Epigenetic Changes in Skeletal Muscle: Does Resistance Exercise Protocol Make A Difference?

Jesslyn Hendrickson

April 25, 2019

Acknowledgments:

Dr. Michael Lyons

Dr. Jonathan Mochel

Dr. Steve Carlson

Abstract

Performing regular resistance exercise training has been found to improve an individual's health, including improved metabolism and ability to perform maximal contractile force in skeletal muscle. Certain epigenetic changes are believed to provide a positive impact on individuals and resistance exercise is also known to cause epigenetic changes to occur, including DNA methylation, changes in mRNA expression, differential microRNA expression, and histone modifications. There is debate as to whether these changes are beneficial but with chronic exercise, these changes do appear to provide a benefit to the individual. All these changes play a role in gene expression and the changes that occur with resistance exercise can possibly cause an advantage to an individual, such as increased insulin sensitivity and muscle hypertrophy. Possible mechanisms causing these changes in skeletal muscle include changes in calcium influx, ATP depletion, alterations in metabolic cycle intermediates, and oxidative stress. Understanding the mechanisms by which these changes occur in skeletal muscle due to resistance exercise can have implications in human medicine, such as interventions to treat metabolic disease including type 2 diabetes mellitus. This review examines epigenetic changes that occur with specific resistance exercises related to duration, intensity, and frequency and discuss possible mechanisms.

Introduction

Numerous studies have shown the benefits of resistance training, which is physical exercise that causes muscles to contract against an external force. The specific exercise, intensity, training volume, frequency, as well as the half-life of the proteins created by training can have positive as well as negative impacts on skeletal muscle and their cellular homeostasis.[1] Positively, resistance training can stimulate muscle contractile protein synthesis in vivo to cause muscle hypertrophy and maximal contractile force output. Negatively, incorrect or extreme resistance training can cause skeletal muscle damage and more severely, breakdown of proteins and muscle fibers called rhabdomyolysis.[1] Skeletal muscle has great plasticity, having adaptive responses with exercise including improved metabolic efficiency and contractility.[2] It has the ability to alter levels of proteins as well as what types of proteins are produced with disruption to cellular homeostasis, likely due to the occurrence of epigenetic changes occuring.[1] There is interest in the study of epigenetic changes in skeletal muscle due to the capability of altering phenotype by environmental factors, including resistance exercise which can induce changes in metabolism as well as mechanical abilities of muscle.

If skeletal muscle were able to be "programmable" in some way, such as through knowing how to elicit specific epigenetic changes, exploitation of these changes could result in additional benefits to those looking to improve the performance of skeletal muscle and increase hypertrophy and therefore, muscle mass.[8] Research has shown that resistance exercise training could possibly reprogram skeletal muscle through epigenetic changes, conferring a benefit to the individual, and possibly even have a memory of epigenetic changes occurring with exercise. This can have huge implications for elite athletes but also in preventative medicine to promote a longer lifespan.

Although this is a relatively new field of research, the implications of exercise on the epigenome of an individual are becoming quite prominent with many of the epigenetic changes noted to be beneficial. Any duration of exercise, acute and chronic/long-term, have been shown to have an impact on the genome regarding epigenetic changes but in a specific manner, pertaining to specific genes or a type of tissue such as skeletal muscle.[3] When examining what genes or tissues were most commonly impacted by epigenetic changes induced by exercise, the following were most common: metabolism, muscle growth, inflammation, and hematopoeisis.[3] Implications with these noted changes can include the notions that exercise can allow for silencing of certain genes that can have negative implications on metabolism or other factors as well as the idea of trainability of certain genes, such as performing a certain exercise can cause upregulation of a gene to allow for increased insulin sensitivity or increased muscle mass. It is important to consider these factors as skeletal muscle accounts for about 80% of insulin-stimulated glucose removal, showing great relevance to metabolic diseases including diabetes mellitus.[18]

Epigenetic Changes in Skeletal Muscle

Epigenetics can be defined as the study of alteration to DNA without changing the sequence that can produce heritable characteristics, but it is important to note that epigenetic changes vary from individual to individual.[3] In a review performed by Durham, many forms of epigenetic changes occurring from various exercises are reviewed in multiple types of tissue in the body, including skeletal muscle, neurons, germ cells, and cardiac myocytes which can be seen in Figure 1.[5] Within this review, there are several types of epigenetic changes induced by

exercise that are deemed most prominent in skeletal muscle; DNA methylation, changes in regulation of mRNA transcription, histone modifications, and expression of microRNA. When DNA methylation occurs, a methyl group is incorporated into the fifth carbon of the pyrimidine ring of a cytosine base by a DNA methyltransferase and creates 5-methylcytosine.[4] Most often, methylation causes the expression of a specific gene to decrease as it changes the access of chromatin or causes the recruitment of binding proteins but this is dependent on which gene this is occurring.[4] From DNA methylation, there can be further categories including hypermethylation, hypomethylation, and also hydroxymethylation, which is the conversion of 5-methylcytosine to 5-hydroxymethylcytosine via Tet methylcytosine dioxygenases.[5] While hydroxymethylation can occur within the genome, the impact of this epigenetic change is poorly understood at this time. The epigenetic importance of DNA methylation is of great concern as DNA methylation can contribute to an increased risk in metabolic disease such as by altering insulin sensitivity. [2] With resistance exercise, there are changes to the expression of insulin sensitivity genes as well as genes related to muscle hypertrophy which will be further discussed.

