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STRESS AND OTHER FACTORS AND THEIR EFFECT ON SKELETAL GROWTH AND METABOLISM;
STRENGTHS-BASED LAB GROUPS IMPROVE LEARNING OF UNDERGRADUATE ANATOMY AND
PHYSIOLOGY

By

Taylor L. Barnes

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For the Degree of Master of Science

Major: Animal Science

Under the Supervision of Professors Dustin T. Yates and Jessica L. Petersen

Lincoln, Nebraska

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STRESS AND OTHER FACTORS AND THEIR EFFECT ON SKELETAL GROWTH AND METABOLISM;
STRENGTHS-BASED LAB GROUPS IMPROVE LEARNING OF UNDERGRADUATE ANATOMY AND
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University of Nebraska, 2018

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Muscle growth is an important consideration in livestock production that affects producer profits. Producers can maximize muscle growth in animals by decreasing stress and using supplements that increase growth and efficiency. In these studies, we sought to show how stress and growth-stimulating factors affect muscle metabolism in sheep and myoblast function in cattle. First, lambs were fed diets containing no supplement, ractopamine HCl (β 1 agonist), or zilpaterol HCl (β 2 agonist) for 21 d and housed under thermoneutral or heat stress (40°C, 35% RH) conditions (2x3 factorial). At harvest, skeletal muscle strips were used to measure ex vivo glucose oxidation under basal and insulin-stimulated conditions. Surprisingly, we did not observe interactions among environmental treatment, dietary supplement, and incubation media for glucose oxidation rates. However, heat stress decreased ($P \leq 0.05$) muscle glucose oxidation by ~21%, supplementation of β 2 agonist increased ($P \leq 0.05$) muscle glucose oxidation by ~15%, and addition of insulin to media increased ($P \leq 0.05$) glucose oxidation by ~25%. Interestingly, supplementation of β 1 agonist had no effects on muscle glucose oxidation. In our second study, myoblasts were isolated from cattle with high (<40 ng/ml; High A4) or control (< 20ng/ml) follicular fluid androstenedione concentrations. High A4 cows consistently wean heavier calves and thus we hypothesized that they have increased myoblast function which might be passed on to their calves. Primary myoblasts were assessed for proliferative capacity (2-h EdU pulse) in growth media (20% FBS) containing no additive (basal), TNF α , or testosterone. Percentages of myogenin-positive and desmin-positive myoblasts were determined after 4-d incubation in differentiation media (2% FBS) containing no additive (basal) or TNF α . No interactions between A4 classification and incubation conditions were found for any

output. Myoblasts from High A4 cows exhibited ~9% slower ($P \leq 0.05$) proliferation rates but ~25% greater ($P \leq 0.05$) percentages of myogenin-positive nuclei and ~15% greater ($P \leq 0.05$) percentages of desmin-positive nuclei after 4-d differentiation. These data show that physiological conditions responsible for high levels of A4 in follicular fluid may also be directing skeletal myoblasts to prematurely exit the cell cycle and begin precocious differentiation. Together these studies further demonstrate that stress has a negative impact on skeletal muscle growth, but β_2 adrenergic agonists improve muscle growth and that altered androstenedione concentrations also impede proper myoblast function effectively limiting muscle growth.

A final study examined how strengths-based groups affected learning in an undergraduate anatomy and physiology laboratory course. This project aimed to determine if these groups would improve grades and to assess student perceptions about learning and group work. Utilizing the CliftonStrengths for Students assessment students were classified into one of four domains based on their top identified strengths: executing, influencing, strategic thinking or relationship building. Balanced groups ($n=9$) contained one randomly-selected student from each of the four domains. Unbalanced groups ($n=13$) contained four students from the same domain. At the conclusion of the semester students completed a final practical exam and a survey of their perceptions on learning and their assigned group. Balanced groups scored greater ($P < 0.05$) on the final practical exam and overall lab scores. However, student perceptions did not differ between the groups and was generally negative. Students in the strategic thinking domain scored highest ($P < 0.05$) whereas relationship builders scored lowest ($P = 0.05$) on the final practical exam as well as the overall laboratory grade. These findings indicate that balancing student strengths in lab groups improves learning outcomes and we believe this simple approach could be applied to in-class group work across many STEM disciplines.

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Chapter 1

Literature Review

Introduction

In 2017 more than 81.7 billion pounds of meat was produced in the US, totaling \$88.9 billion in gross income for producers (NASS, 2017). Producing meat efficiently requires maximizing muscle development and growth in these animals in order to provide adequate animal protein to the growing population and shrinking available agriculture land to the world. Many factors affect muscle growth and metabolic efficiency of skeletal muscle, but ultimately efficiency is determined by how well skeletal muscle is able to metabolize glucose through stepwise pathways to produce the energy required for development and growth of muscle mass (Bouche et al., 2004). Livestock producers have the option to choose feedstuffs and supplements they feed to their livestock with the goal of maximizing lean muscle to the animal. One of the most common supplement types during finishing are β adrenergic agonists, with over 70% of feedlot cattle currently being fed these supplements (Centner et al., 2014). β adrenergic agonists increase lean muscle mass and decrease fat deposition without an increase in dry matter intake (Yang and McElligott, 1989; Mersmann, 1998).

The hormone profile within the body and changes from normal physiological concentrations can cause major anabolic and catabolic changes in muscle. Androgen steroids in particular are known to increase muscle protein synthesis, growth, and strength (Rasmussen et al., 2000), although the impact of some precursors such as androstenedione on skeletal muscle growth is not clear. Additionally, stressors such as

illness or harsh environmental conditions have long been known to decrease metabolic efficiency (McVeigh et al., 1982) and carcass quality (Kreikemeier et al., 1998), although their mechanisms and potential interactions with normal metabolism are still not well understood. Ultimately, gaining a better understanding the mechanisms of the factors affecting muscle development and growth will allow producers to increase muscle growth while decreasing negative effects of stress leading to more profit and pounds of meat produced per animal. To understand how stressors affect skeletal muscle growth and metabolism, we first must consider how these processes are working.

Skeletal Muscle Development and Growth

Early Skeletal Muscle Fiber Formation

Skeletal muscle makes up about 40% of total body weight (Brown, 2014), making it logical to consider when examining changes in total body mass. Development of muscle, or myogenesis, begins with myogenic precursor cells migrating from the somite into the limb and begin to proliferate (Oksbjerg et al., 2004). The proliferating myoblasts begin to fuse together to form myofibers. The first myotubes form early in gestation and give rise to the first wave of myogenesis, the primary myofibers, which will serve as scaffolding for subsequent waves of fiber formation (Wilson et al., 1992). These primary myofibers stretch the entire length of the muscle, tendon to tendon (Picard et al., 2002). As the primary wave nears completion, secondary fibers form along the length of the primary fibers (Wilson et al., 1992). These secondary myofibers are smaller in diameter,

but they are far greater in number (Zhang et al., 1998). Similar to secondary fibers forming on primary fibers, a tertiary wave of fibers will form along the secondary fibers (Figure 1)(Wilson et al., 1992). The total number of fibers becomes static late in gestation for most mammalian species including sheep and cattle (Wigmore and Stickland, 1983; Robelin et al., 1991; Greenwood et al., 2000) although the rabbit, pig, and rodent continue hyperplasia into early postnatal life (Etude, 1972).

Myoblast Facilitated Muscle Hypertrophy

Myoblast function is mediated by a family of transcription factors called myogenic regulatory factors (MRFs)(Figure 2). Paired box 7 (pax7) is expressed by myoblasts throughout the proliferating phase and begins down regulation as differentiation begins (Bentzinger et al., 2012). Myogenic factor 5 (myf5) is a biomarker for activation of myoblasts and is expressed throughout proliferation as well but must be downregulated for differentiation to occur (Ustanina et al., 2007). MyoD functions to remove myoblasts from the cell cycle but is expressed during, and serves as a biomarker for proliferation as cells begin the transition into differentiation (Singh et al., 2015). Those cells that terminally differentiate continue myoD expression and also upregulate myogenin, which promotes the morphological changes necessary for differentiation and fusion with existing fibers (Day et al., 2009). These changes include expression of desmin and Myosin Heavy Chain (MyHC) proteins later in differentiation prior to fusing with fibers. Desmin is expressed in all muscle cells and is a type III intermediate filament that anchors the sarcomere to the nucleus and helps regulate structural integrity in

muscle fibers. Conversely, MyHC isoforms are specific to each muscle fiber type (Talmadge and Roy, 1993). They are part of the contractile unit in skeletal muscle and contraction is fueled by different metabolic processes in the differing fiber types (Szalay et al., 1997).

Myoblast Self Renewal

A subset of fetal progenitor cells do not form muscle fibers and instead establish populations of quiescent satellite cells (Morgan and Partridge, 2003). Postnatal muscle growth requires myoblasts to proliferate, differentiate, and fuse with existing fibers in a mechanism similar to the later waves of myogenesis (Almeida et al., 2016). When these cells fuse with existing muscle fibers, the increase in myonuclear content facilitates increased protein synthesis capacity needed for hypertrophic growth (Clifton et al., 2006).

Skeletal Muscle Glucose Metabolism

Glucose Uptake

Glucose is the major energy substrate for skeletal muscle in mammalian species including ruminants, who shuttle volatile fatty acids produced by microbes in the rumen to the liver to produce glucose via gluconeogenesis (Huntington, 1997). Circulating glucose is taken up by the cell by transporters on the cell membrane. The most common type of glucose transporters are the GLUT proteins (Brown, 2000). Tissues that

must sense glucose levels for regulatory purposes such as liver or pancreatic β cells primarily express GLUT2, which has a lower affinity for glucose (Bell et al., 1990). In response to high blood glucose concentrations in the blood, insulin secreted from pancreatic β cells and upon binding its tyrosine kinase receptor on skeletal muscle, a signal cascade follows with phosphorylation of insulin receptor substrate 1 (IRS-1), Phosphoinositide 3-kinase (PI3K), and Akt (White, 2003). This cascade ultimately causes GLUT4 to translocate to the cell surface allowing clearance of glucose from circulation (Thorell et al., 1999).

Glucose Oxidation

Once inside skeletal muscle cells, glucose can be stored as glycogen or metabolized via one of several enzymatic pathways. Intracellular glucose is first irreversibly converted to glucose-6-phosphate and can no longer leave the cell, as skeletal muscle lacks glucose-6-phosphatase (Nelson et al., 2008). Glucose-6-phosphate or glycogen storage can then be metabolized by glycolysis, yielding pyruvate (Brooks, 1998). Under anaerobic conditions pyruvate is metabolized to lactate (Xu et al., 2008). Alternatively, pyruvate can be taken into the mitochondria and used in the TCA cycle yielding NADH and FADH₂ to utilize in the electron transport chain for oxidative phosphorylation (Xu et al., 2008). In the electron transport chain slower but more efficient oxidative phosphorylation occurs as this process yields ~36 ATP per mole of glucose metabolized compared to glycolysis, which only produces ~2 ATP (Lodish et al., 2008).

Skeletal muscle is a dynamic tissue that can express a range of metabolic phenotypes as determined by the ratio of three different fiber types. Type I fibers, also called red or slow twitch fibers, produce energy almost exclusively through oxidative phosphorylation facilitated by a high density of mitochondria and myoglobin, whereas Type IIx fibers, also called white or fast twitch fibers, produce energy almost exclusively through glycolysis (Essen et al., 1975). These fibers have fewer mitochondria as well as less myoglobin and capillary content, which causes them to fatigue more quickly (Lane et al., 1998). Type IIa fibers, or fast oxidative fibers, are the intermediate between the two other fiber types and are capable of both oxidative and glycolytic metabolism (Essen et al., 1975). The primary fibers formed in the primary wave of myogenesis are typically larger and become Type I fibers, whereas later waves typically become Type II fibers (Maier et al., 1992). Because of their differing metabolic phenotypes and characteristics, different fiber types predominate in different muscle groups, depending on their purposes (Monster et al., 1978). In general, muscles that play a part in posture and are engaged nearly all the time contain more Type I fibers as they need endurance. Meanwhile, those needed for short bursts of powerful movement contain more Type II fibers (Schiaffino and Reggiani, 2011). Although fiber number is set at birth, metabolic phenotype has more plasticity. Many factors can affect the phenotypes of these fibers such as, but not limited to, diet, environment, supplements, age, and fetal programming.

Regulation of Glucose Metabolism

Glucose homeostasis is highly regulated within the body, with skeletal muscle accounting for approximately 65% of total glucose utilized (Brown, 2014). Circulating glucose concentrations are mainly regulated by the hormone insulin and its effect on skeletal muscle and other insulin-sensitive tissues (Henquin, 2000). When blood glucose concentrations are high, this protein hormone is secreted from β cells within the islets of Langerhans residing in the pancreas (Gustafsson and Islam, 2007). As previously discussed, once released, insulin binds to its tyrosine kinase receptor on skeletal muscle, which initiates the recruitment of IRS-1 (White, 2003). This molecule can activate multiple pathways, but the canonical molecule phosphorylated is Akt (Cheng and White, 2012). Akt stimulates the translocation of GLUT4 to the plasma membrane, facilitating glucose uptake (Thorell et al., 1999).

Although insulin signaling is the primary regulator of skeletal muscle glucose metabolism, other signaling factors including known as cytokines affect this regulation as well. Cytokines mainly are released from white blood cells, but can be released from other tissues as well such as adipose and skeletal muscle. Within skeletal muscle, most cytokines are secreted from resident macrophages which can express either pro- or anti-inflammatory characteristics (Wijesundera et al., 2014). The M1 phenotype of macrophages is pro-inflammatory secreting inflammatory cytokines, whereas the M2 phenotype is known to be anti-inflammatory (Badylak et al., 2008). Inflammatory cytokines, such as tumor necrosis factor-alpha (TNF α) and interleukin-6 (IL-6) inhibit translocation of GLUT4 transporters stimulated by insulin (Lorenzo et al., 2008).

Additionally, they may inhibit downstream targets of insulin such as Akt, though these mechanisms are not well understood (Bach et al., 2013).

