MTOR SIGNALING PATHWAY ASSOCIATED WITH SKELETAL MUSCLE HYPERTROPHY FOLLOWING RESISTANCE EXERCISE TRAINING.

by

Mark A. Newman

BS, Indiana University of Pennsylvania, 1998

MS, Indiana University of Pennsylvania, 2000

MPH, University of Pittsburgh, 2007

Submitted to the Graduate Faculty of

the School of Education in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

University of Pittsburgh

2008

UNIVERSITY OF PITTSBURGH

SCHOOL OF EDUCATION

This dissertation was presented

by

Mark A. Newman

It was defended on

July 14, 2008

and approved by

Steven E. Riechman, PhD, Assistant Professor

Bret H. Goodpaster, PhD, Associate Professor

Robert J. Robertson, PhD, Professor

Dissertation Advisor: Frederic L. Goss, PhD, Associate Professor

MTOR SIGNALING PATHWAY ASSOCIATED WITH SKELETAL MUSCLE HYPERTROPHY FOLLOWING RESISTANCE EXERCISE TRAINING.

Mark A. Newman, PhD

University of Pittsburgh, 2008

Objective: The purpose of this investigation is to examine the association of genetic variations in 4 single nucleotide polymorphisms (SNPs) of the AKT1 gene and 3 SNPs of the AMPKα2 gene with percent change in lean muscle mass, arm mass-adjusted strength, and leg mass-adjusted strength following a resistance exercise training (RET) program while controlling for the effects of age, gender, and RET history. These genes are part of the mTOR signaling pathway, which has been shown to be an important mediator of protein synthesis in adult animals. Design: Participants were young men and women (age 18-31) from the Molecular Epidemiology of Resistance Exercise Training (MERET) study who completed 10 weeks of RET. Participants trained 3 days per week at 75% of one repetition maximum, performing 3 sets (6-10 repetitions) of 13 resistance exercises. Results: There were no significant associations between the individual AKT1 and AMPKα2 SNPs examined in this investigation to percent changes in lean muscle mass, arm mass-adjusted strength, or leg mass-adjusted strength following RET. However, significant interactions between various SNPS of the AKT1 and AMPKα2 genes and measures of muscle mass and strength were observed. Conclusion: The results of this investigation suggest that future research involving the mTOR signaling pathway and its association to variations in the individual response of skeletal muscle response to standardized RET is warranted.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PREFACE

I would like to thank my committee members for their guidance and support in both the development and review process of this manuscript. I couldn't have found a more dedicated and patient group of professionals with which to work.

First, I would like to thank Dr. Steven Riechman. It was through Dr. Riechman that I became interested in researching the role genetic variation plays in resistance exercise training. His expert knowledge of both exercise physiology and molecular epidemiology guided me throughout the completion of this project.

I would like to thank my advisor, Dr. Frederic Goss. Dr. Goss has always been there to offer me his expertise; advising me on my coursework and throughout the dissertation process. The support he has given me during my time at the University of Pittsburgh allowed me to make the most of my educational experience. Working with him has truly been a pleasure.

In addition, I would like to thank Dr. Robert Farrell for his advice and for allowing me access to his laboratory to complete the genotyping of the participants in my study. I would also like to thank Nancy Petro for her expertise and patience while teaching me genotyping methods.

1.0 INTRODUCTION

Skeletal muscle is the most abundant tissue in the human body, and maintenance of its mass is essential for such basic functions as ambulation, metabolism, and respiration. Resistance exercise, involving either free weights or specifically designed machines, is the most common way to maintain or increase muscle mass. Resistance exercise causes an increase in the recruitment rate of motor units and a marked increase in the load against which the recruited motor units contract (1). The human body adapts to resistance exercise training (RET) by increasing the cross-sectional area of the muscle, this is called hypertrophy (1). The fibers of untrained muscle vary considerably in diameter. In adult human beings, the cross-sectional area of an individual muscle fiber ranges from approximately 2000μm^2 to 7500μm^2 with the mean and median in the 3000-4000μm^2 range (1). RET brings the smaller muscle fibers up to the size of the larger ones (2). Fast-twitch muscle fibers (type II), which are known to supply quick bursts of power but fatigue quickly, typically hypertrophy to a greater extent than slow twitch fibers (type I), which are more suited for endurance activities and fatigue at a much slower rate (3). In a longitudinal study that compared the cross-sectional area of the triceps brachii muscle before and after six months of resistance training, the type II fibers hypertrophied by 33% and the type I fibers by 27%(4). The nervous system also plays a role in the human body's adaptation to RET. The nervous system adapts to RET by increasing the number of motor units being activated, producing an improved recruitment pattern and synchronization of these motor

units, thus more power can be generated due to the increased number of muscle fibers involved (2).

1.1 RET AND PROTEIN SYNTHESIS

Protein synthesis is the process in which cells build protein. Protein synthesis is a multi-step process which begins with amino acid synthesis, followed by transcription and subsequently translation. Amino acid synthesis is a set of biochemical processes which build the amino acids from carbon sources like glucose (5). Transcription is the process in which deoxyribonucleic acid (DNA) is transferred into ribonucleic acid (RNA). RNA then serves as the template for the translation of genes into proteins, transferring the amino acids to the ribosome of the cell where they are assembled into proteins (5).

The primary acute response to resistance exercise is an increase in the rate of protein synthesis. The protein content of skeletal muscle is in a constant state of change. The rates of protein synthesis and degradation change based on the demands placed on the body. Muscular hypertrophy results from an accumulation of proteins, this can happen because of an increased rate of production, a decreased rate of degradation, or a combination of the two (4). During resistance exercise, protein synthesis decreases while protein degradation increases. However, protein synthesis has been shown to increase during the recovery phase of resistance exercise (6). Protein synthesis in response to RET is stimulated by the combination of insulin, along with an increase in amino acid availability. This process in turn stimulates the translation of messenger RNA, leading to protein synthesis (7). Regulation of these translation initiation steps is controlled through the phosphorylation of initiation factors and protein kinases that are

controlled by signaling pathways downstream of the phosphatidylinositol 3-kinase (PI3K), which is a gene that is part of the mTOR signaling pathway (6).

1.2 MTOR SIGNALING PATHWAY AND PROTEIN SYNTHESIS

Modifications in the magnitude, frequency, duration, and intensity of mechanical stress can each cause changes in patterns of gene expression in muscle, influence protein synthesis and stability, and affect muscle metabolism to produce adaptations in muscle mass (8). These adaptations are accomplished through signal transduction by which an extra cellular signal interacts with receptors at the cell surface, activating signaling pathways which act on myofibers by changing gene expression (9). Genes that are part of a particular signaling pathway interact with each other in various ways before a biological outcome is produced. RET is well known to result in marked increases in muscle mass and strength but the responses to a standardized program vary considerably among individuals (10). The reasons for this inter-individual variability are largely unknown. However, the answer may lie in part, among variability in signaling pathways that control protein synthesis.

The mammalian target of rapamycin (mTOR) signaling pathway (Figure 1) has been shown to be an important mediator of protein synthesis and subsequently muscle fiber size in adult animals (11). However, molecular signaling pathways are rarely linear and often have many branch points and multiple places at which signaling events can activate the cascade of reactions. Genes that are part of a signaling pathway stimulate other genes that lie downstream in the pathway by either activating or inhibiting their effects. Under normal conditions, this cascade of reactions produces the biological outcome of the signaling pathway, in the case of the

mTOR signaling pathway this outcome is protein translation and cell growth. However, if any of the genes in the signaling pathway are not functioning properly, the process of signal transduction could be compromised. Genetic variations in the mTOR signaling pathway may cause one individual to respond more favorably to a RET program than another person, environmental conditions being equal.

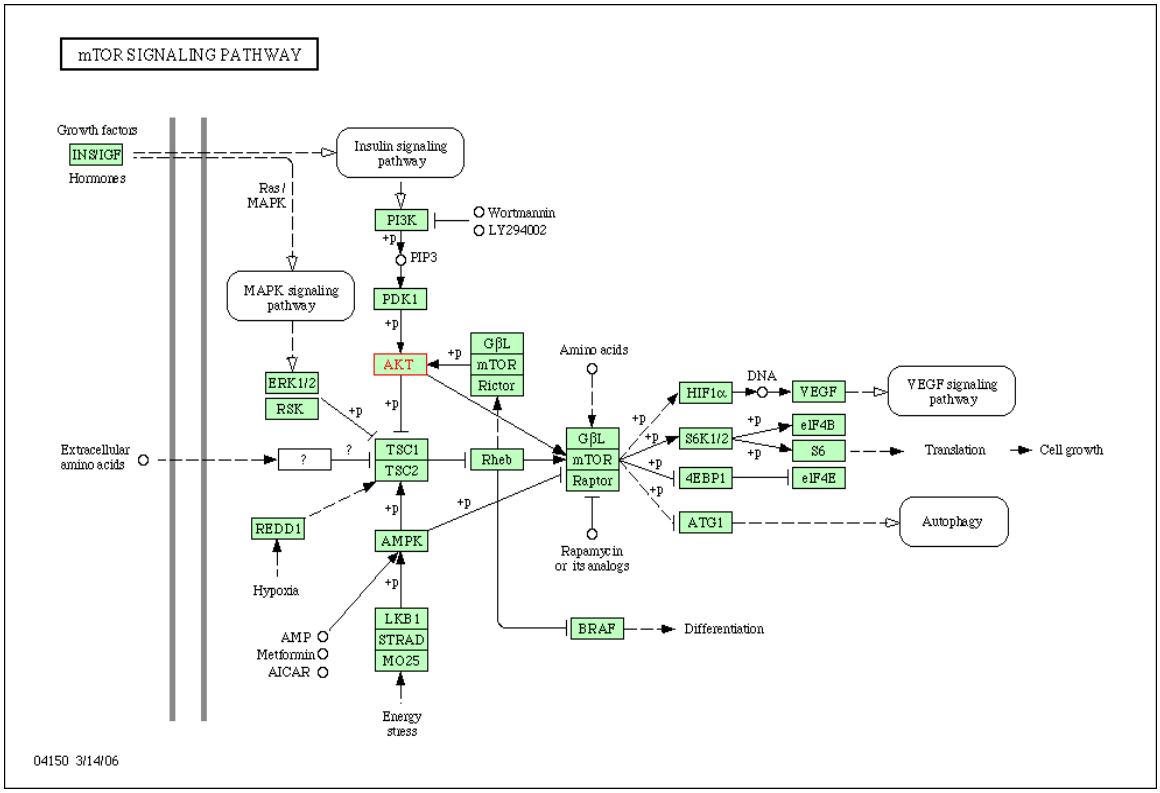

Figure 1 mTOR Signaling Pathway. Genes connected by ---> represent activating steps and genes connected by ---| represent inhibitory steps. Source: National Center for Biotechnology Information.

1.3 SIGNIFICANCE

It is of interest to study the cellular mechanisms behind muscle hypertrophy to understand how muscle mass may be maintained or increased but also to find ways to combat muscle atrophy. Muscle may begin to atrophy as a result of muscle wasting diseases like cancer and AIDS, immobilization due to an orthopedic injury, or the normal aging process. Gaining a better understanding of the signaling pathways involved with muscle hypertrophy will not only increase the knowledge of basic muscle biology but may be the essential step in the discovery of anti-atrophy drugs. Currently, there are no safe, effective, and approved drugs to treat muscle atrophy. The relatively new technique of studying a gene pathway, as opposed to a single gene, will allow for the examination of how particular genetic variations in the mTOR signaling pathway interact to effect muscle mass increases with resistance exercise training.

Genes that are part of the mTOR signaling pathway interact in a cascade of reactions to produce cell growth. Some of the genes in this pathway act on each other by regulating activity. These genes act like a light switch, turning each other on and off as the cascade of reactions goes from beginning to end. Studying several genes in the pathway that are known from previous investigations to interact will allow a more comprehensive view of the process. Variations in these genes could compromise protein synthesis and cell growth, the biological outcomes of the mTOR signaling pathway. These variations could also be the target of interventions designed to treat disease and aging associated muscle atrophy.

1.4 STATEMENT OF THE PROBLEM

The primary purpose of this investigation was to examine the association of genetic variations in selected genes of the mTOR signaling pathway with percent change in lean muscle mass following a RET program while also controlling for any covariance due to gender, age (<19.5 years or >19.5 years) and RET history (0 hours per week, 0.1-1.8 hours per week or >1.8 hours per week during the past year). A secondary analysis examined the association of genetic variations in selected genes of the mTOR signaling pathway with percent changes in arm and leg mass-adjusted strength following an RET program while also controlling for the effects of gender, age, and RET history.

2.0 CONCEPTUAL FRAMEWORK

2.1 GENETIC VARIATION

A single nucleotide polymorphism (SNP) is a DNA sequence variation that occurs when a single nucleotide: adenine (A), thymine (T), cytosine (C), or guanine (G) in the genome differs between individuals. For example, two DNA fragments from different individuals AAGC**C**TA to AAGC**T**TA, contain a difference in a single nucleotide. This combination produces two alleles C and T, almost all common SNPs have two common alleles. An allele is one of any number of viable DNA combinations occupying a locus (position) on a chromosome. An individuals genotype for that gene is the set of alleles it happens to possess (12).

Humans are diploid organisms; that is they have paired homologous chromosomes in their cells, and these contain two copies of each gene. Organisms in which two copies of the gene have the same alleles are homozygous for that gene. In contrast, an organism which has two different alleles for that gene is called heterozygous. Phenotypes are the expressed characteristics associated with a certain allele and can be dominant, recessive, or neither. A dominant phenotype will be expressed when at least one allele of its associated phenotype is present, whereas a recessive phenotype will only be expressed when both alleles are of its associated type (12).

Genetic variations can come in the form of mutations which produce amino acid sequence changes (nonsynonomous), in genes, and those that may not (synonomous). Synonymous mutations change the nucleotide base triplet (codon) produced but do not alter the amino acid encoded. These mutations are usually neutral if they occur in a noncoding region of DNA. However, if they occur in a coding region of DNA they could influence gene expression by altering a DNA sequence that regulates how a particular gene is expressed. Nonsynonymous variations can result in the occurrence of a missense mutation or a nonsense mutation. A missense mutation occurs when a nucleotide substitution results in an incorrect amino acid change. These effects can be minimal if the amino acid substituted is chemically similar to the one specified (12).

If the amino acids are chemically dissimilar, gene function could be compromised. A nonsense mutation is more serious and occurs when a normal codon specifying an amino acid is replaced by a stop codon, which may impact the signaling pathway by prematurely ending protein translation; this may result in a nonfunctional protein. A third type of mutation, called frameshift mutation, occurs when the insertion or deletion of one or more nucleotides results in the disruption of the normal translational reading frame of three nucleotides per codon, to a multiple other than three. This shift can also cause the introduction of a stop codon instead of a normal codon, prematurely ending protein translation and compromising gene function (12). Any of the above mentioned genetic variations could alter the signaling response of a gene, in addition to a signaling pathway, which in the case of mTOR may result in differences among individuals in hypertrophy with RET.

2.2 THE MTOR SIGNALING PATHWAY

In humans, skeletal muscle hypertrophy is characterized by an increase in the size of individual myofibers. Hypertrophy is an adaptation to resistive exercise and is the result of an increase in protein synthesis (10). Previous research has shown that the mTOR signaling pathway plays a role in the control of cell size (11). This process can be explained, in part, by this signaling pathway's role in activating proteins needed for protein synthesis. An increase in protein synthesis allows new contractile filaments to be added to the muscle fiber, thus increasing its size. RET stimulates the expression of insulin-like growth factor 1 (IGF-1) which has been shown to stimulate skeletal muscle hypertrophy by activating the mTOR pathway (13).

The interaction of insulin and growth factors with their receptors leads to the activation of phosphoinositide 3-kinase (PI3K). PI3K is a lipid kinase; its lipid products provide a site for assembling signaling proteins at certain locations in the membrane in response to cell stimulation. PI3K phosphorylates phosphatidylinositol (4,5)-bisphosphate which results in the production of phosphatidylinositol-3,4,5-triphosphate [PtdIns(3,4,5)P_3]. This reaction provides a membrane-binding site for two kinases: AKT, a serine-threonine kinase also known as protein kinase B (PKB) and phosphoinositide-dependent protein kinase (PDK1)(14).

PDK1 plays an important role in the mTOR pathway because it phosphorylates and activates several key genes which are important for cell growth, proliferation, and survival. These genes include AKT, S6 kinase (S6K), p70 S6 kinase ($p70^{s6k}$, RPS6KB2), and p90 S6 kinase (RSK) (15). Song et *al.* (16) observed that in the hypertrophic muscle of transgenic mice over expressing IGF-1, phosphorylation of PDK1 increased by 53%, mTOR by 112% and p70S6K by 254%. These results suggest that over expression of IGF-1 in mice results in hypertrophy, with possible mediation from the mTOR signaling pathway. Variations in PDK1

could affect its ability to activate these downstream components of the pathway, compromising the physiological processes it helps regulate.

In order for the catalytic activity of AKT to begin, it must first be phosphorylated by PDK1. AKT lies at the center of the mTOR pathway and its activation regulates genes involved in protein synthesis while its inactivation allows the expression of genes known to be associated with muscle atrophy, called atrogenes (17). In humans, there are three forms of AKT, (i.e. AKT1-3) encoded by distinct genes, with AKT1 being most active in skeletal muscle (18). The central role that AKT1 plays in skeletal muscle is that of a coordinator of protein synthesis and protein degradation, ensuring that the cell manages its energy utilization efficiently (17). AKT1 phosphorylation and expression are elevated during muscle hypertrophy and reduced during atrophy (11). Further, more direct evidence, comes from a study involving resistance exercise in humans in which AKT1, mTOR, and $p70^{s6k}$ increased significantly in association with protein synthesis 1-2 hours post-exercise (19). Additionally, a 2003 study by Bolster et *al.* (20) found that AKT activation due to exercise was specific to the response generated from RET as opposed to other contractile activity such as endurance exercise training. Despite the compelling evidence of an association of AKT1 to protein synthesis and skeletal muscle hypertrophy, there is currently a lack of studies investigating associations of SNPs in the AKT1 gene with skeletal muscle hypertrophy.

Experiments in *Drosophila melanogaster* have suggested that the inhibition of mTOR or $p70^{s6k}$ was sufficient to promote decreases in cell size (22). The mTOR kinase lies in a protein complex that brings together two major anabolic components for muscle, the branch-chained amino acids and IGFs (23). The function of mTOR in muscle has been examined by utilizing rapamycin, a chemical that binds mTOR (also known as FRAP) and inhibits its function.

Activation of mTOR results in an increase in protein translation by activating $p70^{s6k}$, a positive regulator of protein translation. In addition, mTOR inhibits the activity of eukaryotic translation initiation factor 4E binding protein 1 (4EBP1), a negative regulator of protein initiation factor, eIF-4E (24). When skeletal muscle myotubes were treated with rapamycin, *in vivo,* compensatory hypertrophy was completely blocked and $p70^{s6k}$ activation was inhibited (21).

Recently, research has identified two structurally and functionally distinct mTOR containing complexes (25). The first is the raptor-mTOR complex which mediates cell growth through S6K and 4EBP1. The second and least understood is the rapamycin insensitive rictor-mTOR complex. How the rictor-mTOR complex is regulated is not completely understood but it is believed to facilitate regulation of AKT by PDK1 (26). Thus, mTOR appears to have a very complex and important role in integrating a variety of growth signals, from basic nutritional stimulation to activation by protein growth factors, resulting in protein synthesis.

AMP-activated protein kinase (AMPK) is an important regulator of cellular metabolism and is regulated in muscle during exercise (27). AMPK is a heterotrimeric complex made up of a catalytic subunit (AMPKα) and two regulatory subunits (AMPKβ and AMPKγ) (28). AMPK fits into the mTOR signaling pathway through its relationship to AKT and tuberous sclerosis complex 2 (TSC2). Hahn-Windgrassen *et al* (29) found that AKT inhibits TSC2, a negative regulator of mTOR, and activates mTOR by inhibiting the AMPK mediated phosphorylation of TSC2. Winder *et al.* (30) have implicated AMPK as being responsible for attenuating the anabolic processes that require adenosine tri-phosphate (ATP) and promoting the catabolic processes that generate ATP.

Past research has shown that during an acute bout of resistance exercise, muscle protein synthesis appears to be suppressed, it then increases within 2-3 hours post-exercise and remains

elevated for 24-48 hours (27). A 2006 study by Dreyer *et al* (19), found that a decrease in muscle protein synthesis during resistance exercise was associated with an increase in AMPKα2 (PRKAA2) activity and a decrease in 4E-BP1. Specifically, these authors found AMPKα2 activity increased 75% following an acute bout of resistance exercise and 4E-BP1 activity decreased by 36%, which may have contributed to the 32% reduction in muscle protein synthesis that occurred during resistance exercise in this study. These findings provide a strong link to AMPKα2 activity in skeletal muscle specifically, making it an interesting candidate gene to examine for association to skeletal muscle hypertrophy and strength gains following RET.

Currently there is a lack of research on how specific SNPs in the AMPK gene may be associated with skeletal muscle phenotypes, specifically skeletal muscle hypertrophy and strength gains following RET. Therefore it is necessary to look at other investigations involving SNPs in the AMPK gene which may be associated with biological processes that could contribute to protein synthesis, resulting in skeletal muscle hypertrophy. Spencer-Jones et *al*. (31) hypothesized that genetic variation in the AMPKα2 gene is associated with the regulation of lipid profile, affecting body fat deposition and insulin sensitivity. These authors found five common SNPs in the AMPKα2 gene (rs1124900, rs2796516, rs2746342, rs2796498, and rs1418442) which were significantly associated with lower total cholesterol and lower low-density lipoprotein cholesterol (LDL). In addition, these authors also found that one AMPKα2 haplotype was associated with lower total cholesterol and lower LDL cholesterol. A haplotype is a set of SNPs on a single chromatid, or one of two strands of DNA that make up a chromosome.