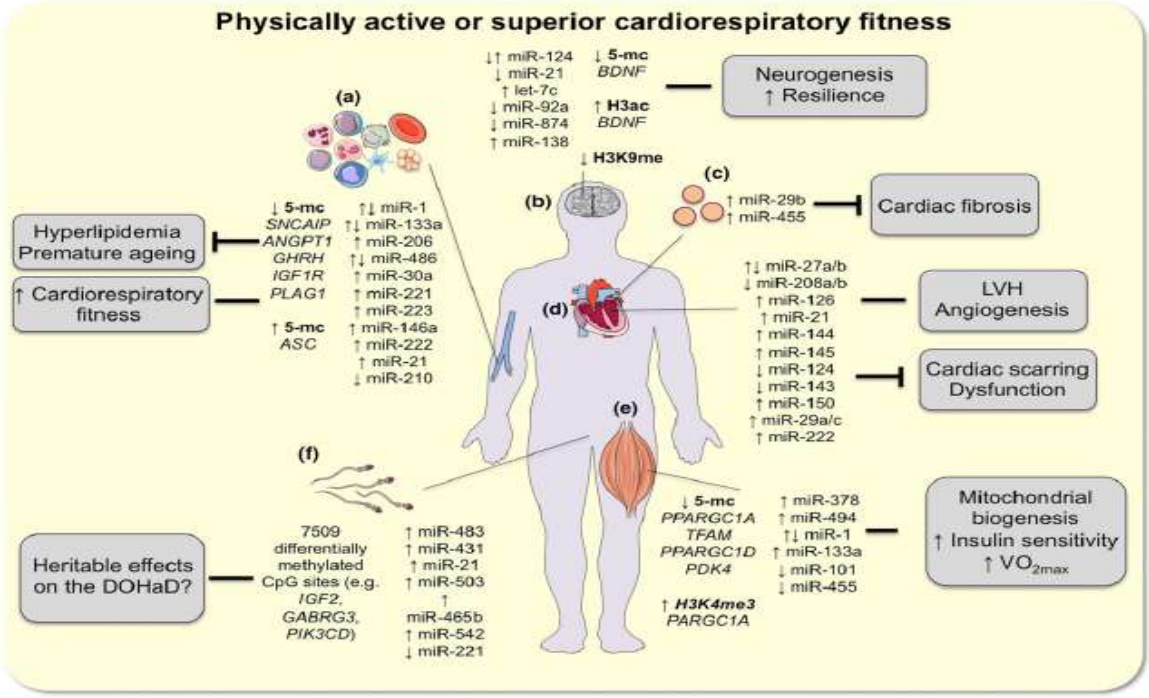

Figure 1 Epigenetic modifications linked to exercise-induced adaptations. Chronic exercise training regulates epigenetic modifications, including DNA methylation, histone post-translational modifications (histone methylation and acetylation) and microRNAs. The epigenetic landscapes of peripheral blood (a), brain (b), cardiac exosomes (c), heart (d), skeletal muscle (e) and sperm (f) cells respond to chronic exercise training. The epigenetic modifications caused by exercise training are associated with health benefits, such as blood lipid and glucose control, LVH, psychological resilience and neurogenesis, the prevention of premature ageing, and it is hypothesized that sperm and oocyte, and *in utero* epigenetic changes influences embryogenesis and affects the developmental origins of health and disease in the offspring (DOHaD). Legend: ↑, increased; ↓, decreased; 5-mc, 5-methylcytosine; *SNCAIP*, synuclein-alpha interacting protein; *ANGPT1*, angiopoietin 1; *GHRH*, growth hormone-releasing hormone; *IGF1R*, insulin-like growth factor 1; *PLAG1*, pleomorphic adenoma gene 1; *ASC*, PYD and CARD domain containing; miR, microRNA; H3, histone 3; ac, acetylation; K9, lysine 9; me, methylation; LVH, left ventricular hypertrophy; K4, lysine 4; VO_{2max}, maximum oxygen uptake (cardiorespiratory fitness); me3, trimethylation; CpG, cytosine neighbouring a guanine dinucleotide; *IGF2*, insulin-like growth factor 2; *GABRG3*, gamma-aminobutyric acid type A receptor gamma3 subunit; *PIK3CD*, phosphatidylinositol-4, 5-bisphosphate 3-kinase catalytic subunit delta; DOHaD, developmental origins of health and disease. This figure was produced using Servier Medical Art (http://www.servier.com/).

Image obtained from: Denham, J. "Exercise and Epigenetic Inheritance of Disease Risk." *Acta Physiologica*, vol. 222, no. 1, 2018, doi:10.1111/apha.12881.

Changes in regulation of mRNA transcription, while not immediately thought of as an epigenetic change, is an important factor to consider as changes to these transcripts can alter protein levels in a tissue. Changing mRNA transcription can also result in creation of isoforms of a specific gene, such as with insulin-like growth factor-1.[6] There are several types of histone modifications that are known, including phosphorylation, acetylation, and methylation of amino acid residues within histone tails but the most common histone modification mentioned in literature pertaining to epigenetic changes with exercises is histone acetylation and histone deacetylation. With histone acetylation, an acetyl group is attached by histone acetyltransferases (HAT's) to lysine residues on the following histone proteins: H2A, H2B, H2AX, or H3.[5] Histone acetylation is often reported to cause an increase in gene expression. This acetylation can be removed by histone deacetylases (HDAC's) as well. The last category of epigenetic changes known to occur with exercise refers to microRNA's (miRNA's), which are small noncoding RNA's that are only about 22 nucleotides in length and are important regarding silencing of genes.[5] Specifically, miRNA's regulate gene expression post-transcriptionally, creating their silencing effect by negative regulation of a post-transcriptional gene or by degradation of the mRNA.[5] miRNA's are important with gene expression as even one single miRNA can alter the expression of multiple mRNA's and their associated proteins which can also lead to tissue specific changes in an individual. One such alteration reported with resistance exercise from miRNA's is related to muscle hypertrophy.

These specific epigenetic changes that have been shown to occur with exercise can have huge implications in the overall wellbeing of an individual, and possibly even in the progeny of the individual. Evidence of these heritable epigenetic changes have been seen in rodent models up to generation 4 (F4) but further research is required to understand more about how and why these heritable epigenetic changes persist in later generations.[5] By examining further what specific changes occur with specific resistance exercises, we can begin to develop training routines for individuals, and this can have a role in preventative medicine in humans and animals as well as other fields of science including meat science in livestock. In order to be able to create these programs and understanding the possible implications in inheritance, the mechanisms of how these epigenetic changes occur due to resistance exercise training must be examined further as they are not well understood.

DNA Methylation

The impact of epigenetic modifications on skeletal muscle has been a growing area of study with the consideration of environmental impact being examined. The implications of these epigenetic modifications occurring in skeletal muscle due to resistance training is also a growing area of science which has shown great promise in learning more about the benefits of regular physical exercise on the individual but also the individual's progeny. Of all the epigenetic modifications that can occur in skeletal muscle as well as other tissues, DNA methylation has shown to be one modification of great importance and prominence that occurs during resistance exercise.

With acute exercise, epigenetic changes have been noted to occur in skeletal muscle. When comparing genomes between the baseline of an individual and after an acute bout of incremental cycling with resistance exercise until fatigue, methylation throughout the genome was decreased in skeletal muscle biopsies.[2] What is most interesting about this study is that the epigenetic response to exercise is intensity dependent with higher intensity exercise leading to

more pronounced demethylation, meaning that exercise can induce a dose-dependent expression of certain genes.[2] Specifically, hypomethylation was noted on the promoter region of the following genes: PGC-1α, PDK4, and PPAR-δ. These genes were examined in this study as these genes are important in regulation of mitochondrial function and fuel usage, including ATP, in cells.[2] Alteration of mitochondrial function and fuel usage in cells by hypomethylation is thought to be beneficial to an individual as this can improve efficiency of mitochondria and how fuels are utilized in metabolism, including glycolysis. It was hypothesized that by looking at genes dealing with these physiological functions in relation to exercise, possible mechanisms of how the epigenetic changes occur with resistance exercise could be formulated.

In an investigative study regarding the methylome of human skeletal muscle after variations in resistance training, evidence suggests that there is a "phenomenon of epigenetic memory in skeletal muscle".[4] Participants in this study were subjected to an acute, single bout of resistance exercise, chronic resistance exercise of 2 sessions per week over a course of 7 weeks, complete cessation for 7 weeks for detraining, and then followed by a subsequent retraining period of 3 sessions per week for 7 weeks.[9] Lower limb exercises were performed in this study, including behind the head barbell squats, leg press, leg extensions, leg curls, Nordic curls, weight lunges, and calf raises. Upper limb exercises included flat barbell bench press, machine shoulder press, latissimus dorsi pull downs, bent over dumbbell row, and triceps cable extensions. Each exercise was performed with increasing weight load until the subject could no longer complete 10 full repetitions. Biopsies of the vastus lateralis muscle were obtained at an untrained baseline as well as 30 minutes after each period of acute exercise, training, detraining, and retraining. With a single, acute bout of resistance exercise of lower limb resistance exercises, genome wide DNA methylation was present. With chronic resistance training over a course of 7 weeks, it is shown that DNA hypomethylation does occur. But with the occurrence of retraining, there is a curious phenomenon that increased frequency of hypomethylation occurs, showing that indeed there is a possibility of epigenetic memory present within skeletal muscle.[9] The most noticeable and statistically significant epigenetic change occurred with retraining, showing primarily increased levels of hypomethylation. With initial training, lean leg muscle mass increases and returns toward baseline with detraining but when retraining occurs, muscle mass is increased past the level that occurred with initial training which also shows the possibility of the presence of epigenetic memory in skeletal muscle. As DNA methylation disappears with chronic/persistent resistance exercise, DNA methylation seems to confer a benefit to an individual in the short term with resistance exercise but long term, it is detrimental as hypomethylation occurs with long term resistance exercise.

