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# Effect of Endurance Exercise Training and High-Fat Diet on Muscle Fiber Type and Aerobic Performance in Tibialis anterior of Mice

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Distinguished Thesis Paper

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## Abstract

Our world has an increasing demand for finding different exercise and diet combinations that lead to greater aerobic performance. Previous studies show that independently, endurance training and high-fat diets increase aerobic muscle fibers and fatigue resistance of muscle. My experiment tested whether the combination of high-fat diet and endurance training would produce greater increases in aerobic muscle fibers and fatigue resistance than either treatment alone. I predicted that there would be an increased proportion of type I and type IIa, oxidative fibers, in addition to an increase time for muscle to fatigue and a decrease in lactic acid concentrations after exercise. Mice were broken into four groups. One experimental group was a control, one ate a high-fat diet, one followed an exercise regime, and one group did both. Weights and lactic acid levels were monitored throughout the experiment. The *tibialis anterior* muscle was analyzed with *in vitro* tests and microscopic analysis. All mice experienced weight gain, but it was most pronounced in the two high-fat groups. *In vitro* properties, such as contraction time and time to fatigue, showed no significant difference between groups. There were also no significant changes in fiber type ratios. Conversely, fiber cross-sectional area significantly decreased for groups undergoing the exercise regime. Lactic acid concentrations following standardized exercise decreased significantly for the High-Fat & Exercise group after the 28-day experiment but were unchanged for all other groups. The lower lactic acid levels could have been the result of a decrease in fiber area and an increase in capillarization of the tissue. This would have increased the oxidative capacity of the cells, reducing the need for anaerobic respiration. The timeframe, sample size (N=32)

and exercise intensity appeared insufficient to induce significantly noticeable changes in muscle function, fiber type, and lactic acid dynamics.

## Introduction

Humans live in an ever-changing and chaotic world. New stressors and influences on the human body continuously appear. It is important to know how to maintain proper health during our lifetime despite these external influences. Muscles are highly plastic, so they can deteriorate or be strengthened in response to external factors. For example, astronauts need to maintain muscle mass and strength during long-term space travel to prevent issues upon returning to Earth and increased gravity (Braddock 2017). As people age, they should be prepared to combat sarcopenia, or the age-related deterioration in muscle mass and strength (Welch 2013). Athletes need to know how to train their muscles for activities such as a triathlon or marathon to help improve their aerobic capacity and fatigue resistance (Röckl 2007). We must know how to maintain and improve muscle structure and function in our environments throughout our lifetimes since muscles are vital to our everyday function, posture, and movement. Scientists continue to seek information on how the environment they are subjected to influences muscles. Through scientific discovery, the public can be best informed on how to care for their body and help it function to the best of its ability to meet their individual needs.

Muscles, like all tissues, are complex. Each muscle is composed of smaller subunits called motor units, which are then each made up of many muscle fibers (Hill et al. 2016). There are two main categories of muscle fibers found in the mammalian body: type I, or slow twitch fibers, and type II, or fast twitch fibers (Hill et al. 2016; Schiaffino & Reggiani 2011). Type I fibers are commonly referred to as slow oxidative fibers. Type II fibers are broken down further into two types: IIa, fast oxidative fibers, and IIb, fast glycolytic fibers. Each fiber type has distinguishing components to help with its specific function (Schiaffino & Reggiani 2011).

Type I, or slow oxidative, muscle fibers are used for sustained, aerobic activity. These cells contain increased mitochondria to perform aerobic respiration and extra capillaries to supply the necessary oxygen to the fibers (Röckl et. al 2007). As supposed by the name, these fibers are often slower to contract than the type II, fast twitch fibers (Hill et al. 2016). Fast twitch fibers, as previously mentioned, have two types: IIa and IIb. Type IIa is a fast, oxidative fiber meaning that it sustains activity aerobically more often than it does anaerobically, similar to type I fibers (Talbot & Maves 2016). Type IIa fibers bridge the difference between Type I and Type IIb fibers. Type IIb fibers are fast, glycolytic which operate mainly anaerobically and produce the quickest contraction. They contain fewer capillaries and mitochondria because of their lessened demand for oxygen for aerobic respiration. Since type IIb fibers operate anaerobically, they are also quickest to produce lactic acid as a by-product of fermentation which is then released into the blood stream (Gladden 2000). Both type II fibers contract more quickly than type I fibers. Each fiber type works uniquely and has specific characteristics that help an organism move, maintain posture, and sustain life (Table 1).

The differences in lactate production in each muscle fiber type should be highlighted further. Muscle produces lactic acid, also known as lactate, when there is not enough oxygen present for ATP production via aerobic respiration (Hill et al. 2016). In this situation, muscle begins to produce lactic acid due to glycolysis, or anaerobic ATP production. The rate of conversion and the overall product from lactate is dictated directly by muscle fiber types (Gladden 2000). Oxidative muscle fibers have access to more oxygen and are less likely revert to anaerobic respiration, while glycolytic fibers often need to rely on it to function. Because of this, one would think that there will tend to be less lactic acid in muscles with more oxidative muscle fibers, like type I or type IIa muscle fibers. Lactic acid levels are useful to monitor for the duration of an experiment since they may offer predictive indicators as to relative amounts of each fiber type when compared with other subjects. Subjects with high lactic acid levels in the blood may have more glycolytic fibers than those this lower lactic acid levels. If continually

monitored, one would also suppose that muscles with a greater oxidative capacity would be able to clear lactate from the blood faster than glycolytic fibers. Clearly, muscle fibers play a large role in determining the specific function of a muscle.

When muscles are used for contraction, the motor units composed of muscle fibers are typically recruited for use according to the Hennemen Size Principle (Gregory & Bickel 2005). This principle states that slow twitch, small motor units will be recruited first, which refers to slow oxidative, type I fibers. This is typically the case in less strenuous activities like walking or picking up a cup. As the required contraction strength increases more muscle fiber contraction is needed. Therefore, the type IIa, fast oxidative fibers, are then recruited in addition to the type I fibers. During a very strong contraction, the type IIb, fast glycolytic fibers, which are made of large motor units, are also recruited (Gregory & Bickel 2005). Examples of this include sprinting or lifting heavy weights, activities which require quick and strong muscle contraction. Each fiber type has a specific function, and they can be used together but it is not always necessary to do so.

Muscle plasticity is important to note when considering the operation of muscle fibers. As defined in an article by Gransee et al. 2015, muscle plasticity is the adaptation and change of muscles and their fibers in response to the needs of the individual and the environment it is a part of. Muscles have a keen ability to change, deteriorate, and adapt. For example, there is evidence that muscle fibers decrease in quantity and cross-sectional area size as people reach their elderly years (Slivka et al. 2008). Bed rest, space weightlessness, and other times of immobility are also connected with losses in muscle mass and decreases in maximal contractile strength (Fitts. et al. 2007). These times of decreased use and lessened strain on the muscles allows them to change since they are not being used at the same intensity.

To counter muscle deterioration, people use exercise. This is because training and athletics take advantage of muscle plasticity. Athletes stress the muscle and induce extra strain. Because of this, the muscle fibers adapt and adjust to meet the new environmental demands. Exercise can be broken down into two main categories: resistance training which is high-load with few repetitions and endurance training which is lower-load with more repetitions (Hoppeler et al. 2011). An example of strength training is weightlifting, which involves short, explosive, high-intensity movements. Strength training has local effects on the muscle that alters muscle size, volume, and strength primarily through increased synthesis of contractile proteins like myosin and actin (Hoppeler et al. 2011). Although strength training is a fundamental way to alter muscle, for the purposes of this study, we will be focusing on endurance training and the changes it initiates in muscle. Endurance training entails performing sustained aerobic activities for long intervals, such as biking, swimming, and running (Hoppeler et al. 2011; Röckl et al. 2007). Endurance training impacts the body as a whole to help improve $VO_2$ max, cardiovascular function, and muscular function for sustained activity (Hoppeler et al. 2011). Endurance training encourages increased fatigue resistance in muscles. Athletic activities demand strength and endurance from a muscle in various ways causing it to change and adapt to meet these demands.