Heat Stress and β Adrenergic Agonists

Adrenergic System

The adrenergic system is a stress system that has two natural ligands, catecholamines called norepinephrine and epinephrine that are produced in the adrenal medulla (Nelson, 1980). During stress epinephrine is released at higher concentrations. This response re-directs available glucose from skeletal muscle to more critical tissues via inhibition of insulin and stimulation of glucagon secretion (Limesand et al., 2006). Additionally, epinephrine activates glycogenolysis in skeletal muscle to increase blood glucose concentration (Perseghin et al., 1997).

β -adrenergic agonists mechanism of action

There are two classes of G protein coupled receptors in the adrenergic system, α and β , each with multiple subtypes (Mersmann, 1998). Targeted stimulation of β adrenergic receptors (β -AR) is thought to increase lean muscle and total weight via increased lipolysis and muscle protein synthesis while simultaneously decreasing protein turnover within muscles (Ricks et al., 1984). β -AR have three subtypes, β -1, β -2, and β -3. The distribution of these subtypes of β -AR greatly depends on species and tissue type (Barnes, 1995). Cardiac muscle has more β -1 receptors and skeletal muscle has more β -

2 receptors (Kim et al., 1991). Adipose tissue greatly varies between species, but usually contains a mix of the three β subtypes (Johnson et al., 2014). Sheep and cattle appear to have a majority of β -2 receptors in skeletal muscle and approximately 75% β -2 and 25% β -1 receptors in adipose tissue (Bowen et al., 1992; Sillence and Matthews, 1994). β -AR are over 400 amino acids in length with three extracellular loops associated with the N-terminus and 3 intracellular loops associated with the C-terminus (Mills and Mersmann, 1995). The β -1, β -2, and β -3 subtypes of β -AR respond to many ligands as a 7-transmembrane G-coupled protein receptor using the G_s protein (Lefkowitz et al., 1983), but only β -1 and β -2 agonists are fed to livestock. When epinephrine or an agonist binds the receptor, a conformational change in the receptor allows release of GDP and a GTP molecule can bind (Strosberg, 1993). This activation allows for the disassociation of the α -subunit of the G protein which activates adenylylate cyclase, which converts ATP to cAMP (Lefkowitz et al., 1983). Protein Kinase A (PKA) is then activated by increased levels of cAMP binding to the regulatory unit of PKA, releasing the catalytic unit, allowing it to phosphorylate many different molecules causing downstream cellular actions (Figure 3)(Das et al., 2007). A primary target phosphorylated by PKA is the cAMP response element binding protein (CREB)(Barnes, 1999). CREB has the ability to bind to the response element of specific genes or sets of genes and stimulate transcription, leading to increased mRNA transcripts in cells and ultimately increased in the protein (Mayr and Montminy, 2001). In a study by Parr et al. (1992 #79), supplementation of β -2 adrenergic agonist increased muscle mass by approximately 37%, calpain II activity by approximately 27% and calpastatin activity by approximately 76% in skeletal muscle.

Calpastatin works as an inhibitor to calpains, which in turn, decreases protein turnover in skeletal muscle (Zaidi and Narahara, 1989). With this increase in activity, mRNA abundance for each increased as well, leading to the hypothesis that β -2 agonists enhances transcription of these mRNAs or post-transcriptional processes that stabilize them to increase calpain II and calpastatin in these steers (PARR et al., 1992). This would lead to the increased muscle hypertrophy consistent with β -2 agonist supplementation (Reeds et al., 1986). Studies by Yates and others examining the effects of β -adrenergic agonists on skeletal muscle fiber type proportions and fiber area, show an increase of Type II fiber area, but no significant changes in fiber type proportion (Smith et al., 1995; Beermann, 2002(Kim, 1987 #83).

Presence of fewer β -1 receptors in skeletal muscle may be the main cause of decreased efficacy of ractopamine's ability to increase muscle efficiency, lean muscle gain, and total muscle mass (Robles-Estrada et al., 2009; Barnes et al., 2017). At high doses, ractopamine can bind β -2 receptors and elicit a biological response, but both the cost of that level of supplementation and possible side effects keep this from being a viable option (Vogel et al.). Ultimately, the mechanism of action within skeletal muscle would function in the same manner as β -2 receptors. Though it may not be eliciting much effect on skeletal muscle, there have been reports of decreased fat when feeding ractopamine HCl (Gruber et al., 2007). This, along with studies showing little effect on skeletal muscle itself, could indicate that β -1 agonists are working to increase lipolysis while decreasing lipogenesis, which ultimately decreases fat mass and gives a larger lean to fat ratio (Engeseth et al., 1992).

Supplementation of β -adrenergic agonists

Targeted stimulation of the β -ARs has been shown to improve growth performance and efficiency in livestock through an increase in lean muscle mass (Buntyn et al., 2016) which is reflected in greater total body weight (Elam et al., 2009; Montgomery et al., 2009). Currently, two dietary supplements containing β adrenergic agonists are approved by the FDA for livestock: ractopamine HCl, a β_1 agonist, and zilpaterol HCl, a β_2 agonist (Delmore et al., 2010; Boler et al., 2012). These are sold under the trade names Optaflexx, ractopamine HCl for cattle, Paylean, ractopamine HCl for pigs, and Zilmax, zilpaterol HCl for cattle.

Interactions between Heat Stress and β Adrenergic Agonists

Heat stress is the biggest cause of mortality for livestock producers (Norris et al., 2003), but for those animals that survive, heat stress still causes problems as it limits muscle growth and efficiency. Heat stress results from the combination of ambient conditions and individual animal tolerance of these conditions and causes an increase in body heat outside of the animal's normal physiological range (Young, 1993). Heat stress is characterized by an increase in rectal temperature and respiration rate, decreased feed intake, and overall loss of efficiency which, together, limit growth in livestock (Beatty et al., 2006). Stress is known to decrease growth efficiency by increasing glycogenolysis, a less efficient process compared to glucose oxidation, within skeletal muscle (McVeigh et al., 1982; Lahucky et al., 1998; Komatsu et al., 2014). This energy sparing phenotype causes heat stressed animals to have lower quality carcasses

showing less marbling, decreased hot carcass weight, and a greater number of dark cutters (Kreikemeier et al., 1998).

Both heat stress and β adrenergic agonists increase activity of the adrenergic system, and thus it is reasonable to postulate that these factors might have interacting effects on skeletal muscle. Others have hypothesized that feeding β adrenergic agonist supplementation would worsen or exacerbate the stress response caused by hyperthermia (Grandin, 2013; Loneragan et al., 2014). An alternative hypothesis is that stimulation of the adrenergic system during heat stress could lessen the efficacy of β adrenergic agonists. There have been studies highlighting physiological changes in animals experiencing heat stress and β adrenergic agonist supplementation such as respiratory rates, final body weights, and carcass characteristics, but mechanisms behind these changes have yet to be examined.

Effects of Anabolic Steroids on Muscle Growth and Efficiency

Overview of Androgens

Androgens are steroid hormones that promote anabolic growth in skeletal muscle and other tissues and also promote the development of secondary male characteristics (Sriram). Additionally, androgens serve as precursors for estrogens and other steroids (Nelson and Bulun, 2001). Androgens include testosterone, dihydrotestosterone (DHT), and androstenedione (A4), and are primarily produced by

the Leydig cells of the testes in the male, but smaller amounts are also produced by the ovaries of the female and by the adrenal glands of both sexes (Zouboulis, 2004). Like all steroids, androgens are derived from cholesterol via enzymatic conversion by p450a (Nebert and Russell, 2002). As lipids, these products travel through the body bound to the carrier plasma protein, sex-hormone binding globulin (SHBG) (Anderson, 1974). Steroidogenesis is primarily regulated by the hypothalamic-pituitary-gonadal (HPG) axis as described in detail by Peng et al. (Peng, 1973). Gonadotropic releasing hormone (GnRH) synthesized and secreted from the hypothalamus stimulates the release of follicle stimulating hormone (FSH) and luteinizing hormone (LH)(Schaeffler et al.) from the anterior pituitary gland, which in turn stimulate the Leydig cells of the testes to produce and secrete testosterone and other androgens (Dufau et al., 1971). These steroids then activate a negative feedback loop on both the hypothalamus and anterior pituitary such that high circulating testosterone concentrations suppress their own HPG stimulation (DAMASSA et al., 1976).

Androgens stimulate target tissues primarily by activating androgen receptors (AR), which are nuclear receptors that preferentially bind testosterone but also have great affinity for DHT and androstendione (Heinlein and Chang, 2002). The ligand binding site of AR not only binds testosterone and DHT, but other steroidal agonists and antagonists as well (Goa, 2007). Upon binding its ligand outside of the nucleus, this nuclear receptor complex changes conformation which allows it to enter the nucleus and interact directly with specific binding sites on DNA (Breiner et al., 1986) via its centrally-located DNA-binding domain (Lorente et al., 2015). Through this mechanism,

AR stimulation upregulates genes associated with muscle growth as well as development of masculine features (McGINNIS and DREIFUSS, 1989). Antagonists such as methyltrienolone, metribolone keep endogenous androgens from binding, therefore preventing many anabolic processes (Danhaive and Rousseau, 1986).

Androgen-stimulated Muscle Growth

Anabolic steroids, especially testosterone, increase muscle mass in humans and animals (Bhasin et al., 2001). High circulating androgen levels lead to increased expression of the AR which increases muscle cell differentiation, decreases fat cell differentiation (Singh et al., 2015), and promotes greater protein synthesis (Bhasin et al., 2001). Moreover, these steroids also increase fusion of myoblasts with muscle fibers to increase hypertrophic muscle growth at rates that are proportional to cellular AR content (Vlahopoulos et al., 2004). Typically, testosterone exerts its most profound hypertrophic effects on skeletal muscle fibers in adult animals, however it is able to increase the number of muscle fibers in the developing fetus to contribute to muscle growth (Cellotti and Cesi, 1992). In cattle, exogenous sources of testosterone such as implants that release the hormone into circulation over a period of weeks are commonly used to increase growth performance, leading to better feed-to-gain ratios and increased lean muscle mass (Cranwell et al., 1996). In humans, anabolic steroids are used in clinical hormonal therapy for patients with disorders such as hypogonadism, anemia, or osteoporosis (Chrousos, 2009) and occasionally by athletes for increased muscle mass and power (Bhasin et al., 2001). In addition to promoting muscle growth

directly, anabolic steroids also decrease catabolic breakdown of muscle by inhibiting cortisol and other glucocorticoids that promote catabolism (Fahey, 1998).

During steroid biosynthesis, androstendione is the immediate precursor of testosterone (James, 1998). This hormone possesses only 10-20% of the androgenic activity of testosterone (Nelson, 1980), but it is hypothesized that exogenous androstendione is converted to testosterone after entering the body, thus gaining the anabolic effects of testosterone on skeletal muscle (Rasmussen et al., 2000). The alternative hypothesis would be that androstendione itself induces these anabolic processes independent of testosterone (Rasmussen et al., 2000). However, these assumptions have not been substantiated as plasma testosterone concentrations were not increased with androstenedione supplementation, nor was there an increase in anabolic effect (Leder et al., 2000; Rasmussen et al., 2000). Alternatively, some of these studies have shown an increase in estrogen, which may have a more catabolic effect on skeletal muscle (King et al., 1999; Leder et al., 2000).

Elevated Androstenedione in Cattle model for Polycystic Ovarian Syndrome

Cows with naturally high follicular fluid A4 concentrations share many of the same pathologies as women with Polycystic Ovarian Syndrome (Azziz et al.) and thus represent a valuable animal model for this disease. Ehrmann (2005)(Ehrmann, 2005) identifies PCOS among the most prevalent endocrine disorders in premenopausal women, affecting approximately 5% of females worldwide. Clinical diagnosis of PCOS requires presence of at least two of the three characteristic symptoms: polycystic

ovaries, anovulation, and hyperandrogenism/hyperandrogenemia (Lobo and Carmina, 2000). Polycystic ovaries are characterized by increased ovarian volume and area as diagnosed by ultrasound (Fulghesu et al., 2001). Anovulation may include decreased frequency of menstrual cycles or complete absence of cyclicity (Franks, 2007).

Hyperandrogenemia must be diagnosed by lab tests that show elevated levels of androgens in circulation but may be indicated by additional clinical symptoms consistent with androgen excess (hyperandrogenism) such as hirsutism, acne, and alopecia (Ehrmann, 2005). These symptoms cause subfertility in women and can lead to lower quality of life by increasing depression and anxiety as well as other health disorders (Bazarganipour et al., 2013). Greater than 30% of women suffering from PCOS are obese (Azziz et al., 2001) and/or have impaired glucose tolerance (Ehrmann et al., 1999).

Additionally, approximately 10% have type 2 diabetes (Legro et al., 1999). Women diagnosed with PCOS are more insulin resistant than those women with the same body composition showing dysfunction in the insulin signaling pathway in both primary targets of the hormone: adipose and skeletal muscle (Dunaif et al., 2001). Cows have been previously shown to be a relevant biomedical model, and the high A4 cow is perhaps the only naturally-occurring animal model for PCOS (Mahesh). Researchers at UNL have shown that these cows are characterized by greater A4 concentrations in follicular fluid of dominant ovarian follicles (Summers et al., 2013), exhibit reduced reproductive efficiency, produce a 17% lower calving rate (Summers et al., 2014). However, the weaning weight of calves born to high A4 cows is 12 kg heavier than control counterparts (Summers et al., 2014).

As discussed previously, it is unclear whether A4 stimulates muscle growth directly (Rasmussen et al., 2000), but direct anabolic effects on skeletal muscle would explain heavier weaning weights in calves born to high A4 cows. Conversely, if A4 is antagonistic to muscle growth, it is possible that calves from high A4 cows are desensitized to these effects due to chronic in utero exposure. Thus basal circulating levels of A4 would have a less inhibitory tone. In human studies, it has been hypothesized that A4 is converted to estrogen, which has catabolic effects, instead of testosterone (Rasmussen et al., 2000). If A4 is indeed catabolic, then the greater net gain in calves of high A4 cows would most likely be explained by decreased responsiveness to A4.

