Does the intensity of deliberate late-gestation exercise impact body composition and milk components in dairy heifers?

by

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Abstract

The initiation of lactation in dairy cattle often results in adipose and skeletal muscle mobilization. Our objective was to determine if exercise affects body composition, total milk production, or milk components in nulliparous pregnant heifers. We hypothesized that exercise may improve physiological responses to increased nutrient demands during lactation by mildly and regularly altering homeostasis and improving adipose or skeletal muscle storage and subsequent catabolism. Nulliparous, pregnant Holstein heifers (n=34) were enrolled in 2 cohorts based on the expected calving date (90.27 ± 6.35 d prepartum) for two concomitant 8-week exercise trials. Heifers in each cohort were stratified into one of three treatment groups based on pre-trial subcutaneous (SQ) fat over the proximal gluteobiceps (thurl)). Treatments were administered Mon, Wed and Fri with an 8-panel motorized free-walker, and included sedentary (SED; penned adjacent to free-walker), low intensity (LI; pace estimated to achieve 70% lactate threshold), and high intensity (HI; pace estimated to achieve 130% of lactate threshold in short intervals). Both LI and HI groups walked the same distance for each exercise bout, ranging from 1km to 3.04km. Treatment ceased at 33.96 ± 6.92 d prepartum. Weekly during treatment, heifers were weighed, and ultrasound was used to quantify SQ adipose thickness, and semitendinosus depth, width, and area. Postpartum carryover effects on milk yield (daily) and components (1 d/wk) were assessed for 10 wk. Cohort data were combined; data were analyzed using a one-way repeated measures SPSS (Version 29.0.2.0) with significance determined at $P < 0.05$. Exercise, regardless of intensity, caused significantly reduced SQ adipose thickness throughout the exercise period compared to SED. Exercise also increased semitendinosus dimensions ($P = .03$), with HI exercise increasing (Tukey separated means; $P < 0.05$) muscle area (.042 vs. .058 and .987), depth (.053 vs. .334 and .548), and width (.003 vs. .104 and. .242cm) compared with SED and LI. During the first 10 weeks of lactation, heifers that underwent high intensity exercise exhibited less rapid SQ fat loss. Heifers that underwent high intensity exercise also retained greater semitendinosus area (.004; .02 vs. .137 and .135 cm) during the first 10 wks of lactation. Both HI and LI retained greater semitendinosus depth (<.001 vs. .009 and .055 mm) and semitendinosus width (P = XX; <.001 vs. <.001 and .094 mm) compared to SED. Exercise, regardless of intensity, impacted body weight and BCS through the first 10 weeks of lactation, with heifers that underwent high intensity exercise losing both weight and condition less rapidly, along with recovering body weight and body condition more quickly. Exercise did not affect milk yield, composition, or component yield. In summary, primarily high-intensity late-gestation exercise increased overall muscle size prepartum and prevented loss of fat thickness and muscle size postpartum without affecting total production and milk components. We conclude that strategic exercise regimens could improve adaptation to the catabolic demands of lactation in nulliparous heifers.

Key Words**:** Dairy Heifers, Exercise, Body Composition, Milk Composition

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Chapter 1 - Literature Review

Introduction to the Transition Period in Dairy Cattle

The Transition Period

The transition period, broadly defined as the three weeks preceding calving through the three weeks following calving, is one of the most metabolically and physiologically challenging phases in the dairy cow's production cycle (Grummer, 1995; Drackley, 1999). During this window, cows undergo interconnected changes in metabolism, physiology, and endocrine function to support parturition, the onset of lactation, and, in many cases, continued fetal development. These adaptations are essential for meeting the rapidly increasing nutrient demands of colostrum production, milk synthesis, and fetal growth, while accommodating the reduced rumen capacity of late gestation (Bell, 1995; Ingvartsen and Andersen, 2000). A hallmark of the transition period is the shift from a pregnant, non-lactating state to a non-pregnant, lactating state, requiring dramatic realignment of nutrient partitioning. Energy, protein, minerals, and water must be redirected from supporting maternal maintenance and gestational tissues to fueling the mammary gland for colostrum and milk production (Bell, 1995; Bauman and Currie, 1980). This process is complicated by a natural reduction in dry matter intake (DMI) beginning several weeks pre-calving, increasing the risk of negative energy balance (Hayirli et al., 2002).

Some degree of negative energy balance is a normal physiological occurrence as cows mobilize adipose reserves to meet energy needs (Grummer, 1995). However, excessive mobilization of fat and muscle can overwhelm metabolic capacity, leading to health disorders such as subclinical and clinical ketosis, fatty liver, displaced abomasum, and impaired immune function (Bobe et al., 2004; Roche et al., 2009). These disorders are linked to economic losses through reduced milk yield, impaired reproduction, increased culling, and higher veterinary costs

(Overton and Waldron, 2004; Seifi et al., 2011). From a production standpoint, the transition period is a bottleneck influencing the success of the entire lactation. Cows that transition smoothly tend to produce more milk, have fewer postpartum health problems, and conceive sooner compared to those experiencing metabolic disturbances (Goff and Horst, 1997; LeBlanc, 2010). Consequently, research has focused on strategies to optimize metabolic adaptation, limit excessive tissue mobilization, and maintain comfort.

While nutritional management remains the primary focus, emerging evidence supports the role of controlled physical activity in improving transition cow outcomes. For example, Goselink et al. (2020) demonstrated that walking prepartum cows twice daily for 45 minutes improved adipose loss and reduced postpartum liver triglyceride accumulation without negative impacts on intake or yield. Similarly, Davidson and Beede (2003) developed and validated an exercise and fitness assessment protocol for dairy cows, showing that prepartum training improved physiological fitness markers and reduced exertional stress responses. These findings highlight the potential for integrating structured exercise into prepartum management programs to complement nutritional strategies and enhance metabolic resilience during the transition period.

Adipose Tissue Mobilization During the Transition Period

Adipose tissue plays a central role in meeting the energetic demands of the transition dairy cow when dietary intake is insufficient. In late gestation, as negative energy balance develops, adipose tissue becomes the primary reservoir of stored energy, mobilized through lipolysis to release non-esterified fatty acids (NEFA) into the circulation (Grummer, 1995; Bauman and Currie, 1980; Bell, 1995). The mobilized NEFA can be oxidized by peripheral tissues, including muscle, or taken up by the liver for β-oxidation or triglyceride synthesis

(McNamara, 2013; Drackley, 1999). While moderate adipose mobilization is an adaptive and necessary process, excessive mobilization has been linked to metabolic disturbances such as hepatic lipidosis, ketosis, impaired immune function, and reduced reproductive performance (Bobe et al., 2004; Roche et al., 2009; Ospina et al., 2010). The magnitude and rate of adipose mobilization depend on several factors, including the severity of negative energy balance, hormonal regulation, body condition score (BCS) at calving, and overall metabolic health status (Bünemann et al., 2019. Pascottini et al., 2020). Understanding these regulatory mechanisms is essential for developing management strategies that encourage controlled mobilization without exceeding the liver's metabolic capacity

Lipolysis and NEFA Release

Lipolysis is the enzymatic breakdown of triglycerides stored in adipocytes into glycerol and NEFA. This process is primarily regulated by hormonal cues, including increased catecholamines and growth hormone, decreased insulin, and elevated cortisol concentrations in late gestation (Vernon, 2005; Bauman and Vernon, 1993; Smith et al., 1973). Hormone-sensitive lipase (HSL) and adipose triglyceride lipase (ATGL) are the key enzymes responsible for hydrolyzing stored triglycerides (Zimmermann et al., 2004; Kawano and Ishikawa, 2018). Once released, NEFA enter the bloodstream bound to albumin and are transported to tissues for oxidation or re-esterification (McNamara, 2012). Peripheral tissues such as skeletal muscle and heart preferentially oxidize NEFA to spare glucose for the fetus and mammary gland (Bell, 1995; Drackley, 1999). The glycerol released during lipolysis serves as a gluconeogenic substrate in the liver, supporting blood glucose maintenance (Bergman, 1990). In well-managed cows, lipolysis proceeds at a rate that meets energy needs without causing excessive hepatic lipid accumulation. However, when NEFA supply chronically exceeds the liver's oxidative and export capacity, triglycerides accumulate, predisposing the cow to fatty liver disease (Bobe et al., 2004).

Factors Influencing the Magnitude of Adipose Mobilization

The extent of adipose mobilization is influenced by both physiological and management factors. Cows with higher body condition scores (BCS ≥ 3.75) at calving tend to experience greater and more rapid NEFA release postpartum compared to cows in moderate condition, as over-conditioned cows exhibit reduced DMI and heightened lipolytic responses (Rukkwamsuk et al., 1998; Garnsworthy and Topps, 1982; Roche et al., 2009). Hormonal adaptations in late gestation—most notably the shift toward insulin resistance—heighten the rate and extent of lipolysis in transition cows. Postpartum increases in GH disrupt insulin's lipogenic effects and stimulate lipid mobilization (Lucy et al., 2016). Overweight cows, already predisposed to insulin insensitivity, exhibit exaggerated NEFA release and hepatic lipid accumulation (Rico et al., 2017). Inflammatory stress further compromises insulin signaling in adipose tissue, exacerbating NEFA mobilization (LPS-induced IR) while metabolic inflammation creates a vicious cycle of hormonal dysregulation and fat breakdown (Qiao et al., 2024). Together, these findings underscore the central role of endocrine and inflammatory dynamics in driving transition-associated adipose mobilization. Nutritional factors, including prepartum diet composition and energy density, can either mitigate or exacerbate NEFA release (Douglas et al., 2006; Grummer, 1995). Management stressors — such as regrouping, overcrowding, or environmental heat stress — may further elevate NEFA through increased cortisol secretion (Collier et al., 2012, Contreras, 2018). Additionally, genetic variation influences lipolytic capacity, with some cows inherently mobilizing more adipose in response to negative energy balance (van Knegsel et al., 2014). Collectively, these factors underscore the importance of individualized and proactive transition cow management

Metabolic Fates of NEFA

Once in circulation, NEFA have three principal metabolic fates: oxidation for energy, re-esterification into triglycerides for storage, or incorporation into milk fat (Drackley, 1999; McNamara, 2012). In the liver, NEFA can be fully oxidized via the tricarboxylic acid (TCA) cycle to produce ATP or partially oxidized to ketone bodies — β-hydroxybutyrate (BHB), acetoacetate, and acetone — which are then released into circulation for peripheral energy use (Herdt, 2000). If hepatic NEFA uptake exceeds the oxidative capacity, excess NEFA are re-esterified into triglycerides. In monogastrics, these triglycerides can be packaged into very-low-density lipoproteins (VLDL) and exported, but in ruminants, hepatic VLDL export is relatively inefficient, making the liver particularly susceptible to fat accumulation (Bauchart et al., 1990; Grummer, 1993). A portion of circulating NEFA can also be taken up by the mammary gland and incorporated directly into milk fat, particularly in early lactation when mobilization is high (Palmquist et al., 1993). The balance among these metabolic fates is crucial for maintaining energy supply without inducing metabolic disorders.

Links to Metabolic Disorders

Excessive adipose mobilization is a major risk factor for transition cow metabolic diseases. When NEFA oxidation in the liver exceeds the capacity of the TCA cycle, ketone bodies accumulate, leading to subclinical or clinical ketosis (McArt et al., 2012; Duffield et al., 2009). Alternatively, when re-esterification exceeds hepatic VLDL export capacity, triglycerides accumulate within hepatocytes, resulting in hepatic lipidosis (Bobe et al., 2004; Grummer, 1993). Both ketosis and fatty liver reduce feed intake, depress immune function, and impair reproductive performance (LeBlanc, 2010; Roche et al., 2009). These disorders can also have long-term consequences for productivity, as affected cows are more likely to be culled in the first 60 days in milk (McArt et al., 2012; Seifi et al., 2011). Prevention hinges on strategies that moderate the rate of adipose mobilization during the critical pre- and post-calving period.

Controlled Mobilization as a Management Goal

From a management perspective, the goal is not to eliminate adipose mobilization but to control its magnitude and timing. Moderate prepartum mobilization can be beneficial, as it provides the metabolic substrates needed for early lactation without overwhelming hepatic function (Grummer, 1995; Drackley, 1999). Controlled mobilization may also “prime” the metabolic system, improving the cow’s ability to handle the high lipid flux that occurs postpartum (van Knegsel et al., 2014). Strategies to achieve controlled mobilization include maintaining optimal pre-calving BCS (2.75–3.5), preventing excessive DMI declines prepartum, and avoiding environmental or social stressors that exacerbate lipolysis (Overton and Waldron, 2004; Roche et al., 2009). Emerging evidence suggests that structured prepartum exercise may serve as an additional tool to regulate adipose mobilization, enhance muscle tone, and improve metabolic flexibility without compromising welfare (Goselink et al., 2020). This approach aligns with the broader objective of preparing the cow’s metabolic machinery for the demands of early lactation while minimizing the risk of metabolic disorders.

Skeletal Muscle Mobilization and Protein Metabolism During the Transition Period

While adipose tissue mobilization is the primary strategy for meeting energy deficits during the transition period, skeletal muscle serves as a critical reserve of amino acids for both maternal and fetal metabolism. In late gestation and early lactation, muscle protein catabolism provides substrates for gluconeogenesis, supports colostrum and milk protein synthesis, and helps maintain essential body functions when dietary amino acid supply is inadequate (Bell, 1995; van der Drift et al., 2012). Controlled mobilization of skeletal muscle is a normal adaptive process; however, excessive or prolonged muscle breakdown can reduce maternal muscle mass, compromise physical strength, delay postpartum recovery, and negatively affect long-term productivity (Block et al., 2001). The magnitude of muscle mobilization is influenced by hormonal regulation, nutrient availability, physiological state, and management conditions, like the factors affecting adipose tissue mobilization (Bauman and Currie, 1980; Drackley, 1999). Recent longitudinal research has reinforced that the amount of muscle a cow carries at calving can have significant implications for milk yield patterns throughout lactation (Hanno et al., 2025). Understanding the mechanisms of muscle protein turnover, the consequences of depletion, and how to measure and manage muscle reserves is essential for supporting both metabolic health and production.

Protein Catabolism in Late Gestation

In late gestation, insulin resistance and endocrine shifts (e.g., reduced insulin signaling and elevated cortisol or growth hormone) initiate skeletal muscle proteolysis, yielding amino acids essential for gluconeogenesis and early lactation demands. This catabolism is regulated hormonally, with insulin resistance lifting inhibition on proteolysis pathways (De Koster & Opsomer, 2013), while transition-associated hormones further amplify protein breakdown (Sadri et al., 2023). Amino acids—especially alanine and glutamine—generated from muscle breakdown are transported to the liver and converted into glucose via gluconeogenesis, supporting both maternal energy needs and fetal development (Aschenbach et al., 2010). Muscle-derived amino acids also contribute to the synthesis of colostrum and milk proteins (Boerman, 2021). However, when proteolysis exceeds adaptive levels, structural muscle reserves can be compromised, reducing physical capacity and immune responsiveness—underscoring the importance of preserving muscle integrity during the transition period (Kuhla et al., 2011). The balance between necessary mobilization and preservation of structural muscle is therefore a key element of transition cow physiology.

Hormonal and Metabolic Regulation of Muscle Mobilization

The hormonal environment of late gestation and early lactation is designed to favor nutrient mobilization over tissue accretion. Reduced insulin sensitivity limits glucose uptake by muscle, sparing glucose for fetal and mammary use while promoting proteolysis (Bauman and Vernon, 1993; Vernon, 2005). Elevated cortisol enhances protein breakdown through activation of the ubiquitin-proteasome and lysosomal proteolytic pathways (Smith et al., 1973; Bradford and Allen, 2005). Growth hormone and insulin-like growth factor-1 (IGF-1) also influence protein turnover, with GH promoting mobilization of body reserves and IGF-1 modulating anabolic processes in response to nutrient status (Etherton and Bauman, 1998; Lucy et al., 2001). Pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) can further accelerate proteolysis during periods of metabolic or inflammatory stress (van der Drift et al., 2012; Bradford et al., 2015). This endocrine profile ensures nutrient delivery to the mammary gland and fetus but increases the risk of excessive muscle loss if negative energy balance is severe or prolonged.

Consequences of Excessive Muscle Loss

Loss of skeletal muscle mass beyond normal physiological levels has both immediate and lasting consequences for dairy cattle. In the immediate postpartum period, depleted muscle reserves can impair locomotion, reduce metabolic resilience, and hinder adaptation to the energetic demands of lactation (Block et al., 2001; Roche et al., 2009). Long-term, excessive muscle loss is associated with delayed resumption of ovarian activity and poorer reproductive performance (Butler, 2003; Wathes et al., 2007). Cows experiencing greater postpartum muscle mobilization are also at greater risk for immune suppression, increasing susceptibility to diseases such as mastitis and metritis (Bradford et al., 2015; LeBlanc, 2010). Recent evidence underscores the link between prepartum muscle status and lactation performance. In a 300-day longitudinal study, Hanno et al. (2025) categorized multiparous cows as high-muscle (HM; longissimus dorsi depth [LDD] > 5.0 cm) or low-muscle (LM; LDD ≤ 5.0 cm) based on ultrasound measurements within 24 hours postpartum. HM cows produced on average 1.9 kg/day more milk during early to mid-lactation than LM cows, though LM cows had a slight late-lactation advantage (+1.8 kg/day). These findings indicate that higher muscle reserves at calving may support improved early lactation performance but may also influence nutrient partitioning and tissue mobilization patterns later in lactation

Measuring Muscle Mobilization in Dairy Cattle

Quantifying muscle mobilization in dairy cattle can be achieved through both invasive and non-invasive methods. Invasive techniques such as carcass dissection and muscle biopsies provide direct measurements of protein content and composition (Bequette et al., 1998; Cantalapiedra-Hijar et al., 2018) but are impractical for routine use. Non-invasive approaches, particularly ultrasonography, are now widely used to measure muscle depth and cross-sectional area at specific anatomical landmarks such as the longissimus dorsi or semitendinosus muscles (van der Drift et al., 2012; Swanson et al., 2000). Ultrasound offers a repeatable and sensitive means of monitoring muscle changes in vivo and can be applied throughout lactation. Hanno et al. (2025) demonstrated the value of this method in their 300-day study, where differences in LDD between HM and LM cows persisted throughout lactation (~1 cm difference), highlighting the stability of this measurement and its potential as a predictor of production outcomes. Blood biomarkers such as 3-methylhistidine and creatinine can also indicate protein breakdown (Lobley et al., 2000; van der Drift et al., 2012), but ultrasound remains the preferred method for longitudinal, non-invasive monitoring.

Strategies to Preserve Muscle Mass Pre- and Post Partum

Maintaining muscle mass during the transition period requires a multifaceted approach involving nutrition, management, and possibly targeted physical activity. Diets should provide adequate metabolizable protein from both rumen-degradable and rumen-undegradable sources to support maternal needs, fetal growth, and colostrum synthesis (NRC, 2001; Lapierre et al., 2012). Managing prepartum BCS within the optimal range (2.75–3.5) can reduce the severity of negative energy balance and limit excessive mobilization of both adipose and muscle tissue (Roche et al., 2009; Overton and Waldron, 2004). Minimizing environmental and social stressors — such as overcrowding, regrouping, or heat stress — helps reduce cortisol-mediated proteolysis (Collier et al., 2012; Bradford and Allen, 2005). There is also emerging interest in structured prepartum exercise as a tool to maintain or enhance muscle reserves. Evidence from beef cattle, sheep, and preliminary dairy studies suggests that controlled physical activity can improve muscle tone, increase cardiovascular efficiency, and enhance metabolic flexibility (Lamb et al., 1979; Holcomb et al., 2001; Andueza et al., 2014). By promoting mild hypertrophy and preserving muscle depth, prepartum exercise may help cows enter lactation with greater reserves, potentially leading to improved early lactation milk yield as observed in HM cows by Hanno et al. (2025). Integrating exercise into transition cow programs will require careful evaluation of intensity, duration, and welfare considerations, but its potential role in supporting both metabolic health and production warrants further investigation.

