ABSTRACT

Title of Dissertation: INSULIN-LIKE GROWTH FACTOR 1

GENOTYPE INFLUENCES MUSCLE STRENGTH

RESPONSE TO STRENGTH TRAINING IN OLDER

ADULTS

Matthew Christopher Kostek, Doctor of Philosophy, 2004

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Strength training (ST) is considered an intervention of choice for the

prevention and treatment of sarcopenia. The insulin-like growth factor 1 protein

(IGF-I) plays a major role in ST-induced skeletal muscle hypertrophy and strength

improvements. A microsatellite repeat in the promoter region of the IGF1 gene has

been associated with IGF-I blood levels and phenotypes related to IGF-I in adult men

and women. To examine the influence of this polymorphism on muscle hypertrophic

and strength responses to strength training (ST), we studied 67 Caucasian men and

women before and after a 10-week single leg knee extension ST program. One

repetition maximum (1RM) strength, muscle volume (MV) via computed tomography

(CT), and muscle quality (MQ) were assessed at baseline and after 10 weeks of training. The IGF1 repeat promoter polymorphism and three single nucleotide polymorphisms (SNP) were genotyped. For the promoter polymorphism, subjects were grouped as homozygous for the 192 allele, heterozygous, or non-carriers of the 192 allele. After 10 weeks of training, 1RM, MV, and MQ increased significantly for all groups combined (P < 0.001). However, carriers of the 192 allele gained significantly more strength with ST than non-carriers of the 192 allele (P = 0.02). There was also a non-significant trend for a greater increase in MV in 192 carriers than non-carriers (P = 0.08). No significant associations were observed for the other polymorphisms studied. Thus, these data suggest that the IGF1 promoter polymorphism may influence the strength response to ST. Larger sample sizes should be used in future studies to verify these results.

INSULIN-LIKE GROWTH FACTOR 1 GENOTYPE INFLUENCES MUSCLE

STRENGTH RESPONSE TO STRENGTH TRAINING IN OLDER ADULTS

by

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INTRODUCTION

Human muscle strength declines at the rate of ~12-14% per decade after the age of ~50 yr. (116,121). This loss of strength with age is due to many factors, but is primarily attributed to a loss of muscle mass (60,167), termed sarcopenia. Because sarcopenia is related to a loss of functional abilities (162), dependency (163), increased risk of falls and fractures (4,118), and decreased bone mineral density (191), it has negative consequences for the health status and functional abilities of older adults.

Several interventions have been proposed for the prevention and treatment of sarcopenia, but it appears strength training (ST) is the most effective with the least negative side effects (15,81). Large increases in strength and muscle mass can result in a relatively short time with ST, but these changes are highly variable (83). For example, in a ST study from our laboratory, 65-75 year old men and women varied in their strength increases from 5 – 59% (5 – 86 lbs.) and muscle volume (MV) increases from 1 – 20% (19 – 344 cubic cm) (83,109). This variation in muscle adaptation to training may suggest a genetic influence. Further support for genetic associations come from twin studies showing > 50% of the variance in baseline strength and lean body mass is heritable (20,57,143,190). Adaptation of muscle to ST has also been shown to be highly heritable (200,201). Yet, there is little information on how specific polymorphisms may affect this response.

Circulating levels of insulin-like growth factor 1 protein (IGF-I) decline with advancing age and IGF-I is thought to be causally related to the loss of muscle mass and strength that occurs with age (70,205). Furthermore, exogenous growth hormone (HGH) administration increases circulating IGF-I levels, which has been shown to increase muscle mass and possibly strength (30,185). Studies with transgenic mice have shown a direct autocrine/paracrine effect of increased levels of IGF-I expression increasing skeletal muscle mass (52). This same transgenic line of mice, when compared to controls, shows reduced age-related losses of skeletal muscle motor neurons and type IIB muscle fibers (127). This preservation of motor neurons and fast twitch fibers is likely to preserve strength in aging muscle. In addition, Boonen et al. (16) observed an increase in muscle strength in older women following recombinant-human IGF-I administration.

Due to their known association with muscle hypertrophy, genes coding for proteins that regulate or are regulated by growth hormone or its mediators (IGF-I and II) are prime candidate genes for influencing muscle mass and strength. Indeed, a report from the HERITAGE Family Study revealed an association between an IGF1 gene marker and baseline fat free mass (FFM) (25). Furthermore, this same marker has been shown to be linked and associated with the change in FFM due to exercise training (25,198). However, the training modality used in the HERITAGE study (25) was one that is not commonly associated with significant increases in FFM. Nevertheless, an area near the IGF1 gene locus was implicated in these studies for influencing FFM both at baseline and in response to training, thus making it a strong candidate gene for muscle mass, as well as strength.

Recent studies have shown that a CA dinucleotide repeat polymorphism in the promoter region of the IGF1 gene can affect blood levels of IGF-I (58,99,132,169,177,214,216). Other CA repeats near gene promoters have been shown to alter gene expression in rats and humans (2,181). The polymorphism in the IGF1 gene is typically between 16 and 22 CA repeats and is commonly referred to by the base pair length of the amplified DNA fragment (e.g. 192 bp). Homozygosity for the 192 allele (19 CAs at nucleotide position 1087-1127 in the human IGF1 DNA sequence Genbank accession number AY260957) has been associated with decreased blood levels of IGF-I and decreased bone mineral density (177). At least two other studies support the possible influence of the 192 allele on decreasing blood levels of IGF-I (58,169); whereas, others show significantly higher blood levels of IGF-I in the presence of the 192 allele (99,132,214). While the functionality, if any, of this polymorphism remains unknown, it appears that it may be associated with the functional properties of and phenotypes related to IGF1. To date, the tissues studied in relation to this polymorphism have been of the type to be primarily affected by the endocrine action of IGF-I. Skeletal muscle, however, is known to be affected by the autocrine/paracrine action of IGF-I, making it a unique tissue in this respect. Yet, even with the known effects of IGF-I on muscle, this polymorphism has never been studied in relation to skeletal muscle phenotypes.

It is possible that the 192 polymorphism is in linkage disequilibrium with another functional polymorphism, thus resulting in the associations seen in the aforementioned studies. Although, no nonsense, missense, or functional polymorphisms have been identified for the IGF1 gene (165), recently identified

polymorphisms in IGF1 may serve as useful candidate markers for phenotypes related to expression levels of IGF1. Because of the relationships described above between a polymorphism in IGF1 and expression levels of the IGF-I protein and between the IGF-I protein and muscle function and muscle motor neurons, we hypothesize that IGF1 genotype will influence skeletal muscle strength and mass response to ST.

Although there are preliminary data from our laboratory to support this hypothesis, no published studies have examined this relationship. Understanding this relationship could eventually lead to individualized exercise prescriptions for older adults. Therefore, it is the purpose of this study to determine whether IGF1 genotype in the promoter region, or in three recently identified SNPs within the IGF1 locus, influence muscle strength and hypertrophy in response to ST in middle-aged and older Caucasian men and women.

METHODS

Subjects: Healthy, inactive Caucasian men (n = 32) and women (n = 35) volunteers between the ages of 52 and 83 yr. were recruited through local senior newspaper advertisements and bulk mailings. A portion of the subjects (n=18) in this investigation are from a cohort that had already been studied in our laboratory prior to this study (83). All subjects from both cohorts underwent a phone-screening interview, received medical clearance from their primary care physician and completed a detailed medical history prior to participating in this study. Only those who had not exercised more than once a week during the six months prior to the study were allowed to participate. All subjects from both cohorts were nonsmokers, and were free of significant cardiovascular, metabolic, or musculoskeletal disorders that

would affect their ability to safely perform heavy resistance exercise. After all methods and procedures were explained, the subjects read and signed a written consent form, which was approved by the Institutional Review Board of the University of Maryland, College Park. All subjects were reminded throughout the study not to alter their regular physical activity levels or dietary habits for the duration of the investigation.

Genotyping of (CA) microsatellite within the IGF1 promoter: Genomic DNA was prepared from EDTA-anticoagulated whole blood samples by standard salting-out procedures (Puregene DNA Extraction, Gentra Systems Inc.). The microsatellite was amplified by PCR of genomic DNA using fluorescence-tagged primers. Previously published PCR primers (177) flanking the CA polymorphism were used with the forward primer FAM-labeled: 5'-GCTAGCCAGCTGGTGTTATT -3' (sense) and 5'-ATGGGAAGAGGGTCTCACCA-3' (antisense).

Standard polymerase chain reactions were performed on a PTC-100 Thermal Cycler (MJ Research, Inc.), annealing steps of 1.0 min at 37°C, and elongation steps 1.0 min at 72°C for 35 cycles was standardized for PCR reactions. All PCR reactions were carried out in a 25 microliter volume containing ~20 ng of genomic DNA template, 14.15 ml dH$_2$O, 2.5 ml 10X PCR buffer, 4.0 ml 1.25 mM dNTP, 0.375 ml forward primer (20 μM), 0.375 ml reverse primer (20 μM), 0.1 ml *Taq* DNA Polymerase (5 U/ml), and 1.5 ml of 25 mM MgCl$_2$). Following amplification, samples were diluted with distilled, deionized water and mixed with deionized formamide and 1 U internal size standard labeled with ROX fluorescent dye (Genescan-500, PE Applied Biosystems), then denatured at 90°C for 2 minutes and

immediately transferred onto ice. Samples were electrophoresed through a capillary on the ABI 3100 DNA sequencer (PE Applied Biosystems, Foster City, Calif). The ABI Genescan/Genotyper 2.5 software programs (PE Applied Biosystems) were used to determine repeat length of each microsatellite by comparison with the calibration curve of the internal standard. Genotypes of three homozygotes were verified via direct sequencing on the ABI 3100. Additionally, positive controls were used during the sequencing runs. As previously reported, use of the forward primer results in the PCR product appearing four bases shorter than actual size; therefore, a correction factor was applied (177). Briefly, use of the forward primer resulted in PCR bands that moved through the gel at the rate of a band four base pairs shorter than actual size. This was confirmed by direct sequencing and was consistent throughout all samples. Therefore, four bases was added to the genotyping of each PCR product (i.e. 188 + 4 = 192 which = 19 CA repeats).

Subject genotypes are based on the PCR fragment length, which represents the actual number of CA repeats present at this locus, typically 16-27. The convention for reporting the genotype of the IGF1 promoter is based on the PCR fragment length, which was variable depending on the number of repeats (e.g., 19 CA repeats = 192 base pairs) (177). Thus, PCR base pair length number was reported to remain consistent with existing conventions.

PCR and RFLP genotyping for SNPs: All PCR reaction mixtures were run in a PTC 100 thermal cycler (MJ Research) using standard conditions. Templates were ~20 ng of genomic DNA, annealing steps were 1.0 min and elongation steps 30 sec at 72°C. Standard polymerase chain reactions were carried out in a 25 microliter

volume containing ~20 ng of genomic DNA template, 14.15 ml dH$_2$O, 2.5 ml 10X

PCR buffer, 4.0 ml 1.25 mM dNTP, 0.375 ml forward primer (20 mM), 0.375 ml

reverse primer (20 mM), 0.1 ml *Taq* DNA Polymerase (5 U/ml), and 1.5 ml of 25

mM MgCl$_2$. All primers were designed based on SNP location and ordered from IDT

DNA technologies. Primers and annealing temperatures are listed in Table 6. All

PCR amplification checks were performed in 2% agarose gels until optimized.

Restriction enzymes were selected for each SNP by Webcutter 2.0 and

purchased from MBI Fermentas. All digests were run according to manufacturers'

recommendations. Restriction enzymes and digestion temperatures are listed in table

6. Briefly, The 19779 PCR fragment was digested in a 20 microliter dilution of 2

units of enzyme, 2.0 μl of manufacturers standard buffer, 2.8 μl of dH20, and 15 μl of

the PCR product. All digests were allowed to proceed overnight and were run on a

2% gel after 24 hours, with the exception of 40395, which was run on a 4% gel to

achieve optimal separation of bands. Two independent investigators determined

genotypes. Direct sequencing of three PCR products from each SNP was performed

to verify genotypes.

Body Composition Assessment: Body weight was determined to the nearest

0.1 kg with subjects dressed in medical scrubs, and height was measured to the

nearest 0.1 cm using a stadiometer (Harpenden, Holtain, Wales, UK) and BMI was

calculated (kg/m^2).

Dual-energy x-ray absorptiometry (DXA): Body composition was

estimated by DXA using the fan-beam technology (model QDR 4500A, Hologic,

Waltham, MA). A total body scan was performed at baseline and again 24 to 48

hours after the final session of the single leg ST program. A standardized procedure for patient positioning, attire and utilization of the QDR software was used. Briefly, subjects would lie supine on the scan table with their arms at their sides, their palms pronated and flat on the table, and their body straight on the mat, as gauged against the center lines at the head and foot ends of the mat. The subject's feet and thighs were slightly separated tilted inward and taped, to prevent movement and insure consistency from scan to scan. Total body FFM, fat mass, and % fat were analyzed using Hologic's version 8.21 software for tissue area assessment. Total body FFM is defined as lean soft tissue mass plus total body bone mineral content (BMC). The coefficients of variation for all DXA measures of body composition were calculated from repeated scans of 10 subjects who were scanned three consecutive times with repositioning (FFM CV < 1%, % Body fat CV = 1.0%). The scanner was calibrated daily against a spine calibration block and step phantom block supplied by the manufacturer. In addition, a whole body phantom was scanned weekly to assess any machine drift over time.

One repetition maximum (1RM) strength test: The 1RM (i.e. the highest resistance at which one repetition could be successfully completed) strength test was assessed in the knee extensors before and after training. Three low resistance familiarization-training sessions were conducted prior to baseline 1RM strength testing, so that subjects would be familiar with the equipment and proper exercise techniques. In addition, this procedure was used to help to control against both large initial gains in strength occurring due to motor learning and to help to prevent injuries. After the three familiarization training sessions and before the regular

training sessions began, knee extensor 1RM strength of each leg individually was assessed on the training apparatus (Keiser A-300 Leg Extension machine). After a warm-up consisting of 2-3 min of light cycling, subjects were positioned with a pelvis strap (seat belt) to minimize the involvement of other muscle groups. Based on observations during the orientation period, age, body weight, and data from our previous studies, the knee extension 1RM test started with a resistance level estimated to be about 20 to 30% of each subject's predicted 1 RM. After ~ 60 s of rest upon a successful completion of a repetition, subsequent trials were performed at progressively higher resistance levels, to minimize the total number of trials required before the true one repetition maximum value was obtained. Approximately the same number of trials was used for the after training test as was used for baseline testing. All aspects of measuring 1-RM strength before and after 10 weeks of single leg training were standardized for each subject, including specific seat adjustments, body position, and level of vocal encouragement.

Muscle Volume: To quantify quadriceps MV, computed tomography (CT) imaging of the trained and untrained thighs was performed (GE Lightspeed Qxi, General Electric, Milwaukee) at baseline and during the last weeks of the 10-week unilateral ST program. Axial sections of both thighs were obtained starting at the most distal point of the ischial tuberosity down to the most proximal part of the patella while subjects were in a supine position. Measurements of MV in the untrained leg served as a control for seasonal, methodological, and biological variation of MV, by subtracting the changes in the control leg from the training-induced changes in the trained leg . Section thickness was fixed at 10 mm, with 40

mm separating each section, based on previous work in our laboratory by Tracy et al. (207). Quadriceps MV was estimated based on using a 4 cm interval between the center of each section. Each CT image was obtained at 120 kVp with the scanning time set of 1 s at 40 mA. A 48-cm field of view and a 512 X 512 matrix was used to obtain a pixel resolution of 0.94 mm. Two technicians performed analyses of all images for each subject using Medical Image Processing, Analysis, and Visualization (MIPAV) software (NIH, Bethesda). Briefly, for each axial section, the cross-sectional area (CSA) of the quadriceps muscle group was manually outlined as a region of interest. The quadriceps CSA was manually outlined in every 10 mm axial image from the first section closest to the superior border of the patella to a point where the quadriceps muscle group is no longer reliably distinguishable from the adductor and hip flexor groups. The same number of sections proximal from the patella was measured for a particular subject before and after training, to ensure within subject measurement replication. Investigators were blinded to subject identification, training status, and genotype, for both baseline and after training analysis. Repeated measurement coefficient of variation was calculated for each investigator based on repeated measures of selected axial sections of one subject on two separate days. Average intra-investigator CV was 1.7% and 2.3% for investigator one and two respectively. The average inter-investigator CV was 3.8%. Final MV was calculated with the use of a truncated cone formula as reported by Tracy et al. (207) and described by Ross et al. (180). Calculation of MQ was strength (kg) / MV (cm^3).

Training Program: The training program consisted of unilateral (single leg) training of the knee extensor of the dominant leg, three times per week, for approximately 10 weeks. Training was performed on a Keiser A-300 air powered leg extension machine that allows ease of changing the resistance without interrupting the cadence of the exercise. The untrained control leg was kept in a relaxed position throughout the training program.

Prior to the regular training sessions, subjects underwent three familiarization sessions during which they completed a typical training sequence with little or no resistance. Subjects would warm-up on a bicycle ergometer for ~ two min prior to each training session. The training consisted of five sets of knee extension exercise for those < 75 yrs of age and four sets for those > 75 yrs of age. The protocol was designed to include a combination of heavy resistance and high volume exercise. The first set was considered a warm-up and consisted of five repetitions at 50% of the 1RM strength value. The second set consisted of five repetitions at the current 5RM value. The 5RM value was increased continually throughout the training program to reflect increases in strength levels. The first four or five repetitions of the third set were performed at their current 5RM value, then the resistance was lowered just enough to complete one or two more repetitions before reaching muscular fatigue. This process was repeated until a total of 10 repetitions were completed. This same procedure was then used in the fourth and fifth sets, but the total number of repetitions increased in each set. The fourth set consists of four or five repetitions at the 5RM resistance followed by 10 more repetitions for a total of fifteen repetitions carried out in the same manner as described above for the 10 repetition set. The fifth

set consisted of four or five repetitions at the 5RM resistance, followed by 15 more repetitions for a total of 20 repetitions performed in the same manner as the other sets. This procedure allowed subjects to use near maximal effort on every repetition while maintaining a relatively high training volume. The second, third, fourth, and fifth sets are preceded by rest periods lasting 30, 90, 150, and 180 seconds, respectively. The shortening phase of the exercise took ~ two seconds, and the lengthening phase took ~ three seconds. Subjects performed supervised stretching of the knee extensors and hip flexors following each training session.