If AMPKα2 is indeed associated with the regulation of cholesterol, it is possible that it may also be associated with the regulation of the anabolic hormone testosterone. Cholesterol is the major precursor for the synthesis of various steroid hormones, including testosterone. The

anabolic effects of testosterone include the growth of muscle mass, increased strength and greater bone density. If increased AMPKα2 activity is associated with lower total and LDL cholesterol then it is possible that less substrate is available for the production of testosterone under conditions when AMPKα2 activity is stimulated, for instance during RET. A reduction in the amount of testosterone produced by the body could be associated with a reduced potential for skeletal muscle growth achieved following RET. Although these SNPs were associated with lower total and LDL cholesterol both individually and as part of a protective haplotype showing lower total and LDL cholesterol, their association with muscle hypertrophy and strength following RET has yet to be determined. In addition rs2796516 was also shown to be significantly associated with obesity-related variables of total fat (%), central abdominal fat (kg), and central fat (%).

The above mentioned genes are all part of the mTOR signaling pathway. This complex set of reactions follows a signaling pathway which begins with IGF-1 and ends in protein translation, resulting in skeletal muscle hypertrophy. RET produces the stimulus to trigger IGF-1 and the subsequent cascade of reactions. Genetic variations in any of the above genes could result in variation in the signaling efficacy of this pathway.

2.3 MERET STUDY

The Molecular Epidemiology of Resistance Exercise Training (MERET) study was designed to examine environmental and genetic contributions to variable muscle responses in young men and women. An investigation using the MERET dataset examined the effects of steroid sulfatase (STS) gene variation on circulating levels of the adrenal steroid dehydroepiandrosterone

(DHEA) and its sulfated conjugate DHEAS following RET (32). Results showed that RET resulted in significant gains in strength, weight, lean muscle mass and body mass index (BMI) in both men and women; as well as a significant decrease in fat percent. Results also showed that participants with the STS "G" allele exhibited greater acute changes in DHEA and the ratio of DHEA:DHEAS than those participants with only an STS "A" allele. These findings suggest that RET induced STS activation can be influenced by genetic variations in the STS gene.

In another study using the MERET dataset, Riechman and colleagues (33) examined the association of interleukin-15 (IL-15) protein and IL-15 receptor-α (IL15RA) genetic variation with RET responses. The IL15RA-Pst1 genotype was associated with significantly greater increases in lean muscle mass as well as arm and leg circumference in those participants with an A allele. IL-15 was also shown to be an important mediator in the variability of muscle mass gains in response to RET. More specifically, single nucleotide polymorphisms in exons 4 and 7 of the IL-15RA gene accounted for a significant proportion of this variability. Additionally, a polymorphism in exon 4 of the IL15RA gene was independently associated with muscle hypertrophy following RET. The above mentioned results suggest that genetic variation is associated with variations in muscle mass following RET. Therefore, the purpose of the present investigation is to utilize the MERET dataset to examine possible genetic variations in selected genes of the mTOR signaling pathway that could be responsible for the individual variations in muscle mass gain following a RET program.

2.4 COMPONENTS OF A RET PROGRAM

The American College of Sports Medicine (ACSM) recommends that RET be performed in an individualized, progressive manner in order to achieve optimal results (34). The ACSM defines progression in terms of RET as "the act of moving forward or advancing toward a specific goal." The optimal characteristics of strength-specific programs include both the use of concentric and eccentric muscle actions and the performance of both single and multiple joint exercises. The trainable characteristics that can be developed as part of an RET program are muscular strength, muscular hypertrophy, muscular power, and muscular endurance. Based on the goals of the participant, RET programs can be individualized to focus on any one, or a combination of, those trainable characteristics.

Progressive overload is the gradual increase in load placed on the body during exercise training. During RET overload may be introduced by increasing the resistance, increasing the number of repetitions performed, altering the speed at which repetitions are performed, or by increasing the total volume of work performed, in any combination. RET programs should be tailored to meet specific training goals. Physiological adaptations to training are specific to the muscle actions involved (34), speed of movement (34), range of motion (35), muscle groups trained (36), energy systems involved (37), and the intensity and volume of training (38, 39). RET programs should also change over time in order for the training stimulus to remain optimal. Previous research has shown that periodically varying the volume and intensity of a RET program is the most effective for long-term progression (40).

2.5 RET AND TRAINING HISTORY

Training history plays an important role in the rate of progression during RET. The rate of strength increase varies considerably between trained and untrained individuals. Trained individuals have much slower rates of improvement (41). Previous research has shown muscular strength increases approximately 40% in untrained, 20% in moderately trained, 16% in trained, 10% in advanced, and 2% in elite individuals in periods ranging from 4 weeks to 2 years. Trained individuals are defined as having approximately six months of consistent RET experience. Advanced individuals are defined as those with years of RET experience who also experienced significant improvements in muscular strength. Elite individuals are athletes who are highly trained and have achieved a high level of competition (34). Previous investigations have shown that changes in muscular strength are most prevalent early in training (42, 43). Studies using an 11-16 week training period have shown the majority of strength increases occurred during the first 4-8 weeks (44, 45). A study employing a 12 month training period showed similar results (43). It is for this reason RET history (0 hours per week, 0.1-1.8 hours per week or >1.8 hours per week during the past year) was chosen as a covariate in this study's analysis of percent change in lean muscle mass, arm muscle quality, and leg muscle quality following RET.

2.6 RET AND HYPERTROPHY

Numerous types of RET programs have been shown to stimulate muscle hypertrophy in men and women (46). RET programs targeting muscle hypertrophy utilize moderate to heavy loads, are

typically high in volume and employ shorter rest periods (47). These programs have been successful because they have been shown to acutely increase testosterone and growth hormone levels to a greater degree than high-load, low-volume programs (48). Traditional strength training programs which utilize high-load, low-repetition training, and longer rest periods are used to optimize strength gains whereas RET programs that utilize a high-load, high-volume, and shorter rest periods have been shown to maximize hypertrophy (49). The ACSM recommends a program which incorporates both concentric and eccentric muscle actions using moderate loads (70-85% of 1 repetition maximum) for 8-12 repetitions per set for 1-3 sets per exercise. The ACSM also recommends utilizing both single and multiple joint exercises with 1-2 minutes of rest between sets. RET frequency should be 2-3 days per week (34).

2.7 RET AND GENDER

Men have greater muscle size and strength than women, primarily due to greater body size and higher levels of anabolic hormones (49). Previous research comparing the effect of RET on muscle development in men and women have concluded that despite similar relative increases in strength, absolute muscle hypertrophy resulting form RET has been higher in men than women (47). Cureton et al. (47) found that after 16 weeks of heavy resistance training percent increases in strength were greater than percent changes in muscle cross-sectional area (CSA) in both men and women, suggesting that strength improvements resulted from a combination of hypertrophic and neural adaptations regardless of gender. Among participants in this study, the relative changes in CSA were similar in men and women, although the absolute changes tended to be higher in men (46).

2.8 RET AND BODY COMPOSITION

RET has a favorable impact on body composition by increasing fat free mass and decreasing fat mass (1). Fat free mass is the body's fat free weight and includes the skeleton, water, muscle, connective tissue, organ tissues and teeth. The ideal body fat percentages are generally between 16% and 25% for women and under 20% for men (1). RET is also an integral part of weight management programs as it increases lean muscle mass. Lean mass is the most important component of resting metabolic rate (RMR); a higher RMR allows a person to expend more energy at rest (1). RMR decreases with age as the percentage of fat increases and lean body mass decreases. RET can help slow the age associated decrease in lean body mass by helping to preserve muscle mass (1).

2.9 RET AND AGING

Previous investigations involving the MERET cohort have noted significant differences in percent change in lean muscle mass, percent change in arm muscle quality, and percent change in leg muscle quality following RET among those participants who are over the age of 19.5 years as opposed to those participants who were under 19.5 years of age (32, 33). While not fully understood, developmental aging may be a possible explanation for the age–related differences observed in the muscle phenotype responses to RET in the MERET cohort. Specifically, there could be a variance in the circulating hormone levels of those MERET participants aged 18-19.5 years versus those MERET participants over the age of 19.5 years. Hormone levels, specifically androgens, can have a direct effect on one's ability to build lean muscle mass following RET. It

is for this reason, age (<19.5, >19.5 years) will be used as a covariate in all analyses preformed in this investigation.

3.0 METHODS

3.1 PARTICIPANTS

154 participants completed 10 weeks of RET (76 men and 78 women). Eligibility was based upon: age between 18 and 31 years, no medical history for disorders involving the cardiovascular, endocrine, or skeletal muscle systems, less than three hours RET activity per week in the past year, normal caloric consumption and no report of taking anabolic steroids or other putative performance enhancing supplements. Medical history was assessed by questionnaire (Appendix A) and RET history was assessed using the Modifiable Activity Questionnaire (Appendix B). Participants were instructed not to perform RET activity outside of the supervised program and to maintain all other non-RET activities at the same level. After all procedures were explained, subjects gave written informed consent prior to participation in the study under protocols approved by the University of Pittsburgh Institutional Review Board (Informed Consent in Appendix C).

3.2 PROTOCOL

Three familiarization-training sessions were conducted prior to the initiation of the program to orientate the participants to the equipment and instruct them on how to perform each exercise

properly. After these familiarization sessions, one repetition maximums (1 RMs) were determined following a warm-up that included three-minutes of exercise on either a treadmill or cycle ergometer and static stretching of the shoulders, chest, back, quadriceps, hamstrings and calves. Prior to a 1 RM attempt, subjects performed three repetitions at a weight equivalent to 50% of the estimated 1 RM. The weight was then increased to 90% of the estimated 1 RM, and 1 repetition was performed. After 60 seconds of rest, the weight was increased to 100% of the estimated 1 RM. Thereafter, successful attempts were followed by attempts made with a higher resistance in a manner that minimized the total number of attempts required before the 1 RM value was obtained.

This procedure was performed on all exercise machines used for the RET program in the same order for all participants. The order in which 1 RM exercises were completed was: chest press, seated row, lat pull downs, leg extensions, triceps extension, arm curl, shoulder press, hamstring curl, low back extension, abdominal crunch and incline press. Leg press and calf raises were performed on a Universal leg press machine. All assessments of 1 RM were completed within 4 days of starting and 4 days of completing the RET program.

3.3 BODY COMPOSITION

Baseline and post-RET measures were obtained for the following: height (wall-mounted stadiometer), weight (Detecto Clinical scale), body composition by hydrostatic weighing (50), and circumference measurements of arms and thighs. Residual lung volume was estimated prior to the hydrostatic weighing assessments according to tables derived from formulas based on the participant's height and age (51, 52). Participants wore a bathing suit during these assessments

which were administered within 2-4 days of starting or completing the RET program. Trials were performed until three valid underwater weight measurements were achieved. Valid measurements were defined as those that were within the margin of error of the computer software program. The mean of these three underwater weight measurements was then determined and percent body fat was calculated according to the formula of Siri (50).

Arm circumference was measured on the right side of the body at the middle of the bicep with the arm relaxed at the side of the body. Thigh circumference was measured on the right side of the body at the midpoint of the femur with the participant in the standing position. All circumferences were measured using a Gullick measuring tape. Duplicate measures were made of each circumference and recorded to the nearest 0.1 cm, an average of these two measures was then calculated. All measurements were recorded on a data collection form (Appendix D).

3.4 RET PROGRAM

The ten-week, three sessions per week RET program included 13 exercises encompassing all major muscle groups with weights set at 80% of 1 RM. These sessions were supervised by exercise physiologists (participant to trainer ratio 2:1 to 3:1). The RET program included the following exercises performed in the following order on the Strive^{TM} (Canonsburg, PA) weight machines: chest press, seated row, lat pull downs, leg extensions, triceps extension, arm curl, shoulder press, hamstring curl, low back extension, abdominal crunch and incline press. Leg press and calf raises were performed on a Universal leg press machine.

A warm up consisting of exercise on either a treadmill or stationary bike for five minutes followed by stretching of all major muscle groups was performed before each day of RET. The

stretches included the shoulders, chest, back, quadriceps, hamstrings and calves. Participants performed 3 sets of 6-10 repetitions for each exercise. Participants were given thirty seconds of rest between each set and one minute between exercises. Workout charts for recording resistance and number of repetitions completed for each weight machine were given to each participant. In order to maintain the relative difficulty at the same level for all participants throughout the study, resistance settings were increased when participants were able to complete 10 repetitions on a particular set.

3.5 GENE SELECTION

Appendix E contains all genes from the mTOR pathway for which SNPs, with at least two known genotypes with a frequency over 15%, were available using the publicly available database *hapmap.org*. SNPs are listed by reference SNP number (rs), genotype frequency from reference homozygotes, heterozygotes and other homozygotes, as well as allele frequency data for the reference allele and other allele. This reference data was taken from Utah residents with ancestry from northern and western Europe which best reflects most MERET subjects.

While the current literature shows how the genes of the mTOR signaling pathway may influence skeletal muscle hypertrophy, the literature is lacking on which particular SNPs may influence this outcome, either positively or negatively, following RET. Therefore it was necessary to choose those genes, which based on the current literature, have the highest potential to be associated with skeletal muscle hypertrophy and strength gains following RET. It was then necessary to choose SNPs from those genes which are common in most individuals based on the

current literature and the genotype and allele frequency data presented in Appendix F and may have the highest potential to be associated with skeletal muscle strength and hypertrophy.

When examining the mTOR signaling pathway for potential candidate genes for this study of genetic variability in muscle response to RET, the literature made an especially strong case for several genes; mTOR, $p70^{s6k}$, PDPK1, AKT1, and AMPKα2. The mTOR gene is an interesting candidate because its inhibition has been linked to decreases in cell size (22). Furthermore, mTOR activates $p70^{s6k}$, a positive activator of protein translation (21). The mTOR gene was not chosen for this investigation due to its large size, it has 23 known SNPs and the mTOR containing complexes RICTOR and RAPTOR have 14 and 63 respectively (Appendix E). Since mTOR was not chosen to be examined in this investigation $p70^{s6k}$ was disqualified as well, due to the fact that the interaction between these two genes could not be evaluated.

PDPK1 was another very interesting candidate gene considered for this study. PDPK1 has a vital role in the mTOR signaling pathway as it phosphorylates several genes related to cell growth and proliferation; AKT1, S6K, $p70^{s6k}$, and RSK (15). It is quite possible that any variations in this gene could affect the efficacy of the mTOR signaling pathway. The reason PDPK1 was not chosen for this study was that it has only two known SNPs that lie in very close proximity to one another on the gene, which makes it difficult to get a true representation of their affect on the function of this gene (Appendix E). Therefore, AKT1 and AMPKα2 were chosen to be examined for possible association to lean muscle mass and muscle strength gains following RET in the MERET cohort.

3.5.1 AKT 1

AKT1 was selected for this investigation because of the important role this gene appears to play among the intracellular signaling mechanisms controlling protein synthesis, which leads to skeletal muscle hypertrophy (11, 13, 21). In the present investigation, four common SNPs that span the AKT1 gene will be examined for possible associations to skeletal muscle hypertrophy and strength gains following RET in the MERET study. Table 1 contains the AKT1 SNPs selected for this investigation. SNPs are listed by reference SNP number (rs), genotype frequency from reference homozygotes, heterozygotes and other homozygotes, as well as allele frequency data for the reference allele and other allele as found in the genetic database *hapmap.org*. This reference dataset was obtained from Utah residents with ancestry from northern and western Europe which best reflects most MERET subjects.

3.5.2 AMPKα2

The AMPKα2 gene was selected for this investigation because of the interactions it has with the AKT1 gene in the mTOR signaling pathway and the vital role it plays as a key regulator of whole body metabolism (27). For the purposes of this investigation, the possible association of three common AMPKα2 SNPs (rs1124900, rs2796516 and rs1418442) with muscle hypertrophy and strength gains following RET in the MERET study will be examined. Table 1 contains the AMPKα2 SNPs selected for this investigation. SNPs are listed by reference SNP number (rs), genotype frequency from reference homozygotes, heterozygotes and other homozygotes, as well as allele frequency data for the reference allele and other allele as found in the genetic database

hapmap.org. This reference dataset was obtained from Utah residents with ancestry from northern and western Europe which best reflects most MERET subjects.

Table 1 Genes and SNPs of the mTOR Signaling Pathway selected for this investigation

Gene	SNP RS #	Reference-Homozygote	Reference-Heterozygote	Other-Heterozygote	Reference-Allele	Other-Allele
AKT 1	rs2494735	A/A (0.414)	A/G (0.517)	G/G (0.069)	A (0.672)	G (0.328)
	rs1130233	G/G (0.677)	A/G (0.258)	A/A (0.065)	G (0.806)	A (0.194)
	rs3001371	C/C (0.467)	C/T (0.417)	T/T (0.117)	C (0.675)	T (0.325)
	rs1130214	G/G (0.517)	G/T (0.417)	T/T (0.067)	G (0.725)	T (0.275)
AMPK α 2	rs1124900	T/T (0.300)	G/T (0.533)	G/G (0.167)	T (0.567)	G (0.433)
	rs2796516	G/G (0.737)	A/G (0.193)	A/A (0.070)	G (0.833)	A (0.167)
	rs1418442	A/A (0.367)	A/G (0.500)	G/G (0.133)	A (0.617)	G (0.383)

3.6 GENETIC ANALYSIS

Blood samples were collected in 10-ml EDTA anticoagulant vials (Vacutainer) immediately before and after the first and last RET sessions. Samples were immediately placed on ice and were subsequently centrifuged at 1,200 *g* for 25 minutes. Plasma was collected and stored at -70°C in 1-ml aliquots. Genomic DNA was isolated from the white blood cells of these samples using standard methods (53). DNA fragments surrounding the chosen SNPs of the AKT1 and AMPKα2 genes were amplified using standard polymerase chain reaction (PCR) methods. Detection of each participant's genetic variation was conducted using either restriction fragment length polymorphism analysis (RFLP), or fluorescence polarization (FP), according to the nature of the specific genetic variation. A Peltier PTC-100 Thermal Cycler (MJ Research, Waltham, MA) was used for all PCR and FP reactions requiring the use of a thermal-cycler.

3.6.1 rs2494735 (AKT1)

Amplification of each participant's genomic DNA was performed using a PCR reaction. The 25-µl per sample final reaction volume containing 1.3µl of DNA consisted of 2.5µl of 10x PCR buffer, 0.75µl $MgCl_2$, 4.0µl deoxyribonucleotide triphosphate (DNTP) mixture, 0.375µl of forward primer: gttcctgctgagttaggg, 0.375µl of reverse primer: ctccgagtcaggtagtc, 0.1µl of *Taq* DNA polymerase, and 16.9µl of dH_2O. The reaction mixture was held in the thermal-cycler at 95°C for 5 minutes followed by 35 cycles of 95°C for 30 seconds, 54°C for 15 seconds and 72°C for 30 seconds, followed by 72°C for 5 minutes. FP was then used to determine each participant's genotype using the following procedures. The first step of FP involves the removal of unincorporated primers and dNTPs from the PCR product and was accomplished in the following manner. Following the PCR reaction, 10µl of an enzymatic mixture containing 1µl of shrimp alkaline phosphatase, 1µl of shrimp alkaline phosphatase buffer, 0.1µl of *Escherichia coli* exonuclease I, and 7.9µl of dH_2O was added to 10-µl PCR product. The mixture was incubated in the thermal-cycler at 37°C for 90 minutes before the enzymes were heat inactivated at 95°C for 15 minutes.

The third step of FP is a single base extension reaction and was accomplished in the following manner. The samples from the previous step were added to a 10µl cocktail containing 1µl of thermosequanase buffer, 1µl of internal primer: cttccacctgtcccggga, 0.05µl of allele-specific dye-labeled dideoxy-NTP, 0.1µl of thermosequanase, and 7.85µl of dH_2O. These samples were then incubated in the thermal-cycler for 94°C for 1 minute followed by 40 cycles of 94°C for 10 seconds and 52°C for 30 seconds then at 72°C for 10 minutes. FP measurement was then performed on a LJL Analyst HT fluorescence reader (LJL Biosystems, Sunnyvale, CA) controlled by LJL Criterion software. The average FP value was then compared with the

negative control samples using AlleleCaller software (LJL Biosystems). Subjects were then genotyped for the SNP rs2494735 (AKT1) as either AA, AG, or GG.