Exercise Physiology in Ruminants

Exercise in ruminants has traditionally received far less research attention than in non-ruminant livestock or companion animals, but available evidence indicates that controlled physical activity can induce beneficial changes in cardiovascular fitness, muscle function, metabolic efficiency, and overall resilience (Lamb et al., 1979; Holcomb et al., 2001; Andueza et al., 2014). In production species, physical conditioning has been explored mainly in beef cattle, sheep, and goats, often with objectives related to improving muscle tone, calving ease, and feedlot performance (Parker et al., 1984; Thompson et al., 1983). While the metabolic demands of exercise are superimposed on those of late gestation, structured prepartum activity may help prepare transition cows for the challenges of early lactation by promoting controlled mobilization of adipose tissue, preserving muscle mass, and enhancing nutrient partitioning.

In the context of dairy cattle, exercise physiology research is emerging as a potential frontier for non-nutritional management strategies, aligning with the broader goal of optimizing metabolic health, production, and welfare in the transition period (Andueza et al., 2014; Holcomb et al., 2001).

Physiological Adaptations to Exercise in Ruminants

Exercise stimulates a series of systemic adaptations that can benefit late-gestation cows. Cardiovascular changes include increased stroke volume, improved oxygen delivery to tissues, and enhanced capillary density within skeletal muscle (Essén-Gustavsson and Lindholm, 1985;

Andueza et al., 2014). Musculoskeletal adaptations include increased oxidative enzyme activity, mitochondrial density, and myofibrillar protein synthesis, which can improve muscle endurance and strength (Holcomb et al., 2001; Andueza et al., 2014). These adaptations can contribute to greater muscular resilience during parturition and early lactation, potentially reducing injury risk and improving mobility. Metabolically, exercise increases insulin sensitivity in skeletal muscle, enhances fatty acid oxidation, and can modulate hormonal profiles in ways that improve nutrient use efficiency (Henriksen, 2002; Holcomb et al., 2001). For transition cows, these effects may translate to improved capacity to oxidize NEFA, thereby lowering the risk of hepatic lipidosis and excessive ketone production.

Intensity and Type of Exercise

The physiological responses to exercise are dose-dependent, with intensity, duration, and frequency influencing outcomes. **Low-intensity exercise** (e.g., extended walking at a slow pace) can increase energy expenditure modestly without causing significant metabolic stress, supporting moderate adipose mobilization (Blake et al., 1982; Goselink et al., 2020, Lu et al., 2022). **High-intensity exercise**, while less studied in dairy cattle, has demonstrated in other livestock species the ability to promote greater muscle hypertrophy, enhance cardiovascular conditioning, and stimulate more robust metabolic adaptations (Essén-Gustavsson and Lindholm, 1985; Thompson et al., 1983).

A potential advantage of high-intensity exercise in the prepartum dairy cow is its capacity to mobilize adipose reserves while simultaneously stimulating or preserving muscle mass — a key goal given the association between greater prepartum muscle reserves and improved early lactation performance observed by Hanno et al. (2025). However, intensity must be carefully managed to avoid excessive stress or energy deficits that could impair fetal growth or colostrum quality

Effects on Muscle Preservation

Maintaining muscle mass through the transition is critical for supporting metabolic health, locomotion, and milk protein synthesis. Exercise has been shown to counteract muscle atrophy by stimulating protein synthesis pathways, enhancing amino acid uptake, and reducing proteolysis (Hawley et al., 2014; Andueza et al., 2014). Given the findings of Hanno et al. (2025), which link higher postpartum longissimus dorsi depth (LDD) to increased early lactation milk yield, prepartum exercise could serve as a proactive means of ensuring cows enter lactation with optimal muscle reserves. This may be particularly valuable in herds where low-muscle cows (LDD ≤ 5.0 cm) are over-represented, as these animals could be targeted for conditioning programs to close the performance gap with high-muscle cows.

Exercise and Energy Partitioning

Prepartum exercise can alter nutrient partitioning by increasing peripheral tissue uptake of glucose and fatty acids, improving mitochondrial oxidative capacity, and modulating hormonal signals such as insulin, growth hormone, and cortisol (Henriksen, 2002; Holcomb et al., 2001). These changes may allow for more efficient use of mobilized NEFA and reduce the accumulation of triglycerides in the liver. Additionally, exercise-induced improvements in cardiovascular function can enhance nutrient and oxygen delivery to both maternal and fetal tissues, supporting overall health and performance (Essén-Gustavsson and Persson, 1985).
By “priming” these metabolic systems before calving, exercise may enable a smoother transition into lactation, characterized by less severe negative energy balance, lower circulating NEFA and

BHB concentrations, and improved milk component stability — all of which align with the metabolic resilience observed in cows with greater muscle reserves (Hanno et al., 2025).

Exercise as a Prepartum Management Strategy

The transition period is widely recognized as the most metabolically challenging stage of the dairy cow's production cycle, and while nutritional strategies remain the cornerstone of management, there is growing interest in complementary, non-nutritional interventions to support metabolic health (Overton and Waldron, 2004; Roche et al., 2009; Andueza et al., 2014). Structured physical activity during late gestation has been proposed as one such intervention, with the potential to enhance metabolic flexibility, regulate adipose mobilization, preserve muscle mass, and improve overall resilience during early lactation (Holcomb et al., 2001; Lamb et al., 1979; Andueza et al., 2014). Unlike dietary modifications, exercise directly engages musculoskeletal and cardiovascular systems, inducing systemic adaptations that may yield both short- and long-term benefits for productivity and welfare. Prepartum exercise could be particularly relevant considering the findings by Hanno et al. (2025), who demonstrated that cows with greater postpartum longissimus dorsi depth (LDD > 5.0 cm) produced more milk in early and mid-lactation compared with cows with lower muscle reserves. This association suggests that maintaining or increasing muscle mass before calving may offer a performance advantage in the critical first months of lactation, where milk yield peaks and metabolic stress is highest. In herds with a high proportion of low-muscle cows, targeted exercise could serve as a conditioning strategy to build reserves before calving, potentially closing the gap in early-lactation performance.

Mechanisms of Benefit

The benefits of prepartum exercise likely arise from multiple, interrelated mechanisms:

1. **Controlled Adipose Mobilization** – Exercise increases energy expenditure, promoting gradual adipose mobilization before calving. This may "precondition" hepatic lipid metabolism, reducing the severity of negative energy balance and lowering circulating NEFA and BHB concentrations postpartum (Holcomb et al., 2001; Drackley, 1999).
2. **Muscle Preservation and Development** – Exercise stimulates myofibrillar protein synthesis and reduces proteolysis, helping to maintain or increase muscle reserves before calving (Andueza et al., 2014; Hawley et al., 2014). Given the relationship between higher LDD and improved early lactation milk yield shown by Hanno et al. (2025), this could translate into a production advantage.
3. **Improved Cardiovascular and Metabolic Efficiency** – Physical activity enhances cardiovascular function, oxygen delivery, and mitochondrial oxidative capacity, improving the cow's ability to utilize mobilized substrates efficiently (Essén-Gustavsson and Lindholm, 1985; Henriksen, 2002).
4. **Potential Welfare Improvements** – Stronger musculoskeletal conditioning may support mobility, reduce injury risk during parturition, and improve cow comfort postpartum (Parker et al., 1984; Lamb et al., 1979, Daigle et al., 2017)

Lactate Threshold and Dairy Cattle Fitness Parameters

The lactate threshold (LT) is defined as the point during incremental exercise where blood lactate concentration begins to rise exponentially, indicating that lactate production exceeds the rate of clearance and oxidation. In human and equine physiology, LT is a widely accepted measure of aerobic capacity and endurance fitness (Esfarjani & Laursen, 2007; Heck et al., 1985; Poole & Gaesser, 1985; Eaton & Rose, 2006; Gondim et al., 2007). Although VO_2max testing is considered the gold standard for aerobic fitness, it is logistically challenging to perform in large ruminants, making LT a more feasible and field-applicable alternative for dairy cattle. Lactate is continuously produced from pyruvate by lactate dehydrogenase (LDH) isoforms, even under aerobic conditions (Brooks et al., 1999; Gladden, 2004). The role of lactate as both a metabolic byproduct and a fuel source depends on the relative abundance of LDH isoforms and the density of monocarboxylate transporters, particularly MCT1 and MCT4, in skeletal muscle fibers. Endurance training increases mitochondrial density, capillary networks, and expression of MCT1 and MCT4, enabling greater lactate uptake and oxidation, especially in oxidative muscle fibers (Dubouchaud et al., 2000; Brooks, 2020; Zhang et al., 2022). These adaptations elevate the lactate threshold, reflecting improved oxidative capacity and reduced dependence on anaerobic glycolysis at a given workload (Li et al., 2019).

Foundational Study: Davidson & Beede (2003)

Davidson and Beede (2003) developed and validated a system to assess physical fitness in dairy cows through structured exercise regimens. In their study, nonpregnant, nonlactating Holstein cows were assigned to either a sedentary control group or exercised using a mechanical walker for 1 or 2 hours every other day over a 60-day period. To evaluate physiological fitness, all cows underwent standardized treadmill tests at four timepoints (days 15, 30, 45, and 60), during which heart rate and plasma lactate concentrations were recorded. While single-point measurements of peak heart rate and lactate did not consistently distinguish treatment groups, repeated-measures analysis revealed that cows in the exercise groups exhibited significantly lower heart rates and plasma lactate responses by day 60, indicating improved cardiovascular and metabolic fitness. These findings demonstrate that repeated physiological testing can effectively quantify training adaptations in dairy cattle and offer a framework for evaluating the impacts of prepartum exercise interventions

Effects of Exercise and Pregnancy: Davidson & Beede (2009)

Building on the earlier work, Davidson and Beede (2009) explored the feasibility and effects of moderate-intensity prepartum exercise in pregnant Holstein cows. Over a 60-day period, cows in the exercise group walked every other day in a mechanical walker, while control cows remained confined. To assess physical fitness, all cows performed a standardized treadmill test at the end of the study. Trained cows demonstrated significantly greater endurance, lasting an average of 22.7 minutes compared to 18.3 minutes in controls. They also exhibited lower peak heart rates (144 vs. 156 bpm) and reduced blood lactate concentrations (1.4 vs. 3.2 mmol/L), indicating enhanced aerobic capacity and reduced physiological strain during exertion. Additionally, exercised cows maintained more stable blood pH, bicarbonate, and base excess values, reflecting improved acid–base regulation. Importantly, the study found that pregnancy did not diminish the positive effects of exercise, suggesting that structured physical activity can safely enhance fitness in late-gestation cows. The authors concluded that prepartum exercise may improve physiological resilience during the transition period without adverse health effects.

Lactate Threshold Parameters in the Current Study: Wilson et al. (2015) as a Model

The present trial adopted the LT determination approach outlined by Wilson et al. (2015), where incremental exercise testing is used to pinpoint the lactate threshold heart rate (LTHR). In their work, Holstein heifers were exercised in a horse walker with blood lactate (mmol/L) and heart rate (bpm) measured at each speed. LT was identified at **6.44 km/h**, where mean blood lactate reached **2.2 mmol/L** and heart rate averaged **145 bpm**.

In the current study, these parameters informed the classification of exercise intensities:

- **Low-Intensity Exercise (LIE):** ≤ 80% of LTHR (< 116 bpm)
- **Moderate-Intensity Exercise (MIE):** 81–99% of LTHR (117–144 bpm)
- **High-Intensity Exercise (HIE):** ≥ 100% of LTHR (≥ 145 bpm)

This method ensured that intensity prescriptions were based on physiological thresholds rather than arbitrary time or speed metrics, allowing for a standardized, individualized workload relative to each animal's aerobic–anaerobic transition point. By aligning intensity zones with LT physiology, the trial could more precisely evaluate how different exercise loads affect adipose mobilization, muscle preservation, and milk composition in the transition period.

Impacts of Exercise on Milk Yield and Components

Milk yield and composition during early lactation are determined by the interplay between nutrient availability, mammary gland function, and the cow's metabolic health during the transition period (Bauman and Currie, 1980; Bell, 1995; Palmquist et al., 1993). Exercise during late gestation has the potential to influence these parameters by modulating nutrient partitioning, enhancing metabolic flexibility, and altering tissue mobilization patterns. By promoting controlled adipose mobilization and preserving muscle mass before calving, prepartum exercise may improve substrate supply for milk synthesis in the critical early weeks postpartum. Conversely, if exercise is excessive or inadequately supported by diet, it could compromise milk yield or alter milk component concentrations due to competition for nutrients between maintenance, recovery, and lactation. Existing literature in dairy cattle is limited but suggests that structured exercise can yield favorable effects on metabolic indicators without negatively impacting milk production (Goselink et al., 2020; Lamb et al 1979). Furthermore, the relationship between muscle reserves at calving and early lactation performance, as shown by Hanno et al. (2025), provides an indirect rationale for exercise-based conditioning to influence milk yield and composition outcomes.

Effects on Milk Yield

Milk yield is largely a function of nutrient intake, mobilization, and utilization efficiency. Cows in better metabolic health at calving generally exhibit higher peak yields and improved lactation persistency (Roche et al., 2009; LeBlanc, 2010). Exercise could influence yield through two primary pathways: (1) improved metabolic capacity to handle lipid flux and maintain energy balance, and (2) preservation of muscle reserves, which may enhance early lactation performance. Hanno et al. (2025) reported that high-muscle (HM) cows — those with longissimus dorsi depth (LDD) > 5.0 cm postpartum — produced an average of 1.9 kg/day more milk during early and mid-lactation compared with low-muscle (LM) cows. While exercise was not part of their study, this association suggests that prepartum programs designed to preserve or build muscle could yield measurable milk production benefits. In beef and dairy studies where cows engaged in moderate physical activity prepartum, no reduction in yield was observed, and in some cases, yield increased due to improved metabolic adaptation ((Goselink et al., 2020; Lamb et al 1979). These findings collectively indicate that prepartum exercise, if managed properly, is unlikely to impair and may even enhance milk production in early lactation.

Effects on Milk Fat

Milk fat originates from two primary sources: de novo synthesis within the mammary gland from short-chain volatile fatty acids such as acetate and butyrate, and direct incorporation of preformed long-chain fatty acids derived from circulating non-esterified fatty acids (NEFA) (Palmquist et al., 1993). In cows experiencing severe negative energy balance, elevated NEFA levels increase the contribution of preformed fatty acids to milk fat but can suppress de novo synthesis, alter the fatty acid profile and potentially reduce overall milk fat concentration (Bauman and Griinari, 2003; Gross et al., 2011). By promoting earlier and more controlled adipose mobilization, prepartum exercise may help stabilize postpartum NEFA levels, thereby supporting more consistent de novo fatty acid synthesis and a more favorable milk fat composition. Although direct research on exercise and milk fat production in dairy cows is limited, Johnson et al. (2019) observed that cows subjected to high-intensity exercise during late gestation produced milk with higher fat concentrations compared to sedentary counterparts. These findings align with the metabolic priming hypothesis, suggesting that structured prepartum conditioning may enhance postpartum lipid metabolism and help maintain stable milk fat percentages—though further empirical validation is needed.

Effects on Milk Protein and Solids-Not-Fat (SNF)

Milk protein synthesis relies on an adequate supply of amino acids to the mammary gland, derived from both dietary protein and mobilized skeletal muscle tissue (Bequette et al., 1998; Lapierre et al., 2012). Once absorbed, these amino acids are transported to the mammary gland and incorporated into casein and whey—the primary protein fractions in milk. Glucose also contributes indirectly by supporting lactose synthesis, which regulates milk volume and can influence protein concentration through dilution effects (Bell, 1995). Cows entering lactation with greater skeletal muscle reserves may be better equipped to sustain milk protein output without excessive catabolism of structural protein. This is supported by Hanno et al. (2025), who reported that high-muscle (HM) cows produced more milk in early lactation, potentially due to greater amino acid availability for milk protein synthesis. Although milk protein percentage was not directly measured, the link between muscle reserves and lactation performance suggests that improved prepartum muscle status reduces metabolic constraint. Notably, Johnson et al. (2019)—the foundational study expanded upon in our work—found that cows undergoing high-intensity exercise in late gestation exhibited increased milk solids-not-fat content and milk protein concentrations, potentially driven by enhanced muscle derived amino acid availability Prepartum exercise could influence milk protein in two main ways:

1. **Preservation or Enhancement of Muscle Mass** – By stimulating mild hypertrophy and reducing prepartum proteolysis, structured exercise may ensure a larger amino acid pool is available for milk protein synthesis postpartum (Andueza et al., 2014; Hawley et al., 2014).
2. **Improved Metabolic Efficiency** – Exercise-induced improvements in insulin sensitivity and nutrient uptake can facilitate more efficient transfer of amino acids to the mammary gland (Holcomb et al., 2001; Henriksen, 2002).

Solids-not-fat (SNF), which includes milk protein, lactose, and minerals, is also sensitive to metabolic health. Cows in severe negative energy balance often exhibit declines in SNF percentages, partly due to reduced lactose synthesis and altered protein metabolism (Gross et al., 2011; Roche et al., 2009). By improving metabolic stability, prepartum exercise could help maintain SNF levels. This is particularly relevant for processors and product quality, as SNF concentration affects cheese yield and other dairy product manufacturing characteristics. Given the relationship between muscle reserves and production performance shown by Hanno et al. (2025), it is plausible that prepartum exercise could contribute to more stable or improved milk protein percentages in early lactation, especially in cows starting with lower baseline muscle depth.

Impacts of Exercise on Animal Behavior and Welfare

Any prepartum exercise program must be evaluated not only for its metabolic and production outcomes but also for its impact on cow welfare and behavior. Welfare encompasses physical health, freedom from injury and disease, and the ability to express natural behaviors, as well as avoidance of unnecessary stress (Fraser et al., 1997; von Keyserlingk et al., 2009). Prepartum exercise interventions must therefore be designed to support both physical and psychological well-being while minimizing the risk of injury, social disruption, or excessive fatigue.

From a behavioral perspective, increased movement—such as access to exercise paddocks or pasture—allows dairy cows to exhibit more natural locomotor behaviors, which in turn supports improved muscle tone, joint function, and mobility. For example, cows with exercise paddock access during the dry period displayed reduced lying time and more allogrooming bouts (Cai et al., 2024). Additionally, pasture-housed cows demonstrated significantly higher stride frequency and standing activity compared to confined cows, indicative of better locomotor engagement (Alsaaod et al., 2022). Evidence also shows that even limited movement opportunities (e.g., one hour of exercise access) can increase daily steps by over 50% (Cellier et al., 2025). These studies highlight the welfare and physiological benefits of enhancing movement opportunity in dairy cow housing systems. In cattle and sheep studies, structured prepartum exercise has improved calving ease and postpartum recovery without evidence of increased distress or injury (Lamb et al., 1979; Parker et al., 1984). However, in dairy operations, logistical factors such as flooring type, group size, and handling frequency can influence whether exercise is experienced as a beneficial stimulus or a stressor. The study by **Daigle et al. (2017)** provides relevant evidence that exercise, when properly implemented, can be integrated without compromising cattle welfare. In their work evaluating behavioral and locomotion responses to structured activity, cattle exhibited no increase in indicators of stress, such as restlessness or avoidance behaviors, and maintained normal lying times and rumination patterns. Importantly, exercise programs that included gradual acclimation and low-stress handling not only avoided negative impacts but also showed potential to enhance mobility and maintain hoof and leg health over time. This suggests that well-managed prepartum exercise may have dual benefits — supporting both welfare and metabolic adaptation.

It is critical, however, to recognize that poorly designed exercise regimens can have the opposite effect. Overly strenuous or prolonged activity, inadequate nutrition to match increased energy demands, or conditions that cause slipping or crowding can result in lameness, fatigue, or elevated stress hormone levels (Cook and Nordlund, 2009; Tucker et al., 2021). Therefore, welfare-focused implementation should prioritize:

1. **Safe Walking Surfaces** — Non-slip flooring and comfortable footing to reduce injury risk.

2. **Gradual Introduction** — Allowing cows to adapt to increased activity over days to weeks.
3. **Adequate Rest Opportunities** — Ensuring lying times are not compromised, as rest is essential for rumination and hoof health.
4. **Nutritional Support** — Adjusting dietary energy and protein to meet both gestational and exercise-related demands.

