77	27.11	26.51	25.81	0.49	NS	0.091 NS

¹ Values are least squares means.

² Probability values of main effects of exercise treatment (T), pregnancy status (PS), and the interaction of T by PS (T x PS), and NS, $P > 0.10$.

³ Pooled least squares mean regardless of pregnancy status, or week of experiment. NE = non-exercised cows; E = exercised cows.

⁴ Pooled least squares mean regardless of exercise treatment, or week of experiment. NP = non-pregnant cows; P = pregnant cows.

⁵ Pooled least squares mean regardless of week of experiment. NP, NE = non-pregnant, non-exercised cows; NP, E = non-pregnant, exercised cows; P, NE = pregnant, non-exercised cows; P, E = pregnant, exercised cows.

⁶ Wk = week of experiment. Wk 2 to 4, cows were fed a diet with a DCAD of 20.2 meq/100 g of DM. Wk 6 to 10, cows were fed a diet with a DCAD of -15.2 meq/100 g of DM.

Table 8.8. Concentrations of base-excess of blood (BE-B) and base-excess of extracellular fluid (BE-ECF) on a rest day (Rd) and immediately after exercise training (Ed) during weeks 2 to 4 and 6 to 10 of the experiment.¹

	Treatment		Pregnancy		Treatment and Pregnancy					P-value ²			
	NE ³	E ³	NP ⁴	P ⁴	NP,	NP, E ⁵	P,	P, E ⁵	SEM	T	PS	T x PS	
<u>BE-B, mmol/L</u>													
wk 2 to 4 ⁶													
	Rd	4.34	4.80	5.13	4.01	4.86	5.40	3.82	4.19	0.29	NS	< 0.001	NS
	Ed	4.17	3.37	4.40	3.13	4.99	3.82	3.35	2.92	0.34	0.030	< 0.001	NS
wk 6 to 10 ⁶													
	Rd	1.56	1.44	2.10	0.90	1.97	2.23	1.14	0.65	0.39	NS	0.001	NS
	Ed	1.11	1.85	1.66	1.30	1.12	2.20	1.10	1.50	0.45	0.086	NS	NS
<u>BE-ECF, mmol/L</u>													
wk 2 to 4 ⁶													
	Rd	3.84	4.29	4.72	3.41	4.42	5.02	3.26	3.57	0.34	NS	< 0.001	NS
	Ed	3.66	2.68	3.93	2.40	4.63	3.23	2.68	2.13	0.40	0.021	< 0.001	NS
wk 6 to 10 ⁶													
	Rd	0.78	0.63	1.40	0.010	1.29	1.50	0.26	-0.24	0.43	NS	0.001	NS
	Ed	0.30	0.92	0.86	0.37	0.34	1.38	0.26	0.47	0.50	NS	NS	NS

¹ Values are least squares means.

² Probability values of main effects of exercise treatment (T), pregnancy status (PS), and the interaction of T by PS (T x PS), and NS, P > 0.10.

³ Pooled least squares mean regardless of pregnancy status, or week of experiment. NE = non-exercised or E = exercised cows.

⁴ Pooled least squares mean regardless of exercise treatment, or week of experiment. NP = non-pregnant or P = pregnant cows.

⁵ Pooled least squares mean regardless of week of experiment. NP, NE = non-pregnant, non-exercised; NP, E = non-pregnant, exercised; P, NE = pregnant, non-exercised; or P, E = pregnant, exercised cows.

⁶ Wk 2 to 4, cows were fed a diet with a DCAD of 20.2 meq/100 g of DM. Wk 6 to 10, cows were fed a diet with a DCAD of -15.2 meq/100 g of DM.

Table 8.9. Blood concentrations of potassium (K⁺), chloride (Cl⁻), and ionized Ca²⁺ (iCa²⁺) on a rest day (Rd) and immediately after exercise training (Ed) during weeks 2 to 4 and 6 to 10 of the experiment.¹

	Treatment			Pregnancy			Treatment and Pregnancy						P-value ²		
	NE ³	E ³		NP ⁴	NP ⁴	P ⁴	NP ⁵	NP, E ⁵	P, NE ⁵	P, E ⁵	SEM	T	PS	T	T x PS
<u>K⁺, mmol/L</u>															
wk 2 to 4 ⁶															
Rd	4.50	4.47		4.54	4.43	4.43	4.52	4.56	4.48	4.38	0.05	NS	0.029	NS	NS
Ed	4.56	4.46		4.57	4.46	4.46	4.61	4.53	4.51	4.40	0.06	NS	0.060	NS	NS
wk 6 to 10 ⁶															
Rd	4.62	4.64		4.64	4.62	4.62	4.62	4.66	4.62	4.61	0.06	NS	NS	NS	NS
Ed	4.65	4.51		4.59	4.57	4.57	4.64	4.54	4.67	4.48	0.66	0.030	NS	NS	NS
<u>Cl⁻, mmol/L</u>															
wk 2 to 4 ⁸															
Rd	102.6	102.5		101.5	103.7	103.7	101.4	101.5	103.8	103.5	0.33	NS	< 0.001	NS	NS
Ed	102.8	103.1		101.9	104.0	104.0	101.6	102.2	104.1	104.0	0.36	NS	< 0.001	NS	NS
wk 6 to 10 ⁸															
Rd	104.9	105.2		103.5	106.6	106.6	103.4	103.6	106.5	106.8	0.45	NS	< 0.001	NS	NS
Ed	105.2	105.1		103.8	106.5	106.5	103.8	103.8	106.5	106.4	0.49	NS	< 0.001	NS	NS

Table 8.9. (continued).

	Treatment		Pregnancy		Treatment and Pregnancy						P-value ²		
	NE ³	E ³	NP ⁴	P ⁴	NP, ⁵	NP, E ⁵	P, NE ⁵	P, E ⁵	SEM	T	PS	T x PS	
<u>iCa²⁺, mmol/L</u>													
wk 2 to 4 ⁶													
Rd	1.228	1.233	1.231	1.230	1.219	1.242	1.236	1.225	0.0079	NS	NS	0.040	
Ed	1.223	1.220	1.223	1.221	1.216	1.230	1.231	1.211	0.0087	NS	NS	0.055	
wk 6 to 10 ⁶													
Rd	1.242	1.245	1.243	1.245	1.232	1.254	1.253	1.237	0.0085	NS	NS	0.030	
Ed	1.249	1.213	1.230	1.232	1.241	1.219	1.258	1.207	0.0081	< 0.001	NS	0.078	

¹ Values are least square means.

² Probability values of main effects of exercise treatment (T), pregnancy status (PS), and the interaction of T by PS (T x PS), and NS, *P* > 0.10.

³ Pooled least squares mean regardless of pregnancy status, or week of experiment. NE = non-exercised cows; E = exercised cows.

⁴ Pooled least squares mean regardless of exercise treatment, or week of experiment. NP = non-pregnant cows; P = pregnant cows.

⁵ Pooled least squares mean regardless of week of experiment. NP, NE = non-pregnant, non-exercised cows; NP, E = non-pregnant, exercised cows; P, NE = pregnant, non-exercised cows; P, E = pregnant, exercised cows.

⁶ Wk 2 to 4, cows were fed a diet with a DCAD of 20.2 meq/100 g of DM. Wk 6 to 10, cows were fed a diet with a DCAD of -15.2 meq/100 g of DM.

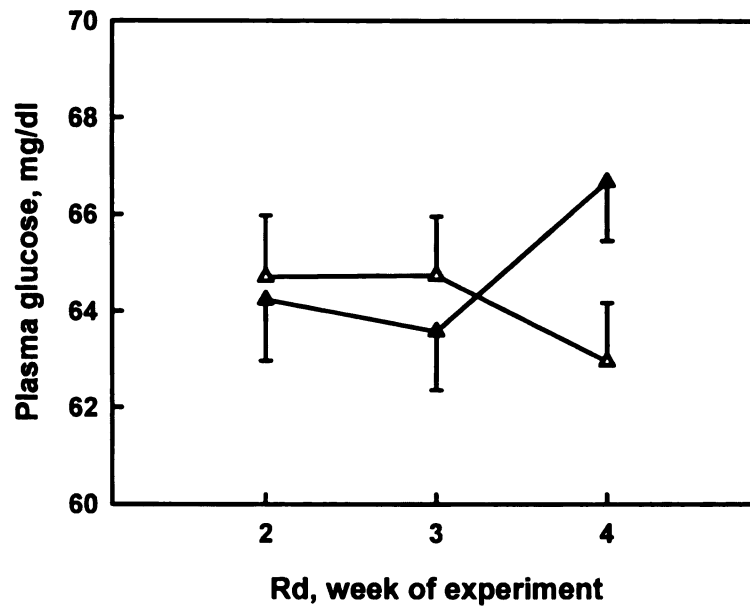


Figure 8.1. Plasma glucose concentrations during non-exercise (rest day, Rd) of wk 2 to 4 of experiment. Results were pooled across exercise treatment: non-pregnant (Δ) and pregnant (▲). Interaction of pregnancy status by week of experiment affected glucose concentrations ($P = 0.04$).

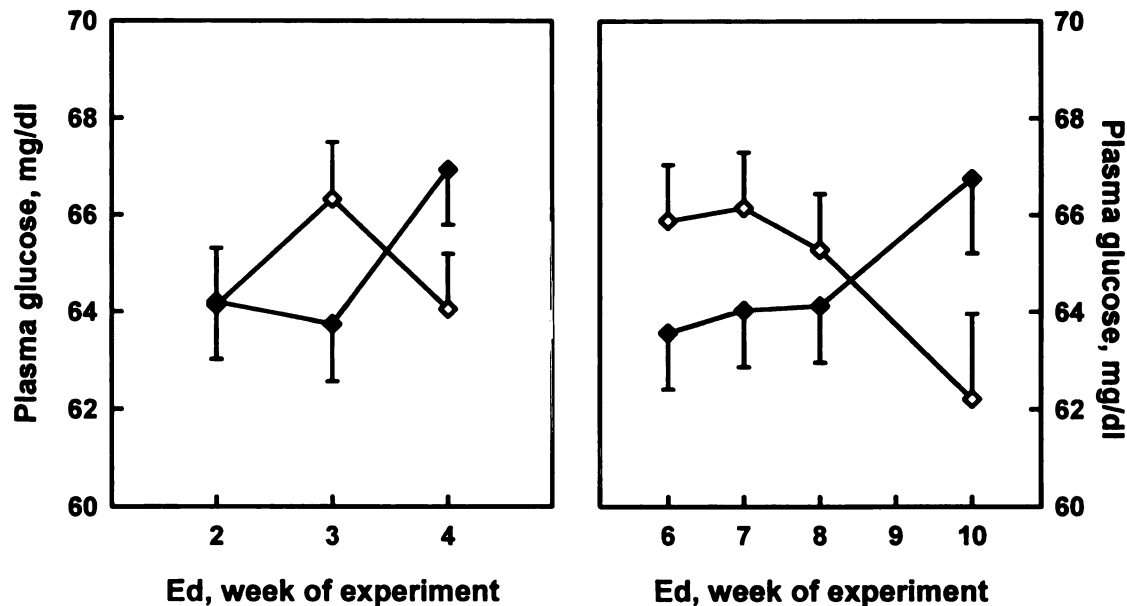


Figure 8.2. Plasma glucose concentrations after exercise training (Ed) of wk 2 to 4 and wk 6 to 10 of experiment. Results were pooled across pregnancy status: non-exercise (◇) and exercise (◆). Interaction of exercise treatment by week of experiment affected glucose concentrations ($P < 0.01$).

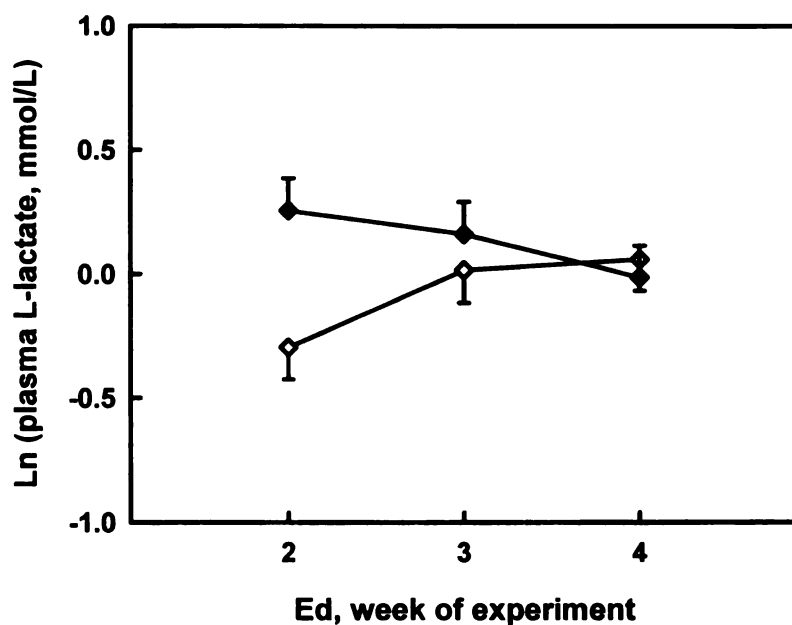


Figure 8.3. Natural logarithmically transformed plasma L-lactate concentrations during post-exercise of wk 2 to 4 of experiment. Results were pooled across pregnancy status: non-exercise (◇) and exercise (◆). Interaction of exercise treatment by week of experiment tended to affect lactate concentrations ($P = 0.06$).

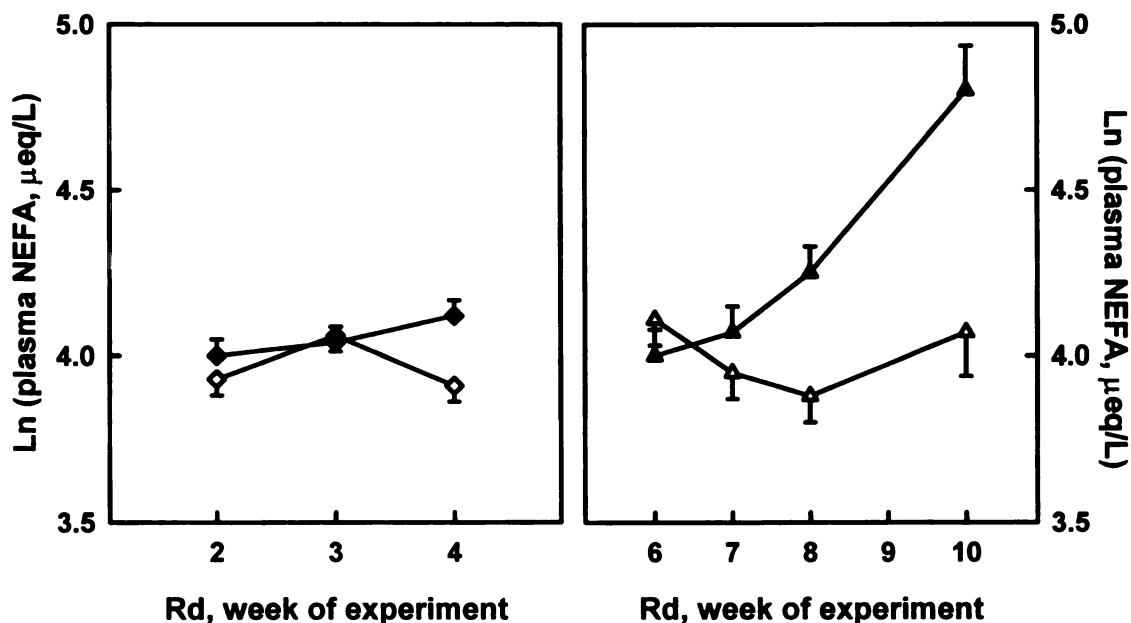


Figure 8.4. Natural logarithmically transformed plasma NEFA concentrations during wk 2 to 4 and 6 to 10 of experiment on a rest day (Rd). During wk 2 to 4, results were pooled across pregnancy status: non-exercise (◇) and exercise (◆). Interaction of exercise treatment by week of experiment affected NEFA concentrations ($P = 0.05$). During wk 6 to 10, results were pooled across exercise treatment: non-pregnant (Δ) and pregnant (▲). Interaction of pregnancy status by week of experiment affected NEFA concentrations ($P = 0.005$).

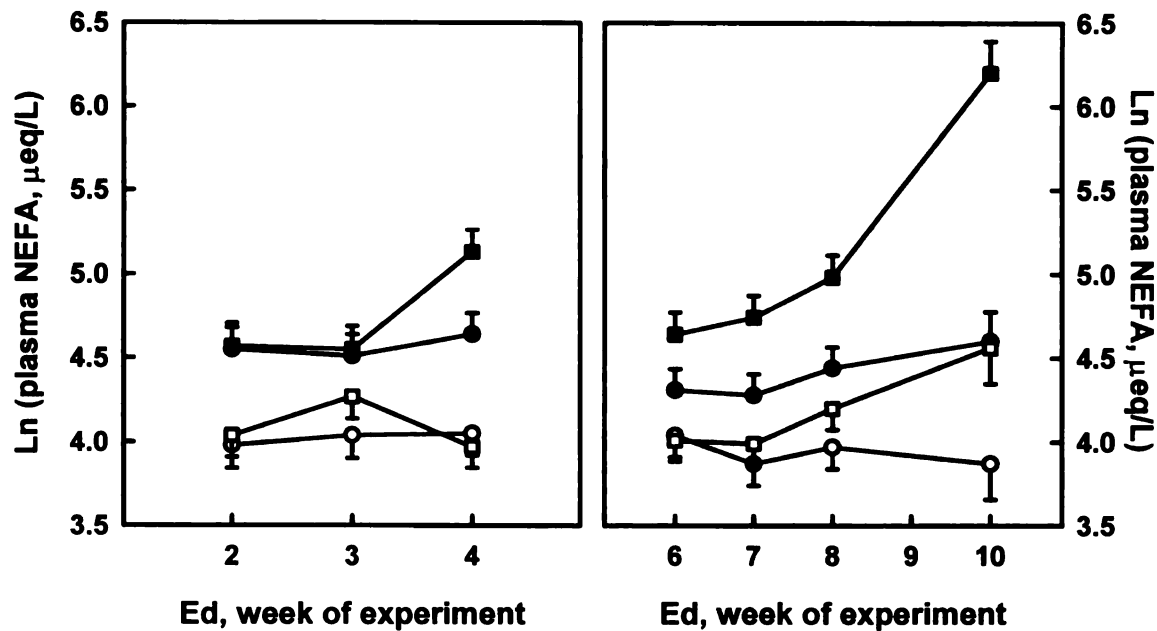


Figure 8.5. Natural logarithmically transformed plasma NEFA concentrations during wk 2 to 4 and 6 to 10 of experiment after exercise (Ed). Treatments were non-pregnant, non-exercise (○); non-pregnant, exercise (●); pregnant, non-exercise (□); pregnant, exercise (■). During wk 2 to 4, interaction of exercise treatment by pregnancy status by week of experiment affected NEFA concentrations ($P = 0.02$). During wk 6 to 10, interaction of exercise treatment by week and interaction of pregnancy status by week of experiment affected NEFA concentrations ($P < 0.01$).

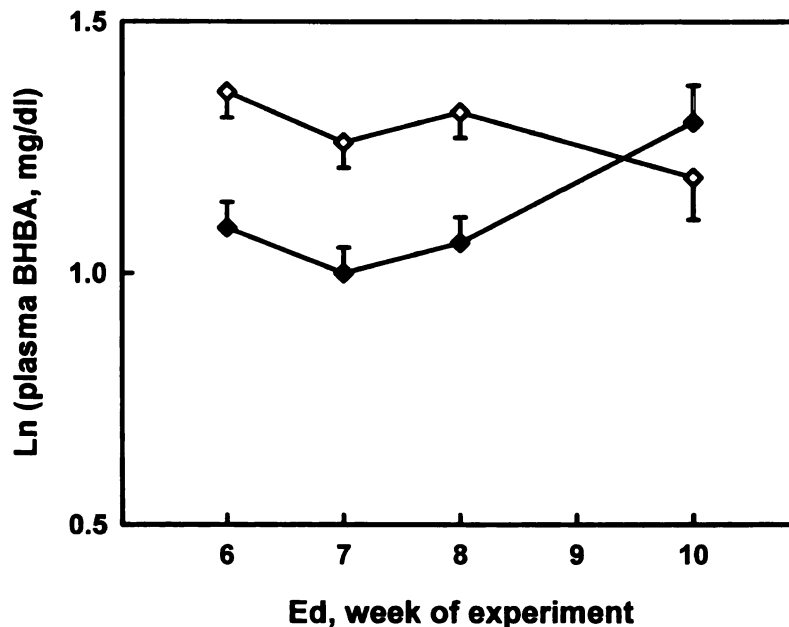


Figure 8.6. Natural logarithmically transformed plasma BHBA concentrations during wk 6 to 10 of experiment after exercise (Ed). Results were pooled across pregnancy status: non-exercise (◇) and exercise (◆). Interaction of exercise treatment by week of experiment affected BHBA concentrations ($P = 0.01$).

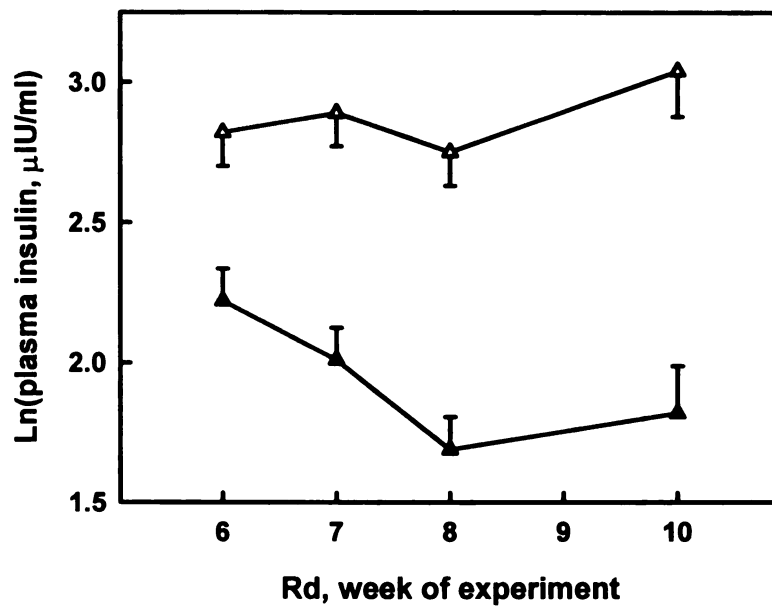


Figure 8.7. Natural logarithmically transformed plasma insulin concentrations during wk 6 to 10 of experiment on rest day (Rd). Results were pooled across exercise treatment: non-pregnant (Δ) and pregnant ($\blacktriangle$). Interaction of pregnancy status by week of experiment affected insulin concentrations ($P = 0.016$).

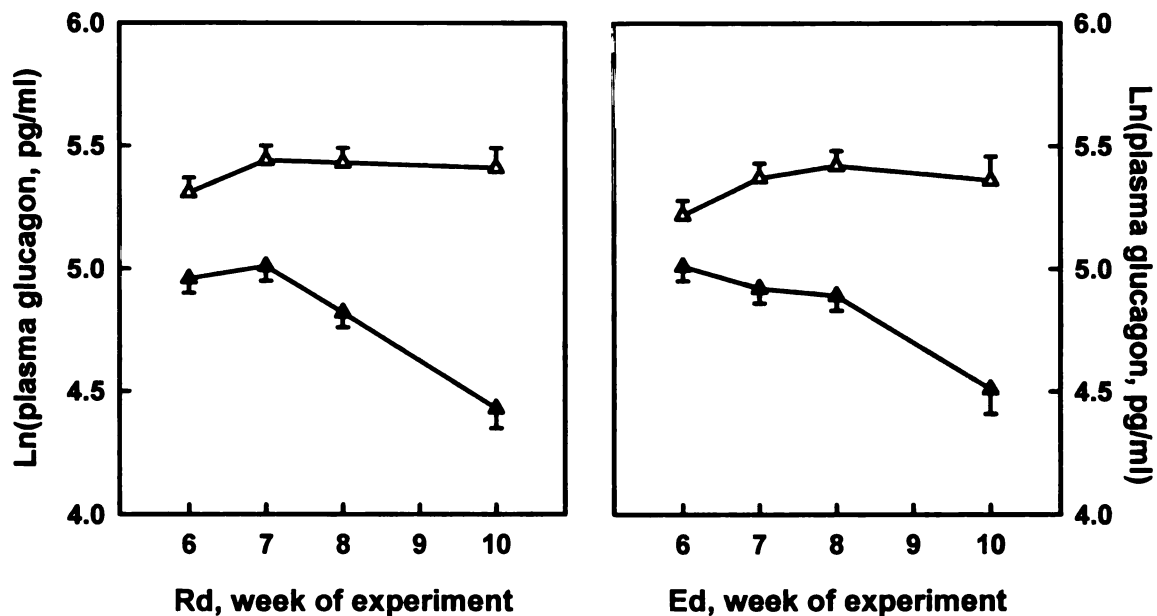


Figure 8.8. Natural logarithmically transformed plasma glucagon concentrations during resting and post-exercise of wk 6 to 10 of experiment. Results were pooled across exercise treatment: non-pregnant (Δ) and pregnant ($\blacktriangle$). Interaction of pregnancy status by week of experiment affected glucagon concentrations ($P < 0.0001$).

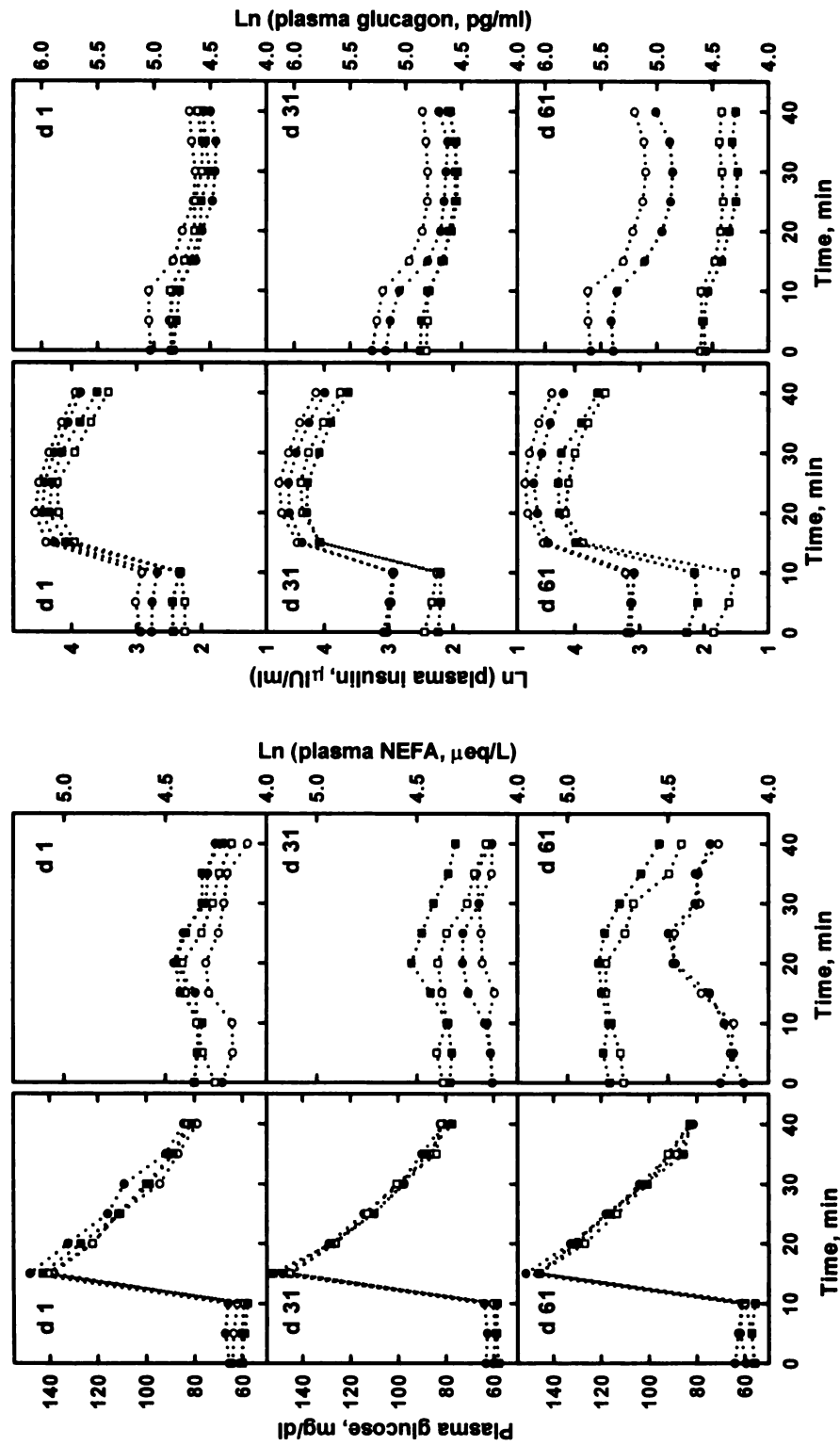


Figure 8.9. Plasma concentrations of glucose and natural logarithmically transformed NEFA, insulin and glucagon concentrations during glucose tolerance test on d 1, 31, and 61 of experiment. Glucose infusion (150 mg/100 kg of BW) occurred at min 10. Treatments were non-pregnant, non-exercise (○); non-pregnant, exercise (●); pregnant, non-exercise (□); pregnant, exercise (■). The SEM were 3.1 for glucose, 0.11 for NEFA, 0.14 for insulin, and 0.08 for glucagon. Pregnancy status tended to affect glucose concentrations ($P = 0.09$) and affected NEFA concentrations ($P < 0.007$). Interaction of exercise treatment by pregnancy status by day of experiment by minute of test affected plasma insulin concentrations ($P < 0.001$). Interaction of pregnancy status by day of experiment by minute of test affected plasma glucagon, whereas exercise treatment tended to affect glucagon concentrations ($P = 0.08$).

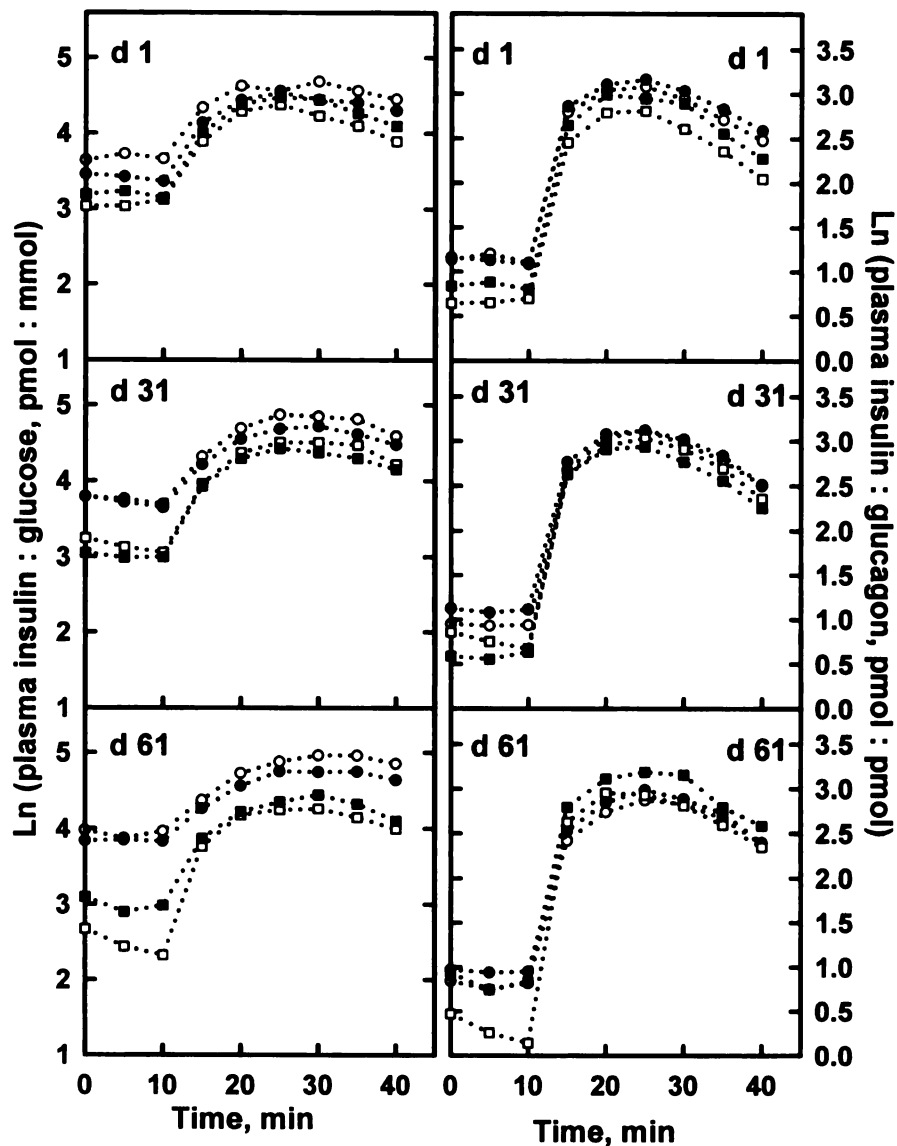


Figure 8.10. Natural logarithmically transformed plasma molar ratios of insulin : glucose and insulin : glucagon during glucose tolerance test on d 1, 31, and 61 of experiment. Treatments were non-pregnant, non-exercise (○); non-pregnant, exercise (●); pregnant, non-exercise (□); pregnant, exercise (■). Glucose infusion (150 mg/100 kg of BW) occurred at min 10. The SEM were 0.14 for insulin : glucose and 0.15 for insulin : glucagon. Interaction of exercise treatment by pregnancy status by day of experiment by minute of test affected both ratios ($P < 0.05$).

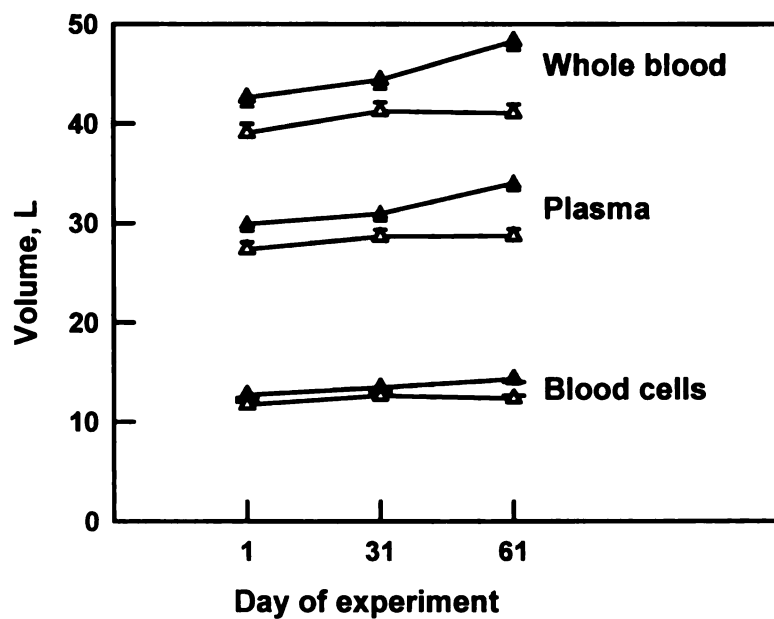


Figure 8.11. Blood cell, plasma, and whole blood volumes on d 1, 31, and 61 of exercise training experiment (least squares means and SEM). Interaction of pregnancy status by day of experiment affected volumes ($P < 0.001$). Results were pooled across exercise treatments: non-pregnant (Δ) and pregnant ($\blacktriangle$).