3.6.2 rs1130233 (AKT1)

Amplification of each participant's genomic DNA was performed using a PCR reaction. The 25-µl per sample final reaction volume containing 1.3µl of DNA consisted of 2.5µl of 10x PCR buffer, 0.75µl $MgCl_2$, 4.0µl deoxyribonucleotide triphosphate (DNTP) mixture, 0.375µl of forward primer: caccttcatcatccgctg, 0.375µl of reverse primer: gactgggccagtttccaa, 0.1µl of *Taq* DNA polymerase, and 16.9µl of dH_2O. The reaction mixture was held in the thermal-cycler at 95°C for 5 minutes followed by 35 cycles of 95°C for 30 seconds, 54°C for 15 seconds and 72°C for 30 seconds, followed by 72°C for 5 minutes. FP was then used to determine each participant's genotype using the following procedures. Following the PCR reaction, 10µl of an enzymatic mixture containing 1µl of shrimp alkaline phosphatase, 1µl of shrimp alkaline phosphatase buffer, 0.1µl of *Escherichia coli* exonuclease I, and 7.9µl of dH_2O was added to 10-µl PCR product. The mixture was incubated in the thermal-cycler at 37°C for 90 minutes before the enzymes were heat inactivated at 95°C for 15 minutes. The samples from the previous step were added to a 10µl cocktail containing 1µl of thermosequanase buffer, 1µl of internal primer: ggcggccaggccaggc, 0.05µl of allele-specific dye-labeled dideoxy-NTP, 0.1µl of thermosequanase, and 7.85µl of dH_2O. These samples were then incubated in the thermal-cycler for 94°C for 1 minute followed by 40 cycles of 94°C for 10 seconds and 52°C for 30 seconds then at 72°C for 10 minutes. FP measurement was then performed on a LJL Analyst HT fluorescence reader (LJL Biosystems, Sunnyvale, CA) controlled by LJL Criterion software. The average FP value was then compared with the negative control samples using AlleleCaller

software (LJL Biosystems). Subjects were then genotyped for the SNP rs1130233 (AKT1) as either GG, AG, or AA.

3.6.3 rs3001371 (AKT1)

Amplification of each participant's genomic DNA was performed using a PCR reaction. The 25-µl per sample final reaction volume containing 1.3µl of DNA consisted of 2.5µl of 10x PCR buffer, 0.75µl $MgCl_2$, 4.0µl deoxyribonucleotide triphosphate (DNTP) mixture, 0.375µl of forward primer: atgttggcaggctagtctcaactc, 0.375µl of reverse primer: gtgctcccaggccctcctcag, 0.1µl of *Taq* DNA polymerase, and 16.9µl of dH_2O. The reaction mixture was held in the thermal-cycler at 95°C for 5 minutes followed by 35 cycles of 95°C for 30 seconds, 56°C for 15 seconds and 72°C for 30 seconds, followed by 72°C for 5 minutes. .FP was then used to determine each participant's genotype using the following procedures. Following the PCR reaction, 10µl of an enzymatic mixture containing 1µl of shrimp alkaline phosphatase, 1µl of shrimp alkaline phosphatase buffer, 0.1µl of *Escherichia coli* exonuclease I, and 7.9µl of dH_2O was added to 10-µl PCR product. The mixture was incubated in the thermal-cycler at 37°C for 90 minutes before the enzymes were heat inactivated at 95°C for 15 minutes. The samples from the previous step were added to a 10µl cocktail containing 1µl of thermosequanase buffer, 1µl of internal primer: cagcctcagtttccccacccaaaacccca, 0.05µl of allele-specific dye-labeled dideoxy-NTP, 0.1µl of thermosequanase, and 7.85µl of dH_2O. These samples were then incubated in the thermal-cycler at 94°C for 1 minute followed by 40 cycles of 94°C for 10 seconds and 52°C for 30 seconds then at 72°C for 10 minutes. FP measurement was then performed on a LJL Analyst HT fluorescence reader (LJL Biosystems, Sunnyvale, CA) controlled by LJL Criterion software. The average FP value was then compared with the negative control samples using AlleleCaller

software (LJL Biosystems). Subjects were then genotyped for the SNP rs3001371 (AKT1) as either CC, CT, or TT.

3.6.4 rs1130214 (AKT1)

Amplification of each participant's genomic DNA was performed using a PCR reaction. The 25-µl per sample final reaction volume containing 1.3µl of DNA consisted of 2.5µl of 10x PCR buffer, 0.75µl $MgCl_2$, 4.0µl deoxyribonucleotide triphosphate (DNTP) mixture, 0.375µl of forward primer: tgctcctcactgacggacttgtct, 0.375µl of reverse primer: cat gaggaagacaggaccagg atg, 0.1µl of *Taq* DNA polymerase, and 16.9µl of dH_2O. The reaction mixture was held in the thermal-cycler at 95°C for 5 minutes followed by 35 cycles of 95°C for 30 seconds, 58°C for 15 seconds and 72°C for 30 seconds, followed by 72°C for 5 minutes. RFLP analysis was used to determine each participant's genotype using the following procedures. Amplified PCR-product (15µl) was added to 5µl cocktail that contained 1µl of the restriction enzyme XcmI, 2µl of NE Buffer and 2µl of dH_2O; then incubated overnight at 37°C. Each digested sample was then loaded onto a 2.0% agarose gel containing ethidium bromide and electrophoresed for 2 hours at 120 volts. After electrophoresis, the DNA fragments were visualized by ultraviolent illumination (Eagle Eye), and fragment sizes were estimated by comparison to a 1-kb ladder run on the same gel. The presence of a polymorphic restriction site (XcmI) at the rs1130214 (AKT1) locus is specified as G base, whereas the absence of this site is a T base. Participants were then categorized as exhibiting GG, GT, or TT.

3.6.5 rs1124900 (AMPKα2)

Amplification of each participant's genomic DNA was performed using a PCR reaction. The 25-µl per sample final reaction volume containing 1.3µl of DNA consisted of 2.5µl of 10x PCR buffer, 0.75µl $MgCl_2$, 4.0µl deoxyribonucleotide triphosphate (DNTP) mixture, 0.375µl of forward primer: cagactgcctacccagca ttt ct, 0.375µl of reverse primer: gaggatttgaggctgaggaggtc, 0.1µl of *Taq* DNA polymerase, and 16.9µl of dH_2O. The reaction mixture was held in the thermal-cycler at 95°C for 5 minutes followed by 35 cycles of 95°C for 30 seconds, 50°C for 15 seconds and 72°C for 30 seconds, followed by 72°C for 5 minutes. FP was then used to determine each participant's genotype using the following procedures. Following the PCR reaction, 10µl of an enzymatic mixture containing 1µl of shrimp alkaline phosphatase, 1µl of shrimp alkaline phosphatase buffer, 0.1µl of *Escherichia coli* exonuclease I, and 7.9µl of dH_2O was added to 10-µl PCR product. The mixture was incubated at 37°C for 90 minutes before the enzymes were heat inactivated at 95°C for 15 minutes. The samples from the previous step were added to a 10µl cocktail containing 1µl of thermosequanase buffer, 1µl of internal primer: att ctg gagagaaggcaggttaattaaatc, 0.05µl of allele-specific dye-labeled dideoxy-NTP, 0.1µl of thermosequanase, and 7.85µl of dH_2O. These samples were then incubated in the thermal-cycler at 94°C for 1 minute followed by 40 cycles of 94°C for 10 seconds and 52°C for 30 seconds then at 72°C for 10 minutes. FP measurement was then performed on a LJL Analyst HT fluorescence reader (LJL Biosystems, Sunnyvale, CA) controlled by LJL Criterion software. The average FP value was then compared with the negative control samples using AlleleCaller software (LJL Biosystems). Subjects were then genotyped for the SNP rs3001371 (AKT1) as either TT, GT, or GG.

3.6.6 rs2796516 (AMPKα2)

Amplification of each participant's genomic DNA was performed using a PCR reaction. The 25-µl per sample final reaction volume containing 1.3µl of DNA consisted of 2.5µl of 10x PCR buffer, 0.75µl $MgCl_2$, 4.0µl deoxyribonucleotide triphosphate (DNTP) mixture, 0.375µl of forward primer: ctgttggttataatacttgggagtg, 0.375µl of reverse primer: aggtgctcaatatcatgggtcatc, 0.1µl of *Taq* DNA polymerase, and 16.9µl of dH_2O. The reaction mixture was held in the thermal-cycler at 95°C for 5 minutes followed by 35 cycles of 95°C for 30 seconds, 48°C for 15 seconds and 72°C for 30 seconds, followed by 72°C for 5 minutes. RFLP analysis was used to determine each participant's genotype using the following procedures. Amplified PCR-product (15µl) was added to 5µl cocktail that contained 1µl of the restriction enzyme MnlI, 2µl of NE Buffer and 2µl of dH_2O; then incubated overnight at 37°C. Each digested sample was then loaded onto a 2.0% Agarose gel containing ethidium bromide and electrophoresed for 2 hours at 120 volts. After electrophoresis, the DNA fragments were visualized by ultraviolent illumination (Eagle Eye), and fragment sizes were estimated by comparison to a 1-kb ladder run on the same gel. The presence of a polymorphic restriction site (MnlI) at the rs2796516 (PRKAA2) locus is specified as G base, whereas the absence of this site is a A base. Participants were then categorized as exhibiting GG, AG, or AA.

3.6.7 rs1418442 (AMPKα2)

Amplification of each participant's genomic DNA was performed using a PCR reaction. The 25-µl per sample final reaction volume containing 1.3µl of DNA consisted of 2.5µl of 10x PCR buffer, 0.75µl $MgCl_2$, 4.0µl deoxyribonucleotide triphosphate (DNTP) mixture, 0.375µl of

forward primer: gcccatctggtttctaattacag, 0.375µl of reverse primer: tcaagtcctggatcctccatatatc, 0.1µl of *Taq* DNA polymerase, and 16.9µl of dH_2O. The reaction mixture was held in the thermal-cycler at 95°C for 5 minutes followed by 35 cycles of 95°C for 30 seconds, 48°C for 15 seconds and 72°C for 30 seconds, followed by 72°C for 5 minutes. RFLP analysis was used to determine each participant's genotype using the following procedures. Amplified PCR-product (15µl) was added to 5µl cocktail that contained 1µl of the restriction enzyme SfaNI, 2µl of NE Buffer and 2µl of dH_2O; then incubated overnight at 37°C. Each digested sample was then loaded onto a 2.0% Agarose gel containing ethidium bromide and electrophoresed for 2 hours at 120 volts. After electrophoresis, the DNA fragments were visualized by ultraviolent illumination (Eagle Eye), and fragment sizes were estimated by comparison to a 1-kb ladder run on the same gel. The presence of a polymorphic restriction site (SfaNI) at the rs1418442 (PRKAA2) locus is specified as A base, whereas the absence of this site is a G base. Participants were then categorized as exhibiting AA, AG, or GG.

3.7 STATISTICAL ANALYSIS

SPSS software (SPSS Inc., Chicago, IL, v11.5) was used for all analyses. One-way analysis of variance (ANOVA) was used to analyze differences in baseline characteristics of the participants' individual genetic constitution, or genotype. Chi-square analysis was used to determine if the frequencies of the genotypes were in Hardy-Weinberg equilibrium. Hardy-Weinberg equilibrium is the principle of 'genetic equilibrium' that exists between the frequency of alleles and the genotype of the population. Genetic equilibrium is the foundation of population genetics. In previous studies using the MERET dataset, Riechman and colleagues (32, 33) found significant percent gains in muscle mass and strength based on participants gender, age, and RET history. Therefore, analysis of covariance (ANCOVA) was used to control for differences in the relative percent change in lean muscle mass and the percent change in mass-adjusted strength to RET by genotype, with reference to gender, age (<19.5 years or >19.5 years) and RET history (0 hours per week, 0.1-1.8 hours per week or >1.8 hours per week during the past year).

Percent gains will be calculated as 100 x (postmeasurement – premeasurement) /premeasurement. Arm mass-adjusted strength was calculated as [1 RM biceps (kg) + 1 RM triceps (kg)/arm circumference (cm)]. Leg mass-adjusted strength was calculated as [1 RM hamstring curl (kg) + 1 RM leg extension (kg)/thigh circumference (cm)].

4.0 RESULTS

4.1 PARTICIPANTS

The baseline characteristics of the MERET cohort are presented in Table 2. Of these participants, 14 (9.3%) of those who completed the RET were not Caucasian (i.e. African American, Hispanic, Asian). In all, 28 men and 28 women were non-compliant with the RET and were excluded from the study. Non-compliance was defined as the inability to complete 30 RET sessions in 10 weeks. If participants missed a scheduled session, a make-up session was arranged. In order to prevent the same muscle being exercised on consecutive days, make-up sessions included split workouts in which participants completed 5 sets of upper and lower body exercises on consecutive days. Participants were limited to five split workouts before they were excluded.

Table 2 Baseline Characteristics of the MERET Cohort

Men/Women (n)	**76 / 78**
Age (years)	**20.9 ± 2.5**
Height (cm)	**170.5 ± 9.0**
Weight (km)	**73.0 ± 15.2**
Fat (%)	**22.8 ± 7.4**
Lean Mass (kg)	**56.1 ± 11.7**
Activity history (hrs-wk)	**6.3 ± 8.7**
RET history (hrs-wk)	**1.0 ± 1.6**

Values for age, height, weight, fat %, lean mass, activity history, and RET history are means ± SD

Of the 154 MERET participants who completed RET, not all participants were able to be genotyped for the seven SNPs chosen for this investigation, despite several attempts. The total N varied by SNP and ranged from 115 to 121 participants (75-79%). Participant characteristics by genotype for each of the 7 SNPs chosen for this investigation are presented in Tables 3-9. Frequencies of all genotypes presented were in Hardy-Weinberg equilibrium and did not differ between gender and race categories ($P > 0.05$).

Table 3 Participant Characteristics by rs2494735 (AKT 1) Genotype

	AA	AG	GG	P-value
Men/Women (n)	21/23	30/29	8/8	0.95
Age (years)	21.4±3.2	20.7±2.1	20.7±2.0	0.34
Height (cm)	170.1±9.4	170.4±8.2	172.2±8.9	0.70
Weight (kg)	72.4±16.7	72.9±13.8	77.2±13.9	0.52
Fat (%)	22.1±7.4	22.4±8.1	25.9±8.8	0.24
Lean mass (kg)	55.9±11.9	56.2±11.0	56.7±9.5	0.97
Act. history (hrs-wk)	6.3±6.2	5.7±5.6	3.2±2.0	0.15
RET history (hrs-wk)	1.1±1.7	1.0±1.7	0.4±0.6	0.33

Values are means ± SD. Frequencies of genotypes are in Hardy-Weinberg equilibrium and are similar between gender and race (P > 0.05).

Table 4 Participant Characteristics by rs1130233 (AKT 1) Genotype

	GG	AG	AA	P-value
Men/Women (n)	35/29	20/28	4/4	0.40
Age (years)	21.3±3.0	20.7±2.1	20.5±1.9	0.42
Height (cm)	170.6±9.0	169.9±8.3	173.7±8.0	0.51
Weight (kg)	72.7±15.2	73.7±14.6	75.5±14.4	0.85
Fat (%)	21.5±7.6	24.4±8.3	25.2±6.3	0.11
Lean mass (kg)	56.7±11.4	55.3±10.7	56.3±11.3	0.80
Act. history (hrs-wk)	5.5±5.5	5.4±4.6	4.1±2.7	0.74
RET history (hrs-wk)	1.0±1.5	0.9±1.6	1.2±2.0	0.87

Values are means ± SD. Frequencies of genotypes are in Hardy-Weinberg equilibrium and are similar between gender and race (P > 0.05).

Table 5 Participant Characteristics by rs3001371 (AKT 1) Genotype

	CC	CT	TT	P-value
Men/Women (n)	28/27	21/23	11/11	0.95
Age (years)	21.2±3.0	21.0±2.1	20.5±2.3	0.55
Height (cm)	170.4±9.1	169.7±8.1	172.5±8.6	0.46
Weight (kg)	72.5±15.8	72.1±14.8	77.7±14.9	0.30
Fat (%)	21.8±7.4	22.1±8.1	26.4±8.3	0.06
Lean mass (kg)	56.4±11.7	55.7±11.2	56.9±9.4	0.91
Act. history (hrs-wk)	5.8±5.8	6.4±6.0	3.5±2.6	0.12
RET history (hrs-wk)	1.1±1.6	1.2±2.0	0.4±0.5	0.17

Values are means ± SD. Frequencies of genotypes are in Hardy-Weinberg equilibrium and are similar between gender and race (P > 0.05).

Table 6 Participant Characteristics by rs1130214 (AKT 1) Genotype

	GG	GT	TT	P-value
Men/Women (n)	33/22	23/30	4/9	0.80
Age (years)	21.0±2.6	21.0±2.7	21.0±2.3	0.99
Height (cm)	171.4±9.5	169.9±7.6	169.8±7.0	0.65
Weight (kg)	76.2±17.2	71.1±12.3	70.1±12.5	0.15
Fat (%)	22.8±7.7	22.1±8.4	24.7±6.8	0.57
Lean mass (kg)	58.1±11.5	55.2±10.2	52.7±10.6	0.18
Act. history (hrs-wk)	5.7±5.2	6.0±6.3	3.7±2.6	0.41
RET history (hrs-wk)	0.9±1.2	1.1±1.9	1.0±2.4	0.81

Values are means ± SD. Frequencies of genotypes are in Hardy-Weinberg equilibrium and are similar between gender and race (P > 0.05).

Table 7 Participant Characteristics by rs1124900 (AMPKα2) Genotype

	TT	GT	GG	P-value
Men/Women (n)	17/16	28/28	15/16	0.97
Age (years)	21.4±3.4	20.8±2.3	21.0±2.2	0.54
Height (cm)	168.5±9.3	171.2±8.2	171.6±8.5	0.25
Weight (kg)	73.1±14.9	73.0±15.5	73.8±14.5	0.97
Fat (%)	22.7±6.5	22.6±8.9	22.6±7.4	0.99
Lean mass (kg)	56.3±11.6	56.0±10.9	56.8±11.1	0.94
Act. history (hrs-wk)	4.4±3.2	5.3±5.2	7.1±7.2	0.14
RET history (hrs-wk)	0.6±0.9	1.0±1.8	1.3±2.0	0.30

Values are means ± SD. Frequencies of genotypes are in Hardy-Weinberg equilibrium and are similar between gender and race (P > 0.05).

Table 8 Participant Characteristics by rs2796516 (AMPKα2) Genotype

	GG	AG	AA	P-value
Men/Women (n)	40/45	18/15	1/0	0.46
Age (years)	20.9±2.5	21.6±2.8	18.0	0.19
Height (cm)	171.1±8.6	169.4±8.6	173.5	0.60
Weight (kg)	73.7±14.8	71.1±14.1	106.0	0.06
Fat (%)	23.0±7.7	21.6±8.4	30.4	0.42
Lean mass (kg)	56.3±11.2	55.4±10.5	73.7	0.26
Act. history (hrs-wk)	5.9±6.0	4.4±3.7	5.0	0.41
RET history (hrs-wk)	1.0±1.6	1.0±1.8	0.8	0.97

Values are means ± SD. Frequencies of genotypes are in Hardy-Weinberg equilibrium and are similar between gender and race (P > 0.05).

Table 9 Participant Characteristics by rs1418442 (AMPKα2) Genotype

	AA	AG	GG	P-value
Men/Women (n)	15/19	33/28	9/11	0.59
Age (years)	21.0±2.9	21.0±2.5	21.0±2.5	0.99
Height (cm)	169.1±8.8	170.8±7.9	171.4±9.9	0.55
Weight (kg)	71.8±14.5	72.4±15.2	76.0±16.2	0.58
Fat (%)	22.3±6.5	22.7±8.5	23.1±7.7	0.93
Lean mass (kg)	55.7±11.6	55.5±10.6	58.1±11.6	0.65
Act. history (hrs-wk)	4.6±3.6	6.2±6.6	6.0±5.1	0.42
RET history (hrs-wk)	0.6±0.9	1.1±1.8	1.0±1.3	0.33

Values are means ± SD. Frequencies of genotypes are in Hardy-Weinberg equilibrium and are similar between gender and race (P > 0.05).

4.2 MUSCLE PHENOTYPE RESPONSES TO RET

Muscle phenotype responses of the MERET cohort to RET by SNP are presented in Tables 10-16. Percent change in lean muscle mass, percent change in arm mass-adjusted strength, and percent change in leg mass-adjusted strength are presented as means ± the standard error of the estimate. The covariates used in each statistical model were gender, age (<19.5 years or >19.5 years) and RET history (0 hours per week, 0.1-1.8 hours per week or >1.8 hours per week during the past year).

Table 10 Muscle Phenotype Responses to RET by rs2494735 Genotype.

	AA	AG	GG
Lean muscle mass gain, %	1.951 ± 0.502	2.601 ± 0.418	3.370 ± 0.807
Arm mass-adjusted strength gain,%	39.197 ± 3.175	39.548 ± 2.645	31.331 ± 5.099
Leg mass-adjusted strength gain, %	39.614 ± 3.268	34.567 ± 2.722	36.244 ± 5.248

Values are means ± SE. Arm mass-adjusted strength = (1 RM biceps + 1 RM triceps) / arm circumference. Leg mass-adjusted strength = (1 RM hamstring curl + 1 RM leg extension) / thigh circumference. P > 0.05 for genotype (analysis of covariance).

Table 11 Muscle Phenotype Responses to RET by rs1130213 Genotype.

	GG	AG	AA
Lean muscle mass gain, %	2.399 ± 0.415	2.559 ± 0.468	2.744 ± 1.142
Arm mass-adjusted strength gain, %	40.137 ± 2.797	38.786 ± 3.155	36.050 ± 7.700
Leg mass-adjusted strength gain, %	37.463 ± 2.733	37.708 ± 3.084	30.610 ± 7.526

Values are means ± SE. Arm mass-adjusted strength = (1 RM biceps + 1 RM triceps) / arm circumference. Leg mass-adjusted strength = (1 RM hamstring curl + 1 RM leg extension) / thigh circumference. P > 0.05 for genotype (analysis of covariance).

Table 12 Muscle Phenotype Responses to RET by rs3001371 Genotype.