From a behavioral health perspective, exercise may also enhance mental stimulation and provide variety in the cow's daily routine, potentially mitigating boredom and frustration often seen in confinement systems (Rushen et al., 2008). When combined with low-stress handling, consistent routines, and appropriate group management, prepartum exercise can be implemented as a welfare-positive practice that aligns with industry and consumer expectations for humane animal care.

Research Gaps and Rationale for the Current Study

Despite significant advances in understanding the metabolic challenges of the transition period and the potential role of exercise, several critical knowledge gaps remain:

1. **Lack of High-Intensity Exercise Data in Transition Dairy Cows**

   While moderate exercise has been shown to adipose mobilization and metabolic resilience in dairy cattle (Goselink et al.), there is little evidence evaluating the effects of **high-intensity** prepartum exercise on metabolic outcomes, tissue mobilization, and lactation performance.

2. **Importance of Muscle Reserves for Lactation Performance**

   The longitudinal cohort study by Hanno et al. (2025) revealed that cows with greater longissimus dorsi depth (LDD > 5.0 cm) postpartum produced approximately 1.9 kg/day more milk during early and mid-lactation compared to those with lower muscle depth. This finding highlights the potential productivity advantage of improved muscle reserves at calving.

3. **Lack of Mechanistic Evidence Connecting Prepartum Exercise to Milk Composition and Welfare**

   The Kansas State University study (Johnson et al., 2019) demonstrated that pregnant heifers walked for up to 45 minutes, four days per week during gestation produced higher milk protein and solids-not-fat (SNF) concentrations, as well as increased milk fat and energy-corrected milk during early lactation, with no adverse effects on locomotion, calving ease, or health. However, this study was limited to heifers and did not examine underlying metabolic markers or tissue reserve dynamics.

4. **Underexplored Behavioral and Welfare Dimensions of Prepartum Exercise**

   While Daigle et al. (2017) provided evidence that structured exercise in cattle can be implemented without adverse welfare outcomes (e.g., changes in lying behavior, rumination, or stress-related behaviors), its implications for **pregnant dairy cows** remain largely untested.

Justification

High-intensity prepartum exercise represents an emerging, yet underexplored, management strategy for improving transition cow health and productivity. The present study was designed to address key gaps in the literature by systematically evaluating the effects of late-gestation exercise protocols that exceed the intensity of moderate walking. Specifically, this research investigates the influence of high-intensity prepartum exercise impacts both adipose and skeletal muscle differences, and subsequent early lactation milk yield and composition. A central focus of the study is to determine whether exercise-induced preservation or enhancement of muscle reserves, as identified by Hanno et al. (2025) as a determinant of early lactation performance, translates into measurable improvements in both milk yield and the stability of milk components. Additionally, this work extends the findings of Johnson et al. (2019), who reported that gestational exercise in dairy heifers improved milk fat, protein, solids-not-fat (SNF), and energy-corrected milk yield without compromising health or calving outcomes. By applying these concepts to dairy heifers and incorporating differing body condition assessments, the current study seeks to uncover the mechanistic pathways linking exercise to production outcomes.

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Chapter 2 - Effects of exercise on body composition and milk composition in pregnant Holsteins

Introduction

The transition from late gestation into early lactation represents one of the most physiologically challenging periods in the life of a dairy cow. During this time, nutrient demands rise sharply due to rapid fetal growth, colostrum synthesis, and the impending onset of lactation (Drackley, 1999). However, during early lactation, dry matter intake typically falls short of meeting the nutritional demands of milk production, leading to a state of negative energy balance. As a result, dairy cattle must mobilize internal body reserves—primarily adipose tissue—to meet their energy needs. When this mobilization is excessive or poorly regulated, it increases the risk of metabolic disorders such as ketosis and hepatic lipidosis, particularly in animals entering lactation with elevated body condition scores (Grummer, 1995; Lei and Simões, 2021).

In nulliparous dairy heifers, this transition into a lactation period presents an even more unique metabolic challenge. Compared to multiparous cows, heifers often enter lactation with more stored body fat, higher body condition scores, and less developed metabolic flexibility (Caixeta et al., 2015). These characteristics make heifers particularly vulnerable to metabolic disruption at calving. A higher prevalence of subclinical ketosis and impaired liver function has been observed in animals that were over-conditioned at calving, a condition often linked to abrupt, excessive mobilization of non-esterified fatty acids (NEFAs) and subsequent hepatic triglyceride accumulation (Bobe et al., 2004; Lei and Simões, 2021).

Traditional dry period management has emphasized rest and minimal physical exertion, under the assumption that reduced activity prevents undue stress before parturition. However, controlled physical activity during late gestation provides significant metabolic and physiological benefits (Goselink et al., 2020). In both human and animal models, prepartum exercise has been shown to enhance insulin sensitivity, support muscle maintenance, and prime lipid metabolism by initiating gradual adipose mobilization (Marcinko et al., 2015; Carter et al., 2010; Goselink et al., 2020). In equine studies, for example, structured exercise programs reduced fat mass in a dose-dependent manner, depending on the intensity of training (Carter et al., 2010). Similar work in dairy cattle has shown that even modest levels of late gestation exercise can improve postpartum energy balance and metabolic resilience (Goselink et al., 2020).

Despite these promising results from exercise on metabolic improvements, research on the role of exercise in dairy heifers during late gestation remains limited, particularly in terms of how exercise intensity influences fat mobilization, muscle maintenance, and lactational readiness. Additionally, there is little clarity on how different intensities of prepartum physical activity affect milk composition, transition-related stress, or early lactation outcomes. In a previous study in our laboratory conducted by Johnson et al. (2019), we found that heifers that were exercised at a high intensity for 8 weeks in late gestation had increased milk fat, milk protein, and milk SNF percentages during the first 15 weeks of the subsequent lactation. We speculated that exercise improved milk components as a direct result of fat mobilization and muscle hypertrophy; however, we did not specifically assess body composition in terms of subcutaneous fat or muscle mass.

Given the metabolic vulnerability of nulliparous heifers and the known risks of over-conditioning at calving, there is a critical need to explore non-nutritional interventions that can improve the physiological responses of these animals. Exercise represents a potentially simple, low-cost strategy to improve prepartum metabolic status, but its implementation in modern dairy systems requires evidence-based guidance on frequency, duration, and intensity. This study was designed to investigate how prepartum exercise at varying intensities influences physical parameters and metabolic parameters in dairy heifers, to identify strategies to optimize transition success, reduce metabolic burden, and support long-term performance, and to further explore the findings of Johnson et al., (2019). We tested the null hypothesis that exercise intensity does not impact body composition or subsequent tissue catabolism during early lactation.

Materials and Methods

IACUC Approval

The Kansas State University Institutional Animal Care and Use Committee approved all protocols for experimental use of live animals (**Protocol #4903**).

Housing and Care of Animals

Pregnant Holstein heifers (n=34) were enrolled in 2 cohorts based on the expected calving date (90.27 ± 6.35 d prepartum) for two concomitant 8-week exercise trials. All animals were housed and originated from the Kansas State University Dairy Teaching and Research Center for the entirety of the trial, dating from July 2024 to March 2025. During the months preceding each of the trials, the heifers were housed either in dry lot pens with access to a single-roof shelter or in a multi-acre pasture. During the exercise trials, heifers were housed in a dry lot with access to a single-roof shelter and provided *ad libitum* water and a total mixed ration (TMR) (Appendix A1-A3).. The TMR was delivered twice daily**.** At roughly 4 weeks before their individual expected calving date, all heifers were moved to the maternity barn, which consisted of a single-roofed open pen shelter, equipped with a swamp cooler. After calving, all animals were moved into one singular fresh cow pen that was a sand-bedded free-stall barn, with a sloped roof over their bed. All animals in the fresh pen were 4 weeks or less into their lactation and were offered TMR twice daily, with ad libitum water and mineral access.

After 4 weeks of lactation, the cows were grouped and housed in 3 separate sand-bedded free-stall barns with roofs over their beds. Animals in different treatment groups were randomly distributed among pens, dependent upon their stage of lactation and the availability of certain pens to avoid overstocking in the facility. Each of the pens consisted of a sloped roof structure with sand-bedded freestalls, affixed with shades and sprinklers, with management being consistent among pens. Cows had ad libitum access to food, minerals, and water, with TMR being delivered twice daily. (Appendix A1-A3).

Experimental Design

Heifers in each of the two cohorts were stratified into one of three treatment groups based on pre-trial subcutaneous (SQ) fat over the proximal gluteobiceps (thurl). Stratification consisted of ranking all animals from least to most adipose depth, starting with the three heifers that had the least SQ fat, then drawing their numbers manually. First numbers were always assigned to the sedentary group (SED; n = 12) where animals did not undergo any exercise and were penned adjacent to the free walker. The second identification number was assigned to the low intensity group (LI; n=12), which consisted of exercise 3x a week at a pace estimated to achieve 70% lactate threshold. And the third number was assigned to the high intensity group (HI; n=10), which consisted of exercise 3x a week at a pace estimated to achieve 130% lactate threshold in short intervals. This process was repeated until all animals were assigned to their respective treatment groups, with adipose depth means being as standardized as possible between the three groups.

All exercise treatments were administered Mon, Wed and Fri in an 8-panel motorized free-walker (Priefert, Mt. Pleasant, Texas). Estimation of lactate threshold for high and low intensity exercise were based on the methods of Wilson et al**. (**2015), where 8 non-pregnant Holstein heifers around 9-10 months in age were fitted with indwelling jugular catheters and equine heart rate monitors and exercised at increasing speeds, with 1-minute rests between intervals to obtain HR recording and blood sampling. This was repeated for 10 intervals, with final speeds reaching 10.5km/h. Results of this interval session were then used to establish an exercise baseline in dairy cattle after measuring lactate accumulation in the blood. In our trial, heifers were acclimated to the walker for two weeks, with different intensities beginning on week 3. The full exercise regime can be viewed in table 2.1. Heifers in both the low and high intensity groups walked the same distance for each exercise bout, ranging from 1km to 3.04km. Treatment ceased at 33.96 ± 6.92 d prepartum

Exercise Treatments

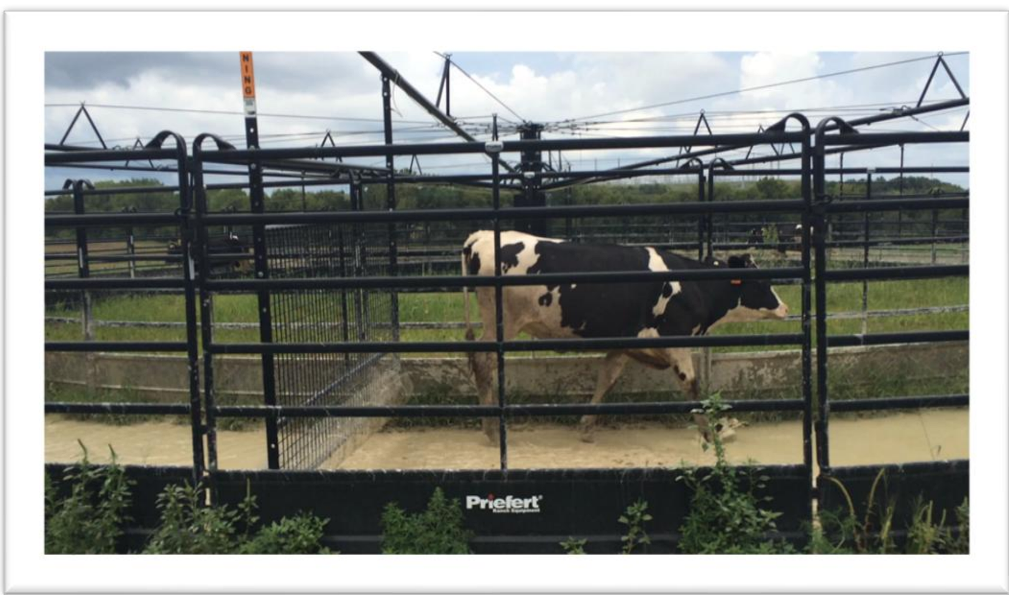

Figure 2.1 The 8-panel exerciser used for exercise treatments (photo by the author).

Before the heifers were moved to the walker, they were pre-sorted into their corresponding treatment groups and moved as a group (*N=5 to 6 animals)* to the walker starting approximately at 0430 on Monday, Wednesday and Friday. All treatment groups were moved to the walker on exercise days, and the SED group resided in a holding pen next to the walker away from food and water.

After arriving at the walker, an individual heifer was placed into each segment of the walker, with first treatment beginning approximately at 0500. The order with which exercise treatments were performed was rotated each exercise day, to avoid confounding issues such as light and temperature. The panels of the exerciser were on occasion electrified to discourage heifers from stopping but this feature was minimally used *(<8 times over the entirety of the trial for 30 seconds*). To provide further encouragement, a research assistant on occasion would accompany the heifer in the bay during the treatment to keep forward momentum, ensuring proper speeds were being obtained. After both LI and HI groups received their exercise treatment for the day, all three groups were moved at the same time back to their dry-lot pen. The time that they returned back to their pens was dependent upon the week of exercise, with the approximate times back to their pen being in week eight by 0830 to 0900.

Table 2.1 Exercise treatment conditions and distances per exercise bout

Week	Low Intensity	High Intensity	Distance per Exercise Bout	
			Low	High
1	5 min w/u at 2.5 km/h 10 min at 3.5 km/h 5 min c/d at 2.5 km/h	5 min w/u at 2.5 km/h 10 min at 3.5 km/h 5 min c/d at 2.5 km/h	1 km	1 km
2	5 min w/u at 2.5 km/h 15 min at 3.5 km/h 5 min c/d at 2.5 km/h	5 min w/u at 2.5 km/h 15 min at 3.5 km/h 5 min c/d at 2.5 km/h	1.285 km	1.285 km
3	5 min w/u at 2.5 km/h 20 min at 3.5 km/h 5 min c/d at 2.5 km/h	5 min w/u at 2.5 km/h 1 min at 5 km/h 2 min at 3.5 km/h 1 min at 5 km/h 2 min at 3.5 km/h 1 min at 5 km/h 2 min at 3.5 km/h 5 min at 2.5 km/h 1 min at 5 km/h 2 min at 3.5 km/h 1 min at 5 km/h 5 min c/d at 2.5 km/h **(*100 % LT Intervals)**	1.58 km	1.51 km
4	5 min w/u at 2.5 km/h	5 min w/u at 2.5 km/h	1.86 km	1.88 km

Week	Low Intensity	High Intensity	Distance per Exercise Bout	
			Low	High
	25 min at 3.5 km/h 5 min c/d at 2.5 km/h	1 min at 5.5 km/h 2 min at 3.5 km/h 1 min at 5.5 km/h 2 min at 3.5 km/h 1 min at 5.5 km/h 2 min at 3.5 km/h 5 min at 2.5 km/h 1 min at 5.5 km/h 2 min at 3.5 km/h 1 min at 5.5 km/h 2 min at 3.5 km/h 1 min at 5.5 km/h 2 min at 3.5 km/h 5 min c/d at 2.5 km/h **(*110 % LT Intervals)**		

Week	Low Intensity	High Intensity	Distance per Exercise Bout	
			Low	High
5	5 min w/u at 2.5 km/h 30 min at 3.5 km/h 5 min c/d at 2.5 km/h	5 min w/u at 2.5 km/h 1.5 min at 5.75 km/h 2 min at 3.5 km/h 1.5 min at 5.75 km/h 2 min at 3.5 km/h 1.5 min at 5.75 km/h 2 min at 3.5 km/h 5 min at 2.5 km/h 1.5 min at 5.75 km/h 2 min at 3.5 km/h 1.5 min at 5.75 km/h 2 min at 3.5 km/h 1.5 min at 5.75 km/h 2 min at 3.5 km/h 5 min c/d at 2.5 km/h **(*115 % LT Intervals)**	2.17 km	2.19 km
6	5 min w/u at 2.5 km/h 35 min at 3.5 km/h 5 min c/d at 2.5 km/h	5 min w/u at 2.5 km/h 1.5 min at 6 km/h 2 min at 3.5 km/h 1.5 min at 6 km/h 2 min at 3.5 km/h 1.5 min at 6 km/h 2 min at 3.5 km/h 5 min at 2.5 km/h 1.5 min at 6 km/h 2 min at 3.5 km/h 1.5 min at 6 km/h	2.46 km	2.49 km

Week	Low Intensity	High Intensity	Distance per Exercise Bout	
			Low	High
		2 min at 3.5 km/h 1.5 min at 6 km/h 2 min at 3.5 km/h 1.5 min at 6 km/h 2 min at 3.5 km/h 5 min c/d at 2.5 km/h **(*120 % LT Intervals)**		
7	5 min w/u at 2.5 km/h 40 min at 3.5 km/h 5 min c/d at 2.5 km/h	5 min w/u at 2.5 km/h 2 min at 6.25 km/h 2 min at 3.5 km/h 2 min at 6.25 km/h 2 min at 3.5 km/h 2 min at 6.25 km/h 2 min at 3.5 km/h 5 min at 2.5 km/h 2 min at 6.25 km/h 2 min at 3.5 km/h 2 min at 6.25 km/h 2 min at 3.5 km/h 2 min at 6.25 km/h 2 min at 3.5 km/h 2 min at 6.25 km/h 5 min c/d at 2.5 km/h **(*125 % LT Intervals)**	2.75 km	2.78 km
8	5 min w/u at 2.5 km/h	5 min w/u at 2.5 km/h 2 min at 6.5 km/h	3.04 km	2.96 km

Week	Low Intensity	High Intensity	Distance per Exercise Bout	
			Low	High
	45 min at 3.5 km/h 5 min c/d at 2.5 km/h	2 min at 3.5 km/h 2 min at 6.5 km/h 2 min at 3.5 km/h 2 min at 6.5 km/h 2 min at 3.5 km/h 5 min at 2.5 km/h 2 min at 6.5 km/h 2 min at 3.5 km/h 2 min at 6.5 km/h 2 min at 3.5 km/h 2 min at 6.5 km/h 2 min at 3.5 km/h 2 min at 6.5 km/h 2 min at 3.5 km/h 5 min c/d at 2.5 km/h **(*130 % LT Intervals)**		

Table 2.1 Exercise treatment conditions and distances per exercise bout. *Lactate Thresholds were estimated based on predetermined Lactate Threshold in non-pregnant Holstein heifers (Wilson, et al., 2015).

w/u = warmup period, c/d= cooldown period

Evaluated Parameters

Weekly during treatment, heifers were weighed, and an ultrasound was used to quantify subcutaneous adipose thickness, and semitendinosus depth, width, and area. A single mammary ultrasound measure, the parenchyma, was scanned at the beginning, middle, and end of the exercise treatment period. Calving observations such as calving date, calving ease, and calf weight were recorded in the maternity barn. Postpartum carryover effects on milk yield (daily) and components (1 d/wk) were assessed for 10 wk. Milk measure monitoring was accompanied by bi-weekly ultrasound scans to track postpartum body composition. To further monitor carryover effects of exercise, all female heifer calves that were born from mothers in this trial were monitored biweekly from birth to weaning (*6 weeks of age)*. Measurements included body weight, and skeletal growth parameters (*Hip height, Wither Height, Hip Width etc.*).

Ultrasound Measures

Ultrasounds were conducted in a covered area that had a holding pen attached adjacent to the ultrasound area. The Exago portable ruggedized ultrasound scanner with a linear transducer (IMV Imaging, Saint-Ouen-sur-Iton, France) was utilized to obtain the subcutaneous fat measure. The frequency was set to 2.5-3.5 MHz, and skin contact with the transducer was made using a vegetable oil solution that was applied directly to the skin over the gluteobiceps. There was no clipping of the hair required to make good contact and obtain easily identifiable images. Once the image was obtained, the ultrasound technician then manually measured adipose thickness starting at the superficial fascia and ending the measurement at the profound fascia. The linear transducer was also utilized to obtain the semitendinosus cross section image following similar protocols manually tracing the semitendinosus area, width, and depth immediately after the image was obtained. To obtain images of the mammary parenchyma the transducer was switched to the micro-convex attachment, and a vitreous ultrasound specific gel was applied at the base of the right rear teat, with ultrasound measurements also being manually traced in real time.