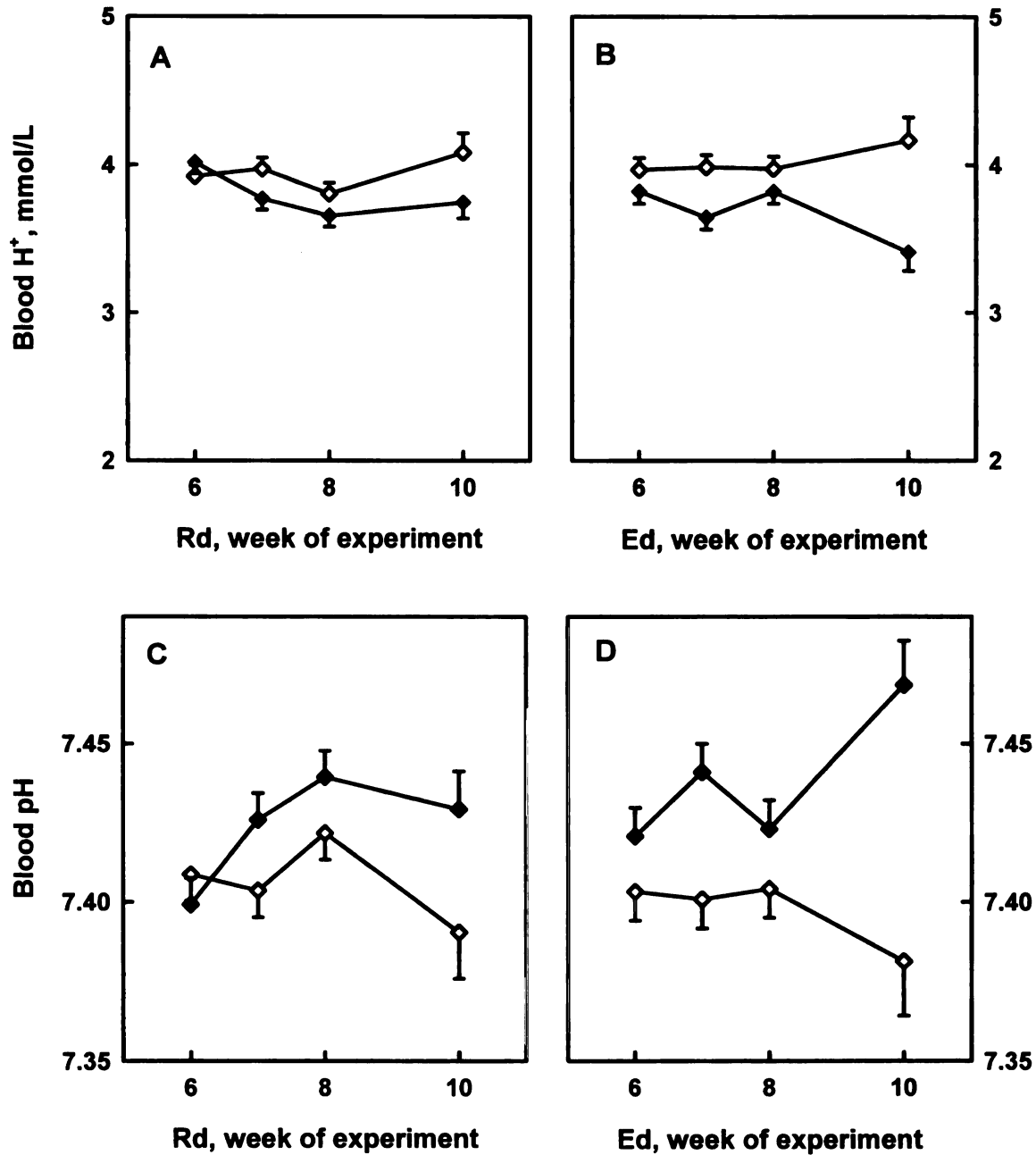


Figure 8.12. Blood H⁺ concentrations (*Panel A and B*) and pH (*Panel C and D*) during wk 6 to 10 of experiment (least squares means and SEM). *Panel A and C* are results from samples collected on a rest day (Rd), whereas *Panel B and D* results were collected immediately after exercise training (Ed). Interaction of exercise treatment by week of experiment affected H⁺ concentrations and pH ($P < 0.05$). Results were pooled across pregnancy status: non-exercise (◇) and exercise (◆).

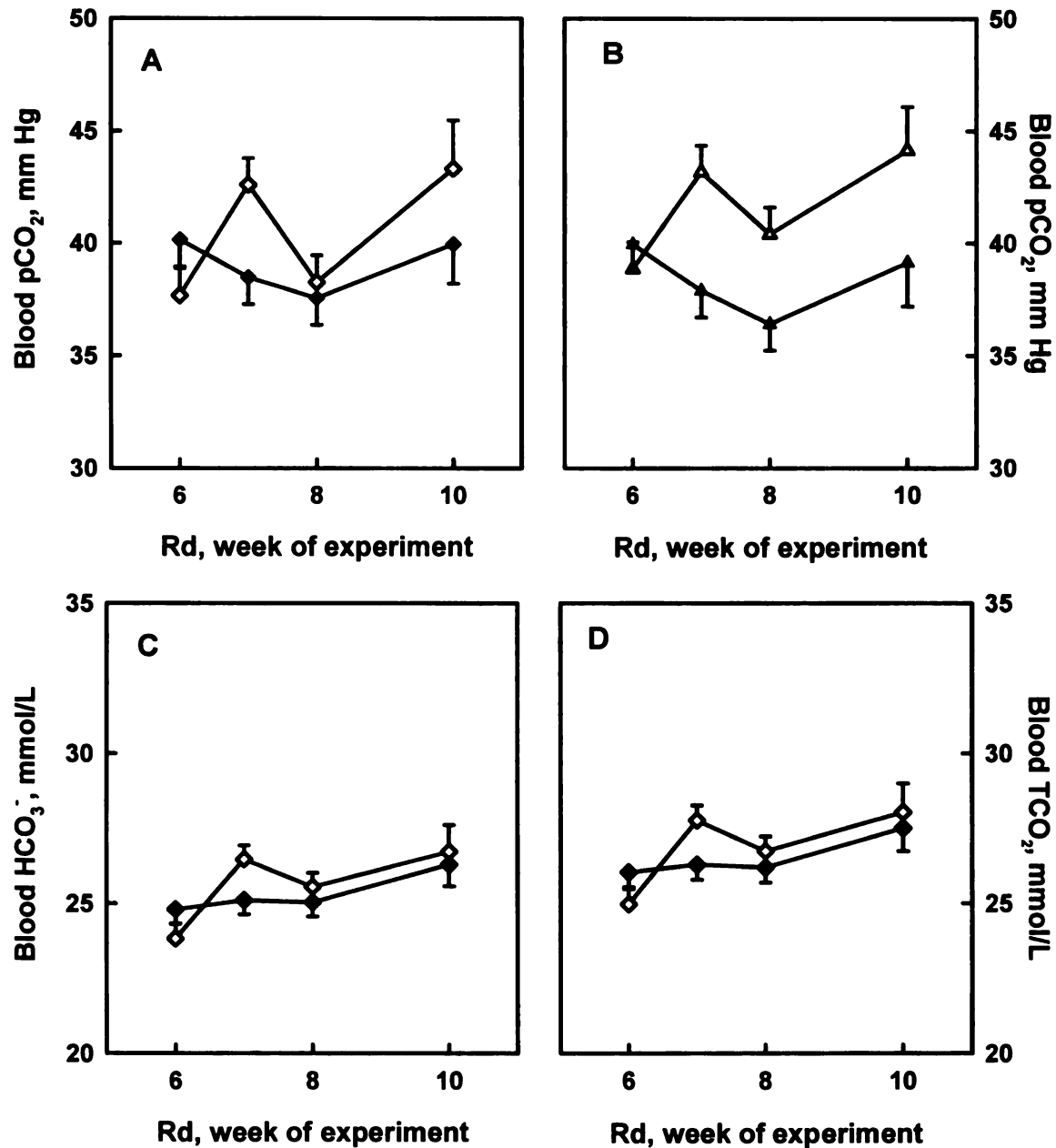


Figure 8.13. Blood partial pressure of carbon dioxide (pCO₂; *Panel A and B*), HCO₃⁻ (*Panel C*), and total carbon dioxide (TCO₂; *Panel D*) concentrations during a rest day (Rd) during wk 6 to 10 of experiment (least squares means and SEM). *Panel A, C, and D* are pooled across pregnancy status: non-exercise (◇) and exercise (◆). *Panel B* are pooled across exercise treatment, non-pregnant (Δ) and pregnant (▲). Interaction of exercise treatment by week of experiment affected pCO₂ (*Panel A*), HCO₃⁻ (*Panel C*), and TCO₂ (*Panel D*, $P < 0.05$). Interaction of pregnancy status by week of experiment affected pCO₂ (*Panel B*, $P < 0.05$).

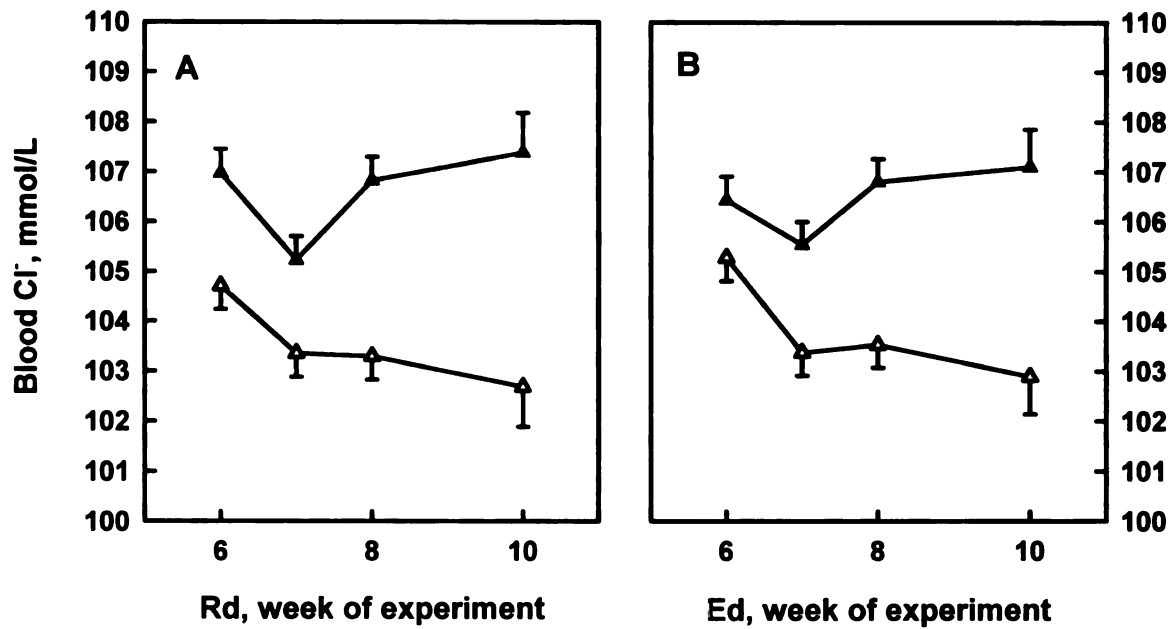


Figure 8.14. Blood Cl^- during rest day (Rd, *Panel A*) and immediately after exercise training (Ed, *Panel B*) during of wk 6 to 10 of experiment (least squares means and SEM). *Panel A and B* are pooled across exercise treatment, non-pregnant ($\diamond$) and pregnant ($\blacklozenge$). Interaction of pregnancy status by week of experiment affected Cl^- concentrations ($P < 0.08$, *Panel A* and $P < 0.05$, *Panel B*).

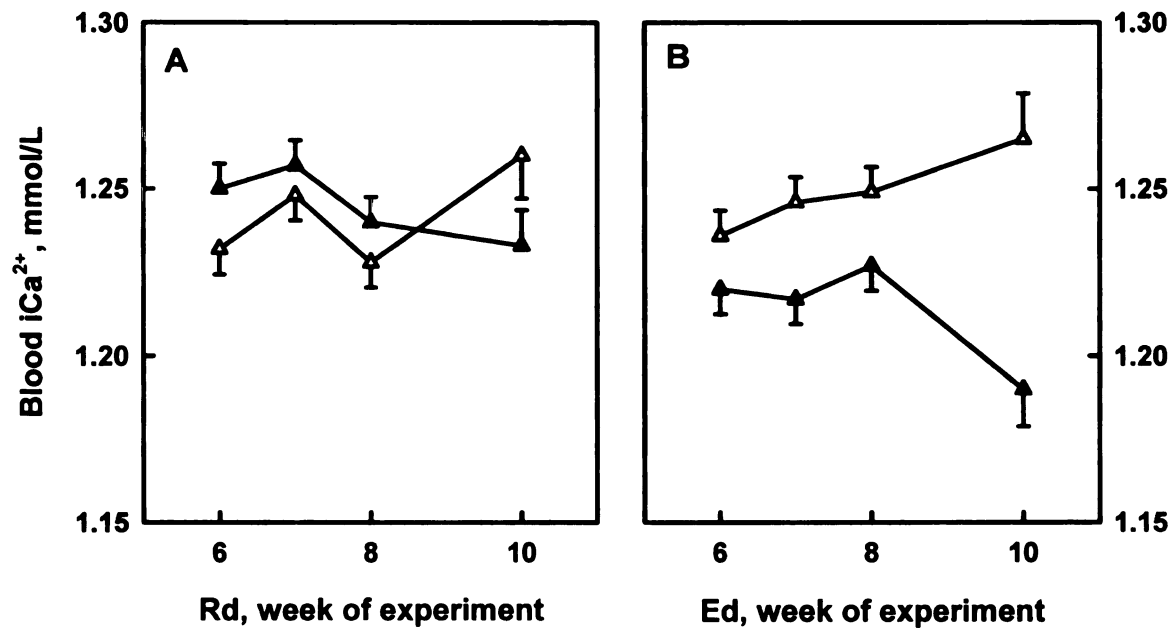


Figure 8.15. Blood iCa^{2+} concentrations during rest day (Rd, *Panel A*) and immediately after exercise training (Ed, *Panel B*) during wk 6 to 10 of experiment (least squares means and SEM). *Panel A and B* are pooled across pregnancy status: non-exercise ($\diamond$) and exercise ($\blacklozenge$). Interaction of exercise treatment by week of experiment affected iCa^{2+} (*Panel A and B*, $P < 0.05$).

CHAPTER 9

EFFECTS OF PREPARTUM EXERCISE TRAINING OF DAIRY COWS ON POSTPARTUM PERFORMANCE, GLUCOSE AND LIPID METABOLISM, AND ACID-BASE STATUS

ABSTRACT

Objective of this experiment was to determine if prepartum exercise training improved postpartum milk production, glucose and lipid metabolism, and acid-base status. Pregnant (n = 26) and non-pregnant (n = 26), non-lactating multiparous Holstein cows were assigned in a factorial arrangement of treatments: exercise training (E) or no exercise (NE), and pregnant (P) or non-pregnant (NP). Exercise training was walking (1.25 to 1.5 h at 3.25 km/h) every other day in an outdoor mechanical walker for 70 d until parturition. During the first week after parturition, cows that were exercised prepartum weighed less, but lost less body weight than NE cows. Prepartum exercise training increased dry matter intake (kg/100 kg BW) of exercised cows during d 1 through 28, but not during d 29 through 120 postpartum. Prepartum exercise training did not affect milk yield, milk composition, and calculated energy balance during d 1 through 120 postpartum. Prepartum exercise training did not affect postpartum plasma concentrations of glucose, NEFA, lactate, or BHBA. During d 0 and 7 postpartum, prepartum exercise training reduced plasma concentrations of insulin and increased glucagon concentrations of pregnant cows. Postpartum liver glycogen concentrations were decreased for P, E cows compared with P, NE cows. However, prepartum exercise training did not affect glucose tolerance; volume of whole blood; or blood acid-base status of cows. Prepartum exercise training modified insulin and glucagon

concentrations during the first week postpartum and improved DMI and BCS during early lactation.

INTRODUCTION

Prepartum daily exercise of dairy cows may be beneficial to metabolism, health, and lactational performance during the periparturient period. During a 4-yr study in Sweden, lactating Red and White cows that were exercised 2 to 3 km/d during summer and 0.4 to 0.8 km/d during winter had fewer calving-related disorders, mastitis, and lameness compared with cows that were not exercised (Gustafson, 1993). However, milk production responses were not described. Pregnant crossbred beef cows, that were forced to exercise (walking 3.2 km/d, 90 d prepartum), had a 14.8% increase in pregnancy rate in the subsequent breeding season compared with non-exercised controls (Bellows et al., 1994).

Additional research of exercise training by walking during the prepartum period with Holstein cattle had variable production responses postpartum. Prepartum exercise for 4, 7, or 8 wk (5 d per wk) did not affect prepartum or postpartum feed intake, or subsequent milk yield of Holstein heifers and cows compared with non-exercised heifers and cows (Anderson et al., 1979; Lamb et al., 1981; Lamb et al., 1979). In contrast with these results, Holstein heifers that exercised 6 wk prepartum (5 d per week) had greater postpartum intake of digestible energy, greater milk yield through 50 DIM and for the entire lactation, fewer days prepartum of udder edema, improved calving ease, and more rapid release of placental tissues compared with non-exercised heifers (Lamb et al., 1979).

Understanding conclusively whether or not exercise training by walking was beneficial to pregnant dairy cattle is not possible from previous research because of

various deficiencies of protocol and data collection within experiments. Independent variables (e.g., age, time of initiation of exercise program relative to expected calving date, and intensity and duration of exercise training) were not similar among various studies. Additionally, in some studies, animals were offered free-choice hay with limited access to grain, so the nutrition program was not optimal to maximize nutrient intake to support subsequent milk yield or reproductive performance. Finally, assessment of altered physical fitness of dairy cows with the exercise programs outlined was inconclusive.

Those studies were not designed to test the cows' actual physical or physiological fitness, or characterize metabolic and physiological adaptations to exercise training. The objectives of the present experiment were to determine if prepartum exercise training affected postpartum body weight changes, dry matter intake, and milk yield to improve postpartum energy metabolism; altered postpartum concentrations of metabolites and hormones; modified muscle and liver tissue composition; affected glucose tolerance and increased postpartum volume of whole blood; and, altered postpartum homeostasis of acid-base status. The hypotheses were that exercise training would improve dry matter intake to improve postpartum energy balance; modify concentrations of metabolites and hormones to improve glucose homeostasis; increase tissue glycogen concentrations; and, increase postpartum whole blood volume and milk yield. Prepartum exercise training would cause physiological adaptations to improve energy metabolism postpartum.

MATERIALS AND METHODS

Animal care and experimental procedures were approved and conducted under standards of the Michigan State University All-University Committee on Animal Use and

Care, application number 05/98-081-00. Guidelines were compliant with National Institutes of Health and the Guide for the Care and Use of Laboratory Animals.

Cows and Exercise training Treatments

Twenty-six pairs of non-pregnant and pregnant multiparous Holstein cows were blocked by parity and expected calving date (ECD) of the pregnant animal. Pairs of cows were assigned randomly to exercise treatments. Treatments in a factorial arrangement were exercise training (E) or no exercise (NE), and pregnant (P) or non-pregnant (NP).

Cows had been dried-off at least 1 wk before the start of the experiment. They also were trained to use the mechanical walker with 2 to 3 short duration (5 min) practice sessions, 1 to 2 wk before the experiment. The start of experiment for a block of cows was approximately 70 d before ECD of the pregnant cows. Exercise training was completed every other day until the parturition of the pregnant cow of the pair.

Exercise training was accomplished in an outdoor mechanical walker with a circular lane that was 90-cm wide and 33.8-m long approximately 36.5 m from the alley in the tie-stall barn where cows were housed (Chapter 3). Cows assigned to NE remained in tie-stalls blocked from feed and water while cows assigned to E were walked. From d 2 through d 28 of experiment (about 68 to 42 d before ECD), E cows walked for 1.25 h at 3.25 km/h and from d 32 to parturition for 1.5 h at 3.25 km/h. On the day of their treadmill test (d 0, 30 and 60), cows were not exercised in the mechanical walker. At parturition of the pregnant cow (P) of each pair, the non-pregnant (NP) cow remained on experiment for 7 additional days, whereas the P cows were on experiment for 120 d postpartum.

Postpartum, all cows were housed in tie-stalls, milked twice daily, and fed a TMR ad libitum, once daily at 0800 (Table 9.1). From d 0 to 27 postpartum, cows were fed a

diet of 1.68 NE_L Mcal/kg, 17.9% CP, dry basis. From d 29 to 120, diet contained 1.61 NE_L Mcal/kg, 18.1% CP, dry basis. Body weights (BW) of individual cows were measured on d 0, 3, 7, 14, 21, 28, 42, 56, 70, 84, 98, 112, and 120 postpartum. Body condition scores (BCS) of individual cows were assigned weekly by three technicians on a 1 to 5 scale (Wildman et al., 1982). Calf birth weights were recorded within 12 h of parturition. Daily DMI and milk yield of individual cows were recorded. Milk samples from each milking were analyzed for percentage of fat, true protein, lactose, and solids non-fat, and somatic cell count on d 7, 14, 21, 28, 56, 84, and 112 (Michigan DHIA, Lansing, MI). Energy-corrected milk yield (kg) was calculated with the equation $0.3246 \times \text{milk yield (kg)} + 12.86 \times \text{fat yield (kg)} + 7.04 \times \text{true protein yield (kg)}$ (Dairy Records Management Systems, 1999).

Daily postpartum net energy balance (Mcal/d) was calculated based on equations from NRC (2001) to determine net energy of intake, net energy requirement of maintenance, net energy loss or gain of body reserves, and net energy requirement of lactation. Positive or negative net energy balance was determined as net energy intake minus net energy requirements of maintenance and lactation.

Occurrences of health related disorders were recorded from d 14 prepartum to d 28 postpartum. Criteria for occurrence of a health related disorder were as follows: retained placenta if placenta was not delivered within 24 h after parturition; clinical mastitis if cow required treatment due to fever and clots in milk; hypocalcemia if blood ionized calcium was less than 0.80 mmol/L; ketosis if readings of a Keto-stik® (Bayer Corporation, Elkhart, IN) were moderate or large; displaced abomasum if surgery was required to correct position of abomasum; and metritis and cystic ovaries were diagnosed with palpation by a veterinarian. Cows were scored at parturition for udder edema with a scale of 1 to 5 with a score of 5 indicating severe edema and hardness of all quarters and score of 1 indicating no visual signs of edema.

Coccygeal Blood Measurements

Coccygeal blood samples were collected before feeding on 0, 7, 14, 21, and 28 d postpartum. Blood was collected in two air-evacuated glass test tubes; one contained Na-heparin and the other contained sodium fluoride/potassium oxalate (**NaF-K Oxalate**; Vacutainer; Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ). Blood (approximately 3 ml) was transferred from Na-heparin tube to a tube with aprotinin (500 KIU/ml of blood) and EDTA (1 mg/ml of blood).

Tubes containing whole blood with NaF-K Oxalate or Na-heparin with aprotinin and EDTA were centrifuged within 15 min after collection. Plasma was harvested and stored at -20°C until analysis. Plasma from tubes with NaF-K Oxalate was analyzed for glucose, lactate, non-esterified fatty acids (**NEFA**), and β -hydroxy butyrate (**BHBA**). Plasma samples collected in tubes with Na-heparin including aprotinin and EDTA were analyzed for glucagon and insulin concentrations. Concentrations of glucose, L-lactate, NEFA, BHBA, insulin, and glucagon were determined as described in Chapter 6. Blood from Na-heparin only tubes was analyzed for pH, partial pressure of carbon dioxide (**pCO₂**), hematocrit (**HCT**), and concentrations of potassium (**K⁺**), ionized calcium (**iCa²⁺**), and chloride (**Cl⁻**; Stat 5 Profiler, Nova Biochemical, Princeton, NJ, see Appendix A). From these measurements, the following variables were calculated: bicarbonate (**HCO₃⁻**), total carbon dioxide (**TCO₂**), hemoglobin (**HGB**), base excess of blood (**BE-B**), and base excess of extracellular fluid (**BE-ECF**).

Liver and Muscle Biopsies

Semitendinous muscle and liver samples were collected to evaluate changes in storage of energy substrates (protein, glycogen, and triglycerides). Additionally, chronic exercise in other species can increase the oxidative capacity of muscle. The activity of

SDH, a mitochondrial membrane bound enzyme of the TCA cycle, was used as an indicator of oxidative capacity of tissue homogenates.

On d 3 and 7 postpartum, liver and semitendinous muscle samples were collected by biopsy from all cows (P and NP) as described (Chapter 7 and Appendix J). Muscle biopsies were performed on alternate hindlimbs on d 3 and 7. Tissues were collected on filter paper and transferred to cryo-genic tubes and placed in liquid N₂. Samples were stored at -80° C until analyses for protein, triglyceride, and glycogen concentrations and succinate dehydrogenase (SDH) activity. Tissues were homogenized (Polytron, Kinematica GMBH, Luzern, Switzerland; 20 µl/mg of wet muscle and 40 µl/ml of wet liver) with 20 mM Na- phosphate buffer, pH 7.4 and 0.5 mM EDTA. Aliquots of homogenates were stored at -20° C until analyses of glycogen and triglycerides (TG) as described in Chapter 7.

Homogenates were assayed immediately after homogenization for SDH activity as an indicator of oxidative capacity and protein concentration (see Appendix L). Protein concentrations were determined in microtiter plates using Bradford reagent (Sigma Chemical, St. Louis, MO). Intra- and inter-assay coefficients of variation were 3.3 and 4.0%, respectively.

Activity of SDH was determined in a kinetic colorimetric assay modified for microtiter plates (Munujos et al., 1993). Assay buffer (made fresh daily) contained 100 mM triethanolamine, pH 8.3 with 0.5 mM EDTA, 2 mM potassium cyanide, 12 g/L Cremophor EL, and 2 mM 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (INT). Reagent buffer also contained 20 mM Na- succinate. Assay buffer and reagent buffer were used at 37° C. To determine non-specific dehydrogenase activity, 50 µl of homogenate, 50 µl of homogenizing buffer, and 150 µl of assay buffer (without Na-succinate) were added to duplicate wells. Activity of SDH was determined in triplicate with 50 µl of homogenate, 50 µl of homogenizing buffer, and 150 µl of reagent

buffer (with Na-succinate). Microtiter plates were incubated for 4 min at 37° C. The reaction rate (mOD/min) for each well was determined by repeated readings every 20 sec for 6 min at 500 nm. Immediately after determination of reaction rate, plates were read to determine pathlength (cm) for each well. The reaction rate was corrected for non-specific activity by subtraction. The activity of SDH was determined using the Beer-Lambert equation. Molar absorbency of INT-formazan product formed was 19,300 (L/mol cm) at 500 nm (Munujos et al., 1993). Activity of SDH ($\mu\text{mol/min}$ per ml of sample) was equal to specific mOD/min $\times$ (1/(pathlength $\times$ 19,300)) $\times$ 250 μl total volume/50 μl of sample.

Assay was validated using multiple liver and muscle samples ($n = 3$) from the same animal at various dilutions. Activity of liver SDH ($\mu\text{mol/min}$) as a function of μg of wet tissue and μg of tissue protein was linear ($r^2 = 0.967$ and 0.972 , respectively). Activity of muscle SDH ($\mu\text{mol/min}$) as a function of μg of wet tissue and μg of tissue protein was linear ($r^2 = 0.849$ and 0.870). Inter-assay coefficients of variation for liver and muscle control tissues were 20% and 28%, respectively. Intra-assay coefficients of variation for liver and muscle control tissues were 10% and 15%, respectively. Tissue homogenates from each block of cows were assayed on the same microtiter plate because of the large inter-assay coefficient of variation.

Glucose Tolerance

Indwelling catheters were inserted on the left and right jugular veins of each cow on d 3 postpartum. A glucose tolerance test was performed on all cows (NP and P), approximately 3 h after feeding on d 3 postpartum. Jugular blood was collected with a syringe containing Na-heparin from one indwelling catheter at min 0, 5, 10, 15, 20, 25, 30, 35, 40, 50, 70, and 90. In the other indwelling catheter, cows were infused at min 10 with a volume of 50% dextrose solution to deliver 150 mg of glucose/kg of BW. Whole

blood was transferred to glass test tubes containing aprotinin (500 KIU/ml of blood) and EDTA (1 mg/ml of blood) or NaF (2.5 mg/ml of blood). As described previously, plasma was analyzed for glucose, NEFA, insulin, and glucagon concentrations (Chapter 6).

Volume of Whole Blood

On d 3 postpartum, approximately 4.5 h after feeding and 1 h after the infusion of glucose for the glucose tolerance test, volume of whole blood was measured for all cows (Chapter 7; Delaquis and Block, 1995a; Hamilton, 1958; Holmes and Weiskopf, 1987). A saline solution with 10 mg/ml Evans blue (Sigma-Aldrich, St. Louis, MO) was filtered through a 0.22 micron sterile filter. Jugular blood was collected with a syringe containing Na-heparin from one indwelling catheter at min 0, 5, 10, 12.5, 15, 20, 30, 40, 50, 70, and 90. In the other indwelling catheter, 50 ml of saline solution containing Evans Blue was infused at min 10. Blood (4 ml) was transferred to glass test tubes and centrifuged. Plasma was harvested and stored at -20°C until analysis for plasma concentrations of Evans Blue. Blood collected at min 0, 5, and 10 was analyzed for HCT (Stat 5 Profiler, Nova Biochemical, Princeton, NJ). Evans Blue concentrations were determined using a colorimetric assay (Hamilton, 1958; Holmes and Weiskopf, 1987; see Appendix K). The intra- and inter-assay coefficients of variation were 1.9 and 4.0%, respectively.

Least squares linear regression of the natural logarithmic transformed plasma dye concentrations versus time of sampling were calculated for each cow on each day. Using these equations, the plasma dye concentration at time 0 was determined. The amount of dye infused was determined as the difference of weight of syringe and solution before and after infusion. Thus, with the known amount of dye infused, the plasma volume at time 0 could be calculated (dye concentration at min 0, $\mu\text{g/ml}$ / $\mu\text{g dye}$ infused). Blood cell volume was calculated as the function of $(\text{HCT} \times \text{plasma volume})/(1$

- HCT). Summation of plasma volume and blood cell volume equaled total whole blood volume.

Statistical Analysis

Data were analyzed using method of ANOVA by mixed model procedures of SAS (1999). The appropriate covariance structure for the repeated measures was determined using the goodness of fit criteria of Aiaike's Information Criterion and Schwarz's Bayesian Criterion. Plasma L-lactate, NEFA, BHBA, insulin, glucagon, and molar ratios of insulin : glucose and insulin : glucagon concentrations had unequal variances across week of experiment and were transformed using the natural logarithm before analyses. Data were analyzed for the time periods: d 0 through 7 postpartum for all cows (NP and P) and d 0 through 28, d 29 through 120, or d 0 through 120 postpartum for P cows. Unless otherwise described, results are presented as generalized least squares means with standard error of mean. Statistical significance was declared at $P < 0.05$ and between $P > 0.05$ and $P < 0.10$ denoted as a tendency.

Dependent variables of d 0 through 7 postpartum (BW, BCS, DMI, volumes of whole blood, and tissue and plasma variables) were analyzed with a mixed model including the random effect of cow nested within treatment (T) and pregnancy status (PS), the repeated measures effect of day postpartum, fixed effects of T, PS, and block. All two- and three-way interactions of T, PS, and day postpartum were included in the model. The covariance structures of repeated measures effect of day postpartum were auto-regressive first order or spatial power. Cumulative milk and components yields, calf birth weights, and udder edema score were analyzed with a mixed model including the random effect of cow nested within T and the fixed effects of T and block.

Data of dependent variables (BW, BCS, DMI, blood and plasma variables, milk composition, milk yield, milk : DMI, and energy balance variables) collected from d 0

through 28, d 29 through 120, or d 0 through 120 postpartum were analyzed with a mixed model including the random effect of cow nested within T, the repeated measures effect of day postpartum, fixed effects of T and interaction of T by day postpartum and block. The covariance structures of the repeated measures effect of day postpartum were auto-regressive first order or spatial power.

During the glucose tolerance test, glucose, NEFA, insulin, and glucagon concentrations were analyzed with a mixed model including the random effect of cow nested within T and PS, the repeated measures effect of minute relative to glucose infusion, fixed effects of T, PS, and block. All two- and three-way interactions of T, PS, and minute relative to infusion were included in the model. The covariance structure of the repeated measures effect of minute relative to infusion was auto-regressive first order.

Basal, peak, and change of glucose and insulin concentrations during the glucose tolerance test, and volume of blood cells, plasma, and whole blood were analyzed with a mixed model including the random effect of cow nested within T and PS, fixed effects of T, PS, and block, and the interaction of T by PS.

Incidences of cows with 1 or more health related disorder were analyzed with catmod procedures of SAS (1999). Probability of chi-square was used to determine if T affected incidence of cows experiencing one or more health related disorder. Type II error rate was calculated assuming α of 0.05 and the incidence rates and number of cows per treatment group reported in the current experiment.

RESULTS AND DISCUSSION

Responses of BW, BCS, and DMI

During d 0 through 7 postpartum, cows that exercised prepartum had lower BW than non-exercised cows (719 vs. 755 ± 10.6 kg, $P = 0.02$). Pregnant cows lost weight during the first week postpartum, whereas NP cows did not (interaction of PS by day postpartum, $P = 0.002$, Table 9.2). Additionally, calf birth weights were 45 and 48 ± 1.6 kg for NE and E cows, respectively. Prepartum exercise training did not affect BCS during first week postpartum (Table 9.2). Pregnant cows had lower BCS on d 7 compared with d 0 and compared with NP cows ($P < 0.001$, Table 9.2).

During the prepartum period, exercised cows had lower BW and BW gain than NE cows (Chapter 5). Therefore, cows that exercised prepartum had less BW at parturition than NE cows. In other studies, prepartum exercise of multiparous dairy cows and heifers also reduced BW gain compared with non-exercised cows and heifers (Anderson et al., 1979; Lamb et al., 1981; Lamb et al., 1979).

Prepartum exercise training did not affect loss of BW of pregnant cows during wk 0 through 17 postpartum (Figure 9.1). Pregnant cows regardless of prepartum exercise had similar reductions of BW from wk 0 through 17 postpartum (effect of day postpartum, $P < 0.001$; Figure 9.1). Similarly, prepartum exercise training did not affect postpartum BW changes of multiparous or primiparous dairy cows (Anderson et al., 1979; Lamb et al., 1979; Lamb et al., 1981). All cows had reduced BCS as week postpartum increased ($P < 0.001$; Figure 9.1). However, the interaction of prepartum exercise training by day postpartum tended to affect BCS ($P = 0.085$; Figure 9.1). From wk 0 through 7 postpartum, cows that exercised tended to have slightly greater BCS postpartum compared with non-exercised cows. From wk 7 through 17, cows regardless of prepartum exercise had similar reductions of BCS (Figure 9.1).

During d 1 through 6 postpartum, prepartum exercise training did not affect DMI (kg/d or kg/100 kg BW) of NP or P cows (Table 9.3). Pregnant cows had less DMI (kg/d or kg/100 kg BW) than NP cows at d 1 postpartum, but DMI were similar on d 6 for P and NP cows (interaction of PS by day postpartum, $P < 0.001$, Table 9.3). Prior to parturition, P cows also had less DMI (kg/d or kg/100 kg BW) than NP cows (Chapter 5).

As day postpartum increased from d 1 through 28, DMI (kg/d and kg/100 kg BW) increased ($P < 0.001$; Figure 9.2 A and C). Prepartum exercise training did not affect DMI (kg/d) during d 1 through 28 postpartum, but tended to increase DMI as a function of BW for E cows compared with NE cows ($P = 0.099$; Figure 9.2 C). During d 29 through 120, prepartum exercise training did not affect DMI (kg/d or kg/100 kg of BW; Figure 9.2 B and D). During d 29 through 120, DMI (kg/d and kg/100 kg of BW) increased as day postpartum increased ($P < 0.001$; Figure 9.2 B and D).

In other studies, prepartum exercise training did not affect prepartum or postpartum DMI (kg/d) of multiparous or primiparous dairy cows (Anderson et al., 1979; Lamb et al., 1979; Lamb et al., 1981). In these studies, cows were not given 24 h access to feed as a TMR in contrast to the current studies. During the first 4 wk, DMI as a percent of BW was greater for cows that exercised prepartum than non-exercised cows. Exercised cows seemed to have better appetites during early lactation potentially due to differences in glucoregulatory hormones or improved transition after parturition to lactation.

Responses of Milk Yield and Milk Composition

Prepartum exercise training did not affect postpartum milk yield (kg/d), energy-corrected milk yield (kg/d), and percent of milk fat, true protein, lactose, and solids non-fat during days 1 through 120 postpartum (Table 9.4). Prepartum exercise did not affect somatic cell count (Table 9.4). Milk yield and energy-corrected milk yield increased as

day postpartum increased until d 30 followed by slight reductions of yield until d 120 postpartum ($P < 0.001$; Figure 9.3). As would be expected, the percent of milk fat, true protein, lactose, and solids non-fat decreased until wk 4 postpartum and remained similar during wk 6 through 17 postpartum ($P < 0.001$; Figure 9.4). Prepartum exercise training did not affect cumulative yields of milk, energy-corrected milk, fat, true protein, lactose, and solids non-fat (kg) during d 1 through 28 or d 1 through 120 postpartum (Table 9.5).

In contrast, prepartum exercise training increased milk yield 2.55 kg/d for the subsequent complete lactation of primiparous dairy cows (Lamb et al., 1979). In other studies with multiparous or primiparous cows milk yield and milk composition of E cows were not greater than NE animals (Anderson et al., 1979; Lamb et al., 1979; Lamb et al., 1981). Even though E cows weighed less than NE cows, they had similar DMI and milk yields as NE cow throughout the entire postpartum period.

Energy Balance and Efficiency

Prepartum exercise training did not affect the weekly average of daily net energy intake, net energy requirement for maintenance, net energy requirement for lactation, loss or gain of reserve body net energy during wk 1 through 4 and wk 5 through 15 postpartum (Table 9.6). Prepartum exercise training did not affect measures of efficiency such as milk yield : DMI (kg/kg); energy-corrected milk yield : DMI (kg/kg); or energy-corrected milk yield : energy intake (kg/Mcal, Table 9.6).

During wk 1 through 4, week postpartum affected average daily net energy intake, net energy requirement for maintenance, and net energy requirement for lactation ($P < 0.05$; Figure 9.5 A). During wk 1 to 4, net energy of body reserve losses were reduced from 14.6 to 7.6 ± 2.72 Mcal/d, (effect of week, $P = 0.13$). During wk 5 through 15, week postpartum affected average daily net energy intake, net energy

requirement for lactation, loss or gain of reserve body net energy, but not net energy requirement of maintenance (Figure 9.5 A).