	CC	CT	TT
Lean muscle mass gain, %	2.334 ± 0.443	2.037 ± 0.485	3.496 ± 0.689
Arm mass-adjusted strength gain, %	39.694 ± 3.034	39.474 ± 3.319	35.559 ± 4.720
Leg mass-adjusted strength gain, %	38.668 ± 2.888	39.516 ± 3.159	28.471 ± 4.493

Values are means ± SE. Arm mass-adjusted strength = (1 RM biceps + 1 RM triceps) / arm circumference. Leg mass-adjusted strength = (1 RM hamstring curl + 1 RM leg extension) / thigh circumference. P > 0.05 for genotype (analysis of covariance).

Table 13 Muscle Phenotype Responses to RET by rs1130214 Genotype.

	GG	GT	TT
Lean muscle mass gain, %	2.005 ± 0.445	2.706 ± 0.446	2.941 ± 0.898
Arm mass-adjusted strength gain, %	40.542 ± 2.852	36.539 ± 2.858	35.116 ± 5.749
Leg mass-adjusted strength gain, %	38.722 ± 2.943	35.795 ± 2.949	34.497 ± 5.934

Values are means ± SE. Arm mass-adjusted strength = (1 RM biceps + 1 RM triceps) / arm circumference. Leg mass-adjusted strength = (1 RM hamstring curl + 1 RM leg extension) / thigh circumference. P > 0.05 for genotype (analysis of covariance).

Table 14 Muscle Phenotype Responses to RET by rs1124900 Genotype.

	TT	GT	GG
Lean muscle mass gain, %	2.188 ± 0.577	2.757 ± 0.432	2.286 0.581
Arm mass-adjusted strength gain, %	42.046 ± 3.893	35.680 ± 2.913	41.976 ± 3.923
Leg mass-adjusted strength gain, %	37.408 ± 3.846	37.723 ± 2.878	35.644 ± 3.875

Values are means ± SE. Arm mass-adjusted strength = (1 RM biceps + 1 RM triceps) / arm circumference. Leg mass-adjusted strength = (1 RM hamstring curl + 1 RM leg extension) / thigh circumference. P > 0.05 for genotype (analysis of covariance).

Table 15 Muscle Phenotype Responses to RET by rs2796516 Genotype.

	GG	AG	AA *
Lean muscle mass gain, %	2.440 ± 0.351	2.447 ± 0.594	4.394 ± 3.278
Arm mass-adjusted strength gain, %	39.008 ± 2.361	36.655 ± 4.005	80.087 ± 22.083
Leg mass-adjusted strength gain, %	35.152 ± 2.265	39.254 ± 3.841	67.104 ± 21.183

Values are means ± SE. Arm mass-adjusted strength = (1 RM biceps + 1 RM triceps) / arm circumference. Leg mass-adjusted strength = (1 RM hamstring curl + 1 RM leg extension) / thigh circumference. P > 0.05 for GG and AG genotypes (analysis of covariance). * AA genotype N = 1.

Table 16 Muscle Phenotype Responses to RET by rs1418442 Genotype.

	AA	AG	GG
Lean muscle mass gain, %	2.098 ± 0.576	2.794 ± 0.420	2.240 ± 0.729
Arm mass-adjusted strength gain, %	37.481 ± 3.984	38.875 ± 2.903	40.481 ± 5.040
Leg mass-adjusted strength gain, %	35.372 ± 3.815	37.693 ± 2.779	34.945 ± 4.826

Values are means ± SE. Arm mass-adjusted strength = (1 RM biceps + 1 RM triceps) / arm circumference. Leg mass-adjusted strength = (1 RM hamstring curl + 1 RM leg extension) / thigh circumference. P > 0.05 for genotype (analysis of covariance).

4.3 BETWEEN SNP INTERACTIONS - PERCENT CHANGE IN LEAN MUSCLE MASS

There were no statistically significant differences observed between each individual SNP and percent change in lean muscle mass following RET. However, the statistical model examining the interaction between two SNPs, rs2494735 (AKT1) and rs3001371 (AKT1) showed a statistically significant difference in percent change in lean muscle mass (P=0.036). Therefore, a least significant difference *post-hoc* test was completed to show which genotypes were associated with percent changes in lean muscle mass following RET. The covariates used in each statistical model were gender, age (<19.5 years or >19.5 years) and RET history (0 hours per week, 0.1-1.8 hours per week or >1.8 hours per week during the past year). Table 17 shows the significant interactions between various rs2494735 (AKT1) and rs3001371 (AKT1) genotypes.

Table 17 Percent Change In Lean Muscle Mass by rs2494735 and rs3001371Genotype

Genotype	N	Mean	Standard Error	Significance
AA/CC AG/CC	41 9	2.565	1.143	0.027
AA/CC GG/CT	41 3	5.975	2.099	0.005
AA/CC GG/TT	41 11	2.788	1.054	0.009
AG/CT GG/CT	39 3	3.766	1.886	0.048
AG/CT GGTT	39 11	2.432	1.061	0.024
AG/TT GG/TT	11 11	2.655	1.332	0.049
GG/CT GG/TT	3 11	6.198	2.042	0.003

Genotypes presented are interactions between various rs2494735 and rs3001371 genotypes. Values are marginal means ± SE. Covariates are gender, age and strength training history.

4.4 BETWEEN SNP INTERACTIONS - PERCENT CHANGE IN ARM MASS-ADJUSTED STRENGTH

Similar to the results for percent change in lean muscles mass, there were no significant differences between participant genotypes for each individual SNP and percent change in arm mass-adjusted strength following RET. Arm mass-adjusted strength was calculated as [1 RM biceps (kg) + 1 RM triceps (kg)/arm circumference (cm)]. Appendix F contains pre and post RET results for MERET participants by genotype for each component of the arm mass-adjusted strength calculation; 1 RM biceps, 1 RM triceps and arm circumference. Two significant interactions did exist in the statistical models comparing rs1130214 (AKT1) with rs1124900

(AMPKα2) (P=0.042) and also rs1124900 (AMPKα2) with rs2796516 (AMPKα2) (P=0.009). A least significant difference *post-hoc* test was used to examine the interactions between each individual genotype with percent change in arm mass-adjusted strength following RET. Tables 18 and 19 show the significant interactions between rs1130214 (AKT1) and rs1124900 (AMPKα2) and rs1124900 (AMPKα2) and rs2796516 (AMPKα2), respectively, by genotype.

Table 18 Percent Change in Arm Mass-Adjusted Strength by Genotype rs1130214 and rs1124900

Genotype	N	Mean	Standard Error	Significance
GT/GG GT/GT	12 25	15.480	7.099	0.031

Genotypes presented are interactions between various rs1130214 and rs1124900 genotypes. Arm mass-adjusted strength = (1 RM biceps + 1 RM triceps) / arm circumference. Values are marginal means ± SE. Covariates are gender, age and strength training history.

Table 19 Percent Change in Arm Mass-Adjusted Strength by Genotype rs1124900 and rs2796516

Genotype	N	Mean	Standard Error	Significance
TT/GG TT/AG	15 13	22.841	8.110	0.006
TT/GG GT/GG	15 40	19.659	6.352	0.003
GG/GG TT/AG	28 13	13.931	7.147	0.054
GG/GG GT/GG	28 40	10.749	5.174	0.040

Genotypes presented are interactions between various rs1124900 and rs2796516 genotypes. Arm mass-adjusted strength = (1 RM biceps + 1 RM triceps) / arm circumference. Values are marginal means ± SE. Covariates are gender, age and strength training history.

4.5 BETWEEN SNP INTERACTIONS - PERCENT CHANGE IN LEG MASS-ADJUSTED STRENGTH

Leg mass-adjusted strength was calculated as [1 RM hamstring curl (kg) + 1 RM leg extension (kg)/thigh circumference (cm)]. Appendix G contains pre and post RET results for MERET

participants by genotype for each component of the leg mass-adjusted strength calculation; 1 RM hamstring curl, 1 RM leg extension and thigh circumference. There were no significant differences between participant genotypes for each individual SNP and percent change in leg mass-adjusted strength following RET. The statistical model comparing the interaction between rs2494735 (AKT1) and rs1130214 (AKT1) did, however, produce a significant association (P=0.036). To further examine this result, a least significant difference *post-hoc* test was used to probe for differences in participant genotypes and percent change in leg mass-adjusted strength following RET. Table 20 shows the significant interactions between rs2494735 (AKT1) and rs1130214 (AKT1) with percent change in leg mass-adjusted strength by participant genotype.

Table 20 Percent Change in Leg Mass-Adjusted Strength by Genotype rs2494735 and rs1130214

Genotype	N	Mean	Standard Error	Significance
AA/GG GG/TT	24 5	19.271	9.931	0.055
AG/TT AG/GT	5 28	19.519	9.769	0.048
AG/TT GG/TT	5 5	30.469	12.795	0.019
GG/GG AA/GG	3 24	27.844	12.451	0.027
GG/GG AA/GT	3 14	28.406	12.909	0.030
GG/GG AA/TT	3 3	41.053	16.619	0.015
GG/GG AG/GG	3 25	35.497	12.407	0.005
GG/GG AG/GT	3 28	36.165	12.347	0.004
GG/GG GG/GT	3 8	35.226	13.680	0.011
GG/GG GG/TT	3 5	47.115	14.876	0.002

Genotypes presented are interactions between various rs2494735 and rs1130214 genotypes. Leg mass-adjusted strength = (1 RM hamstring curl + 1 RM leg extension) / thigh circumference. Values are marginal means ± SE. Covariates are gender, age and strength training history.

5.0 DISCUSSION

The mammalian target of rapamycin (mTOR) signaling pathway has been shown to be an important mediator of protein synthesis and skeletal muscle hypertrophy in adult animals (11). Previous investigations using the MERET cohort have shown that gender, age and RET history are significant predictors of percent gains in lean muscle mass, muscle strength, and muscle quality following standardized RET (32, 33). The aim of this investigation was to examine if specific SNPs of two genes that are an integral part of the mTOR signaling pathway could help explain some of the variability in skeletal muscle response to RET. Therefore, we examined four SNPs from the AKT1 gene and 3 SNPs from the AMPKα2 gene for possible associations with percent changes in lean muscle mass, arm mass-adjusted strength, and leg mass-adjusted strength, following a standardized 10-week RET program. Although none of the SNPs were independently associated with significant percent changes in lean muscle mass, arm mass-adjusted strength, or leg mass-adjusted strength following RET, several interactions between SNPs were found which could prove to be interesting candidates for future investigations.

5.1 PERCENT CHANGE IN LEAN MUSCLE MASS

It is reasonable to assume, given the complexity of the mTOR signaling pathway, that any variability in the normal cascade of reactions this pathway undergoes in order to trigger protein

synthesis could help explain some of the variability in skeletal muscle response to RET. Previous research has shown that AKT1 is involved in the intracellular signaling mechanisms controlling protein synthesis, a vital precursor of muscle growth (11, 13, 21). However, the current literature is dominated with studies that show a significant increase in AKT1 expression following RET (11, 19, 20), and lacking on studies examining which specific AKT1 SNPs may be associated with the variability in skeletal muscle response to RET. The significant interaction effect observed in percent change in lean muscle mass in this investigation occurred between two SNPs from the AKT1 gene, rs2794735 and rs3001371. Given AKT1's reputation as a controller of protein synthesis and protein degradation, any polymorphisms in this gene may possibly play a role in an individual's rate of protein synthesis and or protein degradation following RET (17). An individual's ability to synthesize protein could have a direct effect on their ability to build lean muscle mass following RET. For example, a previous investigation showed AKT1 expression increased significantly in association with protein synthesis 1-2 hours post-RET (19). It is possible that the interaction observed between rs2794735 and rs3001371 could influence the rate of AKT1 expression post-RET. This, in-turn, could affect protein synthesis and subsequently the ability to build lean muscle mass post-RET.

5.2 PERCENT CHANGE IN ARM MASS-ADJUSTED STRENGTH

A secondary analysis performed in this investigation was to examine any associations between the selected SNPs of the AKT1 and AMPKα2 genes and percent changes in arm mass-adjusted strength following RET. None of the seven SNPs examined were independently associated with significant percent changes in arm mass-adjusted strength following RET. However, there were

two significant interactions between SNPs that were associated with percent change in arm mass-adjusted strength following RET. The first was a significant gene-gene interaction between rs1130214 (AKT1) and rs1124900 (AMPKα2). In contrast to AKT1, which acts on the mTOR signaling pathway to increase protein synthesis, the goal of the AMPK gene is to keep the cell in homeostasis. AMPK is up-regulated during times of increased protein synthesis, in an effort to return the cell to homeostasis, by signaling the mTOR pathway to repress protein synthesis. The appearance of an adversarial relationship between AKT1 and AMPK, makes the significant interaction observed in percent change in arm mass-adjusted strength following RET between rs1130214 (AKT1) and rs1124900 (AMPKα2) an interesting one (27). While much more work needs to be done, a possible explanation for this finding is that this particular rs1130214 (AKT1) and rs1124900 (AMPKα2) genotype combination (GT/GG GT/GT) could allow AKT1 signaling to influence the mTOR pathway more strongly than AMPK, resulting in an increase in protein synthesis, as opposed to a repression of protein synthesis.

The second significant interaction observed in percent change in arm mass-adjusted strength following RET was between rs1124900 and rs2796516, two SNPs from the AMPKα2 gene. This finding was of interest because under normal conditions, AMPK works to suppress energy-consuming processes, like protein synthesis, while enhancing energy producing processes. The finding that some of the genotypes between these two SNPs interacted to significantly increase the percent change in arm mass-adjusted-strength following RET could be explained by the possibility that these genotypes may alter the normal function of this gene. If these genotype interactions compromise AMPK's ability to properly signal the mTOR pathway to repress protein synthesis under conditions of increased stress, such as RET, then perhaps

protein synthesis is allowed to continue for a longer period of time, resulting in increased cell growth for individuals possessing these particular genotype combinations.

5.3 PERCENT CHANGE IN LEG MASS-ADJUSTED STRENGTH

The interaction between rs2494735 and rs1130214, both AKT1 SNPs, was significantly associated with an increase in percent change in leg mass-adjusted strength following RET. These findings are similar to those observed for percent change in lean muscle mass following RET, in that both sets of SNPs are from the AKT1 gene. Individuals who possess particular AKT1 genotypes, like those in Tables 10 and 13, may be more efficient at synthesizing protein following RET as opposed to those individuals with other AKT1 genotypes. Additionally, these AKT1 genotypes may affect the rate of AKT1 expression following RET, which could also affect the rate of protein synthesis. This may explain why some of the MERET participants had more favorable muscle mass and muscle strength responses following RET than other MERET participants.

The significant between SNP interactions for percent change in lean muscle mass, percent change in arm mass-adjusted strength, and percent change in leg mass-adjusted strength following RET noted in this report should be viewed as preliminary due to the inequality in the number of participants being compared. In many cases there were large differences in the number of participants with a particular genotype that were being compared with participants with another genotype (Tables 17-20). This was due to the many genotype combinations that exist when examining participant data for interactions between two different SNPs.

5.4 LIMITATIONS

There are several limitations associated with this investigation. First, the number of participants in the between SNP interaction analyses is relatively small. A larger number of participants is needed in order to achieve sufficient statistical power to examine the AKT1 and AMPKα2 between SNP interactions in relation to percent change in lean muscle mass, arm mass-adjusted strength, and leg mass-adjusted strength following RET. Second, there are currently more accurate ways to examine changes in muscle mass following RET. Dual-energy x-ray absorptiometry (DEXA) is one method that would allow for a more accurate quantification of muscle mass gains following RET. Third, muscle biopsies would allow for the analysis of AKT1 and AMPKα2 gene expression. An analysis could then be performed to examine any interactions between AKT1 and AMPKα2 SNPs and changes in the expression of these two genes pre and post-RET.

5.5 CONCLUSION

In conclusion, none of the AKT1 and AMPKα2 SNPs examined in this investigation were independently associated with percent change in lean muscle mass, arm mass-adjusted strength, or leg mass-adjusted strength following RET. However, there is preliminary evidence of associations to lean muscle mass and muscle strength responses of the MERET cohort to RET based on interactions between the SNPs of the AKT1 and AMPKα2 genes examined in this report. The individual response to standardized RET varies greatly from person to person. As future research begins to unravel the complexity of the mTOR signaling pathway and the specific

role its genes play in the development of muscle hypertrophy following RET new light will be shed on the inter-individual response to standardized RET.

5.6 RECCOMMENDATIONS FOR FUTURE RESEARCH

While additional studies with larger sample sizes are needed to confirm any associations between the SNPs examined in this investigation and variations in percent changes in lean muscle mass, and muscle strength following RET, the information presented here provides a solid base for future research to expand upon. Future research involving the AKT1 and AMPKα2 genes could take the approach of focusing more specifically on the above mentioned genotypes to further examine any associations to the variability of skeletal muscle response to RET. Future studies could also examine the potential interactions between the AKT1 and AMPKα2 genes with other genes of the mTOR signaling pathway in relation to the individual variability of muscle response to RET. Though complex, the mTOR signaling pathway contains numerous genes that could prove to be excellent candidates for studies examining the variability of muscle response to RET. As new SNPs from the genes of the mTOR pathway are discovered, particularly those that result in amino acid changes, future studies should examine the possible role they may play in the individual response to RET. Given the sheer size and scope of the mTOR signaling pathway, combined with its biological outcome of protein translation and cell growth, there is near unlimited potential for future investigations to examine this pathway's relationship to muscle hypertrophy and strength gains following RET.

APPENDIX A

MEDICAL HISTORY

1. Name _____

2. Date of Birth / /
month / day / year

3. Gender Male Female

Medical History

4. Are you presently taking any prescribed or over the counter medication? (including birth control pills, insulin, allergy shots or pills, asthma inhalers, vitamin or mineral supplements including iron, anti-inflammatories including aspirin)

Yes List Name of Medication Dose Frequency of Use

1._____
2._____
3._____
4._____

NO

5. Do you have an allergy to any:

			Specify Allergy:
a. drug or medicine (over the counter or prescribed)	Yes	No	
b. foods	Yes	No	
c. insects or animals	Yes	No	
d. plants, grasses, pollen, dust or other environmental factors	Yes	No	
e. other			

6. Has a doctor ever told you that you have had any of the following medical problems?(if you don't know, mark 'No')

a. birth defect	Yes No	t. stomach or intestinal ulcer	Yes No
b. frequent urinary infections	Yes No	u. sickle cell anemia	Yes No
c. kidney injury	Yes No	v. other anemia	
d. other kidney disease	Yes No	w. abnormal bleeding or clotting	
e. blood clot or embolism	Yes No	disorder	Yes No
f. diabetes	Yes No	y. leukemia or other blood	Yes No
g. hernia	Yes No	disorder	Yes No
h. jaundice	Yes No		
i. epilepsy	Yes No		
j. tumor, growth, cyst, cancer	Yes No		
k. over-active thyroid	Yes No		
l. under-active thyroid	Yes No		
m. arthritis	Yes No		
n. Marfan syndrome	Yes No		
o. oral herpes (cold sores)	Yes No		
p. genital herpes	Yes No		
q. injury to liver or	Yes No		
r. spleen	Yes No		
s. hepatitis	Yes No		

7. Have you ever had surgery to the following:

		Date (month/year)	If yes, give reason for surgery:
eyes	Yes No	/	
ears/nose/throat	Yes No	/	
heart	Yes No	/	
lungs	Yes No	/	
stomach or bowels	Yes No	/	
kidneys	Yes No	/	
liver/spleen	Yes No	/	
bone	Yes No	/	
muscle/ligament/tendon	Yes No	/	
joint	Yes No	/	
other (please specify)	Yes No	/	

8. During or after exercise, have you ever:

been dizzy or light- headed?	Yes No
passed out (fainted)?	Yes No
had chest pain, discomfort or tightness?	Yes No
found it more difficult to breath than usual?	Yes No
had problems with coughing?	Yes No

9. Have you ever been told that you have a heart murmur? Yes No

10. Have you ever had racing of your heart, irregular or skipped beats? Yes No

11. Have you ever been told by a doctor that you have had:

high blood pressure?	Yes No
pericarditis, myocarditis, endocarditis (infections of the heart)?	Yes No
rheumatic fever?	Yes No
other heart or vascular problems? (please specify)	Yes No

12. Have you ever had any medical tests for your heart (i.e. EKG, echocardiogram)?

Yes (if so, specify test and reason below)
Test Reason

No

13. Have you ever had:

tuberculosis?	Yes No
asthma?	Yes No
wheezing that starts during or just after exercise?	Yes No
pneumothorax (collapsed lung)?	Yes No

14. Have you ever had a concussion (injury to the head) with or without loss of consciousness?

Yes
How many times? 1x 2x 3x 4x 5x >5x

No

15. Have you ever been knocked unconscious?

Yes
How many times? 1x 2x 3x 4x 5x >5x

What is the longest time that you have been unconscious due to a head injury?
A few seconds Up to 5 minutes 6-15 minutes >15 minutes

No

16. Have you ever had any long term problems due to a head injury (e.g. memory loss, headaches)?

Yes No

17. Have you ever had numbness, tingling, or weakness in your:

shoulders/arms/hands?	Yes No
buttocks?	Yes No
legs/feet?	Yes No

18. Have you ever had a seizure? Yes No

19. Do you experience migraine headaches? Yes No

WOMEN ONLY, MEN SKIP TO QUESTION #23

20. When was your most recent menstrual period?

<1 month ago 1-3 months ago 4-6 months ago >6 months ago

21. In the past 12 months:

have you had trouble with heavy menstrual bleeding? Yes No
have you had bleeding between periods? Yes No
have you had menstrual cramps or pain which affected your school or athletic performance? Yes No
what was the longest time between periods?
<1 month 1-3mo 4-6mo >6mo
on average how long has each period lasted?
1-5 days 6-10d 11-15d >15d

22. Are you presently taking any female hormones (estrogen, progesterone, birth control pills) for the purpose of regulating your periods? Yes No

---

Orthopaedic History

23. In the past 12 months have you seen a physician, athletic trainer or other health care professional for a new or ongoing injury?