Lameness Scores

Lameness scores were conducted on all heifers 1 day before exercise treatment started, and then 1x/wk every Thursday before ultrasounds, for the entirety of the 8-week treatment period. Scores were assigned on a 1-5 basis, with 1 being normal and 5 being severely lame. Visual parameters used to assign scores were outlined by Sprecher, Hostetler and Kaneene 1997.

Scores were assigned based on a visual assessment during the walk from their housing pens up to the ultrasound rail on a concrete path by one individual during the entirety of the trial. If a score greater than one was assigned, the assessor would then confirm with a colleague whether the score had merit. Per protocol, if the score of ≥2 was to persist for two weeks, closer inspection of the animal was warranted during the treatment period. This only occurred once during the trial, and a qualified veterinarian familiar with the animals and the facility determined that the lameness was not associated with the exercise treatment but rather an injury that occurred in the pen. After the cessation of the exercise portion of the trial, no further lameness assessments were conducted.

Body Condition Score

Calving ease was assessed by a subjective scale of 1 to 5 by trained dairy personnel at each calving as follows: 1 = no problem, 2 = minor problem, 3 = needed assistance, 4 = considerable assistance, 5 = very difficult (e.g. C-section or veterinarian intervention). The difference in calving day was calculated by subtracting the actual calving day from the predicted calving date based on the breeding date. Birth weights (for both bull and heifer calves) and gender of claves were recorded. Any incidences of metabolic disorders (e.g. hypocalcemia, metritis, ketosis) were observed and recorded by the dairy unit manager during the first week of calving

Calving Observations

Calving ease was assessed by a subjective scale of 1 to 3 by trained dairy personnel at each calving as follows: 1 = no problem, 2 = some assistance required, 3 = needed significant assistance, (e.g. C-section or veterinarian intervention). The difference in calving day was calculated by subtracting the actual calving day from the predicted calving date based on the breeding date. Birth weights (for both bull and heifer calves) and gender of calves were recorded following farm protocols.

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Calf Performance (Birth to Weaning)

Heifer calves (n = 10; *Sed = 3, LI = 4, HI= 3*) from cows in the exercise trial were enrolled at birth in the study from Cohort 1 or Cohort 2 (day 0). At 1 day of age, the following skeletal measurements were collected: Hip Height (HH), Wither Height (WH), Hip Width (HW), and Hip Length (HL). These measurements were recorded bi-weekly, including body weight, until the heifer calves reached 6 weeks of age. All heifer calves throughout the sampling period received the same amount of milk standardized to their age (i.e. the amount of milk increased as the calves aged but was kept the same at each age), while being offered grain and water ad libitum. Bulk tank milk was sampled weekly to monitor any dietary changes between periods, with no differences occurring between weeks.

Milk Sampling and Milk Weight

Daily milk weights were recorded by the milkers by reading the individual milk meter per cow after every milking. Composite milk samples were taken every Monday or Thursday for all three milkings. "Milk sampling days" were predetermined with the help of trained dairy personnel, to occur only on Mondays and Thursdays to maintain the working efficiency of the dairy. Cows were sampled 1x a week, starting after day 3 of lactation, with their calving date determining which of the two pre-determined weekdays they were sampled. Cows that calved earlier in the week were sampled only Thursdays, while cows that calved later in the week were sampled on Mondays to have only one sample per week per cow. To collect the milk sample, the udder was prepped with a pre-milking udder wash to sterilize the teat ends, and the teats were then dried with a hand towel, with all teats being hand stripped prior to sampling. Samples were taken from the teat closest to the technician by removing a sample into a labeled vial and moving from front to back, taking a sample from each quarter (*approximately 1.0 mL)* and inserting it into the same milk vial. Once all quarters were sampled, the cow then underwent the routine milking. This process was then repeated for all three milkings on each sample day (*approximate ending volume being 11.0 mL).* A preservative tablet was immediately placed in each sample, and samples were stored in airtight containers at 4 C before being shipped every Tuesday to AgSource (Marshfield, Wisconsin) for analysis for complete milk components. Milk composition measures included the following; fat, true protein, lactose, SNF, cells, urea, monounsaturated fatty acids, polyunsaturated fatty acids, saturated fatty acids, total unsaturated fatty acids, short chain fatty acids, medium chain fatty acids, long chain fatty acids, trans fatty acids, acetone, BHB, C14_0, C16_0, C18_0, C18_1, denovo fatty acids, mixed fatty acids, and preformed fatty acids.

Statistical Analysis

All data were combined for cohorts 1 and 2 and *analyzed (calf data was input and analyzed independently for heifer calf follow-up*) using the International Business Machine (IBM), Inc. Statistical Package for the Sciences (SPSS), version 29.0.2.0. A two-way repeated measures ANOVA with a stratified randomized design with repeated measures was used, utilizing the fixed effect of the treatment to determine the effect of sedentary, low-intensity, and high-intensity exercise over time on SQ depth, ST area, depth and width, body weight, BCS, milk weight and milk composition. The main effects of time and treatment, and time * treatment was compared for each variable. For the three calving observations (*calving date, calving ease, and calf weight),* a one-way ANOVA was performed using the fixed effect of treatment. For all data analysis, significance was set at $p = \leq .05$

Treatment Period Results

Subcutaneous Backfat Adipose Depth

Eight weeks of exercise caused differential mobilization of subcutaneous adipose reserves that was dependent on exercise intensity (Week × Treatment*:* *P* < 0.001; Table, 2.2). In contrast, low intensity exercise only reduced subcutaneous adipose tissue depth during week 8 compared with heifers that remained sedentary *(*Week × Treatment*:* P < *0.001; Fig 2.3).*

Measurement		Intensity Group			P-value		
		SED	LI	HI	Week	Treatment	Week * Treatment
SubQ Adipose Depth					0.02	0.43	<0.001
	Week 0	32.51 (1.02)	32.4 (1.017)	33.31 (1.14)			
	Week 1	32.62 (.998)	32.7 (.998)	33.86 (1.094)			
	Week 2	32.87(.985)	32.47 (.985)	33.12 (1.079)			
	Week 3	33.1 (.952)	32.3 (.952)	32.47 (1.043)			
	Week 4	33.08 (.985)	32.03 (.985)	31.43 (1.079)			
	Week 5	33.25 (.849)	31.78 (.849)	31.05 (.930)			
	Week 6	33.61 (.850)	31.88 (.850)	30.68 (.931)			
	Week 7	34.06 (.860)^{A}	31.39 (.860)^{A}	29.91 (.942)^{B}			
	Week 8	34.28 (.898)^{A}	30.9 (.898)^{B}	29.93 (.983)^{B}			

Table 2.2 Estimated marginal means of subcutaneous adipose tissue during 8 weeks of exercise in late gestation Holstein heifers

A, B; **Values in a row without common superscripts differ during that week of treatment (P ≤ 0.05).**

SEM values are reported in parentheses

Treatments: Sed = moved to exercise walker but remained in holding pen (n =12); LI = low intensity exercise heifers walked at a consistent speed of 2.5 kilometers per hour at increasing distances per week estimated to achieve 70% lactate threshold (n=12), HI = high intensity exercise heifers exercised in short sprint intervals of up to 6 kilometers per hour with increasing distances and speed per week estimated to achieve 130% lactate threshold by the end of the exercise period (n=10) .

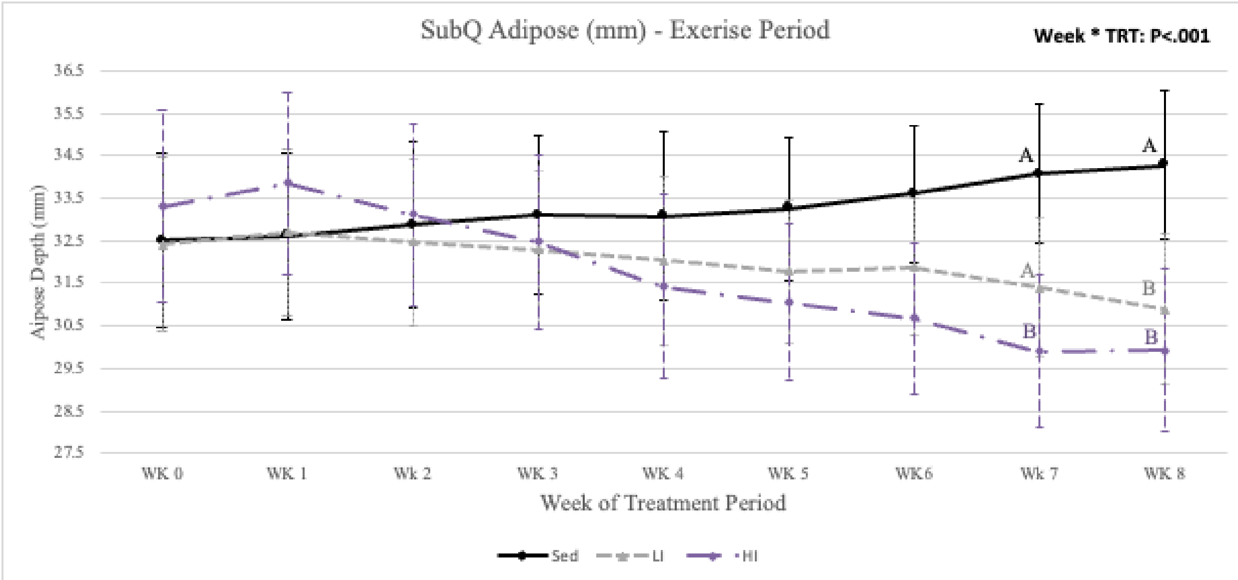

Figure 2.2 Estimated marginal means of subcutaneous adipose tissue during 8 weeks of exercise in late gestation Holstein heifers.

A, B; **Values in a row without common superscripts differ during that week of treatment (P ≤ 0.05).**

Treatments: Sed = moved to exercise walker but remained in holding pen (n =12); LI = low intensity exercise heifers walked at a consistent speed of 2.5 kilometers per hour at increasing distances per week estimated to achieve 70% lactate threshold (n=12), HI = high intensity exercise heifers exercised in short sprint intervals of up to 6 kilometers per hour with increasing distances and speed per week estimated to achieve 130% lactate threshold by the end of the exercise period (n=10) .

Body Condition Score

Exercise for eight weeks, regardless of intensity, caused a decrease in body condition scores during the exercise period (Week × Treatment: $P < 0.001$; Table. 2.3, Fig 2.3). In contrast, heifers who remained sedentary gained body condition during the exercise period.

Measurement		Intensity Group			Pvalue	
	SED	LI	HI	Week	Treatment	Week * Treatment
Body Weight				<0.001	0.12	<0.001
Week 0	3.3 (.123)	3.49 (.123)	3.725 (.134)			
Week 1	3.4(.116)	3.5 (.116)	3.73 (.127)			
Week 2	3.46(.122)	3.5 (.122)	3.73 (.134)			
Week 3	3.46 (.123)	3.5 (.123)	3.68 (.135)			
Week 4	3.6(.100)	3.5 (.100)	3.48 (.110)			
Week 5	$3.71(.081)^A$	$3.38\ (.081)^B$	$3.35\ (.089)^B$			
Week 6	$3.85(.079)^A$	$3.29\ (.079)^B$	$3.23\ (.087)^B$			
Week 7	$4\ (.061)^A$	$3.25\ (.061)^B$	$3.1\ (.067)^B$			
Week 8	$4\ (.065)^A$	$3.17\ (.065)^B$	$2.88\ (.071)^B$			

Table 2.3 Estimated marginal means of body condition score during 8 weeks of exercise in late gestation Holstein heifers.

***A, B*: Values in a row without common superscripts differ during that week of treatment (P ≤ 0.05).**

SEM values are reported in parentheses

Treatments: Sed = moved to exercise walker but remained in holding pen (n =12); LI = low intensity exercise heifers walked at a consistent speed of 2.5 kilometers per hour at increasing distances per week estimated to achieve 70% lactate threshold (n=12), HI = high intensity exercise heifers exercised in short sprint intervals of up to 6 kilometers per hour with increasing distances and speed per week estimated to achieve 130% lactate threshold by the end of the exercise period (n=10) .

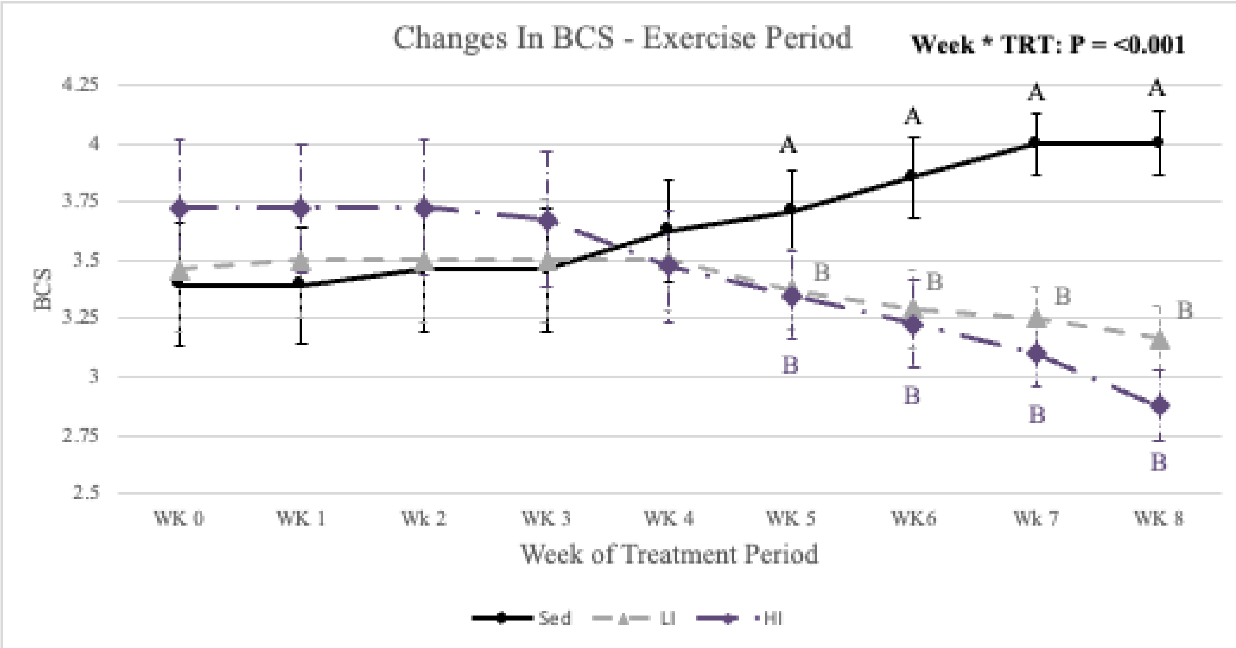

Figure 2.3 Estimated marginal means of body condition score during 8 weeks of exercise in late gestation Holstein heifers.

A, B; **Values in a row without common superscripts differ during that week of treatment ($P \leq 0.05$).**

Treatments: Sed = moved to exercise walker but remained in holding pen (n =12); LI = low intensity exercise heifers walked at a consistent speed of 2.5 kilometers per hour at increasing distances per week estimated to achieve 70% lactate threshold (n=12), HI = high intensity exercise heifers exercised in short sprint intervals of up to 6 kilometers per hour with increasing distances and speed per week estimated to achieve 130% lactate threshold by the end of the exercise period (n=10).

Semitendinosus Area

Eight weeks of exercise did not result in weekly differences in semitendinosus muscle area among exercise intensity groups (Week × Treatment: $P = 0.23$; Table 2.4). However, when averaged across the eight weeks, exercise intensity was a significant source of variation (Treatment: $P = 0.03$). Beginning in week 5, heifers that were exercised at high intensity had greater semitendinosus muscle area than sedentary heifers, and this difference persisted throughout the remainder of the exercise period.

Measurement	Intensity Group			Pvalue		
	SED	LI	HI	Week	Treatment	Week * Treatment
Semitendinosus Area				< 0.001	0.03	0.23
Week 0	247.17 (3.536)	244.68 (3.536)	254.30 (3.874)			
Week 1	246.68 (2.943)	245.42 (2.943)	253.40 (3.224)			
Week 2	247.92 (2.962)	245.75 (2.962)	253.60 (3.245)			
Week 3	247.08 (2.694)	246.92 (2.694)	255.40 (2.951)			
Week 4	247.58 (2.753)	247.58 (2.753)	256.60 (3.016)			
Week 5	246.91 (2.59)^{A}	248.41 (2.590)^{A}	258.60 (2.838)^{B}			
Week 6	246.58 (2.444)^{A}	250.16 (2.444)^{AB}	259.20 (2.677)^{B}			
Week 7	249.16 (2.244)^{A}	250.41 (2.244)^{A}	260.10 (2.458)^{B}			
Week 8	247.58 (1.961)^{A}	252.16 (1.961)^{A}	260.60 (2.149)^{B}			

Table 2.4 Estimated marginal means of semitendinosus muscle area tissue during 8 weeks of exercise in late gestation Holstein heifers.

***A, B*: Values in a row without common superscripts differ during that week of treatment (P ≤ 0.05).**

SEM values are reported in parentheses
Treatments: Sed = moved to exercise walker but remained in holding pen (n =12); LI = low intensity exercise heifers walked at a consistent speed of 2.5 kilometers per hour at increasing distances per week estimated to achieve 70% lactate threshold (n=12), HI = high intensity exercise heifers exercised in short sprint intervals of up to 6 kilometers per hour with increasing distances and speed per week estimated to achieve 130% lactate threshold by the end of the exercise period (n=10) .

Semitendinosus Muscle Depth

Eight weeks of exercise did not result in weekly differences in semitendinosus muscle depth among treatment groups (Week × Treatment: $P = 0.90$; Table 2.5). When averaged across the eight weeks, exercise intensity tended to influence semitendinosus depth variation (Treatment: $P = 0.07$). After seven weeks of exercise, animals in the high-intensity group had greater overall muscle depths when compared with both the low-intensity and sedentary groups.

Measurement	Intensity Group			Pvalue		
	SED	LI	HI	Week	Treatment	Week * Treatment
Semitendinosus Depth				<0.001	0.07	0.90
Week 0	61.66 (1.585)	62.89 (1.585)	64.16 (1.737)			
Week 1	61.06 (1.288)	60.76 (1.288)	63.17 (1.411)			
Week 2	61.55 (1.026)	62.89 (1.026)	64.81 (1.124)			
Week 3	62.42 (.958)	63.81 (.958)	65.43 (1.049)			
Week 4	63.14 (.986)	64.1 (.986)	66.15 (1.080)			
Week 5	63.58 (.970)^{A}	65.39(.970)^{A}	67.22 (1.062)^{B}			
Week 6	63.79 (.896)	65.38(.896)	67.11 (.981)			
Week 7	63.96 (.764)^{A}	65.63(.744)^{A}	67.42 (.837)^{B}			
Week 8	64.02 (.744)^{A}	65.84 (.744)^{A}	67.69 (.815)^{B}			

Table 2.5Estimated marginal means of semitendinosus muscle tissue depth during 8 weeks of exercise in late gestation Holstein heifers.

***A, B*: Values in a row without common superscripts differ during that week of treatment (P ≤ 0.05).**

SEM values are reported in parentheses

Treatments: Sed = moved to exercise walker but remained in holding pen (n =12); LI = low intensity exercise heifers walked at a consistent speed of 2.5 kilometers per hour at increasing distances per week estimated to achieve 70% lactate threshold (n=12), HI = high intensity exercise heifers exercised in short sprint intervals of up to 6 kilometers per hour with increasing distances and speed per week estimated to achieve 130% lactate threshold by the end of the exercise period (n=10) .

Semitendinosus Muscle Width

Eight weeks of exercise did not result in weekly differences in semitendinosus muscle width among all exercise intensity groups (Week × Treatment: *P* = 0.79; Table 2.6). However, when averaged across weeks, exercise intensity was a significant source of variation (Treatment*:* *P* = 0.003). Beginning in week 3, heifers exercised at high intensity had greater semitendinosus muscle width than sedentary heifers, and this difference persisted through the remainder of the exercise period. High-intensity heifers also had greater widths than low-intensity heifers from weeks 3 to 8. In contrast, heifers exercised at low-intensity had greater semitendinosus width compared with sedentary heifers only after eight weeks.