Conclusion

Much is known about the final outcomes of factors that affect muscle growth such as changes in body weight, total muscle growth, and carcass characteristics, but mechanisms of how these effects come about are not yet understood. Additionally, it is becoming more evident that stress and other factors may have interacting effects, leaving a gap in knowledge for research to elucidate regarding how these factors affect physiology, well-being, and performance of animals. By understanding mechanisms of how muscle develops, grows, and metabolizes glucose we can optimize muscle growth in livestock species.

Utilizing the Strengths-based Theory in Teaching

Strengths

In the 1950's Don Clifton started researching why some people excel at their jobs compared to people who do not. By examining common factors in over 100,000 successful individuals out in the work force across many career fields, Clifton and Gallup identified 34 strengths or talents (Clifton and Nelson, 1996). The CliftonStrengths assessment provides the individual the top five strengths (Gallup Inc., 2018). Instead of trying to improve many mediocre skills, having knowledge of your top strengths allows you to be incredibly talented in the areas you naturally excel. Another important consideration is knowing the areas where you require help from others. With this mindset, it makes sense that blending teams with people of varying talents would ultimately be a better, more productive work or learning environment.

Strengths Domains

Each of the 34 identified strengths fits within one of four domains. The domains differ based on being present- or future-oriented and either people- or task-oriented (Figure 4). The four domains are Executing, Strategic Thinking, Relationship Building, and Influencing. Per Gallup, an individual's top domain can be determined by looking at their top five strengths and identifying which domain a majority of those strengths fall in. It is possible to have an even split between two domains and show characteristics of both.

Individuals within the **Executing** domain are the people who are driven by working to complete tasks. They are organized and have strengths such as Arranger,

Deliberative, and Consistency, which Gallup describes as allowing them to make schedules and rules and then strictly adhere to them. Gallup indicates that Achiever, Belief, and Responsibility strengths make them trusted workers or team members that can be counted on to complete an assigned task as they have a deep internal drive to do what they said they would do. They often work long hours to get the task accomplished before the due date.

Strategic Thinkers are able to focus on what could be potential outcomes to push a team towards the future. They excel at making decisions, as they are best able to absorb, consider, and analyze information. Using their Future, Context, and Ideation strengths they focus on making the future better by considering how history has affected the present situation. To complete tasks and solve problems they utilize strengths such as Strategic, Analytical, Learner, and Intellection, which Gallup predicts allows strategic thinkers to enjoy the process of learning and collecting information to better make decisions.

Relationship Builders tend to be less task-oriented and more people-oriented as they excel at forming and valuing relationships with other people. These team members are described by Gallup as often being the glue holding the team together and making it better than simply the sum of its parts. They work well with others, making other team members feel valued through their strengths such as Empathy, Includer, and Positivity. They are able to encourage others to be the best team member possible and to keep the team working smoothly by using their Developer, Connectedness, Harmony, and

Individualization strengths. Relationship builders are adaptable and relatable, making them good team players.

Lastly, **Influencers** are the team members that take charge of a team. Gallup describes them as being able to sell their ideas and convince others to follow them. They encourage others using their Activator, Maximizer, and Woo strengths. Influencers are Self-assured and Commanding, but also communicate well. They are competitive and always forward thinking, which keeps them on the forefront of their desired path.

Strengths-based theory

The strengths-based theory states that capitalizing on strengths and improving weaknesses take similar amounts of effort, but the former allows greater success overall (Clifton and Nelson, 1996). In other words, focusing on individual strengths rather than weaknesses works more effectively to increase productivity and mood. Since 1998, more than 16 million people have taken the CliftonStrengths assessment to identify their natural strengths (Gallup Inc. 2018). Currently, more than 90% of the Fortune 500 companies and greater than 600 colleges and universities utilize CliftonStrengths to identify strengths in individuals to allow better team work and personal performance. Although the traditional CliftonStrengths assessment is useful in an educational setting, it is sometimes difficult to answer career and work-place related questions for students who have not yet experienced careers. To provide easier assessment for students, Gallup released the CliftonStrengths for Students assessment in 2017 (Gallup, 2017). This assessment typically gives the same strengths results, but the assessment questions

are oriented to academic settings. There have been many studies showing that strengths-based approaches work in both the workplace and with individual coaching for students to improve productivity, teamwork, and emotional well-being (Harter et al., 2002; Clifton and Harter, 2003; Clifton, 2004; Lopez and Louis, 2009). However, there is a gap in regarding the impact on teams within an academic setting. Since many STEM based courses utilize group work to enhance learning, it is important to understand how to effectively use strengths knowledge to improve learning in students.

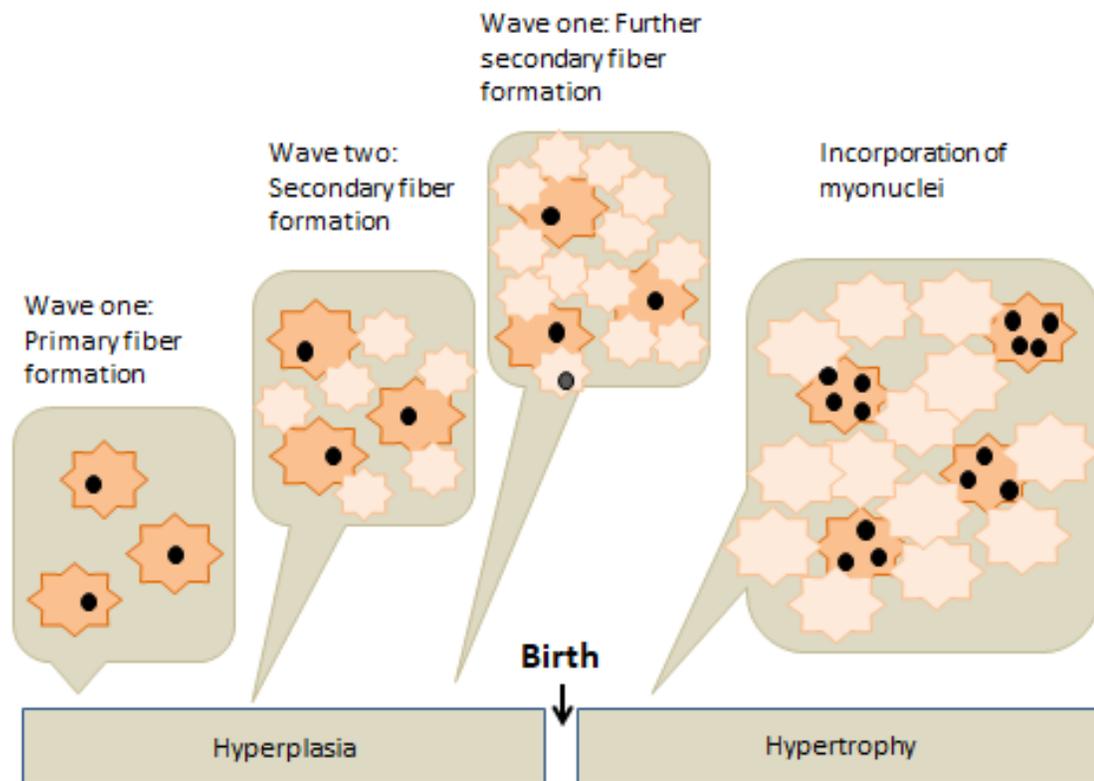


Figure 1. Growth of myofibers happens in waves, each wave providing scaffolding for the next to grow upon. After birth fibers grow in size but no longer in number.

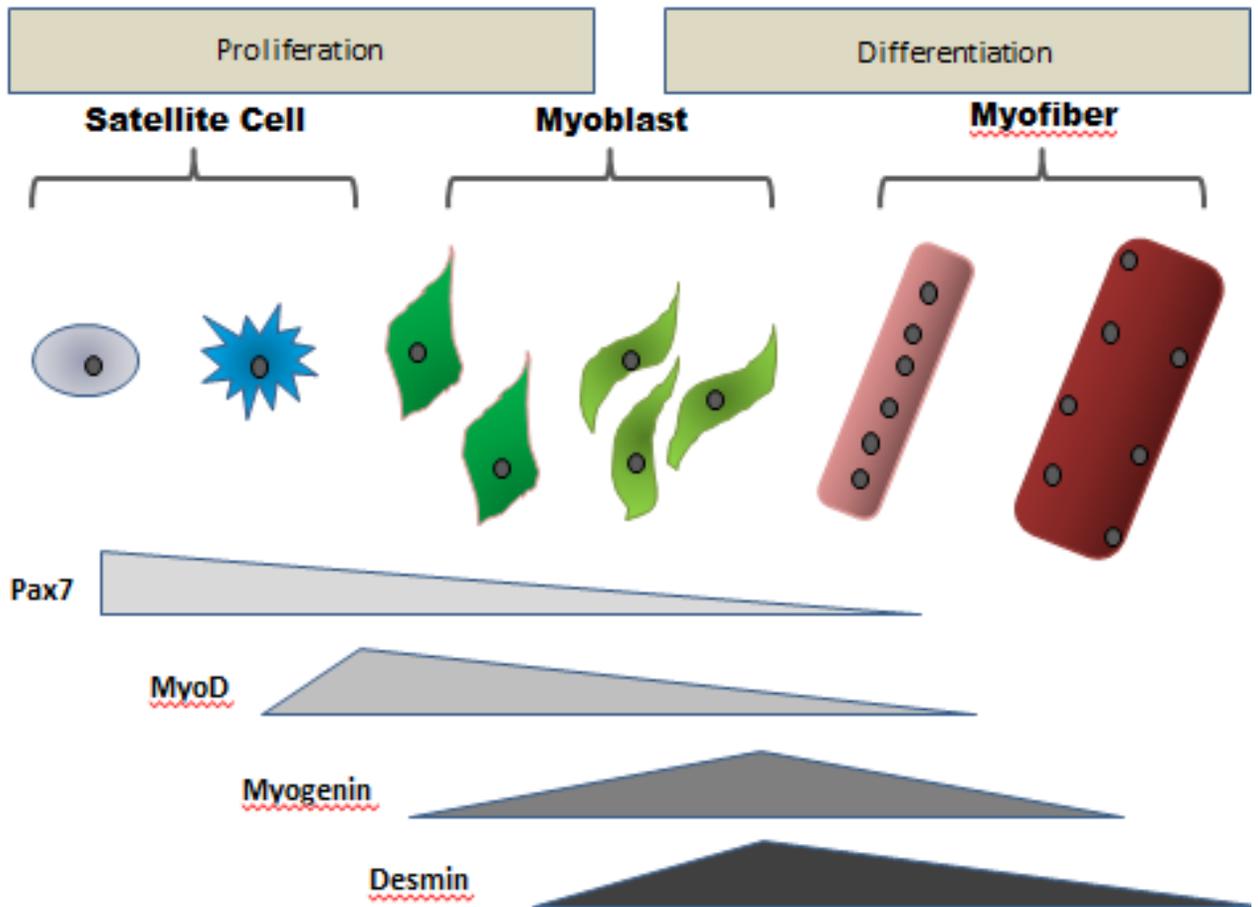


Figure 2. Myogenic regulatory factors serve as biomarkers for different processes the myoblast goes through as it becomes a mature myofiber.