With performance of resistance and endurance exercise over a course of 6 months, genome wide DNA hypomethylation was also reported.[16] Individuals performed one session of spinning class and one session of aerobics class consisting of one hour each with 2 sessions per week. Intensity of the exercise was not explicitly reported in this article, so overall intensity of the exercise appears to be dependent upon the individual performing the exercise. Genome-wide DNA hypomethylation was noted to be present with numerous genes related to metabolism, including retinol metabolism, calcium signaling pathways, and genes related to "known functions in muscle" including MEF2A, RUNXI, NDUFC2, THADA.[16] This study emphasized the importance of alterations in metabolic gene functions in individuals that have a family history of type 2 diabetes mellitus which can have implications in preventative medicine.

Performing one legged knee extension exercise training over a course of 3 months with one leg being trained alone, genome wide DNA methylation was reported only in the trained leg when compared to levels prior to training and the untrained leg.[17] Training only one leg was performed in order to minimize the influence of diet or other environmental factors on an individual. Intensity of the exercise was determined using a one-legged max test, consisting of 2 minutes at a constant load of 10 or 20 watts, based on the measured VO_{2peak} of the subject, followed by 3 or 5 watts increase every 30 seconds until the point of exhaustion. Specifically, DNA methylation was present at 4919 sites across the genome within the trained leg with most methylation present in enhancer regions of genes. Transcriptional analysis of these sites using RNA sequencing showed differential expression of 4076 genes as well.[17] Differential methylation was examined to determine what specific genes were being methylated with the occurrence of resistance exercise. It was noted that the most common genes methylated were genes related to cellular components including mitochondria, molecular functions, and biological processes that are linked to myogenesis, muscle structure, muscle function, and bioenergetics.[17] With the gene specificity, it was also noted that specific differential methylation correlates with changes in expressions of genes and the expression of genes is changed in an exercise-dependent manner, showing greater changes in expression with increased exercise intensity and duration.

mRNA

While DNA methylation is an important epigenetic factor to consider, expression of mRNA's can also be examined to determine up- or down-regulation of specific genes. One study examined the effects of single legged knee extension exercise at 80% of individuals one repetition maximum and changes in mRNA expression of two splice variants of the insulin-like growth factor-I (IGF-I) gene: IGF-IEa and mechano growth factor (MGF).[6] Insulin-like growth factor-I is an important factor with growth and development but little is known about how these two splice variants of IGF-I play a role individually. Prior research has shown that these two isoforms could possibly play a role in increasing muscle mass when mechanical stimulation is applied to skeletal muscle, which can have implications in understanding epigenetic changes on skeletal muscle due to exercise.[6] When comparing between young subjects (ages 25-36 years) and elderly subjects (70-82 years), the baseline of these two isoform mRNA's at rest showed no change in regards to age. With the occurrence of high resistance exercise, there was a "significant increase" in MGF mRNA levels in young subjects but not in the elderly and no noted changes in the mRNA levels of IGF-IEa.[6] Although this study compares young subjects to elderly subjects, there appears to be a correlation between resistance exercise and upregulation of genes important in metabolism, such as the insulin-like growth factor discussed in this study.

Comparing subjects at rest and subjects after an acute bout of low resistance exercise of bilateral knee extension at only 20% of their one repetition maximum with and without blood flow restriction, there were marked changes in levels of several mRNAs pertaining to muscle growth and remodeling.[10] Use of blood flow restriction in this study was utilized to determine if similar changes in mRNA's occurs with low-intensity resistance exercise compared to a state of hypoxia in skeletal muscle with resistance exercise. Increased levels were present with HIF-1α (hypoxia inducible factor-1 alpha), MyoD which regulates muscle differentiation, MuRF 1 (muscle RING finger 1) which is a ubiquitin ligase that likely plays a role in muscle protein degradation, and p21 that is a regulator of the cell cycle progression at G_1 and S phase.[10] Decreased mRNA levels of REDD1 and myostatin was also noted which both play a role in inhibition of myogenesis secondary to stress.[10] As differential expression of specific mRNAs

were noted with low resistance exercise with induced reduced blood flow via pressure cuff placed on the upper leg as well as with high resistance exercise, it can be stated that these epigenetic alterations occurred due to the presence of resistance exercise but changes in oxygen concentration in skeletal muscle could play a role.

microRNA

An often-forgotten area of epigenetics deals with the implications that microRNA's (miRNA or miR) can have on a genome and therefore, an individual. While this is a rather new area of genetics to be investigated, miRNA's can be dismissed in the field as people try to find changes which are more permanent and looking more exclusively at DNA. It is important to examine microRNA's as miRNA's function with RNA silencing and post-translational regulation. Recent research has shown that even miRNA's have a role in epigenetic changes that occur from exercise, including resistance exercise training. In a study performed with 56 young men who proceeded with a 5 days per week resistance training program over a course of 12 weeks, differential regulation of microRNA expression in skeletal muscle was reported compared to baseline.[7] More importantly, one specific miRNA, miR-378, was proven to have a positive correlation with gaining muscle mass in vivo as it was shown that miR-378 levels increased with training and lean body mass increased as well.[7] In this specific study, pushing exercises were performed including military press, bench press, seated chest fly, seated triceps extension. Pulling exercises were also utilized, including seated lateral pull down, seated wide grip row, seated reverse fly, seated biceps curl, a series of abdominal exercises without weights. Leg exercises were also utilized, such as incline leg press at 45 degrees, 2-leg knee extension, 2-leg hamstring curl, and seated calf raises. All exercises were performed in 60 sessions with 20 of the sessions being pushing exercises, 20 sessions being pulling exercises, and the other 20 sessions consisting of leg-resistance exercise workouts. Intensity of the exercise was specified as subjects performing at 80% of one voluntary single repetition maximum. In a separate study performing endurance exercise with resistance exercises incorporated to the point of exhaustion, a decrease in miR-494 was noted after performing these exercises for only 7 days.[5] Increases in mitochondrial content, *Pparfc1a, Tfam,* and *Foxj3* expression were also noted. miR-494 and the specified genes were examined as miR-494 inhibits mitochondrial biogenesis and these genes are important in metabolic and mitochondrial functions.[5]

In skeletal muscle, miRNAs are also known as muscle specific miRNA's, or myomiR's.[15] Differential expression of miRNA's in skeletal muscles compared to other tissues has been shown in prior research, but there is evidence of differential expression with the occurrence of acute and chronic resistance exercise as well as cessation from exercise. With studies examining myomiR's in skeletal muscle, often the following are studied: mir-1, mir-133a, mir-133b, and mir-206. These are noted to be prominent in skeletal muscle, accounting for about 25% of all expression of miRNA's and specifically, myomiR's appear to be increased dramatically during the occurrence of myogenesis.[15] Prior to chronic training, an acute bout of cycling endurance exercise including resistance for 60 minutes at 65% of peak watt showed an increase in mir-1 and mir-133a expression.[15] When having subjects perform cycling endurance exercise with resistance over a course of 12 weeks, myomiR expression was down-regulated with all myomiR's examined (mire-1, mir-133a, mir-133b, mir-206) when compared to baseline levels before the 12 weeks of training. With this chronic exercise training, insulin sensitivity was also noted to improve by 19%.[15] After this 12 week training period, episodes of acute endurance exercise with resistance showed no induced effect on the measured expression of myomiR's.[15]