While muscle plasticity is important for the muscle as a whole, it should also be noted that this plasticity stems from the ability of some muscle fibers to transform from one type of fiber to another. Recent research encourages the idea that muscle fiber plasticity is common and can be influenced by environmental factors. Most existing studies show that change from a type IIb to type IIa or the reverse is a common result of training and exercise (Hoppeler et. al 2011). Currently, researchers are working to discover if transitions between type I and type II fibers can be made. Theoretically, a transition from type IIa to type I fibers would be feasible because it involves complex cellular change, just as in a transition for type IIb to type IIa fibers. Although in the beginning stages, it seems that the conversion may be possible with specific and targeted types of training programs (Wilson et al. 2012).

Training via exercise can influence muscles by changing the components of the muscle fibers. Primarily, specific training over a period of time is known for changing fibers from one type to another that is better suited for the task. From strength training, the number of type IIa and type IIb, both fast twitch muscle fibers, is increased in order to alter muscle response time, mass, and volume of skeletal muscles (Hoppeler et al. 2011). Increased levels of glycolytic muscle fibers are important for strength training as they specialize in anaerobic activity and rapid contraction times (Wilson et al. 2012). Conversely, endurance training builds the proportion of oxidative, type I and type IIa, muscle fibers in the body (Kazior et al. 2016; Röckl et al. 2007). These oxidative fibers function in sustained aerobic muscle activity therefore increasing muscular fatigue resistance (Hoppeler et al. 2011). People who specialize in strength training tend to have higher proportions of type IIb fibers in their muscle. In contrast, those who specialize in endurance training have increased proportions of type I and type IIa fibers (Wilson et al. 2012). Overall, the speculation in current research is that the muscle fiber types switch from one to the other through a change in characteristics, rather than the generation of a completely new fiber as a result of training (Wilson et al. 2012).

In addition to affecting the muscle fibers, training also influences the mitochondria in muscle fiber cells. Oxidative fibers generate ATP for sustained muscle activity primarily through aerobic respiration, which occurs in the mitochondria (Hill et al. 2016). Mitochondria changes can happen in every type of muscle fiber that is experiencing endurance training (Lundby & Jacobs 2015). Increased consumption of ATP during training demands more ATP production from the mitochondria, which may already be operating at full capacity (Hoppeler et al. 2011; Röckl et al 2007). To resist early muscle fatigue and to sustain an increased demand for ATP, mitochondria must increase in number and become more efficient (Zoldaz et al. 2016). After just a few weeks of a training regime, production of new mitochondria can be noticed in the cell (Zoldaz et al. 2016). Biogenesis of mitochondria is a complex

process initiated by the upregulation of transcription of genes for mitochondria components (Hoppeler et al. 2011).

There are multiple ways that signal pathways are triggered to initiate mitochondria biogenesis. During endurance training, the muscle contraction depletes the cells of ATP and they must create more. The by-products of ATP breakdown and muscle contraction accumulate in the cell, including molecules like AMP, $Ca^{2+}$, $H^{+}$, inorganic phosphate, and reactive oxygen species (Hoppeler et al. 2011). Higher concentrations of these by-products lead to the activation of signaling pathways that help to indicate the cell's condition and initiate a response. When AMP:ATP ratio is large, AMP binds with a subunit on AMPK to activate it, a process that is inhibited by the presence of high quantities of ATP. AMPK is an important kinase for many processes, but a key point is that it phosphorylates PGC-1α. PGC-1α is one of the primary activators for the generation of new mitochondria and type I muscle fibers. The increase of these products begins a signaling pathway that alerts the cell that its ATP needs are not being met, and it creates more cellular machinery, like mitochondria, to do so (Hoppeler et al 2011).

Calcium is also a crucial initiator in signaling the need for mitochondria biogenesis. Calcium binds with calmodulin inside the cell, activating enzymes such as kinases and phosphatases (Hoppeler et al. 2011). A key kinase that is activated is CaMPKII. CaMPKII is important in beginning a sequence of events that result in the repression of MEF2, a component in the process that is thought to activate slow twitch muscle fiber gene expression (Hoppeler et al. 2011). This is valuable for creating more oxidative muscles fibers. The $Ca^{2+}$/calmodulin-activated kinases also activates CaMPKIV, a monomer that plays a role in coactivating PGC-1α and stimulating mitochondrial biogenesis (Hoppeler et al. 2011). Essentially, an abundance of the products of ATP breakdown indicate to the cell that more ATP is needed then initiating pathways within the cell that will stimulate more rapid ATP production.

Both the generation of new mitochondria and the increase in volume of existing mitochondria stem from the upregulation of transcription of mRNA sequences for mitochondrial proteins (Hoppeler et al. 2011). Existing mitochondria seem to become more efficient with training. There are an increased number of mitochondrial proteins and enzymes in existing mitochondria that allow for better aerobic respiration performance (Zoldaz et al 2016; Lundby & Jacobs 2015). One study even shows that an increase in the volume of mitochondria in muscle fibers may be correlated with an increased ability to use fat for energy during endurance training to avoid using an organism's carbohydrate stores (Lundy & Jacobs 2015). When training for endurance activities, one is not only preparing their muscles in an organ sense, but down to the cellular component level, their body is adapting and improving.

With an increase in oxidative fibers and their accompanying mitochondria comes an increased demand for oxygen. Capillaries are responsible for supplying muscles with inputs required for aerobic contraction and removing the wastes (Gladden 2000). In general, oxidative muscle fibers have more capillaries supplying them with oxygenated blood than glycolytic muscle fibers. This, in addition to their myoglobin content, is why they tend to be known for their red coloration (Laughlin & Roseguini 2008). The increased capillarization can be found in any muscle fiber types undergoing endurance training but is more noticeable in type I and type IIa fibers, the more oxidative fibers (Laughlin & Roseguini 2008). Once the mitochondria are in place for more aerobic respiration in muscle fibers, then the supplies for the process, such as oxygen, must also be provided. Angiogenesis is the term for the process of capillary formation (Laughlin & Roseguini 2008). This formation occur through the splitting of existing capillaries or the sprouting of new ones (Baum et al 2015). Both paths offer more capillary surface area for gas exchange between the blood and cells.

Angiogenesis is energetically expensive for the body (Bloor 2005). It relies on its signal pathways to be initiated. These pathways are activated under times of low oxygen content in the muscle for

repeated and prolonged periods of time, such as daily endurance exercise. Once cells sense a low oxygen content, a signaling pathway releases VEGF, or vascular endothelial growth factor (Bloor 2005). VEGF initiates angiogenesis by controlling transcription upregulation of mRNA for the sprouting of new capillaries and the components need to create them (Bloor 2005). Other growth factors and signaling components are involved in angiogenesis, but VEGF is the key growth factor for promoting angiogenesis in response to exercise induced oxygen demand (Bloor 2005). Through this process, the number of capillaries reaching the muscle fibers is increased in all muscle fiber types as a result of endurance training, so that more oxygen is continually delivered to the cells during sustained activity and wastes are removed (Hoppeler et al. 2011; Röckl et al 2007). These capillary adaptations favor oxidative fiber type changes in muscles. By fulfilling the need for more oxygen through angiogenesis, cells can continue improving muscle fiber and mitochondrial function for sustained aerobic activities.

It seems to be a consensus that exercise training alters and changes the existing muscle fibers to perform better at desired tasks. However, it is still up for debate as to if and how fibers are able to change from type I to type II and vice versa. There are many studies evaluating the results of strength versus endurance training and how they cultivate muscle fiber types. Still there seems to be no general agreement in the field (Kazior et al. 2016). Repeated experiments and more specific evaluations should be done to continue working towards explaining the phenomenon and what other factors may be affecting muscle fiber changes.