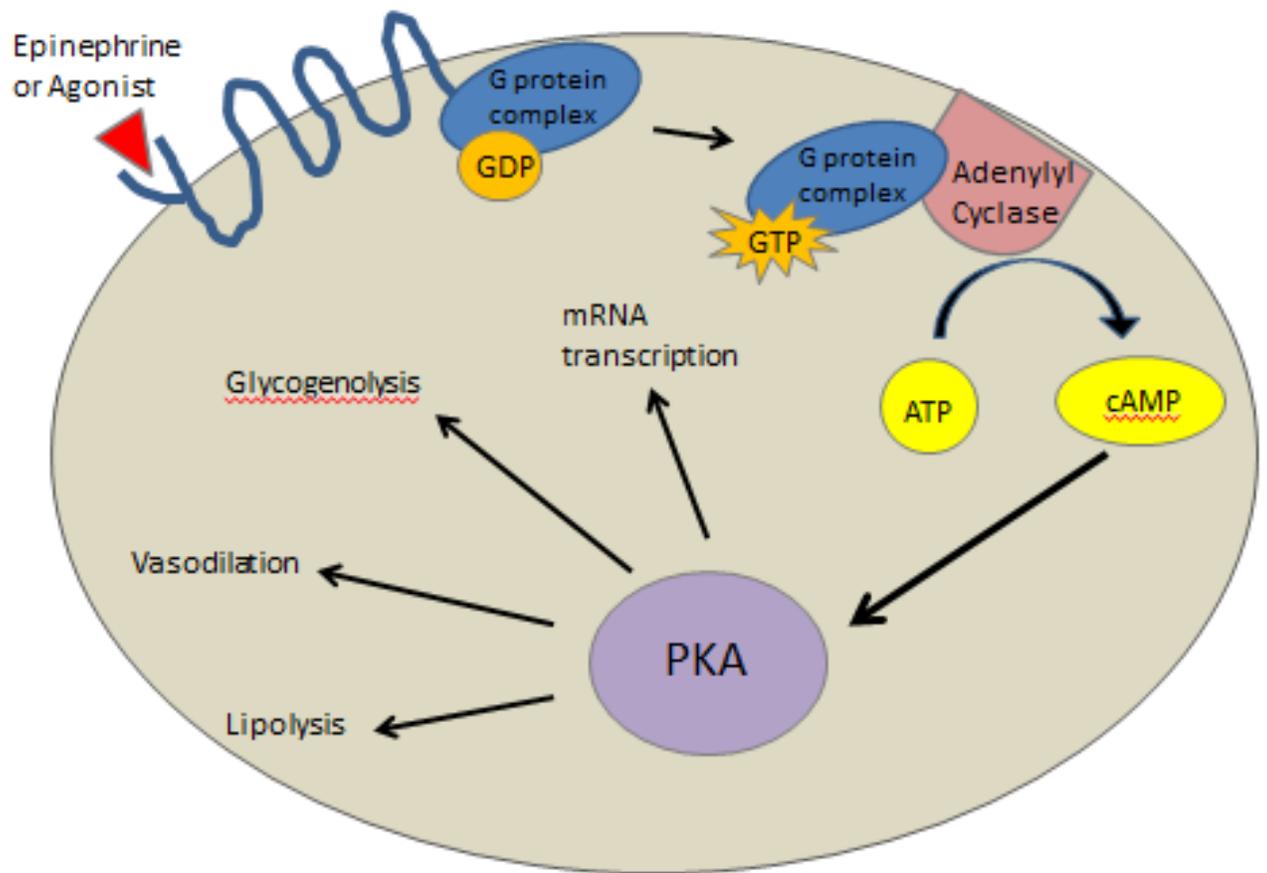


Figure 3. Signal cascade upon binding of β -AR

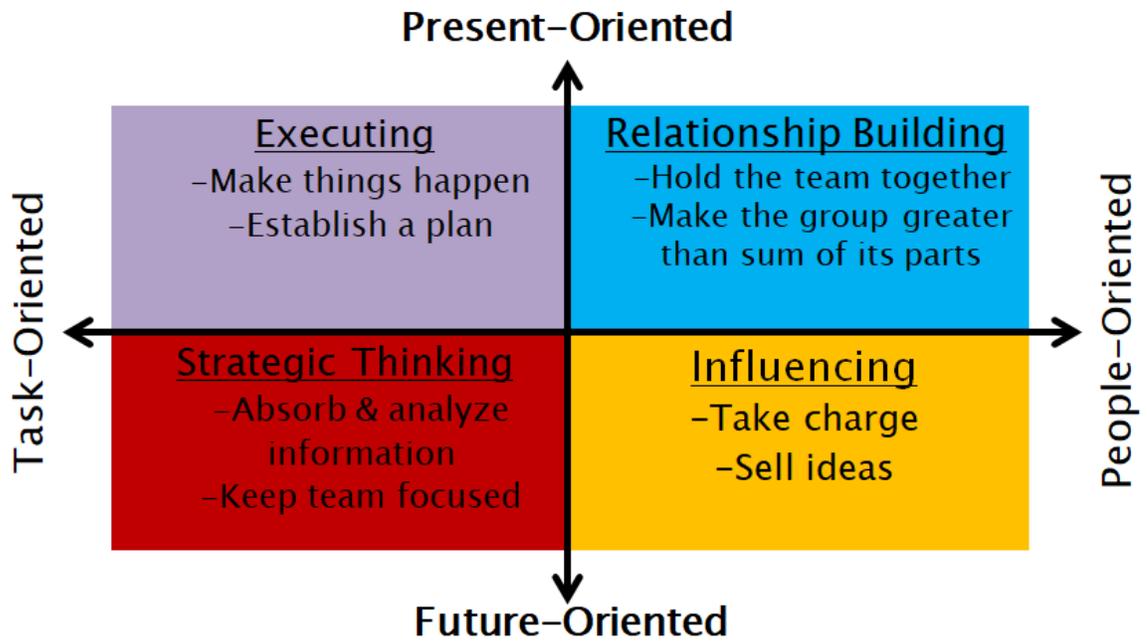


Figure 4. Basic characteristics of the four major domains according to Gallup, Inc.

Chapter 2

Identifying hyperthermia in heat-stressed lambs and its effects on β agonist-stimulated glucose oxidation in muscle

ABSTRACT

Heat stress is known to decrease value and production efficiency in food animals. Conversely, β agonists increase value due to increased muscle growth efficiency, but it is unknown how each of these factors impacts the other. In this study, we sought to determine how heat stress and β agonists affect glucose oxidation in muscle independently and in combination. Crossbred lambs were fed high-energy diets for 21 days containing one of three dietary β agonist treatments: no supplement, ractopamine HCl (β 1 agonist), or zilpaterol HCl (β 2 agonist). In addition, lambs were housed under one of two environmental conditions: thermoneutral (25°C, 15% RH) or heat stress (40°C, 35% RH). On the last day of treatment, two alternative temperature-measuring devices (infrared (IR) thermometer gun and IR camera) were compared to core body temperatures measured by rectal thermometer. Lambs were harvested on day 22 and intact flexor digitorum superficialis muscle strips were used to measure ex vivo glucose oxidation under basal and insulin-stimulated conditions. We found that ear and eye temperatures recorded with the IR camera and skin temperatures (sheared and unsheared) recorded with the IR thermometer guns (at higher emissivity) consistently correlated to core body temperatures measured with the rectal thermometer ($r = \sim 0.6$ to 0.7) and may represent non-invasive alternatives to rectal temperature for detecting hyperthermia in sheep. Surprisingly, we did not observe interactions among environmental treatment, dietary supplement, and incubation media for glucose

oxidation rates. Exposure to heat stress for 21 days decreased ($P \leq 0.05$) skeletal muscle glucose oxidation by ~21%, dietary supplementation of β_2 agonist for 21 days increased ($P \leq 0.05$) muscle glucose oxidation by ~15%, and addition of insulin to media during ex vivo incubation of muscle strips increased ($P \leq 0.05$) glucose oxidation by ~25%.

Interestingly, dietary supplementation of β_1 agonist had no discernable effect on muscle glucose oxidation. These findings show that heat stress reduces muscle glucose oxidation and β_2 agonist increases it, although neither altered the impact of the other. Moreover, these effects were present 24 hours after treatments ended, which shows that heat stress and β agonist supplementation have lasting metabolic effects.

INTRODUCTION

Heat stress and β adrenergic agonists both elicit responses in tissues by activating adrenergic pathways. In livestock, heat stress is known to decrease growth and metabolic efficiency, and β agonist supplementation has been shown to improve growth performance and efficiency (Buntyn et al., 2016). However, little is known about how these two activators of the adrenergic system interact with each other.

Catecholamines, such as epinephrine, are the natural ligand of the adrenergic system. These compounds interact with two classes of receptors, α adrenergic receptors and β adrenergic receptors (Mersmann, 1998). In recent years, supplementation of β -specific adrenergic agonists have benefited the livestock industry due to the increase in lean muscle mass and increase in total body weight that the supplements induce in feedlot animals (Elam et al., 2009; Montgomery et al., 2009). Two β agonist supplements are

presently FDA-approved; the β 1 agonist, ractopamine HCl, and the β 2 agonist, zilpaterol HCl (Delmore et al., 2010; Boler et al., 2012) Boyd et al. (Boyd et al., 2015) hypothesized that the increase in muscle mass in zilpaterol-fed animals may lead to greater heat stress signals such as increased respiration and panting in cattle. However, after analyzing average and maximum body temperatures of these animals, they found that zilpaterol-fed animals actually maintained lower average body temperatures than control animals. These findings show that there is still much to be learned about how these compounds affect muscle metabolic function and growth. Additionally, it is important to understand how environmental stressors such as heat stress affect the efficacy of the supplements. Because of their contrasting individual effects, we hypothesized that heat stress and β agonist supplementation would have interacting influences on skeletal muscle glucose oxidation, a key determinant of metabolic efficiency. Our objective was to determine the impact that heat stress, β 1 agonists, and β 2 agonists have on muscle-specific glucose metabolism and how the effects of these factors interact. Furthermore, we sought to test the ability of alternative temperature-measuring devices to detect hyperthermia in heat stressed animals.

MATERIALS AND METHODS

Animals and experimental design.

This study was approved by the Institutional Animal Care and Use Committee at the University of Nebraska-Lincoln. Studies were performed at the UNL Animal Science Complex, which is accredited by AAALAC International.

Columbia-Suffolk crossbred lambs (n=49) were purchased commercially. The study was performed in random blocks. After a 3-week acclimation period, all lambs were individually penned and fed high-energy diets (Table 1) for 28 days. For the final 21 days, lambs were housed in either thermoneutral (25°C, 15% RH) or heat stress (40°C for 12 h, 35°C for 12 h, 35% RH) conditions and each lamb received either no dietary supplement, ractopamine HCl (0.03996 g/hd/d), or zilpaterol HCl (0.025 g/hd/d) delivered in 200g ground corn added to their daily ration. Lambs were slaughtered on day 29.

Body temperature assessment.

Ambient temperature and relative humidity were continually monitored with a HOBO (Onset Computer Corporation, Bourne, MA) on day 28, two different rectal thermometers (ReliOn, Bentonville, AR) were used to measure rectal temperature, and the readings were averaged. Two infrared (IR) thermometer guns, designated gun A (Model FB61354, Fisher Scientific, Pittsburgh, PA) and gun B (Model TN418LD, Metris Instruments, Los Gatos, CA), and an IR camera (Model A655sc, FLIR Systems Inc., Wilsonville, OR) were used to measure surface body temperatures. Guns were held ~0.5 m from the animal and 10-second average temperatures were recorded at three different locations: the center of nose between the nostrils, sheared loin area of the

back over the 12/13th ribs, and a non-sheared area (~3cm wool length) approximately 5 cm cranial to the sheared area. Temperature at each area was measured across a range of emissivity values, from 0.40-1.00. At the same time, images were captured with the IR camera at two different distances: 1-1.5 m and 2-3 m. Three forward-facing images of each sheep were taken from each distance. Images were analyzed using FLIR ResearchIR Max (FLIR Systems Inc.), and temperatures at the center of the nose between the nostrils, the center of the eye, and the inside center of the ear were each averaged across the three images.

Flexor digitorum superficialis muscle isolation.

At harvest, flexor digitorum superficialis muscles were collected tendon-to-tendon from the left hindlimb and separated into intact longitudinal strips to measure glucose oxidation. After muscle was washed in ice-cold phosphate buffered saline (PBS) and dissected longitudinally, strips were pre-incubated for 1 h at 37° C in gassed (95% O₂, 5% CO₂) Krebs-Henseleit bicarbonate buffer (KHB) containing 0.1% bovine serum albumin (Gibco Life, Grand Island, NY) and 5 mM D-glucose. Media were spiked with either nil (basal) or 5 mU/ml insulin (Humulin-R; Ely Lilly). Strips were then washed in treatment-spiked KHB with no glucose for 20 minutes at 37°C.

Glucose oxidation.

Rates for glucose oxidation were determined by oxidation of [¹⁴C-U]-D-glucose as previously described (Cadaret et al., 2017b) with some modifications. Muscle strips were placed in sealed dual-well chambers and incubated for 2 h at 37°C in treatment-

spiked KHB with 5 mM [^{14}C -U]-D-glucose (0.25 $\mu\text{Ci}/\text{mmol}$). The adjacent well contained 2M NaOH to capture CO_2 . Following incubation, chambers were cooled at -20°C for 2 min, 2M HCl was injected into the media through the rubber seal to release media-bound CO_2 , and the chambers were incubated for 1 hr at 4°C . Following incubation, muscle strips were weighed and NaOH was collected and mixed with UltimaGold scintillation fluid to determine specific activity of $^{14}\text{CO}_2$ using liquid scintillation with a Beckman-Coulter 1900 TA LC counter (Brea, CA). Specific activity of the media was determined from three 10- μl aliquots of unused media mixed with 500 μl distilled water and scintillation fluid. Radioactive compounds and scintillation fluids were purchased from Perkin-Elmer (Waltham, MA).

Akt Phosphorylation.

Insulin activity in muscle strips was estimated from the proportion of phosphorylated Akt to total Akt as previously described (Cadaret et al., 2017b). Muscle strips were incubated in either basal or insulin-spiked KHB media for 20 min at 37°C and then snap frozen in liquid nitrogen and stored at -80°C . Frozen muscle strips were homogenized in manufacturer recommended concentrations of protease (Thermo Fisher, Carlsbad, CA), sonicated for 15 seconds and centrifuged ($14,000 \times g$, 5 min, 4°C). Supernatant was collected and total protein concentration was determined using a Pierce BCA Protein Assay Kit (Thermo Fisher). Equal amounts of protein from each sample were boiled for 5 min at 95°C in BioRad 4X Laemmli Sample Buffer (BioRad, Hercules, CA) and separated by SDS-PAGE. Gels were then transferred to poly-

vinylidene fluoride low fluorescent membranes (BioRad), incubated in Odyssey block solution (Li-Cor Biosciences, Lincoln, NE) for 1 h at room temperature and washed with 1X TBS-T (20 mM Tris-HCl + 150 mM NaCl + 0.1% Tween 20). Membranes were incubated with rabbit antibodies against Akt (1:1,000) or phosphorylated Akt (Ser473) (1:2,000) diluted in Odyssey block solution + 0.05% Tween-20 overnight at 4°C. The following day, membranes were incubated with goat anti-rabbit IR800 IgG secondary antibody diluted in Odyssey block solution containing 0.05% Tween-20 and 0.01% SDS at room temperature for 1 h. Blots were then scanned with the Odyssey Infrared Imaging System and analyzed in Image Studio Lite Software.

Myosin heavy chain electrophoresis

Protein isolated from flexor digitorum superficialis was used to determine myosin heavy chain proportions in these muscles. Protein samples were incubated for 10 min at room temperature, heated to 70°C for 10 min, and then combined with BioRad 4X Laemmli Sample Buffer to make a 1X solution, which was loaded into a gel at 10 µg/well. Myosin Heavy Chain isoforms were separated by SDS-PAGE with a stacking as previously described (Yates et al., 2014). Electrophoresis was performed for 24 h at 150 V and at 4°C. Gels were stained overnight with Gel-Code Blue (Thermo Fisher), washed in distilled water, and imaged on the Odyssey infrared imaging system. MyHC-I and II were measured in Image Studio Lite by densitometry.

Immunohistochemistry

Sections from the mid-portion of the flexor digitorum superficialis were fixed in 4% paraformaldehyde in PBS, embedded in OTC Compound, and frozen at -20°C. Eight- μ m cross sections were then mounted on Fischerbrand Superfrost Plus microscope slides (Thermo Fischer Scientific, Waltham), and immunostaining was performed as previously described (Yates et al., 2014). Briefly, tissues were washed in PBS with 0.1% Triton-X-100 (Sigma-Aldrich) and then boiled in 10 mM citric acid buffer (pH 6, Sigma-Aldrich) for antigen retrieval. Non-specific binding was blocked by incubation with 0.5% NEN blocking buffer (Perkin-Elmer, Waltham, MA) for 1 h at room temperature, and primary antibody diluted in PBS + 1% BSA was applied overnight at 4°C. Fiber type was determined with mouse antibodies against MyHC-I (BA-D5, 1:20; DSHB, University of Iowa, Iowa City, IA), MyHC-IIa (2F7, 1:20; DSHB), and MyHC-IIx (6H1, 1:150; DSHB). Negative controls were incubated in PBS without primary antibody. Fibers were counterstained with rabbit antibody against desmin (1:200; Sigma-Aldrich). Immunocomplexes were detected using immunoglobulin antiserum conjugated with either Alexa Fluor 488 (1:2,000; Jackson ImmunoResearch Laboratories, West Grove, PA) or Alexa Fluor 594 (1:1,000; Invitrogen Life Technologies, Carlsbad, CA) and visualized on an Olympus IX73 microscope. Images were captured with an Olympus CB5S camera and analyzed with CellSens software to determine fiber cross-sectional areas and fiber type ratios. All images were encoded to blind the evaluator of animal and treatment designs to prevent bias.