During wk 1 to 4, net energy requirement of maintenance was slightly greater during wk 1 compared with wk 4, but did not change during wk 5 to 15 postpartum. This was similar to the BW reductions measured during wk 1 to 17 of this study. During wk 1 to 4, the net energy requirement of lactation increased as milk yield increased during wk 1 to 4 postpartum, and was slightly less during wk 13 and 15 compared with wk 5 to 11. During wk 1 to 4 postpartum, cows had a negative net energy associated with body reserves, thus body reserves were used to support milk production (Figure 9.5 A). The calculated loss of body reserves during wk 1 to 4 postpartum were reflective of the measured reduction of BW and BCS during this same time. During wk 5 to 15, net energy of associated with body reserves was only negative during wk 5 postpartum and was did not different from zero during wk 7 to 15. Similarly, BW and BCS did not change greatly after wk 5 postpartum. After wk 7 postpartum, no additional loss or gain of net energy of body reserves was detected, which indicated that cows were in positive energy balance.

In this study, potential errors associated with determination of energy balance may have included measurement of BW due to the effects of gut fill and estimation of energy of body reserves from BCS. Therefore, the difference of net energy intake minus net energy requirements of maintenance and lactation was also used to assess energy balance of cows during wk 1 to 15 (Figure 9.5 B). From this calculation, all cows were had a negative energy balance until wk 14 postpartum. This seems unlikely in that BW and BCS reductions were minimal beyond wk 5 postpartum. Furthermore, milk yield and energy-corrected milk yield had slight reductions after wk 5 postpartum. The estimation of net energy of intake greatly influenced the calculated energy balance as a difference between (intake and requirements). Potential errors using NRC (2001) to

calculate the net energy intake may be determination of the TDN of diet and (or) the estimation of NE_L at actual intake (discount of DMI over maintenance intake for each cow).

Health Related Disorders

The number of cows that experienced 1 or more health related disorder were 8 out of 13 parturitions for NE cows and 3 out of 13 parturitions for E cows ($P < 0.05$, Table 9.7). The type II error rate (β) assuming α of 0.05 with 13 cows per group and incidence rates of 62% and 23% for each treatment group was 0.48 (power = 0.52), which was well below an optimal power of 90%. The incidences of each health disorder are reported in Table 9.7. Exercise treatment reduced the number of cows experiencing 1 or more health related disorders ($P = 0.05$).

Udder edema score was lower for cows that exercised compared with cows that were not exercised ($P = 0.04$, Table 9.7).

During a 4-yr study in Sweden, lactating Red and White cows that were exercised 2 to 3 km/d during summer and 0.4 to 0.8 km/d during winter had fewer calving-related disorders, mastitis, and lameness compared with cows that were not exercised (Gustafson, 1993). Holstein heifers that exercised 6 wk prepartum (5 d per week) had fewer days prepartum of udder edema, improved calving ease, and more rapid release of placental tissues compared with non-exercised heifers (Lamb et al., 1979). In the current studies and these other studies, the number of parturitions were small; therefore, caution should be taken when evaluating these results. As preliminary evidence from these results, prepartum exercise training may reduce the incidences of health related disorders. Additional research with greater numbers of parturitions per treatment are needed to fully understand the influence of prepartum exercise on periparturient cow health.

Plasma Metabolites and Hormones

Postpartum d 0 and 7. On d 0 and 7 postpartum, prepartum exercise training did not affect plasma glucose, lactate, NEFA, and BHBA concentrations (Table 9.8). On d 0, P cows had greater plasma glucose and lactate than NP cows, whereas on d 7, P cows had less glucose and lactate than NP cows (interaction of PS by day postpartum, $P < 0.01$, Table 9.9). Pregnant cows had greater plasma NEFA and BHBA concentrations compared with NP cows (Table 9.8). Similarly, during prepartum period, P cows had greater NEFA and BHBA concentrations than NP cows (Chapter 8). Also, during the 4 wk prior to parturition, exercise training did not affect plasma concentrations of glucose, lactate, and NEFA, whereas BHBA concentrations were slightly reduced for E cows compared with NE cows (Chapter 8). Exercise training did not affect periparturient concentrations of metabolites when cows are not exercising (at rest).

On d 0 and 7 postpartum, plasma concentrations of insulin, glucagon, and molar ratios of insulin : glucose and insulin : glucagon were less for P cows compared with NP cows (Table 9.8). Cows that were exercised prepartum had lower concentrations of insulin compared with non-exercised cows after parturition (Table 9.8). The NP, NE cows had the greater concentrations of glucagon compared with NP, E cows, whereas P, E cows had greater concentrations of glucagon compared with P, NE cows (interaction of T by PS, $P = 0.054$, Table 9.8).

Cows that were exercised had lower molar ratios of insulin : glucose and insulin : glucagon compared with NE cows. Additionally, P cows had reduced ratios of insulin : glucose and insulin : glucagon compared with NP cows (Table 9.8). During the 4 wk prior to parturition, cows that exercised had less plasma insulin and similar glucose and glucagon concentrations than NE cows indicating potential changes of peripheral tissues sensitivity to insulin (Chapter 6). During the postpartum period, the reduction of the molar ratio of insulin : glucose and insulin : glucagon for P, E cows is difficult to

interpret, since both insulin and glucagon were affected in order to maintain glucose homeostasis. Prepartum exercise training affected both glucoregulatory hormones to maintain glucose homeostasis during the first week postpartum. The potential mechanisms involving hepatic output of glucose, pancreatic secretions of glucagon and insulin, and (or) peripheral tissue sensitivity to insulin may have contributed to the differences in glucose homeostasis detected.

Postpartum d 0 through 28. On d 0 through 28 postpartum, prepartum exercise training did not affect plasma metabolites (glucose, lactate, NEFA, and BHBA concentrations) or hormones (insulin and glucagon concentrations) of P cows (Figure 9.6). Day postpartum affected plasma glucose, lactate, NEFA, and glucagon concentrations ($P < 0.05$; Figure 9.6), but did not affect plasma concentrations of BHBA, insulin, and molar ratios of insulin : glucose and insulin : glucagon. Plasma glucose, lactate, and NEFA concentrations were greatest on d 0 compared with other days postpartum, whereas plasma glucagon concentrations were least on d 0 compared with other days.

The increase in plasma glucagon as day postpartum increased could be in response to greater DMI and increased demand of glucose for milk production. Conversely, the decline of concentrations of glucose, lactate, and NEFA could be due to increased tissue uptake for milk production as day postpartum increased. Changes of concentrations of plasma glucose, NEFA, and BHBA during the periparturient period were similar to reports from other nutritional studies (Rukkwamsuk et al., 1998; Stephenson et al., 1997; Vallimont et al., 2001). Additionally, changes of concentrations of plasma insulin and glucagon during the periparturient period also were similar to other studies (Hayirli et al., 2001; Mashek and Beede, 2001). No additional evidence was found to confirm the lack of effect of prepartum exercise on d 0 through 28 postpartum glucose and lipid concentrations or the effects after the cessation of exercise training.

Composition of Muscle and Liver Tissues

Prepartum exercise treatment and PS did not affect muscle protein, glycogen, and triglyceride concentrations, or activity of SDH (Table 9.10). However, the interaction of T by PS affected activity of muscle SDH ($P = 0.04$, Table 9.10). The muscle of NP, E cows had greater activity of SDH than all other groups. No additional information was found to compare with this finding for dairy cows. Prepartum exercise did not greatly affect the oxidative capacity and muscle concentrations of protein, triglycerides, and glycogen of P cows during the postpartum period.

In contrast to our findings, chronic exercise training increased the myofibrillar protein concentrations in muscle and reduced soluble collagen content of lambs (Aalhus et al., 1991). Wethers exercised daily for 1 h, 8 km/h for 5 wk had greater concentrations of glycogen in semimembranous, semitendinous, and longissimus dorsi muscles (Gardner et al., 1998; Pethick and Rowe, 1996). After 18 wk of exercise, the intramuscular fat in semimembranous, vastus lateralis, and gastrocnemius muscles of lambs was reduced (Aalhus and Price, 1990b). Subcutaneous fat was less in exercised wethers compared with non-exercised wethers (Pethick and Rowe, 1996).

In humans, increases in maximal citrate synthase activity (another enzyme of TCA cycle) occurred after only 6 d of exercise training (Green et al., 1999), and succinate dehydrogenase activity increased by 31% after 4 wk of chronic exercise compared with pre-training values (Green et al., 1995). The differences between growing and mature animals and (or) pregnancy status or duration and intensity of exercise training may have affected the measurements of muscle tissue between these studies and the current study.

Prepartum exercise treatment did not affect liver protein concentrations, activity of SDH, or triglyceride concentrations (Table 9.11). However, P cows had greater liver SDH activity indicating greater oxidative capacity (expressed as $\mu\text{mol}/\text{min} \cdot \text{g}$ wet tissue

or $\mu\text{mol}/\text{min} \cdot \text{g}$ tissue protein) than NP cows ($P = 0.01$, Table 9.11). The NP, E cows had greater liver glycogen concentrations than all other groups, whereas P, E cows had the least concentrations of liver glycogen (interaction of T by PS, $P = 0.01$, Table 9.11). Pregnant cows had greater accumulation of triglycerides in liver than NP cows ($P < 0.01$; Figure 9.10). The increased metabolic demands associated with lactation may have affected the concentrations of liver glycogen and triglycerides of P cows compared with NP cows. Increased mobilization of adipose tissue during the periparturient period will increase the accumulation of triglycerides in the liver (Bertics et al., 1992). Furthermore, liver glycogen would be utilized to maintain glucose homeostasis and supply the mammary gland with glucose for milk production (Bauman and Currie, 1980).

During the prepartum period, cows that exercised had greater liver glycogen than cows that did not exercise (Chapter 6). It is not clear as to why P, E cows had the greatest change in liver glycogen during the periparturient period. The P, E cows also had the greatest reduction of molar ratio of insulin : glucagon and insulin : glucose compared with P, NE cows. These changes in glucoregulatory hormones may have affected a greater removal of glycogen from the liver of P, E cows compared with P, NE cows.

Prepartum exercise training did not reduce the accumulation of triglycerides in liver during the periparturient period (Table 9.11). The reduction of DMI of P cows prior to parturition appears to have had a greater influence on lipid mobilization than prepartum exercise (no effect of exercise training on liver TG). In dairy cows, the prepartum reduction of DMI has been previously associated with an increase accumulation of liver triglycerides of dairy cows during the periparturient period (Grummer, 1993).

Glucose Tolerance

On d 3 postpartum, prepartum exercise training did not affect plasma glucose, insulin, glucagon, NEFA or molar ratios of insulin : glucose and insulin : glucagon during a glucose tolerance test. Pregnancy status and minute relative to glucose infusion affected concentrations of plasma glucose, insulin, glucagon, NEFA, and molar ratios of insulin : glucose and insulin : glucagon ($P < 0.001$; Figure 9.7). Pregnant cows had less plasma glucose, insulin, and glucagon prior to infusion and after infusion than NP cows. The molar ratios of plasma insulin : glucose and insulin : glucagon were greater for NP cows compared with P cows. Pregnant cows had greater plasma NEFA concentrations than NP cows. Average basal glucose and insulin concentrations prior to infusion and peak glucose and insulin concentrations after glucose infusion were greater for NP cows compared with P cows ($P < 0.01$, Table 9.12). The increases over basal concentrations of glucose and insulin were greater for NP cows compared with P cows (Table 9.12).

In the current study, PS of cows greatly affected the insulin responsiveness and (or) tissue sensitivity to an intravenous glucose bolus, whereas prepartum exercise training treatment had no effect. In sheep, no differences of plasma insulin concentrations were detected after an intravenous infusion of glucose 18 d before parturition or 36 d postpartum (Bassett, 1989). However, insulin concentrations tended to be reduced during postpartum compared with prepartum values. In dairy cows, peak insulin concentrations and nadir glucagon concentrations in response to intravenous glucose infusion were reduced 28 d postpartum compared with 10 d before parturition (Hayirli et al., 2001) and 14 d postpartum compared with 14 d before parturition (Subiyatno et al., 1996). In another study, postpartum (d 5) glucose and insulin concentrations were decreased compared with 14 d prior to parturition after an intravenous infusion of glucose (Sartin et al., 1985). Non-pregnant and non-lactating cows also were included in the current study; however, groups of cows were fed

different diets, which make it impossible to draw clear conclusions regarding differences in PS.

Similarly, in the current experiment, postpartum peak insulin concentrations were reduced for P cows compared with NP cows and compared with their values 10 d before parturition (Chapter 6). However, it was not clear if this was due to a reduction of insulin secretion in response to glucose bolus or reduced peak glucose concentrations for P cows compared with NP cows. Insulin-independent uptake of glucose by the mammary gland may have reduced the peak concentrations of glucose for P cows compared with NP cows, even though the amount of glucose bolus infused was based on BW of each cows. The glucose utilization and production in response to insulin infusion were similar for non-lactating and lactating sheep (Faulkner and Pollock, 1990). Therefore, in the current study, it is reasonable to consider that the glucose uptake by the mammary gland alone may have affected the differences detected between P and NP cows. The influence of pregnancy status and lactation on insulin resistance either as pancreatic insulin responsiveness or tissue insulin sensitivity cannot be ascertained in the current study.

Furthermore, on d 3 after parturition as time after glucose infusion increased, plasma insulin concentrations decreased quicker for P cows than NP cows, whereas glucose concentrations decreased in a similar manner (Figure 9.7). The clearance rate of insulin of NP cows was less than for P or lactating cows (Sano et al., 1991). In sheep, concentrations of insulin were lower for lactating ewes compared with non-lactating ewes, even though insulin fusion rates were the same (Faulkner and Pollock, 1990). Therefore, it seems probable that PS may have affected the insulin clearance rates, whereas prepartum exercise training did not affect insulin clearance rates.

Also, as time after glucose infusion increased on d 3, plasma NEFA concentrations were reduced for P cows, whereas plasma NEFA concentrations did not

greatly change for NP cows. Prepartum and postpartum concentrations of NEFA declined as time after infusion of glucose increased in dairy cows (Hayirli et al., 2001). The response of NEFA concentrations during glucose infusion may indicate an increased sensitivity of adipose tissue to increased circulating insulin concentrations for P cows compared with NP cows. Faulkner and Pollock (1990) indicated that peripheral glucose utilization was increased more for lactating ewes than non-lactating ewes as insulin concentrations increased on 18 d postpartum. Furthermore, plasma NEFA concentrations were decreased for lactating ewes compared with non-lactating ewes at similar insulin concentrations (Faulkner and Pollock, 1990).

Volumes of Blood Cells, Plasma, and Whole Blood

On d 3 postpartum, prepartum exercise training did not affect volumes of blood cells, plasma, or whole blood of P or NP cows (Table 9.13). During prepartum, exercise training treatment did not greatly affect plasma and whole blood volumes (Chapter 8). Exercise training treatment or training did not affect hematocrit or packed cell volume (Chapter 4 and Blake et al., 1982). In humans, pregnancy and exercise training both increase whole blood volume (McMurray et al., 1993).

On d 3 postpartum, blood cells, plasma, and whole blood volumes were greater for P cows than NP cows ($P < 0.05$, Table 9.13). Plasma volumes (as a percent of BW) of non-lactating cows were less than for lactating cows (Reynolds, 1953). In the current study, P cows had blood cells, plasma, and whole blood volumes of 2.0, 4.7, and 6.7% of BW compared with 1.7, 3.9, and 5.6% of BW for NP cows, respectively. Similar to the current experiment, plasma volumes during three stages of lactation were 30.7, 35.5, and 39.6 L/cow, respectively (6.3, 14.6, and 6.6% of BW, respectively; Delaquis and Block, 1995b). Additionally, Woodford et al. (1984) reported plasma volumes on d 42 postpartum as 37 L/cow (6.9 % of BW).

Acid-Base Status and Mineral Element Concentrations

Postpartum d 0 and 7. On d 0 and 7 postpartum, prepartum exercise training did not affect blood H^+ , pH, pCO_2 , or concentrations of HCO_3^- , and TCO_2 of NP or P cows (Table 9.14). Additionally, prepartum exercise training did not affect mineral element concentrations of K^+ , Cl^- , iCa^{2+} , HCT, or HGB on d 0 or d 7 postpartum (Table 9.14).

Pregnancy status did not affect H^+ concentrations, pH, or pCO_2 (Table 9.14). Interaction of PS by day postpartum affected concentrations of HCO_3^- , TCO_2 , K^+ , Cl^- , iCa^{2+} , HCT, and HGB ($P < 0.05$, Table 9.15). Concentrations of HCO_3^- and TCO_2 were less for P cows on d 0 compared with NP cows. On d 7 postpartum, all cows had greater HCO_3^- and TCO_2 than d 0, and P cows also had greater concentrations than NP cows (Table 9.15).

Cows that were non-pregnant tended to have similar concentrations of K^+ concentrations on d 0 and 7, whereas P cows had reduced K^+ concentrations on d 7 compared with d 0 (interaction of PS by day postpartum, $P = 0.084$, Table 9.15). Concentrations of Cl^- were greater for P cows compared with NP cows on d 0, but not on d 7 (Table 9.15). Ionized Ca^{2+} concentrations were less for P cows compared with NP cows on d 0, but not on d 7 (Table 9.15). Hematocrit and HGB were greater on d 0 for P cows compared with NP cows, but on d 7 concentrations were similar for P and NP cows. From d 0 and 7 postpartum, the acid-base status and mineral element concentrations of NP and P cows changed similarly. Furthermore, the magnitude of changes of HCO_3^- , TCO_2 , K^+ , Cl^- , iCa^{2+} , HCT, and HGB concentrations were greater for P cows compared with NP cows.

Postpartum d 0 through 28. On d 0 through 28 postpartum, prepartum exercise training did not affect blood H^+ , pH, pCO_2 , HCO_3^- , and TCO_2 concentrations of P cows (results not shown). Additionally, prepartum exercise training did not affect mineral

element concentrations of K^+ and iCa^{2+} or HCT and HGB on d 0 through 28 postpartum (results not shown). During d 0 through 28 postpartum, prepartum exercise training did affect concentrations of Cl^- in that E cows had less blood Cl^- than NE cows ($P = 0.013$; Figure 9.8).

Among all P cows during d 0 through 28 postpartum, day postpartum affected blood acid-base status (H^+ , pH, pCO_2 , HCO_3^- , and TCO_2 concentrations), mineral element concentrations (K^+ , Cl^- , and iCa^{2+}) and HCT and HGB ($P < 0.001$; Figure 9.8). Concentrations of H^+ were less (greater blood pH) as day postpartum increased. The pCO_2 , and concentrations of HCO_3^- , and TCO_2 changed in a similar fashion relative to each other in that values increased from d 0 to 7 followed by a slight decline as day postpartum increased beyond d 7. Concentrations of K^+ and Cl^- were greatest on d 0 and declined by d 7 postpartum, whereas iCa^{2+} concentrations were least on d 0 and increased by d 7 postpartum to constant values through 28 d postpartum. The HCT and HGB concentrations were greatest on d 0 and declined on d 7 postpartum to constant values through 28 d postpartum. The changes of pCO_2 , and concentrations of HCO_3^- , TCO_2 , K^+ , and Cl^- may be reflective of increased volume of plasma during d 0 through 7 as indicated by the reduction of HCT during d 0 and 7 postpartum.

Acid-base homeostasis was tightly controlled throughout this experiment in that significant differences were detected even though the differences among treatment groups were small. The change in acid-base status and mineral element concentrations from d 0 to d 7 postpartum may reflect changes of DCAD during the periparturient period. Cows were fed a negative DCAD diet until parturition (d 0) followed by a positive DCAD diet after parturition. Additionally, the reduction of HCT during d 0 and 7 postpartum may indicate an increase of plasma volume, which would have affected acid-base status and mineral element concentrations.

In contrast, exercise training affected prepartum acid-base status and mineral element concentrations on a non-exercise day (Chapter 5). Prepartum, cows that exercised had less H^+ concentrations (greater blood pH) than non-exercised cows, and no differences were detected for PS (Chapter 5). No differences of pCO_2 , and HCO_3^- and TCO_2 concentrations or mineral element concentrations were detected in response to prepartum exercise training. However, prepartum, P cows had less pCO_2 and concentrations of HCO_3^- , and TCO_2 than NP cows, potentially due to differences in whole blood volume. Pregnancy status did not affect mineral element concentrations of K^+ and iCa^{2+} , whereas P cows had greater Cl^- concentrations than NP cows.

Rodriguez (1998) had similar changes in plasma pH, pCO_2 , and concentrations of HCO_3^- , K^+ , Cl^- , and iCa^{2+} during the periparturient period when pregnant cows were fed negative DCAD diets prepartum followed by a positive DCAD diet postpartum. No additional information was available to confirm the lack of effect of prepartum exercise on postpartum acid-base status and mineral element concentrations or effects after the cessation of exercise training. The reasons for the slight reduction of Cl^- concentrations for exercised cows during d 0 to 28 postpartum compared with non-exercised cows are unclear. This response to exercise training was not detected during the prepartum period.

CONCLUSIONS

During the postpartum period, pregnant dairy cows profited from prepartum exercise with increased DMI, reduced BW loss, and greater BCS during the first 4 wk postpartum compared with non-exercised cows. Prepartum exercise training did not affect milk yield or milk composition. Furthermore, the postpartum energy balance calculated from NRC (2001) was not different between E and NE cows. However,

prepartum exercise of pregnant cows reduced plasma insulin and increased plasma glucagon concentrations during the first week after parturition compared with non-exercised cows. Concentrations of liver glycogen were reduced for P, E cows compared with P, NE cows, whereas concentrations in NP, E were greater than in NP, NE cows. Prepartum exercise training affected endocrine control of glucose homeostasis during the first week postpartum. Potentially, dairy cows that were exercised benefitted from increased hepatic glycogenolysis (reduced liver glycogen concentrations) to support the metabolic demands during the first week postpartum until DMI was increased. Prepartum exercise did not affect the concentrations of glucoregulatory hormones in response to an intravenous infusion of glucose.

Prepartum exercise training of cows resulted in cows of less BW at parturition with similar DMI producing similar quantities of milk and milk components as non-exercised cows. Prepartum exercise training may have improved appetite and energy balance enough to improve the transition from non-lactating to lactating physiological states. Additional research of exercise training with increased number of cows is needed to determine if incidences of health disorders can be reduced or if milk yield may actually be affected with prepartum exercise training. Furthermore, the minimum duration and intensity of prepartum exercise required to improve cow performance during the periparturient period is not known.

Table 9.1. Formulated ingredient and analyzed chemical composition of diet fed d 0 through 27 and d 28 through 120 postpartum.

Item	d 0 through 27 postpartum	d 28 through 120 postpartum
	———— % of DM ————	
Corn silage	22.8	29.5
Alfalfa silage	15.5	17.3
Alfalfa hay	12.0	6.8
Corn distillers dried grains	11.6	6.8
Soybean meal	9.3	5.5
Commercial supplement ³	4.2	8.6
High moisture corn	0.4	12.2
Beet pulp	0.7	0
Whole cottonseed with lint	0	6.0
Vitamin-mineral mix with ground corn	23.5 ¹	7.3 ²
Chemical composition		
DM	55.8	51.1
CP	17.9	18.1
ADF	18.8	21.1
NDF	28.7	31.9
EE	4.8	5.9
Ash	7.9	7.1
Ca	0.92	0.87
P	0.57	0.51
Mg	0.44	0.38
K	1.32	1.30
Na	0.28	0.25
Cl	1.06	0.43
S	0.21	0.21
TDN for maintenance ⁴	73.5	73.2
DE for maintenance ⁴ , Mcal/kg	3.32	3.31
NE _L ⁵ , Mcal/kg	1.68	1.61

¹ Contained 7.2% CP, 4.7% ADF, 9.3% NDF, 8.7% ash, 3.0% EE, 1.13% Ca, 0.85% P, 0.98% Mg, 0.38% K, 0.99% Na, 0.12% S, 0.02% Cl, 0.84 ppm of Se, 30 KIU of vitamin A/kg, 6.5 KIU of vitamin D/kg, and 0.49 KIU of vitamin E/kg.

² Contained 18.7% CP, 3.6% ADF, 7% NDF, 17.4% ash, 3.0% EE, 1.47% Ca, 1.15% P, 1.77% Mg, 0.31% K, 2.42% Na, 0.14% S 0.6% Cl, 2.77 ppm of Se, 31 KIU of vitamin A/kg, 9.1 KIU of vitamin D/kg, and 0.52 KIU of vitamin E/kg.

³ Contained 30.1% CP, 9% ADF, 17.9% NDF, 14.7% ash, and 13.7% EE.

⁴ Energy equations and estimates of acid-detergent insoluble CP and neutral-detergent insoluble from NRC (2001).

⁵ Based on equations from NRC (2001). Calculated for each cow's daily intake over maintenance and then averaged.

Table 9.2. Body weight (BW) and body condition score (BCS) during d 0 through 7 postpartum.¹

	Treatments				SEM	P-value ²		
	NP, NE ³	NP, E ³	P, NE ³	P, E ³		T	PS	T x PS
<u>BW, kg</u>								
d 0	775	730	742	705	15.4	0.02	0.007	NS
d 3	787	740	728	707	15.4			
d 7	782	738	718	693	15.4			
<u>BCS</u>								
d 0	4.50	4.25	3.75	3.75	0.10	NS	< 0.001	NS
d 7	4.00	4.25	3.50	3.50	0.15			

¹ Values are least squares means. Body condition scores rounded to nearest 0.25.

² T = effect of exercise treatment; PS = effect of pregnancy status; T x PS = the interaction of exercise treatment by pregnancy status; interaction of PS x day postpartum, $P = 0.002$. NS = non-significant, $P > 0.10$.

³ NP = non-pregnant cows; P = pregnant cows; E = exercised cows; NE = non-exercised cows.

Table 9.3. Dry matter intake on d 1, 3, and 6 postpartum.¹

Dependent Variables	Treatments				SEM	P-value ²		
	NP, NE ³	NP, E ³	P, NE ³	P, E ³		T	PS	PS x D
DMI, kg/day								
d 1	18.2	17.8	11.1	12.0				
d 3	16.7	16.6	12.0	15.6				
d 6	18.4	17.1	16.5	17.1	1.01	NS	0.001	< 0.001
DMI, kg/100 kg BW								
d 1	2.34	2.41	1.55	1.71				
d 3	2.15	2.26	1.73	2.21				
d 6	2.35	2.31	2.32	2.44	0.12	NS	0.021	< 0.001

¹ Values are least squares means.

² T = effect of exercise treatment; PS = effect of pregnancy status; T x D = the interaction of exercise treatment by day postpartum; interaction of T x PS, $P > 0.10$. NS = non-significant, $P > 0.10$.

³ NP = non-pregnant cows; P = pregnant cows; E = exercised cows; NE = non-exercised cows.

Table 9.4. Daily milk yield and weekly milk composition during d 1 through 120 postpartum.¹

Dependent Variables ³	Treatments			P-value ²		
	NE ⁴	E ⁴	SEM	T	D or W	T x D or W
Milk yield, kg/d	43.5	45.7	1.27	NS	< 0.001	NS
Energy-corrected milk yield, kg/d	45.9	46.7	1.73	NS	< 0.001	NS
Fat, %	4.53	4.32	0.19	NS	< 0.001	NS
True protein, %	2.86	2.83	0.04	NS	< 0.001	NS
Lactose, %	4.76	4.75	0.04	NS	< 0.001	NS
Solids nonfat, %	8.54	8.49	0.074	NS	< 0.001	NS
SCC, 1000/ml	578	321	157	NS	NS	NS

¹ Values are least squares means.

² T = effect of exercise treatment; D = effect of day; W = effect of week; T x D or W = the interaction of exercise treatment by day or week postpartum. NS = non-significant, $P > 0.10$.

³ Energy-corrected milk yield = $0.3246 \times \text{milk yield} + 12.86 \times \text{fat yield} + 7.04 \times \text{true protein yield}$ (Dairy Records Management, 1999); SCC = somatic cell count.

⁴ NE = non-exercised cows; E = exercised cows.

Table 9.5. Cumulative milk yield and milk components yield during d 1 through 28 and d 1 through 120 postpartum.¹

Dependent Variables ³	Treatments		SEM	<i>P</i> -value ²
	NE ⁴	E ⁴		T
<u>d 1 through 28</u>				NS
Milk yield, kg	1213	1306	60	NS
Energy-corrected milk yield, kg	1339	1450	91	NS
Fat yield, kg	55.7	59.1	4.74	NS
True protein yield, kg	34.2	37.8	1.9	NS
Lactose yield, kg	55.5	61.7	2.8	NS
SNF yield, kg	100.9	111.8	5.3	NS
<u>d 1 through 120</u>				
Milk yield, kg	5124	5386	152	NS
Energy-corrected milk yield, kg	5364	5507	206	NS
Fat yield, kg	213.1	213.0	12.0	NS
True protein yield, kg	138.2	144.9	4.4	NS
Lactose yield, kg	244.3	256.8	6.0	NS
SNF yield, kg	427.3	448.8	11.0	NS

¹ Values are least squares means.

² T = effect of exercise treatment. NS = non-significant, *P* > 0.10.

³ Energy-corrected milk = 0.3246 x milk yield + 12.86 x fat yield + 7.04 x true protein yield; SCC = somatic cell count.

⁴ NE = non-exercised cows; E = exercised cows.

Table 9.6. Weekly average net energy expenditure and balance and daily milk to DMI ratio.¹

Mcal/d	Treatments			P - value ²		
	NE ³	E ³	SEM	T	W	T x W
<u>wk 1 through 4⁴</u>						
Daily net energy intake ⁵	31.0	32.1	1.1	NS	< 0.001	0.089
Maintenance ⁶	10.9	10.6	0.17	NS	< 0.001	NS
Lactation ⁷	31.9	33.5	1.9	NS	< 0.001	NS
Body Reserves ⁸	-12.9	-11.4	1.4	NS	NS	NS
Net energy for gain ⁹	-11.7	-12.0	1.4	NS	< 0.001	NS
Milk : DMI, kg/kg	2.23	2.25	0.08	NS	NS	0.11
Energy-corrected milk : DMI, kg/kg	2.60	2.55	0.11	NS	< 0.001	NS
Energy -corrected milk : energy intake, kg/Mcal	1.53	1.53	0.06	NS	0.005	NS
<u>wk 5 through 17⁴</u>						
Daily net energy intake ⁵	38.6	37.8	0.5	NS	< 0.001	NS
Maintenance ⁶	10.4	10.1	0.2	NS	NS	NS
Lactation ⁷	32.1	32.3	1.0	NS	0.029	NS
Body Reserves ⁸	0.19	-0.63	0.5	NS	0.0034	NS
Net energy for gain ⁹	-3.8	-4.6	0.9	NS	< 0.001	NS
Milk : DMI, kg/kg	1.87	1.99	0.05	NS	< 0.001	NS
Energy-corrected milk : DMI, kg/kg	1.93	1.98	0.06	NS	< 0.001	NS
Energy -corrected milk : energy intake, kg/Mcal	1.20	1.23	0.04	NS	< 0.001	NS

¹ Values are least squares means.

² T = effect of exercise treatment; W = effect of week or day postpartum; T x W = the interaction of exercise treatment by week or day postpartum; NS = non-significant, $P > 0.10$.

³ Pooled least squares mean for the following groups: NE = non-exercised cows; E = exercised cows.

⁴ Weeks postpartum.

⁵ Daily net energy intake, Mcal/d = average DMI * NE_L of diet as a function of each cow's level of intake over maintenance.

⁶ Maintenance, Mcal/d = $0.08 \times BW^{0.75}$.

⁷ Lactation, Mcal/d = $(0.0929 \times \% \text{ fat} + 0.0563 \times \% \text{ true protein} + 0.0395 \times \% \text{ lactose}) \times \text{milk kg/d}$.

⁸ Body reserves, Mcal/d = $\{[BW \text{ initial} \times BCS \text{ initial} \times 0.51826 + 0.65176 \times BW \text{ initial}] - [(BW \text{ final} \times BCS \text{ final} \times 0.51826 + 0.65176 \times BW \text{ final})]\}$. If body reserves was negative, body reserves was multiplied by 0.82 (efficiency for use of body tissues for milk production), else body reserves was multiplied by 0.64/0.75 (efficiency of gain for lactating cows). Simplified equation derived from equations of NRC (2001).

⁹ Net energy for gain, Mcal/d = daily net energy intake - (maintenance + lactation).

Table 9.7. Incidences of health disorders.

Disorders ¹	Treatment	
	NE ²	E ²
Number of cows with 1 or more health disorder	8	3
Type I error, α ($P = 0.05$) ³		
Type II error, β ($P = 0.48$) ³		
Number cases of health disorders		
Retained placenta	3	0
Periparturient mastitis	5	1
Hypocalcemia	2	0
Ketosis	4	2
Displaced abomasum	1	0
Metritis	3	0
Cystic ovaries	3	1
Number of parturitions	13	13
Udder Edema Score ⁴	2.42	1.27

¹ Occurrences of health related disorders were recorded 14 d prepartum to 28 d postpartum. Criteria for occurrence of a health related disorder were as follows: retained placenta if placenta was not delivered within 24 h after parturition; clinical mastitis if cow required treatment due to fever and clots in milk; hypocalcemia if blood ionized calcium less than 0.80 mmol/L; ketosis if readings of Keto-stik® (Bayer Corporation, Elkhart, IN) were moderate and large; displaced abomasum if surgery was required to correct position of abomasum; and metritis and cystic ovaries were diagnosed with palpation by a veterinarian.

² NE = non-exercised cows; E = exercised cows.

³ Chi-square analysis for incidence rates was used to determine type I error rate. Type II error rate (power) was calculated assuming α of 0.5% with estimated incidence rates and cows per treatment group reported.

⁴ Cows were scored at parturition for udder edema with a scale of 1 to 5 with a score of 5 indicating severe edema and hardness of all quarters and score of 1 indicating no visual signs of edema ($P = 0.04$).

Table 9.8. Plasma metabolite and hormone concentrations pooled across d 0 and 7 postpartum.¹

Dependent Variables ³	Treatments				SEM	P-value ²		
	NP, NE ⁴	NP, E ⁴	P, NE ⁴	P, E ⁴		T	PS	T x PS
Glucose, mg/dl	69.0	68.7	64.4	60.2	1.74	NS	< 0.001	NS
Ln(lactate), mmol/L	-0.10	0.04	0.09	-0.03	0.13	NS	NS	NS
Ln(NEFA), μ eq/L	4.44	4.40	6.41	6.51	0.087	NS	< 0.001	NS
Ln(BHBA), mg/dl	1.47	1.44	1.86	2.13	0.09	NS	< 0.001	NS
Ln(insulin), μ IU/ml	3.65	3.23	1.16	0.92	0.16	0.04	< 0.001	NS
Ln(glucagon), pg/ml	5.67	5.48	4.67	4.78	0.08	NS	< 0.001	0.054
Ln(insulin : glucose), pmol:mmol	4.28	3.87	1.96	1.71	0.16	0.04	< 0.001	NS
Ln(insulin : glucagon), pmol:pmol	-1.30	-1.53	-2.70	-3.14	0.17	0.05	< 0.001	NS

¹ Values are least squares means.

² T = effect of exercise treatment; PS = effect of pregnancy status; T x PS = the interaction of exercise treatment by pregnancy status. NS = non-significant, $P > 0.10$.

³ Ln = natural logarithmically transformed; NEFA = non-esterified fatty acids; BHBA = β -hydroxybutyrate.

⁴ NP = non-pregnant cows; P = pregnant cows; E = exercised cows; NE = non-exercised cows.

Table 9.9. Effect of the interaction of pregnancy status by day postpartum on plasma metabolites and hormones concentrations on d 0 and 7.¹

Dependent Variables ³	Treatments				SEM	P-value ²		
	NP, NE ⁴	NP, E ⁴	P, NE ⁴	P, E ⁴		T	PS	PS x D
Glucose, mg/dl								
d 0	68.3	66.7	76.2	74.0				
d 7	69.6	70.6	52.6	46.5	2.28	NS	NS	< 0.001
Ln(lactate),								
d 0	-0.19	0.13	0.38	0.52				
d 7	-0.02	-0.05	-0.20	-0.59	0.18	NS	NS	0.002

¹ Values are least squares means.

² T = effect of exercise treatment; PS = effect of pregnancy status; T x D = the interaction of exercise treatment by day postpartum. NS = non-significant, $P > 0.10$.

³ Ln = natural logarithmically transformed.

⁴ NP = non-pregnant cows; P = pregnant cows; E = exercised cows; NE = non-exercised cows.

Table 9.10. Activity of succinate dehydrogenase (SDH) and concentrations of protein, glycogen (glucosyl units), and natural logarithmically transformed triglycerides (TG) in semitendinous muscle tissue pooled across d 3 and 7 postpartum.¹

	NP, NE ³	NP, E ³	P, NE ³	P, E ³	SEM	<i>P</i> -value ²			
						T	PS	T x PS	D
SDH, μmol/min per g wet tissue	0.14	0.17	0.16	0.14	0.01	NS	NS	0.04	NS
SDH, μmol/min per g protein	1.25	1.49	1.37	1.33	0.09	NS	NS	0.10	NS
Protein, mg per g wet tissue	116	116	116	109	3.0	NS	NS	NS	NS
Glycogen (glucosyl units), mg per g wet tissue	1.1	1.2	1.1	1.1	0.04	NS	NS	NS	NS
Glycogen (glucosyl units), μg per mg protein	9.9	10.8	9.8	10.4	0.60	NS	NS	NS	NS
Ln(TG, mg per g wet tissue)	1.50	1.45	1.45	1.49	0.20	NS	NS	NS	NS
Ln(TG, μg per mg protein)	3.7	3.6	3.6	3.7	0.20	NS	NS	NS	NS

¹ Values are least squares means.