Yes (if so, please complete a list below)
Specify injury(s): Has this injury healed completely?
Yes No
Yes No
Yes No
Yes No
Yes No

24. Do you presently use for practice or competition:
a. brace, splint, or sleeve? Yes No
b. orthotics (shoe inserts)? Yes No

QUESTIONS 25-36

Have you ever had or do you currently have an injury or problem of the following:
(if you don't know, indicate 'No')

	Yes	No	Place an "x" if condition exists at present		Yes	No	Place an "x" if condition exists at present
25. Neck:							
disc disease				facet disorder			
traumatic fracture				surgery			
stress fracture				other			
whiplash				specify:			
26. Spine / Back:							
cogenital deformity or birth defect				disc disease			
facet disorder				sacroiliac disorder			
traumatic fracture				ciatica			
stress fracture				scoliosis			
back pain				surgery			
back stiffness							
spondyloysis				other			
spondylolisthesis				specify:			
27. Shoulder / Clavicle:							
traumatic fracture				subluxation			
bursitis				dislocation			
acromioclavicular (AC) separation				surgery			
rotator cuff tendonitis/ impingement				other			
instability				specify:			
.28. Upper arm / forearm:							
traumatic fracture				surgery			
muscle injury				other			
tendon injury				specify:			
29. Elbow:							
traumatic fracture				dislocation			
ligament injury				surgery			
tennis (golfer's) elbow				other			
bursitis				specify:			

30. Hand, Wrist, Fingers:

traumatic fracture	dislocation
stress fracture	surgery
ligament injury	other
tendon injury or tendonitis	specify:

31. Pelvis / Hip:

traumatic fracture	tendonitis
stress fracture	contusion/hip pointers
groin strain	surgery
dislocation	other
bursitis	specify:

32. Thigh:

traumatic fracture	quadriceps strain/injury
stress fracture	severe contusion
tendonitis	surgery
bursitis	other
hamstring strain/injury	specify:

33. Knee:

meniscal injury	locking
PCL tear	dislocation of knee or patella (knee cap)
ACL tear	swelling
iliotibial band syndrome	unexplained pain
collateral ligament injury	meniscal surgery
tendonitis	ACL surgery
bursitis	other injury or surgery
pain around knee cap, (patello-femoral pain)	pecify:
sensation of catching, instability, giving away	

34. Lower Leg:

traumatic fracture	shin splints
stress fracture	surgery
muscle strain	other
compartment syndrome	specify:

35. Ankle:

traumatic fracture	bone chip in joint
stress fracture	dislocation
sprain	surgery
tendonitis	other
bursitis	specify:
instability	

36. Foot / Toes:

traumatic fracture	flat arches of feet
stress fracture	dislocation
sprain	surgery
tendonitis or tendon injury	other
bone spur	specify:
plantar fasciitis	

37. In the past 10 years, have you been treated for a serious injury(s) not mentioned above?

Yes (if so, please specify below)
Specify injury(s): Date (month/year):
/
/
/

No

38. Have you ever had a cortisone injection into a tendon, bursa, or joint for an injury or pain?

Yes (if so, please specify below)
Specify injury(s): Date (month/year):
/
/

No

39. Please indicate your ethnic origin (place an 'x' in all that apply)

Native American / Alaska native	Hispanic/Latino/Chicano
Asian	White (non-Hispanic)
Pacific Islander	Other, specify:
Black/African American	Don't know

Health Habits

40. Have you ever been diagnosed as having an eating disorder?

Yes No

41. Have you ever tried to control your weight with:

	Yes	No
fasting?		
vomiting?		
laxatives?		
diuretics?		
diet pills?		

APPENDIX B

MODIFIABLE ACTIVITY QUESTIONNAIRE

Please identify all activities you have done more than 10 times in the past year.

Activity	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	Average # of times each month	Average # of minutes each time
Jogging (outdoor, treadmill)														
Swimming (laps, snorkeling)														
Bicycling (indoor, outdoor)														
Softball/Baseball														
Volleyball														
Bowling														
Basketball														
Skating (roller, ice, blading)														
Martial Arts (karate, judo)														
Tai Chi														

Activity	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	Average # of times each month	Average # of minutes each time
Calisthenics/Toning exercises														
Wood Chopping														
Elliptical Trainer														
Football/Soccer														
Racquetball/Handball/Squash														
Horseback riding														
Hunting														
Fishing														
Aerobic Dance/Step Aerobic														
Water Aerobics														
Dancing (Square,Line,Ballrm)														
Gardening or Yardwork														
Badminton														
Strength/Weight training														
Rock climbing														
Scuba Diving														
Stair Master/Stair Climbing														
Fencing														
Hiking														
Tennis														

Activity	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	Average # of times each month	Average # of minutes each time
Golf														
Canoeing/Rowing/Kayaking														
Water skiing														
Jumping rope														
Snow skiing (X-country/Nordic trk) (downhill)														
Snow shoeing														
Yoga														
Other														
Walking for exercise (outdoor, indoor at mall or fitness center, treadmill														

2. In general, how many HOURS per DAY do you usually spend watching television? _____ hrs

3. Over this past year, have you spent more than one week confined to a bed or chair as a result of an injury, illness or surgery Yes_____ No_____
   If yes, how many weeks over this past year were you confined to a bed or chair? _____ weeks

4. Do you have difficulty doing any of the following activities?
   a. getting in or out of a bed or chair? Yes_____ No_____
   b. walking across a small room without resting? Yes_____ No_____
   c. walking for 10 minutes without resting? Yes_____ No_____

5. Did you ever compete in an individual or team sport (not including any time spent in sports performed during school physical education classes)?
   If yes, how many total years did you participate in competitive sports? _____years

6. Have you had a job for more than one month over this past year, from last _____ to this _____?

APPENDIX C

INFORMED CONSENT

Approved: 01/26/2000
Institutional Review Board
University of Pittsburgh
IRB Number: 991278
Renewal Date: 1/11/2001

CONSENT TO ACT AS A SUBJECT IN A RESEARCH STUDY

TITLE: **Genetic Predisposition to Myosthenic and Sarcopoietic Responses to Resistance Exercise.**

Principal Investigator: Steven E. Riechman, Ph.D.
A 300 Crabtree Hall
Department of Human Genetics
Graduate School of Public Health
University of Pittsburgh
Pittsburgh, PA 15261
(412) 383-8733

Co-investigators:

Robert E. Ferrell, Ph.D.
Department of Human Genetics
Graduate School of Public Health
University of Pittsburgh
Pittsburgh, PA 15261
(412) 624-3018

Stephen M. Roth, Ph.D.
Department of Human Genetics
Graduate School of Public Health
University of Pittsburgh
Pittsburgh, PA 15261
(412) 624-4697

David E. Kelley, M.D.
Division of Endocrinology
School of Medicine, 412-692-2437
University of Pittsburgh
Pittsburgh, PA 15261

Bret Goodpaster, Ph.D.
Division of Endocrinology
School of Medicine, 412-692-2158
University of Pittsburgh
Pittsburgh, PA 15261

Robert J. Robertson, Ph.D.
Health Physical Recreation and Education Dept
Center for Exercise and Health-Fitness Research
University of Pittsburgh, 412-648-8251
113 Trees Hall
Pittsburgh, PA 15261

Ben Hurley, Ph.D.
Department of Kinesiology
University of Maryland
College Park, MD 20742
(301) 405-2486

Source of Support: Developmental and Feasibility Multipurpose Arthritis and Musculoskeletal Disease Center Grant MAMDC

DESCRIPTION:

You are being asked to participate as a volunteer in a research investigation. In this research investigation, you will be on a supervised ten-week strength-training program which requires your participation for one hour, three days per week. You are being asked to participate in this study because you are a healthy male or female between the ages of 18-30 years without any medical conditions. In this study, the researchers are trying to determine if genetic markers are related to the ability to increase muscle size and strength in response to strength training exercise. If you are selected for the training, a DNA sample will be collected and analyzed for common genetic variations thought to contribute to variations in muscle strength and size development. A portion of the sample will be stored for future analyses if new genes involved in muscle development are discovered. All samples will be destroyed after five years. If you agree to participate in this research project, use of your donated DNA sample will be under the control of the principal investigator of this research project. You will not be informed of your genetic results because the genetic data cannot yet be interpreted or applied in a clinically relevant or meaningful manner.

After consent to participate is obtained, you will be asked to spend ten minutes filling out a medical history questionnaire. From the eligible subjects, forty subjects will be randomly selected to participate in the weight training program. If you are not selected you are still eligible to participate in the future. An equal number of males and females will be selected. Approximately 125 subjects are expected to enter this study. 40 subjects will be selected each semester**.** Physical measurements are needed for this study to determine the changes that may occur during the strength training program, and your blood will be drawn and tested three times before you begin and after you have completed the ten week training program (six times total). The DNA for this study will be collected from these blood samples.

If you agree to participate in this research study, you will undergo the following:

1. Under supervision, you will have three days of familiarization with the strength equipment in the Trees Hall weight room at the University of Pittsburgh.
2. Next, you will have a sixty-minute testing session in Trees Hall. Your one repetition maximum (1RM) will be determined for each of the following twelve exercises: leg press, chest press, seated row, lat pull downs, leg extensions, triceps extension, arm curl, shoulder press, leg curl, low back, abdominal crunch and incline press. To determine your 1RM, you will be asked to select the maximum weight that you feel you can lift one (1) time. You will be asked to lift 50% of that weight three times to warm up. After sixty seconds of rest, you will make an attempt to lift 90% of the estimated maximal resistance one time. A refined estimation of maximal strength will then be attempted following 60 seconds of rest. Thereafter, the increases in resistance will be adjusted so as to minimize the total number of trials required before the true 1 RM value is obtained., i.e. the highest resistance at which one repetition can be successfully completed.
3. In order to measure the amount of lean and fat tissue in your body, you will have a scan performed which is called a "DEXA" (dual-energy x-ray absorptiometry) scan. This scan is performed similar to an x-ray study and you will lie on an examination table in a room with

the DEXA scanner for approximately 30 minutes. There are no contrast injections or blood samples, and the test is painless except for any discomfort you may experience because of lying on my back on the firm examination table. This will be performed before and after the strength training.

4. Baseline assessments of the following will be obtained at the Human Energy Research Laboratory (HERL): height, weight, body fat measurement by underwater weighing, bicycle test for assessment of sprinting capacity, and size measurements of shoulders, chest, arms, forearm, thigh, waist, calves and hips, skinfold measurements of chest, abdominal, arms, hip, thigh, calf, back. Blood samples (2-3 tablespoonful) will be collected in the morning before you begin your first workout after at least 6 hours of fasting and tested for: lactate (exercise breakdown product of blood sugar), glucose (blood sugar), testosterone (male hormone), insulin like growth factor I (IGF I-muscle building hormone), IGF-B3 (insulin like growth factor binding protein-transports IGF-I), and creatine kinase (marker of muscle breakdown). These blood measurements are related to exercise and muscle activity and development. Another 2-3 tablespoonful of blood will be drawn after your first training session. A third blood sample of 2 teaspoonfuls will be taken 24 hours after the first workout for the measurement of creatine kinase. Fasting is not required for this sample. During the underwater weighing procedure you will be asked to enter a warm water tank in your bathing suit. You will then be asked to blow out all your air while underwater and hold it for about two seconds. This procedure will be repeated about five times to insure an accurate measurement. You may take a break between attempts for as long as you like. The bicycle test asks that you pedal as hard as you can for thirty seconds.
5. Strength training will be done for one hour at a time, three days per week for ten weeks (30 one-hour sessions total) with at least one day of rest in between sessions.
6. Approximately 2-3 tablespoons of blood will be drawn both before and after your last workout of the 10-week program. Testing on the blood sample will be the same as the blood test prior to the program lactate, glucose, testosterone, Insulin like growth factor I (IGF I), IGF-B3 (insulin like growth factor binding protein), creatine kinase, blood glucose, total cholesterol, triglycerides, total HDL, and HDL2). Physical measurements (height, weight, body fat measurement by hydrostatic (underwater) skinfold measurements of chest, abdominal, triceps, hip, thigh, calf, subscapula, weighing, bicycle ergometer for assessment of anaerobic capacity, and circumference measurements shoulders, chest, arms, forearm, thigh, waist, calves and hips) will also be performed again.

Your participation in this study will require you to spend approximately one hour for the three initial visits, one hour for the 1 RM and physical measurements and one hour for each of the three sessions per week for the ten week weight training program in Trees Hall. One of the investigators will be present to assist if necessary. A spotter will be present if you cannot perform the assigned number of repetitions in each set. If you fail to complete a repetition, the spotter will assist you in that lift. You will be instructed to exhale during the contraction phase of each repetition for every exercise.

The tests are being conducted in a research laboratory that is not certified for diagnostic testing. Therefore the results of these tests will not be given to you and will not become part of your medical record. In addition to the genes named above, research in human genetics and physiology is likely

to identify new genes or new pathways involving known genes that may influence muscle function. These new genes may also be tested without specifically notifying you. Your DNA will not be used for studies other than muscle function without contacting you to obtain your consent.

RISKS AND BENEFITS:

The risks associated with weight training exercise, bicycle sprint test and underwater weighing are low. Weight training, the bicycle sprint test and underwater weighing may be harmful in individuals with high blood pressure, abnormal heart rhythms and musculoskeletal injuries. You may feel light-headed or nauseous after the bicycle test. This is a common response and occurs **in** not more than 25 out of 100 people. You will be asked to continue to cycle to reduce lightheadedness and nausea. You may feel a strong desire to breathe during the underwater test. To minimize these risks, you will complete a medical history questionnaire and an exercise physiologist will conduct these tests. Because you will be required to lift heavy weight and perform unfamiliar exercises, you may experience some muscle soreness or injury to muscle, tendon or ligaments. Initially it is likely you will be sore for 3-5 days (more than 25 out 100 people). The soreness associated with each workout should diminish within two weeks of training. You should alert the investigative team if soreness, pain or other unusual symptoms persist or become intensified during the training. The investigative team will ask you at each training session about these symptoms. . You will be continually monitored and corrected with respect to lifting technique. You will also be asked to warm up and cool down to minimize the risk of injury and soreness. Having blood taken may cause pain, bruising, infection and fainting. These effects are common and occur in **up to** 25 out of 100 people. A person trained to take blood will be used to decrease these risks. You will also be asked to complete a medical history to identify conditions that might be worsened by this training program. If you have one of these conditions, you will not be asked to participate.

This study will provide you with information about your muscular strength, endurance, and body fat. You will be taught proper weight lifting techniques. Strength training can contribute to healthy lifestyle and reduction of risk for many diseases. Participation in this study may contribute to the advancement of scientific knowledge concerning genetic variation and strength training.

Participation in this research study involves a small amount of radiation exposure from the DEXA scans (1 mrem, a unit of radiation exposure to the whole body for each scan). If you participate in both the pre-weight training and post-weight training scans your exposure to radiation will be double (2 mrems). For comparison, radiation workers are permitted by federal regulation, a maximum annual whole body radiation exposure of 5000 mrems. There is no known minimum level of radiation exposure that is recognized as being totally free of the risk of causing genetic defects (cellular abnormalities) or cancer. However, the risk associated with the amount of radiation exposure that you will receive from this procedure is considered to be low and comparable to other everyday risks. If you are a woman of childbearing age you will have a urine pregnancy test performed 24 hours prior to the initial and, if applicable, repeat study and if positive, you will be excluded from the study.

NEW INFORMATION

You have been informed previously that you will not be informed of the genetic testing or other laboratory testing results. You or your representative will be promptly notified if any other information about this research study develops during the course of the study which may cause you to change your mind about continuing to participate.

COSTS AND PAYMENTS:

There will be no charge to you for the participation in this study. You will not receive payment for your participation in this study. Your genetic material may lead, in the future, to new inventions or products. If the research investigators are able to develop new products from the use of your genetic material, there are currently no plans to share with you monies that may result from such products.

COMPENSATION FOR ILLNESS AND INJURY:

University of Pittsburgh investigators and their associates who provide services at the UPMC Health System (UPMC HS) recognize the importance of your voluntary participation to their research studies. These individuals and their staffs will make reasonable efforts to minimize, control, and treat any injuries that may arise as a result of this research.

If you believe that you are injured as the result of the research procedures being performed, please contact immediately the Principal Investigator listed on the cover sheet of this form or the University of Pittsburgh Institutional Review Board (412)-578-3424. Emergency medical treatment for injured solely and directly related to your participation in this research will be provided to you by hospitals of the UPMC HS. It is possible that the UPMC HS may bill your insurance provider for the costs of this emergency treatment, but none of these costs will be billed directly to you. If your research-related injury requires medical care beyond this emergency treatment, you will be responsible for the costs of this follow up care unless otherwise specified below. You will not receive monetary payment for, or associated with, any injury that you suffer in relation to this research.

CONFIDENTIALITY:

All records pertaining to your involvement in this study will be stored in a locked file cabinet at the Human Genetics Department in Parren Hall. Your identity on these case records will be indicated by a case number. This information will only be accessible to the investigators and their research study staff listed on the first page of this document. A code number will indicate your identity on tissue samples and the DNA.

You understand that any information about you or your participation will be handled in a confidential (private) manner consistent with other research records. You will not be identified in any publication of research results. However, in unusual cases, your research records may be inspected by appropriate government agencies or be released in response to an order from a court of law. At the completion of the study, your research records will be kept under lock and key by the primary investigator for a period of five years at which time they will be destroyed.

RIGHT TO WITHDRAW

You understand that you do not have to take part in this study and, should you change your mind, that you can withdraw from the study at any time. Your status with the University and your other care and benefits (i.e. use of the weight room in Trees hall) will be the same whether you participate in this research study or not. You also understand that you may be removed from the research study by the investigators if you do not follow the instructions of the investigators. If you withdrawal or are removed from the study you will be given participation credit for each exercise session completed but not for testing sessions. All other class responsibilities will continue to be your responsibility. Your grade in class will not be influenced by your decision to participate nor by your decision to withdrawal. You retain the right to have your genetic material destroyed.

****

VOLUNTARY CONSENT:

All of the above has been explained to me and all of my questions have been answered. I understand that any future questions I have about this research will be answered by the investigator(s) listed on the first page of this consent document at the telephone numbers given. Any questions have about my rights as a research subject will be answered by the Human Subjects Protection Advocate of the IRB Office, University of Pittsburgh (412-578-8570). By signing this form, I agree to participate in the research study.

_____ _____

Subject's Signature Date

_____ _____

Witness Signature Date

INVESTIGATOR'S CERTIFICATION:

I certify that the nature and purpose, the potential benefits, and possible risks associated with participation in the research study have been explained to the above individual and that any questions about the information have been answered.

_____ _____

Investigator's Signature Date

APPENDIX D

MERET DATA COLLECTION SHEET

Name: _____

Date: __ __/ __ __/ __ __

Test: Pretest Posttest

Age: __ __

Gender: Male Female

RBP: __ __ __/ __ __ __

Race: Afr. Am. Caucn. Oriental Hisp. Oth.