Statistical Analyses: All statistical analyses as described below were performed using SAS software (SAS version 8.2, SAS Institute Inc., Cary NC). All analyses were conducted on the change values in the dependent variables. For MV, the change value was calculated by taking the difference between the change (pre to post training) in MV of the untrained leg and the change in MV (pre to post training) of the trained leg. For strength, the change in 1RM value of the trained leg was used as a dependent variable. Muscle quality was calculated as 1RM kg / MV cm^3 and the change in the trained leg, was used as the dependent variable of MQ. The IGF1 promoter polymorphism was categorized as previously reported in the literature as 192 homozygotes, 192 heterozygotes, or non-carriers of the 192 allele.

To examine the effect of the promoter genotype on response to ST, an analysis of covariance was used for each dependent variable (DV). The three levels of the predefined groupings of genotype at the IGF1 promoter locus was defined as the fixed effect. To reduce error variance and increase precision, covariates were included in the model. Age and gender were included in all models and baseline

values of the dependent variable were included in each model (i.e. baseline value of 1RM of the trained leg was the covariate for the analysis of strength). Preplanned comparisons of 192 carriers versus non-carriers and 192 homozygotes versus heterozygotes and non-carriers were analyzed for each DV.

The single nucleotide polymorphisms were analyzed separately due to a smaller sample size as DNA from 18 subjects was unavailable for SNP genotyping. Due to this smaller sample size and distribution of the three genotype groups, genotype groups with less than two data points (any genotype with one or no subjects) were not included in the analysis. One 2x2x3 reduced model factorial ANOVA for each of the DV's muscle strength and MV was analyzed. The factorial model included main effects and two-factor interactions only, to reduce the total number of comparisons and provide interpretable results. The two levels of the 19779 and 40395 loci, and three levels of the 82686 loci genotypes form the factorial, and dependent variables were calculated as described for the promoter analysis. Age, gender, and baseline values were included as covariates in the factorial ANOVA as described for the promoter analysis. Post-hoc comparisons were made comparing all main effects by LSD post-hoc analysis and significance was accepted when $P < 0.05$ for all analyses.

Hardy-Weinberg equilibrium: The IGF1 genotype distribution was evaluated for conformity to Hardy-Weinberg equilibrium using a chi-square test.

Power analyses: Statistical power for the three primary comparisons was estimated for the 192 genotype effect on each variable. This analysis was done using data from a study in our laboratory, which employed a similar training and testing

protocol. Statistical power for changes in strength a-priori was estimated to be > .80 with alpha set at 0.05 (critical effect size MV = .4, 1RM = .45) and accounting for present sample size and genotype distribution. While for MV power was estimated to be ~ .60 due to the smaller training-induced critical effect size.

RESULTS

Genotype: IGF1 promoter allele and genotype frequencies (Table 1) did not differ significantly from Hardy-Weinberg expectations and were similar to those reported previously (132,177,214). Likewise, IGF1 SNP allele and genotype frequencies did not differ significantly from Hardy-Weinberg expectations (Table 2). IGF1 gene characterization including D-prime and haplotype analysis are included in Appendix B. Briefly, only loci 19779 and 40395 were in significant linkage disequilibrium with an approximate D-prime of .52 (P < 0.05).

Subject characteristics: One female subject's 1RM value from the 192 homozygote group was lost prior to data analysis therefore, her strength values were not included in any analysis. Due to stringent CT scan inclusion criteria, seven subjects were not included in CT analysis (four 192 homozygotes (three females and one male) and three 192 heterozygotes (two females and one male)). Body mass, percent body fat, and FFM for all subjects did not change significantly as a result of training (Table 3). There were no significant differences in age, height, body mass, percent body fat, or FFM at baseline or in response to training among the three IGF1 promoter genotype groups (Table 4).

1RM strength: The 1RM strength of the trained leg did not differ significantly at baseline among IGF1 promoter genotype groups when co-varied for sex and age. The 192 carriers increased their 1RM strength (14.9 ± 1.4 kg) significantly more than non-carriers (9.3 ± 1.8 kg) when co-varied for sex and age ($P < 0.05$; Table 5; Figure 1), but all groups increased significantly with training ($P < 0.01$). There was no significant difference between 192 homozygotes and non-homozygotes for change in 1RM. In response to training, SNP ANCOVA main effects and two-factor interactions revealed no significant differences in the change of 1RM strength (Appendix A).

Muscle volume and muscle quality: Muscle volume increased (i.e., increases in trained leg minus changes in untrained leg) in all groups (192 homozygotes, heterozygotes, and non-carriers) in response to training ($P < 0.01$). Muscle volume and muscle quality did not differ significantly at baseline or in response to training among IGF1 promoter genotype groups when co-varied for sex and age (Table 5; Figure II). However, training-induced increases in MV for 192 carriers (132 ± 12 cm^3) approached significance for being greater than the increases in non-carriers (94 ± 18, $P = 0.08$). The 192 homozygotes were not significantly different from the heterozygotes and non-carriers in MV or MQ response to training. Moreover, there were no significant differences in MV responses to training among any SNP main effect and two-factor interactions.

DISCUSSION

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To our knowledge, this is the first study to examine the influence of IGF1 genotype on strength and MV responses to ST. These results support our hypothesis that IGF1 genotype influences the strength response to ST, but do not support our hypothesis of a similar influence on MV and MQ responses to ST in healthy older adults. However, the genotype association with MV response to training approached significance.

Strength training appears to be the intervention of choice for the prevention and treatment of sarcopenia, based on efficacy and safety concerns with other interventions (5,23). IGF-I has been implicated in the loss of muscle with age (70) and IGF-I expression levels change as a consequence of ST in older adults (49). The results of this study add to this literature by showing that carriers of the 192 allele at the IGF1 locus have greater strength gains than non-carriers with ST.

Although no previous studies have examined the association of the IGF1 gene promoter polymorphism with skeletal muscle phenotypes, IGF1 transgenic expression and exogenously administered IGF-I protein have been shown to increase skeletal muscle size and function (16,138). Additionally, muscle stimulation (mechanical, electrical, and stretch) increases IGF-I protein and overall protein content in skeletal muscle and produces significant increases in muscle size and strength (37,49,123,224). The increase in IGF1 mRNA has been reported to be proportional to the increase in muscle strength in elderly adults following ST (49). Thus, it seems that increases in IGF1 expression may be causally related to increases in muscle size and strength. In the study by Fiatarone et al. (49), IGF1 mRNA increased by an average of ~500% in response to ST in the elderly. However, the variation in IGF1

mRNA expression was more than 500%, suggesting a substantial inter-individual variability in response to training.

Indeed, IGF-I blood levels, muscle size and function are known to be highly heritable traits (20,80,92,190,204). IGF-I blood levels are highly heritable in children, middle-aged, and elderly adults (80,92). In a study of twin children comparing IGF-I plasma levels, monozygotic pairs had a within-pair correlation of $r = 0.91$ and dizygotic pairs of $r = 0.40$ (92). When middle-aged and elderly twins were compared for IGF-I plasma levels, the heritability was found to be less than previously reported, but still quite high at 63% (80). Thus, it appears that at least plasma levels of IGF-I are under considerable genetic influence, though this influence may decrease with age. In this regard, a study examining the same CA repeat IGF1 promoter polymorphism as the one in the current study, demonstrated a significant age related decline in circulating IGF-I levels, which was associated with this polymorphism (169). Consistent with our findings, this would suggest an influence of the IGF1 promoter polymorphism on phenotypes that may be affected by IGF1 expression.

The hypothesis that genetic factors may influence muscular strength is supported by data from both animals (14) and humans (168,201,204). Support from animal studies comes from Biesiadecki et al. (14) who observed a 1.5 to 5.2-fold divergence between the muscular strength of male rat strains with the lowest and highest strength levels and support from human studies come from Reed et al. (168) who observed significant genetic effects for absolute grip strength and grip strength normalized for body weight in 127 identical and 130 fraternal twin pairs. In the latter

study, it was estimated that genetic factors accounted for 65% of the variance in grip strength, even after adjusting for the effects of weight, height, and age (168). In younger men, Thomis et al. (201) reported that up to 82% of the variance in strength was explained by genetic factors. Tiainen et al. (204) examined the strength of the knee extensors (same muscle group tested in the current study) in an older population and attributed 31% of the variance to genetics factors.

To our knowledge, only two studies have attempted a genome-wide search for genes related to phenotypic measures of muscle (24,25). In one of these studies fat free mass, measured by hydrostatic weighing, showed significant linkage with the IGF1 receptor polymorphism in the Quebec Family Study (24). In the HERITAGE Family Study, baseline FFM and the change in FFM due to exercise training both showed evidence of significant linkage with the CA repeat IGF1 promoter polymorphism examined in our current study (25). Additionally, linkage and association of baseline FFM and the change in FFM due to exercise training has been demonstrated with the IGF1 promoter polymorphism (198). Although the training modality used in the HERITAGE study is not usually associated with large changes in FFM, it appears that a polymorphism in the IGF1 gene may be associated with changes in FFM. Likewise, we have demonstrated an association of this polymorphism with muscle function (strength) response to training, which is believed to be under the autocrine/paracrine influence of IGF1.

We are aware of only three studies that have examined the influence of genetic factors on skeletal muscle phenotypic response to ST (56,200,201). Thibault et al. (200) studied ST responses of five monozygotic twin pairs and found no significant

interaction between strength response to training and heredity, which is not surprising given their small sample size. Thomis et al. (201) found evidence for a significant role of heredity in strength responses to ST. These responses were independent of the influence of genetic factors on baseline strength values. However, Folland et al. (56) was the only study that examined the effects of a specific candidate gene polymorphism on strength or muscle mass response to training. They reported a significant relationship between the ACE genotype and strength response to ST (56).

The IGF1 promoter polymorphism has been examined in numerous contexts (58,99,169,177). As in the current study, genotyping of the microsatellite is typically separated into three groups, 192 homozygotes, 192 heterozygotes, and non-carriers of the 192 polymorphism. Whether the 192 polymorphism is causally related to changes in IGF1 function is not known; yet, the 192 allele is the most prevalent allele in the majority of the populations studied to date. Additionally, there is evidence in animals and humans that CA repeats found in gene promoter regions can affect gene expression (2,181). It has been suggested by King (29) that these repeats act as evolutionary tuning knobs to fine-tune gene expression with minor deleterious consequences. Furthermore, genome-wide scans and linkage studies have implicated this allele (192) in phenotypes known to be affected by IGF-I (25,35,198).

As mentioned, the functionality of this polymorphism is not currently known. It is possible that length of the polymorphism could be affecting expression levels or that this polymorphism is in linkage disequilibrium with a functional polymorphism that affects phenotypes related to IGF1 expression. Based on the genome-wide scans that have been reported associating this polymorphism with IGF1 related phenotypes

it appears that it is at least a marker for IGF1 related phenotypes. To address the functionality of this polymorphism *in-vivo* in humans, IGF1 expression levels would be needed along with a large sample size of individuals who are homozygous for this allele. Alternatively, a cell culture system could be used to address this *in-vitro*. Therefore in light of the current lack of data on functionality, the conventional grouping of 192 carriers and non-carriers was used as previously reported in the literature.

Rosen et al. (177) first implicated this polymorphism with serum levels of IGF-I and BMD in a study examining older men and women (177). It was reported that 192 homozygotes had lower blood levels of IGF-I; additionally, and in a group of older men, 192 homozygotes made up a disproportionately large number of those with idiopathic osteoporosis (177). The reports by Frayling et al. (58) and Rietveld et al. (45) concur with Rosen's findings of decreased blood levels of IGF-I in carriers of the 192 allele. In a cross-sectional study of young men and women, Frayling et al. (58) reported that 192 carriers had decreased blood levels of IGF-I, although no other associations were found. The study by Rietveld et al. (45) of older men and women, reported an age- related decline in blood levels of IGF-I in 192 homozygotes. In contrast to Rosen's findings, subsequent studies have demonstrated that 192 *non*-carriers have lower IGF-I blood levels and are more likely to be osteoporotic (99,171). Additionally, a longitudinal study by Missmer et al. (36) in middle-aged and older men and women reported a lower level of IGF-I in control subjects who were non-carriers of 192. Similarly, Vaessen et al. (214) concluded that non-carriers had decreased blood levels of IGF-I and an increased risk for type II diabetes and

myocardial infarction. Finally, Kim et al. (99) reported 192 homozygotes as having the highest blood levels of IGF-I in Korean women.

Some reports have concluded that the 192 allele has no effect on blood levels of IGF-I or only has an effect when combined with oral contraceptive use (89,230). Yet both of these studies found an association with a measured phenotype, breast cancer, or the interaction of blood levels with oral contraceptive use and the 192 allele. There have been two reports of the 192 allele showing no association with IGF-I blood levels or any measured phenotype (3,34). Although conclusive determination of the effect of the 192 genotype from the aforementioned studies is difficult, it seems that the IGF1 192 allele is at least associated with some phenotypes related to IGF1 expression. Skeletal muscle is known to significantly increase IGF1 expression levels and produce unique isoforms of IGF-I in response to ST (49,123). Yet, none of the previous studies has examined phenotypes related to skeletal muscle. In the present study, we report the first intervention study to examine the influence of the 192 allele on muscle phenotypes known to be related to IGF1 expression.

Some limitations of the current study are the lack of IGF1 expression data for muscle, the use of only one ethnic group, and the statistical power for muscle volume. The use of one ethnic group was decided upon to control for the possible varying affects this polymorphism might have in different ethnic (genetic) backgrounds and because it has been shown that the prevalence of the 192 allele differs with ethnicity (34). We are currently examining this relationship in African Americans. The inconclusive results in MV of the present study may be due to a lack of statistical power. The statistical power to detect the genotype difference in MV was ~.60, while

for strength it was > .85. This is primarily due to the smaller (as compared to strength) effect size seen in MV in response to ST. Additionally, the design of the current study may have reduced MV effect size by the use of an untrained control leg. The control leg reduced the absolute change seen in response to ST yet allowed for a more precise determination of the change that could be attributed to ST or genetics by reducing the likelihood that extraneous factors would affect our dependent variable of MV. Thus, future studies should consider changes in muscle mass or MV with a larger sample size and the use of a control leg or group, consider using other ethnic groups, and where the muscle biopsy technique is available, measure mRNA and/or protein levels of IGF-I.

In conclusion, this is the first study to examine the effects of the IGF1 promoter polymorphism on muscle phenotypic responses to ST in older adults. The results suggest that in response to ST, carriers of the 192 allele will have a greater increase in strength and possibly muscle volume when compared to non-carriers. The results of the current study, in combination with future studies, will continue to contribute to the understanding of the role of gene polymorphisms on the responses to exercise training.

Table 1. IGF1 CA promoter allele and genotype frequency

Number of CA repeats	Total (%)	Men	Women
< 17	1 (<1)	0	1
17	3 (2)	2	1
18	8 (6)	5	3
19	82 (62)	38	44
20	27 (20)	12	15
21	12 (9)	6	6
22	1 (<1)	1	0
Range	6 – 22	17 – 22	6 – 21
Genotype			
19/19 (192/192)	24 (36)	10	14
19/ -- (192/ --)	34 (51)	18	16
-- / -- (non-carrier)	9 (13)	4	5

Table 2. IGF1 SNP allele and genotype distribution of all subjects.

	Allele frequency				Genotype frequency					
	T	C	A	G	TT	TC	CC	AA	AG	GG
19779	.78	.22	--	--	.59	.37	.04	--	--	--
40395	--	--	.79	.21	--	--	--	.61	.35	.04
82686	--	--	.39	.61	--	--	--	.19	.40	.40

Table 3. Physical characteristics for all subjects

	Men (n=32)		Women (n=35)	
	Baseline	**After Training**	**Baseline**	**After Training**
Age (yrs)	70 (6)	--	67 (8)	--
Height (cm)	169 (6)	--	161 (8)	--
Weight (kg)	83.5 (12.0)	84.0 (12.7)	71.3 (13.6)	71.2 (14.13)
Fat (%)	29 (5)	29 (4)	39 (5)	39 (6)
FFM (kg)	58.2 (6)	58.5 (7.1)	42.3 (6.1)	42.3 (5.1)
1RM (kg)	74 (14)	89 (22)	45 (8)	55 (11)

Values are means and (SD)
FFM = fat free mass

Table 4. Physical characteristics for subjects by IGF1 promoter genotype

	192 Homozygotes		192 Heterozygotes		All other genotypes	
	Baseline	After Training	Baseline	After Training	Baseline	After Training
Age (yrs)	67 ± 1	--	70 ± 1	--	68 ± 4	--
Height (cm)	169 ± 2	--	167 ± 2	--	165 ± 3	--
Weight (kg)	78.6 ± 2.5	78.7 ± 2.5	75.7 ± 2.0	76.3 ± 2.1	75.8 ± 5.3	76.3 ± 5.6
Fat (%)	34 ± 1	34 ± 1	34 ± 1	34 ± 1	33 ± 2	34 ± 1
FFM (kg)	51.8 ± 1.4	52.0 ± 1.4	50.0 ± 1.0	50.4 ± 1.0	50.0 ± 2.2	50.1 ± 2.4
Male/Female (n)	10/14	--	18/16	--	4/5	--

Values are means ± SE
FFM = fat free mass
None of the After Training values were significantly different from baseline
Weight, % Fat, and FFM are corrected for mean age (68) and sex (50/50 ratio)

Table 5. Muscle volume, 1RM muscle strength, and muscle quality measurements before and after training by IGF1 192 CA repeat polymorphism.