² T = effect of exercise treatment; PS = effect of pregnancy status; T x PS = the interaction of exercise treatment by pregnancy status; D = effect of day of experiment; NS = non-significant, *P* > 0.10.

³ NP = non-pregnant cows; P = pregnant cows; E = exercised cows; NE = non-exercised cows.

Table 9.11. Activity of succinate dehydrogenase (SDH) and concentrations of protein, glycogen (glucosyl units), and natural logarithmically transformed triglycerides (TG) in liver tissue pooled across d 3 and 7 postpartum.¹

	NP, NE ³	NP, E ³	P, NE ³	P, E ³	SEM	<i>P</i> -value ²			
						T	PS	T x PS	D
SDH, μmol/min per g wet tissue	0.88	0.83	1.03	1.11	0.06	NS	0.01	NS	NS
SDH, μmol/min per g protein	4.6	4.5	5.7	5.9	0.3	NS	0.01	NS	NS
Protein, mg per g wet tissue	189	183	179	186	3.7	NS	NS	0.10	NS
Glycogen (glucosyl units), mg per g wet tissue	50.6	58.8	23.2	14.7	3.2	NS	0.01	0.01	NS
Glycogen (glucosyl units), μg per mg protein	267	324	130	79	18	NS	0.01	0.01	NS
Ln(TG, mg per g wet tissue)	0.38	0.78	3.14	3.22	0.15	NS	0.01	NS	0.02
Ln(TG, μg per mg protein)	2.0	2.5	4.9	4.9	0.16	NS	0.01	NS	0.02

¹ Values are least squares means.

² T = effect of exercise treatment; PS = effect of pregnancy status; T x PS = the interaction of exercise treatment by pregnancy status; D = effect of day of experiment; NS = non-significant, *P* > 0.10.

³ NP = non-pregnant cows; P = pregnant cows; E = exercised cows; NE = non-exercised cows.

Table 9.12. Basal, peak, and increase over basal concentrations of glucose and insulin during postpartum glucose tolerance test.¹

	Treatments				SEM	<i>P</i> -value ²		
	NP, NE ³	NP, E ³	P, NE ³	P, E ³		T	PS	T x PS
Basal glucose, mg/dl	66.7	66.6	50.3	45.0	2.0	NS	< 0.001	NS
Peak glucose, mg/dl	146.9	145.2	120.1	118.8	4.0	NS	< 0.001	NS
Increase over basal glucose, mg/dl	80.2	78.7	69.7	73.8	3.1	NS	0.021	NS
Basal insulin, μIU/ml	53.8	48.1	4.2	3.0	6.5	NS	0.013	NS
Peak insulin, μIU/ml	176.6	190.1	55.0	41.2	15.0	NS	< 0.001	NS
Increase over basal insulin, μIU/ml	131.0	142.0	50.6	38.3	11.6	NS	< 0.001	NS

¹ Values are least squares means; samples collected on d 3 postpartum.

² T = effect of exercise treatment; PS = effect of pregnancy status; T x PS = the interaction of exercise treatment by pregnancy status. NS = non-significant, *P* > 0.10.

³ NP = non-pregnant cows; P = pregnant cows; E = exercised cows; NE = non-exercised cows.

Table 9.13. Volumes of blood cells, plasma, and whole blood postpartum.¹

Volume, L	Treatments				SEM	P-value ²		
	NP, NE ³	NP, E ³	P, NE ³	P, E ³		T	PS	T x PS
Blood cells	13.6	12.7	14.3	14.3	0.43	NS	0.013	NS
Plasma volume	29.6	29.4	34.0	33.8	1.17	NS	< 0.001	NS
Whole blood	43.2	42.1	48.3	48.1	1.50	NS	< 0.001	NS

¹ Values are least squares means; blood samples taken on d 3 postpartum.

² T = effect of exercise treatment; PS = effect of pregnancy status; T x PS = the interaction of exercise treatment by pregnancy status. NS = non-significant, $P > 0.10$.

³ NP = non-pregnant cows; P = pregnant cows; E = exercised cows; NE = non-exercised cows.

Table 9.14. Blood acid-base status (H^+ , pH, pCO_2 , concentrations of HCO_3^- , and TCO_2), mineral element concentrations (K^+ , Cl^- , and iCa^{2+}), and cellular concentrations (HCT and HGB) pooled across d 0 and 7 postpartum.¹

Dependent Variables ³	Treatments				SEM	P-value ²		
	NP, NE ⁴	NP, E ⁴	P, NE ⁴	P, E ⁴		T	PS	T x PS
H^+ , mmol/L	3.8	3.7	3.8	3.8	0.06	NS	NS	NS
pH	7.43	7.44	7.43	7.42	0.053	NS	NS	NS
pCO_2 , mm Hg	42.0	38.8	41.3	41.7	1.17	NS	NS	NS
HCO_3^- , mmol/L	27.5	27.1	27.4	27.3	0.37	NS	NS	NS
TCO_2 , mmol/L	28.8	28.3	28.7	28.6	0.32	NS	NS	NS
K^+ , mmol/L	4.4	4.4	4.5	4.4	0.06	NS	NS	NS
Cl^- , mmol/L	101.9	102.0	104.6	103.7	0.45	NS	< 0.001	NS
iCa^{2+} , mmol/L	1.18	1.20	1.12	1.11	0.013	NS	< 0.001	NS
HCT, %	30.8	31.0	31.3	31.3	0.46	NS	NS	NS
HGB, g/dl	10.3	10.3	10.4	10.4	0.15	NS	NS	NS

¹ Values are least squares means.

² T = effect of exercise treatment; PS = effect of pregnancy status; T x PS = the interaction of exercise treatment by pregnancy status. NS = non-significant, $P > 0.10$.

³ H^+ = hydrogen ion; pCO_2 = partial pressure of carbon dioxide; HCO_3^- = bicarbonate ion; TCO_2 = total carbon dioxide; K^+ = potassium ion; Cl^- = chloride ion; iCa^{2+} = ionized calcium ion; HCT = hematocrit; and HGB = hemoglobin.

⁴ NP = non-pregnant cows; P = pregnant cows; E = exercised cows; NE = non-exercised cows.

Table 9.15. Effects of the interaction of pregnancy status by day postpartum for blood acid-base status (concentrations of HCO_3^- and TCO_2), mineral element concentrations (K^+ , Cl^- , and iCa^{2+}), and cellular concentrations (HCT and HGB) on d 0 and 7.¹

Dependent Variables ³	Treatments				SEM	P-value ²		
	NP, NE ⁴	NP, E ⁴	P, NE ⁴	P, E ⁴		T	PS	PS x D
HCO_3^- , mmol/L								
d 0	26.0	25.9	24.3	24.6				
d 7	29.0	28.3	30.6	30.0	0.65	NS	NS	0.005
TCO_2 , mmol/L								
d 0	27.2	27.1	25.4	25.9				
d 7	30.4	29.6	32.0	31.3	0.70	NS	NS	0.012
K^+ , mmol/L								
d 0	4.4	4.6	4.7	4.6				
d 7	4.3	4.3	4.3	4.2	0.09	NS	NS	0.084
Cl^- , mmol/L								
d 0	103.3	103.4	108.8	108.0				
d 7	100.5	100.6	100.4	99.5	0.72	NS	NS	< 0.001
iCa^{2+} , mmol/L								
d 0	1.20	1.22	1.09	1.07				
d 7	1.15	1.19	1.16	1.17	0.02	NS	NS	0.001
HCT, %								
d 0	30.8	31.2	32.5	32.6				
d 7	30.8	30.8	30.2	30.0	0.52	NS	NS	< 0.001
HGB, g/dl								
d 0	10.2	10.3	10.8	10.8				
d 7	10.3	10.3	10.0	10.0	0.18	NS	NS	< 0.001

¹ Values are least squares means.

² T = effect of exercise treatment; PS = effect of pregnancy status; PS x D = the interaction of pregnancy status by day postpartum. Interaction of T by PS was non-significant. NS = non-significant, $P > 0.10$.

³ HCO_3^- = bicarbonate ion; TCO_2 = total carbon dioxide; K^+ = potassium ion; Cl^- = chloride ion; iCa^{2+} = ionized calcium ion; HCT = hematocrit; and HGB = hemoglobin.

⁴ NP = non-pregnant cows; P = pregnant cows; E = exercised cows; NE = non-exercised cows.

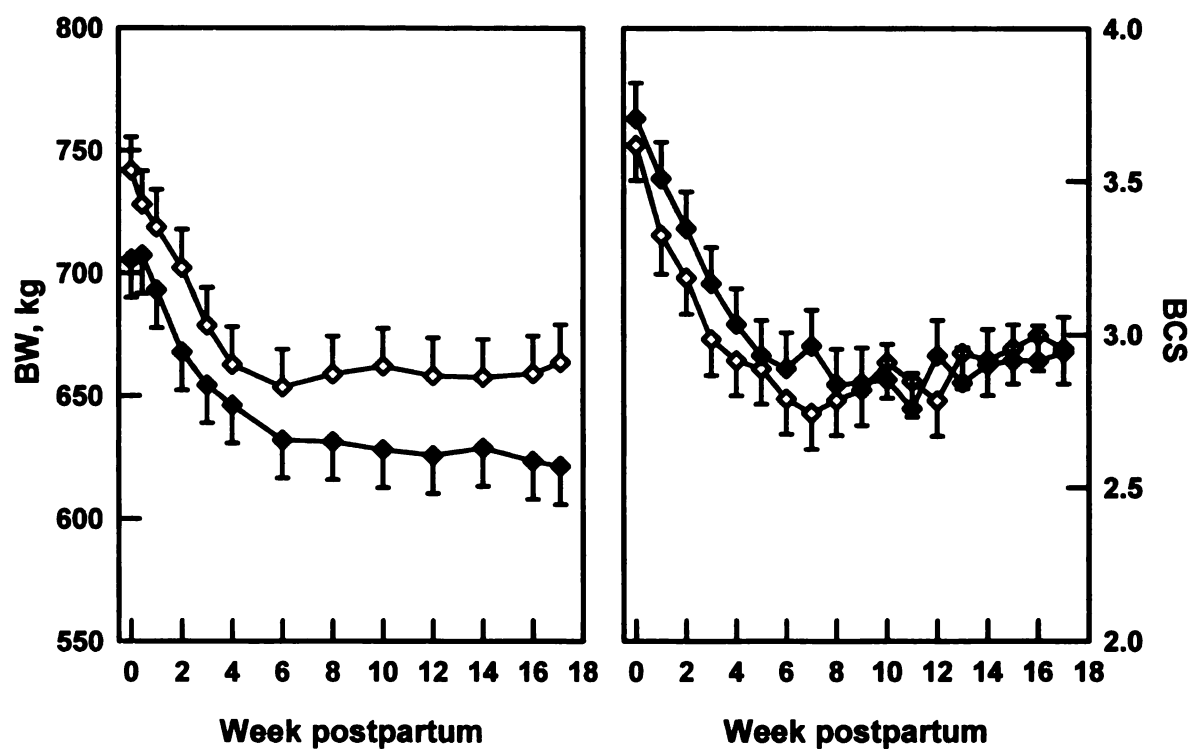


Figure 9.1. BW and BCS of pregnant cows during wk 0 to 17 postpartum. Prepartum exercise training did not affect BW or BCS: non-exercise ($\diamond$) and exercise ($\blacklozenge$). Interaction of exercise by week postpartum tended to affect BCS ($P = 0.085$). Week postpartum affected BW and BCS ($P < 0.001$).

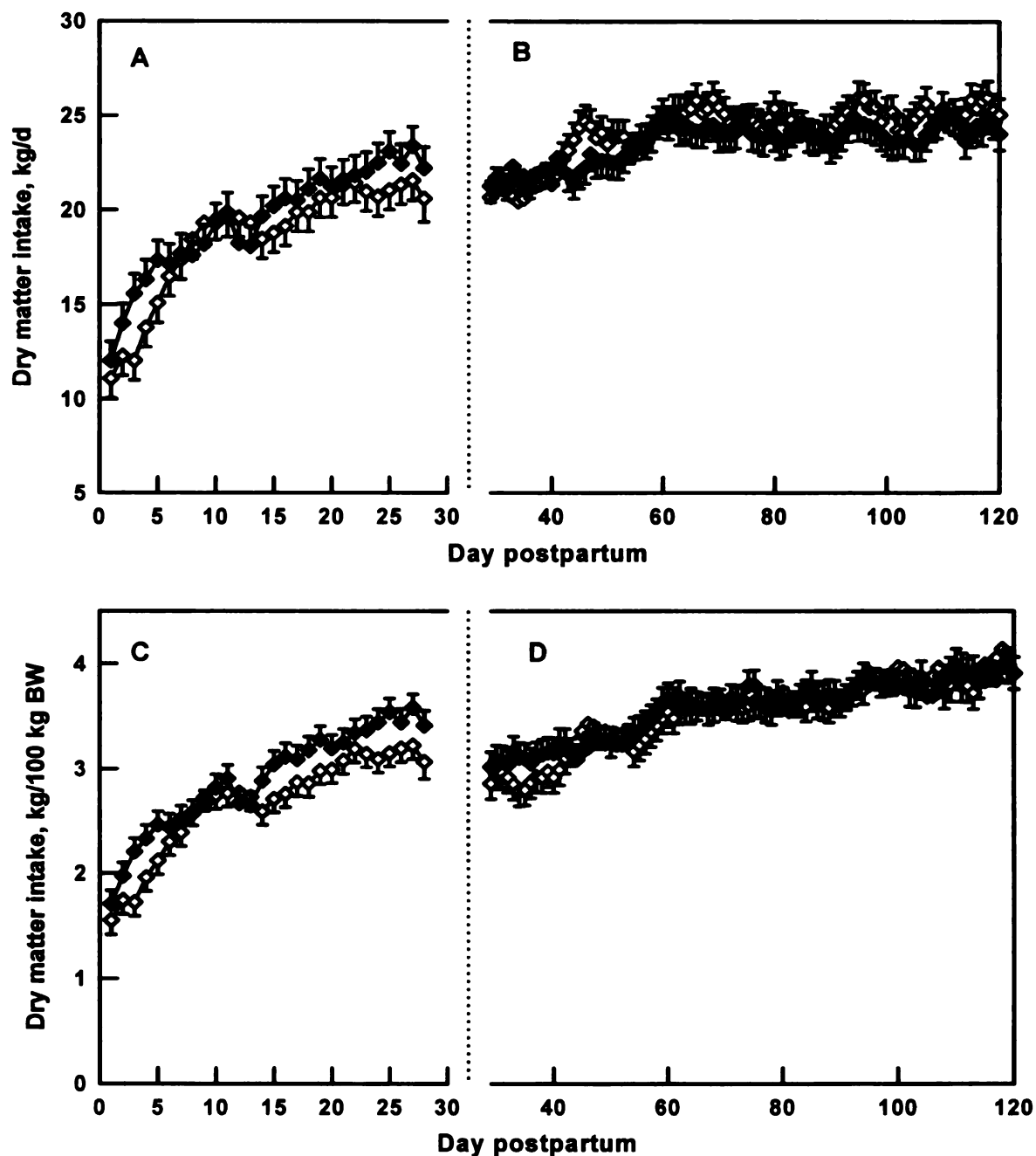


Figure 9.2. DMI (kg/d and kg/100 kg BW) of pregnant cows during d 1 through 120 postpartum: non-exercise (◇) and exercise (◆). *Panel A and C:* Cows were fed a diet of 1.68 Mcal/d. *Panel B and D:* Cows were fed a diet of 1.61 Mcal/d. Prepartum exercise training did not affect DMI (kg/d, *Panel A and B*). Prepartum exercise tended to affect postpartum DMI (kg/100 kg of BW) during d 1 through 28 (*Panel C*, $P = 0.099$), but not during d 29 through 120 (*Panel D*). Day postpartum affected DMI (kg/d and kg/100 kg BW) during d 1 through 28 and d 29 through 120.

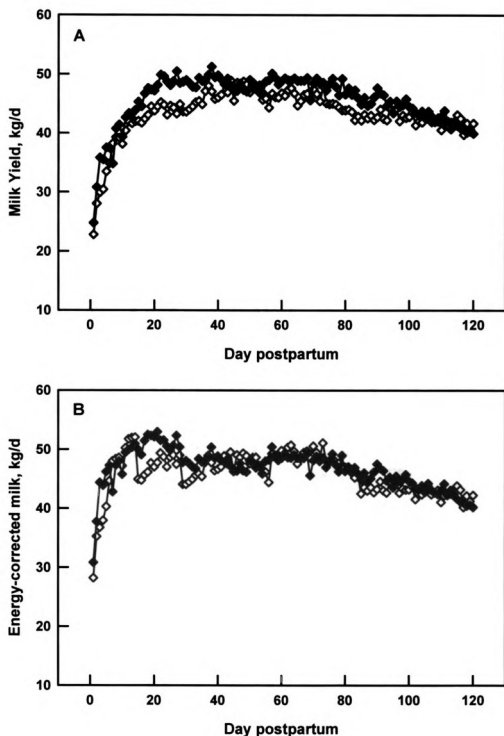


Figure 9.3. Milk yield (*Panel A*, kg/d, pooled SEM = 1.89) and energy-corrected milk yield (*Panel B*, kg/d, pooled SE = 2.60) of pregnant cows during d 1 through 120 postpartum: non-exercise ($\diamond$) and exercise ($\blacklozenge$). Prepartum exercise training did not affect milk yield or energy-corrected milk yield (kg/d). Day postpartum affected milk yield and energy-corrected milk yield during d 1 through 120 ($P < 0.01$).

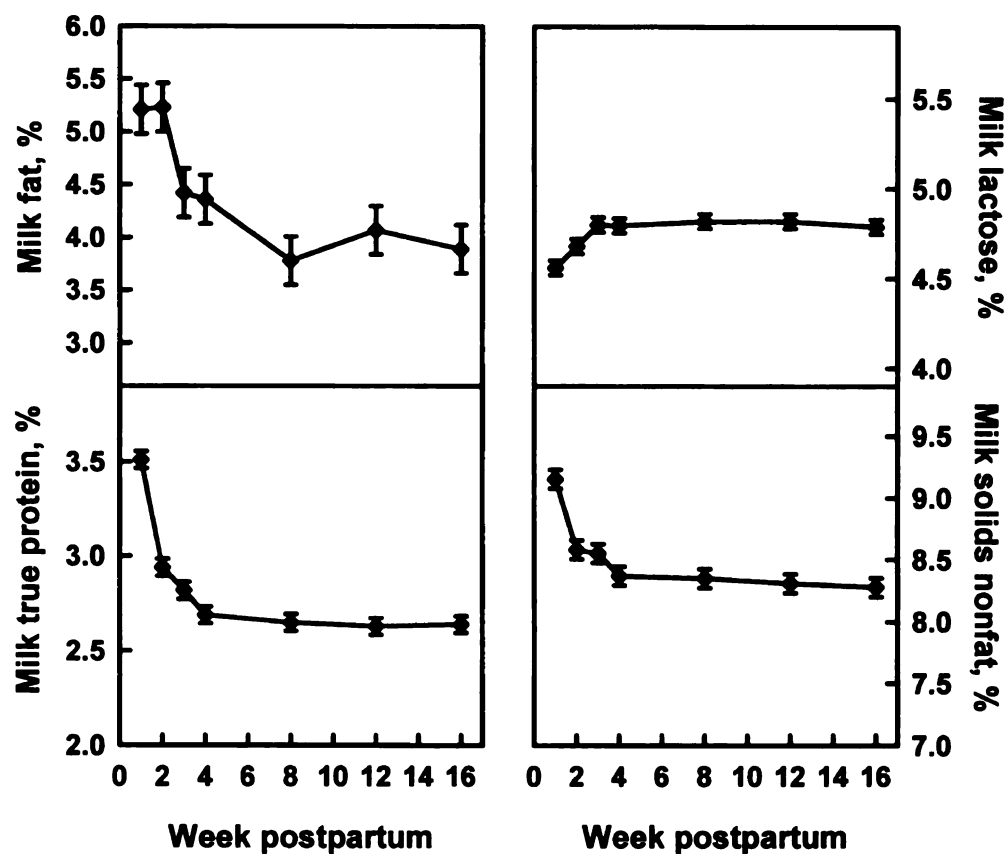


Figure 9.4. Percentage of milk fat, true protein, lactose, and solids non-fat during wk 1 through 16 postpartum. Prepartum exercise training did not affect percentage of any milk component. Week postpartum affected percent of all milk components ($P < 0.01$).

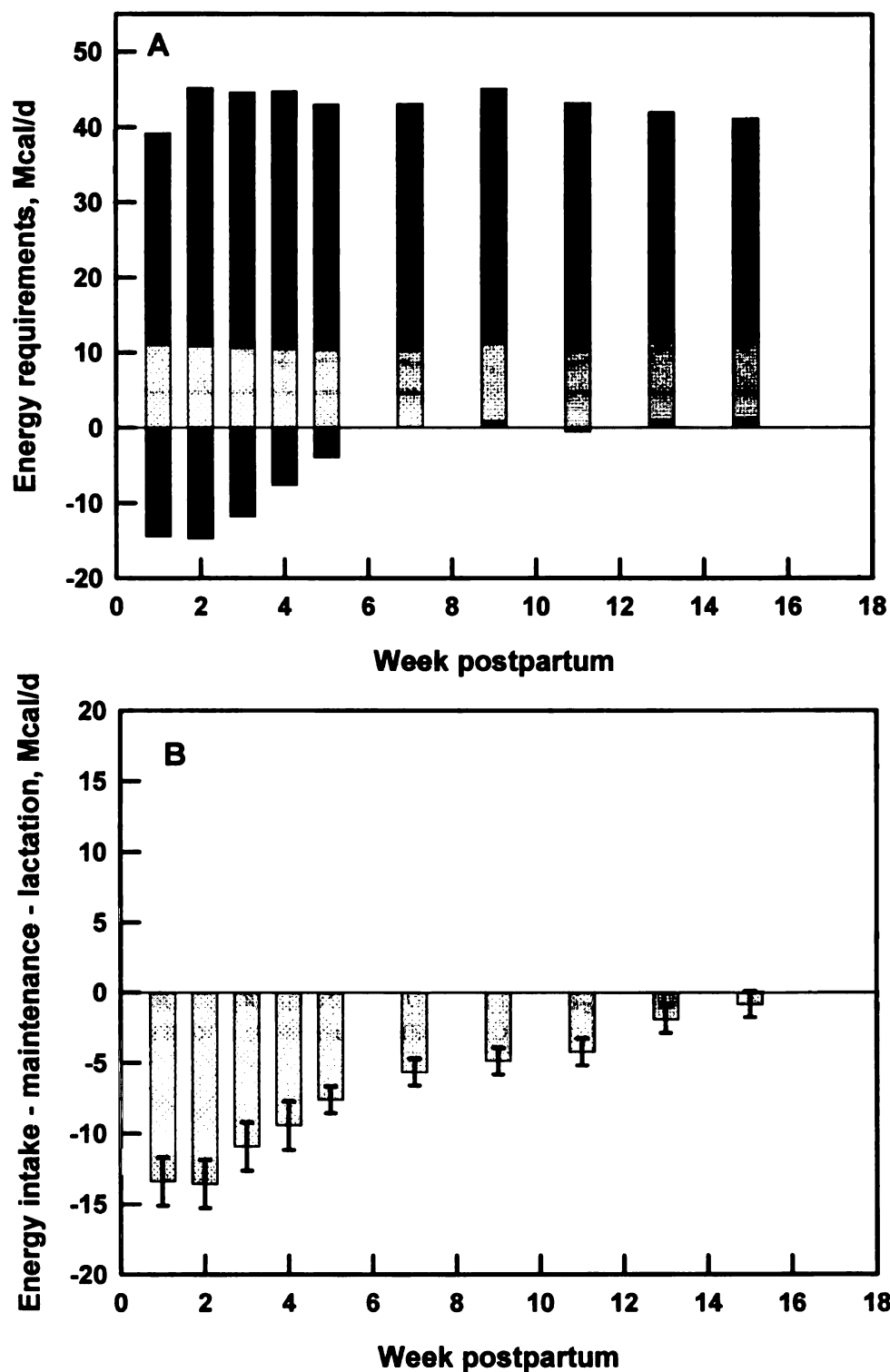


Figure 9.5. Energy requirement of maintenance, lactation, and loss or gain of body reserves wk 1 to 15 postpartum. *Panel A:* maintenance (light gray bar); lactation (medium gray bar); and body reserves (black bar). *Panel B:* Residual energy (difference between energy intake minus energy requirement of maintenance and lactation). Prepartum exercise training did not affect calculations of energy balance. During wk 1 to 4, week affected energy requirement of maintenance, and lactation and residual energy ($P < 0.05$). During wk 5 to 15, week affected energy requirement of lactation and body reserves and residual energy ($P < 0.05$).

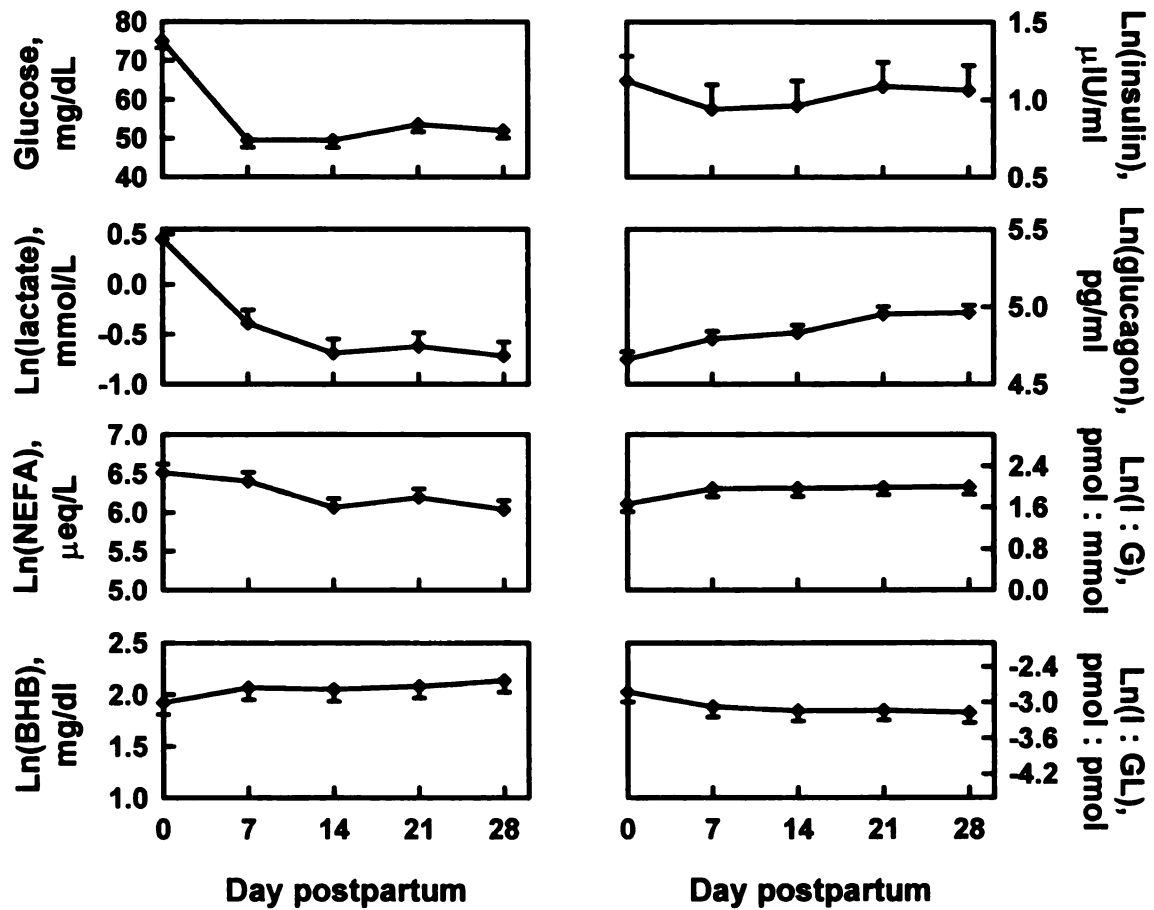


Figure 9.6. Plasma metabolites (glucose, lactate, NEFA, and BHBA concentrations); hormones (insulin and glucagon); and molar ratios of insulin : glucose (I:G) and insulin : glucagon (I:GL) during postpartum d 0 to 28 for pregnant cows. Day postpartum affected concentrations of all variables ($P < 0.001$).

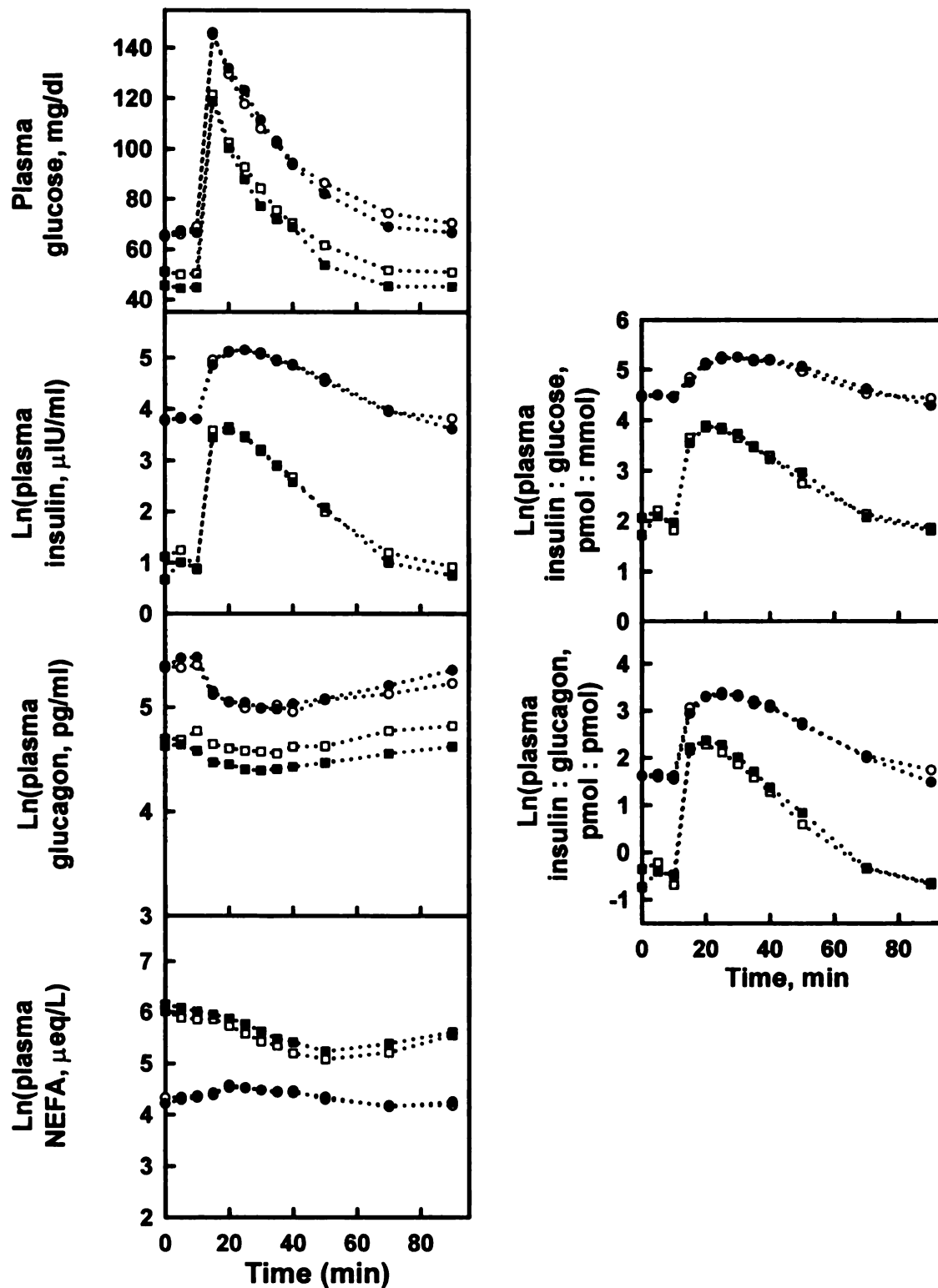


Figure 9.7. Plasma concentrations of glucose, insulin, glucagon, and NEFA, and molar ratios of insulin : glucose and insulin : glucagon on d 3 postpartum glucose tolerance test for all cows. Symbols represent non-pregnant, non-exercise (○); non-pregnant, exercise (●); pregnant, non-exercise (□); and pregnant, exercise (■). Intravenous glucose bolus infusion occurred on min 10. Minute relative to glucose infusion affected concentrations ($P < 0.001$). Interaction of pregnancy status by minute relative to glucose infusion affected plasma concentrations ($P < 0.01$).

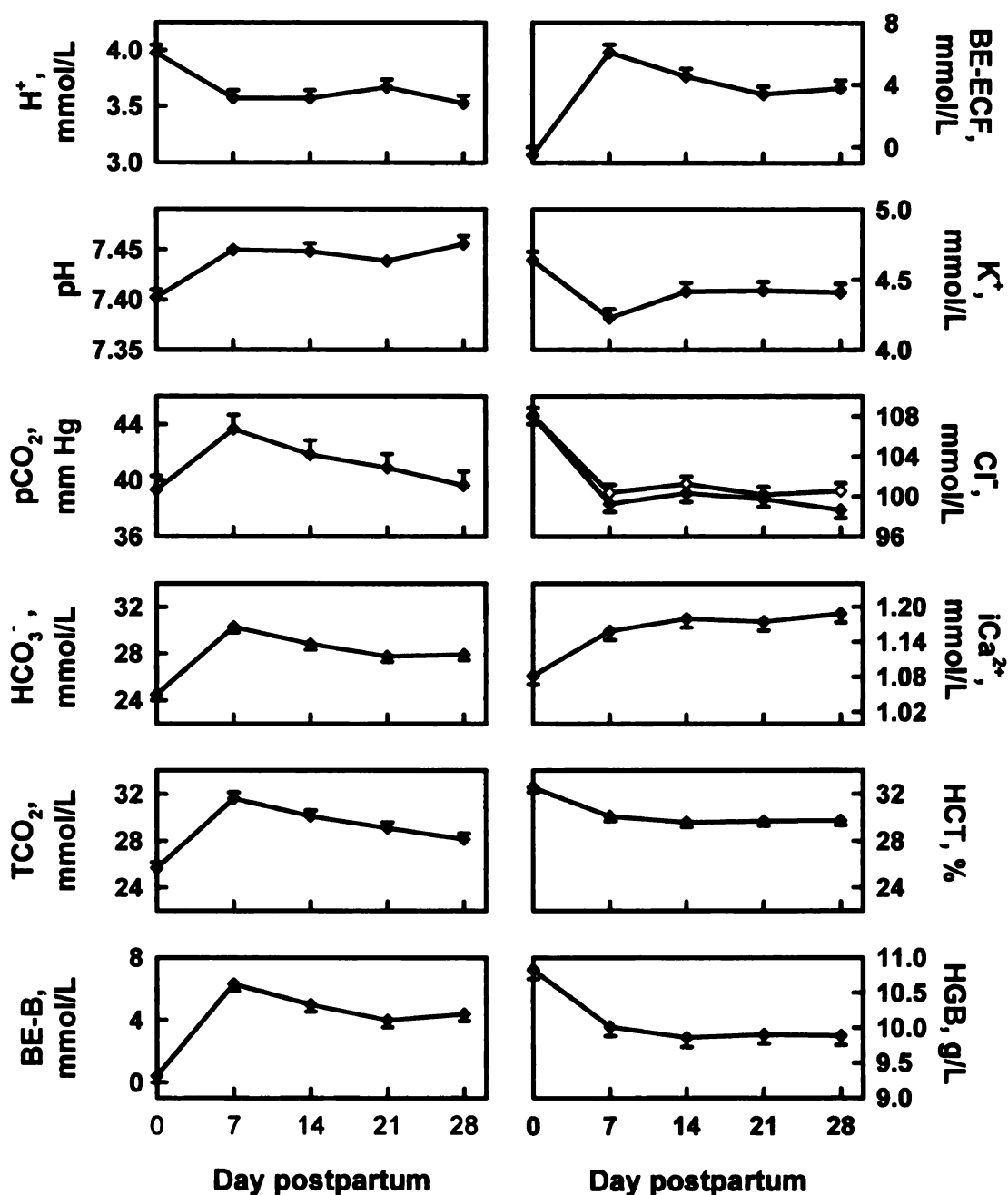


Figure 9.8. Blood hydrogen ion (H^+), pH, partial pressure of carbon dioxide (pCO_2), bicarbonate (HCO_3^-), total carbon dioxide (TCO_2), potassium ion (K^+), chloride (Cl^-), ionized calcium (iCa^{2+}), hematocrit (HCT), and hemoglobin (HGB) during postpartum d 0 to 28 for pregnant cows. Day postpartum affected responses of all variables ($P < 0.001$). Prepartum exercise training treatment also affected Cl^- concentrations: non-exercise (◇) and exercise (◆, $P = 0.013$).