Statistical analysis.

Body temperature data were analyzed by one-way ANOVA for differences between thermoneutral and heat-stressed lambs using the GLM procedure of SAS (SAS Institute, Cary, NC). Pearson's correlation values between IR temperatures and rectal temperatures were calculated using the CORR procedure of SAS. Ex vivo data were analyzed as a 2x3x2 factorial design by ANOVA using the Mixed procedure of SAS, with environmental treatment (n = 24) and dietary supplement (n = 16) in the main plot and incubation media as a repeated measure. All other data were analyzed as a 2x3 factorial using the mixed procedure of SAS. For all outputs, lamb was the experimental unit. Data are presented as means \pm standard error.

RESULTS

Hyperthermic measurements.

Temperatures measured by rectal thermometer, IR thermometer guns, and IR camera were higher ($P \leq 0.05$) in heat stressed lambs than in thermoneutral lambs (Figure 1). Typically, IR temperature measurements taken at the ear, eye, and back correlated well with rectal temperatures. However, IR temperatures measured on the nose of the animal were less correlative with rectal temperatures. Temperatures recorded by IR camera for each area did not differ due to distance from the animal, and correlations to rectal temperature were slightly greater for the ear than for the eye. For temperatures measured by IR thermometer gun, the greatest correlations were observed at higher emissivity levels (above 0.70), and correlations were not significant

below 0.50 emissivity for either gun. Surprisingly, correlations to rectal temperatures were similar between sheared and unsheared areas of the back.

Glucose oxidation

No interactions were observed between environmental treatment, dietary supplement, and incubation media, and thus only main effects are presented. As expected, incubation of muscle strips with insulin increased ($P \leq 0.05$) glucose oxidation rates in all muscle strips compared to incubation without insulin (Figure 2). Exposure to heat stress for 21 days decreased ($P \leq 0.05$) glucose oxidation rates in muscle collected at slaughter the day after ending the environmental treatment. Dietary supplementation of ractopamine for 21 days did not affect muscle glucose oxidation rates, but dietary supplementation of zilpaterol for 21 days increased ($P \leq 0.05$) muscle glucose oxidation.

Insulin signaling responsiveness

No interactions were observed between environmental treatment, dietary supplement, and incubation media, and thus only main effects are presented. As expected, incubation of muscle strips with insulin increased ($P \leq 0.05$) phosphorylated Akt in all muscle strips compared to incubation without insulin (Figure x). Exposure to heat stress for 21 days increased ($P \leq 0.05$) phosphorylated Akt in muscle collected at slaughter the day after ending the environmental treatment. Neither dietary supplement affected muscle phosphorylated Akt.

Myosin heavy chain immunoblot

There were no differences between fiber type ratio between any environmental or supplemental treatments.

Immunohistochemistry

MyHCI positive fibers showed no differences in proportions of MyHC I:II, but area of type I fibers was larger in β -2 supplemented animals than control or β -1 supplemented animals. Within the β -2 supplemented animals heat stressed animals type I fibers were larger.

MyHCII stained fibers tended to have a greater proportion of MyHCII positive fibers under heat stress conditions ($P = 0.07$) with heat stressed animals averaging 37.5% MyHCII positive fibers compared to thermoneutral averaging 30.6% MyHCII positive fibers. There were no differences in area of MyHCII positive fibers between an supplement or environmental treatments.

DISCUSSION

In this study, we show that hyperthermia can be detected in chronically heat-stressed livestock by infrared devices and that it is detrimental to metabolic efficiency. Surprisingly, chronic heat stress did not affect the metabolic benefit of β 2 agonist supplementation, as muscle glucose oxidation was similarly decreased in heat stressed animals regardless of whether they received the dietary supplement or not. Likewise, β 2 agonist supplementation improved muscle glucose oxidation in thermoneutral and heat

stressed animals alike. Moreover, the respective effects of heat stress and β 2 agonist supplementation on muscle glucose oxidation were observed under both basal and insulin-stimulated conditions. On a whole protein level shown by SDS-PAGE, there were no differences between fiber types, but when looking at the immunohistochemistry for type I and II fibers we see that β -2 supplementation increased size of type I oxidative fibers allowing these animals to be more efficient. Meanwhile animals in heat stressed conditions show a greater proportion of type II fibers expressing an energy saving phenotype that uses more glycolytic metabolism compared to oxidative. Together, these findings show that hyperthermic animals exhibit less metabolic efficiency, which helps to explain poorer growth performance under heat stress conditions. Moreover, β 2 agonists are effective promoters of metabolic efficiency even in heat stressed animals, which contributes to their value as growth promoters.

Heat stress, like most physiological stressors, activates the adrenergic system, and thus it would be reasonable to postulate that animals experiencing chronic heat stress would be less responsive to β adrenergic supplements. However, we show that there was no interaction between heat stress and β agonist supplementation on skeletal muscle glucose metabolism. These surprising results indicate that the inhibitory and stimulatory effects of heat stress and β 2 agonist supplementation, respectively, on muscle glucose oxidation occur through independent mechanisms. Although it is safe to assume that the β 2 agonist is functioning solely through β adrenergic pathways, it is unclear whether the effects of heat stress are mediated by other components of the adrenergic system or by other regulatory systems altogether. It is important to note that

the effects from heat stress and zilpaterol were observed 24 hours after ending both treatments. Although the possible effects of residual zilpaterol in the animal's system cannot be dismissed, it would appear from these findings that both factors have lasting effects on muscle glucose metabolism, which is not surprising when the adaptability of skeletal muscle is considered. Future studies may be able to show additional mechanisms by which heat stress and β_2 agonist supplementation work to influence metabolic efficiency in muscle.

In order to evaluate the effects of hyperthermia on metabolic function in livestock, it is important to reliably identify hyperthermic animals. Core body temperature is traditionally estimated by measuring rectal temperature with a thermometer. However, safety concerns and animal disturbance may limit the use of this technique. In this study, we show that hyperthermia can be reliably detected in sheep by measuring body surface temperatures with infrared devices. In general, temperatures measured at the eye, ear, and loin area of the back by IR camera as well as the more affordable IR thermometer guns correlated well with rectal temperatures. Importantly, temperatures measured at the nose were highly inconsistent and did not correlate well with rectal temperatures, and thus should not be considered as appropriate measurements of body temperature. We speculate that the poor results from the nose temperatures were due to the movement of air around the nose and differing amounts of moisture on the nose itself. Nonetheless, these IR devices are potentially useful tools to detect hyperthermia in livestock without entering the animal's pen or making physical contact. Moreover, additional research to normalize

surface body temperature to core body temperature, these devices could be used as an alternative to rectal thermometers in clinical or production settings, which could improve safety and reduce animal stress.

IMPLICATIONS

In this study, we show that infrared devices can be used to detect hyperthermia in heat stressed animals and that hyperthermic animals exhibit reduced skeletal muscle glucose oxidation, which helps to explain performance deficits in hotter conditions. Moreover, β_2 agonists improve muscle glucose oxidation but do not offset the heat stress-induced deficits, meaning that neither chronic heat stress or β_2 agonist supplementation affects the animal's metabolic response to the other factor.

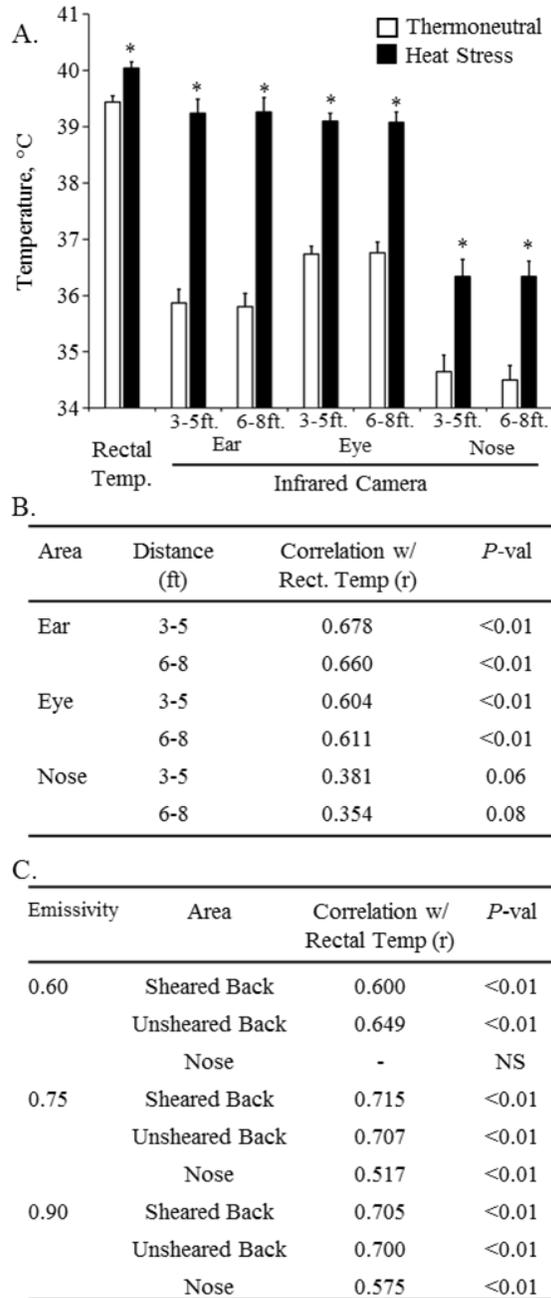


Figure 1. Body temperatures in control and heat-stressed lambs. A. Differences in temperature measured at the ear, eye, and nose of lambs by infrared camera. *Denotes differences ($P \leq 0.05$) between thermoneutral and heat stressed lambs for each area/distance. B. Pearson correlation coefficients between rectal temperature and temperatures measured by infrared camera. C. Pearson correlation coefficients between rectal temperature and temperatures measured by infrared thermometer.

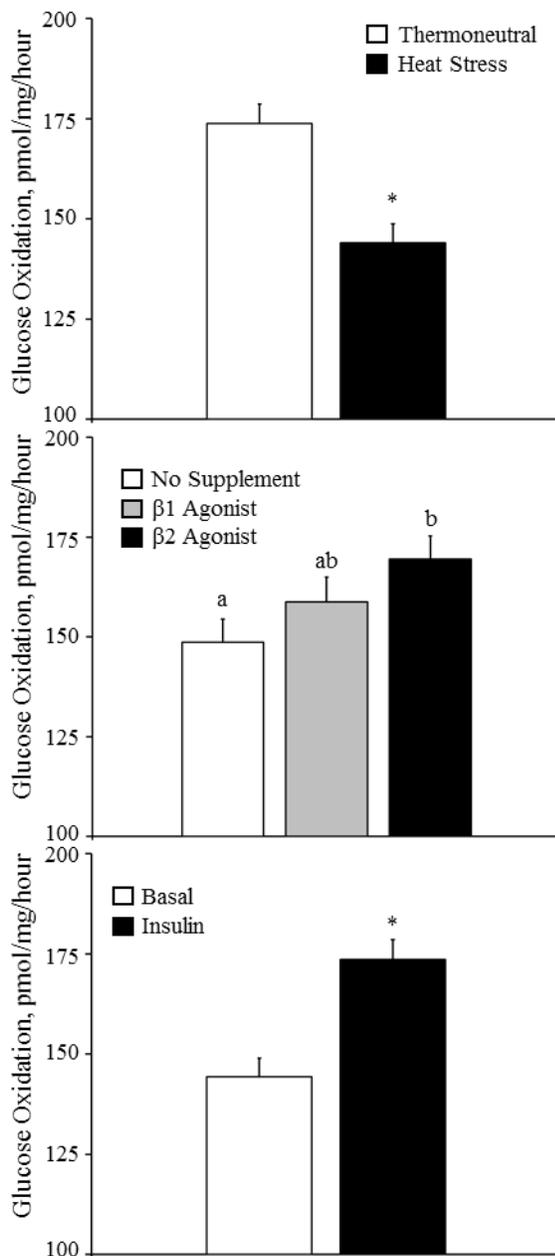


Figure 2. Glucose oxidation in primary flexor digitorum superficialis muscle from lambs housed under thermoneutral or heat stressed (40°C) conditions and fed a diet supplemented with $\beta 1$ or $\beta 2$ agonists. *Denotes differences ($P \leq 0.05$) between thermoneutral and heat stressed lambs in the top box and between basal and insulin-spiked media in the bottom box. ^{a,b}Denote differences ($P \leq 0.05$) among dietary supplements.