When the subjects were allowed a 2 week cessation from training, vastus lateralis muscle biopsies showed a reversion of myomiR levels, specifically mir-1 and mir-133a levels, back to non-significant levels present before training began.[15] Intensity of this cycling exercise with resistance was measured in P_{max}, which is the maximal power that can be generated over at least one full revolution of the pedal with both legs. With the 12 week chronic training program, an intensity level schedule was created; Monday performed a Pmax test, Tuesday performed at 85-91% of Pmax for 70-80 minutes, Wednesday performed at 60-66% of Pmax for 60-80 minutes, Thursday performed at 75-81% of Pmax for 75-81 minutes, and Friday performed at 55-61% of Pmax for 120-150 minutes.[15]

As seen with modifications in mRNA transcription, changes in microRNA expression are noted regarding insulin sensitivity during resistance exercise. Gene interactions are common with many physiological functions, meaning that many genes have some dependence on other genes and multiple genes can alter the level of transcription of others such as with development and growth. One bout of interval training of cycling, including resistance, for 48 minutes was performed on individuals at increasing resistance levels until reaching 90% peak heart rate.[13] With this resistance exercise, increased expression of *PPAR* genes because of an upregulation of was present. *PPAR* genes, or peroxisome proliferator activated receptor genes, are nuclear receptors that have a function of transcription factors which regulate expression of genes.[13] It is believed that *PPAR* genes have a role in regulation of insulin sensitivity genes and by their increased expression, post-translational changes can occur, and increased insulin sensitivity could possibly occur. With this study, it is important to note that changes in miRNAs were not directly correlated with insulin sensitivity.[13]

Histone Modifications

Chromatin remodeling from histone modifications has been known to play a crucial role in regulation of gene activation and silencing. Examination of histone modifications occurring from resistance exercise can be utilized to further understand mechanisms of chromatin remodeling and transcriptional activation in skeletal muscle. Specifically, histone modifications are known to regulate expression of isoforms pertaining to myosin heavy chain in response to muscle unloading, such as muscle atrophy and metabolic perturbations.[12] With "exhaustive exercise" consisting of a single bout of cycling including resistance for 60 minutes at 76 ± 2% of VO_{2peak}, increased histone acetylation was noted at histone 3 lysine 36 (H3K36) which is important for transcriptional activation, specifically with the regulation of transcriptional elongation.[12] Alteration of the function of class IIa histone deacetylases (HDACs) was also reported after the acute bout of 60-minute exercise. Activation of several classes of class IIa HDAC kinases was present after this acute bout of exercise, which is important in phosphorylating HDACs, specifically HDAC 3, 4, and 5, to induce their nuclear export.[12] Also occurring with an acute bout of cycling exercise with resistance was an increase in *Glut4* receptors, allowing for faster and more efficient glucose transport.[12]

HDACs play an important role in gene regulation in skeletal muscle, specifically regarding genes involved in metabolism in skeletal muscle. The most common HDACs found in skeletal muscle are HDAC 4 and 5, often times having a repressive function but they are sensitive to different signaling pathways.[18] HDAC 5 has been found to be a key regulator of the GLUT-4 gene, responsible for formation of the glucose transporter *Glut4* and its receptors.[18] Class IIa HDACs, including HDAC 4 and 5, are reportedly known to be regulated by the

mechanism of phosphorylation-dependent nuclear export, which is known to occur by kinases that are activated with resistance exercise. Activation of these HDACs can have implications in improvement in glucose metabolism due to their relationship with the glucose transporter *Glut4*.

Resistance Exercise and The Possibility of Inheritable Epigenetic Changes

It is known that there are health benefits to individuals performing regular exercise, including resistance training, but there is some evidence that epigenetic changes from resistance exercises can be conferred as heritable benefits to the progeny of the individual.[5] It is difficult to establish this fact though, as during early embryogenesis from week 7-9, the DNA methylomes of gametes, primordial germ cells as well as the zygote undergo erasure of a vast amount of DNA methylation throughout the genome due to base excision repair.[5] Although a large amount of the genome undergoes erasure of epigenetic changes such as DNA methylation, there appear to be specific genes that maintain their epigenetic inheritance throughout fertilization and embryogenesis. Studies performed on mice have shown variability in inheritance of epigenetic changes in progeny in relation to paternal versus maternal exercise.[5] While the influence of exercise maternally on epigenetic inheritance is more established than the influence of exercise paternally, there appears to be a correlation to positive epigenetic changes occurring to zygotes when maternal exercise occurs during embryogenesis.[5] Exercise induced epigenetic changes occurring paternally have been noted to be more likely to remain present after erasure of DNA methylation though.[5] Both maternally and paternally, it is important to understand that epigenetic changes are present in germ cells after the occurrence of resistance exercise as well as other exercises, including aerobic exercise.

Methods of Literature Review

Articles for this review were obtained from the National Center for Biotechnology Information (NCBI) database and the US National Library of Medicine National Institutes of Health (PubMed) database. Keywords used to find articles included: resistance training, epigenetics, DNA methylation, histone modifications, mRNA, microRNA, skeletal muscle, and epigenome. Articles were examined and selected for exercise protocols containing resistance exercise and examination of epigenetic alterations occurring. From the articles, common trends of how the resistance exercise was determined were found to create table categories. Categories for created tables were established by examining the specific resistance exercise type, intensity of the exercise, duration of the exercise, frequency, and epigenetic changes occurring from the exercise to examine separate variables important regarding resistance training. Duration and frequency were examined to compare acute versus chronic resistance training protocols. Intensity was examined to determine if the exercise performed created a work load against the skeletal muscle examined. An exercise was deemed as high intensity if the repetitions were greater than 10 repetitions in 3 or more sets and/or weight load was greater than 50% of an individual's one repetition maximum. Acute resistance exercises in this review was determined to be exercise lasting 7 days or less while chronic resistance exercise training was determined to be persistent, regular exercise occurring for greater than 7 days. Subcategories of tables were created to examine acute versus chronic resistance training protocols, to compare specific categories of epigenetic changes, and to compare specific resistance training protocols. Master tables containing all information to aid in analyses, separated into acute resistance exercise (Table A), chronic resistance exercise (Table B), epigenetic changes occurring (Table C), and specific exercise protocol (Table D), are contained in the Appendix.

Results

Table 1. Epigenetic change occurring with exercise protocol, comparing acute versus chronic exercise.

Epigenetic Change	Exercise Protocol	Acute Effect	Chronic Effect
DNA Methylation	Single leg knee extension (4)	↑ (1)	↓ (2) ↑ (1)
	Cycling with resistance (2)	↓ (1)	↓ (1)
microRNA Expression	Cycling with resistance (3)	↑ (2)	↓ (1)
	Endurance exercise (1)	↓ (1)	NA
	Rotating split-body resistance exercise program (1)	NA	↑ (1)
mRNA Expression	Single leg knee extension (1)	↑ (1)	NA
	Bilateral knee extension (1)	↑ (1)	NA
Histone Modifications	Cycling with resistance (1)	↑ (1)	NA

Epigenetic change occurring compared to exercise protocol to compare effect occurring with acute resistance exercise versus chronic resistance exercise. (#) in exercise protocol indicates number of studies utilizing this protocol and (#) in acute and chronic effects indicates number of articles indicating the specific effect. NA = none available.

When looking at changes in DNA methylation with separate exercise protocols and comparing acute versus chronic resistance exercise, there are conflicting results. With single leg knee extension, there were noted to be increased DNA methylation as well as DNA hypomethylation with acute exercise. For DNA methylation occurrence with chronic exercise utilizing single leg knee extension, two studies found DNA hypomethylation occurring while one study found increased DNA methylation. When examining DNA methylation occurrence with use of cycling including resistance to look at acute versus chronic exercise, DNA hypomethylation was noted in both categories. One study for acute cycling exercise and one study for chronic cycling exercise was found for these results.