RHR:__ __ __

Anthropometrics

Underwater weighing		Skinfolds (right side)	
	__ __		__ __.__/__
Height (cm):	__.__	tricep (mm)	__.__
		Subscap	__ __.__/__
Height (in):	__ __.__	(mm)	__.__
	__ __	Suprailliac	__ __.__/__
Weight (kg):	__.__	(mm)	__.__
	__ __		__ __.__/__
Weight (lb):	__.__	calf (mm)	__.__
	__.__		__ __.__/__
RVpred:	__	thigh (mm)	__.__
	__.__		__ __.__/__
WTUW (kg):	__	Abdom (mm)	__.__
	__		__ __.__/__
% Fat:	__.__	Chest (mm)	__.__
	__ __ .		
water temp	__	Bone Widths (right)	

Fat mass (lb):	__ __.__	humerus (cm)	__ __.__/__ __.__
Lean mass (lb.):	__ __ __.__	femur (cm)	__ __.__/__ __.__

Circumference

Arms (cm):	(R) __ __.__ / __ __.__	(L) __ __.__ / __ __.__
Thigh (cm):	(R)__ __ __.__/ __ __ __.__	(L)__ __ __.__/ __ __ __.__
Calf (cm):	(R) __ __.__ / __ __.__	(L) __ __.__ / __ __.__
Forearm (cm):	(R) __ __.__ / __ __.__	(L) __ __.__ / __ __.__
Arms(Flx):	(R) __ __.__ / __ __.__	male only
shoulders (cm):	__ __ __.__/ __ __ __.__	midax skf __ __.__
Chest (cm):	__ __ __.__/ __ __ __.__	__ __.__
Hip (cm):	__ __ __.__/ __ __ __.__	female only
Waist (cm):	__ __ __.__/ __ __ __.__	bicep skf __ __.__
Femur length	__ __.__/__ __.__	__ __.__

grip strength

	Right	Left
First try		
Second try		

One Repetition Max and Prescription

* Follow instructions for maximal test

	Max	80%		Max	80%
1. Chest press:	__ __	__ __	7. Tric. Extension:	__ __	__ __
2. Seated Row:	__ __	__ __	8. Arm curl:	__ __	__ __
3. Pull Down:	__ __	__ __	9. Low Back:	__ __	__ __
4. Shoulder Press:	__ __	__ __	10. Ab. Crunch:	__ __	__ __
5. Leg Extensions:	__ __	__ __	11. Incline Press:	__ __	__ __
6. Leg curl:	__ __	__ __	12. Leg Press	__ __	__ __

Sum Max _____ Avg% _____

APPENDIX E

GENES OF THE MTOR SIGNALING PATHWAY

Gene	SNP RS #	Ref-Homozygote	Heterozygote	Other-Homozygote	Ref-Allele	Other-Allele
INS	rs3842748	G/G (0.050)	C/G (0.040)	C/C (0.550)	G (0.250)	C (0.750)
IGF1	rs10860864	C/C (0.750)	C/T (0.183)	T/T (0.067)	C (0.842)	T (0.158)
	rs5742667	T/T (0.083)	C/T (0.283)	C/C (0.633)	T (0.225)	C (0.775)
	rs972936	A/A (0.083)	A/G (0.283)	G/G (0.633)	A (0.225)	G (0.775)
	rs2373721	G/G (0.083)	C/G (0.250)	C/C (0.667)	G (0.208)	C (0.792)
	rs11111272	G/G (0.119)	C/G (0.288)	C/C (0.593)	G (0.263)	C (0.737)
	rs2288378	A/A (0.083)	A/G (0.250)	G/G (0.667)	A (0.208)	G (0.792)
	rs10860865	T/T (0.085)	G/T (0.288)	G/G (0.627)	T (0.229)	G (0.771)
	rs7296464	G/G (0.085)	A/G (0.237)	A/A (0.678)	G (0.203)	A (0.797)
	rs7136446	C/C (0.233)	C/T (0.367)	T/T (0.400)	C (0.417)	T (0.583)
	rs10735380	G/G (0.083)	A/G (0.300)	A/A (0.617)	G (0.233)	A (0.767)
	rs9651925	G/G (0.000)	G/T (0.288)	T/T (0.712)	G (0.144)	T (0.856)
	rs9989002	A/A (0.083)	A/G (0.317)	G/G (0.600)	A (0.242)	G (0.758)
	rs4764697	T/T (0.083)	C/T (0.300)	C/C (0.617)	T (0.233)	C (0.767)
	rs5742632	T/T (0.650)	C/T (0.283)	C/C (0.067)	T (0.792)	C (0.208)
	rs2195240	T/T (0.650)	C/T (0.283)	C/C (0.067)	T (0.792)	C (0.208)
	rs2195239	G/G (0.650)	C/G (0.283)	C/C (0.067)	G (0.792)	C (0.208)
	rs5742629	A/A (0.567)	A/G (0.333)	G/G (0.100)	A (0.733)	G (0.267)
	rs7956547	T/T (0.593)	C/T (0.305)	C/C (0.102)	T (0.746)	C (0.254)
	rs10778176	C/C (0.567)	C/T (0.350)	T/T (0.083)	C (0.742)	T (0.258)
	rs1019731	G/G (0.667)	G/T (0.333)	T/T (0.000)	G (0.833)	T (0.167)
	rs10860869	A/A (0.576)	A/T (0.339)	T/T (0.085)	A (0.746)	T (0.254)
	rs17796225	T/T (0.600)	C/T (0.300)	C/C (0.100)	T (0.750)	C (0.250)
	rs12821878	G/G (0.517)	A/G (0.383)	A/A (0.100)	G (0.708)	A (0.292)
	rs2162679	G/G (0.000)	A/G (0.217)	A/A (0.783)	G (0.108)	A (0.892)

Gene	SNP RS #	Ref-Homozygote	Heterozygote	Other-Homozygote	Ref-Allele	Other-Allele
ERK1/2 (MAPK1)	rs9607287	A/A (0.317)	A/G (0.400)	G/G (0.283)	A (0.517)	G (0.483)
	rs3788332	G/G (0.317)	A/G (0.400)	A/A (0.283)	G (0.517)	A (0.483)
	rs2266969	G/G (0.383)	A/G (0.367)	A/A (0.250)	G (0.567)	A (0.433)
	rs2283793	T/T (0.383)	C/T (0.367)	C/C (0.250)	T (0.567)	C(0.433)
	rs9607298	G/G (0.383)	C/G (0.367)	C/C (0.250)	G (0.567)	C(0.433)
	rs1892846	A/A (0.317)	A/C (0.400)	C/C (0.283)	A (0.517)	C(0.483)
	rs1892848	T/T (0.317)	C/T (0.400)	C/C (0.283)	T (0.517)	C (0.483)
	rs9610374	G/G (0.397)	A/G (0.362)	A/A (0.241)	G (0.578)	A (0.422)
	rs9610375	G/G (0.317)	G/T (0.417)	T/T (0.267)	G (0.525)	T (0.475)
	rs5999749	A/A (0.383)	A/C (0.367)	C/C (0.250)	A (0.567)	C (0.433)
	rs5999752	G/G (0.383)	A/G (0.367)	A/A (0.250)	G (0.567)	A (0.433)
	rs17759796	C/C (0.783)	A/C (0.167)	A/A (0.050)	C (0.867)	A (0.133)
	rs9610417	C/C (0.600)	C/T (0.283)	T/T (0.117)	C (0.742)	T (0.258)
	rs5755694	T/T (0.233)	C/T (0.467)	C/C (0.300)	T (0.467)	C (0.533)
	rs8136867	G/G (0.267)	A/G (0.433)	A/A (0.300)	G (0.483)	A (0.517)
	rs2876981	A/A (0.433)	A/C (0.350)	C/C (0.217)	A (0.608)	C (0.392)
	rs9610470	T/T (0.567)	C/T (0.317)	C/C (0.117)	T (0.725)	C (0.275)
	rs11913721	A/A (0.400)	A/C (0.333)	C/C (0.267)	A (0.567)	C (0.433)
	rs2283794	T/T (0.333)	C/T (0.383)	C/C (0.283)	T (0.525)	C (0.475)
	rs9610487	T/T (0.567)	C/T (0.317)	C/C (0.117)	T (0.725)	C (0.275)
	rs4821402	G/G (0.317)	A/G (0.400)	A/A (0.283)	G (0.517)	A (0.483)
	rs9610496	A/A (0.600)	A/T (0.283)	T/T (0.117)	A (0.742)	T (0.258)
	rs5750113	C/C (0.333)	C/T (0.383)	T/T (0.283)	C (0.525)	T (0.475)
RSK (RPS6KA6)	rs1389465	A/A (0.150)	A/G (0.233)	G/G (0.617)	A (0.267)	G (0.733)
	rs11092122	G/G (0.153)	A/G (0.220)	A/A (0.627)	G (0.261)	A (0.739)
	rs1539517	G/G (0.150)	C/G (0.233)	C/C (0.617)	G (0.267)	C (0.733)
	rs1544221	T/T (0.350)	C/T (0.317)	C/C (0.333)	T (0.522)	C (0.478)
RSK (RPS6KA1)	rs921893	G/G (0.100)	A/G (0.300)	A/A (0.600)	G (0.250)	A (0.750)
	rs1466286	C/C (0.567)	C/G (0.383)	G/G (0.050)	C (0.758)	G (0.242)
	rs12723936	A/A (0.480)	A/G (0.500)	G/G (0.020)	A (0.730)	G (0.270)
	rs17162190	G/G (0.667)	A/G (0.317)	A/A (0.017)	G (0.825)	A (0.175)
	rs2278978	A/A (0.100)	A/G (0.300)	G/G (0.600)	A (0.250)	G (0.750)
	rs2278979	G/G (0.542)	A/G (0.441)	A/A (0.017)	G (0.763)	A (0.237)
	rs897641	C/C (0.583)	A/C (0.400)	A/A (0.017)	C (0.783)	A (0.217)
	rs3816540	A/A (0.650)	A/C (0.267)	C/C (0.083)	A (0.783)	C (0.217)
	rs3790645	G/G (0.583)	A/G (0.400)	A/A (0.017)	G (0.783)	A (0.217)
	rs444482	C/C (0.583)	C/G (0.400)	G/G (0.017)	C (0.783)	G (0.217)
	rs392814	A/A (0.600)	A/G (0.380)	G/G (0.020)	A (0.790)	G (0.210)
	rs369385	C/C (0.571)	C/T (0.411)	T/T (0.018)	C (0.777)	T (0.223)
	rs11577405	G/G (0.421)	G/T (0.491)	T/T (0.088)	G (0.667)	T (0.333)
	rs12402449	G/G (0.583)	A/G (0.400)	A/A (0.017)	G (0.783)	A (0.217)
	rs282175	T/T (0.567)	C/T (0.417)	C/C (0.017)	T (0.775)	C (0.225)
	rs188158	T/T (0.540)	C/T (0.440)	C/C (0.020)	T (0.760)	C (0.240)
	rs282176	A/A (0.576)	A/G (0.407)	G/G (0.017)	A (0.780)	G (0.220)
	rs282177	G/G (0.583)	A/G (0.400)	A/A (0.017)	G (0.783)	A (0.217)
	rs190737	G/G (0.271)	G/T (0.542)	T/T (0.186)	G (0.542)	T (0.458)

Gene	SNP RS #	Ref-Homozygote	Heterozygote	Other-Homozygote	Ref-Allele	Other-Allele
REDD1 (DDIT4)	rs1053639	T/T (0.483)	A/T (0.483)	A/A (0.033)	T (0.725)	A (0.275)
PIK3R1 (PI3K)	rs831125	A/A (0.797)	A/G (0.169)	G/G (0.034)	A (0.881)	G (0.119)
	rs3730089	G/G (0.648)	A/G (0.333)	A/A (0.019)	G (0.815)	A (0.185)
	rS12659907	G/G (0.817)	A/G (0.183)	A/A (0.000)	G (0.908)	A (0.092)
	rs895304	G/G (0.673)	A/G (0.309)	A/A (0.018)	G (0.827)	A (0.173)
	rs1445760	T/T (0.200)	C/T (0.517)	C/C (0.283)	T (0.458)	C (0.542)
	rs1043526	A/A (0.750)	A/G (0.250)	G/G (0.000)	A (0.875)	G (0.125)
	rs3756668	G/G (0.267)	A/G (0.533)	A/A (0.200)	G (0.533)	A (0.467)
PDPK1 (PDK)	rs7201584	T/T (0.70)	C/T (0.283)	C/C (0.017)	T (0.842)	C (0.158)
	rs12447555	C/C (0.767)	C/C (0.200)	G/G (0.033)	C (0.867)	G (0.133)
AKT1 (PKB)	rs2494731	G/G (0.467)	C/G (0.400)	C/C (0.133)	G (0.667)	C (0.333)
	rs3803304	G/G (0.633)	C/G (0.283)	C/C (0.083)	G (0.775)	C (0.225)
	rs3001371	C/C (0.467)	C/T (0.417)	T/T (0.117)	C (0.675)	T (0.325)
	rs1130214	G/G (0.517)	G/T (0.417)	T/T (0.067)	G (0.725)	T (0.275)
	rs2494735	A/A (0.414)	A/G (0.517)	G/G (0.069)	A (0.672)	G (0.328)
	rs1130233	G/G (0.677)	A/G (0.258)	A/A (0.065)	G (0.806)	A (0.194)
TSC1	rs1073123	A/A (0.783)	A/G (0.217)	G/G (0.000)	A (0.892)	G (0.108)
	rs7875422	G/G (0.817)	A/G (0.183)	A/A (0.000)	G (0.908)	A (0.092)
	rs7865232	T/T (0.776)	C/T (0.224)	C/C (0.000)	T (0.888)	C (0.112)
	rs7875558	G/G (0.783)	A/G (0.217)	A/A (0.000)	G (0.892)	A (0.108)
	rs4367688	G/G (0.250)	A/G (0.400)	A/A (0.350)	G (0.450)	A (0.550)
	rs7858160	C/C (0.767)	C/T (0.233)	T/T (0.000)	C (0.883)	T (0.117)
	rs13295430	G/G (0.817)	A/G (0.150)	A/A (0.033)	G (0.892)	A (0.108)
	rs13295634	G/G (0.083)	G/T (0.350)	T/T (0.567)	G (0.258)	T (0.742)
	rs10901224	T/T (0.817)	G/T (0.183)	G/G (0.000)	T (0.908)	G (0.092)
	rs3761840	A/A (0.283)	A/G (0.383)	G/G (0.333)	A (0.475)	G (0.525)
	rs3827665	C/C (0.817)	C/T (0.183)	T/T (0.000)	C (0.908)	T (0.092)
	rs10116061	C/C (0.250)	C/T (0.400)	T/T (0.350)	C (0.450)	T (0.550)
	rs4419933	T/T (0.083)	C/T (0.350)	C/C (0.567)	T (0.258)	C (0.742)
	rs7874234	C/C (0.633)	C/T (0.350)	T/T (0.017)	C (0.808)	T (0.192)
	rs4469592	G/G (0.817)	G/T (0.183)	T/T (0.000)	G (0.908)	T (0.092)
	rs883838	A/A (0.250)	A/C (0.400)	C/C (0.350)	A (0.450)	C (0.550)
	rs7026607	A/A (0.633)	A/G (0.350)	G/G (0.017)	A (0.808)	G (0.192)
TSC2	rs2073636	T/T (0.237)	C/T (0.492)	C/C (0.271)	T (0.483)	C (0.517)
	rs2074968	C/C (0.283)	C/G (0.483)	G/G (0.233)	C (0.525)	G (0.475)
	rs2074969	A/A (0.271)	A/G (0.475)	G/G (0.254)	A (0.508)	G (0.492)
	rs8063461	A/A (0.217)	A/G (0.533)	G/G (0.250)	A (0.483)	G (0.517)
	rs17654678	T/T (0.690)	G/T (0.276)	G/G (0.031)	T (0.828)	G (0.172)
	rs30259	G/G (0.661)	A/G (0.322)	A/A (0.017)	G (0.822)	A (0.178)

Gene	SNP RS #	Ref-Homozygote	Heterozygote	Other-Homozygote	Ref-Allele	Other-Allele
PRKAA2 (AMPK)	rs1124900	T/T (0.300)	G/T (0.533)	G/G (0.167)	T (0.567)	G (0.433)
	rs10789038	A/A (0.373)	A/G (0.492)	G/G (0.136)	A (0.619)	G (0.381)
	rs11206889	C/C (0.367)	C/T (0.500)	T/T (0.133)	C (0.617)	T (0.383)
	rs10711393	T/T (0.370)	A/T (0.481)	A/A (0.148)	T (0.611)	A (0.389)
	rs4912408	G/G (0.352)	A/G (0.407)	A/A (0.241)	G (0.556)	A (0.444)
	rs2143749	G/G (0.333)	C/G (0.433)	C/C (0.233)	G (0.550)	C (0.450)
	rs11206890	T/T (0.356)	C/T (0.508)	C/C (0.136)	T (0.610)	C (0.390)
	rs2746349	G/G (0.167)	A/G (0.533)	A/A (0.300)	G (0.433)	A (0.567)
	rs10889008	G/G (0.367)	A/G (0.500)	A/A (0.133)	G (0.617)	A (0.383)
	rs2796516	G/G (0.737)	A/G (0.193)	A/A (0.070)	G (0.833)	A (0.167)
	rs2796519	A/A (0.133)	A/G (0.500)	G/G (0.367)	A (0.383)	G (0.617)
	rs2746342	C/C (0.233)	A/C (0.433)	A/A (0.333)	C (0.450)	A (0.550)
	rs2746339	T/T (0.167)	C/T (0.533)	C/C (0.300)	T (0.433)	C (0.567)
	rs2796495	T/T (0.167)	C/T (0.533)	C/C (0.300)	T (0.433)	C (0.567)
	rs2796498	A/A (0.167)	A/G (0.533)	G/G (0.300)	A (0.433)	G (0.567)
	rs2092595	G/G (0.183)	G/T (0.500)	T/T (0.317)	G (0.433)	T (0.567)
	rs2796512	A/A (0.186)	A/G (0.508)	G/G (0.305)	A (0.441)	G (0.559)
	rs2796509	C/C (0.183)	C/T (0.500)	T/T (0.317)	C (0.433)	T (0.567)
	rs1418442	A/A (0.367)	A/G (0.500)	G/G (0.133)	A (0.617)	G (0.383)
	rs857155	A/A (0.186)	A/C (0.492)	C/C (0.322)	A (0.432)	C (0.568)
	rs932447	A/A (0.367)	A/G (0.500)	G/G (0.133)	A (0.617)	G (0.383)
LKB1	rs664254	G/G (0.330)	C/G (0.500)	C/C (0.200)	G (0.550)	C (0.450)
	rs673951	C/C (0.300)	C/T (0.483)	T/T (0.217)	C (0.542)	T (0.458)
	rs2305052	G/G (0.533)	G/T (0.333)	T/T (0.133)	G (0.700)	T (0.300)
STRAD (LYK5)	rs13380863	G/G (0.450)	A/G (0.450)	A/A (0.100)	G (0.675)	A (0.325)
	rs721575	G/G (0.217)	A/G (0.500)	A/A (0.283)	G (0.467)	A (0.533)
	rs9944483	T/T (0.418)	G/T (0.545)	G/G (0.036)	T (0.691)	G (0.039)
	rs3817182	T/T (0.119)	C/T (0.475)	C/C (0.407)	T (0.356)	C (0.644)
	rs2013288	C/C (0.450)	C/T (0.450)	T/T (0.100)	C (0.675)	T (0.325)
	rs16947051	G/G (0.433)	C/G (0.533)	C/C (0.033)	G (0.700)	C (0.300)
	rs1043127	A/A (0.133)	A/C (0.483)	C/C (0.383)	A (0.375)	C (0.625)
	rs1982400	T/T (0.133)	C/T (0.483)	C/C (0.383)	T (0.375)	C (0.625)
	rs7210724	T/T (0.107)	C/T (0.500)	C/C (0.393)	T (0.357)	C (0.643)
	rs1470697	T/T (0.133)	C/T (0.483)	C/C (0.383)	T (0.375)	C (0.625)
	rs8075422	G/G (0.458)	A/G (0.458)	A/A (0.085)	G (0.686)	A (0.314)
	rs2137144	G/G (0.123)	A/G (0.491)	A/A (0.386)	G (0.368)	A (0.632)
	rs6504177	G/G (0.133)	A/G (0.483)	A/A (0.383)	G (0.375)	A (0.625)
	rs6504179	T/T (0.117)	G/T (0.467)	G/G (0.417)	T (0.350)	G (0.650)

Gene	SNP RS #	Ref-Homozygote	Heterozygote	Other-Homozygote	Ref-Allele	Other-Allele
MO25 (CAB39)	rs10168275	C/C (0.633)	C/T (0.367)	T/T (0.000)	C (0.817)	T (0.183)
	rs2720162	G/G (0.200)	G/T (0.467)	T/T (0.333)	G (0.433)	T (0.567)
	rs1464205	T/T (0.400)	C/T (0.450)	C/C (0.150)	T (0.625)	C (0.375)
	rs7564854	G/G (0.733)	A/G (0.250)	A/A (0.017)	G (0.858)	A (0.142)
	rs6733332	T/T (0.627)	C/T (0.356)	C/C (0.017)	T (0.805)	C (0.195)
	rs6436972	A/A (0.633)	A/G (0.350)	G/G (0.017)	A (0.808)	G (0.192)
	rs6750664	G/G (0.633)	A/G (0.350)	A/A (0.017)	G (0.808)	A (0.192)
	rs2438296	G/G (0.150)	G/T (0.467)	T/T (0.383)	G (0.383)	T (0.617)
	rs3792076	G/G (0.633)	A/G (0.350)	A/A (0.017)	G (0.808)	A (0.192)
	rs3792074	A/A (0.633)	A/G (0.350)	G/G (0.017)	A (0.808)	G (0.192)
	rs2438298	A/A (0.617)	A/G (0.267)	G/G (0.117)	A (0.750)	G (0.250)
	rs3792069	G/G (0.733)	A/G (0.233)	A/A (0.033)	G (0.850)	A (0.150)
	rs3792071	G/G (0.633)	A/G (0.367)	A/A (0.000)	G (0.817)	A (0.183)
GβL	rs26863	G/G (0.267)	C/G (0.500)	C/C (0.233)	G (0.517)	C (0.483)
	rs26862	C/C (0.271)	C/G (0.475)	G/G (0.254)	C (0.508)	G (0.492)
MTOR (FRAP1)	rs11121695	G/G (0.533)	A/G (0.433)	A/A (0.033)	G (0.750)	A (0.250)
	rs11585553	G/G (0.533)	A/G (0.433)	A/A (0.033)	G (0.750)	A (0.250)
	rs10864490	G/G (0.533)	A/G (0.433)	A/A (0.033)	G (0.750)	A (0.250)
	rs6674994	T/T (0.033)	C/T (0.433)	C/C (0.533)	T (0.250)	C (0.750)
	rs4845982	T/T (0.019)	C/T (0.500)	C/C (0.481)	T (0.269)	C (0.731)
	rs2300095	T/T (0.067)	C/T (0.483)	C/C (0.450)	T (0.308)	C (0.692)
	rs1010447	T/T (0.050)	C/T (0.483)	C/C (0.467)	T (0.292)	C (0.708)
	rs4845856	T/T (0.050)	C/T (0.483)	C/C (0.467)	T (0.292)	C (0.708)
	rs7526649	C/C (0.050)	C/T (0.483)	T/T (0.467)	C (0.292)	T (0.708)
	rs7549109	G/G (0.050)	C/G (0.483)	C/C (0.467)	G (0.292)	C (0.708)
	rs10779751	A/A (0.050)	A/G (0.483)	G/G (0.467)	A (0.292)	G (0.708)
	rs12124983	T/T (0.050)	C/T (0.483)	C/C (0.467)	T (0.292)	C (0.708)
	rs1064261	C/C (0.050)	C/T (0.483)	T/T (0.467)	C (0.292)	T (0.708)
	rs2076655	G/G (0.050)	A/G (0.483)	A/A (0.467)	G (0.292)	A (0.708)
	rs11121704	C/C (0.052)	C/T (0.483)	T/T (0.466)	C (0.293)	T (0.707)
	rs718206	T/T (0.050)	A/T (0.483)	A/A (0.467)	T (0.292)	A (0.708)
	rs2024627	T/T (0.050)	C/T (0.483)	C/C (0.467)	T (0.292)	C (0.708)
	rs7544489	C/C (0.050)	C/T (0.483)	T/T (0.467)	C (0.292)	T (0.708)
	rs11121705	A/A (0.050)	A/G (0.500)	G/G (0.450)	A (0.300)	G (0.700)
	rs11121706	G/G (0.050)	A/G (0.483)	A/A (0.467)	G (0.292)	A (0.708)
	rs1205592	C/C (0.050)	C/T (0.483)	T/T (0.467)	C (0.292)	T (0.708)
	rs7364685	C/C (0.050)	C/T (0.483)	T/T (0.467)	C (0.292)	T (0.708)
	rs7525957	C/C (0.050)	C/T (0.483)	T/T (0.467)	C (0.292)	T (0.708)