	192 Homozygotes			192 Heterozygotes			All other genotypes		
	Baseline	After Training	n=	Baseline	After Training	n=	Baseline	After Training	n=
				Trained leg					
Muscle Volume (cm^3)	1467 ± 33	1593 ± 37*	20	1362 ± 38	1508 ± 43*	31	1332 ± 74	1438 ± 75*	9
Muscle Quality Kg/cm^3(X 10^{-2})	4.18 ± .15	4.76 ± .13*	20	4.40 ± .12	5.02 ± .10*	31	4.27 ± .18	4.6 ± .16*	9
1RM (kg)	61 ± 2	76 ± 3*	23	59 ± 2	74 ± 2*	34	57 ± 4	66 ± 4* †	9
				Untrained leg					
Muscle Volume (cm^3)	1415 ± 48	1413 ± 48	20	1306 ± 40	1316 ± 46	31	1322 ± 76	1333 ± 79	9
Muscle Quality (X 10^{-2})	4.18 ± .23	4.58 ± .23	20	4.37 ± .14	4.54 ± .15	31	3.70 ± .29	4.05 ± .24	9
1RM (kg)	58 ± 3	63 ± 3	23	57 ± 3	61 ± 3	34	50 ± 5	55 ± 5	9

Values are means ± SE
*Significantly different than before training, p < 0.01
† Significantly different than 192 homozygotes and 192 heterozygotes, p < 0.05
All values are corrected for age and sex

Table 6. Sequences of PCR primers used for amplification and sequencing of IGF1 gene SNPs

SNP	Forward Primer	Reverse Primer	Annealing temp (C)	Restriction enzyme/ digestion temp (C)
19779	5'-CAGATTTGTGGCACTAATAC	5'-CTATTAAAGGGATGACTGTG	49°	EcoRI / 37°
40395	5'-AAGTTAAGAAGCAGTGTTGC	5'-AGTGGTGCAATTGTGGCTCA	55°	AluI / 37°
82681	5'-ACATACAGGTTCTGTGGAAT	5'-GTTGGAGAGGATTATGTGTT	51°	TaiI / 65°

SNP numbering represents nucleotide position in the human IGF1 DNA sequence.
Genbank accession number AY26095

Fig 1.

Change in 1-RM values with strength training among IGF-I genotype groups

(Figure: bar chart with y-axis "Change in 1-RM (kg)" ranging 0 to 20, x-axis "Genotype"; legend: 192/192, 192/ --, Non-carrier; Non-carrier bar marked with *)
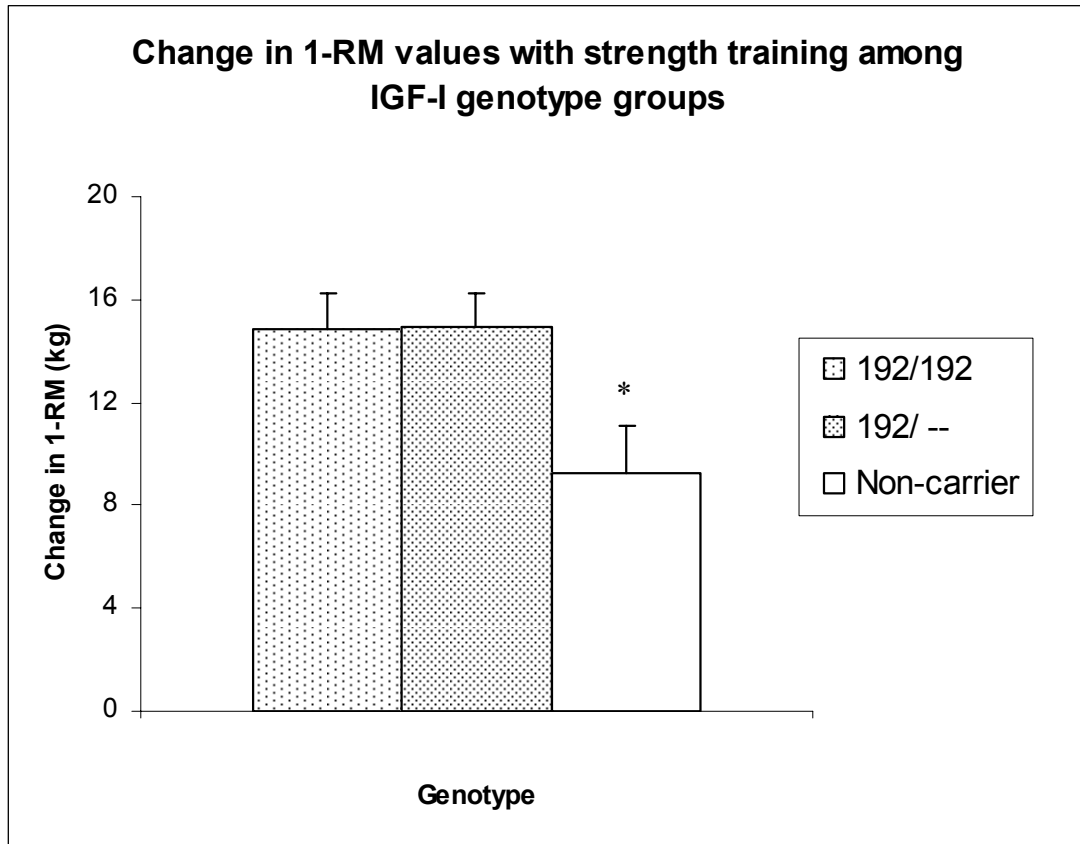
Figure 1. The absolute change in 1RM strength corrected for age and sex for each IGF1 promoter genotype group after 10 weeks of training. Carriers of the 192 allele had significantly greater gains in strength than non-carriers (* $P = 0.02$). Values are means ± SE.

Fig 2.

Change in quadricep muscle volume with strength training among IGF-I genotype groups

Muscle Volume (cubic cm)

160
140
120
100
80
60
40
20
0

☑ 192/192
▨ 192/ --
☐ Non-carrier
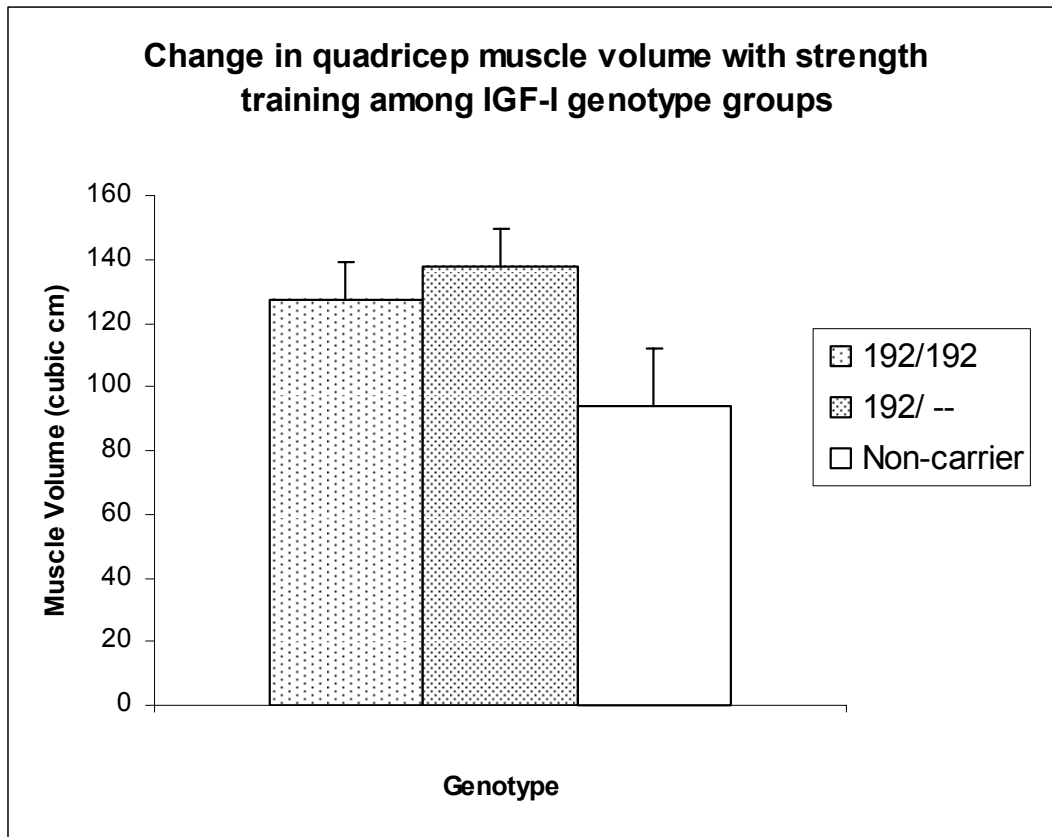
Genotype

Figure 2. The difference between the change in muscle volume of the trained leg and control leg for each IGF1 promoter genotype group corrected for age and sex after 10 weeks of training. Carriers of the 192 allele did not differ significantly from non-carriers in terms of their gain in muscle volume ($P = 0.08$). Values are means ± SE.

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Significance values for SNP 2-way comparisons of changes in strength

19896	40395	82686	Mean change post-training (kg)	19896	40395	82686	Mean change post-training (kg)	P
TC			24	TT			34	0.25
	AA		26		AG		32	0.47
		AA	29			AG	26	0.71
		AA	29			GG	31	0.86
		AG	26			GG	31	0.49
TC	AA		21	TC	AG		27	0.60
TC	AA		21	TT	AA		31	0.32
TC	AG		27	TT	AG		36	0.42
TT	AA		31	TT	AG		36	0.60
TC		AA	22	TC		AG	25	0.83
TC		AA	22	TC		GG	26	0.76
TC		AA	22	TT		AA	37	0.48
TC		AG	25	TC		GG	26	0.93
TC		AG	25	TT		AG	28	0.75
TC		GG	26	TT		GG	36	0.25
TT		AA	37	TT		AG	28	0.60
TT		AA	37	TT		GG	36	0.97
TT		AG	28	TT		GG	36	0.29
	AA	AA	22		AA	AG	30	0.53
	AA	AA	22		AA	GG	27	0.68
	AA	AA	22		AG	AA	37	0.45
	AA	AG	30		AA	GG	27	0.71
	AA	AG	30		AG	AG	23	0.46
	AA	GG	27		AG	GG	35	0.37
	AG	AA	37		AG	AG	23	0.38
	AG	AA	37		AG	GG	35	0.93
	AG	AG	23		AG	GG	35	0.22

Characterization of IGF1 gene

The human IGF1 gene is located on chromosome 12 (12q22-q23). The gene is 88

kb in length and contains six exons and five introns. Partially due to the inconclusive

results of the functionality of the IGF1 promoter microsatellite (CA repeat) and due to the

large size of the IGF1 gene, three SNPs were identified and genotyped in the present

study to search for an association with skeletal muscle phenotypes. Although a small

sample size (n=49) prohibited a haplotype comparison with the measured phenotype, a

characterization of the SNPs was conducted to describe the association of the three SNPs

(LD) and to examine the haplotypes at this gene. This information could be useful to

identify haplotypes useful in other case/control association studies.

A standard measure of the statistical association between a pair of alleles A and B

at two different loci is known as linkage (gametic) disequilibrium (LD), $D_{AB} = (p_{AB}) - (p_A p_B)$. It is defined as the deviation of the haplotype frequency from that expected at

linkage equilibrium (random association of alleles). For a population at linkage

equilibrium $D_{AB} = 0$. If there is no statistical association of alleles in haplotypes, then the

frequency is equal to the product of the corresponding allele frequencies. In this case,

one says that the population is at linkage equilibrium. The coefficient D can be positive

or negative depending on whether alleles A and B are in coupling disequilibrium

(haplotypes AB are overrepresented) or in repulsion disequilibrium (haplotypes AB are

underrepresented).

As mentioned, two loci A and B, are said to be in linkage (gametic)

disequilibrium if their respective alleles do not associate independently in the studied

population. If two genes are independently inherited, expected gametic frequencies, can

be obtained from allelic frequencies in the population. The non-randomness of gametic

frequencies can be quantified by means of a deviation from two locus equilibrium: D is

the gametic disequilibrium coefficient, or measure of deviation from 2 locus equilibrium.

The statistical significance of the observed allelic association under the null hypothesis of

random allelic association can be tested by a chi-square analysis.

The EH program (estimate haplotype frequencies) is a soft-ware program to test

and estimate LD between different markers or between a disease locus and markers

(199,223). Each individual in a sample is classified uniquely in terms of his or her two-

locus genotype and. However, it is often difficult to infer haplotypes from genotype data

alone. This is due to the unknown phase of alleles along a chromosome in individuals.

In individuals who are doubly heterozygous (Aa/Bb) there can be either of two phases,

AB/ab, or Ab/aB. These individuals present a difficulty, but omitting them from

consideration in this analysis can lead to a bias and a loss of information for the test of

LD (199). Methods have been developed to infer haplotype phase via likelihood

methods, as in the EH program or similar programs (199,217). These programs utilize

the EM (expectation maximization) algorithm to resolve phase-ambiguous genotypes

utilizing data from the studied population to estimate haplotype frequencies. The EM

algorithm is a commonly used iterative statistical procedure that uses data parameters

from an existing population to estimate missing or ambiguous data points. In this case,

the EM algorithm resolves the ambiguous haplotypes of double heterozygotes by

utilizing the known genotype data from this population.   Once all haplotypes are known

or estimated, a chi-square analysis is performed to determine the likelihood that the

observed haplotypes would have occurred by chance based on allele frequencies {ott;

terwilliger}(148,199). The EH program accomplishes this in two steps, first by

estimating the frequency of ambiguous haplotypes then by using a chi-square analysis of

all haplotypes compared to haplotypes that would occur with random association of

alleles, which will determine statistical significance of LD {terwilliger}(199). In this

case, the chi-square statistic has 1 df, because under the hypothesis of allelic association,

there are three free parameters (haplotype frequencies), whereas under the hypothesis of

no allelic association, there are only two free parameters (the two gene frequencies). The

difference in free parameters is 1, so the distribution has 1 df. Thus the chi-square

produces a significance (alpha) value for LD, commonly reported as D or D' (112,148).

The disequilibrium coefficient (D) is the simple measure of linkage

disequilibrium ($D_{AB} = p_{AB} - p_A p_B$). However, erosion of LD occurs over time by

recombination. A measure developed by Lewontin, D', will express the coefficient

relative to this initial value (1). In the current study, D' was calculated in an excel

spreadsheet and significance tested by the EH program chi-square analysis (112,199). The Chi-square value calculated by the EH program is the test of significance of LD between the alleles (148,199). The results of the current study show that the 19779 and 40395 SNPs are significantly linked, with a D' of 0.527 suggesting modest LD between these two sites (Chi-square = 12.95, P < 0.05). The 40395 and 82686 SNP haplotypes were not significantly different from what might be expected to happen by chance (chi-square = 2.39, P = 0.12). Additionally, the 19779 and 82686 alleles were not in LD (chi-square = 1.37, P = 0.23) The 19779 and 40395 loci are located closer to each other than the 40396 and 82686, which may partially explain these findings.

Additionally, the Phase program was utilized to estimate haplotype frequencies for all polymorphisms. A total of 15 haplotypes were observed, while 27 were possible. Though these data do not represent an in-depth analysis, they do suggest that recombination has occurred within this gene, more likely near the 3' end. This study represents a short preliminary analysis of the characterization of this gene, while numerous SNPs analyzed independently and as haplotypes and a larger sample size is suggest for future studies for a more thorough analysis.

Haplotype estimations and D prime values for the 19779 and 40395 loci in IGF1 gene.

```
Estimates of Gene Frequencies (Assuming Independence)
----\-----------------------------
locus \ allele    1       2
--------\-----------------------
   1    |          0.7800  0.2200   ◄───── Allele
   2    |          0.7900  0.2100           frequencies
-------------------------------
# of Typed Individuals: 50

There are 4 Possible Haplotypes of These 2 Loci.            Haplotypes
They are Listed Below, with their Estimated Frequencies:    estimated by
-------------------------------------------------------     EM algorithm
| Allele   Allele  |      Haplotype Frequency      |        (Observed)
|   at       at    |                               |
| Locus 1  Locus 2 |   Independent   w/Association  |
-------------------------------------------------------
   1        1         0.616200      0.702536   ◄──
   1        2         0.163800      0.077464          Haplotypes
   2        1         0.173800      0.087464          assuming
   2        2         0.046200 ◄──0.132536           random
-------------------------------------------------------  distribution
# of Iterations = 8                                      (Expected)

                              #param   Ln(L)    Chi-square
----------------------------------------------------------------
H0: No Association               2     -79.83      0.00
H1: Allelic Associations Allowed 3     -73.35     12.95
```

D-prime = 0.5271

Haplotype estimations and D prime values for the 40395 and 82686 loci in IGF1 gene.

```
Estimates of Gene Frequencies (Assuming Independence)
----\-----------------------------
locus \ allele     1        2
--------\-----------------------
   1    |            0.7857  0.2143
   2    |            0.3878  0.6122
--------------------------------
# of Typed Individuals: 49

There are 4 Possible Haplotypes of These 2 Loci.
They are Listed Below, with their Estimated Frequencies:
-------------------------------------------------------
| Allele   Allele  |      Haplotype Frequency        |
|   at       at    |                                 |
| Locus 1  Locus 2 |  Independent   w/Association    |
-------------------------------------------------------
   1        1           0.304665      0.264313
   1        2           0.481050      0.521402
   2        1           0.083090      0.123443
   2        2           0.131195      0.090843
-------------------------------------------------------
# of Iterations = 8

                                #param   Ln(L)    Chi-square
-------------------------------------------------------------
H0: No Association                 2     -90.71      0.00
H1: Allelic Associations Allowed   3     -89.51      2.39
```

Haplotypes estimated by EM algorithm (Observed)

Haplotypes assuming random distribution (Expected)

D-prime = 0.3076

Haplotypes (estimated by Phase)

19 = 19 CA repeats

List of estimated haplotype frequencies.

Haplotypes estimated by Phase based on genotypes.