High-fat diets are of particular interest in reference to muscle fiber changes. Previous studies have shown that initially, a high-fat diet can be beneficial (Eshima et al. 2021). Beneficial changes from high-fat diets better suit the body to perform aerobic-based, endurance activities. Positive changes to improve endurance and aerobic capabilities include increased aerobic capacity of muscle fibers, higher mitochondria content, increased capillarization, and increased amounts of oxidative muscle fibers.

Thomas et al. found that high-fat diets promote a shift to muscle fiber type I (2014). This finding is reaffirmed by Eshima et al. 2021 noting that the aerobic capability of muscle is improved by high-fat diets, pointing to an increased number of oxidative fibers. This shift to oxidative muscle fibers shows that the cells may be more capable of sustained aerobic respiration when consuming a high-fat diet. A shift towards more oxidative muscle operation would also be reflected as a decrease in anaerobic processes. This shift away from anaerobic muscle capabilities would, in theory, lead to a decrease in lactic acid concentrations in the blood during exercise. An increase in oxidative capacity of muscles allows for prolonged activity and less fatigue, which is important for raising the level of fitness and capability during endurance activities.

Like exercise, high-fat diets also influence the mitochondria of the muscle fiber cells. A study by Hancock et al. 2008 showed that a high-fat diet resulted in an increase in mitochondria in rat muscle, contrary to popular assumption (Eshima et al. 2021). Accompanying the increase in mitochondria, this study also found increases in mitochondrial enzymes and proteins, which further points to an increased oxidative capacity of muscle cells experiencing a high-fat diet (Hancock et al 2008; Eshima et al. 2021). It is important to note that research coupling high-fat diets and endurance training is limited. Prominent existing studies, such as Eshima et al. 2021, have tended towards analyzing muscles that were typically oxidative by nature prior to the experiment (Eshima et al. 2021). In summary, high-fat diets can lead to an increase in type I fibers and can also drive increased biogenesis of mitochondria, thereby increasing the muscles' aerobic capacity (Hancock et al 2008).

In contrast, a study by Tallis et al. surmises that obesity from high-fat content diets can deteriorate or improve contractile abilities of muscle, the result depends on the intended function of the muscle (Tallis et al. 2017). Some studies mention that obesity can reduce myogenesis, decreased ability to excite muscles, and poorer cellular usage of calcium (Tallis et al. 2017). However, as common as those results are, other equally occurring studies find that muscles increase in slow twitch and fast twitch

fibers in response to high-fat consumption, and yet more studies have found no change in fibers (Tallis et al. 2017). Other evidence shows that in the short term, obesity can instigate an increase in mitochondria function, increases in concentration of oxidative enzymes, and increases in proportions of slow twitch fiber expression (Tallis et al. 2017). These effects are beneficial for increasing a muscles' ability to perform aerobically. Long term, studies show, these effects under obesity are reversed and the muscle decreases in oxidative capacities for endurance exercise activities (Tallis et al. 2017). Current research lacks cohesive knowledge as to the direct effects of high-fat diets on glycolytic fiber-type based muscles.

The increased numbers of oxidative fibers do not always have noticeable effects, making it harder to track fiber type shifts experimentally. The increase in mitochondria and cellular capability for aerobic respiration is not necessarily coupled with more capillaries in the surrounding tissue to supply oxygen to them. Both an increase in mitochondria within the cells and capillaries to a tissue are needed to increase a muscle's oxidative capability, not just one. Thomas et al. concludes that there is no whole-body change in endurance performance in the initial stages of a high-fat diet, despite compositional changes in muscle fibers (Thomas et al. 2014). Perhaps coupling the high-fat diet with endurance training is necessary to initiate improvement to endurance exercise performance. Alternately, Eshima et al. 2021 contends that the whole-body has a noticeable change as it adapts to better performance for endurance exercises. Yet, another study concluded that the effects of high-fat diet on muscle endurance can only be found when coupled with an endurance training regime (Lee et al. 2001). The data in this area of research is highly contradictory as to how extreme and noticeable the effects of a high-fat diet are. This can be partially attributed to the fact the studies are new and lack repetition. There seems to be at least some positive effects at a cellular level initially from high-fat diets but assessing and evaluating the changes functionally is more difficult than previously thought.

In the sense of muscular operation, there is very little research on the existence of change in contractile force of the muscles and change in fatigue resistance (Eshima et al. 2021). As previously stated, an increase or decrease in contractile strength may depend on the function of the muscle, and which muscle fiber type it operates with at peak performance (Tallis et al. 2017). Muscles mainly using type I muscle fibers tend to see improved contractile strength under a high-fat diet, while muscles primarily using type II fibers see a reduction (Tallis et al. 2017). Regarding fatigue, an obesity and muscle study by Tallis et al. found that the soleus muscle of an obese subject fatigued faster under tetanus and took longer to relax (Tallis et al. 2017). This study concluded that these effects resulted from reduced expression of slow myosin heavy chain (MHC), a major contractile protein in slow twitch fibers (Tallis et al. 2017). Further, the same study pointed out that their results were not reproducible in the other muscles of the same specimens such as the *extensor digitorum longus* muscle (EDL) and diaphragm muscles (DIA) (Tallis et al. 2017). There are very few direct studies on fatigue of muscles in relation to high-fat consumption. The changes in fatigue and contractile force under a high-fat diet may directly correlate with other environmental factors. Differences in muscle function after a high-fat diet is an area that calls for more research and discovery.

Further, it should be noted that lactate production is different in each muscle fiber type. Muscle produces lactic acid, also known as lactate, when there is not enough oxygen present for ATP production via aerobic respiration (Hill et al. 2016). In this situation, muscle begins to produce lactic acid due to glycolysis, or anaerobic ATP production. The rate of conversion and the overall product from lactate are dictated directly by muscle fiber types (Gladden 2000). Oxidative muscle fibers have access to more oxygen and are better equipped to use it because of higher concentrations of capillaries and mitochondria. Therefore, they are less likely to have to revert to anaerobic respiration. The point at which aerobic respiration can no longer keep up with ATP demand and fermentation must be used is called the anaerobic threshold (Poole et al. 2020). Once fermentation begins, so does lactic acid

production, quickly followed by muscle fatigue since anaerobic respiration cannot be sustained long-term (Poole et al. 2020). Muscles with higher oxidative capabilities would reach this threshold at higher intensity of exercise and reach muscle fatigue later, because they are more aerobically equipped. Shifts in fiber types and fiber efficiency would change this threshold for a particular muscle, allowing lactic acid production to start later and extending fatigue resistance. Because of this, one would think that there will tend to be less lactic acid in more oxidative muscle fibers, like type I muscle fibers. Lactic acid levels are useful to monitor for the duration of an experiment since they may offer predictive indicators as to relative amounts of each fiber type when compared with other subjects. Subjects with high lactic acid levels in the blood may have more glycolytic fibers than those this lower lactic acid levels. If continually monitored, one would also suppose that muscles with a greater oxidative capacity would be able to clear lactate from the blood faster than glycolytic fibers. Muscle fibers play a large role in determining the specific function of a muscle.

In summation, more analysis and experimentation are needed to fully understand the impacts of specific training types, high-fat diets, and their direct influence on muscle fiber types. However, the connection between diet, exercise, and muscle remains relatively unexplored. A deeper understanding is needed of these three elements to determine their combined effect. Further, fiber type as a it relates to lactate production has rarely been considered in previous studies on muscles, exercise, and diet. Understanding changes in lactic acid levels in the blood during these experiments can help increase understanding of how oxidative capacities of muscle are being affected under experimental conditions. Clearly, many aspects of this research remain unknown and should be explored deeper.

To analyze all these conditions in conjunction, I created an experiment to test the effects of high-fat diet and exercise both individually and together. When coupled, I predicted that high-fat diet and exercise would cause a shift to more oxidative and slow twitch fibers compared to the control group and either high-fat or exercise groups. The increase in muscle oxidative capacity should also reduce the

lactic acid concentration after a prolonged training regime, enabling greater exercise intensity without fatigue.