Three exercise protocols were found to have examined changes occurring in microRNA expression with the occurrence of resistance exercise. Cycling with resistance was found in three separate studies, showing an increase in miRNA expression with acute exercise in two studies and a decrease in miRNA expression with chronic exercise in one study. With use of endurance exercise including resistance, a reduction in miRNA expression occurred with acute exercise in one study. Reduction of miRNA expression with chronic exercise was found when utilizing a rotating split-body resistance exercise program.

Single leg knee extension was utilized again to examine changes in mRNA expression. In one study, an increase in mRNA expression occurred with the occurrence of acute exercise. An increase in mRNA expression was also noted to occur with acute exercise when performing bilateral knee extension in one study. Only one exercise protocol was found when examining

histone modifications. In one study, an increase in histone modifications was noted to occur with acute exercise when performing cycling with resistance.

Discussion

It is clear to see that there is a statistical significance for the studies shown between resistance exercise training and the presence of epigenetic changes, but the mechanisms that causes these changes is not clear. It is also hard to distinguish currently if these epigenetic changes occurring from exercise are favorable to the individual as the levels of changes occurring varies from individual to individual. Notably, there are recurrent trends of what gene families are up- or down-regulated with the presence of epigenetic changes occurring in skeletal muscle due to resistance exercise training. Most of the gene families that are noted to have epigenetic changes occurring with resistance exercise training pertain to insulin sensitivity and skeletal muscle hypertrophy. This can have huge implications in prevention of metabolic diseases, including type 2 diabetes mellitus, and improvement in athletic performance. By changing the gene expression in skeletal muscle by epigenetic factors, therapies can be created to favor energy dissipation to help with metabolic diseases. More importantly relevant to an athlete, an improvement in the regulation of insulin sensitivity genes can improve the capacity of skeletal muscle to increase in muscle mass as it can be more efficient in glucose metabolism. Thus far, research has seemed to show that these epigenetic changes occurring from performing resistance exercise is favorable to the individual, such as how insulin sensitivity improves with persistence exercise.

Other recurrent trends when examining epigenetic changes in skeletal muscle occurring with resistance exercise have been noted as well. With acute bouts of resistance exercise, there is upregulation in an epigenetic change including DNA methylation and expression of certain miRNAs. But with the occurrence of chronic resistance exercise, these changes, which often cause gene silencing/down-regulation, are reversed to include DNA hypomethylation and down-regulation of specific miRNA expression. With the down-regulation of DNA methylation and miRNA expression, there is higher likelihood for increased gene expression. These changes show that skeletal muscle contains some type of epigenetic memory, altering this in response to changes in physical demands induced by resistance exercise. Why these changes occur, varying from acute to chronic resistance exercise training, shows a likely compensatory impact of epigenetic changes occurring due to varying duration of exercise. When looking at specific resistance exercises and examining for similar epigenetic changes occurring with similar protocols, it is difficult to find a trend at this time as many studies examine separate groups of genes or different forms of epigenetic changes, such as some studies with similar resistance exercise protocols only look at DNA methylation while others look at changes in mRNA expression exclusively. The largest area of epigenetic changes found to be examined was DNA methylation so further research on other areas of epigenetic changes occurring from resistance exercise is needed. With additional studies in several areas of epigenetics, the option for performing a metanalysis can be a plan to further understand how and why these changes occur with resistance exercise.

Epigenetic modifications and the enzymes that create these changes, such as DNA methyltransferases and histone acetyltransferases, are thought to be dependent upon changes in the levels of metabolites present with exercise, including oxygen concentration, intermediates produced during the tricarboxylic acid/Krebs's cycle, and β-hydroxybutyrate.[11] Because of this

dependence, it can be hypothesized that as exercises causes changes in levels of important metabolites in the body, such as oxygen concentration, exercise can have a direct effect on the occurrence of epigenetic modifications and the presence of enzymes that perform these changes. As resistance exercise is an anaerobic exercise, oxygen concentration on the genome likely has a great impact on the occurrence of epigenetic change and therefore, changes in skeletal muscle metabolism or other factors as well. With a restriction of blood flow, causing a reduction in oxygen concentration in skeletal muscle, in combination with low-intensity resistance exercise, there was an increase in the size of skeletal muscle comparable to traditional high-intensity resistance exercise training.[10] β-hydroxybutyrate is believed to play a role in the induction of epigenetic changes occurring with the presence of resistance exercise as it is typically produced with prolonged exercise, such as 2-3 days of strenuous exercise.[11] This molecule has been found to inhibit HDAC1, HDAC3, and HDAC4, increasing inhibition with increase intensity of exercise and with the inhibition of these class I and II HDACs, this allows for epigenetic changes of histone modifications to occur.[11]

Other possible mechanisms of the induction of epigenetic changes occurring due to resistance exercise in skeletal muscle include changes in calcium influx, the AMP:ATP ratio or ATP depletion, and oxidative stress.[1] With continuous generation of action potentials in skeletal muscle, ATP is being depleted in a cell and calcium is released into the sarcoplasmic reticulum with the rate dependent upon contractile activity.[1] As resistance exercise causes frequent and intense contraction of skeletal muscle, it can be conferred that resistance exercise causes a significant increase in calcium release. Increasing intracellular levels of calcium is known to alter protein levels, such as cyclic AMP (cAMP) and adenyl cyclase, which, through second messenger cascades, alters gene transcription as well as other proteins involved in epigenetic changes including phosphorylating proteins. It can be hypothesized that as resistance exercises causes a significant increase in intracellular calcium concentrations which alters proteins involved in gene transcription and epigenetic changes, epigenetic changes are likely to occur because of the occurrence of resistance exercise. All three of these factors, calcium influx, ATP depletion, and oxidative stress, are known to result in high levels of HDAC phosphorylation.[18] HDACs appear to be critical negative regulators of skeletal muscle metabolic gene expression, inhibiting epigenetic changes with histones. For example, HDAC 5 interacts directly with glucose transporter type 4 (GLUT4) which plays a role in glucose transport and metabolism in skeletal muscle.[11] Inhibition of HDAC's could be an effective way to alter metabolic gene expression and further, be an effective therapy to treat metabolic diseases.[18]

It is important to ask if the type of resistance exercise, duration, intensity, and other factors plays a role in what epigenetic changes occur as well as the amount of changes. The epigenetic response to an exercise appears to be intensity dependent, having a correlation which shows that higher intensity exercise leads to more pronounced demethylation.[2] One specific change noted with changes in exercise directly relates to myomiR's as they seem to rapidly adjust to current physical activity level[15]. MyomiR's appear to be induced by endurance exercise with resistance in untrained individuals, suggesting that myomiR's indirectly affect protein abundance by targeting mRNA transcripts following acute exercise only in untrained individuals. Downregulation of these microRNAs seen in several studies with continued resistance exercise could suggest that silencing of specific genes is no longer needed with the persistence of exercise. It is noted that miR-494 is decreased with consistent exercise and miR-494 inhibits mitochondrial biogenesis so with the downregulation of miR-494, mitochondrial biogenesis

could occur. Mitochondrial biogenesis can confer a benefit to the individual as this allows for more rapid ATP creation which can help in metabolic pathways. Studies examining the impact of training, detraining, and retraining has shown that many epigenetic changes do not persistent with the removal of the stimulus. This shows that in order to maintain the epigenetic changes occurring from resistance exercise, there must be consistent exercise training and this will help in maintaining the benefits from these epigenetic changes including alterations to metabolic function. One study examined did report a persistence of hypomethylation after performance of training, detraining, and retraining with an increase in the amount of hypomethylation occurring with retraining. Only several articles reviewed have examined this trained versus untrained impact as well of subjects, meaning that further research is required to fully understand how changes occur between trained and untrained individuals.