Chapter 3

Impaired muscle stem cell function in cows with high concentrations of androstenedione in their follicular fluid

ABSTRACT

Androstenedione (A4) is an anabolic steroid thought to increase muscle mass and strength similarly to testosterone. However, some studies show that it may function more like estrogen, eliciting catabolic effects on muscle. In a herd of cows at University of Nebraska-Lincoln, 2 distinct groups of cows were recognized; those with high levels of A4 in their follicular fluid (>40 ng/ml; High A4) and those with low levels (<20 ng/ml; Control). Although High A4 cows have impaired fertility traits, they consistently wean heavier calves. Thus, we hypothesize that their calves are desensitized to the catabolic effects of A4. The present study sought to test the function of myoblasts (muscle stem cells) from High A4 dams to determine if any intrinsic changes exist that if present in their offspring could help explain weaning weight differences. Primary myoblasts isolated from High and Control cows were tested for proliferative capacity (2-h EdU pulse) in growth media (20% FBS) containing no additive (basal), TNF α , or testosterone. Percentages of myogenin-positive and desmin-positive myoblasts were determined after 4-d incubation in differentiation media (2% FBS) containing no additive (basal) or TNF α . No interactions between A4 classification and incubation conditions were found for any variable. Myoblasts from High A4 cows exhibited ~9% slower ($P \leq 0.05$) proliferation rates but ~25% greater ($P \leq 0.05$) percentages of myogenin-positive nuclei

and ~15% greater ($P \leq 0.05$) percentages of desmin-positive nuclei after 4-d differentiation. $\text{TNF}\alpha$ caused a modest decrease ($P \leq 0.05$) in proliferation rates but not differentiation rates in myoblasts from all cows. Conversely, testosterone had no discernable effect on proliferation of myoblasts. These data show that physiological conditions responsible for high levels of A4 in follicular fluid may also be directing skeletal myoblasts to prematurely exit the cell cycle and begin precocious differentiation. Myoblast function serves as the rate-limiting step in muscle growth, and because calves from High A4 cows are in fact heavier, it is reasonable to postulate that they have developed a desensitivity to the detrimental effect of maternal conditions on myoblast function. Although we cannot know from this study what these maternal conditions are, our findings indicate that it is not associated with altered responsiveness to $\text{TNF}\alpha$ or testosterone. Our next step will be to evaluate myoblasts isolated from offspring of High A4 cows.

INTRODUCTION

It is unclear whether androstenedione (A4) increases muscle mass and strength similar to testosterone or whether it produces primarily catabolic effects on muscle like estrogen (Rasmussen et al., 2000). Summers et al. (Summers et al.) observed two populations of cows that exhibit either high (>40ng/ml; High A4) or low (<20 ng/ml; Control) concentrations of A4 within the fluid of the dominant follicle just prior to ovulation. High A4 cows had decreased reproductive rates and shorter times before

falling out of the herd, but those that did produce calves weaned them ~12 kg heavier than their control counterparts (Summers et al., 2014). It appears that the difference in weights is due to faster-growing and more efficient skeletal muscle. High A4 cows share many characteristics with women suffering from polycystic ovary syndrome (Azziz et al., 2001), whose high levels of circulating androgens are associated with changes in body composition (Kirchengast and Huber, 2001).

Skeletal muscle growth is most impacted by the functional capacity of muscle stem cells known as myoblasts (Cadaret et al., 2017a). Myoblast function can be divided into two distinct processes of proliferation and differentiation. Both processes occur through a carefully orchestrated progression controlled by myogenic regulatory factors (MRF) that are each expressed at specific time points of cellular changes (Molkentin and Olson, 1996). MyoD is expressed primarily when myoblasts are actively proliferating in order to expand their numbers (Gillespie et al., 2009). Myogenin expression is the hallmark MRF biomarker of myoblasts that have exited the cell cycle and begun to differentiate (Hawke and Garry, 2001). As the differentiation process progresses, myoblasts begin to express desmin and continue to do so until fusing with existing muscle fibers to facilitate hypertrophic growth (Ludolph and Konieczny, 1995). The objective of this study was to determine whether differences in myoblast function, specifically proliferation and differentiation, exist between High A4 and Control cows that might explain the greater growth rates of their offspring between birth and weaning. Moreover, we sought to determine if any changes in functional capacity were

related to altered responsiveness to TNF α and/or testosterone, two known regulators of myoblast function and muscle growth.

MATERIALS AND METHODS

Animals and experimental design.

The following experiments were approved by the Institutional Animal Care and Use Committee at the University of Nebraska-Lincoln, which is accredited by AAALAC International.

A subset of mature commercial beef cows from the University of Nebraska Agricultural Research and Development Center were used in these studies. This herd consists of approximately 230 composite beef cows that are 75% Red Angus. Cows were classified as High A4 or Control by human A4 ELISA kit (Alpha Diagnostics International). High A4 cows (n = 10) exhibited >40 ng/ml A4 within the fluid of their dominant follicle just prior to ovulation, and Control cows (n = 10) had <20 ng/ml A4 within their follicular fluid. Myoblasts were isolated from external oblique muscle biopsies collected from randomly selected cows from each classification during ovariectomies for other studies. The biopsy (~5 g) was collected from the incision site, washed in cold PBS, and then finely minced for myoblasts isolation. Myoblasts were liberated via protease digestion and isolated through serial centrifugation as previously described (Yates et al., 2014). Isolates were purified by multiple pre-plate incubations until $\geq 95\%$ pure (pax7-positive).

Myoblast proliferation

Myoblasts were plated on fibronectin coated 6-well plates at a density of 5,000 cells per well and grown in complete growth media (DMEM (Gibco Life Technologies) + 20% fetal bovine serum (FBS, Atlas Biologicals)) for 3 d (media was change at d 2), and then incubated in treatment-spiked growth media for 24 h. Treatment-spiked growth media contained no additive (basal), TNF α (20 ng/ml), or testosterone (10 nM). After 24-h treatment, myoblasts were pulsed with EdU for 2 h, cooled on ice, and fixed in suspension with 4% PFA. Cells were then stained in suspension via Click-iT EdU Staining kit (ThermoFisher) according to manufacturer recommendations. Click-iT EdU cocktail was added at 50 μ M and incubated for 2h at 37° C. Myoblasts undergoing replication during the pulse period were identified with AlexaFluor 555 and counted via flow cytometry (ZEPI; Orflo Technologies).

Myoblast differentiation

Myoblasts were plated on fibronectin coated plates at a density of 30,000 cells per well, grown in complete growth media overnight, and differentiated in treatment-spiked differentiation media (DMEM + 2% FBS) for 4 d (media was changed at d 2) that contained no additive (basal) or TNF α (20 ng/ml). After 4 d, myoblasts were cooled on ice, lifted from the plate with Accutase, washed, and fixed in 4% PFA. Fixed myoblasts were incubated for 1 h at room temperature with primary antibodies against myogenin (1:200; Abcam) or desmin (1:50; GeneTex). Myoblasts were then washed and incubated with PE Conjugate anti-Mouse secondary (1:250; Cell Signaling) for 1 h at room

temperature. Percentages of myogenin-positive and desmin-positive myoblasts were determined by flow cytometry.

Statistical Analysis

Values are expressed as mean percentages \pm SEM. Cow is the experimental unit. Two replicates per incubation condition were performed for each fetus and averaged. Data were analyzed for effects due to A4 classification, incubation condition, and their interaction using the Mixed procedure in SAS (SAS Institute, Cary NC, USA) with culture condition as a repeated variable.

RESULTS

Proliferation.

No interactions were observed between A4 classification and incubation conditions for proliferation rates (Figure 1). Proliferation rates were ~9% less ($P \leq 0.05$) in myoblasts isolated from High A4 cows compared to their Control counterparts regardless of incubation condition. Incubation of myoblasts from all cows in growth media containing TNF α decreased ($P \leq 0.05$) proliferation rates by ~3.5% compared to basal media. Conversely, incubation of myoblasts from all cows in media containing testosterone had no significant effect on proliferation rates.

Differentiation.

No interactions were observed between A4 classification and incubation conditions for percentages of myogenin-positive or desmin-positive myoblasts after 4-d differentiation. The percentage of myogenin-positive cells was ~25% greater ($P \leq 0.05$) in myoblasts isolated from High A4 cows compared to Control cows (Figure 2) regardless of incubation conditions. Likewise, the percentage of desmin-positive cells was ~15% greater ($P \leq 0.05$) in myoblasts isolated from High A4 cows compared to Control cows, regardless of incubation conditions (Figure 3). Unlike proliferation rates, the percentages of myoblasts that were positive for myogenin or desmin did not differ between basal media and TNF α -spiked media.

DISCUSSION

In this study, we show that cows with high A4 concentrations in their follicular fluid also exhibit intrinsic reductions in myoblast proliferative capacity but greater expression of major myoblast differentiation markers. This indicates that conditions responsible for the increase in follicular fluid A4 are also causing precocious differentiation in myoblasts. This may be due simply to impaired proliferation rates that push cells to differentiate because they have exited the cell cycle or due to an unidentified stimulator of differentiation. Regardless, this intrinsic deficit in myoblast function ultimately slows the rate limiting step and presumably restricts muscle growth potential. Because calves born to High A4 cows are of normal size at birth but heavier by weaning, it is reasonable to postulate that exposure to the unidentified conditions of

this “High A4” environment in utero reduces their responsiveness to impediments of myoblast function, allowing greater muscle growth birth to weaning.

Our somewhat surprising observation that testosterone had no effect on myoblast proliferation rates in any cows leads us to believe that A4 (a presumed analog) does not elicit anabolic effects by enhancing myoblast proliferation. Of course, we cannot rule out the potential for different outcomes with different media concentrations than the one used in this study. Many other studies have shown that testosterone works to increase muscle proliferation and muscle protein synthesis (Sinha-Hikim et al., 2003; Kadi, 2008). The small effect of TNF α on proliferation and lack of effect on differentiation was also unexpected compared to findings by Cadaret et al. (Cadaret et al., 2017b), although the fact that these myoblast were from mature rather than growing animals could have contributed to these results.

IMPLICATIONS

The decrease in proliferation and increase in differentiation rates indicates that High A4 cows have intrinsic myoblast dysfunction. As myoblast function serves as the rate-limiting step for muscle growth, this does not explain greater birth-to-weaning growth rates in calves from these High A4 cows. Rather, their calves likely develop a reduced responsiveness to myoblast-restricting conditions of the High A4 cows in utero, which would explain their increased growth between birth and weaning.

Figure 1

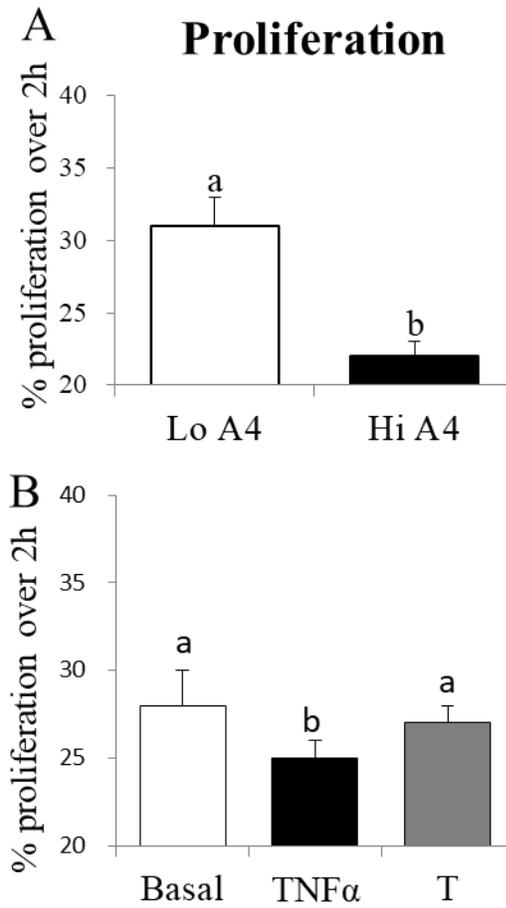


Figure 1. Proliferation rates of myoblasts from High A4 and Control cows during a 2-h EdU pulse. Differences were observed between High A4 and Control cows (A) and among basal, TNF α -spiked, and testosterone-spiked growth media (B). ^{a,b,c} Means with different superscripts differ ($P \leq 0.05$).

Figure 2

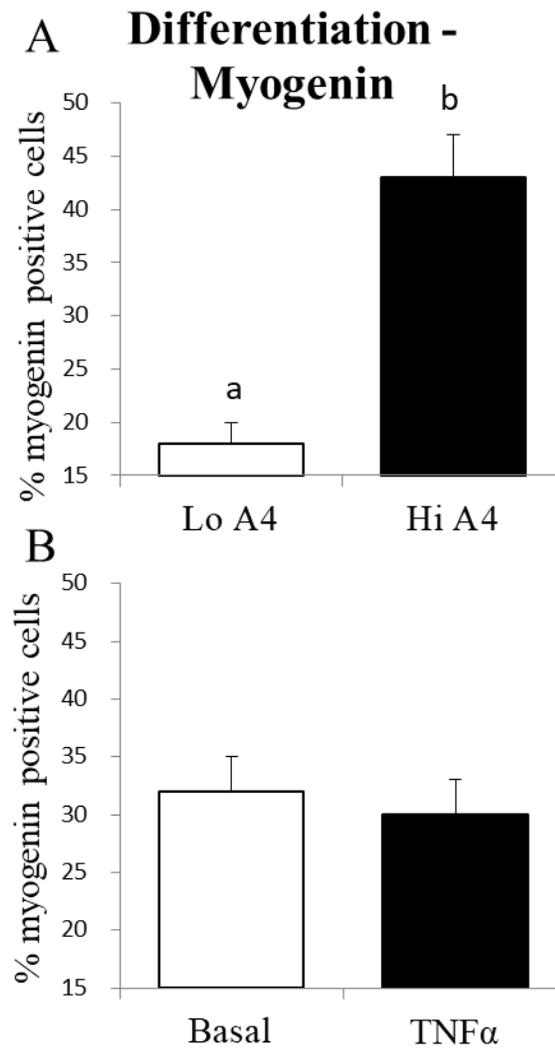


Figure 2. Percent of myoblasts expressing myogenin after four days. Differences were observed between High A4 and Control cows (A) and among basal and TNF α -spiked growth media (B). ^{a,b,c} Means with different superscripts differ ($P \leq 0.05$).