## Methods

I used Charles River, 10-week-old female mice, CD1 strain to test my hypothesis. The mice were provided with *ad libitum* access to food and water. Further, the mice lived in clear, uniformly sized cages with four mice per cage. The holding room was kept on a 12-hour light to dark cycle, with light beginning at 7 am and dark beginning at 7 pm. I weighed the mice initially, and then once per week throughout the experiment.

The mice were split into four groups containing eight mice each. A control group was provided a regular diet and not subjected to an exercise regime. The high-fat group consisted of mice fed a high-fat diet with no exercise regime. The exercise group experienced an exercise regime and was fed a regular diet. The high-fat exercise last group consisted of mice undergoing the exercise regime in addition to eating a high-fat diet. The caloric composition of the regular diet food was 13.5% kcal fat, 28.5% kcal protein, and 58% kcal carbohydrates (Laboratory Rodent Diet 5001*). For the high-fat diet, the caloric composition breakdown was 60% kcal fat, 20% kcal protein, and 20% kcal carbohydrates (D12492).

I drew blood from the mice to measure lactic acid levels at various points in the experiment. Blood was taken from the saphenous vein as described in the paper by Hem et al. 1998. I restrained mice in a holding tube to limit movement with a hole for air flow at the end (Hem et al 1998). Prior to beginning exercise, hair was removed using *Nair* face cream to visibly expose the saphenous vein in anticipation of the blood draw. The result is visible in Figure 1A. In order to carry out the exercise regime, a motor-powered running wheel, acting like a treadmill for mice, was used. The treadmill was an Omnitech Stress Tread XP model made by Omnitech Electronics of Columbus, Ohio. After exercise, a small gauge needle was then used to poke the visible vein and draw a drop of blood (Figure 1B). The

blood was then inserted into the tip of the Nova Biomedical Lactate Plus machine for analysis. Before the start of the experiment, each mouse underwent a 3-day training period on the treadmill to prepare for the initial lactic acid measurement. On day one, mice began with a 10-minute gradual increase from 0 meters per minute (mpm) to 10 mpm and then sustained running at 10 mpm for 30 minutes. The maximum speed was increased to 13.5 mpm on the second day and to 16 mpm on the third day while all other conditions remained the same. After the final round of training exercise, mice were placed in a restraint tube. Blood was drawn immediately to measure post-run lactic acid concentration, as a baseline for exercise lactate levels prior to the experiment. Then, during the experiment, each group also had a resting lactic acid level measured for baseline comparison which was drawn from the mouse in the same manner but without any prior exercise. At the conclusion of the protocol time, there was a final round of exercise of 40 minutes, for every group, with a ramp-up time of 10 minutes at a speed of 16 mpm. After this exercise round, blood was drawn, and lactic acid levels were again measured to record a post-regime exercise lactic acid level. These three measurements were then analyzed for changes in average lactic acid levels between all groups and used to predict muscle composition.

During the 28 days of protocol, mice subjected to the Exercise and High-Fat & Exercise groups ran on the mouse treadmill five days a week. Each round of exercise included 10 additional minutes to increase to desired speed on the treadmill. During week one, the speed was 10 meters per minute (mpm) for 30 minutes, so the mice ran for 40 minutes in total. Week two ran at 12 mpm for 40 minutes, for a total of 50 minutes of running. Week three mice ran at 14 mpm for 50 minutes, 60 minutes in total. And week four ran at 16 mpm for 60 minutes, reaching 70 minutes in all. This regime of exercise was consistent for both exercise and high-fat & exercise groups.

After 28 days, the mice were euthanized with $CO_2$, and the tibialis anterior (TA) muscles were dissected free from underlying tissues. The muscle was then detached on the distal end but left attached to the femur at the proximal end near the knee. During the dissection, Tyrode's buffer was

applied to the muscle to keep it moist and viable between each stage of the experiment. Tyrode's buffer is made up of 1.34mM NaCl, 2.68mM KCl, 1.80mM $CaCl_2$, 1.05mM $MgCl_2$, 0.417mM $NaH_2PO_4$, 11.9mM $NaHCO_3$, 5.56mM glucose, all per 1 liter of water. After dissection, the foot and shin area were placed in a clamp and held steady, as seen in Figure 1C. The distal end of the muscle was attached to a force transducer using string, and an external stimulation probe was placed against the muscle.

The in vitro testing protocol had five stages. Between each stage, I applied Tyrode's Lab buffer using just enough to keep the muscle wet. I determined the stimulus voltage necessary to obtain full recruitment in the first stage. The muscle was stimulated for one millisecond at increasing voltages (30 seconds between stimuli) until a plateau in twitch tension was reached (typically at x volts). For all subsequent in vitro testing, I used the voltage required to obtain full recruitment.

Next, I gradually varied muscle length to determine the optimal length required to produce the strongest contraction. This was done by slightly loosening the tension in force transducer and stimulating the muscle, then tightening it and again stimulating it. For the remainder of the experiment, the muscle was kept at whichever amount of tension elicits the highest contractile strength.

To quantify contraction time and relaxation time, I then recorded three successive muscle twitches (30 seconds apart) at a high recording rate (10000 samples/second). Contraction time was defined as the time from the initial stimulus until full contractile strength was reached. 0.5 relaxation time was defined as the time between the point when a twitch reached its maximum tension and the point at which the muscle was half of the maximum tension.

The fourth stage was a tetanus test. In the fourth stage, I determine maximal contraction strength by stimulating the muscle with a 20-pulse train at increasing frequencies of 20 Hz, 50 Hz, and 100 Hz waiting two minutes between each train.

I measured fatigue by subjecting the muscles to a single one millisecond pulse at 1Hz frequency until twitch tension declined to 25% of its original tension. After stopping stimulation, 20 seconds of the recovery period was also recorded.

While one tibialis anterior muscle was used for the contraction experiments, the other unused tibialis anterior muscle was frozen to be used for muscle fiber typing under a microscope. The muscle was kept in a freezer at -80 degrees Celsius until sectioned for fiber-type analysis. Muscle sections were placed onto slides and stained using a succinate dehydrogenase stain. Microscopic imaging was used to identify Type I, Type IIa, and Type IIb fibers. I identified the large fibers with little to no stain as Type IIb fibers. Fibers with an intermediate stain level and medium size were categorized as Type IIa. The smallest and most darkly stained fibers were marked as Type I fibers. After identification, the area of the fibers for each subject was measured using Image J software. Then, data was averaged and compared between test groups. I analyzed data with a one-way ANOVA and the Tukey post-hoc test to test for significance and represent findings. Lactic acid and weight measurements were analyzed using a one-way ANOVA accompanied by a paired t-test. Results are labeled as significant if $P<0.05$. Graphs and tables were created to allow for visual representation of the results.

## Results

All groups showed significant weight gain during the experiment; however, the increase was much larger for the animals on the high-fat diet compared to the control diet. The high-fat and high-fat exercise groups saw an average of 53% and 38% weight gain, respectively (7.041, 0.005; 8.493, 0.002, Table 2). In contrast, the control and exercise groups only gained 20% and 8% during the experiments ($F=19.906$, $p<0.001$; $F=20.792$, $p<0.001$, Table 2).

All groups showed an increase in lactic acid concentrations compared to resting levels after the first exercise bout ($p<0.001$, $p<0.001$, Figure 2) although the change was not significant for the control

or high-fat groups when analyzed independently (Control: t=0.110, p=0.111; High-Fat: t= 0.175, p=0.134; Figure 3). When measured again after the 28-day experimental period, however, the two groups that weren't exercised didn't show a change in lactic acid levels following the exercise protocol (Control: t=0.175, p=0.134; High-Fat: t=0.079, p=0.446; Figure 3). The exercise only group also did not show any significant changes after revealing a p-value of 0.348, but the t-test did indicate possible significance with a t-value of 0.075. In contrast, the high-fat and exercise group demonstrated a significant decrease in lactic acid concentrations post-exercise compared to their earlier runs (High-Fat Exercise: t=0.075, p=0.023; Figure 3).