Measurement	Intensity Group			Pvalue		
	SED	LI	HI	Week	Treatment	Week * Treatment
Semitendinosus Width				0.58	0.003	0.79
Week 0	79.74 (1.245)	81.158 (1.245)	81.36 (1.364)			
Week 1	80.12 (1.217)	80.95 (1.217)	83.15 (1.333)			
Week 2	79.91 (1.099)	81.47 (1.099)	83.66 (1.204)			
Week 3	79.92 (.965)^{A}	81.14 (1.024)^{A}	84.08 (1.122)^{B}			
Week 4	79.79 (.806)^{A}	81.59 (.965)^{A}	84.34 (1.057)^{B}			
Week 5	79.7 (.648)^{A}	81.59 (.965)^{A}	83.73 (.883)^{B}			
Week 6	79.6 (.648)^{A}	81.66 (.648)^{A}	83.68 (.710)^{B}			
Week 7	79.75 (.656)^{A}	81.15 (.656)^{A}	84.06 (.719)^{B}			
Week 8	78.65 (.629)^{A}	81.28 (.629)^{B}	84.14 (.689)^{C}			

Table 2.6 Estimated marginal means of semitendinosus muscle tissue width during 8 weeks of exercise in late gestation Holstein heifers

***A, B*: Values in a row without common superscripts differ during that week of treatment (P ≤ 0.05).**

SEM values are reported in parentheses

Treatments: Sed = moved to exercise walker but remained in holding pen (n =12); LI = low intensity exercise heifers walked at a consistent speed of 2.5 kilometers per hour at increasing distances per week estimated to achieve 70% lactate threshold (n=12), HI = high intensity exercise heifers exercised in short sprint intervals of up to 6 kilometers per hour with increasing distances and speed per week estimated to achieve 130% lactate threshold by the end of the exercise period (n=10)

Bodyweight

Eight weeks of exercise did not cause differences in weekly weight gain among treatment groups during the exercise period (Week × Treatment: *P* = 0.884; Table 2.7). Similarly, when averaged across all eight weeks, the treatment group alone did not significantly affect overall weight gain (Treatment*:* *P* = 0.21).

Measurement		Intensity Group			Pvalue	
	SED	LI	HI	Week	Treatment	Week * Treatment
Body Weight (kg)				<0.001	0.89	0.50
Week 0	548.09 (29.752)	556.78 (29.752)	554.29 (32.591)			
Week 1	548.85 (28.720)	562.45 (28.720)	558.37 (31.462)			
Week 2	560.56 (28.351)	565.86 (28.351)	565.63 (31.057)			
Week 3	563.97 (28.273)	575.31 (28.273)	574.70 (30.972)			
Week 4	574.55 (29.295)	586.65 (29.925)	586.95 (32.090)			
Week 5	579.09 (27.873)	586.65 (27.873)	586.49 (29.788)			
Week 6	588.16 (27.193)	595.72 (27.193)	592.39 (29.788)			
Week 7	601.39 (27.959)	604.79 (27.959)	600.10 (30.682)			
Week 8	604.03 (28.782)	619.53 (28.782)	610.08 (31.529)			

Table 2.7 Estimated marginal means of body weight during 8 weeks of exercise in late gestation Holstein heifers.

SEM values are reported in parentheses

Treatments: Sed = moved to exercise walker but remained in holding pen (n =12); LI = low intensity exercise heifers walked at a consistent speed of 2.5 kilometers per hour at increasing distances per week estimated to achieve 70% lactate threshold (n=12), HI = high intensity exercise heifers exercised in short sprint intervals of up to 6 kilometers per hour with increasing distances and speed per week estimated to achieve 130% lactate threshold by the end of the exercise period (n=10) .

Mammary Parenchyma

Eight weeks of exercise did not cause any differences in mammary parenchyma area to occur between treatment groups during the exercise period (Week x Treatment: $P = 0.09$; Table 2.8). However, when averaged across weeks, the mammary development did trend towards reduced parenchyma as a direct result of exercise (Treatment: $P = 0.09$).

Measurement	Intensity Group			Pvalue		
	SED	LI	HI	Week	Treatment	Week * Treatment
Semitendinosus Width				<0.001	0.09	0.09
Week 0	126.5 (3.281)	129.167 (3.281)	120.1 (3.594)			
Week 1	124.75 (2.907)	125.00 (2.907)	116.5 (3.184)			
Week 2	123.5 (2.276)	119.167 (2.276)	113.6 (2.493)			

Table 2.8 Estimated marginal means of mammary parenchyma tissue width during 8 weeks of exercise in late gestation Holstein heifers.

SEM values are reported in parentheses

Treatments: Sed = moved to exercise walker but remained in holding pen (n =12); LI = low intensity exercise heifers walked at a consistent speed of 2.5 kilometers per hour at increasing distances per week estimated to achieve 70% lactate threshold (n=12), HI = high intensity exercise heifers exercised in short sprint intervals of up to 6 kilometers per hour with increasing distances and speed per week estimated to achieve 130% lactate threshold by the end of the exercise period (n=10) .

Calving Observations

Eight weeks of prepartum exercise did not result in differences in average calving outcomes, including calving score, calving difficulty, or calf birth weight (Table. 2.9).

Measurement		Intensity Group		Pvalue
	SED	LI	HI	Treatment
Calving Score	1.17 (.135)	1.20 (.148)	1.1 (.148)	0.89
Calving Difference (Days)	-10.17 (3.239)	-5.67 (1.256)	-.4.00 (1.304)	0.17
Calf Weight	39.68 (2.325)	37.50 (2.325)	37.42 (2.325)	0.55

Table 2.9 Estimated marginal means of various calving observation averages, in cows that underwent 8 weeks of late gestation exercise

SEM values are reported in parentheses

Treatments: Sed = moved to exercise walker but remained in holding pen (n =12); LI = low intensity exercise heifers walked at a consistent speed of 2.5 kilometers per hour at increasing distances per week estimated to achieve 70% lactate threshold (n=12), HI = high intensity exercise heifers exercised in short sprint intervals of up to 6 kilometers per hour with increasing distances and speed per week estimated to achieve 130% lactate threshold by the end of the exercise period (n=10) .

Exercise Effect on the Lactation Period

Subcutaneous Backfat Adipose Depth

Eight weeks of exercise during late gestation influenced adipose mobilization during the first ten weeks of lactation (*Week* × *Treatment*: $P = 0.001$; Table 2.10 Fig 2.4). High-intensity exercise caused stabilization of subcutaneous adipose depth, with no significant week-to-week changes. In contrast, low-intensity and sedentary groups had substantial adipose loss during the first four weeks of lactation, followed by a slower rate of loss in later weeks.

Measurement		Intensity Group			Pvalue	
	SED	LI	HI	Week	Treatment	Week * Treatment
Backfat				<0.001	0.95	<0.001
Week 0	32.69 (.732)^{A}	29.9 (.732)^{B}	28.91 (.801)^{B}			
Week 2	28.59 (.568)	28.78 (.568)	27.60 (.622)			
Week 4	26.21 (.613)	26.78 (.613)	26.93 (.672)			
Week 6	25.78 (.541)	26.07 (.541)	26.75 (.592)			
Week 8	25.07 (.601)	25.28 (.601)	26.73 (.658)			
Week 10	24.40 (.682)	25.20 (.682)	26.47 (.747)			

Table 2.10 Estimated marginal means of subcutaneous adipose during the first 10 weeks of lactation, following eight weeks of late gestation exercise in Holstein heifers.

SEM values are reported in parentheses

***A, B*: Values in a row without common superscripts differ during that week of lactation (P ≤ 0.05).**

Treatments: Sed = moved to exercise walker but remained in holding pen (n =12); LI = low intensity exercise heifers walked at a consistent speed of 2.5 kilometers per hour at increasing distances per week estimated to achieve 70% lactate threshold (n=12), HI = high intensity exercise heifers exercised in short sprint intervals of up to 6 kilometers per hour with increasing distances and speed per week estimated to achieve 130% lactate threshold by the end of the exercise period (n=10)

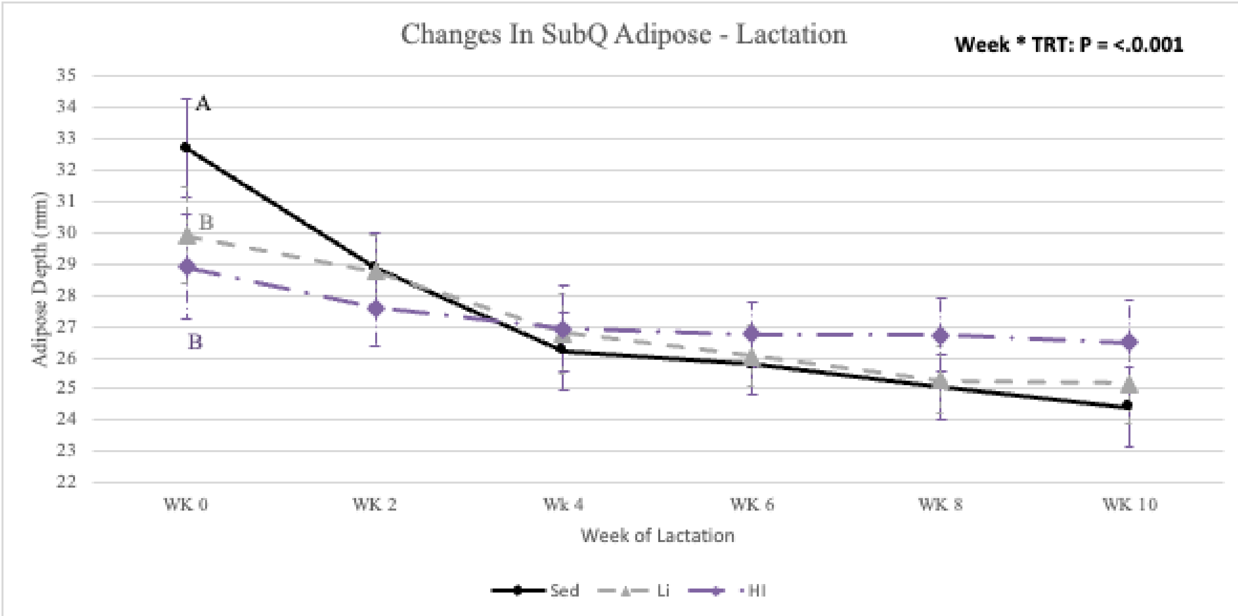

Figure 2.4 Estimated marginal means of subcutaneous adipose during the first 10 weeks of lactation, following eight weeks of late gestation exercise in Holstein heifers.

***A, B*: Values in a row without common superscripts differ during that week of lactation ($P \leq 0.05$).**

Treatments: Sed = moved to exercise walker but remained in holding pen (n =12); LI = low intensity exercise heifers walked at a consistent speed of 2.5 kilometers per hour at increasing distances per week estimated to achieve 70% lactate threshold (n=12), HI = high intensity exercise heifers exercised in short sprint intervals of up to 6 kilometers per hour with increasing distances and speed per week estimated to achieve 130% lactate threshold by the end of the exercise period (n=10).

Body Condition Score

Eight weeks of exercise during late gestation, regardless of intensity, caused differences in body condition score during the first ten weeks of lactation (Week × Treatment: $P < 0.001$; Table. 2.11, Fig. 2.5). Heifers in both high- and low-intensity exercise groups calved with lower body condition scores compared with sedentary animals, and their scores remained relatively stable throughout early lactation. In contrast, sedentary animals experienced a significant decline in body condition during the first four weeks of lactation, after which scores stabilized, resulting in similar overall body condition by week six.

Measurement		Intensity Group			Pvalue	
	SED	LI	HI	Week	Treatment	Week * Treatment
BCS				<0.001	<0.001	<0.001
Week 0	4.02 (.069)^{A}	3.25 (.069)^{B}	3.05 (.076)^{B}			
Week 2	3.60 (.057)^{A}	3.08 (.057)^{B}	2.95 (.062)^{B}			
Week 4	3.17 (.056) ^{A}	2.92 (.056)^{B}	2.80 (.062)^{B}			
Week 6	2.96 (.056)	2.90 (.056)	2.85 (.062)			
Week 8	2.86 (.068)	2.75 (.068)	2.90 (.074)			
Week 10	2.83 (.063)	2.77 (.063)	2.93 (.069)			

Table 2.11 Estimated marginal means of body condition score during the first 10 weeks of lactation, following eight weeks of late gestation exercise in Holstein heifers.

SEM values are reported in parentheses

***A, B*: Values in a row without common superscripts differ during that week of lactation (P ≤ 0.05).**

Treatments: Sed = moved to exercise walker but remained in holding pen (n =12); LI = low intensity exercise heifers walked at a consistent speed of 2.5 kilometers per hour at increasing distances per week estimated to achieve 70% lactate threshold (n=12), HI = high intensity exercise heifers exercised in short sprint intervals of up to 6 kilometers per hour with increasing distances and speed per week estimated to achieve 130% lactate threshold by the end of the exercise period (n=10)

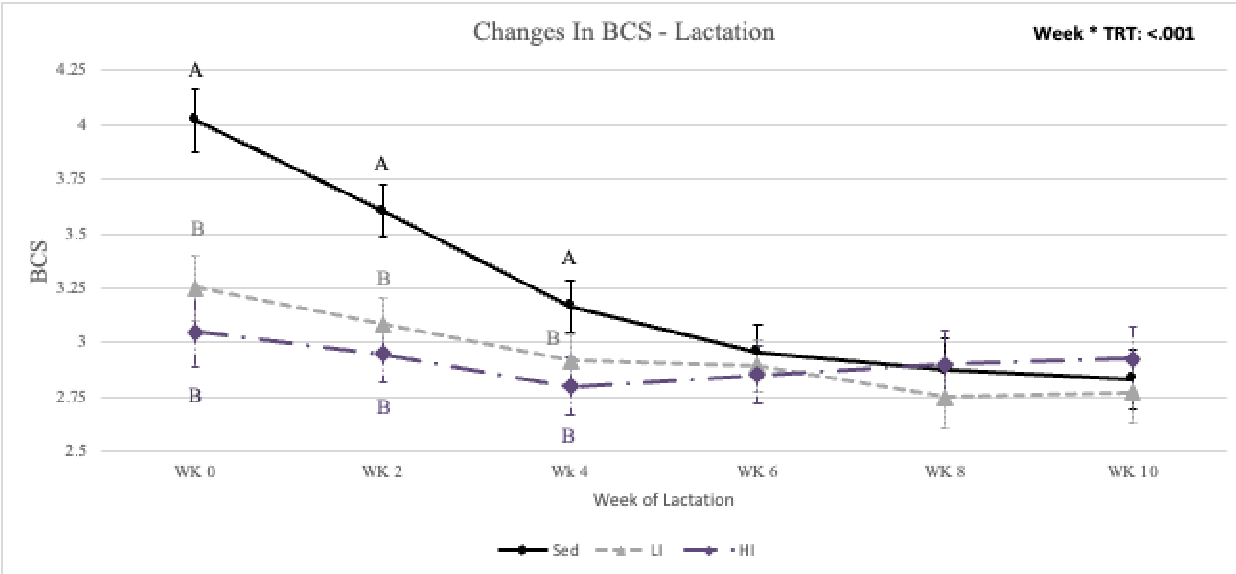

Figure 2.5 Estimated marginal means of body condition score during the first 10 weeks of lactation, following eight weeks of late gestation exercise in Holstein heifers.

***A, B*: Values in a row without common superscripts differ during that week of lactation (P ≤ 0.05).**

Treatments: Sed = moved to exercise walker but remained in holding pen (n =12); LI = low intensity exercise heifers walked at a consistent speed of 2.5 kilometers per hour at increasing distances per week estimated to achieve 70% lactate threshold (n=12), HI = high intensity exercise heifers exercised in short sprint intervals of up to 6 kilometers per hour with increasing distances and speed per week estimated to achieve 130% lactate threshold by the end of the exercise period (n=10).

Semitendinosus Muscle Area

Eight weeks of exercise during late gestation did not alter the weekly rate of semitendinosus muscle area among treatment groups during the first ten weeks of lactation (Week × Treatment: *P* = 0.55; Table. 2.12). However, when averaged across weeks, exercise intensity was a significant source of variation (Treatment*:* *P* = 0.002). Therefore, a carryover effect of prepartum exercise on postpartum muscle status did occur. At calving, the semitendinosus area differed among all three exercise intensity groups, although no distinction occurred between the low- and high-intensity groups following week eight of the exercise period. Across all 10-weeks of lactation, high-intensity animals consistently maintained greater semitendinosus muscle area compared with sedentary animals.

Measurement		Intensity Group			Pvalue	
	SED	LI	HI	Week	Treatment	Week * Treatment
Semitendinosus Area				0.002	0.002	0.55
Week 0	248.42 (1.846)^{A}	252.00 (1.846)^{B}	259.9 (2.022) ^{C}			
Week 2	248.25 (2.023)^{A}	253.00 (2.023)^{AB}	259.30 (2.216)^{B}			
Week 4	248.33 (2.067)^{A}	254.58 (2.067)^{AB}	260.00 (2.264)^{B}			
Week 6	249.58 (2.157)^{A}	255.42 (2.157)^{AB}	260.40 (2.362)^{B}			
Week 8	250.25 (2.279)^{A}	259.92 (2.279)^{AB}	262.80 (2.497)^{B}			
Week 10	249.917 (2.381)^{A}	258.70 (2.381)^{AB}	262.20 (2.608)^{B}			

Table 2.12 Estimated marginal means of semitendinosus muscle area during the first 10 weeks of lactation, following eight weeks of late gestation exercise in Holstein heifers.

SEM values are reported in parentheses

***A, B, C*: Values in a row without common superscripts differ during that week of lactation (P ≤ 0.05).**

Treatments: Sed = moved to exercise walker but remained in holding pen (n =12); LI = low intensity exercise heifers walked at a consistent speed of 2.5 kilometers per hour at increasing distances per week estimated to achieve 70% lactate threshold (n=12), HI = high intensity exercise heifers exercised in short sprint intervals of up to 6 kilometers per hour with increasing distances and speed per week estimated to achieve 130% lactate threshold by the end of the exercise period (n=10)

Semitendinosus Depth

Eight weeks of exercise during late gestation did not alter the weekly rate of semitendinosus muscle depth growth among exercise intensity groups during the first ten weeks of lactation (Week × Treatment: *P* = 0.64; Table 2.13). Furthermore, when averaged across weeks, exercise intensity was not a significant source of variation (Treatment*:* *P* = 0.42).

Measurement	Intensity Group			Pvalue		
	SED	LI	HI	Week	Treatment	Week * Treatment
Semitendinosus Depth				0.17	0.42	0.64
Week 0	70.29 (2.266)	73.36 (2.266)	75.82 (2.483)			
Week 2	70.10 (2.314)	73.32 (2.314)	74.77 (2.535)			
Week 4	69.98 (2.383)	72.58 (2.383)	74.95 (2.610)			
Week 6	70.42 (2.430)	73.31 (2.430)	75.31 (2.662)			
Week 8	71.10 (2.491)	73.68 (2.491)	75.21 (2.729)			
Week 10	71.15 (2.584)	73.93 (2.584)	74.98 (2.83)			

Table 2.13 Estimated marginal means of semitendinosus muscle depth during the first 10 weeks of lactation, following eight weeks of late gestation exercise in Holstein heifers.

SEM values are reported in parentheses

Treatments: Sed = moved to exercise walker but remained in holding pen (n =12); LI = low intensity exercise heifers walked at a consistent speed of 2.5 kilometers per hour at increasing distances per week estimated to achieve 70% lactate threshold (n=12), HI = high intensity exercise heifers exercised in short sprint intervals of up to 6 kilometers per hour with increasing distances and speed per week estimated to achieve 130% lactate threshold by the end of the exercise period (n=10)

Semitendinosus Width

Eight weeks of exercise during late gestation did not alter the weekly rate of semitendinosus muscle growth in width among all exercise intensity groups during the first ten weeks of lactation (Week × Treatment: $P = 0.50$; Table. 2.14). However, when averaged across weeks, exercise intensity was a significant source of variation (Treatment: $P < 0.001$). At calving, semitendinosus width was greater in the high-intensity group when compared with both low-intensity and sedentary groups. Following eight weeks of exercise, low-intensity animals also had greater semitendinosus widths than sedentary animals, but this difference was no longer apparent after calving.