Gene	SNP RS #	Ref-Homozygote	Heterozygote	Other-Homozygote	Ref-Allele	Other-Allele
RICTOR	rs13161443	A/A (0.615)	A/G (0.346)	G/G (0.038)	A (0.788)	G (0.212)
	rs13166875	A/A (0.400)	A/G (0.367)	G/G (0.233)	A (0.583)	G (0.417)
	rs1239274	G/G (0.339)	C/G (0.407)	C/C (0.254)	G (0.542)	C (0.458)
	rs1239276	T/T (0.367)	C/T (0.383)	C/C (0.250)	T (0.558)	C (0.442)
	rs10472314	A/A (0.085)	A/G (0.542)	G/G (0.373)	A (0.356)	G (0.644)
	rs9292729	C/C (0.103)	C/T (0.517)	T/T (0.379)	C (0.362)	T (0.638)
	rs7713236	G/G (0.100)	A/G (0.533)	A/A (0.367)	G (0.367)	A (0.633)
	rs6878291	G/G (0.373)	A/G (0.390)	A/A (0.237)	G (0.568)	A (0.432)
	rs10078168	G/G (0.100)	G/T (0.533)	T/T (0.367)	G (0.367)	T (0.633)
	rs13168966	C/C (0.367)	A/C (0.383)	A/A (0.250)	C (0.558)	A (0.442)
	rs7719775	A/A (0.100)	A/G (0.533)	G/G (0.367)	A (0.367)	G (0.633)
	rs12233987	T/T (0.417)	C/T (0.350)	C/C (0.233)	T (0.592)	C (0.408)
	rs10473128	A/A (0.083)	A/G (0.550)	G/G (0.367)	A (0.358)	G (0.642)
	rs13160161	G/G (0.417)	A/G (0.400)	A/A (0.183)	G (0.617)	A (0.383)
Rheb	rs3753151	C/C (0.167)	C/T (0.517)	T/T (0.317)	C (0.425)	T (0.575)
	rs13224450	G/G (0.070)	A/G (0.316)	A/A (0.614)	G (0.228)	A (0.772)
	rs2299965	C/C (0.217)	C/T (0.517)	T/T (0.267)	C (0.475)	T (0.525)
	rs7810890	T/T (0.217)	C/T (0.517)	C/C (0.267)	T (0.475)	C (0.525)
	rs11772458	G/G (0.231)	C/G (0.577)	C/C (0.192)	G (0.519)	C (0.481)
	rs3087741	A/A (0.150)	A/G (0.500)	G/G (0.350)	A (0.400)	G (0.600)
	rs2299967	T/T (0.220)	C/T (0.508)	C/C (0.271)	T (0.475)	C (0.525)
	rs3789817	G/G (0.217)	A/G (0.517)	A/A (0.267)	G (0.475)	A (0.525)
	rs758666	C/C (0.217)	C/T (0.517)	T/T (0.267)	C (0.475)	T (0.525)
	rs736645	G/G (0.217)	G/T (0.517)	T/T (0.267)	G (0.475)	T (0.525)
	rs4298422	G/G (0.211)	A/G (0.526)	A/A (0.263)	G (0.474)	A (0.526)
	rs2374261	C/C (0.217)	C/T (0.517)	T/T (0.267)	C (0.475)	T (0.525)
	rs12112989	C/C (0.217)	C/T (0.517)	T/T (0.267)	C (0.475)	T (0.525)
	rs6980020	T/T (0.217)	G/T (0.517)	G/G (0.267)	T (0.475)	G (0.525)
	rs12112134	A/A (0.224)	A/G (0.500)	G/G (0.276)	A (0.474)	G (0.526)
	rs6948196	T/T (0.217)	C/T (0.517)	C/C (0.267)	T (0.475)	C (0.525)
	rs6943752	A/A (0.237)	A/G (0.475)	G/G (0.288)	A (0.475)	G (0.525)
Raptor	rs7213696	G/G (0.317)	A/G (0.517)	A/A (0.167)	G (0.575)	A (0.425)
	rs4602089	A/A (0.491)	A/G (0.439)	G/G (0.070)	A (0.711)	G (0.289)
	rs12942526	A/A (0.190)	A/G (0.517)	G/G (0.293)	A (0.448)	G (0.552)
	rs12601089	C/C (0.483)	C/T (0.433)	T/T (0.083)	C (0.700)	T (0.300)
	rs9899782	G/G (0.600)	A/G (0.300)	A/A (0.100)	G (0.750)	A (0.250)
	rs4396582	A/A (0.183)	A/G (0.533)	G/G (0.283)	A (0.450)	G (0.550)
	rs9902639	A/A (0.183)	A/G (0.533)	G/G (0.283)	A (0.450)	G (0.550)
	rs4969230	T/T (0.350)	C/T (0.517)	C/C (0.133)	T (0.608)	C (0.392)
	rs2291359	G/G (0.492)	A/G (0.441)	A/A (0.068)	G (0.712)	A (0.288)
	rs4969235	A/A (0.350)	A/T (0.517)	T/T (0.133)	A (0.608)	T (0.392)
	rs9895584	A/A (0.600)	A/G (0.291)	G/G (0.109)	A (0.745)	G (0.255)

Gene	SNP RS #	Ref-Homozygote	Heterozygote	Other-Homozygote	Ref-Allele	Other-Allele
Raptor						
	rs12950541	G/G (0.483)	A/G (0.433)	A/A (0.083)	G (0.700)	A (0.300)
	rs12450464	G/G (0.607)	A/G (0.304)	A/A (0.089)	G (0.759)	A (0.241)
	rs9913009	T/T (0.617)	C/T (0.283)	C/C (0.100)	T (0.758)	C (0.242)
	rs9901049	T/T (0.610)	G/T (0.288)	G/G (0.102)	T (0.754)	G (0.246)
	rs12943552	C/C (0.483)	C/T (0.450)	T/T (0.067)	C (0.708)	T (0.292)
	rs9915518	G/G (0.610)	A/G (0.288)	A/A (0.102)	G (0.754)	A (0.246)
	rs7213638	C/C (0.600)	C/T (0.300)	T/T (0.100)	C (0.750)	T (0.250)
	rs9890313	C/C (0.600)	C/T (0.300)	T/T (0.100)	C (0.750)	T (0.250)
	rs9989484	T/T (0.593)	G/T (0.305)	G/G (0.102)	T (0.746)	G (0.254)
	rs9891139	A/A (0.600)	A/G (0.300)	G/G (0.100)	A (0.750)	G (0.250)
	rs9894401	G/G (0.600)	A/G (0.283)	A/A (0.117)	G (0.742)	A (0.258)
	rs9897830	A/A (0.579)	A/G (0.316)	G/G (0.105)	A (0.737)	G (0.263)
	rs9895847	G/G (0.625)	A/G (0.268)	A/A (0.107)	G (0.759)	A (0.241)
	rs4380096	A/A (0.467)	A/G (0.467)	G/G (0.067)	A (0.700)	G (0.300)
	rs7208831	G/G (0.600)	G/T (0.300)	T/T (0.100)	G (0.750)	T (0.250)
	rs7216268	C/C (0.600)	A/C (0.300)	A/A (0.100)	C (0.750)	A (0.250)
	rs9989446	A/A (0.600)	A/G (0.300)	G/G (0.100)	A (0.750)	G (0.250)
	rs7216295	C/C (0.593)	C/T (0.305)	T/T (0.102)	C (0.746)	T (0.254)
	rs4969404	C/C (0.333)	C/T (0.519)	T/T (0.148)	C (0.593)	T (0.407)
	rs12947507	G/G (0.500)	A/G (0.448)	A/A (0.052)	G (0.724)	A (0.276)
	rs9900956	T/T (0.600)	C/T (0.300)	C/C (0.100)	T (0.750)	C (0.250)
	rs7221492	C/C (0.600)	A/C (0.300)	A/A (0.100)	C (0.750)	A (0.250)
	rs12941973	C/C (0.500)	C/T (0.448)	T/T (0.052)	C (0.724)	T (0.276)
	rs8064506	T/T (0.600)	C/T (0.300)	C/C (0.100)	T (0.750)	C (0.250)
	rs12452243	A/A (0.593)	A/G (0.305)	G/G (0.102)	A (0.746)	G (0.254)
	rs8077901	G/G (0.621)	G/T (0.293)	T/T (0.086)	G (0.767)	T (0.233)
	rs7208536	C/C (0.610)	C/T (0.288)	T/T (0.102)	C (0.754)	T (0.246)
	rs7208971	A/A (0.593)	A/T (0.305)	T/T (0.102)	A (0.746)	T (0.254)
	rs9910524	C/C (0.600)	C/G (0.300)	G/G (0.100)	C (0.750)	G (0.250)
	rs9898483	T/T (0.586)	C/T (0.310)	C/C (0.103)	T (0.741)	C (0.259)
	rs9898212	A/A (0.600)	A/C (0.300)	C/C (0.100)	A (0.750)	C (0.250)
	rs9898441	A/A (0.600)	A/C (0.300)	C/C (0.100)	A (0.750)	C (0.250)
	rs6565480	A/A (0.600)	A/G (0.300)	G/G (0.100)	A (0.750)	G (0.250)
	rs11150746	A/A (0.473)	A/G (0.364)	G/G (0.164)	A (0.655)	G (0.345)
	rs4969266	C/C (0.583)	C/T (0.400)	T/T (0.017)	C (0.783)	T (0.217)
	rs11150747	C/C (0.612)	C/T (0.347)	T/T (0.041)	C (0.786)	T (0.214)
	rs4969426	A/A (0.125)	A/T (0.411)	T/T (0.464)	A (0.330)	T (0.667)
	rs719781	G/G (0.527)	A/G (0.418)	A/A (0.055)	G (0.736)	A (0.264)
	rs4969429	A/A (0.050)	A/G (0.367)	G/G (0.583)	A (0.233)	G (0.767)
	rs8069962	G/G (0.172)	A/G (0.534)	A/A (0.293)	G (0.440)	A (0.560)
	rs12953234	G/G (0.298)	A/G (0.509)	A/A (0.193)	G (0.553)	A (0.447)
	rs12601434	C/C (0.517)	C/G (0.433)	G/G (0.050)	C (0.733)	G (0.267)
	rs2589138	C/C (0.576)	C/T (0.390)	T/T (0.034)	C (0.771)	T (0.229)
	rs11654508	A/A (0.517)	A/G (0.433)	G/G (0.050)	A (0.733)	G (0.267)

Gene	SNP RS #	Ref-Homozygote	Heterozygote	Other-Homozygote	Ref-Allele	Other-Allele
Raptor						
	rs9905648	C/C (0.733)	C/G (0.200)	G/G (0.067)	C (0.833)	G (0.167)
	rs2672886	T/T (0.267)	C/T (0.550)	C/C (0.183)	T (0.542)	C (0.458)
	rs734338	G/G (0.300)	A/G (0.417)	A/A (0.283)	G (0.508)	A (0.492)
	rs2138125	T/T (0.283)	C/T (0.417)	C/C (0.300)	T (0.492)	C (0.508)
	rs2271612	C/C (0.133)	C/T (0.483)	T/T (0.383)	C (0.375)	T (0.625)
	rs4969311	C/C (0.117)	C/G (0.483)	G/G (0.400)	C (0.358)	G (0.642)
	rs9899051	A/A (0.118)	A/G (0.529)	G/G (0.353)	A (0.383)	G (0.618)
	rs868432	C/C (0.417)	C/T (0.433)	T/T (0.150)	C (0.633)	T (0.367)
HIF1α	rs2301106	T/T (0.810)	C/T (0.172)	C/C (0.017)	T (0.897)	C (0.103)
	rs1951795	A/A (0.017)	A/C (0.271)	C/C (0.712)	A (0.153)	C (0.847)
	rs12435848	A/A (0.017)	A/G (0.267)	G/G (0.717)	A (0.150)	G (0.850)
	rs12434438	G/G (0.017)	A/G (0.283)	A/A (0.700)	G (0.158)	A (0.842)
	rs11158358	G/G (0.017)	C/G (0.233)	C/C (0.750)	G (0.133)	C (0.867)
	rs2301111	G/G (0.017)	C/G (0.283)	C/C (0.695)	G (0.161)	C (0.839)
	rs10873142	C/C (0.018)	C/T (0.263)	T/T (0.719)	C (0.149)	T (0.851)
	rs2301113	C/C (0.017)	A/C (0.333)	A/A (0.650)	C (0.183)	A (0.817)
RPS6KB2 (P70S6K)	rs1476792	T/T (0.267)	C/T (0.55)	C/C (0.183)	T (0.542)	C (0.458)
	rs917570	C/C (0.317)	C/G (0.517)	G/G (0.167)	C (0.575)	G (0.425)
	rs1638588	C/C (0.267)	A/C (0.55)	A/A (0.183)	C (0.542)	A (0.458)
	rs1790753	G/G (0.267)	A/G (0.55)	A/A (0.183)	G (0.542)	A (0.458)
ATG1 (ULK3)	rs2290573	C/C (0.237)	C/T (0.424)	T/T (0.339)	C (0.449)	T (0.551)
	rs2290572	T/T (0.167)	C/T (0.400)	C/C (0.433)	T (0.367)	C (0.633)
	rs936227	A/A (0.153)	A/G (0.407)	G/G (0.441)	A (0.356)	G (0.644)
	rs936229	A/A (0.000)	A/G (0.327)	G/G (0.673)	A (0.163)	G (0.837)
	rs12908814	C/C (0.167)	C/G (0.426)	G/G (0.407)	C (0.380)	G (0.620)
VEGF-CD (FIGF)	rs6527518	G/G (0.500)	G/T (0.150)	T/T (0.350)	G (0.578)	T (0.422)
	rs6629030	A/A (0.717)	A/G (0.117)	G/G (0.167)	A (0.778)	G (0.222)
	rs6632474	T/T (0.717)	C/T (0.117)	C/C (0.167)	T (0.778)	C (0.222)
	rs6418686	T/T (0.633)	C/T (0.133)	C/C (0.233)	T (0.700)	C (0.300)
	rs6629049	G/G (0.732)	G/T (0.125)	T/T (0.143)	G (0.791)	T (0.209)
	rs4830939	A/A (0.300)	A/G (0.233)	G/G (0.467)	A (0.422)	G (0.578)
	rs6632519	C/C (0.660)	C/T (0.170)	T/T (0.170)	C (0.763)	T (0.237)
VEGF-AB (PGF)	rs1042886	C/C (0.778)	A/C (0.204)	A/A (0.019)	C (0.880)	A (0.120)
	rs8185	A/A (0.678)	A/G (0.305)	G/G (0.017)	A (0.831)	G (0.169)
	rs12411	T/T (0.018)	A/T (0.327)	A/A (0.655)	T (0.182)	A (0.818)
	rs2359192	C/C (0.018)	A/C (0.357)	A/A (0.625)	C (0.196)	A (0.804)
	rs2268614	C/C (0.367)	C/T (0.517)	T/T (0.117)	C (0.625)	T (0.375)
	rs2268615	C/C (0.350)	A/C (0.517)	A/A (0.133)	C (0.608)	A (0.392)

Gene	SNP RS #	Ref-Homozygote	Heterozygote	Other-Homozygote	Ref-Allele	Other-Allele
EIF4B						
	rs17691581	A/A (0.448)	A/G (0.448)	G/G (0.103)	A (0.672)	G (0.328)
	rs2037415	G/G (0.842)	A/G (0.158)	A/A (0.000)	G (0.921)	A (0.079)
	rs12582526	C/C (0.667)	C/T (0.300)	T/T (0.033)	C (0.817)	T (0.183)
	rs17122378	T/T (0.842)	C/T (0.158)	C/C (0.000)	T (0.921)	C (0.079)
S6 (RPS6)	rs957	C/C (0.817)	C/T (0.183)	T/T (0.000)	C (0.908)	T (0.092)
EIF4E	rs4699369	G/G (0.517)	A/G (0.433)	A/A (0.050)	G (0.733)	A (0.267)
	rs4699689	C/C (0.564)	C/T (0.382)	T/T (0.055)	C (0.755)	T (0.245)
	rs6830125	G/G (0.362)	C/G (0.517)	C/C (0.121)	G (0.621)	C (0.379)
	rs13110878	A/A (0.517)	A/C (0.433)	C/C (0.050)	A (0.733)	C (0.267)
	rs11735758	T/T (0.517)	A/T (0.433)	A/A (0.050)	T (0.733)	A (0.267)
	rs11736580	T/T (0.517)	C/T (0.433)	C/C (0.050)	T (0.733)	C (0.267)
	rs10023577	G/G (0.367)	A/G (0.500)	A/A (0.133)	G (0.617)	A (0.383)
	rs12498533	A/A (0.367)	A/C (0.500)	C/C (0.133)	A (0.617)	C (0.383)
	rs17570252	T/T (0.525)	C/T (0.424)	C/C (0.051)	T (0.737)	C (0.263)
	rs17028241	A/A (0.845)	A/G (0.155)	G/G (0.000)	A (0.922)	G (0.078)
	rs17583053	C/C (0.517)	C/T (0.433)	T/T (0.050)	C (0.733)	T (0.267)
	rs6852715	C/C (0.367)	C/T (0.500)	T/T (0.133)	C (0.617)	T (0.383)
	rs11727079	A/A (0.508)	A/G (0.441)	G/G (0.051)	A (0.729)	G (0.271)
	rs11727086	A/A (0.517)	A/G (0.433)	G/G (0.050)	A (0.733)	G (0.267)
					Source: Hapmap.org	

APPENDIX F

INDIVIDUAL COMPONENTS OF ARM MASS-ADJUSTED STRENGTH BY GENOTYPE

Table 21Individual Components of Arm Mass-Adjusted Strength by rs2494735 Genotype

	AA	AG	GG
Pre-RET 1 RM Biceps (kg)	40.34 ± 21.74	40.08 ± 22.12	40.00 ± 19.32
Post-RET 1 RM Biceps (kg)	53.40 ± 23.67	55.76 ± 25.27	54.69 ± 23.05
Pre-RET 1 RM Triceps (kg)	49.10 ± 21.00	51.78 ± 23.30	51.25 ± 20.78
Post-RET 1 RM Triceps (kg)	68.06 ± 23.78	70.25 ± 26.15	68.43 ± 23.86
Pre-RET Arm Circ. (cm)	29.68 ± 4.41	29.98 ± 3.55	30.67 ± 3.61
Post-RET Arm Circ. (cm)	30.70 ± 3.97	31.18 ± 3.48	32.24 ± 3.55

Arm mass-adjusted strength = (1 RM biceps + 1 RM triceps) / arm circumference.
Values are Means ± SD

Table 22 Individual Components of Arm Mass-Adjusted Strength by rs1130233 Genotype

	GG	AG	AA
Pre-RET 1 RM Biceps (kg)	42.34±22.50	37.29±20.50	38.13±18.31
Post-RET 1 RM Biceps (kg)	56.72±24.42	52.29±23.43	53.75±26.56
Pre-RET 1 RM Triceps (kg)	51.88±21.87	48.96±22.50	50.00±21.88
Post-RET 1 RM Triceps (kg)	70.55±23.99	67.50±25.76	68.75±25.17
Pre-RET Arm Circ. (cm)	29.83±4.07	30.04±3.55	30.67±3.99
Post-RET Arm Circ. (cm)	30.95±3.75	31.25±3.43	32.10±4.27

Arm mass-adjusted strength = (1 RM biceps + 1 RM triceps) / arm circumference.
Values are Means ± SD

Table 23 Individual Components of Arm Mass-Adjusted Strength by rs3001371 Genotype

	CC	CT	TT
Pre-RET 1 RM Biceps (kg)	41.81±22.45	38.98±21.66	38.86±17.59
Post-RET 1 RM Biceps (kg)	55.45±23.93	54.55±25.19	53.86±22.14
Pre-RET 1 RM Triceps (kg)	51.27±21.91	50.68±23.09	49.55±20.70
Post-RET 1 RM Triceps (kg)	70.18±24.38	68.64±26.09	69.09±23.89
Pre-RET Arm Circ. (cm)	29.86±4.26	29.64±3.78	31.02±2.89
Post-RET Arm Circ. (cm)	30.95±3.95	30.68±3.54	32.74±3.07

Arm mass-adjusted strength = (1 RM biceps + 1 RM triceps) / arm circumference.
Values are Means ± SD

Table 24 Individual Components of Arm Mass-Adjusted Strength by rs1130214 Genotype

	GG	GT	TT
Pre-RET 1 RM Biceps (kg)	43.09±21.07	38.77±21.03	33.84±23.02
Post-RET 1 RM Biceps (kg)	58.36±24.51	52.45±23.40	47.30±24.29
Pre-RET 1 RM Triceps (kg)	54.45±22.35	48.40±21.16	44.62±22.50
Post-RET 1 RM Triceps (kg)	75.00±24.57	65.85±24.54	59.62±22.95
Pre-RET Arm Circ. (cm)	30.49±4.33	29.65±3.49	29.28±3.29
Post-RET Arm Circ. (cm)	31.60±4.07	30.95±3.42	30.39±3.16

Arm mass-adjusted strength = (1 RM biceps + 1 RM triceps) / arm circumference.
Values are Means ± SD

Table 25 Individual Components of Arm Mass-Adjusted Strength by rs1124900 Genotype

	TT	GT	GG
Pre-RET 1 RM Biceps (kg)	38.64±19.25	42.05±25.54	38.71±19.92
Post-RET 1 RM Biceps (kg)	54.69±24.14	56.16±24.42	52.90±23.87
Pre-RET 1 RM Triceps (kg)	50.76±21.18	52.14±23.25	48.22±21.31
Post-RET 1 RM Triceps (kg)	70.76±26.31	69.55±24.70	67.90±24.31
Pre-RET Arm Circ. (cm)	30.37±3.75	29.80±4.03	29.78±3.86
Post-RET Arm Circ. (cm)	31.64±3.83	31.05±3.72	30.84±3.73

Arm mass-adjusted strength = (1 RM biceps + 1 RM triceps) / arm circumference.
Values are Means ± SD

Table 26 Individual Components of Arm Mass-Adjusted Strength by rs2796516 Genotype

	GG	AG	AA*
Pre-RET 1 RM Biceps (kg)	39.76±21.17	41.36±22.44	50.00
Post-RET 1 RM Biceps (kg)	54.35±23.85	55.15±23.89	100.00
Pre-RET 1 RM Triceps (kg)	50.58±22.68	50.75±20.35	70.00
Post-RET 1 RM Triceps (kg)	69.52±25.60	67.58±21.87	110.00
Pre-RET Arm Circ. (cm)	30.01±3.76	29.71±4.04	36.45
Post-RET Arm Circ. (cm)	31.16±3.53	30.91±3.84	38.60

Arm mass-adjusted strength = (1 RM biceps + 1 RM triceps) / arm circumference. Values are Means ± SD. * AA genotype N = 1.