There are concerns when reviewing the literature, as many studies have wide variability of resistance exercise training procedures performed which makes comparing the occurrence of epigenetic changes with certain exercises and procedures difficult. The most common form of exercise examined in studies reviewed utilized cycling, which does have a resistance exercise aspect to it but is also primarily an aerobic exercise, but this is variable with changing intensity. It is important to note that cycling is not an aerobic exercise though when performed at high intensity. Intensity with cycling is often determined by percent VO_{2max} but can also be determined by percent at peak watt and Pmax. With variations in protocol of varying intensity used during cycling, conflicting results can occur considering resistance exercise is often an anaerobic exercise. The other most common resistance exercise included in protocols included single leg knee extensions which is truly a resistance exercise but again, there are variations in protocol in what intensity is used for this exercise such as different percent of an individual's one repetition maximum. In several studies examined, there is no discussion regarding recovery time between sets when performing the exercises which can have a huge implication in the persistence of epigenetic changes. It is important to note though that most studies examined specified the presence of an initial no to low load, familiarization period before beginning the resistance exercise training in order to make sure study participants could perform the examined exercises appropriately. All muscle samples from every study examined also obtained biopsies from the vastus lateralis muscle so there could be other variable epigenetic changes occurring in other muscles performing resistance exercises.

Research considering the role of resistance exercise and retaining epigenetic changes in germ cells has been lacking but there is evidence of inheritable epigenetic changes after the occurrence of exercise. There is the belief that preconception paternal and maternal exercise influences embryogenesis and the developing zygote through DNA methylation and miRNA regulation as these two factors are known to have some persistence with conception and embryogenesis. There are few studies to establish this evidence fully and of the lacking research present, there is more established effects of maternal exercise on germ cells than paternal exercise. With maternal exercise, there appears to be critical points in time when maternal exercises causes epigenetic changes to have an impact on their progeny but the exact details on this still remains unclear.[5] One study reviewed suggested that hypomethylation was maintained during training cessation and with retraining, hypomethylation frequency was enhanced which gives evidence for persistence of epigenetic changes occurring with exercise.[9] This could have implications in inheritance because if these changes persist in an individual, there is a possibility of progeny inheritance. In general, further research regarding the implications of heritable

epigenetic changes occurring from resistance exercise is greatly needed in order to understand the impact exercise can have on progeny.

Although the implications of resistance exercise on producing favorable epigenetic changes in livestock has not been examined, there is great possibility in improving tenderness of meat by involving exercise if the epigenetic changes that occur from it are well established. In a study investigating the impact of progressive-resistance exercise on meat quality, growth, and development in sheep by performing jumping exercises, increased tenderness of meat from subjects was reported but there was no evidence of increased muscle hypertrophy.[19] This is an interesting finding as often the main impact of resistance exercise training is increased muscle hypertrophy, but this could show evidence that epigenetic changes occurring from resistance exercise can have additional implications in meat science, including improvement in tenderness of meat to improve the quality of the product. Further research is needed at this time in order to understand more about the implications of resistance exercise and what epigenetic changes can occur in livestock to improve meat quality.

Conclusion

There is a statistical significance between performing resistance exercise and the occurrence of epigenetic changes in an individual, including DNA methylation and hypomethylation, histone modifications, changes in mRNA levels, and variations in microRNA levels. It is difficult to say if these changes are truly beneficial but many of the genes noted to be altered by these epigenetic changes appear to have a positive impact on individuals, including changes in insulin sensitivity and muscle hypertrophy. Understanding the mechanisms by how these changes occur in skeletal muscle can help with the creation of interventions to help individuals with metabolic diseases, including altering certain metabolites to cause specific epigenetic changes to occur, and to improve athletic performance such as to create more efficient muscle mass gain. There is evidence that epigenetic changes induced by resistance exercise can be heritable to the progeny of an individual which can alter human evolution over time, but this is a developing field of research. In conclusion, further research must be performed in order to understand the mechanisms by which these epigenetic changes occurring from resistance exercise are produced.

Literature Cited:

1. Coffey, Vernon G, and John A Hawley. "The Molecular Bases of Training Adaptation." *Sports Medicine*, vol. 37, no. 9, 2007, pp. 737–763., doi:10.2165/00007256-200737090-00001.

2. Barres, Romain, et al. "Acute Exercise Remodels Promoter Methylation in Human Skeletal Muscle." *Cell Metabolism*, vol. 15, no. 3, 7 Mar. 2012, doi:https://doi.org/10.1016/j.cmet.2012.01.001.

3. Archer, Trevor. "Epigenetic Changes Induced by Exercise." *Journal of Reward Deficiency Syndrome*, vol. 1, no. 2, 19 June 2015, doi:10.17756/jrds.2015-011.

4. Seaborne, R. A., et al. "Methylome of Human Skeletal Muscle after Acute & Chronic Resistance Exercise Training, Detraining & Retraining." *Scientific Data*, vol. 5, 30 Oct. 2018, p. 180213., doi:10.1038/sdata.2018.213.

5. Denham, J. "Exercise and Epigenetic Inheritance of Disease Risk." *Acta Physiologica*, vol. 222, no. 1, 2018, doi:10.1111/apha.12881.

6. Hameed, M., et al. "Expression of IGF-I Splice Variants in Young and Old Human Skeletal Muscle after High Resistance Exercise." *The Journal of Physiology*, vol. 547, no. 1, 19 July 2004, pp. 247–254., doi:10.1111/j.1469-7793.2003.00247.x.

7. Davidsen, Peter K., et al. "High Responders to Resistance Exercise Training Demonstrate Differential Regulation of Skeletal Muscle MicroRNA Expression." *Journal of Applied Physiology*, vol. 110, no. 2, 1 Feb. 2011, pp. 309–317., doi:10.1152/japplphysiol.00901.2010.

8. Sharples, Adam P., et al. "Does Skeletal Muscle Have an 'Epi'-Memory? The Role of Epigenetics in Nutritional Programming, Metabolic Disease, Aging and Exercise." *Aging Cell*, vol. 15, no. 4, 22 Aug. 2016, pp. 603–616., doi:10.1111/acel.12486.

9. Seaborne, Robert A, et al. "Human Skeletal Muscle Possesses an Epigenetic Memory of Hypertrophy." *Scientific Reports*, 30 Jan. 2018, doi:10.1038/s41598-018-20287-3.

10. Drummond, Micah J., et al. "Human Muscle Gene Expression Following Resistance Exercise and Blood Flow Restriction." *Medicine & Science in Sports & Exercise*, vol. 40, no. 4, Apr. 2008, pp. 691–698., doi:10.1249/mss.0b013e318160ff84.

11. Pareja-Galeano, Helios, et al. "Physical Exercise and Epigenetic Modulation: Elucidating Intricate Mechanisms." *Sports Medicine*, vol. 44, no. 4, 8 Jan. 2014, pp. 429–436., doi:10.1007/s40279-013-0138-6.

12. Mcgee, Sean L., et al. "Exercise-Induced Histone Modifications in Human Skeletal Muscle." *The Journal of Physiology*, vol. 587, no. 24, 2 Nov. 2009, pp. 5951–5958., doi:10.1113/jphysiol.2009.181065.