Neither twitch tension (F=1.375, p=0.349), contraction time (F=0.408, p=0.749), 0.5 relaxation time (F=0.867, p=0.470), or tetanic tension (F=0.724, p=0.546) differed between the four groups (Table 3).

All groups reached 50% muscle fatigue between 180 and 196 seconds and 25% fatigue between 266 and 333 seconds. For the 50% fatigue measurements, the F-value was 0.364 and the p-value was 0.779. The 25% fatigue measurement had a p-value of 0.194 and had an F-value of 1.680 (Table 4).

Statistically significant differences were not found between any of the groups' number of a given muscle fibers. The F-value comparing type IIb fibers was 0.374, 0.589 for type IIa, and 0.790 for type I. Cross-sectional areas of type I ($F=9.952$, $p<0.001$), type IIa ($F=33.469$, $p<0.001$), and type IIb fibers ($F=26.414$, $p<0.001$) were significantly different between groups (Figure 6). When broken down by subject group, the control and high-fat groups were significantly different from the exercise and high-fat exercise groups for type IIb, type IIa, and type I fiber types but not each other. The exercise and high- fat exercise groups were also not significantly different from each other for any of the fiber types.

## Discussion

Both high-fat diets and endurance training are commonly studied topics in physiology for their impact on the body, particularly in skeletal muscles. Gauging how environmental factors impact muscles is helpful to people trying to prevent muscle deterioration due to weightlessness in space, bed rest, aging, or for those trying to enhance muscle function for athletic activities. Despite the fact that high-fat diets and forms of endurance training are highly researched, there is very little research pairing the two and assessing their combined value. My experiment used mice to try to determine if there could be any effect on muscles from using a high-fat diet and endurance training simultaneously. Throughout the experiment, weights and various measures of lactic acid levels were monitored. After the protocol, the *tibialis anterior* muscle was used to assess contractile properties such as twitch tension, 0.5 relaxation time, contraction time, tetanus contraction, and time to fatigue. Lastly, the fiber type composition of each muscle was recorded to assess for fiber type changes between subject groups.

The weight measurements were one area where the predicted differences between means were accurate at the culmination of the experiment. All groups showed statistically significant weight change between the initial weights and the final weights in the experiment. Part of this effect can be attributed to the use of young mice for this experiment. Therefore, they were in the growth and maturation phase of their life, so weight gain is expected independent of experimental conditions if they still receive adequate food and water. According to the Charles River company, the mice see an average of 12.5% increase in weight between 10 and 14 weeks of age (*CD-1® IGS Mouse*). This is much lower than the weight gain seen by animals in my study that consumed a high-fat diet. Both groups eating the high-fat diet showed a significantly higher weight gain than animals eating the normal diet. The high-fat group had an even higher gain than high-fat and exercise, about 53% and 38% percent gain respectively. This was predictable since the mice were eating a diet with a higher fat content but not exercising any

amount; therefore, they probably retained more of the weight (Katan 2010). The control and exercise groups ate a regular diet, preventing excess weight gain beyond what is expected from normal growth. The control gained 20%, and the exercise gained 8%, both levels much closer to the expected gain from growth (*CD-1® IGS Mouse*). These weight changes over the course of the experiment were consistent with our predictions.

The lactic acid measurements also showed significant changes between some groups. I anticipated seeing a significant difference between resting lactic acid levels and the post-exercise lactic acid measurements. Although lactic acid studies with mice are limited, if the studies with other mammalian muscles hold true, then lactate levels in the blood increase with during intense forms of exercise (Gladden et al. 2000). A difference in lactic acid levels would prove that the mice were being exercised at a high enough intensity to activate anaerobic pathways and raise blood lactate. This difference between resting and initial exercise tests was noticeable when all groups were averaged, as seen in Figure 2. Individually, this change between resting and initial exercise was not as apparent in the control and high-fat groups. The high variability within groups could be due to some animals "cheating" on the exercise by jumping on the treadmill instead of running and therefore were not working their muscles as intensely. In future experiments, the exercise should be controlled more tightly so that variances in exercise intensity do not impact the results with artificially low exercising lactic acid levels. Changes to the exercise protocol might include only assessing animals that run, having subjects swim, using puffs of air to encourage animals to run continuously, and using a flat treadmill.

I also predicted that the high-fat and exercise group would show lower lactic acid levels after their final run than during their initial run, which held true for this study. This lower level of lactic acid would result from a shift during the experiment from anaerobic, fast twitch fibers to more oxidative fibers during the study. Oxidative fibers use more aerobic respiration, which does not create lactic acid, in place of using anaerobic respiration and producing lactic acid (Hill et al. 2016). Additionally, oxidative

fibers are faster at resorbing lactic from the blood stream, according to a study by Gladden which analyzed how muscle produces lactate and can reuptake it from blood as well (2000). For all of these reasons, lactic acid levels should be lower at a given exercise intensity if the proportions of oxidative fibers are higher or if mitochondrial density or capillarization increases. The results for final versus initial exercise lactic acid levels in the high-fat & exercise group trend towards having more aerobic capacity. The other groups, the control, high-fat, and exercise groups did not experience any significant change between initial and final post-exercise lactic acid levels. Since a significant reduction in lactic acid was part of our results, I conclude that the muscle fibers have an increase in oxidative capacity after undergoing the high-fat diet and endurance training regime. But it would be interesting to follow the trend of the high-fat & exercise group to see if more time undergoing the experimental procedure would have led to further significance.

One difficulty with using mice to understand lactic acid changes is the high amount of noise in the dataset. The sample size was small and variable, though trends in lactic acids could be noticed. Gathering blood from mice for a lactate measurement is stressful and chaotic for the investigator and the animals. This stress induced on the mice from blood draws has been shown to artificially inflate lactic acid levels (Haugen et al. 2020). This, in addition to the small sample size, makes the lactic acid data slightly less reliable, although the trends are interesting and should be studied in more subjects.

Outside of weights and a portion of the lactic acid level results, a majority of the data showed no statistically significant differences between means of test groups. The in vitro tests did not prove that a high-fat diet and exercise had any impact on the muscle's twitch tension, contraction time, relaxation time, tetanus contraction, or fatigue resistance. Previous research by Tallis et al. from 2017 showed that some contractility changes occur under a high-fat diet, such as shifts in contraction force and fatigue resistance (Tallis et al. 2017). The study indicated that a high-fat diet may affect each muscle fiber differently by improving contractile twitch strength of type I fibers and decreasing it in type II fibers

(Tallis et al 2017). But it should be noted that their results were not consistent across different muscles, as some showed no change (Tallis et al 2017). Additionally, the study by Eshima et al. found a change in fatigue resistance after high-fat diet consumption, which they attributed to increased oxidative capacity (2021). This increase in oxidative capacity which enables an increase in fatigue resistance, resulting in an increase in mitochondria and increased capillarization (Hoppeler et al. 2011). Because of this, we predicted that a shift towards increased proportions of type I muscle fibers, as a result of high-fat diet and exercise, would also be accompanied by a change in fatigue resistance of the muscle. Since slow twitch fibers contract more slowly than fast twitch fibers, we expected to find a decrease in the average contraction time of the muscles as a whole if muscles did increase in type I fibers, but this was not the result of this experiment. Further, there was no change in the contraction time or other contractile measures like 0.5 relaxation time and twitch tension. These findings are slightly counter to the point made by Tallis et al. that showed that more slow twitch muscle would have been predicted to have a longer relaxation time, and to the expected increase in contraction time (Tallis et al 2017). Further, it was recorded by previous studies that high-fat diet affects contractile strength because of the fiber type shifts it encourages (Tallis et al. 2017). Our experiment did not reinforce the few and limited findings of these previous experiments about changes in muscle fiber type resulting from diet and exercise changes.