Measurement	Intensity Group SED	LI	HI	Pvalue Week	Treatment	Week * Treatment
Semitendinosus Width				<0.001	<0.001	0.50
Week 0	78.08 (.706)^{A}	80.44 (.706)^{A}	82.58 (.773)^{B}			
Week 2	78.40 (.521)^{A}	81.15 (.521)^{B}	81.67 (.571)^{B}			
Week 4	78.08 (.526)^{A}	81.52 (.526)^{B}	81.96 (.576)^{B}			
Week 6	78.51 (.623)^{A}	81.24 (.623)^{B}	82.28 (.683)^{B}			
Week 8	79.61 (.547)^{A}	81.88 (.547)^{B}	83.19 (.599)^{B}			
Week 10	79.73 (.547)^{A}	81.81 (.702)^{A}	83.83 (.769)^{B}			

Table 2.14 Estimated marginal means of semitendinosus muscle width during the first 10 weeks of lactation, following eight weeks of late gestation exercise in Holstein heifers.

SEM values are reported in parentheses

***A, B*: Values in a row without common superscripts differ during that week of lactation (P ≤ 0.05).**

Treatments: Sed = moved to exercise walker but remained in holding pen (n =12); LI = low intensity exercise heifers walked at a consistent speed of 2.5 kilometers per hour at increasing distances per week estimated to achieve 70% lactate threshold (n=12), HI = high intensity exercise heifers exercised in short sprint intervals of up to 6 kilometers per hour with increasing distances and speed per week estimated to achieve 130% lactate threshold by the end of the exercise period (n=10)

Bodyweight

Eight weeks of exercise did not cause differences in weekly weight among treatment groups during the exercise period (Week × Treatment: $P = 0.884$; Table. 2.15). Similarly, when averaged across all eight weeks, exercise treatment alone did not significantly affect overall weight gain (Treatment: $P = 0.21$).

Measurement		Intensity Group			Pvalue	
	SED	LI	HI	Week	Treatment	Week * Treatment
Body Weight				<0.001	0.8	0.29
Week 0	570.77 (28.715)	595.34 (28.715)	581.05 (31.455)			
Week 2	539.77 (28.523)	548.85 (28.523)	547.94 (31.246)			
Week 4	543.18 (27.411)	539.4 (27.411)	545.67 (30.027)			
Week 6	541.67 (24.480)	552.63 (24.480)	550.21 (26.816)			
Week 8	547.71 (25.805)	546.58 (25.805)	554.29 (28.268)			
Week 10	536.75 (27.860)	553.38 (27.860)	562.45 (30.518)			

Table 2.15Estimated marginal means of body weight during the first 10 weeks of lactation, following eight weeks of late gestation exercise in Holstein heifers.

SEM values are reported in parentheses

Treatments: Sed = moved to exercise walker but remained in holding pen (n =12); LI = low intensity exercise heifers walked at a consistent speed of 2.5 kilometers per hour at increasing distances per week estimated to achieve 70% lactate threshold (n=12), HI = high intensity exercise heifers exercised in short sprint intervals of up to 6 kilometers per hour with increasing distances and speed per week estimated to achieve 130% lactate threshold by the end of the exercise period (n=10).

Exercise Effect on Milk Composition Results

Milk weight

Eight weeks of exercise during late gestation did not alter the weekly rate of milk production among treatment groups during the first ten weeks of lactation (Week × Treatment: $P = 0.42$; Table 2.16). Furthermore, when averaged across weeks, exercise intensity alone was not a significant source of variation in milk yield (Treatment: $P = 0.40$).

Measurement	Intensity Group			Pvalue		
	SED	LI	HI	Week	Treatment	Week * Treatment
Milk Weight (Kg)				<0.001	0.4	0.56
Week 1	24.18 (3.132)	24.30 (3.132)	22.69 (3.430)			
Week 2	30.79 (2.544)	31.14 (2.544)	28.49 (2.781)			
Week 3	34.99 (2.744)	33.30 (2.744)	32.79 (2.745)			
Week 4	37.53 (2.706)	36.54 (2.706)	35.37 (3.006)			
Week 5	39.98 (2.784)	37.97 (2.784)	37.60 (3.005)			
Week 6	41.51 (2.937)	39.51 (2.937)	38.68 (3.218)			
Week 7	42.70 (2.617)	40.36 (2.617)	40.07 (2.867)			
Week 8	43.95 (2.793)	41.19 (2.793)	40.14 (3.059)			
Week 9	44.5 (2.793)	41.42 (2.793)	40.93 (3.059)			
Week 10	42.67 (3.379)	42.53 (3.379)	41.22 (3.701)			

Table 2.16 Estimated marginal means of milk weight during the first 10 weeks of lactation following eight weeks of late gestation exercise in Holstein heifers.

SEM values are reported in parentheses

Treatments: Sed = moved to exercise walker but remained in holding pen (n =12); LI = low intensity exercise heifers walked at a consistent speed of 2.5 kilometers per hour at increasing distances per week estimated to achieve 70% lactate threshold (n=12), HI = high intensity exercise heifers exercised in short sprint intervals of up to 6 kilometers per hour with increasing distances and speed per week estimated to achieve 130% lactate threshold by the end of the exercise period (n=10).

Milk fat concentrations

Eight weeks of exercise during late gestation did not alter the weekly rate of milk fat production among treatment groups during the first ten weeks of lactation (Wee*k* × Treatment*:* *P* = 0.89; Table 2.17). Furthermore, when averaged across weeks, exercise intensity alone was not a significant source of variation in milk fat concentrations (Treatment: *P* = 0.81).

Measurement		Intensity Group			Pvalue		
		SED	LI	HI	Week	Treatment	Week * Treatment
Milk Fat					<0.001	0.81	0.89
	Week 1	5.57 (.321)	5.54 (.321)	5.3 (.352)			
	Week 2	4.86 (.263)	4.61 (.263)	4.58 (.288)			
	Week 3	4.84 (.238)	4.83 (.238)	4.44 (.260)			
	Week 4	4.33 (.232)	4.31 (.232)	4.37 (.254)			
	Week 5	4.19 (.166)	4.15 (.166)	4.33 (.182)			
	Week 6	4.12 (.216)	3.73 (.216)	4.2 (.236)			
	Week 7	3.92 (.164)	3.8 (.164)	3.85 (.179)			
	Week 8	4.03 (.195)	3.96 (.195)	4.19 (.214)			
	Week 9	4.13 (.178)	3.87 (.178)	4.04 (.195)			
	Week 10	3.89 (.177)	3.93 (.177)	4.14 (.194)			

Table 2.17 Estimated marginal means of milk fat during the first 10 weeks of lactation following eight weeks of late gestation exercise in Holstein heifers.

SEM values are reported in parentheses

Treatments: Sed = moved to exercise walker but remained in holding pen (n =12); LI = low intensity exercise heifers walked at a consistent speed of 2.5 kilometers per hour at increasing distances per week estimated to achieve 70% lactate threshold (n=12), HI = high intensity exercise heifers exercised in short sprint intervals of up to 6 kilometers per hour with increasing distances and speed per week estimated to achieve 130% lactate threshold by the end of the exercise period (n=10)

Milk Protein concentrations

Eight weeks of exercise during late gestation did not alter the weekly rate of milk protein percentages among all exercise intensity groups during the first ten weeks of lactation (Week × Treatment*:* *P* = 0.45; Table 2.18). Furthermore, when averaged across weeks, exercise intensity alone was not a significant source of variation in milk protein concentrations (Treatment*: P* = 0.19).

Measurement		Intensity Group			Pvalue	
	SED	LI	HI	Week	Treatment	Week * Treatment
Milk Protein				<0.001	0.19	0.45
Week 1	3.73 (.088)	3.64 (.088)	3.77 (.097)			
Week 2	3.22 (.043)^{A}	3.22 (.043)^{A}	3.39 (.047)^{B}			
Week 3	2.97 (.042)^{B}	2.91 (.042)^{A}	. 3.10 (.046)^{B}			
Week 4	2.82 (.041)	2.82 (.041)	2.94 (.045)			
Week 5	2.86 (.040)	2.81 (.040)	2.93 (.044)			
Week 6	2.85 (.041)	2.85 (.041)	2.88 (.045)			
Week 7	2.84 (.043)	2.83 (.043)	2.85 (.047)			
Week 8	2.87 (.063)	2.87 (.049)	2.87 (.054)			
Week 9	2.87 (.063)	2.89 (.063)	2.82 (.069)			
Week 10	2.84 (.046)	2.85 (.046)	2.96 (.051)			

Table 2.18 Estimated marginal means of milk protein during the first 10 weeks of lactation, following eight weeks of late gestation exercise in Holstein heifers

***A, B*: Values in a row without common superscripts differ during that week of lactation (P ≤ 0.05).**

SEM values are reported in parentheses

Treatments: Sed = moved to exercise walker but remained in holding pen (n =12); LI = low intensity exercise heifers walked at a consistent speed of 2.5 kilometers per hour at increasing distances per week estimated to achieve 70% lactate threshold (n=12), HI = high intensity exercise heifers exercised in short sprint intervals of up to 6 kilometers per hour with increasing distances and speed per week estimated to achieve 130% lactate threshold by the end of the exercise period (n=10).

Milk Solids Not Fat Concentrations

Eight weeks of exercise during late gestation did not alter the weekly rate of milk solids-not-fat concentrations among all exercise intensity groups during the first ten weeks of lactation (Week × Treatment*:* *P* = 0.42; Table 2.19). Furthermore, when averaged across weeks, exercise intensity alone was not a significant source of variation in milk solids-not-fat concentrations (Treatment: *P* = 0.55).

Measurement		Intensity Group			Pvalue	
	SED	LI	HI	Week	Treatment	Week * Treatment
Milk SNF				<0.001	0.55	0.42
Week 1	9.29 (.095)	9.31 (.095)	9.37 (.104)			
Week 2	8.98 (.067)	9.03 (.067)	9.13 (.074)			
Week 3	8.80 (.058)^{AB}	8.74 (.058)^{A}	8.98 (.063)^{B}			
Week 4	8.70 (.058)	8.73 (.058)	8.86 (.064)			
Week 5	8.77 (.065)	8.74 (.065)	8.83 (.071)			
Week 6	8.75 (.070)	8.79 (.070)	8.80 (.077)			
Week 7	8.78 (.063)	8.79 (.063)	8.78 (.069)			
Week 8	8.83 (.071)	8.88 (.071)	8.79 (.077)			
Week 9	8.80 (.075)	8.56 (.075)	8.83 (.082)			
Week 10	8.73 (.070)	8.716 (.070)	8.86 (.077)			

Table 2.19 Estimated marginal means of milk solids not fat during the first 10 weeks of lactation, following eight weeks of late gestation exercise in Holstein heifers.

***A, B*: Values in a row without common superscripts differ during that week of lactation (P ≤ 0.05).**

SEM values are reported in parentheses

Treatments: Sed = moved to exercise walker but remained in holding pen (n =12); LI = low intensity exercise heifers walked at a consistent speed of 2.5 kilometers per hour at increasing distances per week estimated to achieve 70% lactate threshold (n=12), HI = high intensity exercise heifers exercised in short sprint intervals of up to 6 kilometers per hour with increasing distances and speed per week estimated to achieve 130% lactate threshold by the end of the exercise period (n=10).

Milk Short Chain Fatty Acids (SCFA) Concentrations

Eight weeks of exercise during late gestation did not alter the weekly rate of SCFA concentrations among all exercise intensity groups during the first ten weeks of lactation (Week × Treatment: $P = 0.91$; Table 2.20). Furthermore, when averaged across weeks, exercise intensity alone was not a significant source of variation in SCFA concentrations (Treatment: $P = 0.11$).

Measurement		Intensity Group			Pvalue	
	SED	LI	HI	Week	Treatment	Week * Treatment
Milk SCFA				<0.001	0.11	0.91
Week 1	0.51 (.024)	0.47(.024)	0.49 (.026)			
Week 2	0.44 (.025)	0.40 (.025)	0.43 (.027)			
Week 3	0.42 (.021)	0.40 (.021)	0.42 (.022)			
Week 4	0.38 (.020)	0.36 (.020)	0.40 (.021)			
Week 5	0.38 (.16)	0.35 (0.16)	0.41 (.017)			
Week 6	0.38 (.015)	0.33 (.015)	0.38 (.016)			
Week 7	0.37 (.015)	0.34 (.015)	0.35 (.016)			
Week 8	0.36 (.016)	0.35 (.016)	0.38 (.017)			
Week 9	0.37 (.016)	0.36 (.016)	0.37 (.017)			
Week 10	0.35 (.018)	0.35 (.018)	0.38 (.019)			

Table 2.20 Estimated marginal means of milk short-chain fatty acid during the first 10 weeks of lactation, following eight weeks of late gestation exercise in Holstein heifers.

SEM values are reported in parentheses

Treatments: Sed = moved to exercise walker but remained in holding pen (n =12); LI = low intensity exercise heifers walked at a consistent speed of 2.5 kilometers per hour at increasing distances per week estimated to achieve 70% lactate threshold (n=12), HI = high intensity exercise heifers exercised in short sprint intervals of up to 6 kilometers per hour with increasing distances and speed per week estimated to achieve 130% lactate threshold by the end of the exercise period (n=10).

Milk Medium Chain Fatty Acids (MCFA) Concentrations

Eight weeks of exercise during late gestation did not alter the weekly rate of MCFA concentrations among all exercise intensity groups during the first ten weeks of lactation (Week × Treatment: *P* = 0.93; Table 2.21). Furthermore, when averaged across weeks, exercise intensity alone was not a significant source of variation in MCFA concentrations (Treatment: *P* = 0.39).

Measurement	Intensity Group			Pvalue		
	SED	LI	HI	Week	Treatment	Week * Treatment
Milk MCFA				0.04	0.39	0.93
Week 1	2.16 (.156)	1.95 (.156)	2.08 (.171)			
Week 2	1.99 (.131)	1.81 (.131)	1.83 (.144)			
Week 3	2.15 (.123)	1.94 (.123)	1.89 (.135)			
Week 4	1.92 (.137)	1.88 (.137)	1.95 (.150			
Week 5	1.85 (.107)	1.70 (.107)	1.94 (.117)			
Week 6	1.85 (.117)	1.63 (.117)	1.87 (.128)			
Week 7	1.81 (.095)	1.72 (.095)	1.68 (.104)			
Week 8	1.87 (.112)	1.803 (.112)	1.93 (.123)			
Week 9	1.91 (.110)	1.73 (.110)	1.93 (.121)			
Week 10	1.90 (.116)	1.83 (.116)	1.88 (.127)			

Table 2.21 Estimated marginal means of milk medium-chain fatty acid during the first 10 weeks of lactation, following eight weeks of late gestation exercise in Holstein heifers.

SEM values are reported in parentheses

Treatments: Sed = moved to exercise walker but remained in holding pen (n =12); LI = low intensity exercise heifers walked at a consistent speed of 2.5 kilometers per hour at increasing distances per week estimated to achieve 70% lactate threshold (n=12), HI = high intensity exercise heifers exercised in short sprint intervals of up to 6 kilometers per hour with increasing distances and speed per week estimated to achieve 130% lactate threshold by the end of the exercise period (n=10).

Milk Long Chain Fatty Acid (LCFA) Concentrations

Eight weeks of exercise during late gestation did not alter the weekly rate of LCFA concentrations among all exercise intensity groups during the first ten weeks of lactation (Week × Treatment: *P* = 0.73; Table 2.22). Furthermore, when averaged across weeks, exercise intensity alone was not a significant source of variation in LCFA concentrations (Treatmen*t*: *P* = 0.87).

Measurement		Intensity Group			Pvalue	
	SED	LI	HI	Week	Treatment	Week * Treatment
Milk LCFA				<0.001	0.87	0.73
Week 1	2.08 (.178)	2.23 (.178)	2.03 (.0195)			
Week 2	1.79 (.094)	1.72 (.094)	1.64 (.103)			
Week 3	1.65 (.101)	1.78 (.101)	1.53 (.111)			
Week 4	1.49 (.085)	1.53 (.085)	1.47 (.094)			
Week 5	1.44 (.063)	1.50 (.063)	1.46 (.094)			
Week 6	1.39 (.084)	1.29 (.094)	1.41 (.094)			
Week 7	1.31 (.057)	1.28 (.057)	1.33 (.063)			
Week 8	1.36 (.071)	1.34 (.071)	1.39 (.078)			
Week 9	1.41 (.058)	1.34 (.058)	1.30 (.064)			
Week 10	1.27 (.059)	1.30 (.059)	1.38 (.064)			

Table 2.22 Estimated marginal means of milk long-chain fatty acid during the first 10 weeks of lactation, following eight weeks of late gestation exercise in Holstein heifers.

SEM values are reported in parentheses

Treatments: Sed = moved to exercise walker but remained in holding pen (n =12); LI = low intensity exercise heifers walked at a consistent speed of 2.5 kilometers per hour at increasing distances per week estimated to achieve 70% lactate threshold (n=12), HI = high intensity exercise heifers exercised in short sprint intervals of up to 6 kilometers per hour with increasing distances and speed per week estimated to achieve 130% lactate threshold by the end of the exercise period (n=10).

Denovo Milk Fat Synthesis Concentrations

Eight weeks of exercise during late gestation did not alter the weekly rate of de novo milk fat synthesis among treatment groups during the first ten weeks of lactation (Week × Treatment: *P* = 0.96; Table 2.23). When averaged across weeks, exercise intensity showed a tendency to affect de novo milk fat concentrations (Treatment: *P* = 0.07), with low-intensity animals having lower concentrations compared with both high-intensity and sedentary animals. Overall, prepartum exercise did not modify the rate of de novo milk fat synthesis; however, low-intensity exercise may have reduced the magnitude of de novo fat production during early lactation.

Measurement	Intensity Group			Pvalue		
	SED	LI	HI	Week	Treatment	Week * Treatment
Milk DeNovo FA				<0.001	0.07	0.96
Week 1	1.18 (.058)	1.03 (.058)	1.12 (.063)			
Week 2	0.97 (.066)	0.89 (.066)	1.00 (.072)			
Week 3	1.04 (.054)	0.92 (.054)	1.00 (.060)			
Week 4	0.93 (.052)	0.88 (.052)	1.00 (.057)			
Week 5	0.92 (.043)	0.87 (.043)	1.03 (.047)			
Week 6	0.94 (.041)	0.84 (.041)	0.98 (.045)			
Week 7	0.91 (.043)	0.87 (.043)	0.90 (.047)			
Week 8	0.92 (.044)	0.89 (.044)	0.98 (.049)			
Week 9	0.94 (.044)	0.90 (.044)	0.98 (.049)			
Week 10	0.93 (.049)	0.91 (.049)	1.01 (.054)			

Table 2.23 Estimated marginal means of denovo milk fat synthesis changes during the first 10 weeks of lactation, following eight weeks of late gestation exercise in Holstein heifers.

SEM values are reported in parentheses

Treatments: Sed = moved to exercise walker but remained in holding pen (n =12); LI = low intensity exercise heifers walked at a consistent speed of 2.5 kilometers per hour at increasing distances per week estimated to achieve 70% lactate threshold (n=12), HI = high intensity exercise heifers exercised in short sprint intervals of up to 6 kilometers per hour with increasing distances and speed per week estimated to achieve 130% lactate threshold (n=10).

Mixed Fatty Acids Concentrations

Eight weeks of exercise during late gestation did not alter the weekly rate of mixed fatty acids concentrations among all exercise intensity groups during the first ten weeks of lactation (Week × Treatment: *P* = 0.89; Table 2.24). Furthermore, when averaged across weeks, exercise intensity alone was not a significant source of variation in mixed fatty acid concentrations (Treatment: *P* = 0.39).