13. Mclean, Carrie S., et al. "Gene and MicroRNA Expression Responses to Exercise; Relationship with Insulin Sensitivity." *Plos One*, vol. 10, no. 5, 18 May 2015, doi:10.1371/journal.pone.0127089.

14. Ntanasis-Stathopoulos, J, et al. “Epigenetic Regulation on Gene Expression Induced by Physical Exercise.” *Journal of Musculoskeletal Neuronal Interaction*, vol. 13, no. 2, 26 Feb. 2013, pp. 133–146.

15. Nielsen, Søren, et al. “Muscle Specific MicroRNAs Are Regulated by Endurance Exercise in Human Skeletal Muscle.” *The Journal of Physiology*, vol. 588, no. 20, 19 Aug. 2010, pp. 4029–4037., doi:10.1113/jphysiol.2010.189860.

16. Nitert, Marloes Dekker, et al. “Impact of an Exercise Intervention on DNA Methylation in Skeletal Muscle From First-Degree Relatives of Patients With Type 2 Diabetes.” *Diabetes*, vol. 61, no. 12, 15 Dec. 2012, pp. 3322–3332., doi:10.2337/db11-1653.

17. Lindholm, Maléne E, et al. “An Integrative Analysis Reveals Coordinated Reprogramming of the Epigenome and the Transcriptome in Human Skeletal Muscle after Training.” *Epigenetics*, vol. 9, no. 12, 7 Dec. 2014, pp. 1557–1569., doi:10.4161/15592294.2014.982445.

18. Mcgee, Sean L, and Mark Hargreaves. “Histone Modifications and Skeletal Muscle Metabolic Gene Expression.” *Clinical and Experimental Pharmacology and Physiology*, vol. 37, no. 3, 23 Feb. 2010, pp. 392–396., doi:10.1111/j.1440-1681.2009.05311.x.

19. Aalhus, J. L., and M. A. Price. “The Effect Of A Progressive-Resistance Exercise On Growth, Development And Meat Quality Of Sheep.” *Canadian Journal of Animal Science*, vol. 70, no. 1, 1990, pp. 89–95., doi:10.4141/cjas90-010.

Appendix

Table A. Acute resistance exercise and the epigenetic changes.

Exercise	Intensity	Frequency	Duration	Epigenetic Change
Single, acute bout of resistance exercise[2]: cycling ergometer with resistance	Incremental exercise until volitional fatigue	One bout	One bout	Whole genome hypomethylation compared to untrained baseline
Single bout of exercise of lower limb resistance exercise[9]	Increasing weight load until subject could no longer complete 10 full repetitions	One bout	One bout	Genome wide DNA methylation
A single bout of resistance training/" exhaustive exercise": cycling[12]	76 ± 2% of VO_{2peak}	One bout	One bout: 60 minutes	-Increase in the amount of *Glut4* receptors, increased H3K36 acetylation -alteration of class IIa HDAC function -activation of multiple class IIa HDAC kinases
One bout of interval training[13]: cycling	4 sets each composed of 8 minutes of cycling at 70% peak heart rate, 2 minutes 90% peak, and 2 minutes rest	One bout	One bout: 48 minutes	Increased *PPAR* genes via upregulation of miR-378
Acute endurance exercise (cycling ergometer with resistance)[15]	At 65% of peak Watt	60 minutes	One bout	Increase in myomiR's mir-1 and mir-133a
Single leg knee extension exercise[6]	80% of individuals one repetition maximum	10 sets of 6 repetitions	One bout	Increase in MGF mRNA in young individuals, not elderly
One acute bout of bilateral knee extension resistance exercise with and	20% of one repetition maximum	One set of 30 repetitions and 3 sets of 15 repetitions	One bout	-increased HIF-1alpha, p21, MyoD, and MuRF 1 mRNA expression

without induced reduced blood flow via pressure cuff placed on legs[10]				-decreased REDD1 and Myostatin mRNA expression
Endurance exercise[5]	To point of exhaustion	Once per day for one week	7 days	-Increased skeletal muscle mitochondria content, *Ppargc1a*, *Tfam*, and *Foxj3* expression -Decrease miR-494 abundance

Table B. Chronic resistance exercise and the epigenetic changes.

Exercise	**Intensity**	**Frequency**	**Duration**	**Epigenetic Change**
Chronic resistance exercise program[9]: lower limb exercises and upper limb exercises	Increasing weight load until subject could no longer complete 10 full repetitions	4 sets of 10 repetitions, 90-120 seconds between sets and about 3 minutes between exercises	60-minute training session, 2 times per week for 7 weeks	Genome wide DNA hypomethylation
Retraining after cessation for 7 weeks from resistance exercise[9]: lower limb exercises	Increasing weight load until subject could no longer complete 10 full repetitions	4 sets of 10 repetitions, 90-120 seconds between sets and about 3 minutes between exercises	60-minute training session, 2 times per week for 7 weeks	Increased frequency of DNA hypomethylation across the genome compared to ending chronic training

Endurance exercise training (cycling ergometer with resistance)[15]	Monday-Pmax test performed, Tuesday 85-91% of Pmax 70-80 minutes, Wednesday at 60-66% Pmax for 60-80 minutes, Thursday 75-81% of Pmax for 75-81 minutes, Friday 55-61% of Pmax for 120-150 minutes	5 times per week	12 weeks	myomiR expression down-regulated with all myomiR's examined (mire-1, mir-133a, mir-133b, mir-206)
Resistance and endurance exercise: one-hour spinning class + one-hour aerobics class [16]	Dependent upon individual	2 sessions per week	6 months	Genome-wide DNA hypomethylation and gene expression with numerous genes related to metabolism: retinol metabolism, calcium signaling pathways, MEF2A, RUNXI, NDUFC2, THADA

Single leg knee extension exercise[17]	One legged max test: 2 minutes at a constant load of 10 or 20 watts (based on measured VO_{2peak}) followed by 3 or 5 watts increase every 30 seconds until exhaustion	4 sessions per week for 45 minutes	3 months	Skeletal muscle transcriptome and altered genome-wide DNA methylation (4919 sites in genome): Oxidative phosphorylation and blood vessel development
Rotating split-body resistance exercise program [7]	80% of one voluntary single repetition maximum	5 days per week	12 weeks	-miR-378 upregulation: correlation positive with muscle mass gains in vivo -differential regulation of skeletal muscle miRNA expression

Table C. Similar epigenetic effects occurring from resistance exercise, divided into categories of DNA methylation, microRNA expression changes, mRNA expression changes, and histone modifications.