Figure 3

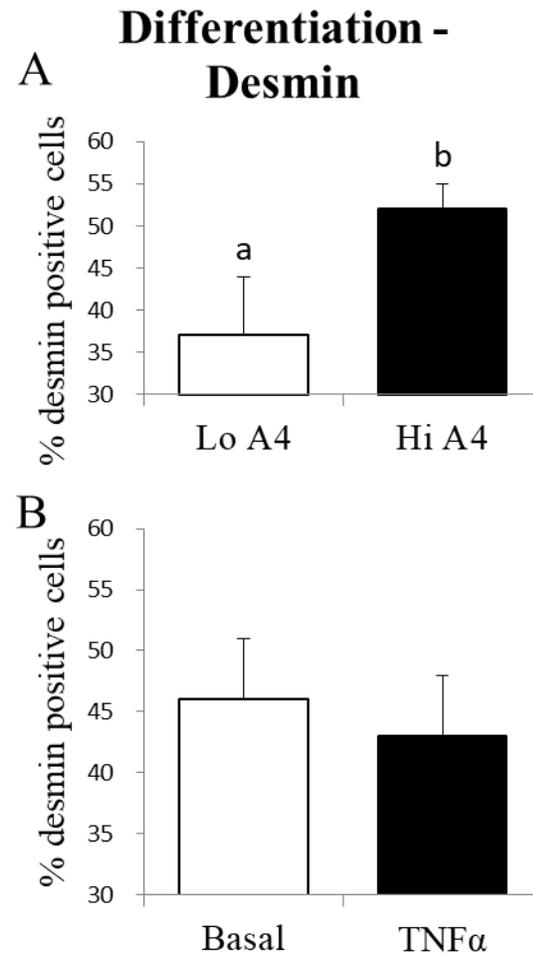


Figure 3. Percent of myoblasts expressing desmin after four days. Differences were observed between High A4 and Control cows (A) and among basal and TNF α -spiked growth media (B). ^{a,b,c} Means with different superscripts differ ($P \leq 0.05$).

Chapter 4

Assigning Strengths-based lab groups improves learning of undergraduate anatomy and physiology concepts

ABSTRACT

Previous research on implementing strengths-based approaches has focused primarily on improving performance in the work place, but the same approaches could apply to classroom settings in higher education. The CliftonStrengths for Students assessment was developed to identify strengths related to academia, and this project aimed to determine if assigning students to groups based on strengths identified by this assessment improved learning in a sophomore-level anatomy & physiology laboratory course. We also assessed student perception of their assigned strengths-based groups. Students were classified into one of four domains based on their top identified strengths: executing, influencing, strategic thinking, or relationship building. For the first half of the semester, students worked in peer-chosen groups of four. Following the midterm practical exam, students were reassigned to groups of four based on their identified strengths domains. Balanced groups (n=9) contained one randomly-selected student from each of the four domains. Unbalanced groups (n=13) contained four students from the same domain. At the end of the semester, students individually completed a final practical exam that assessed their knowledge of the topics covered after assigning their strengths-based groups. They also assembled an anatomical model of the ear with their group members and their performance was timed and graded for

accuracy. After the final practical exam, students completed a survey of their perception of the group to which they were assigned, how they learn, and their expected grade on the final practical exam. Students assigned to balanced groups scored greater ($P < 0.05$) on the final practical exam by $\sim 4.3\%$ and on their total lab grade by $\sim 3.1\%$ compared to students in unbalanced groups. Midterm practical exam scores completed prior to strengths-based group assignment did not differ between groups. Although all students over-estimated their practical and overall lab scores, the difference between self-predicted and actual scores was less ($P = 0.05$) for students in balanced groups. No differences were observed among groups for ear model accuracy score, time to completion, or efficiency (score/ time). Despite improved performance in the balanced strengths-based groups, attitude/perception of the assigned groups did not differ between group types and was in fact generally negative. Students in the strategic thinking domain scored highest ($P < 0.05$) on the final practical exam and in their overall lab grade, and students in the relationship building domain scored lowest ($P = 0.05$). However, students in the relationship building domain were best able to correctly predict their domain with greater ($P = 0.05$) frequency than students in the other domains. These findings indicate that balancing student strengths in lab groups improves learning outcomes but not student perception of the experience. Because identified strengths were not specific to anatomy and physiology but rather academia in general, we believe this simple approach could be applied to in-class group work across many STEM disciplines.

INTRODUCTION

The strengths-based theory states that capitalizing on strengths and improving weaknesses takes a similar amount of effort, but the former allows greater success overall (Clifton and Nelson, 1996). In other words, focusing on individual strengths rather than weaknesses works more effectively to increase productivity and mood. Studies conducted in workplace settings show that identifying and blending individual strengths within teams led to a 6% increase in per-person productivity over a 6-month period (Connelly, 2002). The impact of strengths-based approaches on improving performance in the work place indicates that the approach may have similar impact on increased learning from group activities in higher education, which also requires teamwork. Although strengths-based research has been performed in K-12 children for some time (Hurlock, 1925; Terman and Oden, 1947; Clifton et al., 2006) it has only more recently been investigated in college students on an individual basis and not related to in-class group work. Previous academic studies of individualized strengths-based coaching have observed improvements in both high school students and college undergraduates, including better GPAs and attendance records (Harter, 1998; Williamson, 2002).

To help facilitate this coaching style, Gallup Inc. developed the CliftonStrengths for Students assessment using questions related to academia to identify strengths of students (Clifton, 2017). Based on the improvements observed in team productivity in the workplace, and considering that group work is an integral part of many collegiate science-based laboratory courses, we hypothesized that forming groups of students

with distinct strengths would improve academic performance in a science-based laboratory setting. This project aimed to determine if working in blended strengths-based groups improved learning outcomes compared to traditional peer-chosen groups and unblended strengths-based groups of a sophomore-level anatomy & physiology laboratory course. In addition, we examined student perceptions of their groups to determine if this approach was well-accepted by students.

MATERIALS AND METHODS

Informed consent of participants

All procedures for data collection in this study were approved by the Human Subjects Institutional Review Board at the University of Nebraska-Lincoln. Prior to data collection, students were informed of their option to participate in this study by an independent party without instructors or teaching assistants present. Students, instructors, and teaching assistants were not informed of the specific objectives of the study to ensure a double-blind design. All students were required to complete the assignments and assessments as a part of the course, but only data from consenting individuals were utilized for the study. Students that agreed to participate received a 1.5% bonus on their overall course grade as compensation for participation in the study. Students who chose not to participate were offered the same bonus for an equal alternative effort. Of the 85 students enrolled in the course, 79 were included in analyses. The consent form and recruitment script are included in Appendix A.

Class and assignments

The ASCI 240: Anatomy and Physiology of Domestic Animals Laboratory was split into four sections that convened each Tuesday of the semester. Each section was led by the same instructor, but had a different teaching assistant. Typical lab sessions started with a quiz over the previous week's information, followed by an explanation of the week's activity and accompanying lab assignment. The remainder of the lab period consisted of a hands-on learning activity performed in groups with the teaching assistant and instructor available to answer questions. At mid-semester and the end of the semester, students completed in-class laboratory practical exams over the topics from the preceding weeks.

Strengths assessment and group structure

At the beginning of the semester, students were required as part of the course to complete the CliftonStrengths for Students assessment at no cost to them and to inform their instructor of their top five strengths. Students who had completed the assessment within the last year were allowed to report those results. Students who did not complete this assignment were not included in the study. Each week before the midterm practical exam, students worked in peer-chosen groups. Each week after the midterm practical exam, students worked in their assigned strengths-based groups. Each of the 34 possible strengths described in the CliftonStrengths for Students assessment corresponds to one of four domains: executing, influencing, relationship building, or strategic thinking (Figure 1). Using the top five strengths from the

assessment, students were assigned by the investigators to one of four domains by identifying which domain a majority their strengths fit. Balanced groups (n=9) contained one randomly-selected student from each domain and unbalanced groups (n=13) groups contained four randomly-assigned students from the same domain.

Puzzle assessment

As part of the final practical exam, groups performed an in-class assembly of the anatomical components of the ear using a plastic model. The elapsed time from when each group started the puzzle to the time at which they informed the instructor they were finished was recorded. The model was then assessed by the instructor for accuracy and scored based on the percentage of correctly-placed parts.

Survey

Students completed a written survey in class that assessed their perceptions of learning and group work. Questions regarding learning perception covered the ways in which students felt they learned best such as hands-on anatomical dissections or LabTutor computer modules, and whether they thought the knowledge gained in this lab would help them in future classes or careers. Students were asked to predict both their final practical exam and over-all lab grades. Lastly, students answered questions about working in their strengths-based groups after the midterm practical exam to assess their perceptions of working in these groups. These questions addressed preparation, equal contribution among group members, and if this group helped their understanding of the information presented. The survey is provided in Appendix B.

Statistical analysis

Data for the mid-term and final practical exam scores, the difference between predicted and actual final practical exam scores and the puzzle activity time, accuracy, and efficiency (accuracy/time) were analyzed by ANOVA using the mixed procedure of SAS (SAS Institute, Cary NC, USA) for differences between balanced and unbalanced groups. Additionally, these data were analyzed by ANOVA using the mixed procedure of SAS for differences among identified strengths domains. Students' predictions of their own domains were analyzed by Chi-square frequency test using the freq procedure of SAS. Student was considered the experimental unit, and any students not successfully completing the course, providing strengths assessment results, or consenting to inclusion were discluded from the data set

RESULTS

Students assigned to balanced strengths-based groups scored greater ($P < 0.05$) on the final practical exam by 4.3% and on their total lab grade by 3.1% (Figure 2). Midterm practical exam scores completed prior to strength-based group assignment did not differ between groups. Students in both groups over-estimated their practical and overall lab scores, but the difference between predicted and actual score was less ($P = 0.05$) for those in balanced groups (Figure 3).

Students in the strategic thinking domain scored the highest ($P = 0.05$) on the final practical exam and overall in the lab, and students in the relationship building domain scored the lowest ($P = 0.05$) on both (Figure 4). Students in the relationship building

domain were most frequently ($P=0.05$) able to correctly predict which domain they fit compared to students in the other domains. There were also no differences between the treatment groups for the puzzle assessment for time, accuracy, or efficiency (grade/time).

There were no differences in students' perceptions of group work after being put into their strengths-based groups. Additionally, there were no differences on students' thoughts on learning or the strengths assessment itself.

DISCUSSION

In this study we show that structuring groups based on the blending of individual strengths improves learning of anatomy and physiology concepts in undergraduate students despite there being generally negative perceptions from students. Surprisingly, we saw no differences in performances of the timed team project between balanced and unbalanced strengths-based groups. Additionally, we observed differences in performance among the individual domains. Strategic thinkers scored highest in the class and relationship builders self-identified their own domain. Together, these findings demonstrate that utilizing knowledge of students' individual strengths helped facilitate better learning in a science-based lab.

The increase in performance in the balanced strengths-based groups was most likely a result of students working more cohesively and helping the other members of their group by filling in gaps in understanding of the activities/assignments presented in lab. This increase simply highlights how those who learn well most likely were helping

other students who were struggling, whether intentionally or simply by making sure assignments were answered correctly to allow better studying in the long-run. Similar increases in productivity and moral were seen in workplace teams when blended by individual strengths (Connelly, 2002; Harter et al., 2002; Clifton and Harter, 2003). Interestingly, the advantage of blended strengths was not evident in an isolated challenge, as blended-strengths groups did not out-perform single-strength groups in the ear model puzzle. The lack of differences in time, accuracy, or efficiency may be a product of assessing group-work in a single, isolated event. Moreover, the differences between groups may have become more evident had there been more qualitative measurements or questions asked about this assignment itself such as: equal contribution, mood during assignment, or other factors that would be interesting considerations in how strengths-based groups work together.

Despite improved performance in the balance strengths-based groups, student perception of assigned groups was generally negative, which we attribute to the disruption in their routine caused by separating them from their peer-chosen groups. Students prefer to choose those they have successfully worked with before or actively avoid those they did not work well with in the past rather than be place in randomized groups (Colbeck et al., 2000). Additionally, older students prefer not to work in groups, while younger students are more often indifferent to groupwork (Barfield, 2003)

We observed clear differences among strengths domains regarding how students learn. With strengths such as analytical, which focuses on looking for reasons and causes

in information and concepts, and learner, which includes people who are energized by learning new concepts, in the strategic thinking domain it is understandable that these students in this domain would perform well in a science-based lab. Strategic thinkers constantly analyze and absorb information focusing on what the team could be. They are talented at evaluating the past to push towards a more productive future.

Alternatively, students in the relationship building domain may have simply lacked the analytical and technical problem solving skills needed to understand difficult concepts in this science-based lab. Instead, relationship builders are the team members who bring the group together allowing the team to be more than just the sum of its parts.

In conclusion, this study indicates that balancing students individual strengths in laboratory groups improved the overall effectiveness of groupwork on learning of life sciences information at the undergraduate level. By increasing efforts to learn more about student strengths, instructors can better guide them through their college experiences and improve their academic performance. Going forward, it will be important to determine how students in each domain can identify strengths in other students, which would have implications in improving self-selected groups. We would expect that those individuals in relational domains of relationship building and influencing would more accurately identify domains of others, as they were better able to self-identify in our study. Understanding how each domain learns, thinks about learning, and feels about working within a group could be valuable information that allows tailoring of student's education to their specific learning needs. We believe this improvement can be extended to in-class group work across all STEM disciplines.

DOMAIN	EXECUTING	INFLUENCING	RELATIONSHIP BUILDING	STRATEGIC THINKING
SPECIFIC STRENGTHS WITHIN EACH DOMAIN	Achiever	Activator	Adaptability	Analytical
	Arranger	Command	Connectedness	Context
	Belief	Communication	Developer	Futuristic
	Consistency	Competition	Empathy	Ideation
	Deliberative	Maximizer	Harmony	Input
	Discipline	Self-Assurance	Includer	Intellection
	Focus	Significance	Individualization	Learner
	Responsibility	Woo	Positivity	Strategic
	Restorative		Relator	

Figure 1. The 34 strengths identified by the CliftonStrengths for Students assessment sorted into the four major domains that each best fits.