	Haplotype	Frequency (Observed)
1	18 TAG	6.0
2	19 TAA	9.0
3	19 TAG	39.0
4	19 CAA	1.0
5	19 CGA	9.0
6	19 CGG	4.0
7	20 TAA	13.0
8	20 TAG	3.0
9	20 TGA	3.0
10	20 CAA	1.0
11	20 CAG	6.0
12	21 TAA	3.0
13	21 TGG	3.0
14	21 CGA	1.0
15	22 TGG	1.0

Expected frequency of haplotypes assuming random association of alleles

	Haplotype	Frequency (Expected)	SE
1	18 TAA	0.006678	0.008298
2	18 TAG	0.050241	0.009076
3	18 TGA	0.000815	0.002707
4	18 TGG	0.000795	0.002675
5	18 CAG	0.000295	0.001675
6	19 TAA	0.116501	0.022571
7	19 TAG	0.345778	0.024625
8	19 TGA	0.004312	0.008766
9	19 TGG	0.005693	0.007927
10	19 CAA	0.009287	0.010787
11	19 CAG	0.008383	0.008315
12	19 CGA	0.076888	0.016437
13	19 CGG	0.041003	0.010246

14	20 TAA	0.111485	0.016532
15	20 TAG	0.054389	0.017168
16	20 TGA	0.020039	0.011016
17	20 TGG	0.003828	0.006544
18	20 CAA	0.013045	0.004896
19	20 CAG	0.051620	0.007064
20	20 CGA	0.000497	0.002150
21	21 TAA	0.019629	0.009833
22	21 TAG	0.006788	0.008437
23	21 TGA	0.002071	0.004429
24	21 TGG	0.026060	0.005128
25	21 CGA	0.009539	0.008663
26	21 CGG	0.004540	0.004889
27	22 TGG	0.009214	0.002332
28	22 CGG	0.000590	0.002332

Research Hypotheses and Significance of the proposed study

Research Hypotheses

1.   Muscle volume changes, estimated by computed tomography and due to strength training, will vary among individuals depending on their IGF1 geneotype.

2.   Muscle strength changes, estimated by 1RM, due to strength training will vary among individuals depending on their IGF1 geneotype.

3.   Muscle quality changes, estimated by measures of muscle volume and strength, due to strength training will vary among individuals depending on their IGF1 geneotype.

Significance:

Numerous studies to date have found an association of the IGF1 polymorphism with blood levels of IGF-I and/or specific health parameters that could be related to the production of IGF-I protein. However, no studies to date have examined the association of this polymorphism with changes in muscle volume (due to strength training) which is known to be affected by production of IGF-I protein. The findings of this study may help to determine whether variation in the promoter region of IGF-I gene locus has an effect on skeletal muscle in response to strength training in older adults. Furthermore, these findings may help to identify individuals who are more vulnerable to the consequences of sarcopenia or those more likely to respond to ST. With this information, and information on other genes found to influence sarcopenia, vulnerable individuals may be targeted as candidates for strength training, physical therapy, nutritional, or other interventions based

on their genetic makeup. This could allow individualized interventions to maximize health benefits in a particular individual.

Delimitations, Limitations & Operational Definitions

Delimitations

1. The scope of this study will be delimited to 67 men and women between the ages of 50 and 85 who volunteer as participants and complete the study protocol.

2. Participation in the study will be limited to healthy participants free of musculoskeletal or cardiovascular disease.

3. Polymorphisms in the regions flanking the 192 polymorphism will not be identified or assessed in the genomic material for this study. It is therefore possible that any reported genotype effect is due to linkage disequilibrium between the 192 polymorphism and a distinct and putative polymorphism.

4. Based on previous research subjects will be divided into three groups in determining the effect of this genotype. The groupings will be based on homo- and hetero-zygosity for the 192 polymorphism.

Limitations

1. The participants will be volunteers and not randomly selected from the general population. Therefore, the results of this study cannot be generalized to individuals who do not possess characteristics such as age, body size, physical activity, etc. similar to those of subjects in the study.

2.	Subjects will self-report many factors related to health and lifestyle such as physical activity habits, dietary habits, medication regimens, and medical conditions. It is possible that inaccurate self-reports may adversely affect the results of this study.

Operational Definitions:

IGF-I protein: A polypeptide similar in structure to insulin with autocrine/paracrine effects on muscle during growth and differentiation and in adult life.

IGF-I gene: A gene of at least 45 kb containing five exons and four introns. The location of the human IGF-I gene is 12q22–q23.

192 polymorphism (IGF-I gene): This polymorphism is identified by the length of a CA dinucleotide repeat found in the promoter region of the IGF-I gene. It can be 16 to 22 dinucleotides in length (99% of population) and is located at nucleotide position 947–984 in the original human IGF-I DNA sequence Genbank accession number M12659, M77496.

Sedentary: A description for individuals who are not physically active. In the proposed study this term describes individuals who, on average, have exercised aerobically for less than 20 minutes per day less than 3 times per week and have not performed any type of regular resistance training over the past six months.

Sarcopenia: A condition characterized by the loss of muscle size, quality, and function that occurs with aging. This typically leads to or exacerbates ailments such as osteoporosis and loss of functional independence.

Dual-energy x-ray absorptiometry (DXA): A technique for assessing whole and regional body composition that considers the body to be composed of three compartments: bone mineral mass, soft tissue, and lean tissue. Tissue amounts are based on the attenuation of x-rays as they pass through the body.

Computed tomography (CT): A technique for assessing regional muscle size based on the examination of axial scans of the thigh. Visual images are created from the measurement of the intensity of x-rays and analyzed to measure cross-sectional area. The images are based on the attenuation of x-rays as they pass through the body. Attenuation scores are measured in hounsfield units, which depend upon the level of absorption of emitted x-ray beams, -1000 air to +1000 bone. AT is usually -190 to -30.

Muscle volume: Muscle volume is an estimate of the volume of the quadriceps muscle group and will be determined by the MIPAV software and equations used by Tracy (207). Briefly, this involves an equation that utilizes the 8-10 axial thigh slices that are obtained from the CT scan.

Muscle quality: Also known as specific tension or specific force, is the strength of a muscle divided by the cross-sectional area to estimate the amount of force production per unit area of muscle tissue. This has been shown to decrease with age and increase with resistance training.

5-RM: Refers to the maximum amount of resistance an individual can move through a complete range of motion only five times

APPENDIX D: FORMS
Consent form

CONSENT TO PARTICIPATE IN A RESEARCH PROJECT

Project Title: Effects of Gene Variations on Age- and Strength Training-Induced Changes in Muscular Strength, Body Composition, Blood Pressure, Glucose Metabolism, and Lipoprotein-lipid Profiles

I state that I am over 18 years of age, in good physical health, and have elected to participate in a program of research being conducted by Dr. Ben Hurley in the Department of Kinesiology at the University of Maryland, College Park, MD 20742.

I understand that the primary purpose of this study is to assess the role that genetics may play in causing losses of muscular strength and muscle mass with age and gains in strength and muscle mass as a result of strength training. I understand that another purpose of the study will be to assess the influence of genes on changes in body composition, blood pressure, blood sugar metabolism, blood fats muscle power, and performance of common physical tasks with age and strength training.

I understand that the procedures involve three phases. During the first phase, I will undergo testing, which will include a blood draw to analyze my DNA (genetic material), blood sugar and fats, and other blood proteins. My blood pressure, body composition, bone mineral density, leg muscle volume, muscle strength, muscle power, and ability to complete selected tasks similar to common activities of daily living will also be assessed during this first phase. The second phase of the study involves my participation in a strength training program three times a week for approximately six months. The third and final phase will be a repeat of all previously taken measures, except analysis of my DNA, which will not need to be repeated. Some of the tests will be repeated both after ~ 10 weeks of training and again after the entire training program. These repeat tests will include blood pressure, strength, power, muscle volume and body composition. Other tests will be repeated only after the entire training program.

I understand that the blood draw will require providing about 2 to 3 tablespoons of blood. I understand that there is a risk of bruising, pain and, in rare cases, infection or fainting as a result of blood sampling. However, these risks to me will be minimized by allowing only qualified people to draw my blood. A portion of this blood sample will be sent to the University of Pittsburgh to analyze my DNA. I understand that the remainder will be stored at the University of Maryland for later analysis of my blood sugar, the hormone that regulates my blood sugar (insulin), blood fats, and other blood proteins. I understand that a portion of this sample may also be used for potential future studies, but only as such studies examine strength, body composition (i.e., fat, muscle & bone), metabolism of blood sugar, and blood pressure. I understand that I may contact the principal investigator at any future point in time to request that any stored blood sample be destroyed immediately.

I understand that while I am lying on a padded table, my leg muscle and fat mass will be measured by computed tomography (CT). The CT scan will be performed at the Washington Adventist Hospital. My percent body fat and bone mineral density measurements will be performed at the United States Department of Agriculture in Beltsville, Maryland by dual-energy x-ray absorptiometry (DXA). This will require my lying still on a padded exam table wearing metal-free clothing for about 10 minutes at a time, totaling less than 30 total minutes for the entire procedure.

I understand that there will be a total radiation dose of approximately 1 Rem to the whole body (effective dose equivalent) from each CT scan. This amount is well below the maximal annual radiation dose (5 Rems) allowed for exposure in the workplace. The body composition and bone density testing completed by DXA involves a small radiation exposure. The radiation exposure I will receive from DXA is equal to an exposure of less than 50 millirems to the whole body. Naturally occurring radiation (cosmic radiation, radon, etc.) produces whole body radiation of about 300 millirems per year. Therefore, the total dose of radiation exposure due to the DXA measurement is minimal and the combined dose of DXA and CT is considered low. The major risk from high radiation exposure is passing on damaged genes (genetic mutations) to offspring. Consequently, this risk is typically of less concern to those who are beyond childbearing age.

I understand that strength and power assessments will be performed on machines that measure how much force and how fast I can exert force through a typical range of knee extension motion. Strength testing will also be performed on the same exercise machines used for training by measuring the maximal amount of force that I can move through the full range of an exercise. During each strength training session I will be asked to exercise on machines which offer resistance against extending and flexing my arms, legs, and trunk region for approximately 40 minutes or less a day, three times a week for up to six months. I understand that I may experience some temporary muscle soreness as a result of the testing sessions. There is also a risk of muscle or skeletal injury from strength and power testing, as well as from strength training. The investigators of this study will use procedures designed to minimize this risk.

I understand that I will be asked to complete some tasks to measure my ability to carry out normal daily activities. These tasks include rising from a chair, short brisk walks and climbing a flight of stairs. Any risk of injury during the completion of these tasks will be minimized by having all sessions supervised by an exercise physiologist qualified to direct this type of testing and wearing a safety harness during the short brisk walks and climbing a flight of stairs.

I understand that it is also possible that heart or blood vessel problems could arise during my participation in the testing or training involved in this study. Although unusual, it is possible that these problems could lead to a heart attack or even death. Therefore, prior evaluation and permission from my physician will be required to participate in this study. I also understand that it is possible that these risks will not be eliminated completely, even with a medical evaluation prior to participation in the study. However, we believe the risk of harm from study participation is small and that the benefits of the study will likely outweigh any probable risks.

I understand that all information collected in this study is confidential, and that my name will not be identified at any time to anyone other than the investigators of the study.

I understand that this study is not designed to help me personally, but may help the investigators better understand who is likely to be most and least susceptible to losing strength, power, and muscle mass with advanced age and who is most and least likely to benefit from strength training.

I understand that it is my decision and my decision alone whether or not I consent to participate in this study. I understand that I am free to ask questions about this study before I

decide whether or not to consent to participate in it. I understand that if I consent to participate in the study I am free to withdraw from participation at any time without penalty or coercion, or without any requirement that I provide an explanation to anyone of my decision to withdraw.

For my participation in the study I will receive information after the study is completed about my blood pressure, blood test results, bone mineral density, body composition, and functional ability, free of charge. However, I understand that I will not receive any financial compensation in exchange for my participation in this study.

In the event of physical injury resulting from participation in this study, upon my consent, emergency treatment will be available at the medical center of Washington Adventist Hospital with the understanding that any injury that requires medical attention becomes my financial responsibility. I understand that the University of Maryland at College Park will not provide any medical or hospitalization insurance coverage for participants in this research study, nor will they provide compensation for any injury sustained as a result of this research study, except as required by law.

I understand that if I am injured while participating in this research project as a result of the negligence of a United States Government employee who is involved in this research project, I may be able to be compensated for my injury in accordance with the requirements of the Federal Tort Claims Act. If I am a federal employee acting within the scope of my employment, I may be entitled to benefits in accordance with the Federal Employees Compensation Act.

I understand that I can discuss this research study at any time with the principal investigator, Dr. Ben Hurley at (301) 405-2486 or with the study coordinator, Matt Delmonico, at (301) 405-2569.

I have read and understand the above information and have been given an adequate opportunity to ask the investigators any questions I have about the study. My questions, if any, have been answered by the investigators to my satisfaction. By my signature I am indicating my decision to consent to participate voluntarily in this study.

Principal investigator: Ben Hurley, Ph.D., Dept of Kinesiology, HLHP Building, University of Maryland, College Park, MD 20742-2611, Ph: (301) 405-2486.

Printed Name of Subject_____

Signature of Subject_____ Date_____

IRB APPROVED
VALID UNTIL

NOV 3 0 2004

UNIVERSITY OF MARYLAND
COLLEGE PARK
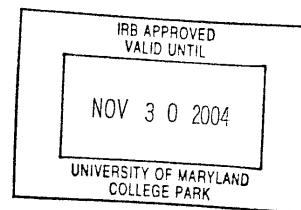
Training log

Unilateral Strength Training

Subject's Name: _____ Seat position _____ 1 RM value _____ Leg _____

Training Session #	FAM I	FAM II	1	2	3	4	5	6
Date								
Pre-Ex .BP (mm Hg)								
5 RM*Resistance (lbs)								
Peak Ex.BP (mm Hg)								
Post Ex.BP (mm Hg)								
Weight (lbs)								

*= Weight adjusted as needed to maintain 5 RM

Training Session #	10	11	12	13	14	15	16	17	18	19	20
Date											
Pre-Ex .BP (mm Hg)											
5 RM*Resistance (lbs)											
Peak Ex.BP (mm Hg)											
Post Ex.BP (mm Hg)											
Weight (lbs)											

*= Weight adjusted as needed to maintain 5 RM

Training Session #	21	22	23	24	25	26	27	28	29	30	31
Date											
Pre-Ex .BP (mm Hg)											
5 RM*Resistance (lbs)											
Peak Ex.BP (mm Hg)											
Post Ex.BP (mm Hg)											
Weight (lbs)											

*= Weight adjusted as needed to maintain 5 RM

Training

Λ 5 reps @ 50% of 1 RM resistance- 30 sec rest
ΛΛ 5 reps @ 5 RM resistance- 1.5 min rest
ΛΛΛ ~5reps @ 5 RM resistance, then lower weight just enough to do 1-3 reps, repeat process until 10 total reps -2.5 min rest.
ΛΛ ~5reps @ 5 RM resistance, then lower weight just enough to do 1-3 reps, repeat process until 15 total reps -3 min rest.
Λ ~5reps @ 5 RM resistance, then lower weight just enough to do 1-3 reps, repeat process until 20 total reps

CT request

The GUSTO Study

"**G**enes **U**nderlying **S**trength **T**raining adaptations in **O**lder adults"

UNIVERSITY OF MARYLAND

College Park

<u>To</u>: Washington Adventist Hospital, Centralized Records & Admitting

<u>Fax #</u>: (301) 891-6149

<u>From</u>: Ben Hurley, Ph.D., Professor, Department of Kinesiology

<u>Fax #</u>: (301) 405-5578   <u>Phone #</u>: (301) 405-2569

<u>RE</u>: Scheduling of patients for CT muscle mass study

Patient Name _____

Previously a patient at Washington Adventist Hospital: ___Yes ___No

Date/Time for CT scan _____ DOB: _____ Age _____ Sex ___

CT scanner: ___Old scanner ___Newer scanner ___Either

Address _____   Phone # _____

           _____

           _____

Diabetes: __Yes __No   If yes, type 1 or type 2? _____ Meds: _____

Scan type: <u>Extremity (bilateral thigh)</u>   <u>Contrast</u>: **NO**

Emergency Contact (relationship) _____ Phone # _____

Initial telephone interview

University of Maryland at College Park
Department of Kinesiology

The GUSTO Study

Data Sheet for Initial Subject Telephone Interview

➤ Contact Information

Name: _____

Address: _____

_____

Phone #: _____ E-Mail: _____

Best way and time to contact: _____

How did you hear about the GUSTO Study? _____

➤ Age _____yrs   Date of Birth: _____/_____/_____
                                   MM   DD   YY

➤ Smoker? ___Yes ___No

➤ Any history of heart disease, stroke, or other cardiovascular diseases?
   ___Yes ___No
      If yes, briefly describe: _____

➤ History of diabetes? ___Yes ___No
   If yes, type I or type II? _____ Take insulin? __Yes __

➤ Currently involved in any regular exercise or fitness routine or program?
   ___Yes ___No
      If yes, briefly describe: _____

➤ What racial group best identifies you?

      ___ American Indian or Alaskan Native
      ___ Asian or Pacific Islander
      ___ Black, not of Hispanic origin
      ___ Hispanic
      ___ White, not of Hispanic origin
      ___ Other/Unknown

"Thanks for your interest in the GUSTO Study. We will contact you within a week
you know whether you may be qualified for the GUSTO study."

Medical Clearance

Medical Clearance to Participate in Research Project

It is my understanding that _____ (name of the volunteer), a patient under my care, has volunteered to participate in the study entitled, *"Do Genes Influence Responses to Strength Training?"* The volunteer must have the approval of her or his physician to participate in this study.

Exclusionary criteria for eligibility are listed below. If you believe that your patient named above has any of the medical conditions indicated below, please place a check in front of the condition(s) indicated:

_____ Severe cardiovascular disease, such as ___unstable angina, ____uncontrollable hypertension, ____uncontrolled dysrhythmias, ____severe stenotic or regurgitant valvular disease, _____ hypertrophic cardiomyopathy and _____ symptomatic peripheral arterial disease.

____Severe COPD or other signs of significant pulmonary dysfunction

____Intracranial aneurysm

____Musculoskeletal diseases that cause severe joint pain at rest or upon exertion

____Diseases that promote muscle protein breakdown

____Joint, vascular, abdominal or thoracic surgery in the past year

____History of bone fragility fractures

____Having any condition that is likely to be aggravated by muscular exertion

____Being unable to engage safely in mild to moderate exercise, such as independently walking up at least one flight of stairs or walking two blocks on level ground.

Although we are unaware of any cardiac complications that have resulted from strength testing or strength training, there is only a limited amount of data available in people over the age of 75. There is one report of non-fatal subarachnoid hemorrhage associated with strength training in three patients who had pre-existing intracranial aneurysms. For this reason, any patient who has known or suspected intracranial aneurysm or who is at high risk for having an intracranial aneurysm, should not participate in this study.