CHAPTER 10

CONCLUSIONS AND IMPLICATIONS

The confinement systems of many of today's dairy farms limit the activity or exercise of cows during the prepartum period. The lack of activity may be reducing the physical fitness of cows, particularly during the prepartum period. Physical fitness may be critical to the cow's ability to respond to the physiological and metabolic stressors of the periparturient period. In a dramatic change over just a few days, cows that have had relatively small nutritional and metabolic demands must meet tremendous demands at parturition and the onset of lactation. For cows to be productive and healthy in the face of physiological and metabolic stressors, many adaptations of metabolism must occur during the periparturient period.

The use of prepartum exercise may be a management tool to stimulate physiological and metabolic processes prior to parturition to ease the transition from the non-lactating to lactating state. Cows may be able to adapt more quickly to metabolic and physiological demands of parturition and early lactation if fitness is improved with prepartum exercise. Furthermore, the ability to recover from the physical exertion of parturition may be related to prepartum activity and improved physical fitness. This may carryover to improved postpartum health during early lactation, greater milk yield, and more efficient reproductive performance.

A system to exercise dairy cows during the periparturient period was developed at Utah State University (Arave et al., 1978; Blake et al., 1982). Responses to prepartum exercise of pregnant dairy cows and heifers at various speeds and durations of time (week) were variable (Anderson et al., 1979; Anderson et al., 1979; Lamb et al., 1981; Lamb et al., 1979). Milk yield and DMI were not consistently different for cows or heifers

exercised prepartum compared with those that were not exercised. Udder edema was reduced and subsequent reproductive performance improved for exercised animals compared with non-exercised animals. These studies did not evaluate the interaction between exercise and pregnancy on physiological fitness responses or adaptation of carbohydrate and lipid metabolism. Prepartum exercise may improve the maintenance of homeostasis of carbohydrate and lipid metabolism during the periparturient period. Changes of management such as the incorporation of an exercise training regimen has the potential to cause physiological adaptations that could improve milk yield, health, and reproduction during the subsequent lactation.

In other species, increasing exercise workloads (acute exercise) caused linear increases of heart rate. Heart rate was used to indicate the amount of exertion a cow experiences during activity and was linearly related to estimates of power in the current research. In the current research, heart rates of dairy cows were greater while standing compared with lying regardless of other activities such as eating, drinking, and ruminating. Additionally, heart rates of cows co-mingling in turn-out lots were not greater than heart rates while standing in tie-stalls. From these results, it became apparent that to increase activity of cows sufficiently to increase their heart rates, cows must be forced to walk. A mechanical walker with circular lane was used to increase activity (exercise training). Heart rates of cows walking at 4.0 km/h were greater than for cows walking at 3.55 km/h, whereas the duration of exercise of 1 or 2 h did not affect heart rate. Furthermore, while cows walked on a treadmill at 5.0 km/h, heart rate increased more with 1.6% increase of slope compared with 0.8 % increase of slope. Speed and incline of exercise work had greater effects on the heart rate than time to end of exercise and activities of eating, ruminating, drinking, standing, lying, or co-mingling.

Using heart rate as the basis to describe the amount of exertion of cows, a exercise training protocol (exercise in the mechanical walker every other day) was completed to affect the physical fitness of dairy cows. An acute exercise test (cardiovascular treadmill test) was used to assess changes of physical fitness of dairy cows after exercise training. After exercise training treatment, physical fitness of non-lactating, non-pregnant dairy cows was improved. The time to end of the treadmill test was greater and heart rate and plasma lactate concentrations were reduced during exercise compared with non-exercised cows. Exercise training increased physical fitness of dairy cows. The main study of the research project was undertaken to assess the potential benefits of improved physical fitness of dairy cows during the periparturient period.

As described in Chapters 5, 6, 7, 8, and 9, an experiment was completed in which pregnant and non-pregnant cows received no exercise or exercise training every other day. Key findings from this research and potential areas of additional research are outlined in Figure 10.1. Prepartum exercise training was required to force cows to exert themselves as indicated by increased heart rates during exercise compared with heart rates during typical routine activities. Additionally, pregnancy increased heart rates compared with no pregnancy, regardless of exercise training treatment (A, Figure 10.1).

Physical fitness improved for NP and P cows that were exercised (B, Figure 10.1). The ability to continue the treadmill test for greater lengths of time, as well as, the reduction of heart rate and plasma lactate concentrations during submaximal workloads were indicative of improved physical fitness after 60 d of exercise training. The reduction of plasma lactate (less metabolic acidosis) at given workloads of the acute exercise test for programmed-exercised cows may influence the maintenance of blood acid-base status, regardless of pregnancy status (C, Figure 10.1).

Adaptations of the respiratory system after exercise training also may contribute to the maintenance of acid-base status to compensate for metabolic acidosis. Increased H^+ concentrations of metabolic acidosis would stimulate an increase of ventilation rates during exercise to compensate for the acid-base disturbance (respiratory alkalosis). Cows in the exercise training treatment had greater pCO_2 and HCO_3^- concentrations during the treadmill test on d 60 than non-exercised cows which is additional evidence that disturbances of acid-base status during acute exercise were reduced after exercise training treatment.

Exercise training affected glucose and lipid metabolism of NP and P cows during acute exercise. Cows that were exercise trained compared with non-exercised cows had lower plasma glucose concentrations (maintained plasma glucose homeostasis) and reduced plasma lactate (less anaerobic metabolism) during acute exercise. Exercise training did not greatly affect circulating plasma concentrations of NEFA and BHBA during acute exercise. Most dramatically, glucagon concentrations were reduced for E cows compared with NE cows. Additionally, pregnancy greatly reduced plasma glucose, insulin, and glucagon concentrations, whereas plasma NEFA and BHBA were increased compared with no pregnancy. Insulin and glucagon concentrations required to maintain glucose homeostasis were further reduced for P, E compared with P, NE cows.

Exercise training treatment did not greatly affect acid-base homeostasis or mineral element concentrations during rest or immediately after exercise training. Furthermore, exercise training treatment did not greatly affect blood mineral element concentrations of Cl^- or iCa^{2+} , but did alter K^+ concentrations during the treadmill test on d 60 of the exercise training treatment (D, Figure 10.1). The concentrations of K^+ at given workloads of acute exercise were lower for exercised cows compared with non-exercised cows, which may be reflective of fluid shifts between extracellular and

intracellular compartments. However, exercise training treatment did not affect the plasma and whole blood volumes regardless of pregnancy status, whereas P cows had greater whole blood volumes than NP cows.

Exercise training treatment reduced the BW gain during the experiment, but did not alter water or feed intake (E, Figure 10.1). Exercise training did not alleviate the reduction of DMI typically measured near parturition. During the prepartum period, energy requirements and energy balance were affected more by the pregnancy status than the exercise training treatment. All cows were in positive energy balance and gained BW; however, exercised cows had slightly lower gains and energy balance than non-exercised cows. Thus, exercise training treatment in this study reduced the partitioning of energy to BW gain for NP and P cows. Furthermore, exercise training did not affect the birth weight of calves.

During rest (the day between exercise days of the exercise training treatment), pregnancy status closer to parturition caused reductions of plasma concentrations of insulin and glucagon and increased concentrations of NEFA, whereas glucose, lactate, and BHBA concentrations were not affected. Exercise training did not greatly affect circulating plasma concentrations of glucose, lactate, NEFA, and BHBA, but reduced insulin and glucagon concentrations in a similar pattern as pregnancy status (F and G, Figure 10.1).

Non-exercised cows required greater insulin and glucagon concentrations to maintain plasma glucose concentrations compared with exercised cows. Prepartum liver glycogen concentrations also were increased for cows that exercise compared with NE cows. During a glucose tolerance test, the increase of insulin concentrations over basal were reduced for exercised cows indicating that the responsiveness to increased glucose concentrations or the tissue sensitivity to increased insulin concentrations was greater. The implications are that cows that exercised compared with non-exercised

cows could have a greater capacity to maintain and control glucose supply during the periparturient period to support subsequent health and lactation.

Exercise training increased prepartum plasma concentrations of NEFA and muscle and liver triglycerides, whereas plasma BHBA concentrations were reduced. The decline of plasma insulin for exercised cows may have increased lipolysis which would explain the increased plasma NEFA concentrations. The decline of BHBA may indicate increased fatty acid oxidation.

During the postpartum period, pregnant dairy cows profited from prepartum exercise compared with those not exercised with increased DMI, reduced BW loss, and greater BCS during the first 4 wk postpartum. Prepartum exercise training did not affect postpartum blood acid-base status, mineral element concentrations, volume of whole blood, or milk yield on composition. Furthermore, the postpartum energy balances calculated based on NRC (2001) were not different between E and NE cows.

Prepartum exercise training affected endocrine control of glucose homeostasis during the first week postpartum. Prepartum exercise of pregnant cows reduced plasma insulin and increased plasma glucagon concentrations during the first week after parturition compared with non-exercised cows. Concentrations of liver glycogen were reduced for P, E cows compared with P, NE cows during the first week postpartum. Dairy cows that were exercised possibly benefitted from increased hepatic glycogenolysis (reduced liver glycogen concentrations) to support the metabolic demands during the first week postpartum until DMI increased. These responses may be involved with improved appetite and reduced body tissue loss of cows exercised during the prepartum period.

Additional research is warranted to fully explore the potential beneficial effects of incorporating prepartum exercise training into conventional dairy management schemes. The variation and status of physical fitness of dairy cows at the beginning of the dry

period in typical management systems such as free stall barns is not known.

Development of a large-scale exercise training protocol and a simpler method to evaluate physical fitness in the field are needed to begin to determine the minimum amount and frequency of exercise training needed to cause physiological adaptations beneficial to the periparturient dairy cow. Based on findings of the current research, heart rate could be used as a non-invasive measure that is closely related to physical fitness.

From the experiments in this dissertation and previous research, it was evident that prepartum exercise training has the potential to reduce the incidence of periparturient metabolic disorders and increase milk production. Pregnant Holstein heifers exercised for 4 wk prepartum (5 d per wk) for 1.6 km at 5.46 km/h had fewer days prepartum of udder edema, improved calving ease, and more rapid release of placental tissues compared with non-exercised heifers (Lamb et al., 1979). Prepartum exercise training of cows resulted in smaller cows (less BW at parturition) with similar DMI producing similar quantities of milk and milk components as non-exercised cows. Prepartum exercise training may have improved appetite and energy balance enough to improve the transition from non-lactating to lactating physiological states. Additional experiments with larger numbers of cows are required to conclusively determine if prepartum exercise training can consistently affect these responses. This information is critical for the adoption of exercise training into current management practices within modern dairy systems.

The potential beneficial effects of greater physical fitness of pregnant cows during the parturition has not been fully examined. During parturition, the amount of exertion that a cow experiences and amount of work done (energy expended) has not been fully described. Cows with a greater physical fitness may have quicker delivery, less dystocia or quicker recovery after parturition. In humans, exercise training during first

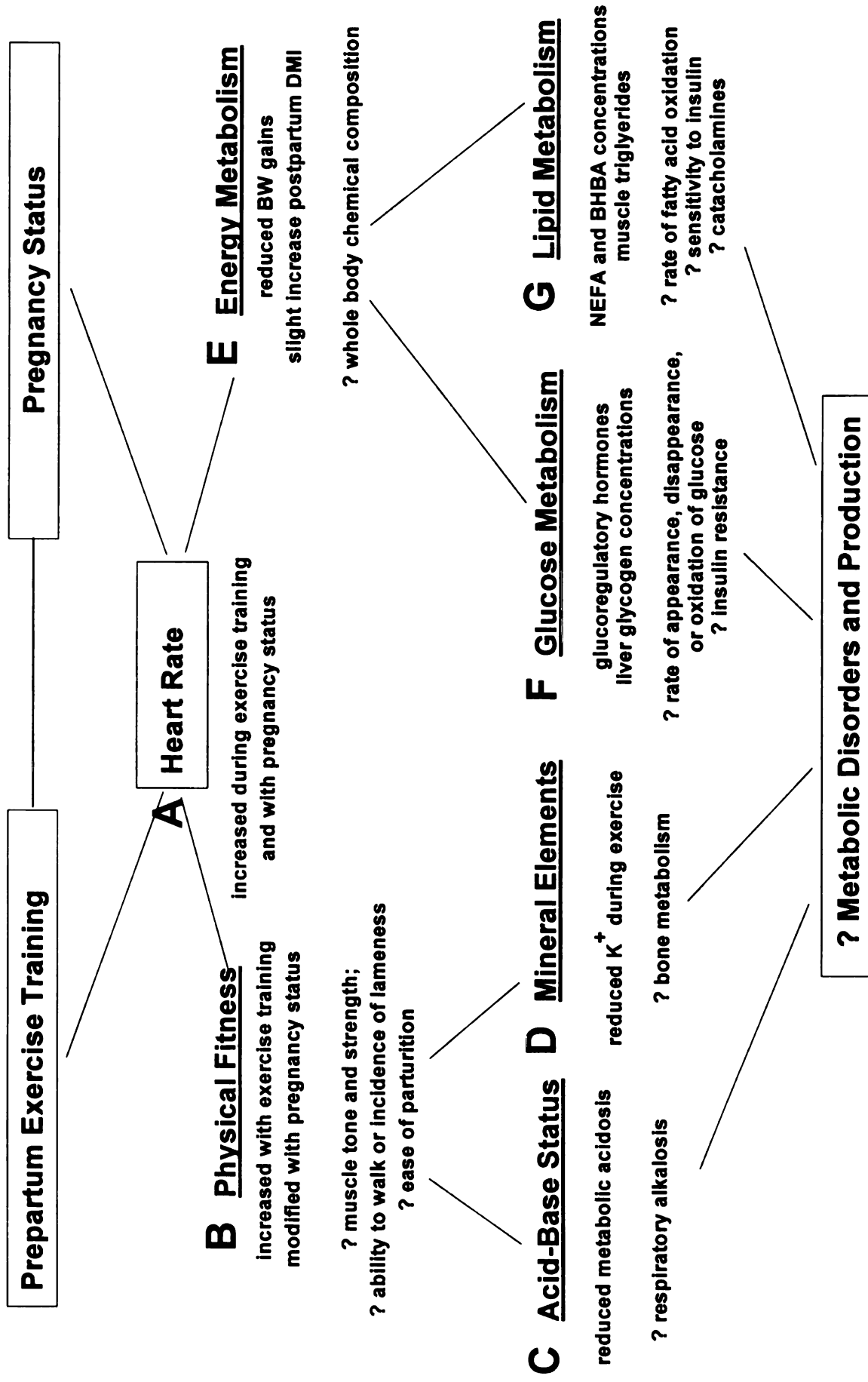
two trimesters reduced the odds for cesarean delivery in nulliparous women compared with sedentary women (Bungum et al., 2000). Women who exercised during pregnancy also had reduced pain perception and stress during labor (less cortisol and greater β -endorphin concentrations) than sedentary women (Varrassi et al., 1989). Pregnant crossbred beef cows that were forced to exercise (walking 3.2 km/d, 90 d prepartum) had a 15% increase in pregnancy rate in the subsequent breeding season compared with non-exercised controls (Bellows et al., 1994).

Physiological adaptations after prepartum exercise training examined in this dissertation were blood acid-base status, and glucose and lipid metabolism. Other body systems which may be also affected after repeated exercise training include muscle strength, bone metabolism, the respiratory and immune systems, stress endocrinology including glucocorticoids and catecholamines, and protein metabolism. Little information to fully elucidate the responses of these physiological systems is available for ruminants.

Finally, prepartum exercise training caused changes in the concentrations of glucoregulatory hormones and liver glycogen concentrations during the periparturient period. However, the potential adaptations of insulin sensitivity or responsiveness, increased hepatic gluconeogenesis and glycogenolysis capacity, hepatic fatty acid metabolism, or adipose fatty acid mobilization have not been fully described. The effects of exercise training on the body composition of pregnant cows has not been fully described. Additional research in ruminants to explore glucose and lipid metabolism at the molecular basis could help explain the role of insulin sensitivity or responsiveness during physiological adaptations associated with periparturient period. Collectively, all of these physiological adaptations may reduce the incidence of metabolic disorders and increase productivity of the periparturient dairy cow.

Figure 10.1. Key findings from the current research and potential areas of additional research are summarized. **A)** Prepartum exercise training was required to force cows to significantly increase activity (increase of heart rate). Additionally, pregnancy increased heart rates. **B)** Exercise training improved the physical fitness of pregnant and non-pregnant cows in that duration of acute exercise was greater, and heart rate and plasma lactate concentrations at given workloads were reduced. However, additional research is needed to fully understand the potential benefits related to muscle strength or ease of parturition. **C)** Exercise training caused a reduction in plasma lactate (tendency for less metabolic acidosis) at given workloads which influenced the maintenance of blood acid-base status, regardless of pregnancy status. Adaptations of the respiratory system after exercise training also may contribute to the maintenance of acid-base status. **D)** Exercise training did not greatly affect mineral element concentrations. The concentrations of K^+ at given workloads were lower for exercised cows compared with non-exercise cows, which may be involved with fluid balance or shifts during the periparturient period. Additionally, bone metabolism or turnover also may be affected with exercise training. **E)** The increased exercise also affected the energy partitioning of cows in that BW gains at similar DMI were less for exercised cows, regardless of pregnancy status. Additionally, postpartum DMI during the first 4 wk were slightly greater for exercised cows compared with non-exercised cows. However, the effects of exercise training on the body composition of pregnant cows has not been described. **F)** Potential adaptations of the control of substrate availability or utilization included reductions of plasma insulin for exercised cows. During acute exercise, plasma glucose and glucagon concentrations were reduced for cows that exercised compared with non-exercised cows. Prepartum liver glycogen concentrations also were increased for cows that exercised, whereas postpartum liver glycogen concentrations were reduced for pregnant, exercised cows. It is not clear if rate of appearance, or disappearance, or oxidation of glucose were affected after exercise training treatment. **G)** Exercise training increased prepartum plasma concentrations of NEFA and muscle and liver triglycerides, whereas plasma BHBA concentrations were reduced. Additional research is warranted to fully understand the contributions of the prepartum changes in glucose and lipid metabolism to the differences detected during postpartum period. Collectively, all of these physiological adaptations may reduce the incidence of metabolic disorders and increase productivity of the periparturient dairy cow.

Figure 10.1.



APPENDICES

A. EQUATIONS USED BY STAT 4 PROFILER

Concentration of bicarbonate [HCO_3^-], mmol/L

$\text{Log}_{10}[\text{HCO}_3^-] = \text{pH} + \text{Log}_{10}(\text{pCO}_2) - 7.604$, where pCO_2 was partial pressure of carbon dioxide.

Total carbon dioxide content (TCO_2), mmol/L

$\text{TCO}_2 = [\text{HCO}_3^-] + \alpha(\text{pCO}_2)$, where α was 0.0307, solubility coefficient of CO_2 in plasma at 37°C .

Hemoglobin (HGB), g/dl

$\text{HGB} = \text{Hematocrit \%} + 3.0$

Base excess of blood (BE-B), mmol/L

$\text{BE-B} = (1 - 0.014[\text{HGB}])([\text{HCO}_3^-] - 24 + (1.43[\text{HGB}] + 7.7)(\text{pH} - 7.4))$

Base excess of extra-cellular fluid (BE-ECF), mmol/L

$\text{BE-ECF} = [\text{HCO}_3^-] - 25 + 16.2(\text{pH} - 7.4)$

B. LACTATE ASSAY

Supplies:

Sigma Chemical Co.

735-10 Lactate Reagent (Lactate Oxidase)
Reconstituted with 9 ml de-ionized water for 1X concentration
826-10 Lactate Standard (40 mg/dl L-Lactate)
735-10 Lactate Standards Set (20, 80, 120 mg/dl L-lactate)

University Stores

150-8644 Co-star 96-well flat bottom tissue culture plates

Bovine Plasma Pools

High and low concentrations of plasma lactate (before and after activity)
Stored -20° C, 50 µl per microcentrifuge tubes

Plasma Samples

Whole blood collected using NaF/KOxalate anticoagulant on ice to prevent red blood cell glycolysis, centrifuged (Allegra 6R, Beckman Instruments, Palo Alto, CA) at 3000 rpm, for 15 min, transfer plasma at least 500 µl per microcentrifuge tubes, store -20° C.

96-well plate layout:

20 µg/ ml	10 µg/ ml	5 µg/ ml	2.5 µg/ ml	30 µg/ ml	15 µg/ ml	7.5 µg/ ml	High Pool	Low Pool	20 CTR	40 CTR	BLK
1	2	3	4	5	6	7	8	9	10	11	12
1	2	3	4	5	6	7	8	9	10	11	12
13	14	15	16	17	18	19	20	21	22	23	24
13	14	15	16	17	18	19	20	21	22	23	24
25	26	27	28	29	30	31	32	33	34	35	36
25	26	27	28	29	30	31	32	33	34	35	36
20 µg/ ml	10 µg/ ml	5 µg/ ml	2.5 µg/ ml	30 µg/ ml	15 µg/ ml	7.5 µg/ ml	High Pool	Low Pool	20 CTR	40 CTR	BLK

µg/ml: diluted standards see below

Pool and CTR: collected pools and purchased controls (CTR, mg/dl)

BLK: blank 50 µl of de-ionized water

1-36: Samples in duplicate

Lactate µg/ml = 10 mg/dl Lactate mg/dl * 0.111= Lactate mmol/L

Instructions for pipetting into plate:

Standards:

Serial dilution (rows A and H of plate) in the plate using deionized water (Final volume = 50 µl/well).

Well 1 (10 µl of 80 mg/dl standard) + 90 µl of deionized water)

Well 5 (10 µl of 120 mg/dl standard) + 90 µl of deionized water)

Plate 50 µl of deionized water in wells 2 through 4 and 6 through 10

To serial dilute:

remove 50 µl, add it to the next well, mix and repeat across plate,
in the wells 5 and 9 discard 50 µl.

Controls:

High pool of plasma: 10 µl/well plus 40 µl of deionized water

Low pool of plasma: 25 µl/well plus 25 µl of deionized water

10 µl of 20 mg/dl standard plus 40 µl of deionized water

10 µl of 40 mg/dl standard plus 40 µl of deionized water

Samples:

10 µl/well plus 40 µl of deionized water

If samples have low concentration of lactate, dilute with 20 µl of sample/well plus
30 µl of deionized water.

Assay Reaction:

Reagent: Add 150 µl/well

Read 15 minutes after reagent addition at 540 nm in microtiter plate reader using lactate assay protocol.

Additional validation of assay: All final volumes should be 50 µl /well

Parallelism: High Pool (50 µl/well)

Low Pool (50 µl/well)

Serially dilute pools with 50 µl of deionized water

Final dilution will contain 100 µl, remove 50 µl

Corrected for dilution factors.

Recovery: High Pool (5 µl + 5 µl of 20 mg/dl standard)

High Pool (5 µl + 5 µl of 40 mg/dl standard)

High Pool (5 µl + 5 µl of 80 mg/dl standard)

Low Pool (10 µl + 5 µl of 20 mg/dl standard)

Low Pool (10 µl + 5 µl of 40 mg/dl standard)

Low Pool (10 µl + 5 µl of 80 mg/dl standard)

Bring total volume to 50 µl with the appropriate amount of deionized water

Actual lactate concentration is calculated from mg of lactate in pool and standard divided by volume.

$$(1 - (\text{Actual} - \text{measured}) / \text{actual}) * 100 = \text{Recovery percent}$$

Parallelism: Coefficient of variation = 3.62 %

Recovery: 106.1% of spike recovered (range 90 to 118%)

C. GLUCOSE ASSAY

Supplies:

Sigma Chemical Co.

510-A Glucose Oxidase, Colorimetric Endpoint Kit

G-3761 Glucose standard solution (500 mg/dl) OR

G-7528 D-glucose anhydrous (3 g/l in deionized water)

University Stores

150-8644 Falcon 96-well flat bottom tissue culture plates

Bovine plasma pools

High and low concentrations of plasma glucose

Stored -20° C, 100 µl per microcentrifuge tubes

Plasma Samples

Whole blood collected using NaF/KOxalate anticoagulant on ice to prevent red blood cell glycolysis, centrifuged (Allegra 6R, Beckman Instruments, Palo Alto, CA) at 3000 rpm, for 15 min, transfer plasma at least 500 µl per microcentrifuge tubes, store -20° C.

Calibration Standard Curve Dilution:

Dilute 300 mg/dl or 500 mg/dl standard with deionized water according to the table, store at 4°C in microcentrifuge tubes, use within 2 weeks:

Standard, mg/dl	µg/ml	µl of 300 mg/dl glucose	µl of deionized water (300 mg/dl)	µl of 500 mg/dl glucose	µl of deionized water (500 mg/dl)
10	100	50	1450	20	980
20	200	100	1400	40	960
30	300	200	1800	60	940
50	500	300	1500	100	900
75	750	250	750	150	850
100	1000	500	1000	200	800
120	1200	400	600	240	760
150	1500	1000	1000	300	700
180	1800	1200	800	360	640

Glucose mmol/L = (mg/dl)/18

Reagents:

Dissolve the contents of 2 capsules of PGO enzyme from kit 510-A in 100 ml of deionized water,

Add 3.2 ml of o-Dianisidine Di-HCl from kit 510-A (re-constituted with 20 ml deionized water),

Store in a foil-wrapped bottle or amber bottle at 4°C.

(20 ml/ plate)

96-Well Plate:

10 mg/ dl	20 mg/ dl	30 mg/ dl	50 mg/ dl	75 mg/ dl	100 mg/ dl	120 mg/ dl	150 mg/ dl	180 mg/ dl	Pool 1	Pool 2	BLK
1	2	3	4	5	6	7	8	9	10	11	12
1	2	3	4	5	6	7	8	9	10	11	12
13	14	15	16	17	18	19	20	21	22	23	24
13	14	15	16	17	18	19	20	21	22	23	24
25	26	27	28	29	30	31	32	33	34	35	36
25	26	27	28	29	30	31	32	33	34	35	36
10 mg/ dl	20 mg/ dl	30 mg/ dl	50 mg/ dl	75 mg/ dl	100 mg/ dl	120 mg/ dl	150 mg/ dl	180 mg/ dl	Pool 1	Pool 2	BLK

mg/dl: diluted standards

Pool: collected high and low plasma pools

BLK: blank 5 µl of deionized water

1-36: Samples in duplicate

Plating:

Vortex standard, controls, and samples prior to pipetting.

5 µl of standard, controls, or samples following plate layout in table.

Add 200 µl of enzyme reagent, (100 µl twice using multiple channel pipette)

Shake plate and incubate 30 min, at 37° C (small table top oven).

Read at 450 nm with the glucose protocol on microtiter plate reader.

Additional validation of assay:

Prepared in microcentrifuge tubes and assay 5 µl/well

Parallelism: Serially dilute plasma pool with deionized water
1:0, 1:1, 1:0.5, 0.5:1, 1:3, 2:1 of µl of plasma:µl of water
Correct for dilution factors.

Recovery: Add the following standards to plasma pool
in a 1:1 ratio of µl of plasma:µl of standard
20, 30, 50, 75 mg/dl standard)
Actual concentration is calculated from mg of glucose in pool and mg of
standard divided by volume.
 $(1 - (\text{Actual} - \text{measured}) / \text{actual}) * 100 = \text{Recovery percent}$

Validation:

Parallelism: Coefficient of variation = 3.98%;

Recovery = 100.4% of spike recovered.

D. LACTATE ANALYZER

Supplies:

YSI Sport Lactate Analyzer 1500 with PC data acquisition
(See manual for additional maintenance and troubleshooting)

YSI

No. 2357 Buffer packet
No. 2327 Lactate standard, 5 mmol/L
No. 2328 Lactate standard, 15 mmol/L
No. 1530 Lactate standard 30 mmol/L
Syringepet

Sigma Chemical co.

735-10 Lactate Standards Set (20, 80, 120 mg/dl L-lactate)

Bovine plasma pools

High and low concentrations of plasma lactate
Stored -20° C, 100 µl per microcentrifuge tubes

Plasma Samples

Whole blood collected using NaF/KOxalate anticoagulant on ice to prevent red blood cell glycolysis, centrifuged (Allegra 6R, Beckman Instruments, Palo Alto, CA) at 3000 rpm, for 15 min, transfer plasma at least 500 µl per microcentrifuge tubes, store -20° C.

Acceptable ranges for pools and standards:

5 mmol/L lactate standard: 44.1 to 45.9 mg/dl; 4.9 to 5.10 mmol/L
15 mmol/L lactate standard: 128.3 to 141.9 mg/dl; 14.25 to 15.75 mmol/L
30 mmol/L lactate standard: 256.5 to 283.5 mg/dl; 28.5 to 31.5 mmol/L
High Pool Plasma: 30.7 to 37.8 mg/dl; 3.41 to 4.2 mmol/L
Low Pool Plasma: 6.4 to 7.9 mg/dl; 0.71 to 0.88 mmol/L
Sigma standards: 120 mg/dl: 12.65 to 13.99 mmol/L
 80 mg/dl: 7.44 to 9.32 mmol/L
 20 mg/dl: 2.11 to 2.33 mmol/L

Lactate µg/ml = 10 mg/dl Lactate mg/dl * 0.111= Lactate mmol/L

Solutions:

Reference solution: 1 buffer packet and 500 ml pure water with 4500 µl of 30 mmol/L lactate standard

Buffer solution: 1 buffer packet and 500 ml pure water

Waste solution: Empty containers as needed.

Analysis:

Boot IBM PC, open Procomm Software, Turn on analyzer

NOTE: Rinse syringepet with deionized water three-four times between different solutions and samples. Not required between duplicates. Make sure that the chamber of syringepet does not contain air bubbles; that the syringepet is delivering 25 µl of volume. See manual if problems or call tech support. Clean occasionally with detergent and fine pipe cleaner.

To calibrate analyzer:

1. To run (press 0 followed by enter)
2. To calibrate (press 1 followed by enter)
3. Inject 25µl of 5 mmol/L lactate standard using syringepet
4. Check calibration with another injection of 5 mmol/L lactate
5. If not within acceptable ranges, re-calibrate

To check linearity:

1. Sample (press 1 followed by enter)
2. Sample ID number (press 0, 0, 0)
3. Inject 25 µl of 30 mmol/L lactate standard using syringepet
4. Make sure within acceptable ranges
5. Press menu, to return to sampling screen

Note: Each analysis takes 96 seconds, check linearity after 36 analysis. Re-calibrate and check linearity after 72 analysis approximately every 2 hours.

To Sample:

1. Open file to capture data on PC (Alt - F1)
2. Note filename on worksheet
3. Analyze sample (press 1 followed by enter)
4. Enter ID number - 001, etc.
5. Inject 25 µl of sample using syringepet
6. Record concentration of lactate displayed with correct sample identification and number; Press menu.
7. Once all samples are analyzed, close data file on PC (Alt - F1)

Additional validation of assay: Prepared in microcentrifuge tubes and assayed

Parallelism: Serially dilute plasma pool with buffer solution
1:0, 1:1, 1:0.5, 1:2, 1:3, 1:4, 2:1 of µl of plasma:µl of buffer
Correct for dilution factors.

Recovery: Add the following standards to plasma pool
in a 1:1 ratio of µl of plasma:µl of standards 20, 80, 120 mg/dl
Actual concentration is calculated from mg of lactate in pool and mg of standard divided by volume.
$$(1 - (\text{Actual} - \text{measured}) / \text{actual}) * 100 = \text{Recovery percent}$$

Validation: Parallelism: Coefficient of variation = 4.98%;
Recovery = 97 % of spike recovered.

E. NON-ESTERIFIED FATTY ACID ASSAY

Supplies:

Wako Chemicals USA, Inc.
No. 994-75409 NEFA-C Kit

Fisher Scientific
No. 12-565-226 Nunc® 96-well flat bottom tissue culture plates

University stores
155 85120 NaPO_4 monobasic
155 84360 NaPO_4 dibasic

Bovine plasma pools
High pools of plasma NEFA (from early lactating cows)
Stored -20°C , 100 μl per microcentrifuge tubes

Plasma Samples
Whole blood collected using NaF/KOxalate anticoagulant on ice to prevent red blood cell glycolysis, centrifuged (Allegra 6R, Beckman Instruments, Palo Alto, CA) at 3000 rpm, for 15 min, transfer plasma at least 500 μl per microcentrifuge tubes, store -20°C .

Solutions:

Phosphate buffer 50 mM, pH 6.9: 6.9 g of NaH_2PO_4 monobasic
7.1 g of Na_2HPO_4 dibasic
pH with 1 M Na_2HPO_4 dibasic
1 L deionized water

Reagents, Store in amber bottles, 4°C :

	Color A	Color B
Reagent *	powder A	powder B
Solvent*	10 ml of A	20 ml of B
Phosphate buffer	13.3 ml	33.3 ml

*included in kit

Calibration Standard Curve Dilution:

Dilute standard solution (1 meq/L) in kit with deionized water, (1mmol/L = 1 meq/L)
store at 4°C in microcentrifuge tubes, use within 2 weeks:

Standard, mg/dl	μl of 1000 $\mu\text{meq/L}$ NEFA	μl of deionized water
1000	1000	0
750	750	250
500	500	500
250	250	750
100	100	900
50	50	950
0	0	1000

Plating:

Vortex standard, controls, and samples prior to pipetting.

20 µl of standard, controls, or samples following plate layout on attached sheet

Add 75 µl of color A (using multiple channel pipette)

Shake plate on reader and incubate 30 min, at room temperature

Add 150 µl of color B (75 µl twice using multiple channel pipette)

Shake plate on reader and incubate for 30 min at room temperature.

Read plate at 550 nm using the NEFA protocol

96-Well Plate:

1000 µeq/L	750	500	250	100	50	0	Pool 1	1	2	3	4
5	6	7	8	9	10	11	12	13	14	15	16
5	6	7	8	9	10	11	12	13	14	15	16
17	18	19	20	21	22	23	24	25	26	27	28
17	18	19	20	21	22	23	24	25	26	27	28
29	30	31	32	33	34	35	36	37	38	39	40
29	30	31	32	33	34	35	36	37	38	39	40
1000 µeq/L	750	500	250	100	50	0	Pool 1	1	2	3	4

µeq/L: diluted standards

Additional validation of assay: Prepared in microcentrifuge tubes and assay 20 µl/well

Parallelism: Serially dilute plasma pool with deionized water
1:0, 1:1, 1:0.5, 1:2, 1:3, and 1:4 of µl of plasma:µl of water
Correct for dilution factors.

Recovery: Add the following standards to plasma pool
in a 1:1 ratio of µl of plasma:µl of standard
(250, 100, 50, µeq/L standard)
Actual concentration is calculated from mg of NEFA in pool and standard
divided by volume.
 $(1 - (\text{Actual} - \text{measured}) / \text{actual}) * 100 = \text{Recovery percent}$

Validation: Parallelism: Coefficient of variation = 7.5 %;
Recovery = 100.3 % of spike recovered.

For samples with less than 50 µeq/L of NEFA:

Low - Calibration Standard Curve Dilution:

Dilute standard solution (1 meq/L), store at 4°C in microcentrifuge tubes, use within 2 weeks:

Standard, mg/dl	µl of 1000 µmeq/L NEFA	µl of deionized water
300	300	700
250	250	750
100	100	900
25	25	975
10	10	990
5	5	995
0	0	1000

Plating:

Vortex standard, controls, and samples prior to pipetting.

20 µl of standard, controls, and samples following plate layout on attached sheet

Add 75 µl of color A (using multiple channel pipette)

Shake plate on reader and incubate 30 min, at room temperature

Add 150 µl of color B (75 µl twice using multiple channel pipette)

Shake plate on reader and incubate for 30 min at room temperature.

Read plate at 550 nm using the NEFA protocol

96-Well Plate:

300 µeq/L	250	100	25	10	5	0	Pool 1	1	2	3	4
5	6	7	8	9	10	11	12	13	14	15	16
5	6	7	8	9	10	11	12	13	14	15	16
17	18	19	20	21	22	23	24	25	26	27	28
17	18	19	20	21	22	23	24	25	26	27	28
29	30	31	32	33	34	35	36	37	38	39	40
29	30	31	32	33	34	35	36	37	38	39	40
300 µeq/L	250	100	25	10	5	0	Pool 1	1	2	3	4

µeq/L: diluted standards

Validation with low curve:

Parallelism: Coefficient of variation = 2.5 %;

Recovery = 102.0 % of spike recovered.

F. β -HYDROXY BUTYRATE ASSAY

Supplies:

Sigma Chemical Co.

310-A β -hydroxybutyrate

310-11 β -OHbutyrate calibrators solution

University stores

150 8644 96-well flat bottom tissue culture plates

Bovine plasma pools

High pools of plasma BHBA(from early lactating cows)

Stored -20°C , 100 μl per microcentrifuge tubes

Plasma Samples

Whole blood collected using NaF/KOxalate anticoagulant on ice to prevent red blood cell glycolysis, centrifuged (Allegra 6R, Beckman Instruments, Palo Alto, CA) at 3000 rpm, for 15 min, transfer plasma least 500 μl per microcentrifuge tubes, store -20°C , at

Calibration Standard Dilution:

Dilute standard calibrator solutions, store at 4°C in microcentrifuge tubes, use within 2 weeks:

Standard, mg/dl	μl of 75 mg/dl	μl of 25mg/dl	μl of 10 mg/dl	μl of 0.9%
30	400	0	0	600
25	333	0	0	667
15	0	600	0	400
10	0	400	0	600
5	0	0	500	500
2.5	0	0	250	750
0	0	0	0	1000

Reagent:

Dissolve powder in reagent bottle of kit with 10.2 ml (3.4 ml, three times) of deionized water, use same day. Place in oven at 37°C for 10 minutes prior to use.