The other in vitro tests continue the pattern of no significant differences between test groups. Tension measurements during high-frequency stimulations of 20Hz, 50Hz, and 100Hz were all within the same range and indicated no statistical differences. We predicted that the group subjected to both a high-fat diet and an exercise program would see an increase in twitch tension under various frequencies of stimulation. This is because an increased proportion of slow twitch fibers would be slower to contract and relax again between each of the high-frequency stimulations, so slow twitch fibers would reach tetanus at a lower frequency of stimulations than fast twitch (Tallis et. al 2017). Instead of having each

contraction be independent of the others, oxidative fibers would begin summation earlier and contract stronger due to being unable to fully relax before the next stimulation. However, our results showed that all groups had the same rate of increased summation as the frequency of stimulations increased, as shown in the same changes in average tension during contractions. On the same note, we predicted that time to fatigue would increase with a shift to type I fibers. This is because oxidative fibers are meant to sustain activity long-term and have higher endurance than glycolytic fibers (Schiaffino & Reggiani 2011) Their ability to sustain aerobic activity is enabled by increased mitochondria and mitochondrial proteins to help improve efficiency in each cell (Hoppeler et al. 2011). Increased capillarization for supplying oxygen to the fibers and upregulation of transcription of genes for type I muscle fiber proteins also stimulates these muscles' higher oxidative capacity (Hoppeler et al. 2011). Multiple previous studies have shown that with endurance training muscles exhibit an increased time to fatigue (Hoppeler et al. 2011). This fatigue resistance would indicate a shift to more oxidative muscle fibers or an increase in the efficiency of existing ones due to an increase in mitochondria or capillarization (Zoladz et al. 2016; Laughlin & Roseguini 2008). We tested both time to 50% muscle fatigue and time to 25% fatigue. The results showed no significant difference between groups when analyzed with a one-way ANOVA. However, there was a slight trend towards longer times to 25% fatigue for the exercising groups. Once again, this concludes that functionally we cannot say that any shift in muscle fiber type occurred as a result of a high-fat diet and exercise.

Since there was no significant change to relaxation time, contraction strength, or any other muscular contraction properties, there must be a reason. Athletes with long-term experience in endurance exercises show increased proportions of type I muscle fibers (Hawley 2002). I had expected to find higher proportions of type I fibers in the groups undergoing regular exercise but did not find any significant changes. The primary explanation is that the high-fat diet and exercise combination did not

lead to a shift in muscle fiber types. Therefore, I would find no differences between the test groups and their in vitro performance. This may have occurred because my study was too short.

Thomas et. al wrote that there appeared to be no functional differences in initial stages of a high-fat diet study (Thomas et. al 2014). My study was only four weeks in length. Other similar studies allowed specimens to undergo experimental regimes for six and twelve weeks for mice, and six weeks for rats (Lundby & Jacobs 2015; Eshima et. al 2021; Lee et. al 2001). All of these experiments exceed my experimental timeline. Perhaps allowing the mice to grow and develop for a longer period of time, while on the particular diet and undergoing exercise would display more prominent changes in the muscle's properties.

Additionally, there is the potential that the muscle fibers had begun to adapt to become type I, but because the capillaries have not yet adapted to supply them with increased amounts of oxygen there was no change in animal performance. In a 2008 study, Laughlin & Roseguini mentioned that a shift towards more aerobic muscle fibers must be accompanied by increased capillary density in order to demonstrate a functional change (Laughlin & Roseguini 2008). However, previous studies were close to the same length in time as mine, so I could have been able to see some evidence of precursors to change. My experiment could not assess functional changes in capillarization because the *in vitro* tests did not have any cardiac output. However, the running lactic acid levels were affected by cardiac output. Increased levels of capillarization in the muscles may be a cause for the increased aerobic efficiency seen in the exercising groups with lower lactic acids. My experiment may have had limited results because of the short duration of the experiment did not allow for complete changes in capillarization and the inability to fully assess the changes that have taken place in the body outside of the muscle fibers.

Upon dissecting and viewing the muscles under a microscope, there was no significant difference in the amount of slow twitch fibers between experimental groups. All muscles had similar proportions of each muscle type, so no muscles were beginning to trend towards proportion shifts different from the control. This evidence, combined with the results of the in vitro tests, shows no change in the muscles on a cellular level since there was no functional or visible change.

When fiber typing muscles, the cross-sectional area of each fiber was measured. This data showed significant differences between non-exercising and exercising subject groups. In all fiber types, the average cross-sectional area was significantly smaller for exercising groups than non-exercising groups. Differences in cross-sectional areas may have been precursory to fiber type changes. More oxidative fiber types are typically smaller than anaerobic fibers, since this provides a larger surface area to volume ratio for the necessary gas exchange of aerobic respiration (van Wessel et al. 2010). This allows oxygen to move into the cell and wastes out of the cell quicker, which enables the cell to keep up with cellular respiration function in muscle contraction. Perhaps if muscle fibers labeled as type II were decreasing in area relative to the type II muscle in the control groups, then they are starting a slow transition towards becoming type I muscle fibers, or at least towards more oxidative function. In my experiment, fibers for the exercise and high-fat exercise were significantly different in area from those in the control and high-fat condition. Since there was a significant change in the cross-sectional area, I suppose that fiber type changes could have appeared if the experiment ran had run longer. The data revealed that all groups had relatively the same proportions and size of each fiber type in their *tibialis anterior* muscle, regardless of diet and exercise level.

It is unique that the only functional change found in the experiment was the change in lactic acid levels. The muscles were viewed under a microscope and there was no difference in proportions of fiber types. The only viable explanation for these results is that the existing muscle fibers became more efficient over the course of the experiment. This result agrees with other studies, such as Hawley,

Eshima, and Laughlin & Roseguini (2002, 2021, 2008) . Efficiency could be increased in one of two ways: increase mitochondria in each fiber cell or increase capillary supply of oxygen (Zoldaz et al. 2016; Laughlin & Roseguini 2008). These two methods may also be combined to lead to these decreases in post-exercise lactic acid levels. Further experimentation and testing would be required to know which processes affected the muscles. Even though there was not a fiber type change, the muscles still displayed small changes that favor increased oxidative capacities.

## Conclusion

Although our study did not support our hypothesis that a high-fat diet and endurance training would lead to an increase in type I, slow twitch oxidative muscle fibers, the results did confirm that the high-fat diet and exercise combination was effective in reducing lactic acid levels following a bout of moderate-intensity exercise. Repeating my experiment over a longer duration to allow for the subjects to adjust more fully to the exercise regime and diet would likely yield more dramatic changes in the skeletal muscles since slight shifts in fiber type size were detected in this short period of time. An adjustment to duration of the experiment could lead to more perceptible and possibly significant changes in the body. Further, the training and diet could be changed to different levels and intensities which might result in more noticeable changes to the muscle cells. This study was limited not only by the short experimental timeframe and an extremely small sample size of just 32 specimens. In another experiment, this amount should be increased as much as possible. Lastly, there was a limited ability to force the mice to exercise consistently. Some mice often would jump on the treadmill wheel instead of running, therefore “cheating” themselves out of their true exercise. Future experiments should look to find ways to make sure mice are fully participating in and being worked by the type of exercise they experience. To conclude, this study demonstrated that the correlation between high-fat diets, specific types of exercise, and their effects on the body still remain largely unknown. This experiment showed

that the endurance training and high-fat combination may improve efficiency of a muscle's oxidative abilities as demonstrated by an improved reduction in lactic acid levels and the decrease in muscle fiber size in the exercise and high-fat exercise groups as compared to the control and high-fat groups. This combination leaves an open avenue for future research and experimentation as the contractile properties of the muscle and lactic acid effects under these conditions remain relatively unstudied.