Please check one of the following:

_____ Clearance granted

_____ Clearance not granted

_____ Please send me the following information about the study:

Volunteers in this study will participate in resistance exercise under the supervision of exercise specialists trained specifically for this study under the direction of the Principal Investigator, Ben Hurley Ph.D., Professor, Department of Kinesiology, College of Health and Human Performance, University of Maryland, College Park, Maryland 20742(email: bh24@umail.umd.edu; tele: 301-405-2486; fax: 1-301-405-5578).

Physician's signature: _____   Date_____

1RM data collection form

University of Maryland / National Institute on Aging
GUSTO

Symptom-limited Baseline Knee Extension 1-RM

Arms across chest	_____
Seat Belt	_____
Remember to breathe	_____
CHECK EACH LINE BEFORE TEST	

Examiners Name_____

Name_____ Date_____

Time _____ Location_____

Body weight_____ Age_____ Predicted 1-RM_____

Seat_____ Leg_____ Blood Pressure_____ *Right leg / Left leg*

	Resistance	P/D scale	RPE scale
Rest	-----	_____	_____
Set 1	0	_____	_____
Set 2	_____	_____	_____
Set 3	_____	_____	_____
Set 4	_____	_____	_____
Set 5	_____	_____	_____
Set 6	_____	_____	_____
Set 7	_____	_____	_____
Set 8	_____	_____	_____
Set 9	_____	_____	_____
Set 10	_____	_____	_____
Set 11	_____	_____	_____
Set 12	_____	_____	_____

Most severe P/D: _____ Subject's initials: _____

Post BP_____ 3 min. post BP_____

Notes:_____

_____

_____

DXA record
DEXA Body Scan – USDA / University of Maryland
Conway/Hurley/Kostek

Date:_____ Time:_____am/pm

Name:_____ Gender: M / F

Date of Birth:_____

Height:_____ inches   _____ cm

Weight:_____ lbs.   _____ kg

Subject number:_____

Dominant leg: R / L

Time and composition of last meal (or snack):

_____

_____

_____

_____
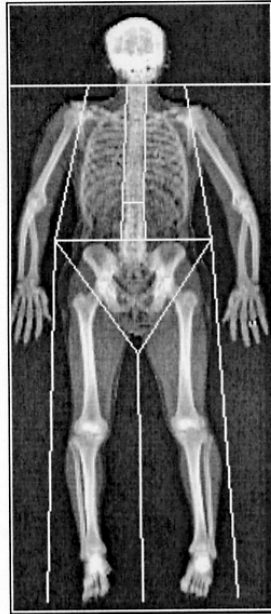

Comments:_____

_____

Initials of examiner and DXA technician:_____

DXA output

HOLOGIC

oJan 26 16:24 2004   [327 x 150]
Hologic QDR-4500A (S/N 45816)
Whole Body Fan Beam V8.26a:3*

A01130403
Name:
Comment: post whole body
I.D.:   gusto   Sex: M
S.S.#:   - -   Ethnic: W
ZIP Code: Height: '   "
Operator: KRS Weight: 172
BirthDate: 06/04/35   Age: 68
Physician: GUSTO
Image not for diagnostic use
  TBAR1790 - 1
  F.S. 68.00% 0(10.00)%
  Head assumes 17.0% brain fat
  LBM 73.2% water

Region	Fat (grams)	Lean+BMC (grams)	% Fat (%)
L Arm	862.4	3675.6	19.0
R Arm	900.3	3498.9	20.5
Trunk	10310.3	29972.9	25.6
L Leg	2325.1	9636.4	19.4
R Leg	2985.4	9181.3	24.5
SubTot	17383.4	55965.1	23.7
Head	1072.3	4378.2	19.7
TOTAL	18455.7	60343.2	23.4

HOLOGIC

Representation of RFLP genotyping gels
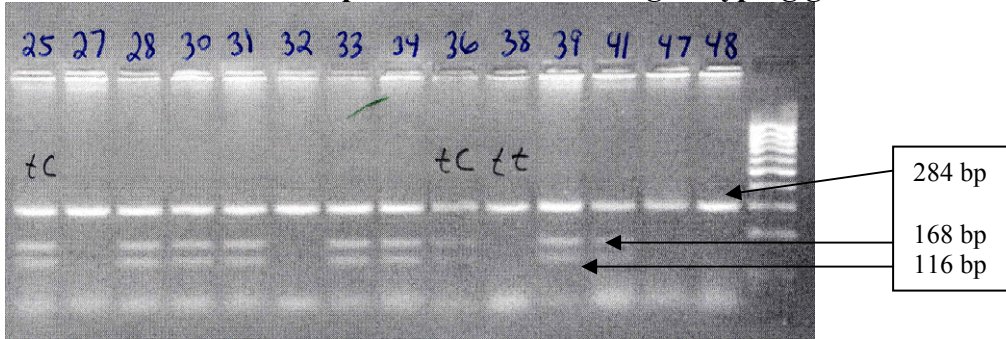
19779
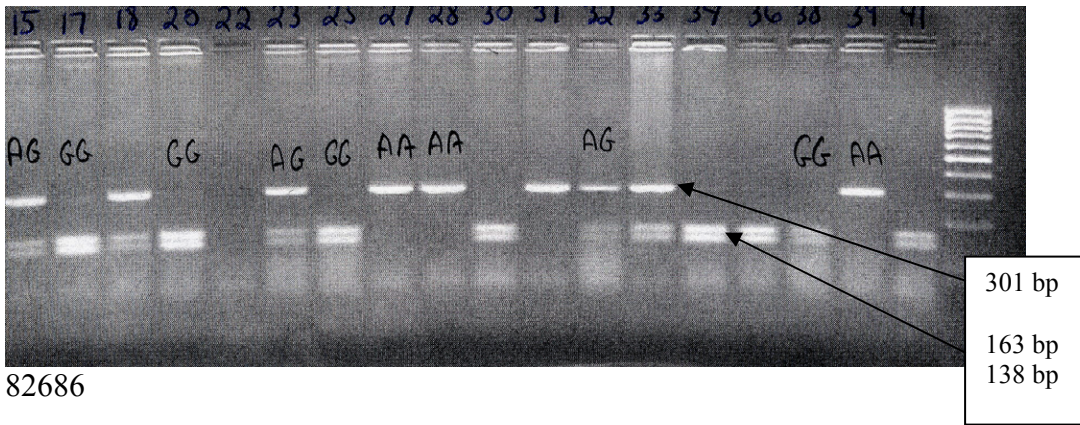
82686

40395

Representation of FAM labeled CA repeat on ABI 3100
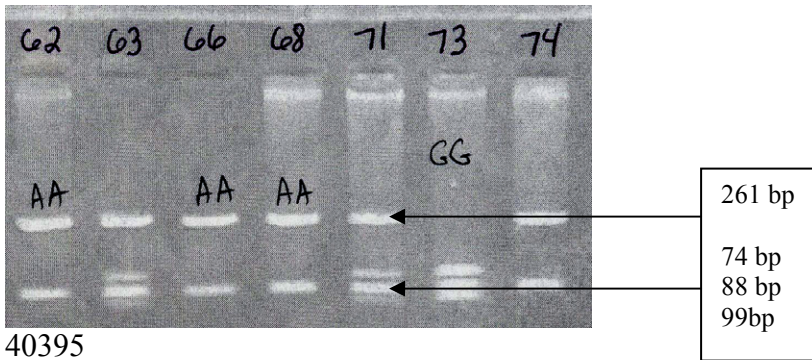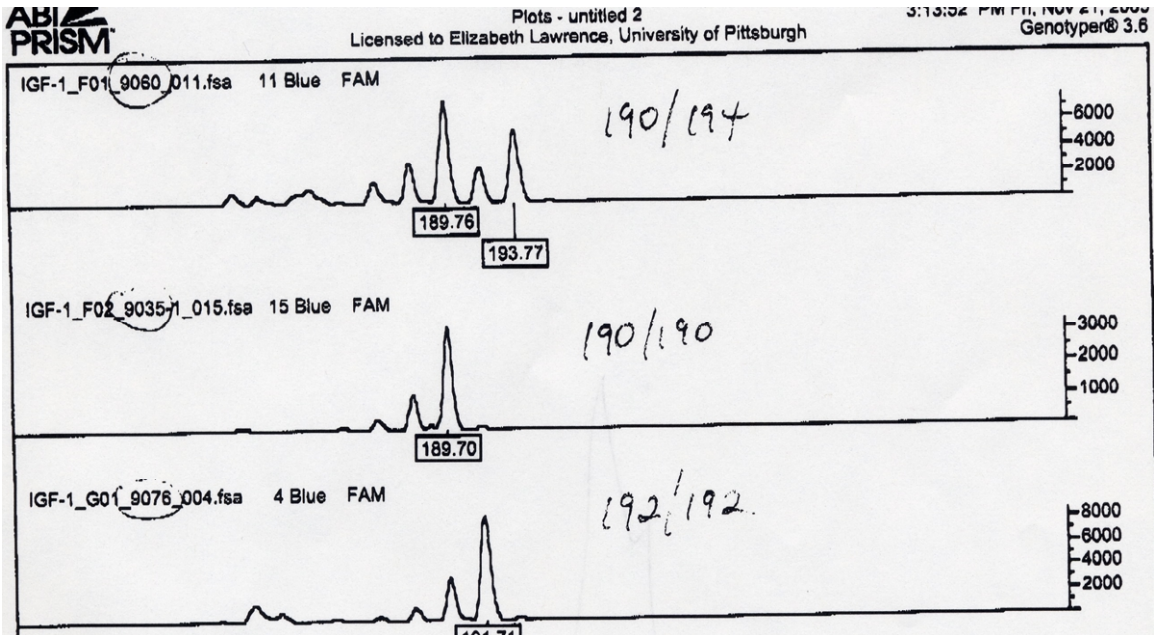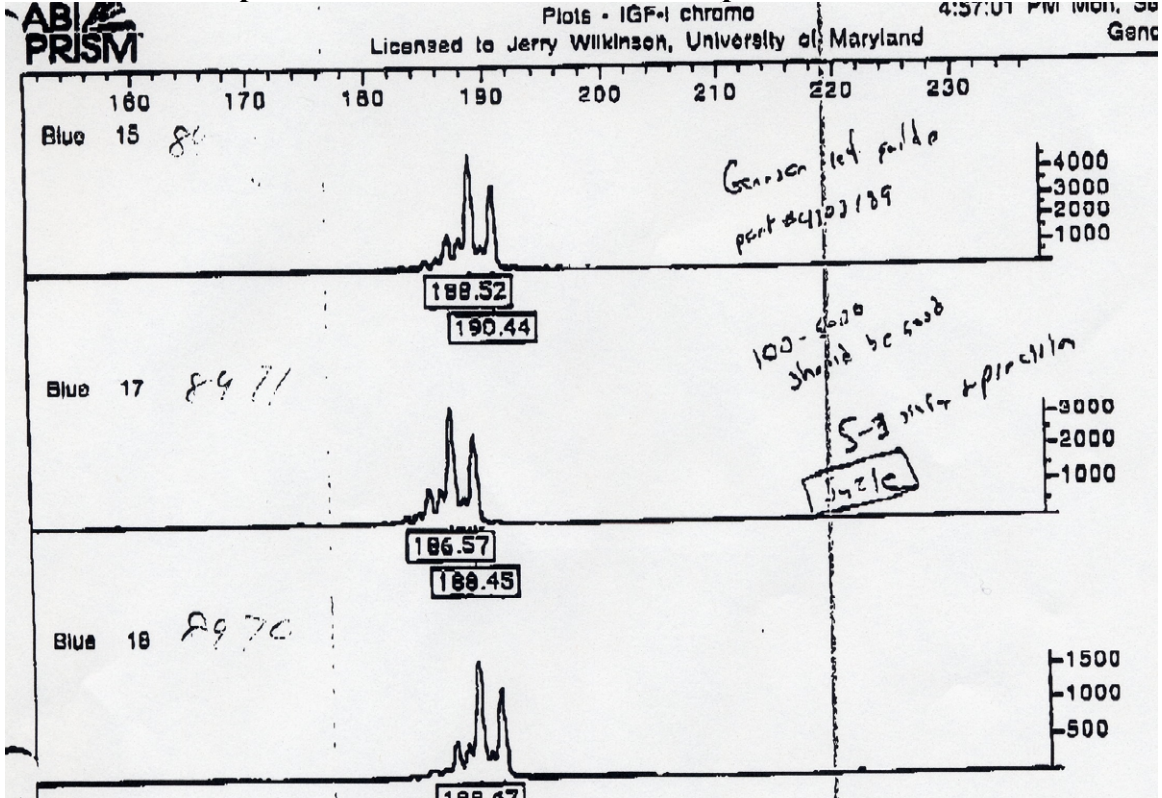
ID Number	IGF1 promoter genotype	IGF category	Age	SEX	19779	40395	82686
HUR 015	192/194	2	79	F	TT	AA	AG
HUR 017	190/192	2	80	M	TT	AA	GG
HUR 018	192/194	2	77	M	TT	AG	AG
HUR 020	190/194	3	60	F	TC	AA	GG
HUR 023	192/192	1	61	F	TT	AA	AG
HUR 025	192/196	2	57	F	TC	GG	GG
HUR 027	194/194	3	52	F	TT	AA	AA
HUR 028	192/192	1	64	F	TC	AG	AA
HUR 030	192/194	2	57	F	TC	AA	GG
HUR 031	192/192	1	60	F	TC	AG	AA
HUR 032	190/194	3	54	M	TT	AG	AG
HUR 033	192/192	1	62	F	TC	AA	AG
HUR 034	192/192	1	65	F	TC	AG	GG
HUR 036	192/194	2	75	M	TC	AA	GG
HUR 038	192/194	2	61	M	TT	AA	GG
HUR 039	194/194	3	77	M	TC	AA	AA
HUR 041	192/192	1	63	M	TC	AG	GG
HUR 047	192/196	2	54	M	TT	AG	GG
HUR 048	192/192	1	53	F	TT	AA	GG
HUR 049	192/194	2	77	F	TT	AA	AG
HUR 052	192/192	1	78	F	TT	AA	GG
HUR 055	192/194	2	61	F	TC	AG	AA
HUR059	192/196	2	78	F	TT	AA	AG
HUR060	192/196	2	76	F	TT	AA	AG
HUR 062	192/194	2	69	M	TT	AA	GG
HUR 063	192/196	2	66	M	TC	AG	AA
HUR 065	192/192	1	65	M	TC	AG	AG
HUR 066	192/194	2	71	F	TT	AA	
HUR 068	194/194	3	66	M	CC	AA	GG
HUR 071	192/192	1	75	M	TC	AG	AA
HUR 073	192/196	2	72	M	CC	GG	AG
HUR 074	192/192	1	65	F	TT	AA	GG
HUR 075	190/192	2	71	M	TT	AA	AG
HUR 076	192/194	2	59	F	TT	AG	AG
HUR 077	192/192	1	70	F	TT	AA	GG
HUR 078	192/192	1	71	M	TT	AA	GG
HUR 079	192/196	2	72	M	TT	AG	GG
HUR 080	188/194	3	82	M	TT	AA	AG
HUR 081	192/194	2	83	F	TT	AA	AG
HUR 084	194/194	3	81	F	TT	AA	AA
HUR 085	192/192	1	71	M	TC	AG	AG
HUR 087	188/192	2	62	M	TT	AA	GG
HUR 091	192/192	1	61	F	TC	AG	AG
HUR 092	192/194	2	68	M	TC	AA	AG

HUR 093	192/192	1	65	F	TT	AA	AG
HUR 094	192/194	2	65	F	TT	AA	GG
HUR 097	192/196	2	85	F	TT	AG	AG
HUR 098	192/194	2	72	M	TT	AA	AG
HUR 099	192/192	1	70	M	TC	AG	AA
1	192/194	2	68	M			
2	190/192	2	71	M			
3	192/198	2	66	M			
4	192/196	2	69	M			
5	190/192	2	71	M			
6	192/192	1	72	M			
7	192/196	2	68	M			
9	192/192	1	72	M			
10	192/192	1	73	M			
11	192/192	1	65	M			
14	190/192	2	67	F			
16	192/192	1	64	F			
18	192/196	2	66	F			
20	192/192	1	71	F			
21	192/192	1	70	F			
22	176/188	3	70	F			
23	194/196	3	66	F			
24	190/192	2	73	F			

SD		7.46
Mean		68.36
SEM		0.91

ID Number	Height	Weight baseline	Weight After	Baseline %Fat	After %Fat	Baseline FFM	After FFM
	cm	kg	kg	%		kg	kg
HUR 015	168.5	87.2	86.5	47.64%	46.31%	45.66	46.44
HUR 017	160.5	65.5	64.6	20.90%	22.93%	51.81	49.79
HUR 018	168.6	78.4	78.9	28.11%	26.92%	56.36	57.66
HUR 020	162	54.8	53.8	33.52%	31.38%	36.43	36.92
HUR 023	165.1	62.2	62.3	35.05%	33.75%	40.4	41.3
HUR 025	169.6	90.2	93.4	41.31%	46.84%	52.94	49.65
HUR 027	161.6	100.9	101.4	46.48%	46.64%	54	54.1
HUR 028	160	62.6	64.8	39.31%	33.23%	37.99	43.24
HUR 030	161.8	60	60.4	30.67%	29.67%	41.6	42.45
HUR 031	165	87.9	89.2	45.11%	46.02%	48.25	48.17
HUR 032	168.6	95	95.4	34.21%	34.86%	62.5	62.16
HUR 033	178	60.4	60.3	28.44%	30.13%	43.22	42.13
HUR 034	175.5	88.5	88.7	39.40%	39.44%	53.63	53.73
HUR 036	169	87.42	86.9	25.50%	26.00%	65.13	64.31
HUR 038	164.9	63.5	63.5	21.10%	21.15%	50.1	50.07
HUR 039	179.5	97	99.0	33.30%	32.19%	64.7	67.13