96-Well Plate:

30 mg/dl	25 mg/dl	15 mg/dl	10 mg/dl	5 mg/dl	2.5 mg/dl	0 mg/dl	Pool	1	2	3	4
5	6	7	8	9	10	11	12	13	1	15	16
5	6	7	8	9	10	11	12	13	1	15	16
17	18	19	20	21	22	23	24	25	2	27	28
17	18	19	20	21	22	23	24	25	2	27	28
29	30	31	32	33	34	35	36	37	3	39	40
29	30	31	32	33	34	35	36	37	3	39	40
30 mg/dl	25 mg/dl	15 mg/dl	10 mg/dl	5 mg/dl	2.5 mg/dl	0	Pool	1	2	3	4

Plating: NOTE: ultraviolet reaction, no visible color change
Vortex standard, controls, and samples prior to pipetting.
10 µl of standard and 20 µl of controls or samples
Add 220 µl of reagent, (110 µl twice using multiple channel pipette)
Shake plate on reader and incubate 3-4 min, at (small table top oven).
Pre-read at 340 nm with the β-OHButyrate protocol on microtiter plate reader
Save plate pre-read data to hard drive in protocol folder
Dilute 2 ml of enzyme solution (entire bottle) with 2.4 ml of deionized water,
Add enzyme to plate (10 µl/ well using multiple channel pipette)
Shake plate on reader and incubate for 1 h at 37° C in oven.
Open plate file in protocol folder on computer and read at 340 nm

Additional validation of assay: Prepared in microcentrifuge tubes and assay 20 µl/well

Parallelism: Serially dilute plasma pool with deionized water
1:0, 1:0.5, 1:1, 1:1.5, and 1:2 of µl of plasma:µl of water
Correct for dilution factors.

Recovery: Add the following standards to plasma pool
in a 1:1 ratio of µl of plasma:µl of standard
(25, 10, 5 mg/dl standard)
Actual concentration is calculated from mg of BHBA in pool and mg of
BHBA standard divided by volume.
 $(1 - (\text{Actual} - \text{measured}) / \text{actual}) * 100 = \text{Recovery percent}$

Validation: Parallelism: Coefficient of variation = 8.5 %;
Recovery = 105.7 % of spike recovered.

BHBA mmol/L = mg/dl * 0.096

G. INSULIN ASSAY

Supplies:

Diagnostic Products Corporation
TKINX (1000 tubes), TKIN5 (500), TKIN2 (200) Insulin Coat-count kit
Store kit at 2-8 °C

Bovine plasma pools
High pools of plasma insulin from cows after feeding
Stored -20° C, 100 µl per microcentrifuge tubes

Plasma Samples
Whole blood collected using Na-heparin anticoagulant on ice, centrifuged
(Allegra 6R, Beckman Instruments, Palo Alto, CA) at 3000 rpm, for 15 min,
transfer plasma at least 500 µl per microcentrifuge tubes, store -20° C.

12 x 75 polypropylene tubes

Calibration curve: Reconstitute each calibrator 30 minute prior to use, can be stored at 4° C for 1 week, otherwise store -20° C. Stable for 30 day at -20° C; avoid freezing and thawing cycles. Lot specific µIU/ml with each kit:

Concentration (µIU/ml)	Dilution (calibrator:deionized water)
0	1 of A:6 ml deionized water
5	1 of B:3 ml deionized water
15	1 of C:3 ml deionized water
25	300 µl of reconstituted G:3900 µl deionized water
50	1 of D:3 ml deionized water
100	1 of E:3 ml deionized water
200	1 of F:3 ml deionized water
350 (not used in assay)	1 of G:3 ml deionized water

Assay Procedure:

Label tubes: 12 x 75 tubes with numbers 1, 2 and coat-a-count tubes with 3-288
Pipette 200 µl of calibrator, pool, or sample to the bottom of tube (3 - 288)
Pipette 1 ml of ¹²⁵I-Insulin to every tube (1-288), vortex

NOTE: no more than 40 minute should elapse from the start of pipetting to finish with the addition of label

Cap tubes 1 and 2; cover tubes with plastic wrap or foil; and incubate 18-24 h at room temperature

After incubation, place tubes in foam racks, and decant tubes thoroughly (except tubes 1 and 2) by inverting, drain 2 to 3 min, shake and tap remaining liquid out on absorbent paper.
Count 1 minute in gamma counter.

Utilize assay software program to calculate standard curve and sample concentrations.

Additional validation of assay:

Parallelism: dilute pool with 0 calibrator
1:0 (100 µl: 0 µl), 1:1 (100 µl:100 µl), 1:1.5 (80 µl:120 µl), µl of
plasma:µl of calibrator
Correct for dilution factors.

Recovery: To plasma pool add 350 µIU/ml calibrator
150 µl of pool + 50 µl of calibrator
Actual concentration is calculated from µIU of insulin in pool and µIU of
standard divided by volume.
 $(1 - (\text{Actual} - \text{measured}) / \text{actual}) * 100 = \text{Recovery percent}$

Validation: Parallelism: Coefficient of variation = 8.0 %;
Recovery = 110 % of spike recovered.

Insulin µIU/ml * 7.217 = Insulin pmol/L

H. GLUCAGON ASSAY

Supplies:

Linco Research Inc.
GL-32K (250 tubes)
Store kit at 2-8 °C

Bovine plasma pools
pools of plasma from cows after feeding
Stored -20° C, 100 µl per microcentrifuge tubes

Plasma Samples

Whole blood collected using Na-heparin anticoagulant on ice with aprotinin 500 KIU/ml of whole blood, centrifuged (Allegra 6R, Beckman Instruments, Palo Alto, CA) at 3000 rpm, for 15 min, transfer plasma at least 500 µl per microcentrifuge tubes, store -20° C.

12 x 75 borosilicate glass tubes

Calibration curve: (in kit, ready to use)

20, 50, 100, 200, and 400 pg/ml

Assay Procedure:

Label tubes: 12 x 75 tubes with numbers 1-250

Pipette 300 µl of assay buffer (in kit, ready to use) to tubes 3 and 4 (non-specific binding); 200 µl of assay buffer to tubes 5 and 6 (total binding); and 100 µl of assay buffer to tubes 7 through 250.

Pipette 100 µl of standards, pools, or samples to tubes 7 through 250.

Pipette 100 µl of glucagon antibody (in kit, ready to use) to tubes 5 through 250.

Vortex, cover, and incubate 20 to 24 h, 4° C.

After incubation, reconstitute ¹²⁵I-glucagon tracer with label hydrating buffer for 30 min at room temperature. Add 100 µl of tracer to all tubes (1 to 250).

Vortex, cover, and incubate 22 to 24 h, 4° C.

After incubation, add 1 ml of cold precipitating reagent to tubes 3 to 250.

Cap tubes 1 and 2, vortex, cover, and incubate 20 min, 4° C.

After incubation, centrifuge for 20 min, 4° C at 3000 g, place tubes in foam racks, and decant tubes thoroughly (except tubes 1 and 2) by inverting, drain 2 to 3 min, shake and carefully tap remaining liquid out on absorbent paper.

Count 1 minute in gamma counter.

Utilize assay software program to calculate standard curve and sample concentrations.

Additional validation of assay:

- Parallelism:** dilute pool with assay buffer
1:0 (100 µl: 0 µl), 1:2 (50 µl:50 µl), 1:3 (25 µl:75 µl), 3:1 (75 µl:
25 µl) µl of plasma:µl of assay buffer
Correct for dilution factors.
- Recovery:** To plasma pool add 20, 50, and 100 pg/ml calibrators
50 µl of pool + 50 µl of calibrator
Actual concentration is calculated from pg of glucagon in pool and pg of
standard divided by volume.
 $(1 - (\text{Actual} - \text{measured}) / \text{actual}) * 100 = \text{Recovery percent}$
- Validation:** Parallelism: Coefficient of variation = 7.2 %;
Recovery = 93.7 % of spike recovered.

glucagon pg/ml * 0.287 = glucagon pmol/L

I. JUGULAR CATHETERIZATION

Supplies:

Item	Supplier	Product number
12 gauge x 2 inch hypodermic	Vetpo Distributors	53112
Instrument Tray with lid	Vetpo Distributors	78468
Vetcassette Poly 2 (Vetafil suture)	Vetpo Distributors	57064
Isopropyl alcohol	University Stores	15549400
Non-sterile gauze 4 x 4 inch	University Stores	15372340
Tygon Tubing (0.275 ID inch)	University Stores	14588160
Elastikon	University Stores	15311700
Masking Tape	University Stores	13093020
10 ml luer-lok syringes	University Stores	15373160
Sterile saline	University Stores	15581330
18 ga x 1.5 inch needles	University Stores	15351300
Betadine Scrub	University Stores	15302000
Porous Tape	University Stores	1537370
Sterile Gloves, Size 8	University Stores	15325060
1 ml slip-lok syringes	University Stores	15373100
Cidex plus	University Stores	15310030
Na-Heparin Injectable	Bergun-Brunswig Medical	536375
Tag Cement	Nasco	C12168N
Tygon Tubing (0.04 ID; 0.07 OD in inches)	Cole-Parmer Instrument	P-06418-04
Pipe cleaners	-	-
scissors	-	-

Preparations:

70% isopropyl alcohol (700 ml isopropyl alcohol and 300 ml deionized water)

Gauze soaked betadine scrub and 70% isopropyl alcohol

(Store in 1 L screw-cap containers)

1cc syringe caps for catheter

(Cut 1 ml syringes at the 0.1 mark through tubing and plunger with a hot knife;
store in Cidex plus to sterilize)

Catheter Collars

(Cut tygon tubing in approximately 2 inch lengths; store in Cidex plus to sterilize)

Tubing adapters for catheter

(Remove point from 18 gauge needles and file sharp edges down; store in Cidex
plus to sterilize)

Elastikon catheter pouches

(Cut 6-8 inch lengths of elastikon, add 2-inch wide masking tape to center of sticky side of elastikon, place 0.25-inch wide velcro strips around outer edge, placing the loop velcro on one side and napped velcro on the other side with staples. When the lengths are folded in half, the velcro should attach and a pouch formed.)

Catheters

(Cut 50 cm lengths of narrow tygon tubing, mark every 10 cm, and sterilize by gas at vet school or by autoclaving)

Heparin Saline

(Using sterile technique, add Na-heparin to saline bag through injection site of bag for 100 IU/ml)

Procedure:

Restrain cow's head in lock-up or handling shoot, tie nose to the side having a bend in cow's neck

Clip hair above the jugular vein

Scrub site with betadine scrub soaked gauze, rinse with 70% alcohol gauze, repeat 2 more times

Pinch off blood flow in vein

Insert 12 ga needle into jugular vein following path of vein and at least a 45° angle to skin, and centered over top of vein with beveled end pointing towards the floor, once in the vein release blood flow

Insert 20 cm of catheter into vein through the 12 ga needle

Remove 12 ga needle, place tubing collar over catheter, insert tubing adapter into catheter, push collar over tubing adapter collar

Check catheter for blood flow and flush catheter with approximately 10 ml Heparin saline

Cap catheter with 1 ml cap

Suture in place:

Using an approximately 4-inch strip of porous tape, make tape tabs both sides of catheter near its entrance into the skin, use a 18 ga needle to pierce tape and skin slightly above the catheter entrance on either side of catheter tubing. Thread a 8-inch length of suture material through 18 ga needle, starting at the beveled end. Remove 18 ga needle holding the suture in place. Tie surgical knot, avoid to pinching catheter by tying too tight.

Cut a hole in the center of one side of the elastikon pouch. Thread the catheter through this hole, elastikon side towards cow. Attach to cow with tag cement and coil catheter length inside pouch, closing pouch with velcro.

Release cow's nose and secure pouch with elastikon wrapped around cow's neck.

Clean instruments with alcohol and betadine scrub using pipe cleaner.

J. LIVER AND MUSCLE BIOPSY

Supplies:

Item	Supplier	Product number
2% Lidocaine- HCl, 100 ml	Vetpo Distributors	14150
Aquacillin, 250 ml	Vetpo Distributors	01302
Instrument Tray with lid	Vetpo Distributors	78468
Betadine Solution	University Stores	15302020
Isopropyl alcohol	University Stores	15549400
Non-sterile gauze 4 x 4 inch	University Stores	15372340
10 ml luer-lok syringes	University Stores	15373160
Sterile Gloves, Size 8	University Stores	15325060
18 ga x 1.5 inch needles	University Stores	15351300
Betadine Scrub	University Stores	15302000
Cidex plus	University Stores	15310030
surgical blade	University Stores	14500480
Whatman filter paper	University Stores	14527360
Spatula	University Stores	14551640
cryogenic vials	University Stores	15098920
Muscle biopsy	Popper & Sons	8067
Liver biopsy	Cook Vet Products	V-QC-14-15.0 20T
liquid nitrogen	Biochemistry stores	-

Preparations:

70% isopropyl alcohol (700 ml isopropyl alcohol and 300 ml deionized water)

Gauze soaked betadine scrub, betadine solution, and 70% isopropyl alcohol
(Store in 1 L screw-cap containers)

Liver biopsy instruments must be gas sterilized or chemical sterilized following Cidex instructions

Muscle biopsy instruments must be autoclaved or chemical sterilized

Sterilize spatula and filter paper to avoid contamination of biopsy instrument during multiple sampling

Pre-weigh cryogenic vials, writing weight on vial

Procedures of Liver Biopsy:

Restrain cow's head in lock-up or handling shoot with access to the right side of cow

On the right side of the cow, clip hair in 8 x 8 square between the 10th and 11th rib, horizontally located on the diagonal from the hooks to the point of the elbow

Inject 10 ml lidocaine with 18 ga needle and syringe between the 10th and 11th rib, on the diagonal from the hook to the point of the elbow (7 ml in the intercostal muscles and 3 ml closer to the skin surface)

Scrub site with betadine scrub soaked gauze, rinse with 70% alcohol gauze, repeat 2 more times, finally leave a betadine solution on biopsy site

With sterile surgical techniques, do a 0.25 inch skin incision at the injection site. Slide the liver biopsy instrument (set to fire) in approximately 4 inches, perpendicular to the skin surface and parallel to the floor. The biopsy instrument should move back and forth with the cow's breathing pattern. Push center of biopsy instrument out, click or trigger spring action to cut a biopsy. Remove from cow and pull back center until clicks, push forward inner core where the tissue will be located. Slide tissue onto sterile filter paper, repeating steps until enough tissue is collected. Place in cryogenic tube with spatula and flash freeze in liquid nitrogen. Using sterile gauze remove any blood on outside of cow and wash with betadine solution. Inject 30 ml of aquacillin (penicillin), only given once for both liver and muscle biopsy.

Procedure for Muscle Biopsy:

Restrain cow's head in lock-up or handling shoot with access to the back side of cow
On the thigh of the cow, clip hair in 8 x 8 square between the pin and hock
Inject 10 ml lidocaine with 18 ga needle and syringe in the center muscle of the thigh (semitendinous muscle) (7 ml in the muscles and 3 ml closer to the skin surface)

Scrub site with betadine scrub soaked gauze, rinse with 70% alcohol gauze, repeat 2 more times, finally leave a betadine solution on biopsy site

With sterile surgical techniques, do a 0.25 inch skin incision at the injection site. Insert muscle biopsy instrument, will take some force (blunt cutting edge). Using hand on the outside of cow, push muscle into center hole of instrument, while pushing in the opposite direction with the biopsy instrument. Push the center cannula in taking a cut of the muscle tissue, repeat until enough tissue is collected. Remove middle portion of instrument and use stylet to retrieve muscle from cannula. Place in cryogenic vials and flash freeze in liquid nitrogen. Using sterile gauze remove any blood on outside of cow and wash with betadine solution. Inject 30 ml of aquacillin (penicillin), only given once for both liver and muscle biopsy.

Clean instruments with alcohol and betadine scrub using pipe cleaner.

K. BLOOD VOLUME WITH EVANS DYE ASSAY

Supplies:

Item	Supplier	Product number
Evans Blue	Sigma Chemical (100g)	E2129
Heparin Salt (181 U/mg)	Sigma Chemical	H-9399
Millipore Stericap Filters	Fisher(24/pk)	SVGPV1024
Heparin 5000 units/ml 10ml vial	Bergen-Brunswig Medical	536375
12 x 75 test tubes	University Stores	15093330
60 ml syringe	University Stores	15373220
10 ml luer-lok syringes	University Stores	15373160
18 ga x 1.5 inch needles	University Stores	15351300
Sterile saline	University Stores	15371860
microcentrifuge tubes	University Stores	1508970
NaCl	University Stores	15581330
microtiter plates	University Stores	15086440

Preparation of dye solution:

Evans blue (1% w/v) in physiological saline:

Dissolve 9 g NaCl in 900 ml purified water

Add 10 g Evans Blue

Bring volume to 1000 ml with purified water

In a sterile laminar flow hood (culture room) and using sterile technique, sterile filter (0.22 micron filter) solution using a vacuum pump into autoclaved glass bottle with injectable cap (1 L bottle).

Heparin-Saline (100 U/ml)

Inject 5 ml of heparin (5000 U/ml) into 0.9% saline 250 ml bag using 10 ml syringe and 18 gauge needle

Procedure:

Indwelling jugular catheters inserted in the left and right jugular vein of the cow towards the heart. One side will be used to infuse the dye solution and the other to collect samples.

Evans blue solution: 50 ml per cow (500 mg of dye per cow) infused with 60 ml syringe. Pre-weigh syringes, weigh syringes with solution, weigh syringes with residual volume to determine actual volume inject into cow.

Infusion of dye at time 0, blood (5 ml per sample) sampled at -5, 0, 2.5, 5, 10, 20, 30, 40, 60, and 80 min relative to infusion.

Collection of blood: Remove 2 ml of saline and blood from catheter with a 10 ml syringe. Use a sampling, clean syringe collect 5 ml of blood. Flush catheter with heparin-saline, re-cap catheter until next sampling

Processing blood sample: Take the 5 ml blood sample in syringe and dispense into 12 x 75 test tube place on ice. Samples collected prior to dye infusion will be analyzed immediately with NOVA Stat Profiler (blood-gases analyzer) to determine hematocrit. All samples will be centrifuged at 3000 rpm for 15 minute. Use a transfer pipette to collect plasma (clear portion of sample) after centrifugation. Be sure to avoid collecting any precipitated cells at the bottom of the vacutainer. To store plasma, split the volume into two pre-labeled microcentrifuge tubes. Store at -20°C, until analysis.

Assay:

Control pool of samples collected

Standard Evans Dye solution (10000 ng/μl)

10g of Evans dye and 9 g NaCl in 1 L of deionized water, sterile filtered

Calibration curve:

Dilution of standard dye solution,

ID	Standard, μg/ml	μl of standard	μl of deionized water
A	125	125	9875
B	100	100	9900
C	62.5	5000 of A	5000
D	50	5000 of B	5000
E	31.25	5000 of C	5000
F	25	5000 of D	5000
G	15.625	5000 of E	5000
H	12.50	5000 of F	5000
I	10	10	9990

96-Well Plate:

A	B	C	D	E	F	G	H	I	Pool	blank	blank
A	B	C	D	E	F	G	H	I	Pool	blank	blank
1	2	3	4	5	6	7	8	9	10	11	12
1	2	3	4	5	6	7	8	9	10	11	12
13	14	15	16	17	18	19	20	21	22	23	24
13	14	15	16	17	18	19	20	21	22	23	24
25	26	27	28	29	30	31	32	33	34	blank	blank
25	26	27	28	29	30	31	32	33	34	blank	blank

Plating:

Vortex standard, controls, and samples prior to pipetting.

Time 0 samples from each cow are run as the blanks.

Pipette 100 μl of sample or standard into each well.

Read at 625, L1 and 740, L2 nm with the Evans dye protocol on microtiter plate reader

Program will correct data with the following equation ($L1 - 1.794 \times L2$)

Additional validation of assay: Prepared in microcentrifuge tubes and assay 20 µl/well

Parallelism: Serially dilute plasma pool with deionized water
1:0, 19:1, 9:1, 5.67:1, and 4:1 of µl of plasma:µl of water
Correct for dilution factors.

Recovery: Add the following standards to plasma pool
in a 9:1 ratio of µl of plasma:µl of standard
(12500 ng/well standard)
Actual concentration is calculated from µg from plasma and µg from
standard divided by total volume.
 $(1 - (\text{Actual} - \text{measured}) / \text{actual}) * 100 = \text{Recovery percent}$

Validation: Parallelism: Coefficient of variation = 2.0%;
Recovery = 96.5 % of spike recovered.

Calculations of plasma volume (PV):

PV= amount of dye injected/ estimated plasma concentration of dye at time 0.

To estimated plasma concentration at time 0 :

Least squares linear regression of ln(plasma dye concentration, Y) versus time, X

Where $b = \ln(\text{plasma dye concentration at time 0})$

$$\ln(Y) = m(X) + b$$

Use inverse of ln to determine plasma dye concentration at time 0 to calculate PV.

Calculations of blood volume from hematocrit and PV:

Total blood volume = PV + cell volume

Cell volume = (hematocrit * PV)/(1-hematocrit)

References:

Delaquis and Block (1995) J. Dairy Sci. 78:2259-2284

McKeever et al. (1988) Equine Vet. Sci. 8:208-212

Holmes and Weiskopf (1987) Am. J. Physiol. 252:R1003-R1008

Hamiltion (1958) J. Lab. Clin. Med. 52:762-767

Campbell et al. (1958) J. Lab. Clin. Med. 52:768-777.

L. SUCCINATE DEHYDROGENASE AND PROTEIN TISSUE ASSAYS

Tissue Homogenization:

Samples: Biopsies collected as described previously and stored -80 °C.
Control tissues collected during slaughter, frozen in liquid N₂, and stored -80 °C.

Buffer:

20 mM Na-Phosphate, pH 7.4
1.08 g of NaH₂PO₄ monobasic
1.73 g of Na₂HPO₄ dibasic
1000 ml of de-ionized water
pH with 1 M Na₂HPO₄ dibasic (141.96 g in 1 L)
0.5 mM EDTA (186 mg in 1 L buffer)

Tare weigh 17 x 100 tube, add sample, determine weight of sample
Homogenizing dilutions: 20 µl per mg of muscle and 40 µl per mg of liver

Add half of the volume of buffer to sample tubes

Grind tissue with polytron approximately 10-15 sec.

Rinse polytron with the remaining portion of buffer, collecting it in sample tube

Rinse polytron with 70% ethanol between samples and dry.

Assay Supplies:

Na-Succinate, Formula weight: 270.1, C₄H₄O₄Na₂·6H₂O Sigma S 2378
Substrate for SDH reaction

INT, 2-(4-iodophenyl)-3-(4-nitophenyl)-5-phenyltetrazolium chloride Sigma I 8377
Formula weight: 505.7
Reduced dye indicator that replaces NAD⁺ in the reaction

Triethanolamine, Formula weight, 185.7 Sigma T 1502
biological buffer pH range 7.8-8.3

Potassium cyanide (KCN), Formula weight, 65.13 Sigma 20,781-0
inhibits other dehydrogenase activities

Cremophor EL Sigma C 5135
solublizes the formazan color - product (water insoluble)

Bradford Reagent Sigma B 6916

96-well flat bottom tissue culture plates University Stores 150 8644

Protein Assay (Bradford)

This method was chosen because BME and EDTA have known interference with the Lowery assay. Adapted from P.J. Hansen, University of Florida.

Standard stock: BSA 50 mg/ml in homogenization buffer above, diluted to 5 mg/ml

Run sample and standards in duplicate.

Standard, µg/ml	BSA Stock, µl	Homogenization buffer, µl
625	200	1400
500	200	1800
400	200	2300
312.5	1:2 dilution of 625	
250	1:2 dilution of 500	
200	1:2 dilution of 400	
125	1:2 dilution of 250	
100	1:2 dilution of 200	
62.5	1:2 dilution of 125	
50	1:2 dilution of 100	
31.25	1:2 dilution of 62.5	
0	0	1000

Must dilute samples in microcentrifuge tubes
another 1:15 or 1:20 to fall on curve (50 µl of sample plus 700 µl of buffer)

Pipette 20 µl of standard and sample into individual wells.
Add 200 µl of Bradford Reagent into all wells and shake plate
Wait 5 min before reading, should be read within 30 min

Read Absorbency at 595 nm (0 µg/ml is a blank).

Additional validation of assay:

Parallelism: Serially dilution of tissue homogenate with buffer
Correct for dilution factors.

Recovery: Add the following standards to tissue homogenate (250, 200, 100,
and 50 µg/ml) in equal volumes

Actual concentration is calculated from µg of protein from tissue and µg
of protein from standard divided by total volume.
 $(1 - (\text{Actual} - \text{measured}) / \text{actual}) * 100 = \text{Recovery percent}$

Parallelism: Coefficient of variation = 4.2%

Recovery = 105 % of spike recovered

Control tissue, µg/ml: Coefficient of variation = 14%

Control tissue, µg of protein/mg wet tissue: Coefficient of variation = 13%

Succinate Dehydrogenase Assay

Adapted from Munujos et al., 1993, Analytical Biochemistry 212:506-509.

Starting Buffer: 100 mM triethanolamine (TEA), pH 8.3
9.285 g in 500 ml di-water, pH 8.3 with 1 M NaOH

Add the following to 500 ml of TEA buffer
0.5 mM EDTA (93.06 mg)
2 mM KCN (65.13 mg)
12 g/L Cremophor EL (6 g)

Make *fresh daily* (enough for 3 plates):

INT buffer: Add 2 mM INT (60.68 mg) to 60 ml

Reagent: Buffer plus 20 mM Na-succinate (0.162 g/30 ml buffer)

Temperature of buffer and reagent should be 37° C before use

Add 50 µl of sample to five wells and 50 µl of homogenizing buffer to each well

Blank wells (B, duplicate): add 150 µl buffer (without Na-succinate)

Activity wells (triplicate): add 150 µl reagent (with Na-succinate)

96-Well Plate:

1	1	1	1B	1B	9	9	9	9B	9B	17	17B
2	2	2	2B	2B	10	10	10	10B	10B	17	17B
3	3	3	3B	3B	11	11	11	11B	11B	17	
4	4	4	4B	4B	12	12	12	12B	12B	18	18B
5	5	5	5B	5B	13	13	13	13B	13B	18	18B
6	6	6	6B	6B	14	14	14	14B	14B	18	
7	7	7	7B	7B	15	15	15	15B	15B		
8	8	8	8B	8B	16	16	16	16B	16B		

Incubate for 0:04:20 at 37° C,

Read using kinetic protocol for 0:06:20 every 20 sec to determine reaction rate (mOD/min) for each well.

Immediately after kinetic protocol, read with endpoint to determine pathlength (cm) for each well.

Activity of SDH µmol/min per ml of sample =
 $\text{mOD/min} \times (1/(\text{pathlength} \times 19,300)) \times 250 \text{ µl total volume}/50 \text{ µl of sample}$

Based on Beer-Lambert equation:

$[\text{INT-formazan}] = \text{OD}/k \times L$

$k = \text{INT-Formazan molar absorbency} = 19,300 \text{ (L/mol cm)}$

OD = optical density at 500 nm

L = path length (corrected to 1 cm with pathlength).

Additional validation of assay:

Parallelism: Serially dilution of control tissue homogenate (n = 3 per tissue) with buffer, Correct for dilution factors.

Liver: R-squared were 0.967 and 0.972 for $\mu\text{mol}/\text{min}$ vs. μg of wet tissue and μg of tissue protein, respectively.

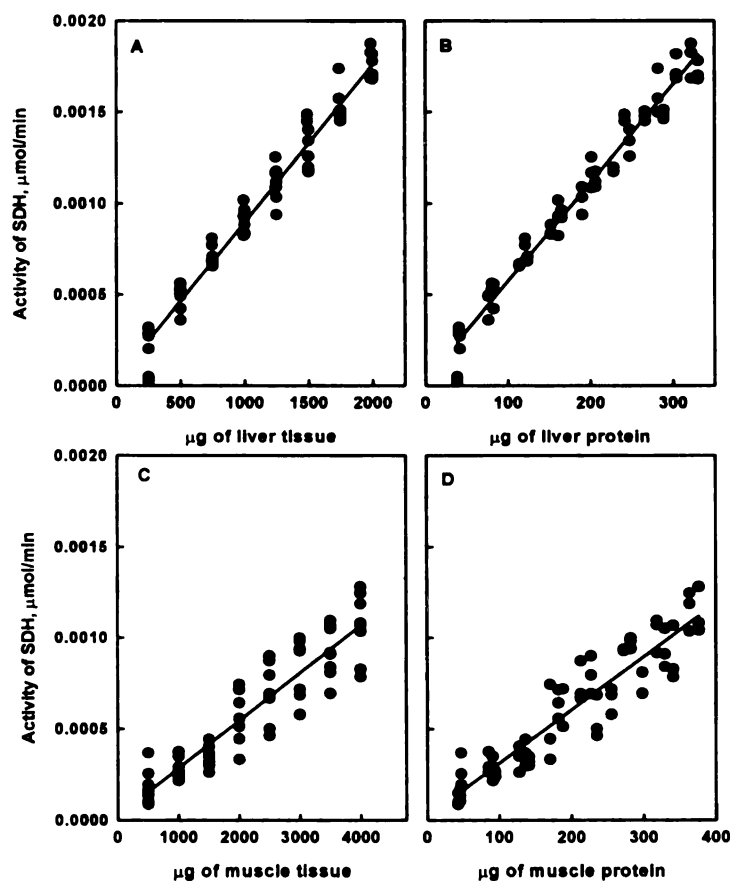
Muscle: R-squared were 0.849 and 0.870 for $\mu\text{mol}/\text{min}$ vs. μg of wet tissue and μg of tissue protein, respectively.

Control liver tissue, $\mu\text{mol}/\text{min}$ g of wet tissue: Coefficient of variation = 22%

Control liver tissue, $\mu\text{mol}/\text{min}$ g of tissue protein: Coefficient of variation = 18.4%

Control muscle tissue, $\mu\text{mol}/\text{min}$ g of wet tissue: Coefficient of variation = 30%

Control muscle tissue, $\mu\text{mol}/\text{min}$ g of tissue protein: Coefficient of variation = 26%



Validation of assay to determine activity of SDH for liver (*Panel A and B*) and muscle (*Panel C and D*) tissues. Activity ($\mu\text{mol}/\text{min}$) was linearly related to μg of wet tissue and μg of tissue protein ($r^2 = 0.97$ and 0.97 for liver and $r^2 = 0.85$ and 0.87 for muscle).

M. GLYCOGEN ASSAY

Modified from Andersson et al., Biochem Pharm, 2000, 59:821-829 and Suzuki et al., Mol Cell Biol, 2001, 21:2683-2694.

Supplies:

12 x 75 borosilicate glass tubes	
potassium hydroxide (KOH, FW 56.11)	University Stores 15569160
sodium sulfate (Na ₂ SO ₄ , FW 142.04)	University Stores 15585880
ethanol (ethyl alcohol)	University Stores 15506080
glacial acetic acid	University Stores 15500760
sodium acetate (FW 82.03)	University Stores 15576000
bovine glycogen	Sigma G-0885
amyloglucosidase	Sigma 10113
Glucose Kit	
Glucose Oxidate, Colorimetric	Sigma 510-A
Five 96-well plate/kit (36 samples/plate)	
Glucose Standard	Sigma G3761

Prepare standards from stock glucose 500 mg/dl = 5 µg/µl

Standard, µg/well	Stock, µl	Acetate buffer, µl
5.0	200	1800
4.5	180	1820
4.0	160	1840
3.25	130	1870
2.5	100	1900
2	80	1920
1.25	50	1950
1.0	40	1960
0.5	20	1980
0	0	2000

Liver samples:

200 µl of homogenate in 12 x 75 tubes was incubated with 400 µl of 2 M KOH for 30 min at 70° C water bath.

Cool to room temperature, add 200 µl of 1 M Na₂SO₄ and 1 ml of cold-95% ethanol

Solution was spun 5 min, 2000 g in RC-3B centrifuge
Invert and shake supernatant from tubes

Dry precipitate, overnight at room temperature.

Add to 400 µl of 0.375% amyloglucosidase (w/v) in 0.3 M sodium acetate buffer (pH 4.8 with acetic acid), vortex
Incubate 1 h at 55° C, checking to make sure precipitate in solution every 20 min.

Muscle samples:

200 µl of homogenate in 12 x 75 tubes incubated with 400 µl of 2 M KOH for 1 h at 70° C water bath

Cool to room temperature. Add 200 µl of Na₂SO₄ and 1 ml of cold-95% ethanol
Solution was spun 5 min, 2000 x g in RC-3B centrifuge

Invert and shake supernatant from tubes.

Dry precipitate, overnight at room temperature.

Add to 400 µl of 0.75% amyloglucosidase (w/v) in 0.3 M sodium acetate buffer (pH 4.8 with acetic acid), vortex

Incubate 1 h at 55° C, checking to make sure precipitate in solution every 20 min.

Glucose units:

Glucose measured using Sigma Kit No. 510-A as previous protocol, with standards (10 µl per well) prepared acetate/acetic acid buffer (5 to 10 µl per liver sample, 20 µl per muscle sample).

Additional validation of assay:

Parallelism: Serially dilution of tissue homogenate and purchased glycogen with buffer, correct for dilution factors.

Recovery: Add the following dilutions of prepared glycogen to tissue homogenate (1:25, 1:200; 1:250) in equal volumes

Actual concentration is calculated from µg from tissue and µg from prepared glycogen divided by total volume.

$$(1 - (\text{Actual} - \text{measured}) / \text{actual}) * 100 = \text{Recovery percent}$$

Parallelism: Coefficient of variation = 3.8%

Recovery = 99.3% of spike recovered

Prepared glycogen measured 87% glucose (Sigma reports as 90% glucose on dry basis

Control liver tissue, mg/g of wet tissue: Coefficient of variation = 15%

Control liver tissue, µg/mg of tissue protein: Coefficient of variation = 11%

Control muscle tissue, µmol/min g of wet tissue: Coefficient of variation = 14%

Control muscle tissue, µmol/min g of tissue protein: Coefficient of variation = 16%

N. TRIGLYCERIDE ASSAY

Adapted from Hara and Radin, Anal Biochem. 1979, 90:420-426 and
Moore et al., J. Dairy Sci., 2000, 83:2095-2104.

Supplies:

Hexane	University Stores 15542560
Isopropyl alcohol (2-propanol)	University Stores 15548640
Triton-X	Sigma x-100
Triglyceride Kit	Sigma 343-25P
Triolein	Sigma T-9275
12 x 75 polypropylene tubes	
12 x 75 borosilicate glass tubes	
compressed nitrogen	

Extraction of lipid:

Efficiency of extraction estimated to be 89% recovered, non-linear with values greater than 1200 µg/ml of triolein. Samples greater than this should be re-extracted.

Prepare standards from triolein stock 910 mg/ml

Dilute 1:91 in hexane (50 µl of triolein plus 4500 µl of hexane: 10,000 µg/ml)

Standard, µg/well	1:91 dilution, µl	Hexane, µl
10	100	1900
8	80	1920
5	1:2 dilution (1000 µl of 10 µg)	1000
4	1:2 dilution (1000 µl of 8 µg)	1000
2.5	1:2 dilution (1000 µl of 5 µg)	1000
2	1:2 dilution (1000 µl of 4 µg)	1000
1.25	1:2 dilution (1000 µl of 2.5 µg)	1000
1.0	1:2 dilution (1000 µl of 2 µg)	1000
0.5	1:2 dilution (1000 µl of 1 µg)	1000
0	0	2000

2 ml of hexane : isopropyl alcohol (3:2, 900 ml hexane plus 600 ml isopropyl alcohol)
was added to 500 µl of homogenate or standards in 12 x 75 polypropylene tubes

Vortex for 5 min using VWR Multi-tube vortexer (2270 Lab)

Add 800 µl of 8% Na₂SO₄ (48 g in 600 ml de-ionized water, w/v)

Allow phases to separate, transfer top layer to 12 x 75 glass tubes

Wash interface with 200 µl of 7:2 hexane : isopropyl alcohol, two times

Add 600 µl of 2% Triton-x100 in 7:2 hexane : isopropyl alcohol

Dry in 45° C water bath under a steady stream of nitrogen

Re-constitute with 500 µl of de-ionized water.

Assayed for triglycerides:

Sigma Kit No. 343
96-well microtiter plate
20 µl of sample or standards in duplicate
Add 150 µl of reagent

Incubate 37° C for 20 min, read microtiter plate at 520 nm

Additional validation of assay:

Parallelism: Serially dilution of tissue homogenate and purchased triolein after extraction, correct for dilution factors.