Measurement		Intensity Group			Pvalue	
	SED	LI	HI	Week	Treatment	Week * Treatment
Milk Mixed FA				<0.001	0.39	0.89
Week 1	2.00 (.112)	1.84 (.112)	1.85 (.122)			
Week 2	1.59 (.100)	1.478 (.100)	1.48 (.109)			
Week 3	1.66 (.088)	1.55 (.088)	1.47 (.097)			
Week 4	1.45 (.101)	1.41 (.101)	1.49 (.111)			
Week 5	1.37 (.075)	1.27 (.075)	1.46 (.082)			
Week 6	1.37 (.079)	1.19 (.079)	1.40 (.087)			
Week 7	1.30 (.072)	1.24 (.072)	1.25 (.078)			
Week 8	1.35 (.079)	1.29 (.079)	1.42 (.087)			
Week 9	1.37 (.077)	1.25 (.077)	1.38 (.085)			
Week 10	1.34 (.081)	1.31 (.081)	1.36 (.089)			

Table 2.24 Estimated marginal means of mixed fatty acids during the first 10 weeks of lactation, following eight weeks of late gestation exercise in Holstein heifers.

SEM values are reported in parentheses

Treatments: Sed = moved to exercise walker but remained in holding pen (n =12); LI = low intensity exercise heifers walked at a consistent speed of 2.5 kilometers per hour at increasing distances per week estimated to achieve 70% lactate threshold (n=12), HI = high intensity exercise heifers exercised in short sprint intervals of up to 6 kilometers per hour with increasing distances and speed per week estimated to achieve 130% lactate threshold by the end of the exercise period (n=10).

Preformed Fatty Acids Concentrations

Eight weeks of exercise during late gestation did not alter the weekly rate of preformed fatty acid concentrations among all exercise intensity groups during the first ten weeks of lactation (Week × Treatment: *P* = 0.71; Table 2.25). Furthermore, when averaged across weeks, exercise intensity alone was not a significant source of variation in preformed fatty acid concentrations (Treatment*:* *P* = 0.71).

Measurement		Intensity Group			Pvalue	
	SED	LI	HI	Week	Treatment	Week * Treatment
Milk Preform FA				<0.001	0.71	0.71
Week 1	2.15 (.235)	2.38 (.235)	2.15 (.258)			
Week 2	2.02 (.120)	1.961 (.120)	1.84 (.132)			
Week 3	1.81 (.124)	2.02 (.124)	1.67 (.136)			
Week 4	1.65 (.090)	1.72 (.090)	1.58 (.099)			
Week 5	1.61 (.077)	1.69 (.077)	1.60 (.084)			
Week 6	1.49 (.100)	1.41 (.100)	1.54 (.109)			
Week 7	1.41 (.066)	1.41 (.066)	1.45 (.072)			
Week 8	1.48 (.086)	1.47 (.086)	1.48 (.094)			
Week 9	1.51 (.068)	1.42 (.068)	1.36 (.074)			
Week 10	1.33 (.075)	1.38 (.075)	1.47 (.082)			

Table 2.25 Estimated marginal means of milk-performed fatty acid during the first 10 weeks of lactation, following eight weeks of late gestation exercise in Holstein heifers.

SEM values are reported in parentheses

Treatments: Sed = moved to exercise walker but remained in holding pen (n =12); LI = low intensity exercise heifers walked at a consistent speed of 2.5 kilometers per hour at increasing distances per week estimated to achieve 70% lactate threshold (n=12), HI = high intensity exercise heifers exercised in short sprint intervals of up to 6 kilometers per hour with increasing distances and speed per week estimated to achieve 130% lactate threshold by the end of the exercise period (n=10).

Milk Lactose Concentrations

Eight weeks of exercise during late gestation did not alter the weekly rate of milk lactose concentrations among all exercise intensity groups during the first ten weeks of lactation (Week × Treatment: $P = 0.83$; Table 2.26). Furthermore, when averaged across weeks, exercise intensity alone was not a significant source of variation in milk lactose concentrations (Treatment: $P = 0.64$).

Measurement		Intensity Group			Pvalue	
	SED	LI	HI	Week	Treatment	Week * Treatment
Milk Lactose				<0.001	0.64	0.83
Week 1	4.33 (.058)	4.42 (.058)	4.33 (.067)			
Week 2	4.55 (.046)	4.59 (.046)	4.50 (.067)			
Week 3	4.69 (.034)	4.65 (.034)	4.68 (.040)			
Week 4	4.69 (.034)	4.78 (.034)	4.74 (.043)			
Week 5	4.73 (.037)	4.77 (.039)	4.74 (.036)			
Week 6	4.77 (.031)	4.83 (.031)	4.78 (.045)			
Week 7	4.83 (.030)	4.83 (.030)	4.80 (.035)			
Week 8	4.82 (.038)	4.87 (.038)	4.79 (.044)			
Week 9	4.80 (.039)	4.85 (.039)	4.78 (.045)			
Week 10	4.77 (.038)	4.75 (.038	4.78 (.044)			

Table 2.26 Estimated marginal means of milk lactose during the first 10 weeks of lactation following eight weeks of late gestation exercise in Holstein heifers.

SEM values are reported in parentheses

Treatments: Sed = moved to exercise walker but remained in holding pen (n =12); LI = low intensity exercise heifers walked at a consistent speed of 2.5 kilometers per hour at increasing distances per week estimated to achieve 70% lactate threshold (n=12), HI = high intensity exercise heifers exercised in short sprint intervals of up to 6 kilometers per hour with increasing distances and speed per week estimated to achieve 130% lactate threshold by the end of the exercise period (n=10).

Milk BHB Concentrations

Eight weeks of exercise during late gestation did not alter the weekly rate of milk BHB concentrations among all exercise intensity groups during the first ten weeks of lactation (Week × Treatment*:* $P = 0.66$; Table 2.27). Furthermore, when averaged across weeks, exercise intensity alone was not a significant source of variation in milk BHB concentrations *(*Treatment*:* $P = 0.13$).

Measurement		Intensity Group		Pvalue		
	SED	LI	HI	Week	Treatment	Week * Treatment
Milk BHB				<0.001	0.13	0.66
Week 1	0.17 (.017)	0.15 (.017)	0.13 (.019)			
Week 2	0.13 (.010)	0.13 (.010)	0.11 (.011)			
Week 3	0.12 (.013)	0.13 (.013)	0.09 (.014)			
Week 4	0.11 (.008)	0.14 (.008)	0.11 (.009)			
Week 5	0.12 (.011)	0.11 (.011)	0.11 (.012)			
Week 6	0.12 (0.10)	0.12 (.010)	0.11 (.011)			
Week 7	0.11 (.011)	0.11 (.011)	0.10 (.012)			
Week 8	0.13 (.008)	0.13 (.008)	0.11 (.008)			
Week 9	0.11 (.008)	0.12 (.008)	0.11 (.008)			
Week 10	0.11 (.011)	0.12 (.011)	0.10 (.012)			

Table 2.27 Estimated marginal means of milk BHB during the first 10 weeks of lactation following eight weeks of late gestation exercise in Holstein heifers.

SEM values are reported in parentheses

Treatments: Sed = moved to exercise walker but remained in holding pen (n =12); LI = low intensity exercise heifers walked at a consistent speed of 2.5 kilometers per hour at increasing distances per week estimated to achieve 70% lactate threshold (n=12), HI = high intensity exercise heifers exercised in short sprint intervals of up to 6 kilometers per hour with increasing distances and speed per week estimated to achieve 130% lactate threshold by the end of the exercise period (n=10).

Various Milk Components

Eight weeks of exercise during late gestation did not alter the rate of weekly production rates of the following components included in the milk analysis; cells, urea, acetone, monounsaturated fatty acids, polyunsaturated fatty acids, saturated fatty acids, total unsaturated fatty acids, C14 fatty acid, C16 fatty acid, C18 fatty acid, and C18_1 fatty acid (Table 2.28). When averaged across weeks, no treatment differences occurred in all the remaining milk components. Throughout the 10 weeks of lactation, all components listed decreased over time, regardless of exercise intensity in the prepartum period.

Measurement			Intensity Group			Pvalue	
		SED	LI	HI	Week	Treatment	Week * Treatment
Cells (*per ML*)					<0.05	0.07	0.58
	Week 1	363.83 (184.191)	356.57 (184.191)	343.667 (201.771)			
	Week 10	41.47 (230.846)	64.42 (230.846)	511.600 (252.800)			
					\		
Urea %					<0.001	0.42	0.62
	Week 1	15.05 (.836)	14.02 (.836)	14.253 (.916)			
	Week 10	11.44 (.634)	10.53 (.634)	11.18 (.694)			
Acetone %					<0.001	0.08	0.53
	Week 1	0.36 (.070)	0.33 (.070)	.21 (.077)			
	Week 10	0.12 (.019)	.02 (.019)	.13 (.025)			
Monounsaturated Fat %					<0.001	0.7	0.61
	Week 1	1.49 (.149)	1.64 (.149)	1.44 (.163)			
	Week 10	0.84 (.049)	0.90 (.049)	0.93 (.054)			
Polyunsaturated Fat %					<0.001	0.33	0.78
	Week 1	.14 (.012)	.14 (.012)	.13 (.013)			
	Week 10	.08 (.007)	.08 (.007)	.11 (.008)			
Saturated Fat %					<0.001	0.44	0.94
	Week 1	3.69 (.224)	3.47 (.224)	3.47 (.246)			
	Week 10	2.75 (.140	2.73 (.140)	2.88 (.154)			
Total Unsaturated Fat %					<0.001	0.88	0.68
	Week 1	1.60 (.171)	1.44 (.171)	1.57 (.187)			
	Week 10	0.95 (.049)	1.00 (.049)	1.06 (.054			
C14 Fatty Acid %					0.22	0.35	0.87
	Week 1	0.45 (.037)	0.40 (.037)	0.41 (.041)			
	Week 10	0.44 (.028)	0.43 (.028)	0.45 (.031)			
C16 Fatty Acid %					0.21	0.56	0.87
	Week 1	1.43 (.130)	1.28 (.130)	1.31 (.142)			
	Week 10	.130 (.087)	.126 (.087)	1.27 (.095)			
C18 Fatty Acid %					<0.001	0..84	0.96
	Week 1	0.78 (.057)	0.79 (.057)	0.77 (,062)			
	Week 10	0.45 (.024)	0.45 (.024)	0.47 (.027)			
C18_1 Fatty Acid %					<0.001	0.75	0.61
	Week 1	1.32 (.119)	1.44 (.119)	1.29 (.131)			
	Week 10	0.71 (.041)	0.76 (.041)	0.81 (.045)			

Table 2.28 Beginning and ending estimated marginal means of various milk components during the first 10 weeks of lactation, following eight weeks of late gestation exercise in Holstein heifers.

SEM values are reported in parentheses

Treatments: Sed = moved to exercise walker but remained in holding pen (n =12); LI = low intensity exercise heifers walked at a consistent speed of 2.5 kilometers per hour at increasing distances per week estimated to achieve 70% lactate threshold (n=12), HI = high intensity exercise heifers exercised in short sprint intervals of up to 6 kilometers per hour with increasing distances and speed per week estimated to achieve 130% lactate threshold by the end of the exercise period (n=10).

Heifer Calf Growth Outcomes:

Eight weeks of exercise in late gestation did not alter the weekly average growth of several performance parameters (*calf weight, hip height, hip width, pin width, hook width, hip length, and wither height)* from birth to weaning in heifer calves whose dams were enrolled in the exercise trial (Table 2.29). When averaged across the weeks, the exercise intensity of the dam prepartum did not affect any of the performance parameters.

Measurement		Intensity Group			Pvalue	
	SED	LI	HI	Week	Treatment	Week * Treatment
Calf Weight (Kg)				<0.001	0.24	0.85
Week 0	39.68 (2.325)	37.50 (2.325)	37.42 (2.325)			
Week 6	61.8 (3.811)	57.23 (3.811)	60.21 (3.811)			
Calf Hip Height (Inches)				<0.001	0.97	0.81
Week 0	31.13 (.512)	31.33 (.482)	31.5 (.512)			
Week 6	34.88 (.364)	34.67 (.344)	34.63 (.364)			
Hip Width (Inches)				0.07	0.11	0.64
Week 0	5.94 (.539)	6.46 (.509)	6.31 (.539)			
Week 6	6.06 (.581)	6.54 (.548)	6.536 (.581)			
Pin Width (Inches)				0.11	0.11	0.7
Week 0	5.94 (.539)	6.46 (.509)	6.31 (.539)			
Week 6	6.06 (.581)	6.54 (.548)	6.56 (.581)			
Hook width (Inches)				0.42	0.41	0.43
Week 0	7.69 (.568)	6.63 (.535)	6.56 (.568)			
Week 6	7.75 (.586)	6.68 (.553)	6.75 (.586)			
Hip Length (Inches)				0.57	0.14	0.92
Week 0	8.375 (.384)	7.67 (.362)	8.94 (.384)			
Week 6	8.56 (.394)	7.75 (.372)	9.00 (.394)			
Wither Height (Inches)				<0.001	0.88	0.1
Week 0	30.00 (.503)	29.92 (474)	29.31 (.503)			
Week 6	35.44 (.555)	34.5 (.523)	34.38 (.555)			

Table 2.29 Beginning and ending estimated marginal means of various heifer calf growth parameters from birth to weaning. Calves are from the heifers that were enrolled in eight weeks of late gestation exercise

SEM values are reported in parentheses

Treatments: Sed = moved to exercise walker but remained in holding pen (n =12); LI = low intensity exercise heifers walked at a consistent speed of 2.5 kilometers per hour at

increasing distances per week estimated to achieve 70% lactate threshold (n=12), HI = high intensity exercise heifers exercised in short sprint intervals of up to 6 kilometers per hour with increasing distances and speed per week estimated to achieve 130% lactate threshold by the end of the exercise period (n=10).

Discussion

The impact of exercise on body composition traits (During exercise and early lactation)

The hypothesis that exercise improves physiological responses to the increased nutrient demands of lactation—through mild and regular alterations to homeostasis that enhance adipose or skeletal muscle storage and subsequent mobilization—is supported by the observed outcomes. Exercise improved multiple body composition measures, with high-intensity protocols producing the most pronounced impacts on subcutaneous adipose depth and body condition scores during both the exercise period and the first 10 weeks of lactation.

Metabolic disorders in dairy cattle can result from both excessive and insufficient body fat reserves. Cows with a body condition score (BCS) of 3.75 or higher at calving are at significantly greater risk of developing clinical and subclinical ketosis compared to cows with moderate BCS values (Fischer et al., 2023). Over-conditioning during the dry period exacerbates this risk, as excessive lipolysis and mobilization of adipose reserves can overwhelm hepatic capacity and lead to fatty liver development (Ospina et al., 2024). Both excessively high and low BCS have been associated with increased incidence of metabolic and immune-related disorders, including displaced abomasum, metritis, and impaired reproductive performance (Dellai and Gasa, 2022). Furthermore, objective assessments of back-fat thickness by ultrasound indicate that cows with greater adipose reserves or more extensive BCS loss early in lactation have an elevated risk of hyperketonemia (Montoro-Dasi et al., 2023). Subclinical ketosis is a globally prevalent and economically significant condition that is often observed in cows undergoing excessive adipose mobilization (Neves, 2019). The increased frequency of ketosis in such cases resulted from rapid adipose tissue mobilization, which elevated circulating NEFA concentrations and imposed a substantial metabolic burden on the liver, which can lead to hepatic steatosis (McArt et al., 2012). Initiating adipose mobilization prior to calving through controlled exercise may help distribute lipid mobilization over a longer time frame, reducing hepatic overload and mitigating the risk of early-lactation ketosis.

In our study, high-intensity exercise led to significant prepartum reductions in subcutaneous adipose depth, with declines evident by week five and remaining lower than both low-intensity and sedentary treatments by week seven. This early mobilization appeared beneficial postpartum, as high-intensity cows lost less adipose overall and maintained depths similar to prepartum values across all 10 weeks of lactation. By initiating lipid mobilization prepartum through exercise, these cows may have reduced the acute metabolic load on the liver postpartum, potentially lowering non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHB) accumulation associated with hepatic steatosis and ketosis risk. Similar exercise benefits have been reported in dairy cattle with high body condition scores, where prepartum adipose mobilization improved metabolic health indicators (Goselink et al., 2020). In that study, late-gestation exercise in Holsteins with high body condition scores led to lower milk fat content immediately postpartum and reduced plasma BHB, demonstrating a carryover effect of prepartum adipose mobilization into early lactation.

McCabe et al. (2021) further demonstrated that ultrasonographic measures of muscle and subcutaneous adipose thickness in late gestation reliably reflect the extent of tissue mobilization during the periparturient period, validating the use of such measurements in assessing exercise-induced metabolic adaptations. Similarly, Culp et al. (2019) found that abdominal adipose depots were preferentially mobilized compared with subcutaneous fat in early lactation, highlighting the importance of depot-specific mobilization patterns—an area in which our findings of subcutaneous stabilization add a novel dimension. Beyond tissue-level changes, Rodríguez et al. (2020) reported that cows with elevated prepartum fat mobilization exhibited decreased inflammatory and oxidative stress profiles postpartum, despite having equal body condition scores, indicating systemic effects of lipid mobilization that extend beyond simple fat loss. Complementary work by Abuelo et al. (2020) linked late-gestation metabolic stress to altered metabolic and immune responses in calves, underscoring the potential intergenerational effects of prepartum lipid metabolism. Qiao et al. (2024) demonstrated that in transition cows, impaired insulin signaling promotes excessive adipose lipolysis, elevating circulating NEFA and triggering hepatic metabolic inflammation. When lipolysis occurs in a moderated, rather than excessive, manner, the resulting metabolic adaptations appear to enhance hepatic function and improve resilience to the nutrient demands of early lactation. Qiao et al.'s (2024) conclusions further support the concept that moderated prepartum lipolysis may prime animals for improved metabolic resilience during early lactation. In our trial, prepartum exercise, especially at high intensity, enabled heifers to begin decreasing adipose before the assumed catabolic demands of lactation, resulting in less dramatic postpartum adipose loss and stabilization within the first 10 weeks. This earlier mobilization may have enhanced their ability to adapt to the metabolic and energetic demands of lactation more efficiently than their low-intensity or sedentary counterparts. Collectively, the present results and supporting literature allow us to extrapolate that controlled prepartum adipose mobilization may provide a metabolic advantage in early lactation, though further research is warranted to directly evaluate its effects on systemic metabolic health, inflammatory status, and reproductive performance.

Body condition score (BCS) trends in our study closely mirrored changes in subcutaneous adipose depth, with exercise producing measurable declines in both variables during the exercise period. This outcome is expected, as the primary determinant of BCS is the amount of subcutaneous fat present at specific anatomical locations. By week 4 of exercise, heifers in the high-intensity group had experienced a significant reduction in BCS (Week 0: 3.73±..40 → Week 4: 3.48± .13), whereas low-intensity heifers showed little change (Week 0: 3.46 ± .45 → Week 8: 3.12± .25) and sedentary heifers gained condition steadily (Week 0: 3.40 ±.35→ Week 8: 4.00±.26). Our findings align in part with Davidson and Beede (2003), who reported modest BCS declines following treadmill exercise regardless of intensity. However, there were no changes in adipose depth in their study, which contrasts with our results. This discrepancy may be explained by differences in exercise intensity and physiological status; Davidson and Beede's study used nonlactating, nonpregnant multiparous Holstein cows, while our trial involved pregnant heifers transitioning into lactation. Additional research, such as the prepartum exercise trial done by Goselink et al. (2020) provided the link between exercise and measurable tissue mobilization. McCabe et al. (2021) demonstrated that ultrasonographic measurements of subcutaneous adipose and muscle thickness during late gestation reliably reflected periparturient tissue mobilization, confirming the validity of our approach. Culp et al. (2019) further showed that abdominal adipose depots tended to mobilize more readily than subcutaneous fat in early lactation, suggesting that the subcutaneous stabilization observed in high-intensity cows may represent an earlier redistribution of lipid mobilization prior to calving. Collectively, these results indicate that prepartum exercise—particularly at high intensity, and cause concurrent reductions in BCS and adipose depth, aligning with and extending the findings of Davidson and Beede (2003), while adding direct evidence of adipose depletion in pregnant, transitioning dairy heifers.