Table 27 Individual Components of Arm Mass-Adjusted Strength by rs1418442 Genotype

	AA	AG	GG
Pre-RET 1 RM Biceps (kg)	37.65±19.90	41.89±22.62	38.00±16.97
Post-RET 1 RM Biceps (kg)	52.35±24.25	55.74±23.54	54.50±24.86
Pre-RET 1 RM Triceps (kg)	48.97±20.07	51.97±23.24	48.50±20.46
Post-RET 1 RM Triceps (kg)	67.94±25.71	69.67±23.77	69.00±26.18
Pre-RET Arm Circ. (cm)	29.80±3.76	29.79±4.17	30.27±3.64
Post-RET Arm Circ. (cm)	30.92±3.60	30.99±3.89	31.46±3.72

Arm mass-adjusted strength = (1 RM biceps + 1 RM triceps) / arm circumference. Values are Means ± SD

APPENDIX G

INDIVIDUAL COMPONENTS OF LEG MASS-ADJUSTED STRENGTH BY GENOTYPE

Table 28 Individual Components of Leg Mass-Adjusted Strength by rs2494735 Genotype

	AA	AG	GG
Pre-RET 1 RM Hamstring (kg)	90.23±32.01	93.56±31.88	91.56±37.22
Post-RET 1 RM Hamstring (kg)	108.07±35.75	113.98±36.89	112.81±34.06
Pre-RET 1 RM Leg Ext. (kg)	165.38±55.55	174.78±62.62	158.48±69.74
Post-RET 1 RM Leg Ext. (kg)	235.48±62.36	247.56±80.09	221.89±78.27
Pre-RET Thigh Circ. (cm)	55.17±5.67	54.96±5.47	55.84±5.91
Post-RET Thigh Circ. (cm)	55.18±5.22	55.97±5.14	57.22±5.71

Leg mass-adjusted strength = (1 RM hamstring curl + 1 RM leg extension) / thigh circumference. Values are Means ± SD.

Table 29 Individual Components of Leg Mass-Adjusted Strength by rs1130233 Genotype

	GG	AG	AA
Pre-RET 1 RM Hamstring (kg)	93.67±31.59	89.79±33.15	91.25±36.42
Post-RET 1 RM Hamstring (kg)	113.67±37.25	109.06±33.30	110.62±39.32
Pre-RET 1 RM Leg Ext. (kg)	173.86±59.38	162.52±58.07	175.08±87.98
Post-RET 1 RM Leg Ext. (kg)	246.38±68.25	233.94±76.00	235.34±100.45
Pre-RET Thigh Circ. (cm)	54.96±5.56	55.71±5.67	54.89±4.48
Post-RET Thigh Circ. (cm)	55.54±5.09	56.38±5.46	56.03±4.50

Leg mass-adjusted strength = (1 RM hamstring curl + 1 RM leg extension) / thigh circumference. Values are Means ± SD.

Table 30 Individual Components of Leg Mass-Adjusted Strength by rs3001371 Genotype

	CC	CT	TT
Pre-RET 1 RM Hamstring (kg)	94.00±32.93	88.63±33.49	95.00±31.32
Post-RET 1 RM Hamstring (kg)	111.73±35.71	111.70±37.69	112.50±34.52
Pre-RET 1 RM Leg Ext. (kg)	170.42±57.74	164.52±58.64	177.60±74.52
Post-RET 1 RM Leg Ext. (kg)	244.93±68.98	236.46±78.66	240.94±80.63
Pre-RET Thigh Circ. (cm)	54.97±5.58	55.04±5.60	56.46±5.42
Post-RET Thigh Circ. (cm)	55.31±5.02	55.71±5.33	57.91±5.18

Leg mass-adjusted strength = (1 RM hamstring curl + 1 RM leg extension) / thigh circumference. Values are Means ± SD.

Table 31 Individual Components of Leg Mass-Adjusted Strength by rs1130214 Genotype

	GG	GT	TT
Pre-RET 1 RM Hamstring (kg)	96.91±34.26	90.19±30.03	81.53±34.84
Post-RET 1 RM Hamstring (kg)	118.18±38.65	107.55±33.71	101.92±30.72
Pre-RET 1 RM Leg Ext. (kg)	182.33±67.34	162.29±53.40	145.36±53.69
Post-RET 1 RM Leg Ext. (kg)	254.49±75.21	235.51±71.30	204.04±72.67
Pre-RET Thigh Circ. (cm)	56.47±5.75	54.72±5.30	52.97±3.61
Post-RET Thigh Circ. (cm)	56.86±5.57	55.45±5.00	54.07±3.56

Leg mass-adjusted strength = (1 RM hamstring curl + 1 RM leg extension) / thigh circumference. Values are Means ± SD.

Table 32 Individual Components of Leg Mass-Adjusted Strength by rs1124900 Genotype

	TT	GT	GG
Pre-RET 1 RM Hamstring (kg)	91.21±33.38	92.68±33.60	92.90±31.80
Post-RET 1 RM Hamstring (kg)	110.90±37.24	113.48±34.94	110.97±37.18
Pre-RET 1 RM Leg Ext. (kg)	179.64±74.43	168.70±59.88	162.88±44.87
Post-RET 1 RM Leg Ext. (kg)	256.53±84.09	238.10±71.60	233.29±66.16
Pre-RET Thigh Circ. (cm)	56.16±4.94	55.00±5.89	54.64±5.68
Post-RET Thigh Circ. (cm)	57.22±4.97	55.61±5.36	55.12±5.21

Leg mass-adjusted strength = (1 RM hamstring curl + 1 RM leg extension) / thigh circumference. Values are Means ± SD.

Table 33 Individual Components of Leg Mass-Adjusted Strength by rs2796516 Genotype

	GG	AG	AA*
Pre-RET 1 RM Hamstring (kg)	92.35±32.84	91.52±31.19	115.00
Post-RET 1 RM Hamstring (kg)	110.71±35.81	113.18±35.51	160.00
Pre-RET 1 RM Leg Ext. (kg)	167.52±60.47	174.39±63.13	205.00
Post-RET 1 RM Leg Ext. (kg)	234.63±72.13	251.45±75.97	373.30
Pre-RET Thigh Circ. (cm)	55.18±5.23	54.69±5.91	69.25
Post-RET Thigh Circ. (cm)	55.66±4.91	55.81±5.41	71.58

Leg mass-adjusted strength = (1 RM hamstring curl + 1 RM leg extension) / thigh circumference. Values are Means ± SD. * AA genotype N = 1.

Table 34 Individual Components of Leg Mass-Adjusted Strength by rs1418442 Genotype

	AA	AG	GG
Pre-RET 1 RM Hamstring (kg)	88.82±32.48	91.39±32.43	98.50±32.89
Post-RET 1 RM Hamstring (kg)	109.41±39.50	112.30±33.97	111.75±38.43
Pre-RET 1 RM Leg Ext. (kg)	174.91±65.12	163.23±57.80	178.26±65.09
Post-RET 1 RM Leg Ext. (kg)	245.15±77.14	229.76±69.23	258.15±80.32
Pre-RET Thigh Circ. (cm)	55.44±4.89	54.54±5.77	56.26±6.16
Post-RET Thigh Circ. (cm)	56.04±4.81	55.24±5.36	56.75±5.74

Leg mass-adjusted strength = (1 RM hamstring curl + 1 RM leg extension) / thigh circumference. Values are Means ± SD.

BIBLIOGRAPHY

1. **Brooks GA, Fahey TD, White TP and Baldwin KM.** Principles Of Skeletal Muscle Adaptations. In: *Exercise Physiology: Human Bioenergenics and Its Applications 3rd. ed.* Mountain View, CA: Mayfield Publishing Company. 2000.

2. **Nieman DC.** The Acute and Chronic Effects of Physical Activity. In: *Fitness and Sports Medicine: A Health-Related Approach 3rd ed.* Mountain View, CA: Mayfield Publishing Company. 188-191, 1995.

3. **McCall GE, Byrnes WC, Dickinson A, Pattany PM and Fleck SJ.** Muscle fiber hypertrophy, hyperplasia, and capillary density in college men after resistance training. *J. Appl. Physiol.* 81:2004-2012, 1996.

4. **MacDougall JD.** Morphological changes in human skeletal muscle following strength training and immobilization. In: *Human Muscle Power,* N.L. Jones, J. MacCartney, and A.J. McComas (Eds.). Champaign Il: Human Kinetics. 269-285, 1986

5. **Voet D, Voet JG, and Pratt CW.** Amino Acid Metabolism. In: *Fundamentals Of Biochemistry.* John Wiley and Sons Inc. New York, NY. 611-654, 1999.

6. **Kimball SR, Farrell PA, and Jefferson LS.** Invited Review: role of insulin in translational control of protein synthesis in skeletal muscle by amino acids or exercise. *J. Appl. Physiol.* 93:1168-1180, 2002.

7. **Goodman MN.** Amino acid and protein metabolism. In: E.S. Horton & R.L. Terjung (Eds.), *Exercise, nutrition, and energy metabolism.* New York: Macmillan. 88-89, 1988.

8. **Tidball JG.** Mechanical signal transduction in skeletal muscle growth and adaptation. *J. Appl. Physiol.* 98:1900-1908, 2005.

9. **Bassel-Duby R, and Olsen EN.** Signaling Pathways in Skeletal Muscle Remodeling. *Annu. Rev. Biochem.* 75:19-37, 2006.

10. **Goldspink DF.** *et al.* Protein turnover measured *in vivo* and *in vitro* in muscles undergoing compensatory growth and subsequent denervation atrophy. *Biochem J.* 210: 89-98, 1983.

11. **Faridi J, Fawcett J, Wang L, and Roth RA.** Akt promotes mammalian cell size by stimulating protein synthesis and inhibiting protein degradation. *Am J Physiol Endocrinol Metab* 285: 964-972, 2003.

12. **Watson JD, Gilman M, Witkowski J, and Zoller M.** DNA is the Primary Genetic Material. In: *Recombinant DNA.* W.H. Freeman and Company, New York, NY. 13-30, 2001.

13. **Rommel C, Bodine SC, Clarke BA, Rossman R, Nunez L, Stitt T, Yancopoulos GD, and Glass DJ.** Mediation of IGF-1-induced skeletal myotube hypertrophy by PI(3)K/AKT/mTOR and PI(3)K/AKT/GSK3 pathways. *Nature Cell Biology* 3: 1009-1013, 2001.

14. **Glass DJ.** Signaling pathways that mediate skeletal muscle hypertrophy and atrophy. *Nature Cell Biology* 5: 87-90, 2003.

15. **Mora A, Komander D, van Aalten D, and Alessi DR.** PDK1, the master regulator of AGC kinase signal transduction. *Seminars in Cell & Developmental Biology* 15: 161-170, 2004.

16. **Song YH, Godard M, Li Y, Richmond SR, Rosenthal N, and Delafontaine P.** Insulin-Like Growth Factor 1-Mediated Skeletal Muscle Hypertrophy Is Characterized by Increased mTOR-p70S6K Signaling without Increased AKT Phosphorylation. *Journal of Investigative Medicine.* 53(3): 135-142, 2005.

17. **Nader GA.** Molecular determinants of skeletal muscle mass: getting the "AKT" together. *The International Journal of Biochemistry & Cell Biology.* 37: 1985-1996, 2005.

18. **Vivanco J, and Sawyers CL.** The phosphatidylinositol 3-kinase AKT pathway in human cancer. *Nature Rev. Cancer* 2: 489-501, 2002.

19. **Dreyer HC, Fujita S, Cadenas JG, Chinkes DL, Volpi E, and Rasmussen BB.** Resistance exercise increases AMPK activity and reduces 4E-BP1 phosphorylation and protein synthesis in human skeletal muscle. *J Physiol.* 576.2:613-624, 2006.

20. **Bolster DR, Kubica N, Crozier SJ, Williamson DL, Farrell PA, Kimball SR** *et al.* Immediate response of mammalian target of rapamycin (mTOR)-mediated signaling following acute resistance exercise in rat skeletal muscle. *J. Physiol.* 553(pt 1): 213-220, 2003.

21. **Bodine SC,** *et al.* AKT/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy *in vivo. Nature Cell Biol.* 3: 1014-1019, 2001.

22. **Hardt SE and Sadoshima J.** Glycogen synthase kinase-3β: a novel regulator of cardiac hypertrophy and development. *Circ Res.* 90: 1055-1063, 2002.

23. **Zhang H, Stallock JP, Ng JC, Reinhard C, and Neufeld TP.** Regulation of cellular growth by the *Drosophila* target of rapamycin dTOR. *Genes Dev.* 14: 2712-2724, 2000.

24. **Ohanna M,** *et al.* Atrophy of S6K1$^{-/-}$ skeletal muscle cells reveals distinct mTOR effectors for cell cycle and size control. *Nature Cell Biology.* 7(3): 286-294, 2005.

25. **Hara K,** *et al.* Regulation of eIF-4E BP1 phosphorylation by mTOR. *J. Biol. Chem.* 272: 26457-26463, 1997.

26. **Sarbassov DD, Ali SM, and Sabatini DM.** Growing roles for the mTOR pathway. *Current Opinion in Cell Biology.* 17: 596-603, 2005.

27. **Sarbassov DD, Guertin DA, Ali SM, and Sabatini DM.** Phosphorylation and Regulation of AKT/PKB by the Rictor-mTOR Complex. *Science.* 307: 1098-1101, 2005.

28. **Birk JP, and Wojtaszewski FP.** Predominant α2/β2/γ3 AMPK Activation during Exercise in Human Skeletal Muscle. *The Journal of Physiology*. 10:1113-1120. 2006.

29. **Hahn-Windgassen A, Nogueira V, Chen CC, Skeen JE, Sonenberg N, and Hay N.** AKT activates the mammalian target of rapamycin by regulating cellular ATP level and AMPK activity. *J Biol Chem.* 280(37): 32081-32089, 2005.

30. **Winder WW, Taylor EB, Thomson DM.** Role of AMP-activated protein kinase and mTOR signaling pathways. *Med. Sci. Sports Exerc.* 38(11): 1945-1949, 2006.

31. **Spencer-Jones NJ, Ge D, Snieder H, Perks U, Swaminathan TD, Spector TD, Carter ND and O'Dell SD.** AMP-kinase α2 subunit gene PRKAA2 variants are associated with total cholesterol, low-density lipoprotein-cholesterol and high-density lipoprotein-cholesterol in normal women. *J Med Genet.* 43: 936-942, 2006.

32. **Riechman SE, Fabian TJ, Kroboth PD, and Ferrell RE.** Steroid sulfatase gene variation and DHEA responsiveness to resistance exercise in MERET. *Physiol Genomics* 17: 300-306, 2004.

33. **Riechman SE, Balasekaran G, Roth SM, and Ferrell RE.** Association of interleukin-15 protein and interleukin-15 receptor genetic variation with resistance exercise training responses
J Appl Physiol. 97: 2214 – 2219, 2004.

34. **Kraemer WJ, Adams K, Cafarelli et*.al.*** American College of Sports medicine Position Stand: Progression Models in Resistance Training for Healthy Adults. *Med. Sci. Sports Exerc.* 34(2): 364-380, 2002.

35. **Dudley GA, Tesch PA, Harris RT, Golden CL, and Buchanan P.** Influence of eccentric actions on the metabolic cost of resistance exercise. *Aviat. Space Environ. Med.* 62:678-682, 1991.

36. **Bandy WD, and Hanten WP.** Changes in torque and electromyographic activity of the quadriceps femoris muscles following isometric training. *Med. Sci. Sports Exerc.* 73:455-467, 1993.

37. **Fleck SJ, and Kraemer WJ.** *Designing Resistance Training Programs,* 2nd Ed. Champaign, IL: Human Kinetics, 1997, 1-115.

38. **Kraemer WJ, Noble BJ, Clark MJ, and Culver BW.** Physiologic responses to heavy-resistance exercise with very short rest periods. *Int. J. Sports Med.* 8:247-252, 1987.

39. **Hakkinen KA, Pakarinen A, Alen M, and Komi PV.** Serum hormones during prolonged training of neuromuscular performance. *Eur. J. Appl. Physiol.* 53:287-293, 1985.

40. **Schlumberger A, Stec J, Schmidtbleicher D.** Single vs. multiple- set strength training in women. *J. Strength Cond. Res.* 15:284-289, 2001.

41. **Stone MH, Potteiger JA, and Pierce KC, et al.** Comparison of the effects of three different weight-training programs on the one repetition maximum squat. *J. Strength Cond. Res.* 14:332-337, 2000.

42. **Hakkinen K.** Factors influencing trainability of muscular strength during short term and prolonged training. *NSCA J.* 7:32-34, 1985.

43. **Morganti CM, Nelson ME, Fiatarone MA, et al.** Strength improvements with 1 yr of progressive resistance training in older women. *Med. Sci. Sports Exerc.* 27:906-912, 1995.

44. **Hickson RC, Hidaka K, and Foster C.** Skeletal muscle fiber type, resistance training, and strength-related performance. *Med. Sci. Sports Exerc.* 26:593-598, 1994.

45. **O'Bryant HS, Byrd R, and Stone MH.** Cycle ergometer performance and maximum leg and hip strength adaptations to two different methods of weight training. *J. Appl. Sport Sci. Res.* 2:27-30, 1988.

46. **Kraemer WJ, and Fleck SJ.** Resistance training: exercise prescription (part 4 of 4). *Phys. Sports Med.* 16:69-81, 1988.

47. **Cureton KJ, Collins MA, Hill DW, and Mcelhannon FM.** Muscle hypertrophy in men and women. *Med. Sci. Sports Exerc.* 20(4): 338-344, 1988.

48. **Kraemer WJ.** Endocrine responses and adaptations to strength training. In: *Strength and Power in Sport,* PV Komi (Ed.). Boston: Blackwell Scientific Publications, 291-304, 1992.

49. **Hubal MJ, Gordish-Dressman H, Thompson PD, et al.**Variability in Muscle Size and Strength Gain after Unilateral Resistance Training. *Med. Sci. Sports Exerc.* 37(6):964-972, 2005.

50. **Siri WE.** The gross composition of the body. In: *Advances in Biological and Medical Physics IV*, edited by C. A. Tobias, and J. H. New York: Academic, 1956.

51. **Weidman DA, Tesh JC, Castek JE, and Buckmeyer PJ.** A formula for the prediction of residual volume in males. *Medicine and Science in Sports and Exercise.* 19(2): S31.

52. **Russell DD.** A new formula for the prediction of residual volume in females. Unpublished Masters Thesis, University of Wisconsin Lacrosse. 1987.

53. **Miller SA, Dykes DD, and Pulesky HF.** A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 16:1215, 1988.