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## Tables and Figures

**Table 1.** Comparison of the characteristics of type I and type II muscle fiber types. Characteristics include cellular components, contraction rate, and sustained duration of contraction.

| Characteristics | Type I | Type II |
|---|---|---|
| **Myosin ATPase** | Slow | Fast |
| **Mitochondria** | Many | Few |
| **Color** | Red | White |
| **Contraction Rate** | Slow | Fast |
| **Duration** | Long | Short |

**Table 2.** Body mass changes as a result of diet and exercise manipulations. Data are presented as means plus/minus standard error of the mean. Asterisks indicate statistical significance, where $p<0.05$.

| Group | Initial Weight (g) | Final Weight (g) |
|---|---|---|
| **Control** | 29.8 ± 0.5 | 35.8 ± 0.8* |
| **High-Fat** | 29.5 ± 0.6 | 45.3 ± 2.6* |
| **Exercise** | 29.6 ± 0.5 | 31.9 ± 0.6* |
| **High-Fat & Exercise** | 28.6 ± 0.6 | 39.5 ± 1.7* |

**Table 3. Effects of diet and exercise on in vitro contractile performance.** Mean values for twitch tension, contraction time, half relaxation time, tetanus tension plus/minus standard error of the mean.

| Group | Twitch Tension (g) | Contraction Time (ms) | 0.5 Relaxation Time (ms) | 100Hz Tension (g) |
|---|---|---|---|---|
| **Control** | 1.13 ± 0.18 | 25.1 ± 0.9 | 2.0 ± 1.0 | 3.51 ± 0.58 |
| **High-Fat** | 1.80 ± 0.26 | 25.0 ± 0.9 | 18.7 ± 1.1 | 4.51 ± 0.73 |
| **Exercise** | 1.58 ± 0.13 | 25.5 ± 0.6 | 19.3 ±0.9 | 4.13 ± 0.35 |
| **High-Fat & Exercise** | 1.59 ± 0.12 | 24.1 ± 1.1 | 20.8 ± 1.0 | 4.28 ± 0.14 |

**Table 4. Effects of diet and exercise on in vitro fatigue time.** Mean values for time in seconds until muscle reached 50% and 25% of original contraction strength. Standard error of the mean is given to indicate range of confidence.

| Group | Time to 50% Fatigue (s) | Time to 25% Fatigue (s) |
|---|---|---|
| **Control** | 180.12 ± 14.24 | 266.00 ± 21.09 |
| **High-Fat** | 181.53 ± 8.47 | 306.16 ± 12.01 |
| **Exercise** | 178.03 ± 15.98 | 332.36 ± 28.58 |
| **High-Fat & Exercise** | 195.53 ± 12.83 | 320.41 ± 12.86 |

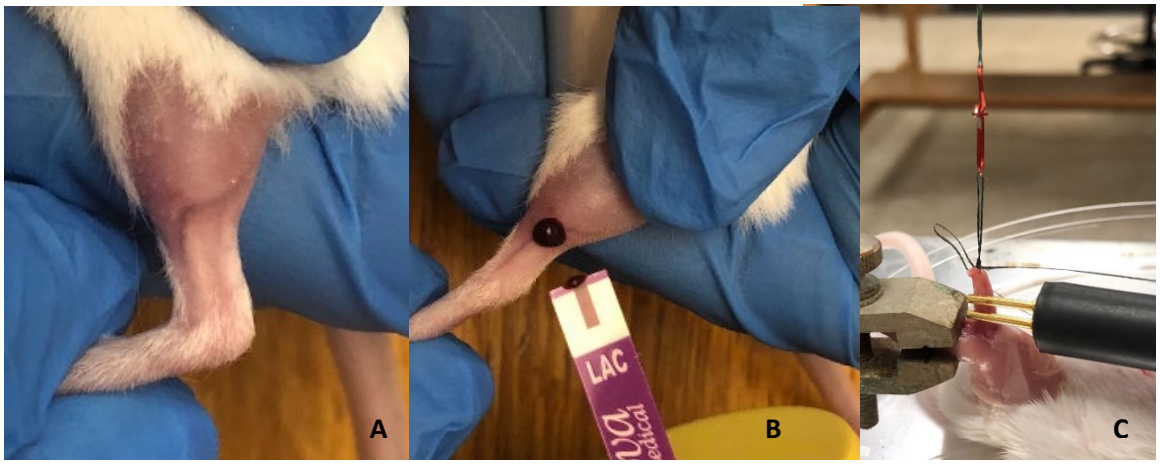


**Figure 1.** A) Mouse leg after Nair is used to remove hair. Saphenous vein is purple and distinct making it easy to draw blood. B) Mouse leg during blood draw from saphenous vein. Biomedical Lactate Plus machine is being used to gather and analyze lactic acid levels in the blood. C) Position of muscle and leg of mouse for in vitro contraction protocol.

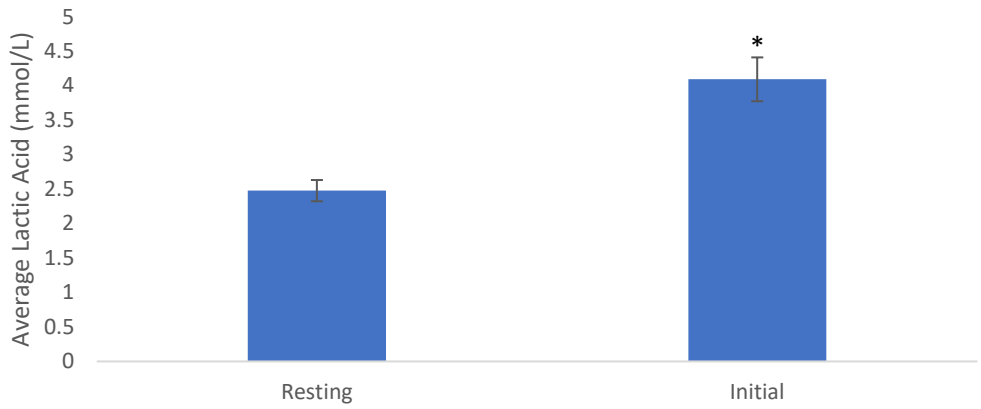


**Figure 2.** Comparison of resting lactic acid level versus lactic acid level after exercise prior to experimental regime. All subject groups were pooled (n=28). Error bars are used to represent the standard error of the mean. Significant differences are indicated by an asterisk. There is a significant difference between groups indicating that mice were intensely exercised enough to influence lactic acid

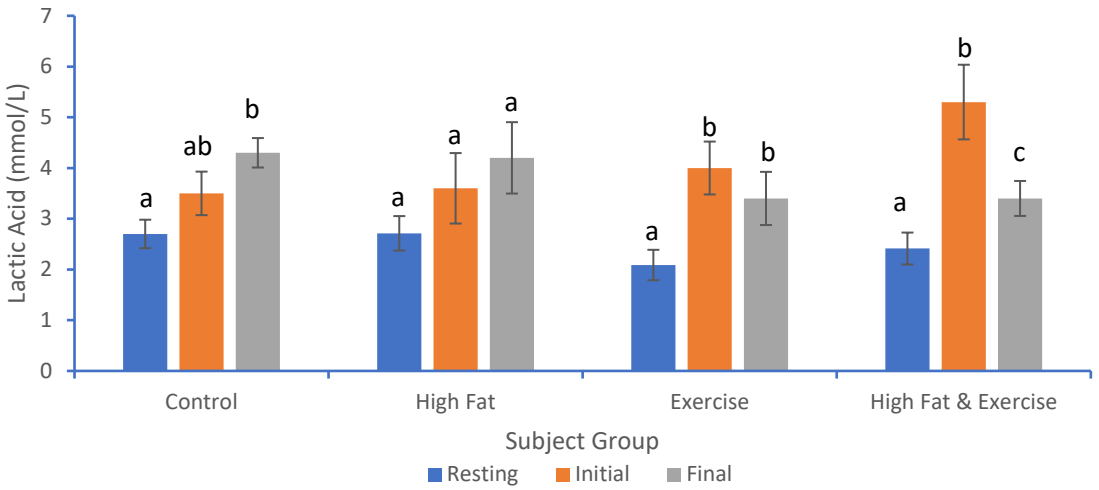


**Figure 3**. Comparison of average lactic acid levels of mice at rest and in various stages of exercise. Mean lactic acid levels during rest, initial exercise run, and final experimental exercise run are displayed. Error bars represent standard error of the the mean. Different letters indicate significance within each group at the 0.05 level. Comparisons were done within each subject group so subjects are monitored for change rather than comparing between groups.