DNA Methylation				
Exercise	**Intensity**	**Frequency**	**Duration**	**Epigenetic Change**
Single leg knee extension exercise[17]	One legged max test: 2 minutes at a constant load of 10 or 20 Watts (based on measured VO_{2peak}) followed by 3 or 5 Watts increase every 30 seconds until exhaustion	4 sessions per week for 45 minutes	3 months	Skeletal muscle transcriptome and altered genome-wide DNA methylation (4919 sites in genome): Oxidative phosphorylation and blood vessel development
Single bout of exercise of lower limb resistance exercise[9]	Increasing weight load until subject could no longer complete 10 full repetitions	One bout	One bout	Genome wide DNA methylation
DNA Hypomethylation				
Single, acute bout of resistance exercise[2]: cycling ergometer with resistance	Incremental exercise until volitional fatigue	One bout	One bout	Whole genome hypomethylation compared to untrained baseline
Retraining after cessation for 7 weeks from resistance exercise: Lower limb resistance exercises [9]	Increasing weight load until subject could no longer complete	4 sets of 10 repetitions, 90-120 seconds between sets and about 3 minutes between exercises	60-minute training session, 2 times per week for 7 weeks	Increased frequency of DNA hypomethylation across the genome compared to ending chronic training

	10 full repetitions			
Chronic resistance exercise program[9]: lower limb exercises and upper limb exercises	Increasing weight load until subject could no longer complete 10 full repetitions	4 sets of 10 repetitions, 90-120 seconds between sets and about 3 minutes between exercises	60-minute training session, 2 times per week for 7 weeks	Genome wide DNA hypomethylation
Resistance and endurance exercise: one-hour spinning class + one-hour aerobics class [16]	Dependent upon individual	2 sessions per week	6 months	Genome-wide DNA hypomethylation and gene expression with numerous genes related to metabolism: retinol metabolism, calcium signaling pathways, MEF2A, RUNXI, NDUFC2, THADA
microRNA				
Endurance exercise training (cycling ergometer with resistance)[15]	Monday-Pmax test performed, Tuesday 85-91% of Pmax 70-80 minutes, Wednesday at 60-66% Pmax for 60-80 minutes, Thursday 75-81% of Pmax for 75-81 minutes, Friday 55-61% of	5 times per week	12 weeks	myomiR expression down-regulated with all myomiR's examined (mire-1, mir-133a, mir-133b, mir-206)

	Pmax for 120-150 minutes			
Endurance exercise[5]	To point of exhaustion	Once per day for one week	7 days	Decrease miR-494 abundance
Rotating split-body resistance exercise program [7]	80% of one voluntary single repetition maximum	5 days per week	12 weeks	-miR-378 upregulation: correlation positive with muscle mass gains in vivo -differential regulation of skeletal muscle miRNA expression
One bout of interval training[13]: cycling	4 sets each composed of 8 minutes of cycling at 70% peak heart rate, 2 minutes 90% peak, and 2 minutes rest	One bout	One bout: 48 minutes	Increased *PPAR* genes via upregulation of miR-378
Acute endurance exercise (cycling ergometer with resistance)[15]	At 65% of peak Watt	60 minutes	One bout	Increase in myomiR's mir-1 and mir-133a
mRNA				
Single leg knee extension exercise[6]	80% of individuals one repetition maximum	10 sets of 6 repetitions	One bout	Increase in MGF mRNA in young individuals, not elderly
One acute bout of bilateral knee extension resistance exercise with and without induced reduced blood flow via pressure cuff placed on legs[10]	20% of one repetition maximum	One set of 30 repetitions and 3 sets of 15 repetitions	One bout	-increased HIF-1alpha, p21, MyoD, and MuRF 1 mRNA expression -decreased REDD1 and Myostatin mRNA expression
Histone Modifications				

A single bout of resistance training/" exhaustive exercise": cycling[12]	76 ± 2% of VO_{2peak}	One bout	One bout: 60 minutes	-Increase in the amount of *Glut4* receptors, increased H3K36 acetylation -alteration of class IIa HDAC function -activation of multiple class IIa HDAC kinases

Table D. Epigenetic effects occurring from similar protocols of resistance exercise.

Single Leg Knee Extension				
Exercise	**Intensity**	**Frequency**	**Duration**	**Epigenetic Change**
Single leg knee extension exercise[17]	One legged max test: 2 minutes at a constant load of 10 or 20 Watts (based on measured VO_{2peak}) followed by 3 or 5 Watts increase every 30 seconds until exhaustion	4 sessions per week for 45 minutes	3 months	Skeletal muscle transcriptome and altered genome-wide DNA methylation (4919 sites in genome): Oxidative phosphorylation and blood vessel development
Chronic resistance exercise program[9]: lower limb exercises and upper limb exercises	Increasing weight load until subject could no longer complete 10 full repetitions	4 sets of 10 repetitions, 90-120 seconds between sets and about 3 minutes between exercises	60-minute training session, 2 times per week for 7 weeks	Genome wide DNA hypomethylation
Retraining after cessation for 7 weeks from resistance exercise[9]: lower limb exercises	Increasing weight load until subject could no longer complete 10 full repetitions	4 sets of 10 repetitions, 90-120 seconds between sets and about 3 minutes between exercises	60-minute training session, 2 times per week for 7 weeks	Increased frequency of DNA hypomethylation across the genome compared to ending chronic training
Single leg knee extension exercise[6]	80% of individuals one repetition maximum	10 sets of 6 repetitions	One bout	Increase in MGF mRNA in young individuals, not elderly

Single bout of exercise of lower limb resistance exercise[9]	Increasing weight load until subject could no longer complete 10 full repetitions	One bout	One bout	Genome wide DNA methylation
One acute bout of bilateral knee extension resistance exercise with and without induced reduced blood flow via pressure cuff placed on legs[10]	20% of one repetition maximum	One set of 30 repetitions and 3 sets of 15 repetitions	One bout	-increased HIF-1alpha, p21, MyoD, and MuRF 1 mRNA expression -decreased REDD1 and Myostatin mRNA expression
Cycling with Resistance				
Endurance exercise training (cycling ergometer with resistance)[15]	Monday-Pmax test performed, Tuesday 85-91% of Pmax 70-80 minutes, Wednesday at 60-66% Pmax for 60-80 minutes, Thursday 75-81% of Pmax for 75-81 minutes, Friday 55-61% of Pmax for 120-150 minutes	5 times per week	12 weeks	myomiR expression down-regulated with all myomiR's examined (mire-1, mir-133a, mir-133b, mir-206)
Resistance and endurance exercise: one-hour spinning class + one-hour aerobics class [16]	Dependent upon individual	2 sessions per week	6 months	Genome-wide DNA hypomethylation and gene expression with numerous genes

				related to metabolism: retinol metabolism, calcium signaling pathways, MEF2A, RUNXI, NDUFC2, THADA
A single bout of resistance training/" exhaustive exercise": cycling[12]	76 ± 2% of VO_{2peak}	One bout	One bout: 60 months	-Increase in the amount of *Glut4* receptors, increased H3K36 acetylation -alteration of class IIa HDAC function -activation of multiple class IIa HDAC kinases
One bout of interval training[13]: cycling	4 sets each composed of 8 minutes of cycling at 70% peak heart rate, 2 minutes 90% peak, and 2 minutes rest	One bout	One bout: 48 minutes	Increased *PPAR* genes via upregulation of miR-378
Acute endurance exercise (cycling ergometer with resistance)[15]	At 65% of peak Watt	60 minutes	One bout	Increase in myomiR's mir-1 and mir-133a
Single, acute bout of resistance exercise[2]: cycling ergometer with resistance	Incremental exercise until volitional fatigue	One bout	One bout	Whole genome hypomethylation compared to untrained baseline
Upper and Lower Limb Resistance Exercises				
Rotating split-body resistance exercise program [7]	80% of one voluntary single repetition maximum	5 days per week	12 weeks	-miR-378 upregulation: correlation positive with muscle mass gains in vivo

				-differential regulation of skeletal muscle miRNA expression
Chronic resistance exercise program[9]: lower limb exercises and upper limb exercises	Increasing weight load until subject could no longer complete 10 full repetitions	4 sets of 10 repetitions, 90-120 seconds between sets and about 3 minutes between exercises	60-minute training session, 2 times per week for 7 weeks	Genome wide DNA hypomethylation
Other/Unspecified				
Endurance exercise[5]	To point of exhaustion	Once per day for one week	7 days	-Increased skeletal muscle mitochondria content, *Ppargc1a*, *Tfam*, and *Foxj3* expression -Decrease miR-494 abundance