Recovery: Add the following dilutions of prepared triolein to tissue homogenate prior to extraction in equal volumes

Actual concentration is calculated from µg from tissue and µg from triolein divided by total volume.

$$(1 - (\text{Actual} - \text{measured}) / \text{actual}) * 100 = \text{Recovery percent}$$

Parallelism: Coefficient of variation = 10%

Recovery = 95% of spike recovered

Control liver tissue, mg/g of wet tissue: Coefficient of variation = 14%

Control liver tissue, µg/mg of tissue protein: Coefficient of variation = 13%

Control muscle tissue, mg/g of wet tissue: Coefficient of variation = 65%

Control muscle tissue, µg/mg of tissue protein: Coefficient of variation = 59%

* control muscle tissue was extremely variable, unclear as to why*

O. ADDITIONAL VARIABLES NOT DESCRIBED IN DISSERTATION

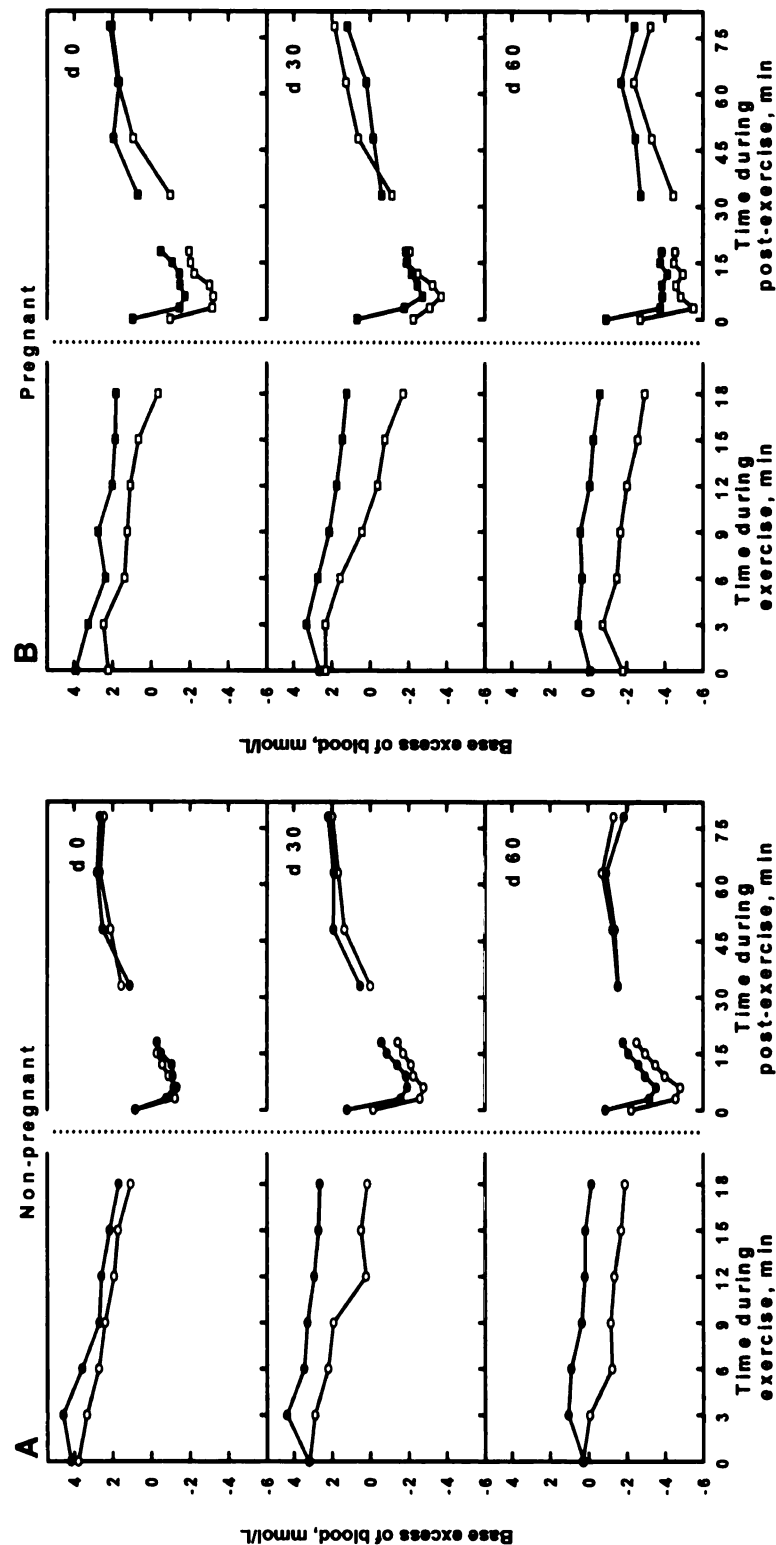


Figure O.1. Least squares means of base excess of blood concentrations during exercise and post-exercise portions of treadmill test on d 0, 30, and 60 of experiment. Treatments were non-pregnant, non-exercise (○); non-pregnant, exercise (●); pregnant, non-exercise (□); pregnant, exercise (■). *Panel A:* Data from non-pregnant cows. *Panel B:* Data from pregnant cows. During exercise, blood base excess of blood concentrations were affected by interaction of treatment by pregnancy status by minute of test ($P = 0.063$) and interaction of pregnancy status by day of experiment by minute of test ($P = 0.062$) and 0.054, pooled SEM = 1.159). During 0 to 18 min post-exercise, interaction of treatment by minute of test ($P = 0.012$, pooled SEM = 0.938) were significant. During 33 to 78 min post-exercise, minute of test ($P < 0.001$) and pregnancy status ($P < 0.001$) were significant (pooled SEM = 0.980).

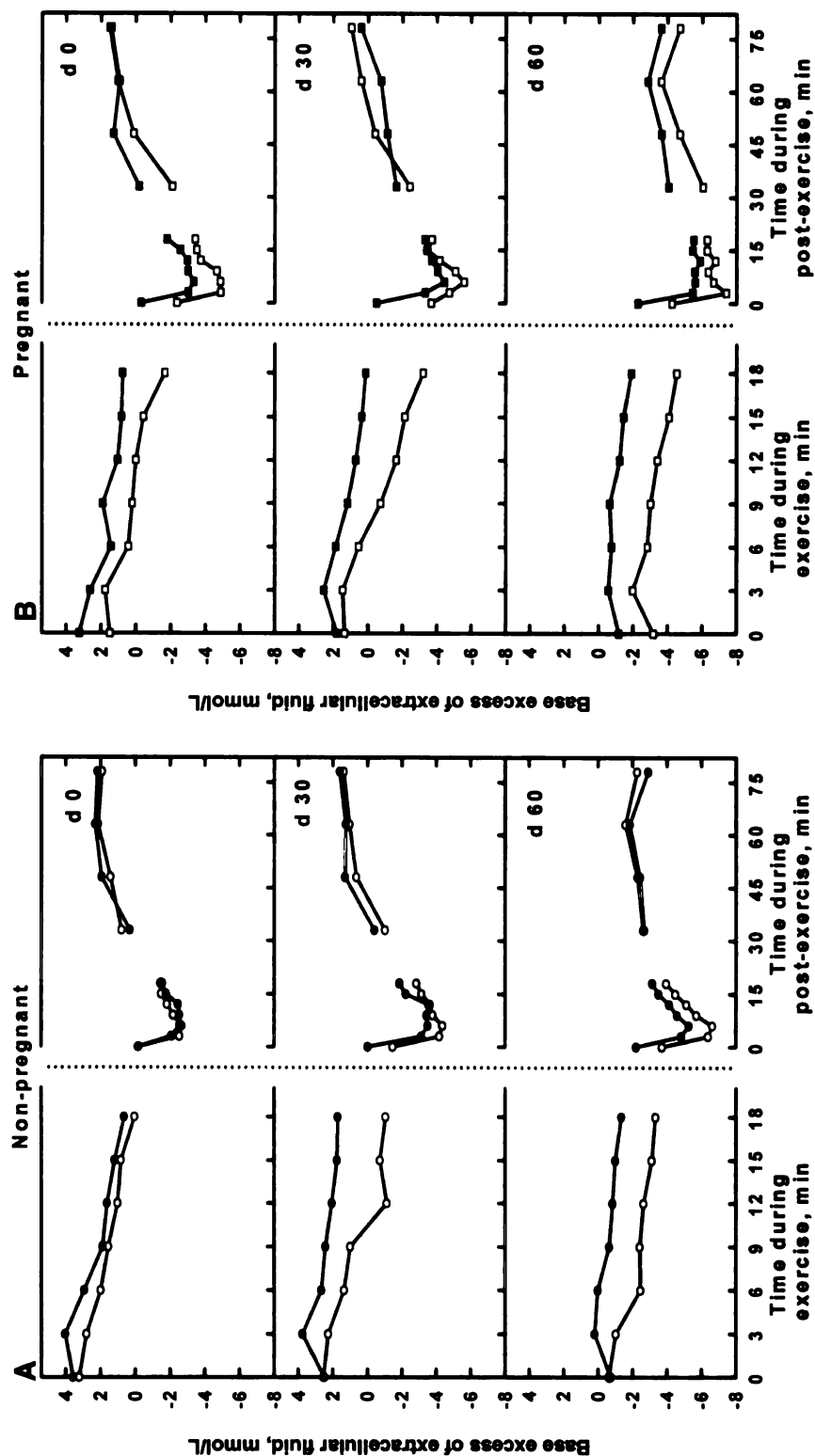


Figure O.2. Least squares means of base excess of extracellular fluid concentrations during exercise and post-exercise portions of treadmill test on d 0, 30, and 60 of experiment. Treatments were non-pregnant, non-exercise ($\square$); non-pregnant, exercise ($\bullet$); pregnant, non-exercise ($\square$); pregnant, exercise ($\bullet$). *Panel A:* Data from non-pregnant cows. *Panel B:* Data from pregnant cows. During exercise, blood base excess of extracellular fluid concentrations were affected by interaction of treatment by pregnancy status by minute of test ($P = 0.085$) and interaction of pregnancy status by day of experiment by minute of test ($P = 0.054$, pooled SEM = 0.996). During 0 to 18 min post-exercise, interaction of treatment by minute of test ($P = 0.071$) and interaction of pregnancy status by day of experiment by minute of test ($P = 0.008$, pooled SEM = 0.812) were significant. During 33 to 78 min post-exercise, minute of test ($P < 0.001$) and pregnancy status ($P < 0.001$) were significant (pooled SEM = 0.860).

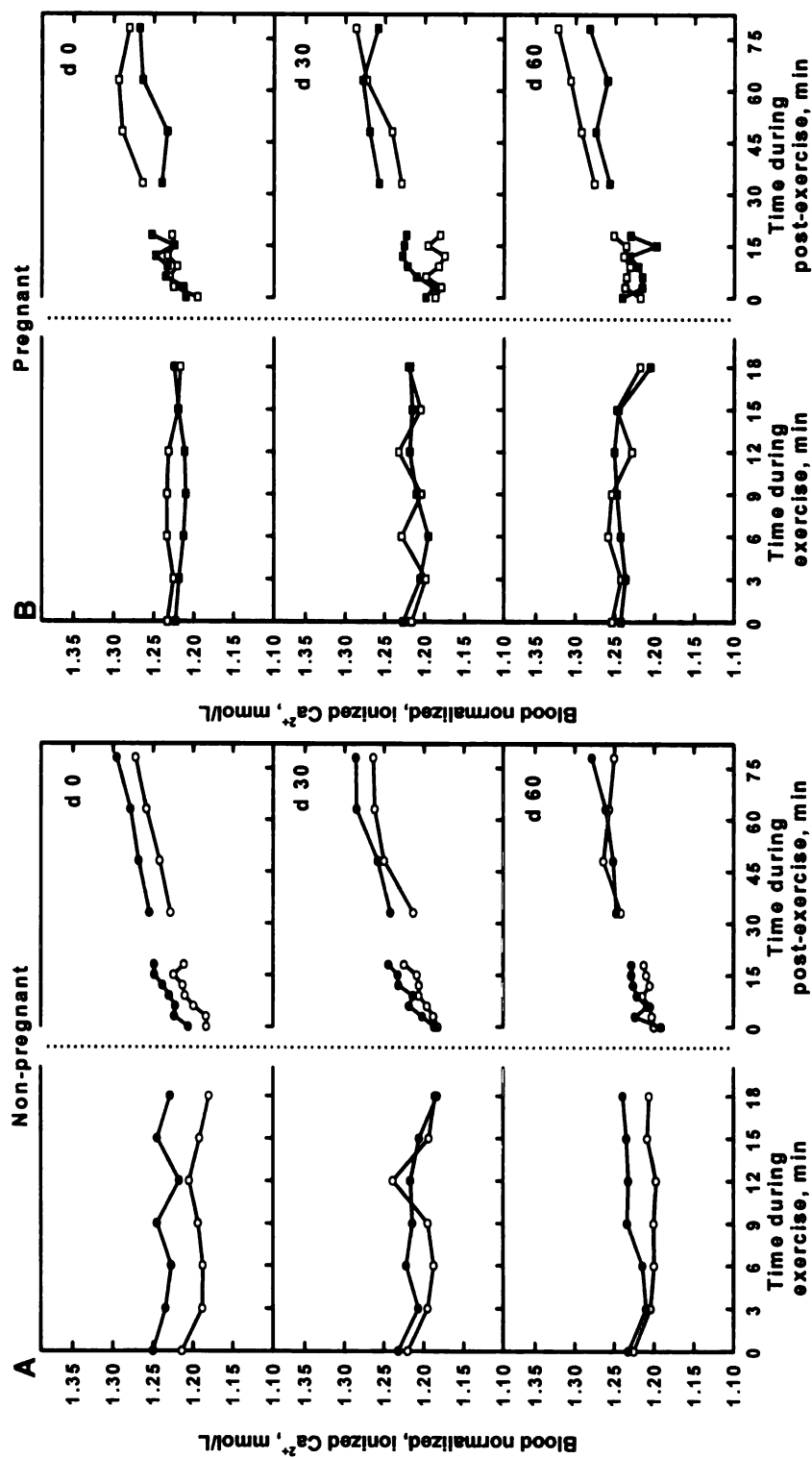


Figure O.3. Blood normalized, ionized Ca^{2+} , adjusted for a pH of 7.4, (nCa^{2+}) concentrations during exercise and post-exercise portions of treadmill test on d 0, 30, and 60 of experiment. Treatments were non-pregnant, non-exercise (○); non-pregnant, exercise (●); pregnant, non-exercise (□); pregnant, exercise (■). *Panel A:* Results from non-pregnant cows. *Panel B:* Results from pregnant cows. During exercise, blood nCa^{2+} concentrations were affected by minute of test ($P = 0.002$, pooled SEM = 0.0285). During 0 to 18 min post-exercise, minute of test ($P < 0.001$, pooled SEM = 0.0260) was significant. During 33 to 78 min post-exercise, minute of test was significant ($P < 0.001$, pooled SEM = 0.0200).

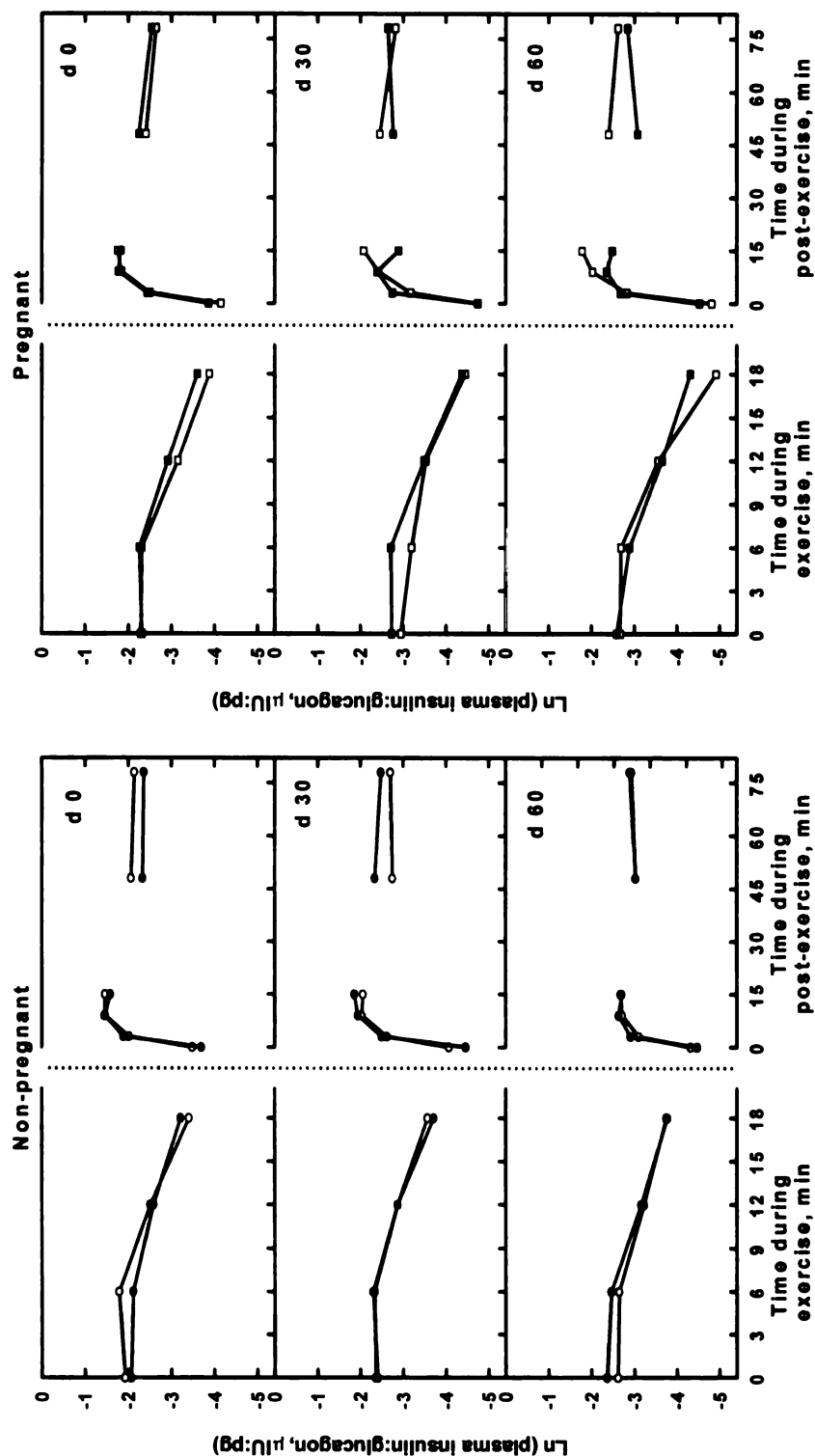


Figure O.4. Natural logarithmically transformed plasma insulin : glucagon ratios during exercise and post-exercise portions of treadmill test on d 0, 30, and 60 of experiment. Treatments were non-pregnant, non-exercise (○); non-pregnant, exercise (●); pregnant, non-exercise (□); pregnant, exercise (■). *Panel A:* Data from non-pregnant cows. *Panel B:* Data from pregnant cows. During exercise, 0 to 18 min post-exercise, and 33 to 78 min post-exercise pooled SEM were 0.349, 0.386, and 0.280, respectively. During 0 to 18 min of exercise, the interaction of T by PS by day of experiment by minute of test ($P = 0.106$) and interaction of T by minute of test ($P = 0.040$) affected plasma insulin : glucagon ratios. During 0 to 18 min post-exercise, plasma insulin : glucagon tended to be affected by the interaction of T by PS by minute of test ($P = 0.097$) and interaction of T by minute of test ($P = 0.087$). During 33 to 78 min post-exercise, there were no differences in the ratios.

Table O.1. Plasma concentrations (natural logarithmically transformed) of molar ratios of insulin : glucose, and insulin : glucagon on a rest day (Rd) and immediately after exercise training (Ed) during wk 2 to 4 and 6 to 10 of experiment.¹

	Treatment		Pregnancy		Treatment and Pregnancy						P-value ²		
	NE ³	E ³	NP ⁴	P ⁴	NP, NE ⁵	NP, E ⁵	P, NE ⁵	P, E ⁵	SEM	T	PS	T x PS	
<u>Ln(insulin : glucose, pm ol/mmol)</u>													
2 to 4 wk ⁶													
Rd	3.00	2.75	3.18	2.57	3.29	3.08	2.72	2.42	0.09	0.009	< 0.001	NS	
Ed	2.95	2.67	3.26	2.36	3.36	3.16	2.54	2.18	0.11	0.017	< 0.001	NS	
6 to 10 wk ⁶													
Rd	3.25	2.98	3.60	2.63	3.72	3.48	2.79	2.47	0.13	0.042	< 0.001	NS	
Ed	3.22	2.76	3.48	2.49	3.70	3.26	2.74	2.25	0.13	0.001	< 0.001	NS	
<u>Ln(insulin : glucagon, pm ol/pmol)</u>													
2 to 4 wk ⁶													
Rd	0.55	0.43	0.71	0.27	0.74	0.68	0.36	0.19	0.11	NS	0.0003	NS	
Ed	0.51	0.38	0.79	0.11	0.78	0.80	0.25	-0.03	0.14	NS	< 0.001	NS	
6 to 10 wk ⁶													
Rd	0.63	0.42	0.70	0.35	0.74	0.66	0.52	0.18	0.14	NS	0.015	NS	
Ed	0.57	0.28	0.67	0.20	0.73	0.58	0.41	-0.01	0.15	0.066	0.004	NS	

¹ Values are least squares means.

² Probability values of main effect of exercise treatment (T), pregnancy status (PS), and the interaction of T by PS (T x PS), and NS = non-significant, $P > 0.10$.

³ Pooled least squares mean regardless of pregnancy status, or week of experiment. NE = non-exercised cows; E = exercised cows.

⁴ Pooled least squares mean regardless of exercise treatment, or week of experiment. NP = non-pregnant cows; P = pregnant cows.

⁵ Pooled least squares mean regardless of week of experiment. NP, NE = non-pregnant, non-exercised cows; NP, E = non-pregnant, exercised cows; P, NE = pregnant, non-exercised cows; P, E = pregnant, exercised cows.

⁶ Wk = week of experiment. Wk 2 to 4, cows were fed a diet with a DCAD of 20.2 meq/100 g of DM. Wk 6 to 10, cows were fed a diet with a DCAD of -15.2 meq/100 g of DM.

CITED REFERENCES

- Aalhus, J. L., and M. A. Price. 1990a. The effect of load carrying on carcass characteristics and composition in sheep. *Can. J. Anim. Sci.* 70:81-88.
- Aalhus, J. L., and M. A. Price. 1990b. The effects of endurance exercise on live animal performance and carcass growth and development in sheep. *Can. J. Anim. Sci.* 70:97-105.
- Aalhus, J. L., and M. A. Price. 1991. Endurance-exercised growing sheep: I. Post-mortem and histological changes in skeletal muscles. *Meat Sci.* 29:43-56.
- Aalhus, J. L., M. A. Price, P. J. Shand, and Z. J. Hawrysh. 1991. Endurance-exercised growing sheep: II. Tenderness increase and change in meat quality. *Meat Sci.* 29:57-68.
- Aguera, E. I., M. D. Rubio, R. Vivo, R. Santisteban, A. Munoz, and F. Castejon. 1995. Blood parameter and heart rate response to training in Andalusian horses. *Rev. Exp. Fisiol.* 51:55-64.
- Anderson, M. J., R. C. Lamb, and J. L. Walters. 1979. Effect of prepartum exercise on feed intake and milk production of multiparous cows. *J. Dairy Sci.* 62:1420-1423.
- Andersson, U., T. D. Butters, R. A. Dwek, and F. M. Platt. 2000. N-Butyldeoxygalactonojirimycin: A more selective inhibitor of glycosphingolipid biosynthesis than N-butyldeoxynojirimycin, in vitro and in vivo. *Biochem. Pharmacol.* 59:2.
- Apple, J. K., J. E. Minton, K. M. Parsons, M. E. Dikeman, and D. E. Leith. 1994. Influence of treadmill exercise on pituitary-adrenal secretions, other blood constituents, and meat quality of sheep. *J. Anim. Sci.* 72:1306-1314.
- Arave, C. W., R. C. Lamb, and J. L. Walters. 1987. Physiological and glucocorticoid response to treadmill exercise of Holstein heifers. *J. Dairy Sci.* 70:1289-1293.
- Arave, C. W., J. L. Walters, and R. C. Lamb. 1978. Effect of exercise on glucocorticoids and other cellular components of blood. *J. Dairy Sci.* 61:1567-1572.
- ARC. 1980. Pages 100-103 in *The Nutrient Requirements of Ruminant Livestock*. Agricultural Research Council, ed. Commonwealth Agricultural Bureaux, Slough, England.
- Art, T., and P. Lekeux. 1993. Training-induced modifications in cardiorespiratory and ventilatory measurements in thoroughbred horses. *Equine Vet. J.* 25:532-536.
- Astrand, P., and K. Rodahl. 1986. Physical training. Pages 412-485 in *Textbook of Work Physiology: Physiological Bases of Exercise*. 3rd ed. McGraw-Hill, Inc., New York, NY.

- Azevedo, J. L., J. K. Linderman, S. L. Lehman, and G. A. Brooks. 1998. Training decreases muscle glycogen turnover during exercise. *Eur. J. Appl. Physiol.* 78:479-486.
- Baird, G. D. 1982. Primary ketosis in the high-producing dairy cow: Clinical and subclinical disorders, treatment, prevention, and outlook. *J. Dairy Sci.* 25:1-10.
- Bassett, J. M. 1989. Metabolic and endocrine responses of pregnant and lactating ewes to intravenous glucose or insulin. *J. Agric. Sci.* 113:173-182.
- Bauman, D. E., and W. B. Currie. 1980. Partitioning of nutrients during pregnancy and lactation: A review of mechanisms involving homeostasis and homeorhesis. *J. Dairy Sci.* 63:1514-1529.
- Bauman, D. E., C. J. Peel, W. D. Steinhour, P. J. Reynolds, H. F. Tyrrell, A. C. G. Brown, and G. L. Haaland. 1988. Effect of bovine somatotropin on metabolism of lactating dairy cows: influence on rate of irreversible loss and oxidation of glucose and nonesterified fatty acids. *J. Nutr.* 118:1031-1040.
- Bayliss, D. A., and D. E. Millhorn. 1992. Central neural mechanisms of progesterone action: application to the respiratory system. *J. Appl. Physiol.* 73:393-404.
- Bell, A. W. 1979. Lipid metabolism in liver and selected tissues and in the whole body of ruminant animals. *Prog. Lipid Res.* 18:117-164.
- Bell, A. W. 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. *J. Anim. Sci.* 73:2804-2819.
- Bell, A. W., K. D. Chandler, and B. J. Leury. 1982. Fetal and uteroplacental energy metabolism in the sheep: Effects of maternal undernutrition and exercise. *Proc. Energy Metab. Farm Anim.* 29:58-61.
- Bell, A. W., J. R. S. Hales, A. A. Fawcett, and R. B. King. 1986. Effects of exercise and heat stress on regional blood flow in pregnant sheep. *J. Appl. Physiol.* 60:1759-1764.
- Bell, A. W., J. R. S. Hales, R. B. King, and A. A. Fawcett. 1983. Influence of heat stress on exercise-induced changes in regional blood flow in sheep. *J. Appl. Physiol.* 55:1916-1923.
- Bellows, R. A., R. E. Short, and R. B. Staigmiller. 1994. Exercise and induced-parturition effects on dystocia and rebreeding in beef cattle. *J. Anim. Sci.* 72:1667-1674.
- Benedetti, E., H. M. Silva, and R. P. Rocha. 1990. Walking trials with dairy cows: effect on voluntary water intake. *Arq. Bras. Med. Vet. Zoot.* 42:39-48.

- Bergeron, R., M. Kjaer, L. Simonsen, J. Bulow, and H. Galbo. 1999. Glucose production during exercise in humans: A-hv balance and isotopic-tracer measurements compared. *J. Appl. Physiol.* 87:111-115.
- Bergman, E. N. 1986. Splanchnic and peripheral uptake of amino acids in relation to the gut. *Fed. Proc.* 45: 2277-2282.
- Bergman, E. N., and R. N. Heitmann. 1978. Metabolism of amino acids by the gut, liver, kidneys, and peripheral tissues. *Fed. Proc.* 37:1228-1232.
- Bergstrom, J., and E. Hultman. 1966. Muscle glycogen synthesis after exercise, an enhancing factor localized to the muscle cells in man. *Nature.* 210:309-310.
- Bertics, S. J., R. R. Grummer, C. Cadorniga-Valino, and E. E. Stoddard. 1992. Effect of prepartum dry matter intake on liver triglyceride concentration and early lactation. *J. Dairy Sci.* 75:1914-1922.
- Bird, A. R., K. D. Chandler, and A. W. Bell. 1981. Effects of exercise and plane of nutrition on nutrient utilization by the hind limb of the sheep. *Aust. J. Biol. Sci.* 34:541-550.
- Blake, J. T., J. D. Olsen, J. L. Walters, and R. C. Lamb. 1982. Attaining and measuring physical fitness in dairy cattle. *J. Dairy Sci.* 65:1544-1555.
- Blum, J. W., W. Bianca, F. Naf, P. Kunz, J. A. Fischer, and M. Da Prada. 1979. Plasma catecholamine and parathyroid hormone responses in cattle during treadmill exercise at simulated high altitude. *Horm. Metab. Res.* 11:246-251.
- Blum, J. W., and H. Eichinger. 1988. Epinephrine and norepinephrine related to cardiorespiratory and metabolic changes in calves during physical exercise. *Horm. Metab. Res.* 20:738-742.
- Bonen, A., P. D. Campagan, L. Gilchrist, and P. Beresford. 1995. Substrate and hormonal responses during exercise classes at selected stages of pregnancy. *Can. J. Appl. Physiol.* 20:440-451.
- Borba-Murad, G. R., H. M. de Souza, G. Lopes, E. B. Ferreira, D. Dambrosio, and R. B. Bazotte. 1998. Changes in glycemia induced by exercise in rats: Contribution of hepatic glycogenolysis and gluconeogenesis. *Res. Commun. Mol. Pathol. Pharmacol.* 102:113-123.
- Bottger, I., E. M. Schlein, G. R. Faloona, J. P. Knochel, and R. H. Unger. 1972. The effect of exercise on glucagon secretion. *J. Clin. Endocrinol. Metab.* 35:117-125.

- Brockman, R. P. 1979a. Effect of somatostatin on plasma glucagon and insulin, and glucose turnover in exercising sheep. *J. Appl. Physiol.* 47:273-278.
- Brockman, R. P. 1979b. Glucagon responses to exercise in sheep. *Aust. J. Biol. Sci.* 32:215-220.
- Brockman, R. P. 1987. Effect of exercise on net hepatic uptake of lactate, pyruvate, alanine, and glycerol in sheep. *Can. J. Physiol. Pharmacol.* 65:2065-2070.
- Brockman, R. P., and R. Halvorson. 1981. Effects of somatostatin and adrenergic blockade on glucagon, insulin and glucose in exercising sheep. *Aust. J. Biol. Sci.* 34:551-5560.
- Brockman, R. P., and R. Halvorson. 1982. Glucose, glucagon, and insulin during adrenergic blockade in exercising sheep. *J. Appl. Physiol.* 52:315-319.
- Burelle, Y., C. Fillipi, F. Peronnet, and X. Leverve. 2000. Mechanisms of increased gluconeogenesis from alanine in rat isolated hepatocytes after endurance training. *Am. J. Physiol. Endocrinol. Metab.* 278:E35-E42.
- Carpenter, M. W., S. P. Sady, M. A. Sady, B. Haydon, D. R. Coustan, and P. D. Thompson. 1990. Effect of maternal weight gain during pregnancy on exercise performance. *J. Appl. Physiol.* 68:1173-1176.
- Chandler, K. D., B. J. Leury, A. R. Bird, and A. W. Bell. 1985. Effects of undernutrition and exercise during late pregnancy on uterine, fetal and uteroplacental metabolism in the ewe. *Br. J. Nutr.* 53:625-635.
- Christensen, N. J., and H. Galbo. 1983. Sympathetic nervous activity during exercise. *Annu. Rev. Physiol.* 45:139-153.
- Clapp, J. F. 1985. Maternal heart rate in pregnancy. *Am. J. Obstet. Gynecol.* 152:659-660.
- Clapp, J. F., M. Wesley, and R. H. Sleamaker. 1987. Thermoregulatory and metabolic responses to jogging prior to and during pregnancy. *Med. Sci. Sports Exer.* 19:124-130.
- Clapperton, J. L. 1964. The energy metabolism of sheep walking on the level and on gradients. *Br. J. Nutr.* 18:47-54.
- Coggan, A. R. 1997. Plasma glucose metabolism during exercise: Effect of endurance training in humans. *Med. Sci. Sports Exer.* 29:620-627.

- Coggan, A. R., W. M. Kohrt, R. J. Spina, D. M. Bier, and J. O. Holloszy. 1990. Endurance training decreases plasma glucose turnover and oxidation during moderate intensity exercise in men. *J. Appl. Physiol.* 68:990-996.
- Coggan, A. R., C. A. Raguso, B. D. Williams, Sidossis L. S., and A. Gastaldelli. 1995a. Glucose kinetics during high-intensity exercise in endurance-trained and untrained humans. *J. Appl. Physiol.* 78:1203-1207.
- Coggan, A. R., S. C. Swanson, L. A. Mendenhall, D. I. Habash, and C. L. Kien. 1995b. Effect of endurance training on hepatic glycogenolysis and gluconeogenesis during prolonged exercise in men. *Am. J. Physiol.* 268:E375-E383.
- Collier, R. J., J. P. McNamara, C. R. Wallace, and M. H. Dehoff. 1984. A review of endocrine regulation of metabolism during lactation. *J. Anim. Sci.* 59:498-510.
- Constantinopol, M., J. H. Jones, E. R. Weibel, C. R. Taylor, A. Lindholm, and R. H. Karas. 1989. Oxygen transport during exercise in large mammals II. Oxygen uptake by the pulmonary gas exchanger. *J. Appl. Physiol.* 67:871-878.
- Courant, G. T., and S. I. Barr. 1990. Exercise during rat pregnancy and lactation: Maternal effects and offspring growth. *Physiol. Behav.* 47:427-433.
- Curtis, C. R., H. N. Erb, C. J. Sniffen, R. D. Smith, and D. S. Kronfeld. 1985. Path analysis of dry period nutrition, postpartum metabolic and reproductive disorders, and mastitis in Holstein cows. *J. Dairy Sci.* 68:2347-2360.
- Dairy Records Management Systems. DHI Glossary. 1999. Fact Sheet: A-4:9. DRMS, Raleigh, NC.
- D'Hour, P., A. Hauwuy, J. B. Coulon, and J. P. Garet. 1994. Walking and dairy cattle performance. *Ann. Zootech.* 43:369-378.
- Delaquis, A. M., and E. Block. 1995a. Acid-base status, renal function, water and macromineral metabolism of dry cows fed diets differing in cation-anion difference. *J. Dairy Sci.* 78:604-619.
- Delaquis, A. M., and E. Block. 1995b. Dietary cation-anion difference, acid-base status, mineral metabolism, renal function, and milk production of lactating cows. *J. Dairy Sci.* 78:2259-2284.
- Derman, K. D., and T. D. Noakes. 1994. Comparative aspects of exercise physiology. Pages 13-25 in *The Athletic Horse: Principles and Practice of Equine Medicine*. D. R. Hodgson and R. J. Rose, ed. W. B. Saunders Co., Philadelphia, PA.

- Di Marco, O. N., and M. S. Aello. 1998. Energy cost of cattle walking on the level and on a gradient. *J. Range Manage.* 51:9-13.
- Dohm, G. L., G. J. Kasperek, and H. A. Barakat. 1985. Time course of changes in gluconeogenic enzyme activities during exercise and recovery. *Am. J. Physiol.* 249:E6-E11.
- Donovan, C. M., and K. D. Sumida. 1990. Training improves glucose homeostasis in rats during exercise via glucose production. *Am. J. Physiol.* 258:R770-R776.
- Donovan, C. M., and K. D. Sumida. 1997. Training enhanced hepatic gluconeogenesis: The importance for glucose homeostasis during exercise. *Med. Sci. Sports Exer.* 29:628-634.
- Drackley, J. K. 1999. Biology of dairy cows during the transition period: The final frontier? *J. Dairy Sci.* 82:2259-2273.
- Drackley, J. K., T. R. Overton, and G. N. Douglas. 2001. Adaptations of glucose and long-chain fatty acid metabolism in liver of dairy cows during the periparturient period. *J. Dairy Sci.* 84 (E. Suppl.):E100-E112.
- Dyk, P. B. 1995. The association of prepartum non-esterified fatty acids and body condition with peripartum health problems on 95 Michigan dairy farms. M. S. Thesis, Michigan State University, East Lansing.
- Ebeling, P., R. Bourey, L. Koranyi, J. A. Tuominen, L. C. Groop, J. Henriksson, M. Mueckler, A. Sovijarvi, and V. A. Koivisto. 1993. Mechanism of enhanced insulin sensitivity in athletes. *J. Clin. Invest.* 92:1623-1631.
- Ehrlein, H. J., H. Honicke, W. V. Engelhardt, and G. Tolkmitt. 1973. Heart frequency during standardised exercise as a measure of the working capacity of horses. *Zentralbl. Veterinaermed. Reihe A.* 20:188-208.
- Erkkola, R. 1976. The influence of physical training during pregnancy on physical work capacity and circulatory parameters. *Scand. J. Clin. Lab. Invest.* 36:747-754.
- Evans, D. L. and R. J. Rose. 1988. Cardiovascular and respiratory responses to submaximal exercise training in the thoroughbred horse. *Pflugers Arch.* 411:316-321.
- Faulkner, A., and H. T. Pollock. 1990. Metabolic responses to euglycaemic hyperinsulinaemia in lactating and non-lactating sheep in vivo. *J. Endocrinol.* 124:59-66.
- Fencel, V., and D. E. Leith. 1993. Stewart's quantitative acid-base chemistry: Applications in biology and medicine. *Respir. Physiol.* 91:1-16.