Heifers in our study were only exercised until approximately four weeks before parturition, after which they rested in the maternity pen without engaging in strenuous activity. During this "maternity period," all treatment groups gained BCS by the time of calving. The onset of lactation, as documented by Gross (2022), Guliński (2021), and Lei and Simões (2021), typically involves rapid mobilization of adipose tissue to meet the energetic demands of milk production. This was evident in our study, as sedentary heifers lost an entire BCS point during the first month of lactation and continued to lose condition until week 10. In contrast, exercised heifers experienced a slower rate of BCS loss, with the greatest resiliency observed in the high-intensity group, which was able to recover condition within the first 10 weeks of lactation. This aligns with Goselink et al. (2020), who concluded that the benefits of prepartum exercise were most pronounced in cows with a BCS ≥ 3.25—a threshold met by all heifers in our study (mean enrollment BCS = 3.53). Furthermore, evidence from human research supports these findings: Marcinko et al. (2015) demonstrated that high-intensity training improved liver and adipose tissue insulin sensitivity, exercise capacity, and whole-body glucose homeostasis independent of changes in body weight or adiposity. These mechanisms may help explain the superior resilience of high-intensity heifers in our study to the catabolic demands of early lactation.

Differences in BCS and adipose mobilization across treatment groups have direct implications for the metabolic challenges of early lactation. Rapid postpartum adipose loss, as observed in sedentary heifers, was a hallmark of negative energy balance (NEB) and was closely linked to elevated non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHB) concentrations, which can increase the risk of hepatic steatosis and ketosis (Drackley, 1999; Overton and Waldron, 2004; Sundrum, 2015). By initiating lipid mobilization prepartum, exercised heifers—particularly those in the high-intensity group, may have distributed fat mobilization over a longer time frame, reducing the acute metabolic load on the liver in early lactation. This aligns with evidence from Goselink et al. (2020), who found that prepartum exercise in high-BCS cows reduced postpartum BHB concentrations and improved metabolic profiles. In the context of our findings, the ability of high-intensity heifers to stabilize BCS and adipose depth early in lactation suggests a protective effect against severe NEB and its associated disorders, setting the stage for improved metabolic resilience.

In theory, exercise should promote muscle hypertrophy, as muscles subjected to sustained tension and intense bouts of contraction adapted by increasing in size (Wilmore et al., 2008). High-intensity exercise is generally expected to yield greater gains than low-intensity activity, because explosive and forceful movements recruited a larger proportion of fast-twitch fibers and generated higher mechanical load—both of which were key drivers of hypertrophy (Wilmore et al., 2008). In contrast, low-intensity endurance (aerobic) exercise promotes enhancements in skeletal muscle oxidative capacity and mitochondrial content, improving endurance performance—without inducing significant muscle fiber hypertrophy or enlargement (Holloszy 1984). In practice, motivating heifers to consistently reach target treatment speeds proved challenging, particularly in the high-intensity group. Refusals were most common in the first cohort, exercised during midsummer in Kansas when relative humidity averaged ~70% and ambient temperatures ranged from 20.5 °C to 32.2 °C. To overcome this, research assistants walked alongside reluctant heifers in the exercise bays to ensure higher work intensity was achieved. It should also be noted that the high-intensity group had two fewer animals than the other treatments, potentially reducing statistical power.

Surprisingly, no significant differences were observed in semitendinosus muscle area gain per week over the eight-week exercise period among treatment groups. However, there was a treatment effect overall, with high-intensity heifers having a larger semitendinosus muscle area than sedentary heifers, though not significantly larger than heifers exercised at low-intensity. This difference may partly reflect baseline variation in frame size (which was not measured and recorded), as heifers were stratified into treatments based solely on subcutaneous adipose depth rather than body size, and the high-intensity group began the trial with a numerically larger average muscle area even though it wasn't significantly different from both heifers within the low intensity exercise and sedentary groups. The exercise effect on semitendinosus muscle size in our study also paralleled the treatment effects observed in semitendinosus depth and width where high intensity exercise had overall greater width and depth during the treatment period, in comparison to low intensity and high intensity animals. These findings align with Rivero et al. (2007), who reported that in horses, high-intensity exercise was necessary to induce muscle hypertrophy due to the greater energetic and mechanical demands of intense work, while low-intensity exercise primarily improved muscular stamina without increasing muscle size. The concept that high-intensity, load-bearing exercise is a prerequisite for measurable hypertrophy has been well established in multiple species, including cattle, where hypertrophy occurred primarily when mechanical overload exceeded habitual activity levels (Wilmore et al., 2008; Fiems, 2012). However, the lack of weekly hypertrophy differences in our trial may also reflect the unique physiology of transition dairy cattle. Hatfield et al. (2022) demonstrated that periparturient dairy cows often experienced muscle mobilization, with longissimus dorsi thickness declining significantly around calving, driven by nutrient partitioning toward fetal growth and mammary development. This natural catabolic shift may have masked any small gains from the exercise regimen. Environmental conditions, such as summer heat stress could have further limited training intensity, as high temperatures have been shown to impair muscle growth responses (Kim et al., 2023). Furthermore, high-intensity prepartum exercise has been associated with maintenance of muscle size and structure during the transition period; however, significant hypertrophy does not typically occur without progressive overload, adequate nutrition, and favorable environmental conditions. Our conclusions, therefore, extend the principles of exercise physiology to a dairy production context, highlighting the complexity of eliciting hypertrophic responses in pregnant, transitioning heifers.

It is possible that both ultrasound probe placement and the selected muscle for monitoring were less than ideal to see growth differences occurring per week, but there is a lack of literature investigating muscle growth with exercise using an ultrasound probe in mature dairy animals. Available literature, including findings by Lamb et al. (2010), which focuses on ultrasound-based assessment of muscle mass for live carcass grading in beef cattle. However, because the semitendinosus muscle lacks commercial significance in yield-based carcass valuation, it is not routinely included in standard ultrasound scanning protocols (Lamb et al., 2010). Furthermore, our protocol was based on selecting the largest and easiest to access leg muscle that best supports the explosive movements associated with exercise. Our ultrasound probe placement was based on a postmortem Holstein dairy calf study that measured muscle depth (David and Rossow, 2018).

It was initially assumed that during early lactation, we would observe extensive catabolism of the semitendinosus muscle, evidenced by reductions in muscle area, depth, and width—due to the diversion of amino acids toward milk protein synthesis (Gross, 2022; Hanno et al., 2025; Thanh et al., 2023). However, this was not apparent in the ultrasound results for any treatment group. Rather, all groups maintained their average semitendinosus muscle area, with no significant week-to-week changes during lactation. This lack of detectable catabolism conflicts with the findings of Hanno et al. (2025), who reported that dairy cattle deplete 30–35% of their skeletal muscle reserves within the first 60 days of lactation, regardless of initial muscling. Several factors likely account for the difference: 1) the Hanno et al. study population consisted of multiparous Holstein cows housed in tie stalls from calving to day 28 of lactation, and 2) their measurements focused on the longissimus dorsi muscle rather than the semitendinosus. Based on these differences, it is plausible that muscle catabolism is more pronounced in spinal musculature, which is less involved in explosive, load-bearing locomotion, than in hind-limb locomotor muscles.

Although overall muscle size remained stable during lactation, both exercise groups, particularly the high-intensity group, retained greater semitendinosus muscle measures (area, depth, and width) through the first 10 weeks of lactation. Sedentary heifers maintained constant measures across the same period, but without the elevated baseline values seen in exercised animals. This pattern suggests that the treatment effect established during the prepartum exercise period persisted into lactation. Notably, Hanno et al. (2025) also observed that cows with higher baseline muscling lost more muscle mass in the longissimus dorsi than cows with lower muscling opposite to our findings. While the previously mentioned differences in study design still apply, an additional explanation is possible: animals in our high-intensity group may have developed muscles more resilient to the metabolic and mechanical stresses of lactation. This consistency of muscle area may have been supported by two factors: 1) the functional role of the semitendinosus as a primary locomotor muscle, which may preserve its structure when regularly engaged in load-bearing work, and 2) improved metabolic adaptation. High-intensity exercise has been shown in other species to enhance skeletal muscle's ability to adapt to metabolic stress through improved insulin sensitivity and nutrient utilization (Marcinko et al., 2015). Because heifers performing high-intensity exercise were able to minimize the catabolic demands of lactation on both subcutaneous adipose and body condition scores, these acclimations likely explain their greater muscle retention during early lactation. The concurrent preservation of muscle mass and controlled stabilization of adipose reserves in heifers subjected to high-intensity exercise reflects a potentially more efficient nutrient partitioning strategy, one that supports both structural integrity and metabolic resilience throughout the transition period. During the eight-week exercise period, one mammary measure of parenchyma area was collected via ultrasound. In theory, mammary parenchyma should increase in size approaching parturition due to continued mammary development (Svennersten-Sjaunja and Olsson, 2005). However, in our study, heifers in the high-intensity group showed a decrease in parenchyma area by week 4 compared with their baseline values. In contrast, sedentary heifers maintained their average parenchyma area over the exercise period (Week 0: 126.5 ± 3.28 cm^2; Week 8: 123.5 ± 2.25 cm^2). The decrease in mammary parenchyma, as a result of high intensity, may be partially explained by scanning inconsistencies. To obtain an image, the ultrasound technician had to position themselves behind each heifer and apply firm pressure to the udder. Being heifers, they were unaccustomed to teat handling and often kicked or moved abruptly, leaving only a brief window to position the probe and capture an acceptable image. Consistency was therefore difficult to maintain. To our knowledge, no published studies have specifically examined changes in mammary parenchyma area in response to exercise in nulliparous heifers. Compounding the challenge, the ultrasound unit and convex probe suffered irreparable breakdown during early lactation, preventing continuation of parenchyma measurements into the

10-week postpartum period. Given the absence of supporting literature and the potential for inconsistencies in image capture, we are unable to draw firm conclusions regarding the interaction between exercise and mammary parenchyma development in this study.

Overall, prepartum exercise, particularly at high intensity, produced coordinated changes in body composition that persisted into early lactation. High-intensity heifers experienced greater reductions in subcutaneous adipose and body condition score prior to calving, yet mobilized these reserves more strategically, allowing for quicker stabilization of adipose depth and faster recovery of body condition postpartum. This controlled lipid mobilization was accompanied by improved retention of semitendinosus muscle area, depth, and width through the transition period, suggesting that high-intensity exercise supported both structural integrity and metabolic resilience during the early lactation negative energy balance phase. In contrast, animals that were sedentary, or performed low intensity exercise, displayed less favorable nutrient partitioning, with greater or prolonged adipose loss and less muscle preservation. Collectively, these results highlight the potential of targeted prepartum exercise programs to optimize nutrient allocation, maintain musculoskeletal strength, and support a more efficient metabolic adaptation to the demands of lactation

The Impact of Exercise on Milk Production and Composition

Prepartum exercise, irrespective of intensity, did not affect milk yield or major milk components (fat, protein, lactose, solids-not-fat) during early lactation. These findings are consistent with Davidson and Beede (2003), who reported no differences in feed intake, milk yield, or composition between exercised and non-exercised cows. Similarly, Lamb et al. (1979)

demonstrated that prepartum exercise improved calving ease and expedited postpartum uterine involution, but milk yield remained and milk composition (*fat corrected milk, milk fat, protein and SNF).* Lamb et al. did also report that the lack of differences may in part be due to dietary factors, such as not feeding consistent feeds throughout all treatments. In our trial, once in lactation due to having multiple cohorts, there was not a consistent ration that was fed across all 10 weeks of lactation, so that may also account for why we didn't see alterations in milk or milk components as well.

The absence of production differences in the present study may reflect the controlled management conditions provided across all groups, including sedentary controls, which minimized negative energy balance through consistent nutritional provision and housing. As emphasized by Overton and Waldron (2004), milk synthesis is primarily constrained by nutrient availability and endocrine regulation, indicating that exercise alone is unlikely to enhance production when energy intake is optimized. More recently, Goselink et al. (2020) also reported that although substantial prepartum activity promoted early metabolic adaptations, it did not alter milk yield or the overall milk composition during the first six weeks postpartum.

In contrast, Johnson et al. (2019), the study upon which the present trial was designed to expand, reported that high-intensity exercise in late gestation increased milk fat, milk protein, and solids-not-fat concentrations. Similar outcomes were anticipated; however, the present results did not replicate these findings. One possible explanation is that the actual exercise intensities achieved may have differed between studies. Despite structured protocols, maintaining consistent motivation and pace among all animals in the current trial was challenging, even with additional encouragement from research personnel. Another potential explanation is that differences in the nutritional plane offered to animals across the two studies may have masked the direct effects of exercise on milk composition.

Altogether, while there is differing milk composition outcomes in response to exercise, combining our study with the conclusions of Goselink et al., (2020), Davidson and Beede (2003) and Lamb et al (1979), we conclude that, under well-managed nutritional and environmental conditions, prepartum exercise does not negatively affect milk yield or composition. Instead, the benefits of high-intensity exercise are more strongly associated with improvements in body composition and metabolic resilience, which may indirectly support productive efficiency over the long term.

Variables related to animal welfare, parturition, and post-partum health

Exercise, regardless of intensity, had no adverse effects on locomotion, parturition, calving ease, calf birth weight, or heifer calf postnatal growth from birth to weaning. These findings are consistent with conclusions from Goeslink et al. (2020) and Blake et al. (1982) that physical activity could be safely implemented in prepartum management without compromising animal welfare. Although Lamb et al. (1979) reported improved calving ease scores in cows exercised up to parturition, the heifers in the present study were exercised only up to four weeks before calving. Nevertheless, calving ease remained unaffected, with an average score of 1 (unassisted) across all groups, indicating no observable loss of parturition efficiency despite the earlier cessation of exercise.

Our findings are also in agreement with Daigle et al. (2017), who investigated the effects of structured and free-form exercise in weaned Bos indicus-cross calves. In that study, neither programmatic walking nor free movement altered average daily gain, immune response, or key behaviors such as feeding, rumination, and social interaction. These similarities in findings, allow us to conclude that well-managed exercise, regardless of format or intensity, does not impair productivity, health, or welfare across different life stages of cattle.

During the eight-week prepartum period, body weight gain remained consistent across all treatment groups, demonstrating that exercise, regardless of intensity, did not alter overall weight gain or loss. This aligns with Andersen et al. (2003), who reported that moderate exercise in dry cows did not impair nutrient intake or fetal development. Similarly, in our trial, calf birth weights did not differ between treatments, reinforcing the conclusion that exercise did not negatively affect fetal growth or maternal nutrient transfer. During the 10-week lactation period, there were no significant differences between groups in weekly body weight loss or recovery, nor were any treatment effects detected. While these results may seem inconsistent with trends observed in subcutaneous adipose and muscle measurements, it is important to recognize that body weight reflects changes in fat, muscle, and water combined. In our trial, fat was mobilized differently among treatment groups, while muscle volume was largely preserved in exercised heifers, particularly in the high-intensity group. Variations in water retention or loss could also contribute to these patterns and should be explored in future studies through monitoring hydration status and water intake.

Locomotion scores, recorded weekly, did not differ between sedentary and exercised heifers, averaging 1 across all groups. Only one heifer (high-intensity) was removed from the study due to an unrelated injury. These results mirror those of Blake et al. (1982) and Daigle et al. (2017), where regular movement was found to maintain or improve musculoskeletal condition without increasing lameness risk. Although our trial was not designed to improve locomotion scores, given all heifers started with optimal mobility, we conclude that appropriately managed physical activity does not harm limb health.

Importantly, our results add to the growing body of proof that exercise does not adversely impact performance or animal welfare outcomes. When implemented with clear protocols and appropriate intensity thresholds, prepartum exercise appears to be a safe management strategy that does not compromise calving outcomes, maternal or neonatal growth, welfare indicators, or early lactation performance. Future research using larger sample sizes and more advanced welfare metrics (e.g., stride analysis, physiological stress markers) could further clarify the benefits during the transition period.

Implications

Late gestation exercise, regardless of intensity or type, induced significant adipose mobilization during late pregnancy. High-intensity exercise provided distinct advantages for both the cow and the producer by promoting prepartum adipose mobilization alongside muscle development, effectively buffering the catabolic demands of lactation and supporting adipose and body condition stabilization. Heifers in the high-intensity group lost less adipose early in lactation and maintained their increased muscle mass, demonstrating a coordinated preservation of tissue reserves. Importantly, exercise even at high intensities did not negatively affect milk yield or milk component percentages. Furthermore, no adverse effects were observed in key animal welfare and productivity parameters, including locomotion, parturition outcomes, calving ease, calf birth weight, and calf growth. Collectively, we conclude that strategic implementation of high-intensity exercise during late gestation is a safe management tool that enhances metabolic preparedness and maintains animal well-being without compromising lactation performance.

Implications

Late-gestation exercise, regardless of intensity or type, was associated with significant adipose mobilization during pregnancy. High-intensity exercise provided additional advantages by promoting both prepartum adipose mobilization and muscle preservation, thereby metabolically "priming" heifers for the demands of lactation. Animals in the high-intensity group had less postpartum adipose loss and body condition loss while maintaining muscle mass, and these acclimations to exercise occurred without detrimental effects on milk yield or composition. Equally important, no negative consequences of exercise occurred in critical animal welfare and productivity outcomes, including locomotion, parturition, calving ease, calf birth weight, calf growth, or overall lactation performance. These outcomes demonstrate that structured exercise can be implemented safely in late gestation without compromising cow or calf health. Therefore, by initiating controlled adipose mobilization before calving, exercise may reduce the severity of negative energy balance and lower the risk of metabolic disorders such as ketosis and fatty liver, ultimately offering benefits to both animal well-being and producer profitability.

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Appendix A - Additional Materials and Methods Information

TMR Rations

Nutrient	Unit	DM
D.M.	%	47.99
CP	%	16.42
CHO C uNDF	%	7.84
aNDFom	%	30.40
Forage aNDFom	%	19.61
NFC	%	40.29
Starch	%	26.60
Sugar (WSC)	%	3.74
EE	%	4.44
TFA	%	3.47
Ash	%	8.84
Ca	%	0.92
Mg	%	0.36
P	%	0.42
K	%	1.30
RUP 3x Level 1	%	6.61
NEl 3x NRC	Mcal/lb	0.73
RD CHO 3x Level 1	%	45.13
RD Starch 3xLevel 1	%	20.08
peNDF	%	19.70
TDN 1x	%	71.23
CHO C uNDF Lig*2.4	%	5.68
RDP 3x Level 1	%	9.81
Monensin	mg/lb	6.22
CF	%	9.42

Appendix Figure A.1 TMR blend that was fed to cows in lactation

Nutrient	Unit	DM
D.M.	%	48.14
CP	%	16.52
CHO C uNDF	%	7.67
aNDFom	%	30.09
Forage aNDFom	%	18.80
NFC	%	40.43
Starch	%	26.47
Sugar (WSC)	%	3.67
EE	%	4.54
TFA	%	3.60
Ash	%	8.75
Ca	%	0.94
Mg	%	0.36
P	%	0.43
K	%	1.38
RUP 3x Level 1	%	6.75
NEl 3x NRC	Mcal/lb	0.74
RD CHO 3x Level 1	%	43.53
RD Starch 3xLevel 1	%	19.47
peNDF	%	19.33
TDN 1x	%	71.79
CHO C uNDF Lig*2.4	%	7.28
RDP 3x Level 1	%	9.77
Monensin	mg/lb	6.22
CF	%	8.71

Appendix Figure A.2 TMR blend that was fed to cows in lactation

Nutrient	Unit	DM
D.M.	%	48.02
CP	%	17.14
CHO C uNDF	%	8.42
aNDFom	%	29.69
Forage aNDFom	%	20.38
NFC	%	39.68
Starch	%	22.90
Sugar (WSC)	%	4.99
EE	%	4.02
TFA	%	2.79
Ash	%	9.47
Ca	%	0.93
Mg	%	0.37
P	%	0.40
K	%	1.48
RUP 3x Level 1	%	7.08
NEl 3x NRC	Mcal/lb	0.72
RD CHO 3x Level 1	%	44.59
RD Starch 3xLevel 1	%	16.87
peNDF	%	20.01
TDN 1x	%	69.77
CHO C uNDF Lig*2.4	%	5.83
RDP 3x Level 1	%	10.06
Monensin	mg/lb	8.02
CF	%	10.06

Appendix Figure A.3 TMR blend that was fed to cows in lactation