HUR 041	163.7	70.84	69.8	30.97%	30.52%	48.9	48.5
HUR 047	179.6	92.64	95.0	30.92%	31.79%	64	64.8
HUR 048	168.3	75	75.0	40.53%	38.93%	44.6	45.8
HUR 049	165.1	87.09	88.8	49.67%	48.13%	43.83	46.08
HUR 052	158	112.5	111.0	A1			
HUR 055	164.7	65.6	64.8	39.02%	37.19%	40	40.7
HUR059	145.4	53.5	51.3	36.64%	37.76%	33.9	31.93
HUR060	159.8	68.12	69.3	34.38%	37.05%	44.7	43.6
HUR 062	173.4	79.12	79.3	29.44%	29.07%	55.83	56.22
HUR 063	171.2	74.32	74.4	30.81%	28.36%	51.42	53.3
HUR 065	178	97.27	99.3	30.42%	31.26%	67.68	68.26
HUR 066	160.02	66.36	66.9	42.13%	41.85%	38.4	38.9
HUR 068	178.2	77.7	78.1	25.62%	28.39%	57.79	55.9
HUR 071	172.3	85.84	87.3	32.67%	33.30%	57.8	58.2
HUR 073	176.1	104.2	105.6	35.99%	34.17%	66.7	69.52
HUR 074	158.5	62.82	58.6	36.33%	33.11%	40	39.2
HUR 075	172.1	88.56	85.6	28.48%	28.86%	63.34	60.9
HUR 076	160.6	60.54	59.8	34.61%	35.54%	39.59	38.56
HUR 077	156.4	67.96	65.8	36.79%	37.52%	42.96	41.1
HUR 078	168.9	74.6	73.2	22.82%	21.20%	57.58	57.71
HUR 079	176.5	92.53	97.9	30.15%	33.67%	64.63	64.94
HUR 080	171	60.32	60.0	21.75%	21.83%	47.2	46.9
HUR 081	143.7	57	58.9	32.28%	33.29%	38.6	39.29
HUR 084	151.4	55.26	53.9	32.14%	31.95%	37.5	36.68
HUR 085	190.4	115.8	115.5	29.71%	29.70%	81.4	81.2
HUR 087	170.2	83.91	89.3	21.92%	24.95%	65.52	66.98
HUR 091	154.9	63.95	65.4	40.73%	41.60%	37.9	38.21
HUR 092	178.7	76.34	77.0	22.31%	23.13%	59.31	59.19
HUR 093	162.1	77.84	77.4	43.65%	45.28%	43.86	42.35
HUR 094	154.7	72.34	72.1	43.35%	45.40%	40.98	39.38
HUR 097	155.6	66.9	70.4	36.41%	40.33%	42.54	42.01
HUR 098	174.1	87.6	86.8	31.37%	34.06%	60.12	57.24
HUR 099	178.5	88.8	89.6	27.82%	28.13%	64.1	64.4
1	162.56	85.3	84.7	40.55%	29.74%	50.712	59.524
2	170.18	65.5	67.1	22.06%	23.38%	51.051	51.38
3	172.72	82.5	81.2	32.07%	30.23%	56.042	56.684
4	175.26	88	89.8	35.93%	34.28%	56.386	58.997
5	180.34	78	78.2	A2			
6	177.8	74.5	75.9	29.35%	29.27%	52.635	53.6715
7	167.64	72.7	73.4	22.82%	22.32%	56.113	57.034
9	175.26	83.4	82.9	29.84%	30.10%	58.512	57.926
10	177.8	80.5	83.0	23.79%	25.31%	61.348	62.012
11	177.8	96.5	97.2	34.81%	35.79%	62.911	62.416
14	165.1	79.1	78.5	43.42%	42.90%	44.752	44.802
16	154.94	69.4	67.9	43.47%	43.50%	39.229	38.381
18	165.1	61	61.1	40.92%	38.71%	36.039	37.453
20	154.94	65.6	65.7	41.78%	41.84%	38.194	38.199
21	175.26	83	81.4	42.93%	39.77%	47.367	49.003
22	149.86	66.3	64.1	43.08%	41.58%	37.738	37.435
23	162.56	69.2	74.7	35.53%	38.66%	44.611	45.829

24	162.56	66.9	66.1	37.54%	35.86%	41.786	42.406
SD	9.3	14.17	14.6	0.07	0.07	10.4	10.6
Mean	167.1	77.20	77.5	0.34	0.34	50.5	50.8
SEM	1.1	1.73	1.8	0.01	0.01	1.3	1.3

A1 = Exceeded weight restrictions for accurate DXA analysis and was not scanned.
A2 = Final body comp not in "Contract study" database, therefore his body comp data not used.

ID Number	Untrained Leg baseline (lbs)	Untrained Leg after 10 wk Training (lbs)	Trained Leg baseline (lbs)	Trained Leg after 10wk Training (lbs)	Change in trained leg after 10wk (lbs)	Percent increase
HUR 015	98	101	107	145	38	35.5%
HUR 017	64	101	120	156	36	30.0%
HUR 018	150	170	170	200	30	17.6%
HUR 020	96	95	101	102	1	1.0%
HUR 023	112	114	112	130	18	16.1%
HUR 025	112	137	84	137	53	63.1%
HUR 027	138	142	152	187	35	23.0%
HUR 028	70	65	95	128	33	34.7%
HUR 030	96	98	108	136	28	25.9%
HUR 031	76	92	97	130	33	34.0%
HUR 032	175	200	188	220	32	17.0%
HUR 033	A3					
HUR 034	127	135	130	160	30	23.1%
HUR 036	117	126	126	155	29	23.0%
HUR 038	123	190	123	200	77	62.6%
HUR 039	152	157	165	181	16	9.7%
HUR 041	105	115	117	158	41	35.0%
HUR 047	200	245	225	275	50	22.2%
HUR 048	115	118	110	152	42	38.2%
HUR 049	95	95	100	100	0	0.0%
HUR 052	120	130	123	150	27	22.0%
HUR 055	117	55	133	170	37	27.8%
HUR059	61	46	65	64	-1	-1.5%
HUR060	25	20	96	125	29	30.2%
HUR 062	206	220	185	225	40	21.6%
HUR 063	145	145	140	170	30	21.4%
HUR 065	154	155	170	190	20	11.8%
HUR 066	A4		85	103	18	21.2%
HUR 068	114	140	158	185	27	17.1%
HUR 071	136	140	175	190	15	8.6%
HUR 073	166	160	130	170	40	30.8%
HUR 074	120	125	125	141	16	12.8%

HUR 075	110	155	110	170	60	54.5%
HUR 076	100	86	105	115	10	9.5%
HUR 077	76	90	87	112	25	28.7%
HUR 078	150	175	174	212	38	21.8%
HUR 079	200	205	200	240	40	20.0%
HUR 080	88	96	91	117	26	28.6%
HUR 081	55	55	65	76	11	16.9%
HUR 084	A5		80	95	15	18.8%
HUR 085	191	200	175	205	30	17.1%
HUR 087	220	246	245	285	40	16.3%
HUR 091	A6		88	103	15	17.0%
HUR 092	125	135	144	185	41	28.5%
HUR 093	103	112	100	130	30	30.0%
HUR 094	114	110	119	120	1	0.8%
HUR 097	105	100	100	113	13	13.0%
HUR 098	155	162	155	188	33	21.3%
HUR 099	216	224	220	264	44	20.0%
1	165	180	175	222	47	26.9%
2	150	155	160	200	40	25.0%
3	210	215	200	235	35	17.5%
4	162	190	165	215	50	30.3%
5	160	165	155	185	30	19.4%
6	168	203	174	225	51	29.3%
7	173	200	153	205	52	34.0%
9	155	165	175	210	35	20.0%
10	125	130	150	180	30	20.0%
11	145	195	145	230	85	58.6%
14	100	105	105	130	25	23.8%
16	91	93	87	100	13	14.9%
18	78	85	75	102	27	36.0%
20	100	115	110	140	30	27.3%
21	105	115	105	145	40	38.1%
22	115	125	95	100	5	5.3%
23	40	60	70	100	30	42.9%
24	95	100	85	125	40	47.1%
SD	43.44	50.45	41.67	50.13	16.29	0.13
Mean	125.87	136.17	131.17	162.33	31.17	0.24
SEM	5.47	6.36	5.13	6.17	2.01	0.02

A3 = After training 1RM sheet was lost before data entry therefore none of her strength values were used.

A4 = Invalid test at post-training for untrained leg. Therefore, untrained strength values are not used.

A5 = Previous injury on untrained knee therefore data for untrained strength not used.

A6 = Invalid post-test due to pain/discomfort during test. Her data for untrained strength not used.

ID Number	Untrained leg baseline	Untrained leg after training	Trained leg baseline	Trained leg after	Δ muscle volume
	cubic cm	cubic cm	cubic cm	cubic cm	cubic cm
HUR 015	1097.2	1100.2	1188.2	1281.6	90.4
HUR 017	1300.0	1224.0	1321.7	1432.7	187.0
HUR 018	1585.2	1578.2	1531.5	1641.0	116.6
HUR 020	1062.7	1065.3	1029.1	1083.1	51.3
HUR 023	680.0	696.0	1084.0	1172.0	72.0
HUR 025	1183.8	1208.9	979.8	1139.7	134.7
HUR 027	1490.0	1527.0	1442.0	1525.0	46.0
HUR 028	A7				
HUR 030	1095.3	1094.9	1265.3	1303.1	38.1
HUR 031	1024.8	1010.7	1203.4	1296.6	107.4
HUR 032	1781.3	1788.6	1839.7	2011.7	164.7
HUR 033	1315.0	1312.0	1245.0	1299.0	51
HUR 034	1556.4	1548.2	1566.2	1705.3	147.3
HUR 036	A8				
HUR 038	1428.0	1417.3	1347.4	1469.2	132.5
HUR 039	1887.3	1882.1	1976.8	2053.5	81.9
HUR 041	1601.0	1619.0	1802.0	1871.0	41.0
HUR 047	2047.5	2095.2	2349.3	2605.1	208.1
HUR 048	A9				
HUR 049	A10				
HUR 052	A11				
HUR 055	1139.2	1091.7	1286.1	1379.7	141.1
HUR059	A12				
HUR060	255.3	258.5	1122.0	1283.0	157.8
HUR 062	1650.7	1678.1	1731.3	1986.5	227.8
HUR 063	1365.9	1435.8	1403.9	1571.4	97.5
HUR 065	A13				
HUR 066	679.0	540.0	754.6	805.2	189.6
HUR 068	1523.7	1476.9	1630.6	1692.0	108.2
HUR 071	1602.1	1565.3	1652.9	1801.1	185.0
HUR 073	1812.6	1772.5	1881.6	2039.1	197.6
HUR 074	1156.4	1134.8	1211.7	1270.3	80.2
HUR 075	1461.5	1422.5	1432.8	1550.9	157.1
HUR 076	967.1	919.4	790.0	916.0	173.7
HUR 077	975.6	965.9	1085.5	1138.1	62.3
HUR 078	1599.3	1560.9	1638.0	1670.9	71.2
HUR 079	1894.3	2035.0	1885.2	2036.9	11.0
HUR 080	1174.8	1185.8	1185.8	1240.7	43.9
HUR 081	903.8	906.2	789.5	854.7	62.8
HUR 084	736.8	723.4	754.0	867.7	127.1
HUR 085	2007.8	2069.1	1847.6	2016.8	108.0
HUR 087	1952.0	1920.0	1820.0	2108.0	320.0
HUR 091	907.9	894.3	900.9	982.0	94.7
HUR 092	1644.5	1591.4	1625.4	1742.2	170.0

HUR 093	1030.0	1008.0	1042.0	1129.0	109.0
HUR 094	1081.0	1037.1	1025.2	1126.5	145.1
HUR 097	958.0	998.0	959.0	1043.0	44.0
HUR 098	1601.0	1624.0	1650.0	1808.0	135.0
HUR 099	1864.0	1845.0	1834.0	2120.0	305.0
1	1768.0	1937.0	1755.3	2132.4	208.1
2	1504.3	1564.9	1528.0	1691.6	103.0
3	1785.4	1798.6	1815.4	2000.3	171.7
4	1803.3	1894.0	1769.8	2055.0	194.5
5	1188.0	1097.0	1718.6	1860.6	233.0
6	1818.0	1834.0	1708.0	1948.1	224.1
7	1683.0	1794.1	1615.0	1858.9	132.9
9	1725.9	1749.5	1893.3	2040.9	124.0
10	1662.9	1635.6	1696.2	1916.5	247.5
11	1987.1	1974.0	1966.1	2085.6	132.5
14	941.2	992.7	1217.2	1305.9	37.2
16	941.2	992.7	1090.2	1245.7	104.0
18	941.2	992.7	890.7	985.0	42.8
20	1133.8	1150.1	1137.5	1257.9	104.1
21	1223.6	1203.4	1258.6	1373.9	135.4
22	1089.6	1127.4	935.7	1132.3	158.8
23	870.3	948.1	920.0	1036.0	38.2
24	1223.2	1226.6	1178.5	1295.7	113.8
SD	402.14	416.73	379.43	420.8	67.4
Mean	1356.17	1362.33	1403.42	1538.2	128.3
SEM	51.92	53.80	48.98	54.3	8.7

A7 – A13 = CT scan misalignment or poor clarity. Therefore, scan analysis not reliable and data for muscle volume and quality not used.

APPENDIX G: LITERATURE REVIEW

The following review of literature provides background information relevant to the understanding of the influence of the IGF1 gene on ST adaptations in older adults. This review will focus on the following topics: 1) causes and consequences of sarcopenia, 2) muscle strength declines with age, 3) muscle mass decline with age, 4) assessment of muscle mass and muscle quality, 5) mechanisms of sarcopenia, 6) strength training adaptations in the elderly, 7) genetic variability in skeletal muscle, 8) IGF-I physiology, and 9) IGF-I promoter polymorphism and the recommendations for future studies.

Causes and consequences of sarcopenia: The term sarcopenia was coined by Rosenberg in 1989 (179). It comes from the Greek words *sarx* (flesh) and *penia* (loss), literally meaning loss of flesh. Sarcopenia refers to the loss of muscle mass with aging, which in turn affects function. Skeletal muscle function, in this case, can be defined as the ability of muscle to do mechanical and/or chemical (metabolic) work. The decline in work capacity of muscle is due to many factors, including a loss or detrimental change in the muscle's total volume, strength, gene expression for synthesis of contractile proteins, metabolic characteristics, nervous system innervation and number and type of muscle fibers. These changing characteristics of skeletal muscle can result in a decline in function with increasing age. It has been reported from both cross-sectional and longitudinal studies that muscle function declines with advancing age (59,115,121,128). Although differences exist, the decline in muscle function is similar between men and women (115). This functional decline in muscle leads to loss of independence, hip

fractures, morbidity and mortality (4,161,163). Depending on the criteria used to classify

sarcopenia, the number of people who are sarcopenic in the US is estimated to be about 9

million (12). With an aging society, this is estimated to expand significantly and become

a major medical cost. In the United States, census data report that 39 million Americans

are over the age of 65. Within six years, this will increase by more than 6 million (US

census Bureau, 2000). Consequently, health care costs will increase for the elderly. In

2001, projects spending totaled 103 billion dollars for nursing home stays, and by 2010,

this will increase by 77% to 183 billion (Health Care financing administration, 2000).

Sarcopenia is believed to be a multifactorial process that occurs, to some degree,

naturally as part of the aging process in humans, but with significant inter-individual

variability. The primary measures of loss of muscle function are muscle strength and

mass (or volume). The loss of both muscle strength and mass are a known consequence

of aging. Numerous cross-sectional and longitudinal studies have demonstrated that

muscle strength and mass decrease with aging (114,115,121,128).

Sarcopenia does occur ubiquitously yet with significant variability in humans.

While the causes are yet to be fully elucidated, it appears that interventions to counteract

sarcopenia hold much promise with ST being the most efficacious. With an aging

society, it becomes more important to address this disease process through a better

understanding of its causes, prevalence, and treatment.

The first study to define sarcopenia in a large group was in The New Mexico

study reported by Baumgartner et al. (12). In this study, sarcopenia was defined as

having two SD below the mean appendicular muscle mass for healthy young adults.

Baumgartner et al. (6) measured 883 elderly Hispanic, white men, and women

appendicular muscle mass by DXA. The prevalence of sarcopenia ranged from 13 to 24

% in persons 65 to 70 yr and was over 50% for those older than 80 yr. Additionally,

sarcopenic women had 3.6 times higher rates of disability and men had 4.1-fold higher

rates compared with those with greater muscle mass. Iannuzzi-Sucich et al. (45) used

DXA to quantify appendicular skeletal muscle mass in 195 women aged 64-93 and 142

men 64-92yr. They defined sarcopenia as two SD below the muscle mass /height (m2)

for young controls. The overall prevalence of sarcopenia was 22.6% in women and

26.8% in men and 31 and 45% respectively for women and men over age 80.

The consequences of sarcopenia range from decreased functional capacity to increased

mortality.   Several studies have reported an association between low muscle strength

and increased mortality rates (64,107,164). It has been shown that corrected arm muscle

area is a better predictor of long term mortality than BMI which is an often used predictor

of mortality in older adults (130). Additionally, Tellado et al. (98) demonstrated that

body cell mass was the best predictor of mortality in an intensive care unit independent of

diagnosis. In a recent report from the BLSA, Metter et al. (69) reported that hand grip

strength was a predictor of mortality as was the change in hand grip strength, independent

of physical activity or muscle mass.

Muscle volume and strength reach a peak between 40 and 50 yrs of age and

remain relatively constant until the fifties. Beginning in the sixties muscle strength will

begin to decline at approximately 12 - 14% per decade and muscle mass will decline at ~

6% per decade (121). This amounts to a loss of muscle function of ~ 40% by the eighth

decade of life and often leads to disability and morbidity and possibly even mortality

(129,131). With an aging population, this is becoming a greater public health concern.

Muscle Strength decline with age: The age related losses in muscle strength are related to changes in neural drive, altered muscle pennation angle, increases in connective tissue, loss of type II fibers, and decreased expression of MHC proteins (6,97,104,114). . Cross-sectional as well as longitudinal studies have consistently demonstrated a significant loss of muscle strength beginning in the 50s or 60s for men, and slightly earlier in women (115,121) . The average reported age-related decrease in strength is 20-40% when young subjects are compared to those in their 70s or 80s (106,228,229). Men have a greater absolute loss than women, yet begin with a greater amount and thus will retain a greater absolute amount of muscle strength than women even after aging. Starting with a lower absolute value combined with the fact that they live longer, women are believed to be more susceptible to sarcopenia and live a longer time in infirmity than men (108).