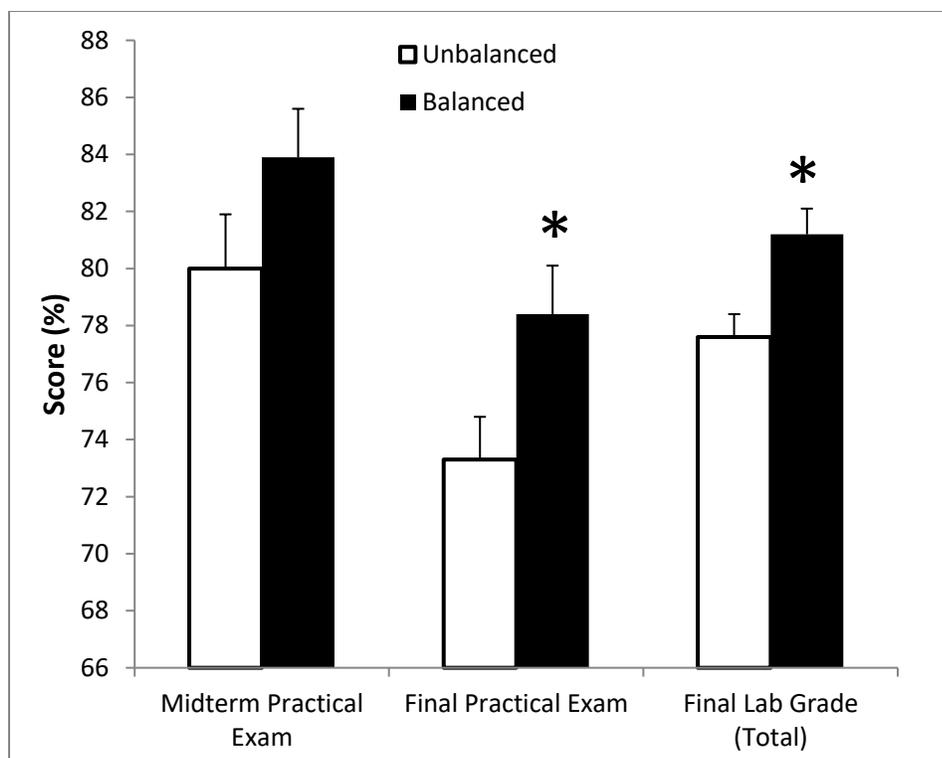


Figure 2. Grade differences by groups. Balanced strengths-based laboratory groups show an increase in final practical exam score and overall final lab score, showing the advantage of blended individual strengths within a group. * indicates difference ($P < 0.05$) between unbalanced and balanced strengths-based groups.

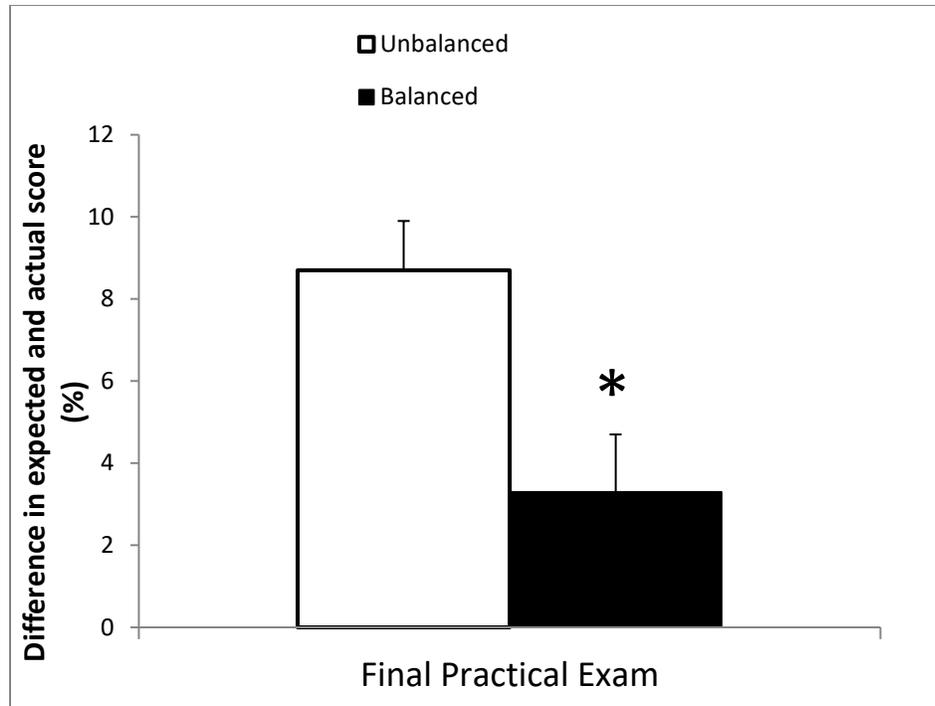


Figure 2. Difference in perceived and actual final exam scores. Both balanced and unbalanced strengths-based groups overestimated their grades on the final practical exam, but students in balanced groups were not as exaggerated in their overestimate. * indicates difference ($P < 0.05$) between unbalanced and balanced groups.

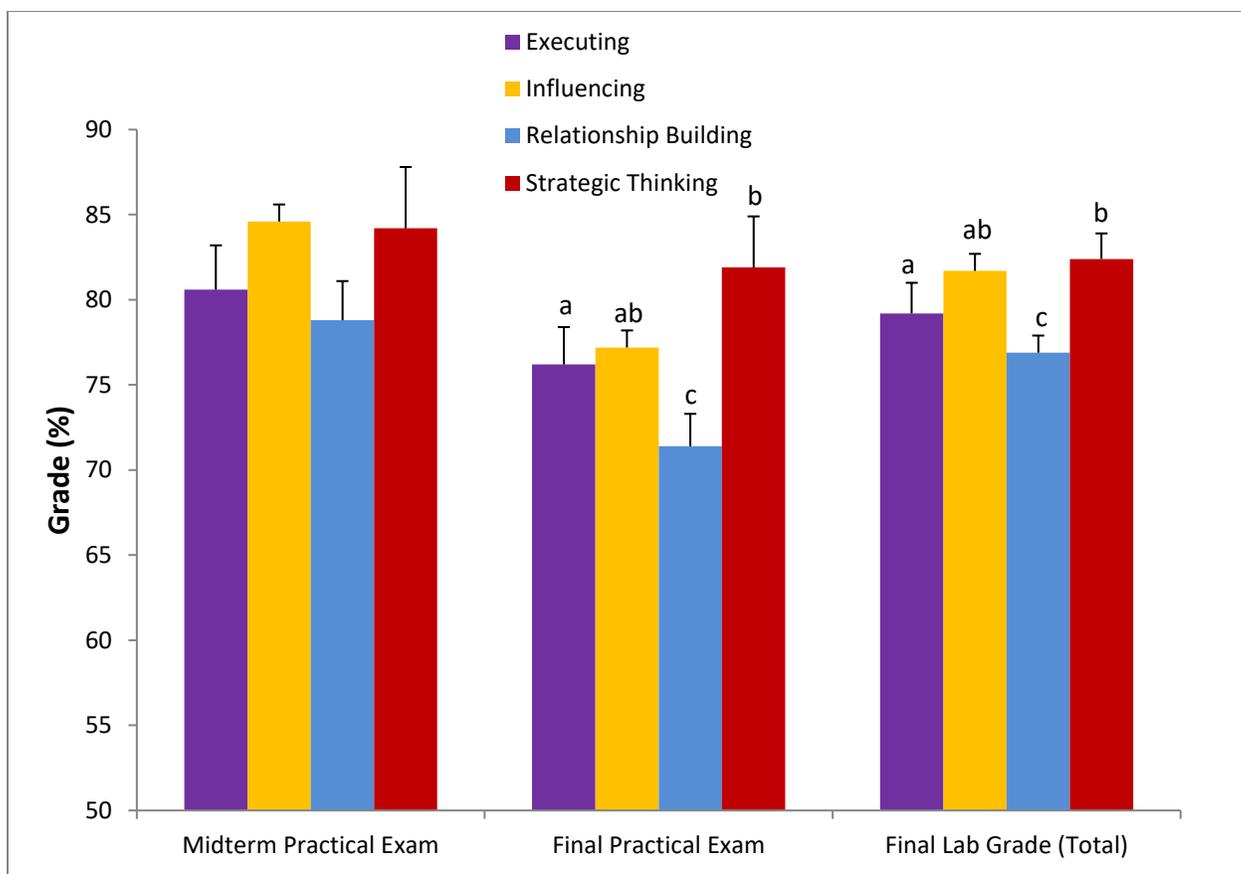


Figure 3. Scores by domain. Strategic thinking students scored highest on the final practical exam and the final lab grade. Relationship building students score lowest. .
 a,b,c indicates difference ($P < 0.05$) between domains for each outcome.

Appendix A

INFORMED CONSENT FORM



INSTITUTE OF AGRICULTURE AND NATURAL RESOURCES
DEPARTMENT OF ANIMAL SCIENCE

Title: Using analytics to structure the learning environment in ASCI 240 lab sections.

Purpose: This research aims to determine how analytical information impacts scientific learning. You are invited to participate in this study because you are a UNL student enrolled in ASCI 240.

Procedures: We are evaluating the long-standing methods for this course, and thus your participation involves no additional requirements beyond your normal participation in the course. **One of your assignments that you are already receiving credit for is a survey at the end of the semester. Results of this survey and your lab practical grades will be disclosed to the investigators at the conclusion of the semester to be analyzed to help in understanding how group work is affected by different analytics in a lab course setting. All records will be encoded to ensure confidentiality.**

Benefits: There are no direct benefits/detriments to you as a research participant. However, the information obtained should improve the future quality of this course.

Risks and/or Discomforts: There are no inherent risks or discomforts associated with this research.

Confidentiality: Any information obtained during this study that could identify you will be kept strictly confidential via the same standards and procedures applied to grade confidentiality. Identifiable data will be maintained by the instructor for this course and will be de-identified by encoding (i.e. names or other student identifiers will be replaced by randomly assigned codes) prior to analysis. The information obtained in this study may be published in scientific journals or presented at scientific meetings but only in a de-identified form (not specified to any student).

Compensation: You will receive a **1.5% bonus added to your final grade** for participating in this project. **Should you choose not to participate, a survey of similar time commitment will be available to you to earn this extra credit for your final grade.**

Opportunity to Ask Questions: You may ask any questions concerning this research and have those questions answered before agreeing to participate or at any time during the study. You may contact the investigator(s) via the information below. Please contact the University of Nebraska-Lincoln Institutional Review Board at (402)472-6965 for any concerns regarding this research or if you have any questions about your rights as a participant.

Freedom to Withdraw: Participation in this study is voluntary. You may decline to participate or withdraw from participation at any time without impacting your standing in this course and the University of Nebraska-Lincoln or risking any other form of penalization. **Records from students auditing the course, taking it pass/fail, or that withdraw from the class prior to its conclusion will not be included in the data set.**

Consent, Right to Receive a Copy: We are asking that you voluntarily make a decision to participate or decline participation in this study. Your signature below will certify that you have decided to participate after having read and understood the information presented. If you would like a copy of this consent form for your records, please notify your instructor or any of the investigators listed below.

Signature of Participant:

Printed Name	Signature of Participant	Date
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Name and Phone number of Investigators:

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RECRUITMENT SCRIPT

“I am here to invite you to participate in a research project that will be carried out this semester by a research team that includes Dr. Dustin Yates, Dr. Dennis Brink, and Ms. Taylor Barnes. Please read through the consent form I am handing out. This study is intended to improve that future quality of this course. It is important for you to know that participation in this study is completely optional. **There will be no additional class work for you to participate. Your survey answers and practical grades will be given to the researchers, but all identifying features on these survey answers and practical grades will be removed before being given to the researchers so they will not know who you are.** Should you choose to participate, you will be given an additional 1.5% bonus added to your final course grade. Should you choose NOT to participate; it will not affect your grade or your learning opportunities in this course, and you may request an opportunity to earn an equivalent bonus by completing a task of similar effort. If you elect to participate in this study, you may return this signed form to your TA by the end of the class period. If you would like more time, you may choose to return the signed form to your TA or instructor during class time within the next week. Alternatively, you may turn in the signed form to our office assistant, Andi Hallberg, in room A224 on the second level of this building. **Records from any students auditing the course, taking it pass/fail, or that withdraw from the class prior to its completion will not be included in the data set.** If you have any questions regarding

your participation in this study, please contact Dr. Yates, Dr. Brink, or Ms. Barnes via the information provided on the consent form.”

Appendix B

END OF CLASS SURVEY

Name: _____

Gender: M F N/A

Grade Level: Fr So Jr Sr N/A

Approximate Size of High School Graduating Class: <15 15-50 50-100 100-300 300+
N/A

Expected grade in **ASCI 240** (overall): A⁺ A A⁻ B⁺ B B⁻ C⁺ C C⁻ D⁺ D D⁻
F

Expected grade on **final lab practical**: A⁺ A A⁻ B⁺ B B⁻ C⁺ C C⁻ D⁺ D D⁻
F

Please rate: (4 = Strongly Agree, 3 = Agree, 2 = Disagree, 1 = Strongly Disagree)

Course Content:

Expectations for lab were clear

1 2 3 4

I enjoyed the lab portion of this course

1 2 3 4

The knowledge gained in lab will help me in future classes

1 2 3 4

The knowledge gained in lab will help me in my career

1 2 3 4

How you learn:

The hands-on dissections helped me learn the most

1 2 3 4

The LabTutor computer modules helped me learn the most

1 2 3 4

I would prefer my other science courses to be taught in this manner

1 2 3 4

I was comfortable asking the instructor/TAs for assistance to improve my understanding

1 2 3 4

Strengths:

Had you taken the CliftonStrengths for Students test before enrolling in this course? Yes
No

If yes, approximately how long ago did you take the assessment? -

If yes, have your strengths have changed since taking the assessment? Yes No

What are your top 5 strengths according to the assessment?

- 1.
- 2.
- 3.
- 4.
- 5.

What strengths domain do you fit best according to CliftonStrengths?

Executing Influencing Relationship Building Strategic Thinking

How strongly do you agree with the CliftonStrengths results regarding your top 5 strengths?

1 2 3 4

What do you consider most inaccurate about the CliftonStrengths results?

Please answer these questions regarding your group work experience:

(4 = Strongly Agree, 3 = Agree, 2 = Disagree, 1 = Strongly Disagree)

Working in a group helped me better understand course materials

1 2 3 4

Our group was almost always well prepared for lab

1 2 3 4

All of our group members participated and contributed a meaningful amount

1 2 3 4

My group almost always worked well together

1 2 3 4

I would work with these same group mates in future courses

1 2 3 4

I could identify most or all of the top strengths for most of my group mates

1 2 3 4

What would you expect the top CliftonStrengths strength domain to be for your group mates?

(Executing, Influencing, Relationship Building, or Strategic Thinking)

Group Mate Name	Strength Domain
1.	

2.	
3	

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