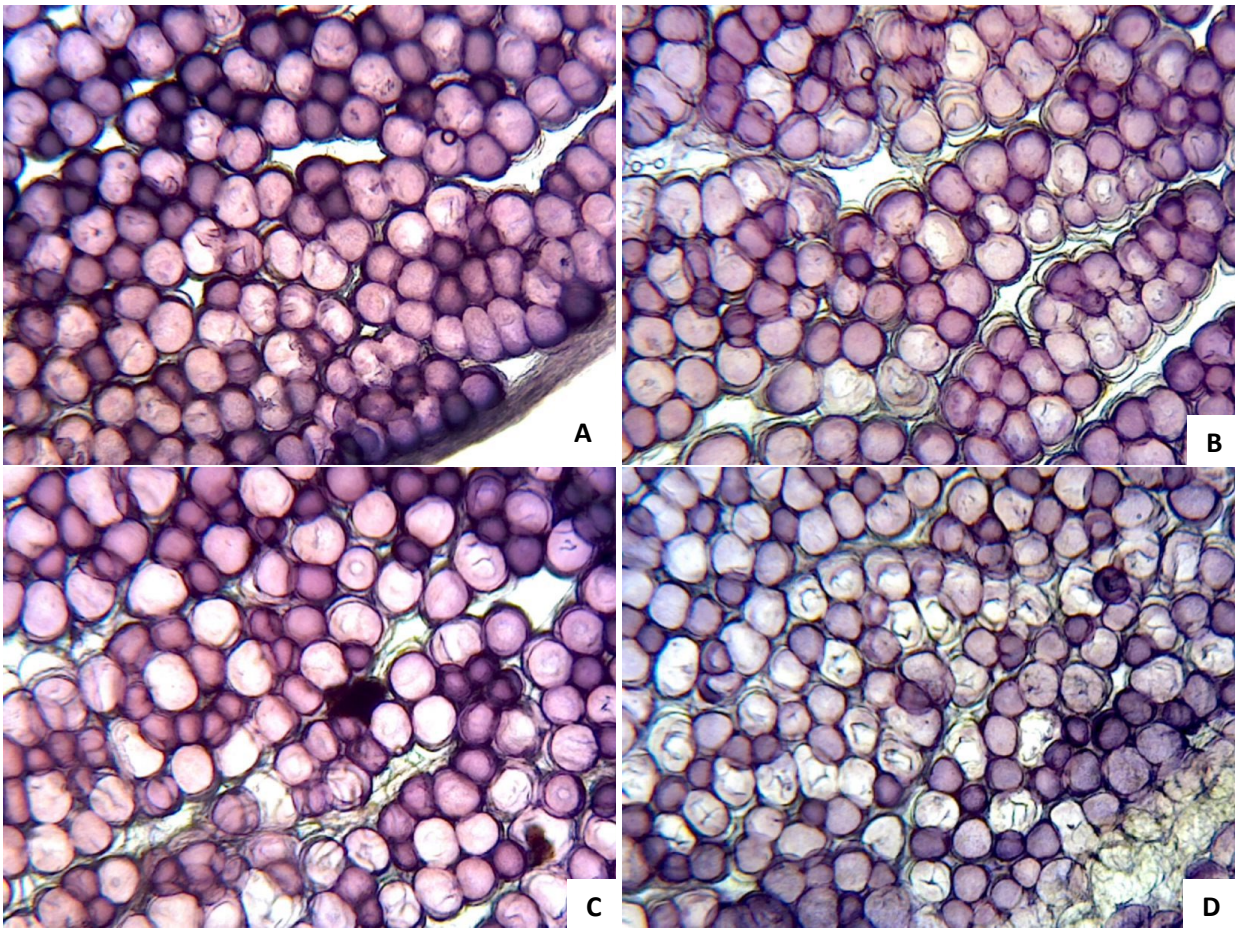


**Figure 4.** Images of section of muscle fibers after sectioning and staining for succinate dehydrogenase. Images from Control (A), High-Fat (B), Exercise (C), and High-Fat & Exercise (D) groups shown. Large white fibers are type IIb. Medium and light stain fibers are type IIa. Dark stain and smallest fibers are type I.

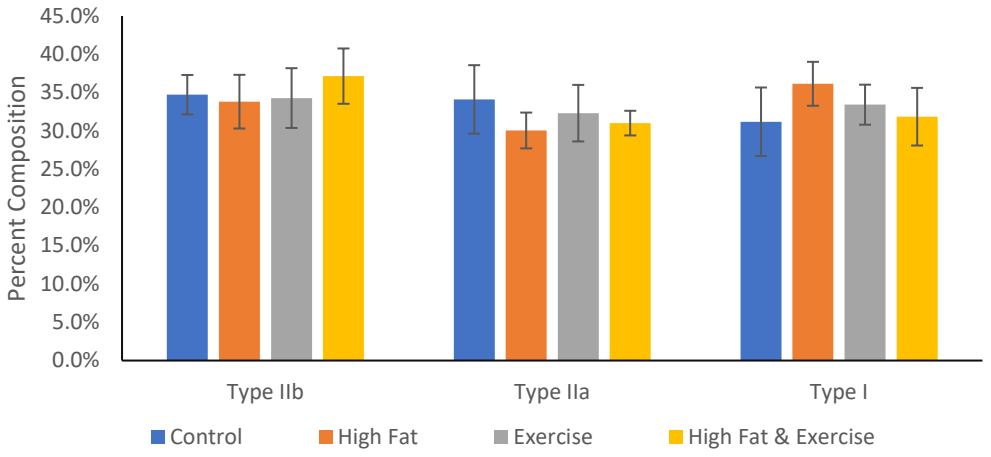


**Figure 5**. Average percent composition of each muscle type per group. Errors bars indicated using standard error calculations. No significant difference detected between the percent of each type of muscle fiber in a particular group.

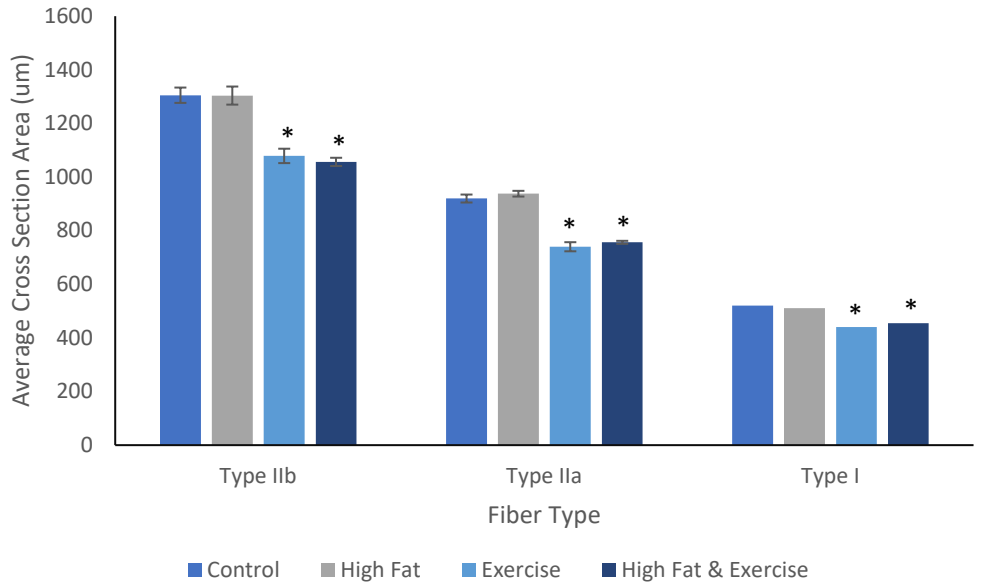


**Figure 6**. Average cross-sectional area of each fiber type compared between subject groups . Standard error is used to create error bars. Errors are so small in for the type I fiber category that it cannot be seen on graph. Significant differences were found with statistics but visually the Exercise and High-Fat & Exercise groups tend to be smaller in size.