Investigators have concluded that concentric strength tends to peak in the 30s, then plateaus until ~50 yr of age and then declines at a rate of ~10-15% per decade (60,106,116). Indeed, this decrement is at least partially due to a decline in force production by muscle fibers. Single muscle fibers from elderly humans have been studied and compared to younger adults cross-sectionally. For example, Frontera et al. reported older men (~75 yr) having a 35% reduction in force generation in type IIa fibers than younger men (~35 yr) (61). In another cross-sectional study, Trappe et al. (208), reported older women having 25-40% less power in single fibers when compared to young women, old men, and young men . Older women also demonstrated a reduced force production in single fibers (208) . Thus, it seems that the reduction in whole muscle strength is at least partially due to decrease in force generating potential of single

muscle fibers. This assumes that all motor units in the muscle are being fully activated.

Yet, if the elderly cannot maximally activate existing musculature then force production

of muscle fibers may not be much of a limiting factor. It does appear that older

individuals can fully and maximally contract their remaining musculature as the twitch

interpolation technique has demonstrated full or near full contraction of muscle in the

elderly (29,82,101). Although some studies have shown less than full activation in older

adults and as low as 69% (75,196). Additionally, ST did not seem to increase maximal

musculature activation in those who could not fully activate their muscles (75). Yet a

recent report suggests that with practice elderly men can fully activate their elbow

flexors and extensors if a deficit was present (86). Previous studies have not allowed a

skill learning or practice period for measured strength tasks. Thus, drawing evidence

from the numerous aforementioned studies of varying design, it would seem that there is

a significant decline in muscular strength with advancing age. Yet individuals do not

experience this decline in strength at identical rates. For example, Kallman et al. in a

cross-sectional analysis of 847 subjects 20 to 100 yr, reported grip strength peaked in

fourth decade and declined in curvilinear fashion thereafter, so that by the ninth decade

strength had declined 37%. Their longitudinal analysis, however, showed that 15% of the

subjects aged 60 yr and over exhibited no strength decline during an average 9-yr follow-

up, suggesting significant interindividual variability in the loss of strength.

Muscle mass decline with age: The loss of muscle mass with age is highly

correlated with and is likely a contributing factor to the loss of muscle strength that

occurs with age (60,167,229). Depending on the measurement technique used, muscle

mass has been reported to decline with age at a slower rate than muscle strength (6,125).

Although various measurement techniques have been used to estimate losses in muscle mass with age (ultra-sound, computed tomography scanning (CT), magnetic resonance imaging (MRI), creatinine excretion, ^{40}K counting, dual-energy x-ray absorptiometry (DXA), and hydrodensitometry), little or no information is available from direct measurement of muscle mass.   Nevertheless, based on estimations, it seems that skeletal muscle mass declines approximately 40% from the 30s to the 90s , with a 10% decline occurring from the 30s to the 60s (87,102,114,125,149,228,229). Using ultrasound, Young et al. reported a 25-35% decrease in quadricep muscle cross-sectional area (CSA) in old men and women when compared with controls (228,229). Similar reports of a decline in muscle mass come from CT scans of older and younger men (102,149). Lexell et al. (114) utilized whole muscle post-mortem analysis to measure size of whole muscle, number of fibers, and fiber size to quantify total changes in aging muscle. He compared CT cross-section of whole muscle post-mortem reporting a 40% decline in CSA from the 30s to the 70s (114).

Assessment of muscle mass For a faster, non-invasive, more economically efficient measurement of muscle mass, dual-energy absorptiometry (DXA) has been employed in body composition studies. However, the technology relies on the assumption of x-ray attenuation through bodily tissues remaining constant from person to person and throughout aging. It has been reported that DXA tends to overestimate the amount of lean tissue in the elderly because DXA cannot distinguish between water and other lean tissues (155). Thus, DXA may underestimate the loss in FFM. Many of the aforementioned studies examined only men and only one or two muscle groups. A few studies have overcome these methodological shortcomings (65,87,121). Lynch et al.

(109) assessed 502 men and women from the BLSA utilizing DXA scans of the upper

and lower body(121). They reported an 18% loss of leg muscle mass in men and a 15%

loss in women from the 40s to the 70s. Arm muscle mass showed a slightly greater

decline (21%) with age but was similar between men and women (121). Examining 148

women and 139 men ages 20-90, Gallagher et al. (65) reported a total appendicular

skeletal muscle mass decline of approximately 15% for men and 11% for women after

adjusting for height, weight, and age. Utilizing MRI, Janssen et al. (87)studied 268 men

and 200 women analyzing whole body skeletal muscle mass ages 18-88. They reported

an approximate 27% decline in skeletal muscle mass in men and slightly less decline in

women but women began with almost 40% less muscle mass (87). Again, it is possible

that DXA is underestimating the loss of FFM with increasing age. Additionally, cross-

sectional studies likely underestimate the loss of FFM because the elderly pool of

subjects for these studies are on average, likely to be healthier than their peers who did

not survive to the time of the study. Thus, only the healthiest individuals may survive to

have all measurements completed. Therefore, it seems that muscle mass is lost at a rate

of at least 6% per decade after the 40's for women and 60's for men.   However, it should

be noted that there is significant variability in the loss of muscle mass with age (121,128).

Muscle Quality: The quality of lean body mass declines with age (51). As

measured by ^{40}K counting, body cell mass declines faster with age, than intercellular

connective tissue and water. Thus, a pound of lean mass from an elderly person contains

less intracellular tissue and more extracellular tissue. In young men (20-25), cell mass

represents 59% of lean body mass while in old men (80-89) cell mass is only 46% of lean

mass (44). An 18 year longitudinal study of 564 men and 61 females reported that body

cell mass, driven by muscle mass, declines linearly with aging (55). Measures of muscle quality (MQ) sometimes referred to as specific tension or strength per unit of muscle volume, takes into account an estimate of skeletal muscle in contrast to lean body mass or fat-free mass. Muscle quality depends on accurate measures of force production and muscle size, full central activation of muscle, ensuring that only the muscles of interest participated in force production, and effects of pain or other neural inhibitory factors are accounted for (38). Early studies were conflicting, i.e. Young et al. (228) found no difference in force production/cross sectional area (CSA) of the knee extensors of older women compared to controls (228). Yet other reports have found a loss of MQ occurring with advancing age (60,167,229). Muscle quality has been shown to decline both at the whole muscle level (121) and at the single muscle cell level (59). Lynch et al. (121) reported a decline in MQ in the leg musculature and that arm muscle quality decreased to a similar extent, but the decline in arm MQ was more pronounced in women. Additionally, Frontera et al. (61) examined single muscle fibers in young and older men and women in a cross-sectional design and reported a decline in muscle fiber quality in men. Thus, it seems that MQ declines with age, but the magnitude of decline depends on gender and individual responses.

Mechanisms for the decline in muscle size and function with age: No single cause has been identified that explains the decline of muscle size or function with age. Yet, many interrelated factors have been identified that likely contribute to sarcopenia . Some of major factors seem to be a loss of alpha motor neurons, declines in testosterone and estrogen, growth hormone, IGF-1, protein synthesis, changes in myosin heavy chain (MHC) gene expression) and an elevation in catabolic stimuli such as TNF alpha.

The total number of central nervous system and muscle neurons is known to decrease with age (19,174). Furthermore, this loss of muscle neurons seems to be predominantly of the fast motor unit (36,40). These losses are related to the loss in muscle strength that occurs with age (39,40). The preferential loss of fast fibers is thought to cause reinervation by slow motor units thus transforming them to the slow myosin type (40). This would explain the increased motor unit size that is observed with advancing age. In total, this translates to a decreased number of the higher force and velocity producing fibers. This could result in reduced force and or power production by the muscle of interest due to the loss of the more powerful muscle fibers. Yet, Urbanchek et al. (107) reported that only 11% of the decrease in muscle force production is attributable to denervated fibers (213). Furthermore, some recent evidence suggests that the loss of motor neurons that occurs with age is not as much as was once thought (133).

The turnover of proteins is a constant metabolic process within the body. High protein turnover helps maintain muscle quality by replacing damaged or deformed proteins. To maintain protein balance, synthesis must be equal to the degradation rates. In normal healthy humans, this is the case and there is a no net change in whole body protein. Muscle contractile and mitochondrial protein synthesis rates are reduced with advancing age (7,139,175,176). A cross-sectional study by Welle et al. (219) measured total and fractional protein synthesis in young men (21-31) and older men (62-81). They demonstrated that the fractional rate of myofibrillar protein synthesis was 28% slower in the older group compared to younger. Total myofibrillar protein synthesis, estimated as total myofibrillar mass (from creatinine excretion) times the fractional synthesis rate, was 44% slower in the older group. Whole body protein synthesis was lower in the older

group even after adjusting for total FFM (219). In contrast, a study by Morais et al. (72) reported that after correction for FFM, whole body protein turnover was not decreased in the elderly. Balagopal et al. (2), reported that whole body muscle protein synthesis declined by about 50% when young were compared to the elderly but whole body non-muscle protein synthesis did not change. This was primarily due to the decrease in synthesis rate of myosin heavy chain proteins. In another study by Balagopal et al. (4), mixed muscle protein synthesis and gene transcript levels of (myosin heavy chain) MHC-IIa and MHC-IIx were shown to decrease with age, whereas, MHC-I did not differ based on age. Consequently, ST increased the levels of MHC-I, but did not change the MHC-IIa and IIx in the same subjects. This finding suggests that protein synthesis may not be different with age in response to ST, but MHC expression levels do differ with age. In a ST study of frail elders, Yarasheski et al. (226) reported that despite a large increase in the fractional rate of protein synthesis in the exercise group, the rate of whole body protein synthesis only tended (not statistically significant)to increase after resistance training. Whole body proteolysis rate and protein synthesis rate were unchanged in the frail elders after 3 months of ST. The exercise did stimulate the in-vivo rate of vastus lateralis (mixed) muscle protein synthesis. It was concluded that skeletal muscle contractile proteins in 76- to 92-yr-old physically frail elders retain the ability to increase the rate of fractional muscle protein synthesis in response to progressive resistance training (226). The non-significant finding of whole body protein synthesis change with ST is not surprising given that skeletal muscle only accounts for ~25% of total body protein synthesis. Therefore, the increases seen in muscle protein synthesis are not likely to be detected at the whole body level.

Hasten et al. (77), examining a group of older men and women, reported the older adults having a lower MHC and mixed vastus lateralis muscle protein synthesis rate than 23-32 yr olds. Baseline actin protein synthesis rates were similar between the two age groups. After a ST program, it was reported that as a result of their lower whole body skeletal muscle mass, 78-84 yr olds had lower absolute rates (g protein synthesized/day) of MHC, mixed, and actin protein synthesis than 23-32 yr olds. Exercise increased mixed muscle and MHC protein synthesis rates in both age groups. Thus, it seems that contractile protein anabolic actions in response to acute resistance exercise are not compromised by advanced age. And in response to ST, rates of whole body proteolysis, protein synthesis, and leucine oxidation, when expressed in kilograms of FFM, are not different between younger and older subjects (77). The preceding studies suggest that a reduction in muscle protein synthesis is likely a contributing factor to sarcopenia, but the decline is reversed by a ST intervention.

The Role of Hormones: Several hormones play a role in muscle protein synthesis, satellite cell activation, and overall muscle growth. Testosterone (T), GH, and IGF-1 are all known to have an anabolic effect on skeletal muscle. However, all of these exhibit a decline with age (10,74) . Administration of GH has been shown to increase lean muscle mass and protein synthesis in humans. (63,185). Yet, although GH may increase whole body protein synthesis it seems to have no effect on skeletal muscle strength (227). Serum T levels decline gradually by the fourth decade of life (74). This decline is even more dramatic for unbound testosterone partly due to the increased sex hormone-binding globulin concentrations being higher in older men and has been associated with a decreased FFM (183). Administration of gonadotropin-releasing

hormone antagonist causes a decrease in FFM, muscle strength, and muscle protein synthesis (122). Epidemiological studies have lent further support to the hypothesis of decreasing muscle mass and strength with decreasing T levels (13,151,183). In a study of hypogonadal men, T increased FFM but not strength after 3 years of administration (193). Some studies have shown that T will increase some measures of strength, although it has not been shown to increase knee extension strength, which is known to be associated with daily function in the elderly. Only two studies to date have used older men not considered hypogonadal in a double blind randomized placebo trial (22,95). Snyder et al. (194) found an increase in FFM, but no change in leg strength, whereas, Ferrando et al. (47) found that some measures of strength increased. Yet the increases in strength were less than typically found in a short-term ST intervention. Some of the possible side effects of T therapy include increased prostate size, increased hematocrit, and altered lipoprotein levels. Thus, its safety is still a concern.

T administration has been shown to increase IGF-1 muscle expression levels (47) and is a possible mechanism by which T may cause muscle hypertrophy. IGF-1 levels decline with advancing age and are thought to be causally related to the decline in muscle function observed with age (69,71). The somatomedin hypothesis suggests that circulating GH is causes expression and secretion of IGF-I by the liver. According to this theory, the liver is the primary source of circulating IGF-I and is under the direct control of GH. GH and circulating IGF-1 levels both decline with age at a similar rate. In young adults who were GH deficient, GH therapy increased circulating GH and IGF-I levels and increased muscle mass (79). Additionally it is known that IGF-I is expressed by muscle and acts in an autocrine/paracrine fashion to simulate muscle protein synthesis (71).

Consequently, a study that administered GH in older men did increase plasma IGF-I concentrations but did not increase the mRNA for IGF-1 in muscle (218).

Strength training adaptations in elderly: Due to the prevalence in the population, total health care costs, and detrimental physical consequences of sarcopenia, it would be beneficial from an economic and public health perspective to find a safe and effective method to increase muscle strength and mass in the elderly. As noted previously, hormone supplementation in the form of HGH has not proved effective and has been shown to be unsafe (15). In addition, the safety and efficacy of T is not yet clear. However, strength training (ST) in the elderly has been shown to be both safe (81,153) and effective in increasing muscle strength and mass in as little as 8 weeks (50). Indeed, several studies have demonstrated the efficacy of ST to increase muscle mass and strength in men and women age 50 – 98 (17,49,50,62,83). Additionally, the muscle adaptations due to ST have been shown to have a positive effect on functional ability in the elderly (18,186).

Muscle strength increases ~25 to 45% in response to ST in the elderly (49,84,95,109,210). At least one study has shown that muscle strength responds to the stimulus of ST up to the age of 98 yrs (50). Most studies would suggest that there is little or no age effect in the muscle strength response to ST. However, Lemmer et al., reported slightly but significantly greater strength gains (34%) in 20-30 year old men and women when compared to 65-75 old men and women (28%) (109). Yet, there was no age difference in the response of muscle volume to the same ST program (83). These data suggest that in response to ST, elderly muscle adapts similarly as younger muscle in response to progressive muscle overload (ST).

Depending on the method of measurement, intensity of the program, and possibly age and sex of the participants, total body muscle mass generally increases, but with a range from no significant change to a 41% increase (22,62). In a 12 week ST program of men and women 56-80 yrs, Campbell et al. (22) reported a small increase in FFM but no change in body cell mass as estimated by hydrostatic weighing and K-40 counting (22). Yet another study utilizing similar methods has reported increases in total body lean tissue of 10% (21). In the latter study, measures of creatinine excretion were used in addition to hydrostatic weighing, potentially explaining the greater increase in lean tissue observed in the latter study. It seems that creatinine excretion yields the highest measurement for changes in lean tissue due to ST in the elderly of 9 - 41% (21,62,142). The difference in measurement techniques is larger than would be expected; yet, each of the measurement techniques relies on the quantification of different factors. Moreover, each has specific assumptions for estimating lean tissue mass. Furthermore, it is likely that whole body lean tissue probably does not change much in a short- term study based on the findings of the change in actual muscle volume.

Change in muscle volume or cross-sectional area allows the measurement of the specific muscle being stimulated and allows the differentiation of muscle bone and fat. Muscle volume or cross-sectional area is typically measured by computed tomography or magnetic resonance imaging. Several studies have shown the effects of ST in the elderly to cause an increase of ~8% while the range is 3 – 23% (50,62,68,173). Frontera et al. (31) reported one of the first studies to use imagining techniques (CT) in response to a ST program. They observed a 9% increase in the CSA of the knee extensors in a group of 60 -72 yr old men who underwent a 12 week ST program. . Two studies have used subjects

in their 90's and showed increases of 3% and 10% respectively (51,76). Some studies have used muscle volume as opposed to CSA to examine the changes in muscle size (83,96,206). These studies have shown similar or greater increases in muscle due to ST. Indeed it has been suggested that muscle volume is a much more accurate method of reporting changes in muscle size due to ST (207).

Yet, even with the accuracy of the measurement of muscle size by medical imaging techniques, it does not measure changes at the myofiber level. At the single muscle fiber level, it appears that changes due to ST are greater when expressed in % increases than when measured by imaging at the level of a whole muscle group. The increases in fiber size are typically at least 10%, but often more than 30%. This is not completely surprising because MRI and CT assessment of whole muscle groups are also measuring connective tissue and other tissues, which are not likely to change much as a result of training. The increase in fiber size is seen in both type I and type II fibers (17,21,49,62,113). Additionally, training studies have been conducted in older adults that examined functional changes at the fiber level. Trappe et al. (211) examined men before and after 12 weeks of ST examining single muscle fibers via muscle biopsy. They found an increase in fiber area of 20% in type I fibers and 13% in type II fibers. Force increased by 35% in type II fibers and by 20% in type I fibers. Shortening velocity increased 75% in MHC I and 45% in MHC IIa. Additionally power increased 56% in both fiber types combined (211). In a similar study of older women (74 ± 2 yr), following 12 weeks of ST, fiber diameter increased by 24% in type I fibers and made no change in IIa fibers. Force increased by 33% in type I and 14% in type II fibers. Shortening velocity was unaltered in both fiber types following the training, yet power

increased 50% in type I and 25% in type II fibers (209). It is concluded from these studies that, although sex differences may exist, muscle strength measured at the whole muscle level and single muscle fiber level increase in response to ST in the elderly.