- Ferrell, C. L. 1991. Maternal and fetal influences on uterine and conceptus development in the cow: II. Blood flow and nutrient flux. *J. Anim. Sci.* 69:1954-1965.
- Fosha-Dolezal, S. R. and M. R. Fedde. 1988. Serum potassium during exercise in Hereford calves: influence of physical conditioning. *J. Appl. Physiol.* 65:1360-1366.
- Fregin, G. F., and D. P. Thomas. 1983. Cardiovascular response to exercise in the horse: a review. Pages 76-90 *in* Equine Exercise Physiology. D. H. Snow, S. G. B. Perrson, and R. J. Rose, ed. Burlington Press Ltd, Cambridge, U.K.
- Friedman, J. E. 1994. Role of glucocorticoids in activation of hepatic PEPCK gene transcription during exercise. *Am. J. Physiol.* 266:E560-E566.
- Galbo, H. 1983. Pages 14-45 *in* Hormonal and Metabolic Adaptation to Exercise. H. Galbo, ed. Thieme-Stratton, Inc., New York.
- Gardner, G. E., D. W. Pethick, and G. Smith. 1998. Effect of chromium chelavite supplementation on the metabolism of glycogen and lipid in adult Merino sheep. *Aust. J. Agric. Res.* 49:137-145.
- Gemeda, T., E. Zerbini, A. G. Wold, and D. Demissie. 1995. Effect of draught work on performance and metabolism of crossbred cows 1. Effect of work and diet on body-weight change, body condition, lactation and productivity. *Anim. Sci.* 60:361-367.
- Genuth, S. M. 1993. Endocrine regulation of calcium and phosphate metabolism. Pages 876-896 *in* Physiology. 3rd ed. R. M. Berne and M. N. Levy, ed. Mobsy-Year Book, Inc., St. Louis, MO.
- Geor, R. J., K. W. Hinchcliff, and R. A. Sams. 2000. Glucose infusion attenuates endogenous glucose production and enhances glucose use of horses during exercise. *J. Appl. Physiol.* 88:1765-1776.
- Goff, J. P., and R. L. Horst. 1997. Physiological changes at parturition and their relationship to metabolic disorders. *J. Dairy Sci.* 80:1260-1268.
- Goodyear, L. J. 2000. AMP-activated protein kinase: A critical signaling intermediary for exercise-stimulated glucose transport? *Exer. Sport Sci. Rev.* 28:113-116.
- Goodyear, L. J., and B. B. Kahn. 1998. Exercise, glucose transport, and insulin sensitivity. *Annu. Rev. Med.* 49:235-261.
- Grant, R. J., and J. L. Albright. 1995. Feeding behavior and management factors during the transition period in dairy cattle. *J. Anim. Sci.* 73:2791-2803.

- Green, H., S. Grant, E. Bombardier, and D. Ranney. 1999. Initial aerobic power does not alter muscle metabolic adaptations to short-term training. *Am. J. Physiol.* 277:E39-E48.
- Green, H. J., S. Jones, M. Ball-Burnett, B. Farrance, and D. Ranney. 1995. Adaptations in muscle metabolism to prolonged voluntary exercise and training. *J. Appl. Physiol.* 78:138-145.
- Greenfield, R. B., M. J. Cecava, and S. S. Donkin. 2000. Changes in mRNA expression for gluconeogenic enzymes in liver of dairy cattle during the transition to lactation. *J. Dairy Sci.* 83:1228-1236.
- Grummer, R. R. 1993. Etiology of lipid-related metabolic disorders in periparturient dairy cows. *J. Dairy Sci.* 76:3882-3896.
- Grummer, R. R. 1995. Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. *J. Anim. Sci.* 73 :2820-2833.
- Gustafson, G. M. 1993. Effects of daily exercise on the health of tied dairy cows. *Pre. Vet. Med.* 17:209-223.
- Hackney, A. C., M. Gall, R. G. McMurray, W. Watson, and V. Katz. 1992. Alpha-fetoprotein, cortisol and prolactin responses to maximal exercise during pregnancy. *Scand. J. Med. Sci. Sports.* 3:41-44.
- Hales, J. R. S., A. W. Bell, A. A. Fawcett, and R. B. King. 1984. Redistribution of cardiac output and skin AVA activity in sheep during exercise and heat stress. *J. Therm. Biol.* 9:113-116.
- Hamilton, L. H. 1958. A spectrophotometric method for the determination of Evans blue dye in the presence of hemolysis and turbidity. *J. Lab. Clin. Med.* 52:762-767.
- Hara, A., and N. S. Radin. 1978. Lipid extraction of tissues with a low-toxicity solvent. *Anal. Biochem.* 90:420-426.
- Harman, N. G., and D. W. Pethick. 1994. The effects of sustained exercise on gluconeogenesis, glycogenolysis and glycogen synthesis in Merino sheep. *Aust. J. Agric. Res.* 45:1189-1202.
- Hay, W. W. Jr. S. J. W., M. Gilbert, F. C. Battaglia, and G. Meschia. 1984. Effect of insulin on glucose uptake by the maternal hindlimb and uterus, and by the fetus in conscious pregnant sheep. *J. Endocrinol.* 100:119-124.
- Hayirli, A., D. R. Bremmer, S. J. Bertics, M. T. Socha, and R. R. Grummer. 2001. Effect of chromium supplementation on production and metabolic parameters in periparturient dairy cows. *J. Dairy Sci.* 84:1218-1230.

- Henning, P. H. 1987. The effect of increased energy demand through walking exercise on intake and ruminal characteristics of sheep fed a roughage diet. *J. Agric. Sci.* 109:53-59.
- Henriksen E. J., and A. E. Halseth. 1994. Early alterations in soleus GLUT-4, glucose transport, and glycogen in voluntary running rats. *J. Appl. Physiol.* 76:1862-1867.
- Henriksson, J. 1977. Training induced adaptation of skeletal muscle and metabolism during submaximal exercise. *J. Physiol.* 270:661-675.
- Hippen, A. R., P. She, J. W. Young, D. C. Beitz, G. L. Lindberg, L. F. Richardson, and R. W. Tucker. 1999. Alleviation of fatty liver in dairy cows with 14-day intravenous infusions of glucagon. *J. Dairy Sci.* 82:1139-1152.
- Hodgson, D. R., and R. J. Rose. 1994. Training regimens: physiologic adaptations to training. Pages 379-385 in *The Athletic Horse: Principles and Practice of Equine Sports Medicine*. 1st ed. D. R. Hodgson and R. J. Rose, ed. W. B. Saunders Co., Philadelphia.
- Hodgson, D. R., R. J. Rose, J. DiMauro, and J. R. Allen. 1985. Effects of a submaximal treadmill training programme on histochemical properties, enzyme activities and glycogen utilisation of skeletal muscle in the horse. *Equine Vet. J.* 17:300-305.
- Hodson, D. R., and R. J. Rose. 1994. Evaluation of performance potential. Pages 231-243 in *The Athletic Horse: Principles and Practice of Equine Sports Medicine*. 1st ed. D. R. Hodgson and R. J. Rose, ed. W. B. Saunders Co., Philadelphia.
- Hokama, J. Y., R. S. Streeper, and E. J. Henriksen. 1997. Voluntary exercise training enhances glucose transport in muscle stimulated by insulin-like growth factor I. *J. Appl. Physiol.* 82:508-512.
- Holloszy, J. O., and E. F. Coyle. 1984. Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. *J. Appl. Physiol.* 56:831-839.
- Holmes, M. A., and R. B. Weiskopf. 1987. Determination of plasma volume in swine by the enzyme-dilution method. *Am. J. Physiol.* 252:R1003-R1008.
- Hoppeler, H. 1990. The different relationship of $\text{VO}_{2\text{max}}$ to muscle mitochondria in humans and quadrupedal animals. *Respir. Physiol.* 80:137-146.
- Hoppeler, H., and E. R. Weibel. 1998. Limits for oxygen and substrate transport in mammals. *J. Exp. Biol.* 201:1051-1064.
- Hopster, H., and H. J. Blokhuis. 1994. Validation of a heart-rate monitor for measuring a stress response in dairy cows. *Can. J. Anim. Sci.* 74:465-474.

- Ingvartsen, K. L., and J. B. Andersen. 2000. Integration of metabolism and intake regulation: A review focusing on periparturient animals. *J. Dairy Sci.* 83:1573-1597.
- Jarrett, I. G., O. H. Filsell, and F. J. Ballard. 1976. Utilization of oxidizable substrates by the sheep hind limb: Effects of starvation and exercise. *Metab.* 25:523-531.
- Jones, J. H., K. E. Longworth, A. Lindholm, K. E. Conley, R. H. Karas, S. R. Kayar, and C. R. Taylor. 1989. Oxygen transport during exercise in large mammals I. Adaptive variation in oxygen demand. *J. Appl. Physiol.* 67:862-870.
- Jones, M. T., R. E. Rawson, and D. Robertshaw. 1992. Determination of maximal oxygen consumption in exercising pregnant sheep. *J. Appl. Physiol.* 73:234-239.
- Judson, G. J., O. H. Filsell, and I. G. Jarrett. 1976. Glucose and acetate metabolism in sheep at rest and during exercise. *Aust. J. Biol. Sci.* 29:215-222.
- Judson, G. J., G. J. Mooney, and R. S. Thornbury. 1983. Plasma biochemical values in thoroughbred horses in training. Pages 354-370 *in* Equine Exercise Physiology. D. H. Snow, S. G. B. Persson, and R. J. Rose, ed. Burlington Press Ltd, Cambridge, U.K.
- Karlstrom, K., B. Essen-Gustavsson, and A. Lindholm. 1994. Fibre type distribution, capillarization and enzymatic profile of locomotor and nonlocomotor muscles of horses and steers. *Acta. Anat.* 151:97-106.
- Kaske, M., K. Herzog, S. Kraeft, and J. Rehage. 2001. Pancreatic insulin response and tissue responsiveness to insulin in dry cows, lactating cows, and cows suffering from fatty liver: results of hyperglycemic and hyperinsulinemic euglycemic clamp experiments. *J. Dairy Sci.* 84 (Suppl. 1):118. (Abstr.).
- Kelly, J. M., M. Summers, H. S. Park, L. P. Milligan, and B. W. McBride. 1991. Cellular energy metabolism and regulation. *J. Dairy Sci.* 74:678-694.
- Kemp, J. G., F. A. Greer, and L. A. Wolfe. 1997. Acid-base regulation after maximal exercise testing in late gestation. *J. Appl. Physiol.* 83:644-651.
- Kiens, B. 1997. Effect of endurance training on fatty acid metabolism: Local adaptations. *Med. Sci. Sports Exer.* 29.
- Knochel, J. P., J. D. Blachley, J. H. Johnson, and N. W. Carter. 1985. Muscle cell electrical hyperpolarization and reduced exercise hyperkalemia in physically conditioned dogs. *J. Clin. Invest.* 75:740-745.

- Kriketos, A. D., D. A. Pan, J. R. Sutton, J. F. Y. Hoh, L. A. Baur, G. J. Cooney, A. B. Jenkins, and L. H. Storlien. 1995. Relationships between muscle membrane lipids, fiber type, and enzyme activities in sedentary and exercised rats. *Am. J. Physiol.* 269:R1145-R1162.
- Kuhlmann, W. D., S. R. Dolezal, and M. R. Fedde. 1985a. Effect of ruminal CO₂ on gas exchange and ventilation in the Hereford calf. *J. Appl. Physiol.* 58:1481-1484.
- Kuhlmann, W. D., D. S. Hodgson, and M. R. Fedde. 1985b. Respiratory, cardiovascular, and metabolic adjustments to exercise in the Hereford calf. *J. Appl. Physiol.* 58:1273-1280.
- Lamb, D. R. 1984. The nature of exercise physiology. Pages 1-9 in *Physiology of Exercise: Responses and Adaptations*. 2nd ed. Macmillan Publishing Co., New York, NY.
- Lamb, R. C., M. J. Anderson, and J. L. Walters. 1981. Forced walking prepartum for dairy cows of different ages. *J. Dairy Sci.* 64:2017-2024.
- Lamb, R. C., B. O. Barker, M. J. Anderson, and J. L. Walters. 1979. Effects of forced exercise on two-year-old holstein heifers. *J. Dairy Sci.* 62:1791-1797.
- Lavoie, C., F. Ducros, J. Bourque, H. Langelier, and L. Chiasson. 1997. Glucose metabolism during exercise in man: The role of insulin in the regulation of glucose utilization. *Can. J. Physiol. Pharmacol.* 75:36-43.
- Lawrence, L. 1994. Nutrition and the athletic horse. Pages 205-227 in *The Athletic Horse: Principles and Practice of Equine Sports Medicine*. 1st ed. D. R. Hodgson and R. J. Rose, ed. W. B. Saunders Co., Philadelphia.
- Lawrence, P. R., and R. J. Stibbards. 1990. The energy costs of walking, carrying and pulling loads on flat surfaces by Brahman cattle and Swamp buffalo. *Anim. Prod.* 50:29-39.
- Lefcourt, A. M., B. Erez, M. A. Varner, R. Barfield, and U. Tasch. 1999. A noninvasive radiotelemetry system to monitor heart rate for assessing stress responses of bovines. *J. Dairy Sci.* 82:1179-1187.
- Leighton, B., E. Blomstrand, R. A. J. Challiss, F. J. Lozeman, M. Parry-Billings, G. D. Dimitriadis, and E. A. Newsholme. 1989. Acute and chronic effects of strenuous exercise on glucose metabolism in isolated, incubated soleus muscle of exercise-trained rat. *Acta. Physiol. Scand.* 138:177-184.
- Leury, B. J., A. R. Bird, K. D. Chandler, and A. W. Bell. 1990a. Glucose partitioning in the pregnant ewe: effects of undernutrition and exercise. *Br. J. Nutr.* 64:449-462.
- Leury, B. J., K. D. Chandler, A. R. Bird, and A. W. Bell. 1990b. Effects of maternal undernutrition and exercise on glucose kinetics in fetal sheep. *Br. J. Nutr.* 64:463-472.

- Lotgering, F. K., R. D. Gilbert, and L. D. Longo. 1983a. Exercise responses in pregnant sheep: blood gases, temperatures, and fetal cardiovascular system. *J. Appl. Physiol.* 55:842-850.
- Lotgering, F. K., R. D. Gilbert, and L. D. Longo. 1983b. Exercise responses in pregnant sheep: oxygen consumption, uterine blood flow, and blood volume. *J. Appl. Physiol.: Respirat. Environ. Exercise Physiol.* 55:834-841.
- Lotgering, F. K., M. B. Van Doorn, P. C. Struijk, J. Pool, and H. C. S. Wallenberg. 1991. Maximal aerobic exercise in pregnant women: heart rate, O₂ consumption, CO₂ production, and ventilation. *J. Appl. Physiol.* 70:1016-1023.
- MacDougall, J. D., A. L. Hicks, J. R. MacDonald, R. S. McKelvie, H. J. Green, and K. M. Smith. 1998. Muscle performance and enzymatic adaptations to sprint interval training. *J. Appl. Physiol.* 84.
- Martin, T. P. 1987. Predictable adaptations by skeletal muscle mitochondria to different exercise training workloads. *Comp. Biochem. Physiol.* 88B:273-276.
- Martin, W. H. 1997. Effect of endurance training on fatty acid metabolism during whole body exercise. *Med. Sci. Sports Exer.* 29:635-639.
- Mashek, D. G., and D. K. Beede. 2000. Peripartum responses of dairy cows to partial substitution of corn silage with corn grain in diets fed during the late dry period. *J. Dairy Sci.* 83:2310-2318.
- Mashek, D. G., and D. K. Beede. 2001. Peripartum responses of dairy cows fed energy-dense diets for 3 or 6 weeks prepartum. *J. Dairy Sci.* 84:115-125.
- Matthewman, R. W., J. Meritt, J. D. Oldham, and G. W. Horgan. 1993a. Effect of exercise on milk yield, milk composition and blood metabolite concentrations in Hereford X Friesian cattle. *Asian-Australas. J. Anim. Sci.* 6:607-617.
- Matthewman, R. W., J. D. Oldham, and G. W. Horgan. 1993b. A note on the effect of sustained exercise on straw intake and body weight in lactating cattle. *Anim. Prod.* 57:491-494.
- McClelland, G., G. Zwingelstein, C. R. Taylor, and J. M. Weber. 1995. Effect of exercise on the plasma nonesterified fatty acid composition of dogs and goats: Species with different aerobic capacities and diets. *Lipids.* 30:147-153.
- McMurray, R. G., W. A. Forsythe, M. H. Mar, and C. J. Hardy. 1987. Exercise intensity-related responses of B-endorphin and catecholamines. *Med. Sci. Sports Exerc.* 19:570-574.
- McMurray, R. G., A. C. Hackney, W. K. Guion, and V. L. Katz. 1996. Metabolic and hormonal responses to low-impact aerobic dance during pregnancy. *Med. Sci. Sports Exer.* 28:41-46.

- McMurray, R. G., A. C. Hackney, V. Katz, M. Gall, and W. J. Watson. 1991. Pregnancy induced changes in maximal oxygen uptake during swimming. *J. Appl. Physiol.* 71:1454-1459.
- McMurray, R. G., M. F. Mottola, L. A. Wolfe, R. Artal, L. Millar, and J. M. Pivarnik. 1993. Recent advances in understanding maternal and fetal response to exercise. *Med. Sci. Sports Exerc.* 25:1305-1321.
- McNamara, J. P. 1991. Regulation of adipose tissue metabolism in support of lactation. *J. Dairy Sci.* 74:706-719.
- Mendez, D. G., O. N. di Marco, and P. M. Corva. 1996. Energy expenditure of cattle walking on a flat terrain. *Anim. Sci.* 63:39-44.
- Mole, P. A., L. B. Oscai, and J. O. Holloszy. 1971. Adaptation of muscle to exercise: Increase in levels of palmityl CoA synthetase, carnitine palmityltransferase, and palmityl CoA dehydrogenase and in the capacity to oxidize fatty acids. *J. Clin. Invest.* 50:2323-2330.
- Moore, S. J., M. J. VandeHaar, B. K. Sharma, T. E. Pilbeam, D. K. Beede, H. F. Bucholtz, J. S. Liesman, R. L. Horst, and J. P. Goff. 2000. Effects of altering dietary cation-anion difference of calcium and energy metabolism in peripartum cows. *J. Dairy Sci.* 83:2095-2104.
- Morton, M. J., N. S. Paul, G. R. Compos, M. V. Hark, and J. Metcalfe. 1985. Exercise dynamics in late gestation: effects of physical training. *Am. J. Obstet. Gynecol.* 152:91-97.
- Mundie, T. G., A. J. Januszkiewicz, D. B. Rayburn, D. G. Marin, and G. R. Ripple. 1991. Effects of conditioning and maximal incremental exercise on oxygen consumption in sheep. *Am. J. Vet. Res.* 52:1019-1023.
- Munujos, P., J. Coll-Canti, F. Gonzalez-Sastre, and F. J. Gella. 1993. Assay of succinate dehydrogenase activity by a colorimetric-continuous method using idonitrotetrazolium chloride as electron acceptor. *Anal. Biochem.* 212:506-509.
- Murakami, T., Y. Shimomura, N. Fujitsuka, N. Nakai, S. Sugiyama, T. Ozawa, M. Sokabe, S. Horai, K. Tokuyama, and M. Suzuki. 1994. Enzymatic and genetic adaptation of soleus muscle mitochondria to physical training in rats. *Am. J. Physiol.* 267:E388-E395.
- Novosadova, J. 1977. The changes in hematocrit, hemoglobin, plasma volume and proteins during and after different types of exercise. *Eur. J. Appl. Physiol. Occup. Physiol.* 36:223-230.
- NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. ed. National Research Council, ed. Natl. Acad. Sci., Washington, DC.

- Palmer, S. M., G. K. Oakes, J. A. Champion, D. A. Fisher, and C. K. Hobel. 1984. Catecholamine physiology in the ovine fetus. III. Maternal and fetal response to acute maternal exercise. *Am. J. Obstet. Gynecol.* 149:426-434.
- Pearson, R. A., and R. F. Archibald. 1989. Biochemical and haematological changes associated with short periods of work in draught oxen. *Anim. Prod.* 48:375-384.
- Perrson, S. G. B. 1983. Evaluation of exercise tolerance and fitness in the performance horse. Pages 441-457 in *Equine Exercise Physiology*. D. H. Snow, S. G. B. Perrson, and R. J. Rose, ed. Burlington Press Ltd, Cambridge, U.K.
- Pethick, D. W. 1991. Comparative aspects of fuel supply during exercise. *Proc. Nutr. Soc. Austr.* 16:197-204.
- Pethick, D. W., N. Harman, and J. K. Chong. 1987. Non-esterified long-chain fatty acid metabolism in fed sheep at rest and during exercise. *Aust. J. Biol. Sci.* 40:221-234.
- Pethick, D. W., C. B. Miller, and N. G. Harman. 1991. Exercise in Merino sheep – the relationships between work intensity, endurance, anaerobic threshold and glucose metabolism. *Aust. J. Agric. Res.* 42:599-620.
- Pethick, D. W., and J. B. Rowe. 1996. The effect of nutrition and exercise on carcass parameters and the level of glycogen in skeletal muscle of Merino sheep. *Aust. J. Agric. Res.* 47:525-537.
- Piguet, M., R. M. Bruckmaier, and J. W. Blum. 1993. Treadmill exercise of calves with different iron supply, husbandry, and work load. *Zentralbl. Veterinaarmed. Reihe A.* 40:456-465.
- Piguet, M., R. M. Bruckmaier, and J. W. Blum. 1994. Cardio-respiratory, haematological and lactate responses of breeding calves to treadmill exercise. *J. Anim. Physiol. Anim. Nutr.* 72:101-114.
- Pivarnik, J. M., W. Lee, J. F. Miller, and J. Werch. 1990. Alterations in plasma volume and protein during cycle exercise throughout pregnancy. *Med. Sci. Sports Exer.* 22:751-755.
- Pivarnik, J. P., N. A. Ayres, M. B. Mauer, D. B. Cotton, B. Kirshon, and G. A. Dildy. 1993. Effects of maternal aerobic fitness on cardiovascular responses to exercise. *Med. Sci. Sports Exerc.* 25:993-998.
- Poliner, L. R., G. J. Dehmer, S. E. Lewis, R. W. Parkey, C. G. Blomqvist, and J. T. Willerson. 1980. Left ventricular performance in normal subjects: a comparison of the responses to exercise in the upright and supine positions. *Circulation.* 62:528-534.

- Putman, C. T., N. L. Jones, E. Hultman, M. G. Hollidge-Horvat, A. Bonen, D. R. McConachie, and G. J. F. Heigenhauser. 1998. Effects of short-term submaximal training in humans on muscle metabolism in exercise. *Am. J. Physiol.* 275:E132-E139.
- Ren, J. M., C. F. Semenkovich, E. A. Guive, J. Gao, and J. O. Holloszy. 1994. Exercise induces rapid increases in GLUT4 expression, glucose transport capacity, and insulin-stimulated glycogen storage in muscle. *J. Biol. Chem.* 269:14396-14401.
- Reynolds, A. J., L. Fuhrer, H. L. Dunlap, M. Finke, and F. A. Kallfelz. 1995. Effect of diet and training on muscle glycogen storage and utilization in sled dogs. *J. Appl. Physiol.* 79:1601-1607.
- Reynolds, M. 1953. Measurement of bovine plasma and blood volume during pregnancy and lactation. *Am. J. Physiol.* 175:118-122.
- Ribeiro, J. M. d. C. R., J. M. Brockway, and A. J. F. Webster. 1977. A note on the energy cost of walking in cattle. *Anim. Prod.* 25:107-110.
- Roberts, T. J., J. Weber, H. Hoppeler, E. R. Weibel, and R. C. Taylor. 1996. Design of the oxygen and substrate pathways II. Defining the upper limits of carbohydrate and fat oxidation. *J. Exp. Biol.* 199:1651-1658.
- Rodiek, A. V., L. M. Lawrence, and M. A. Russell. 1987. Cardiovascular effects of intermittent or continuous treadmill conditioning in horses. *J. Equine Vet. Sci.* 7:14-19.
- Rodriguez-Suarez, L. A. 1998. Periparturient responses of cows fed varying dietary cation-anion differences and calcium contents prepartum. Ph. D. Dissertation, Michigan State University, East Lansing.
- Rose, R. J., and D. R. Hodgson. 1994. Hematology and biochemistry. Pages 63-78 in *The Athletic Horse: Principles and Practice of Equine Sports Medicine*. 1st ed. D. R. Hodgson and R. J. Rose, ed. W. B. Saunders Co., Philadelphia.
- Rowell, L. B. 1986. Pages 213-327. in *Human Circulation: Regulation during Physical Stress*. Oxford University Press, Inc., New York.
- Rukkwamsuk, T., T. Wensing, and M. J. H. Geelen. 1998. Effect of overfeeding during the dry period on regulation of adipose tissue metabolism in dairy cows during the periparturient period. *J. Dairy Sci.* 81:2904-2911.
- Rukkwamsuk, T., T. Wensing, and M. J. H. Geelen. 1999. Effect of fatty liver on hepatic gluconeogenesis in periparturient dairy cows. *J. Dairy Sci.* 82:500-505.

- Ryder, J. W., Y. Kawano, D. Galuska, R. Fahlman, H. Wallberg-Henriksson, M. J. Charron, and J. R. Zierath. 1999. Postexercise glucose uptake and glycogen synthesis in skeletal muscle from GLUT4-deficient mice. *FASEB J.* 13:2246-2256.
- Sady, S. P., M. W. Carpenter, P. D. Thompson, M. A. Sady, B. Haydon, and D. R. Coustan. 1989. Cardiovascular response to cycle exercise during and after pregnancy. *J. Appl. Physiol.* 66:336-341.
- Sano, H., M. Nakai, T. Kondo, and Y. Terashima. 1991. Insulin responsiveness to glucose and tissue responsiveness to insulin in lactating, pregnant, and nonpregnant, nonlactating beef cows. *J. Anim. Sci.* 69:1122-1127.
- Sartin, J. L., K. A. Cummins, R. J. Kemppainen, D. N. Marple, C. H. Rahe, and J. C. Williams. 1985. Glucagon, insulin, and growth hormone responses to glucose infusion in lactating dairy cows. *Am. J. Physiol.* 248:E108-E114.
- SAS. 1999. *in SAS Software, Release 8.2.* SAS, ed. SAS Inst., Inc., Cary, NC.
- Schneider, P. L., D. K. Beede, and C. J. Wilcox. 1988. Nycterohemeral patterns of acid-base status, mineral concentrations and digestive function of lactating cows in natural or chamber heat stress environments. *J. Anim. Sci.* 66:112-125.
- Simmons, C. R., W. G. Bergen, M. J. VandeHaar, D. J. Sprecher, C. J. Sniffen, E. P. Stanisiewski, and H. A. Tucker. 1994. Protein and fat metabolism in cows given somavubove before parturition. *J. Dairy Sci.* 77: 1835-1847.
- Singh, R., and N. Singh. 1986. Blood metabolites as influenced by exercise during management of buffalo heifers. *Indian Vet. Med. J.* 10:224-227.
- Stanton, B. A., and B. M. Koeppen. 1993. Regulation of potassium, calcium, magnesium, phosphate, and acid-base balance. Pages 784-809 *in Physiology*. 3rd ed. R. M. Berne and M. N. Levy, ed. Mosby-Year Book, Inc., St. Louis, MO.
- Stephenson, K. A., I. J. Lean, M. L. Hyde, M. A. Curtis, J. K. Garvin, and L. B. Lowe. 1997. Effects on monensin on the metabolism of periparturient dairy cows. *J. Dairy Sci.* 80:830-837.
- Stroup, W. W. 1999. Mixed model procedures to assess power, precision, and sample size in the design of experiments. *Proc. Am. Stat. Assoc. Biopharm. Sect. Online*. Available: http://biometry.unl.edu/faculty/steve/802/2000/power_sas.pdf.
- Subiyatno, A., D. N. Mowat, and W. Z. Yang. 1996. Metabolite and hormonal responses to glucose or propionate infusions in periparturient dairy cows supplemented with chromium. *J. Dairy Sci.* 79:1436-1445.

- Sumida, K. D., and C. M. Donovan. 1994. Endurance training fails to inhibit skeletal muscle glucose uptake during exercise. *J. Appl. Physiol.* 76:1876-1881.
- Sumida, K. D., and C. M. Donovan. 1995. Enhanced hepatic gluconeogenic capacity for selected precursors after endurance training. *J. Appl. Physiol.* 79:1883-1888.
- Sumida, K. D., J. H. Urdiales, and C. M. Donovan. 1993. Enhanced hepatic gluconeogenesis from lactate in perfused livers after endurance training. *J. Appl. Physiol.* 74:782-787.
- Suzuki, Y., C. Lanner, J. Kim, P. G. Vilardo, H. Zhang, J. Yang, L. D. Cooper, M. Steele, A. Kennedy, C. B. Bock, A. Scrimgeour, J. C. Lawrence, and A. A. DePaoli-Roach. 2001. Insulin control of glycogen metabolism in knockout mice lacking the muscle-specific protein phosphatase PP1G/R_{GL}. *Mol. Cell. Biol.* 21:2683-2694.
- Taylor, L. E., P. L. Ferrante, D. S. Kronfeld, and T. N. Meacham. 1995. Acid-base variables during incremental exercise in sprint-trained horses fed a high-fat diet. *J. Anim. Sci.* 73:2009-2018.
- Thomas, D. P., G. F. Fregin, N. H. Gerber, and N. B. Ailes. 1980. Cardiorespiratory adjustments to tethered-swimming in the horse. *Pflugers Arch.* 385:65-70.
- Thomas, D. P., G. F. Fregin, N. H. Gerber, and N. B. Ailes. 1983. Effects of training on cardiorespiratory function in the horse. *Am. J. Physiol.* 245:R160-R165.
- Turcotte, L. P., E. A. Richter, and B. Kiens. 1992. Increased plasma FFA uptake and oxidation during exercise in trained vs. untrained humans. *Am. J. Physiol.* 262:E791-E799.
- Turner, D. L., H. Hoppeler, H. Claassen, P. Vock, B. Kayser, F. Schena, and G. Ferretti. 1997. Effects of endurance training on oxidative capacity and structural composition of human arm and leg muscles. *Acta. Physiol. Scand.* 161:459-464.
- Vallimont, J. E., G. A. Varga, A. Arieli, T. W. Cassidy, and K. A. Cummins. 2001. Effects of prepartum somatotropin and monensin on metabolism and production of periparturient holstein dairy cows. *J. Dairy Sci.* 84:2607-2621.
- Vandehaar, M. J., G. Yousif, B. K. Sharma, T. H. Herdt, R. S. Emery, M. S. Allen, and J. L. Liesman. 1999. Effect of energy and protein density of prepartum diets on fat and protein metabolism of dairy cattle in the periparturient period. *J. Dairy Sci.* 82:1282-1295.
- Vanheest, J., and C. D. Rodgers. 1997. Effects of exercise in diabetic rats before and during gestation on maternal and neonatal outcomes. *Am. J. Physiol.* 273:E727-E733.

- Veenhuizen, J. J., J. K. Drackley, M. J. Richard, T. P. Sanderson, L. D. Miller, and J. W. Young. 1991. Metabolic changes in blood and liver during development and early treatment of experimental fatty liver and ketosis in cows. *J. Dairy Sci.* 74:4238-4253.
- Vock, R., H. Hoppeler, H. Claassen, Wu D. X. Y., R. Billeter, J. M. Weber, C. R. Taylor, and E. R. Weibel. 1996a. Design of the oxygen and substrate pathways VI. Structural basis of intracellular substrate supply to mitochondria in muscle cells. *J. Exp. Biol.* 199:1689-1697.
- Vock, R., E. R. Weibel, H. Hoppeler, G. Ordway, J. M. Weber, and C. R. Taylor. 1996b. Design of the oxygen and substrate pathways V. Structural basis of vascular substrate supply to muscle cells. *J. Exp. Biol.* 199:1675-1688.
- Wallace, R. L., G. C. McCoy, T. R. Overton, and J. H. Clark. 1996. Effect of adverse health events on dry matter consumption, milk production, and body weight loss of dairy cows during early lactation. *J. Dairy Sci.* 79(Suppl. 1):205. (Abstr.).
- Wasserman, D. H., J. L. Johnson, J. L. Bupp, D. B. Lacy, and D. P. Bracy. 1993. Regulation of gluconeogenesis during rest and exercise in the depancreatized dog. *Am. J. Physiol.* 265:E51-E60.
- Wasserman, D. H., J. A. Spalding, D. Bracy, D. B. Lacy, and A. D. Cherrington. 1989a. Exercise-induced rise in glucagon and ketogenesis during prolonged muscular work. *Diabetes.* 38:799-807.
- Wasserman, D. H., J. A. Spalding, D. B. Lacy, C. A. Colburn, R. E. Goldstein, and A. D. Cherrington. 1989b. Glucagon is a primary controller of hepatic glycogenolysis and gluconeogenesis during muscular work. *Am. J. Physiol.* 257:E108-E117.
- Wasserman, D. H., P. E. Williams, D. B. Lacy, R. E. Goldstein, and A. D. Cherrington. 1989c. Exercise-induced fall in insulin and hepatic carbohydrate metabolism during muscular work. *Am. J. Physiol.* 256:E500-E509.
- Weber, J. M., G. Brichon, G. Zwingelstein, G. McClelland, C. Saucedo, E. R. Weibel, and C. R. Taylor. 1996. Design of the oxygen and substrate pathways IV. Partitioning energy provisions from fatty acids. *J. Exp. Biol.* 199:1667-1674.
- Weber, J. M., T. J. Roberts, and Taylor C. R. 1993. Mismatch between lipid mobilization and oxidation: glycerol kinetics in running African goats. *Am. J. Physiol.* 264:R797-803.
- Weber, J. M., T. J. Roberts, R. Vock, E. R. Weibel, and C. R. Taylor. 1996. Design of the oxygen and substrate pathways III. Partitioning energy provision from carbohydrates. *J. Exp. Biol.* 199:1659-1666.

- Weibel, E. R., C. R. Taylor, J. M. Weber, R. Vock, T. J. Roberts, and H. Hoppeler. 1996. Design of the oxygen and substrate pathways VII. Different structural limits for oxygen and substrate supply to muscle mitochondria. *J. Exp. Biol.* 199:1699-1709.
- Wiklund, E., G. Malmfors, and K. Lundstrom. 1998. The effects of exercise on muscle fibre composition and oxidative capacity in eight bovine skeletal muscles. *Swedish J. Agric. Res.* 28:111-116.
- Wildman, E. E., G. M. Jones, P. E. Wagner, R. L. Boman, H. F. Trout, and T. N. Lesch. 1982. A dairy cow body condition scoring system and it's relationship to selected production characteristics. *J. Dairy Sci.* 65:495-501.
- Winder, W. W., R. C. Hickson, J. M. Hagberg, A. A. Ehsani, and J. A. McLane. 1979. Training induced changes in hormonal and metabolic responses to submaximal exercise. *J. Appl. Physiol.* 46:766-771.
- Wolfer, L. A., P. J. Ohtake, M. F. Mottola, and M. J. McGrath. 1989. Physiological interactions between pregnancy and aerobic exercise. *Exer. Sport Sci. Rev.* 17:295-351.
- Wolff, J. E., E. N. Bergman, and H. H. Williams. 1972. Net metabolism of plasma amino acids by liver and portal-drained viscera of fed sheep. *Am. J. Physiol.* 223:438-446.
- Woodford, S. T., M. R. Murphy, and C. L. Davis. 1984. Water dynamics of dairy cattle as affected by initiation of lactation and feed intake. *J. Dairy Sci.* 67:2336-2343.
- Zanzinger, J., and K. Becker. 1992. Blood parameters in draught oxen during work: relationship to physical fitness. *Comp. Biochem. Physiol.* 102A:715-719.
- Zerbini, E., T. Gameda, D. H. O'Neill, P. J. Howell, and R. C. Schroter. 1992. Relationships between cardio-respiratory parameters and draught work output in F1 crossbred dairy cows under field conditions. *Anim. Prod.* 55:1-10.
- Zerbini, E., T. Gameda, A. G. Wold, S. Nokoe, and D. Demissie. 1995. Effect of draught work on performance and metabolism of crossbred cows 2. Effect of work on roughage intake, digestion, digesta kinetics and plasma metabolites. *Anim. Sci.* 60:369-378.
- Zerbini, E., A. G. Wold, and D. Demissie. 1996. Effect of draught force and diet on dry-matter intake, milk production and live-weight change in non-pregnant and pregnant cows. *Anim. Sci.* 62:225-231.

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