Effects of ST on muscle quality: The loss of muscle strength with age is thought to derive from the loss in muscle mass (91,106), neuromuscular function (91,106), and contractile properties of muscle(59,208). Additionally, changes in connective tissue (149) and the decrease in muscle fiber pennation angle (104) are associated with aging and the loss of strength. Recent studies have demonstrated an increase in MQ following ST (47,101). In response to a 9 week ST program, Tracy et al. (101) reported a 14 and 16% increase in MQ (quantified by 1-RM and muscle volume) for men and women respectively (206). Ivey et al. (47) examined the training and detraining effects on MQ with age and gender comparisons. They found that young and older men and women increased MQ, but after a period of detraining, the older women were the only group to decrease MQ. This is of specific concern because older women begin with lower strength and MQ and live longer than men, making them more susceptible to functional decline and dependence later in life. Furthermore, older women suffer greater disabilities than older men (94). Studies at the single fiber level suggest that men may increase muscle quality while women do not. Yet, Ivey et al. (47) showed just the opposite in the young women.

Genetic variation in muscle at baseline and in response to training: Several interventions have been proposed for the prevention and treatment of sarcopenia, but it appears that ST is the most effective with the least side effects (15,81). Large increases in strength and muscle mass can result in a relatively short time with ST, but these

changes are highly variable (83). For example, in a ST study from our lab, 65-75 year old men and women varied in their strength increases from 5 – 59% (5 – 86 lbs.) and from 1 – 20% (19 – 344 cubic cm) in increases in MV (83,109). The subjects in the aforementioned study were of similar age, health history, and past exercise training status. Additionally these subjects underwent training programs of identical time, intensity and, type. With the controls of this study, it would be expected that individuals would have similar responses. This variation in muscle adaptation in a well-controlled study suggests a genetic determinant. Further support for a genetic association comes from twin studies showing > 50% of the variance in baseline strength and lean body mass is attributed to heredity (20,57,143,190). Adaptation of muscle to ST has also been shown to be highly heritable (200,201).

The estimation of heritability of a specific trait is commonly estimated by the study of monozygotic and dizygotic twins. The most common analysis is the measurement of a phenotype between and among sets of monozygotic and dizygotic twins. Monozygotic twins share identical genetics or 100% of their alleles while dizygotic twins share ~50% of their alleles. In theory, a trait that has a perfect correlation of 1.00 between sets of monozygotic twins and a correlation of .50 between dizygotic twins would suggest that the trait is purely under genetic control. However, many additional factors can affect this estimate among these are similar environment, additive genetic and dominant genetic effects, and unique environments. One approach to account for each factors influence is through a biometric path-analysis, which attempts to analyze all possible contributing factors to the measured phenotype of interest. This method allows distinction between narrow and broad sense heritability.

Narrow sense heritability is defined as the ratio of a trait's additive variance to it's total variance and is a measure of the predictability of offspring trait values which are based on parental trait values (1). Because the additive variance does not always give an adequate assessment of the influence of genetics on a trait, it is important to consider the dominance variance and, hence, the broad sense heritability of traits. Unlike additive variance, which measures the variance due to mean effects of single alleles, the dominance variance of a trait measures the variance due to the interaction effect of the two alleles that constitute the genotype at a locus, summed over the genome (1). The importance of these factors on traits such as fat free mass is demonstrated in the study of a founder population, which originated in the Tyrolean Alps and currently resides in South Dakota on communal farms. With the use of family pedigrees and path analysis, it was estimated that the trait of fat free mass has a narrow heritability of .45 and a broad heritability of .76, suggesting a role for both additive and dominant effects on this trait. This study demonstrates the need for analysis of both broad and narrow sense heritability in that one third of the heritability estimate would have been overlooked if not for the estimation of the broad heritability. It is interesting to note that systolic blood pressure in this population had a narrow heritability close to zero while the broad heritability was .45 (1). Again, this emphasizes the importance of analyzing both the broad and narrow sense heritability.

In a study by Thomis et al., (203) path-analysis models were used to determine the relative genetic and environmental contribution to the observed variation in maximal isometric and dynamic strength and muscle cross-sectional area in twins. Their approach used linear models to represent hypotheses about causal paths (latent to observed

variables) and correlational paths (between the latent variables) to explain the observed

variation in a trait based on observed phenotypes and known relations in genetically

related individuals (141,203). In this model, the latent causal factors can be genetic, with

additive (A) or dominance (D) gene action, environmental causes can be shared by twins

or family members that are reared in the same family (C, from common environment)

and non-shared or specific environmental factors (E, includes also random measurement

error) (141,203). Path-analysis examines variations of the ACDE model to determine the

most parsimonious model fitting the data. In some cases, it may be determined that any

one of factors will be excluded from the model for a particular trait. For example, it was

found that the variation for most strength and anthropometric measurements was best

explained by a model with additive genetic and unique environmental factors only (no

dominant or common environmental factors found to be significant in the model) (203).

The genetic determination of maximal static strength varied between 70 – 78% depending

on the angle of measurement, with unique environmental factors accounting for the

remaining variation (22 – 30%) of maximal torque at these angles. In contrast, adipose

tissue measured by CT scanning had 48% of variability explained by common

environmental effects, 40% by additive genetic effects, 12% by unique environmental

effects, and environmental factors, unique to the individual as well as measurement errors

contributed for the rest (5-14%) (203).

Another study by Thomis et al., utilizing twins found that 66-78% of the variance

in maximal muscle torque was explained by additive genetic factors, whereas unique

environmental factors accounted for 22-34% of the variance (202). In this study,

monozygotic and dizygotic correlations suggested the contribution of nonadditive genetic

factors contributing to the total variance. However, nonadditive factors were not

significant in the model and therefore dropped from the analysis. Similar results were

reported in the only study that has examined the contribution of genetic factors on the

response to strength training. This study utilized twins with the path-analysis model and

again concluded that baseline strength was ~70% due to additive genetic factors and 30%

due to environmental factors (E). Additionally, it was found that training-specific genetic

factors were significant for the post-training measures and were estimated to explain

about 20% of the variation in achieved 1RM and isometric strength measures. Thus, it

seems that different genes may control the response to RT in comparison to those that

control baseline measures of muscle strength.

Only two studies could be found that examined the effects of a specific candidate

gene polymorphism on strength or muscle mass at baseline or in response to training

(56,83). Folland et al., reported a significant relationship between the ACE genotype and

strength response to ST (56). Myostatin is another candidate gene that has been

identified as potentially having an effect on muscle response to RT (83). Although the

effect of myostatin polymorphisms in humans have not been conclusive (48). Due to

their known association with muscle hypertrophy, genes coding for proteins that regulate

or are regulated by growth hormone or its mediators (IGF1 and II) are prime candidate

genes for influencing muscle mass and strength. Indeed, a report from the Heritage

Family Study revealed an association between an IGF1 gene marker and FFM (25).

Furthermore, this same marker has been shown to be linked and associated with the

change in FFM due to exercise training (25,198). However, the training modality used in

the Heritage study (25) was one that is not commonly associated with significant

increases in FFM. Nevertheless, an area near the IGF1 gene locus was implicated in these studies for influencing FFM both at baseline and in response to training, thus making it a strong candidate gene for muscle mass, as well as strength.

IGF1: The IGF1 gene consists of 88,066 base pairs and is located on human chromosome 12 (12q22-q24.1). The gene contains two known promoters, six exons and 5 introns. Depending on tissue of origin and transcriptional splicing the mRNA typically contain 153 amino acids and is eventually translated into a 70 amino acid protein with three disulfide bridges. The mRNA can produce at least three different transcripts, two of which are expressed in skeletal muscle.

The somatomedin hypothesis came from early experiments trying to understand somatic growth caused by the pituitary gland. These experiments used an assay that measured incorporation of $^{35}SO_4$ into chondroitin sulfate from cartilage (184) as a measurement of somatic bone growth. Hypophysectomy of rats reduced incorporation of $^{35}SO_4$ into chondroitin sulfate of cartilage (136), while exogenous injections of pituitary extracts and bovine GH restored $^{35}SO_4$ this incorporation (184). Yet, when GH was placed directly on costal cartilage slices in vitro, only a minimal effect of incorporation was observed (33). Thereby suggesting that GH may be modulating the levels of another hormone in the circulation, which mediated the growth effects. Further experiments helped to solidify the hypothesis which came to be known as "the somatomedin hypothesis" (32). This hypothesis suggests that GH works indirectly by way of mediation factors to cause somatic growth.

Insulin-like growth factor I was eventually purified from rat serum and shown to be the somoatomedic substance regulated by GH (170). It was termed "insulin-like"

because of its ability to stimulate glucose uptake into fat and muscle cells (160).

Additionally IGF-I shares ~50% amino acid identity with insulin (170). The primary

structural difference between insulin and IGF-I is that IGF-I retains the C chain that is

cleaved from proinsulin during post-transcriptional processing (170). Upon the discovery

of IGF-I, the hypothesis was further refined to suggest that GH secreted by the pituitary

would act on the liver as its target organ, where IGF-I would be secreted to act on bodily

tissues causing growth and provide feedback to the pituitary to control the level of GH

secretion.

The first indication that the somatomedin hypothesis was incomplete was when

D'Ecrole et al. in 1980, discovered that explants of fetal mouse tissue maintained in

serum-free media showed higher levels of IGF-I in the culture medium as compared with

extracts of the tissues themselves: liver, intestine, heart, brain, kidney, and lung (31).

Additional studies supported the finding of various tissues expressing IGF-I (172). It was

further demonstrated that IGF-I production by various tissues of the body could be

affected by and also act independent of GH plasma levels (119,120). These tissues

included lung, kidneys, heart, and skeletal muscle (119,120). However, the direct effect

of GH on non-hepatic tissues was still in question and it was subsequently shown that GH

could affect tissues via stimulating local production of IGF-I or act directly on tissues to

cause growth (67). The latter process occurred without a mediating factor but was not as

dramatic as when IGF-I was involved in the process.

IGF-I action: IGF-I displays numerous diverse functions during both embryonic

development and postnatally. Indeed, mice carrying null mutations in the IGF-1 gene are

born small and grow very poorly postnatally (5,154). Naturally occurring mutations in

the IGF-1 gene are rare. Only a single patient, with both intrauterine and postnatal growth retardation has been found who had a deletion of the IGF1 gene (222). The complete physiological functions of IGF-I are beyond the scope of this review. Therefore, a brief background of IGF-I action will be given with specific emphasis on skeletal muscle.

As mentioned previously IGF-I exerts some of its influence as an endocrine hormone circulating in the blood stream until reaching a target tissue. Unlike insulin, IGF-I in the circulation is bound to one of six known IGF binding proteins (90). These binding proteins act as carriers of IGF-I to transport it out of circulation and prolong the half-life by protecting it from proteolytic degradation. In addition to their role in circulation, binding proteins are often expressed by target tissues where they act to regulate IGF-I function further. Binding proteins have been shown to augment and attenuate IGF-I action depending on the target tissue (166).

Once released from the binding protein, IGF-I can bind to the insulin or IGF-I receptor though it has much greater affinity for the IGF-I receptor. It is likely that IGF-I actions vary depending on the receptor to which it attaches. The insulin and IGF-I receptor share certain regions, with homology up to ~85%, including the tyrosine kinase domain (212). The IGF-I receptor is a heterotetrameric transmembrane protein consisting of 2 identical extracellular alpha subunits containing cysteine-rich IGF-I binding sites and 2, primarily intracellular, beta subunits containing ATP-binding sites and a cluster of three tyrosines, which upon IGF binding undergo rapid phosphorylation and are required for activation of tyrosine kinase activity (110,197). Ligand binding results in a conformational change leading to autophosphorylation of critical tyrosine

residues that activate cytoplasmic proteins containing SH2 (src homology 2) domains. However, unlike other receptor tyrosine kinases, the IGF receptor interacts with intermediate signaling proteins, insulin receptor substrate (IRS-1) and src homology containing protein (Shc). Thus giving this receptor more complexity and versatility to modulate cellular transcription and translation (54).

The activation of IRS-1 by IGF-I binding to the IGF-I receptor results in the activation of phosphatidylinositol 3-kinase (PI 3-K). In mammalian muscle, the PI 3-K pathway has been shown to mediate skeletal muscle hypertrophy. In transgenic muscle, a mutant form of the Ras proto-oncogene that was only competent to stimulate the PI 3-K pathway, stimulated the PI 3-K pathway and caused skeletal muscle hypertrophy directly and blocked denervation-induced atrophy (135).

The IGF-I receptor has been shown to mediate amino acid uptake in muscle as well as decrease protein degradation, stimulate proliferation and differentiation of myocytes and increase DNA synthesis in muscle satellite cells (41,45,231). Mice lacking the IGF-I receptor die immediately after birth of respiratory failure and severe growth deficiency (45% of normal) (117). Transgenic studies have concluded that IGF-I mediates its proliferative and differentiative effects via the IGF-I receptor (157). The IGF-I receptor plays a role in the regulation of transcription of a number of genes encoding proteins involved in growth and metabolism. Several of the immediate early genes, c-fos and c-jun are associated with cell proliferation and are activated by IGF-I receptor (27,147). C-fos and c-jun being nuclear transcription factors, likely mediate some of the protein synthesis effects of IGF-I receptor.

IGF-I and insulin have both shared and unique actions. Administration of IGF-I increases whole body protein metabolism by increasing protein synthesis and inhibiting proteolysis (63). These actions are distinct from insulin, which acts primarily to inhibit proteolysis. This evidence suggests that IGF-I acts primarily through the IGF-I receptor specifically as opposed to the insulin receptor in muscle. Yet, IGF-I does enhance glucose uptake into muscle (85) which could be mediated by the insulin or IGF-I receptor. Indeed, IGF-I has been used as an adjunctive therapy in patients who are insulin resistant to lower plasma glucose levels (134,188).

The role of IGF-I in muscle development is exemplified by the studies in mice in which expression of myogenin has been knocked out, in that they show the same result as when IGF-I is knocked out i.e. no functional muscle development (78,117). The opposite approach of transgenic animals expressing IGF-I, found muscle growth to be 30% above normal when IGF-I was expressed at 50% above control values (137). Additionally DNA content increased in these muscles. In muscle, IGF-I has been shown to increase amino acid uptake (126), suppress proteolysis (8), increase thymidine incorporation (66), stimulate myogenic differentiation(53), and stimulate myogenesis (187). Even though, IGF-I unlike other mitogenic factors, will stimulate both proliferation and differentiation of muscle cells in culture (158) it does not stimulate differentiation while it is stimulating proliferation (46). There is a temporal separation between these effects. This may be made possible due to the versatile intracellular signaling of the IGF-I receptor. IGF-I treatment of muscle cells results initially in a proliferative response during which expression of myogenic factors are inhibited, followed by stimulation of differentiation accompanied by down regulation of proliferative signals (54).

Transgenic mice over expressing the IGF1 gene show enhanced myotube formation as well as increased mRNA levels of myogenic factors Myo D and myogenin and elevated mRNA for contractile proteins (28). These mice with a 47-fold increase in IGF-I had hypertrophy of all myofiber classes with no increase in body weight or circulating IGF-I concentrations (28). In addition, autocrine expression of IGF-I has been shown to contribute to myotube formation in embryo (73). Autocrine secretion of IGF-I facilitates muscle regeneration after injury in normal and hypophysectomized rats (88,195). It has been shown that IGF1 mRNA and protein levels increase during muscle regeneration (111). Viral mediated over expression of IGF-I in mouse skeletal muscle has been shown to block the age related loss of skeletal muscle mass and strength. Furthermore, the IGF-I expression completely prevented the significant loss of the fastest most powerful type IIb muscle fibers seen in uninjected controls (11). Transgenic mice over expressing IGF-I in muscle demonstrate protection from the normal loss of muscle mass and strength during senescence(137). Additionally, these same transgenic mice display significantly improved muscle regeneration over control mice when muscle damage is induced via cardiotoxin (137). The aged transgenic mice in this study display muscle characteristics similar to or better than younger control mice.

It has also been demonstrated that muscle innervation may be improved in animals over expressing IGF-I. In transgenic mice, skeletal muscle expressing IGF-I prevented aging alterations in the neuromuscular junction, preserved spinal cord motor neuron innervation of muscle, and decreased the loss of type IIb muscle fibers (127). Additionally, Rabinovsky reported that transgenic mice over expressing skeletal muscle IGF-I have accelerated muscle and motor neuron regeneration after sciatic nerve crush

injury (159). The regeneration or preservation of neural innervation will likely be a causative factor in preventing muscle strength and mass loss with age.

Circulating IGF-I and exercise: IGF-I is produced by various tissues including muscle, however, 98% of the circulating form of IGF-I is produced in the liver. Several studies have examined this form of IGF-I in relation to exercise. Cappon et al., (23) first demonstrated that 10 min of above-lactate threshold cycle ergometer exercise studied in 10 subjects (age 22-35 yr) showed a small but significant increase in circulating IGF-I levels very briefly after exercise but this increase was only evident until 20 minutes after exercise while blood was sampled up to 24 hours after the exercise bout. In a study of young men, 10 minutes of isolated forearm exercise showed a small but significant increase in circulating IGF-I, IGF-I BP3, and GH (43). Indeed, it does seem that circulating GH does increase due to a bout of high intensity exercise (23,103,144). Nicklas et al. (144) reported an 18-fold increase in circulating GH immediately following a high intensity bout of RT in older men. However, a ST program did not elicit a response in the resting circulating IGF-I levels when measured before and after the ST program (103,144). Nindl et al. (145) examined the effect of a high intensity ST bout on circulating levels of IGF-I, IGF binding protein 2 (BP-2) and BP-3, and the acid labile subunit (ALS) immediately following ST and continuously (every hour) for 24 hours after. They reported no change in total or free IGF-I protein immediately after exercise or the 24 hours post. However, small but significant changes were observed in BP-2 and -3 and ALS. The authors concluded that circulating IGF-I does not change with exercise, but that small changes in the IGF-I system may modulate its effects. Two studies demonstrated an increase in muscle IGF-I with no change in circulating levels in

response to exercise (9,42). Eliakim et al. (42) used 5 days of high intensity aerobic training in rats to analyze the change in circulating IGF-I compared to muscle. After 5 days it was found that the exercised rats increased muscle IGF-I protein levels significantly but neither the control nor exercise group increased circulating levels of IGF-I protein or IGF-I mRNA in muscle. In a study examining the different responses to concentric versus eccentric exercise, it was reported that eccentric exercise acutely increased (62%) IGF1 mRNA, 48 hours after the bout of training with no change in circulating levels of IGF-I (9). It can be concluded from these studies that circulating IGF-I levels are likely to play a minimal role in the response to exercise, but that locally expressed IGF-I impact is more dramatic. The exact time course of the change in muscle IGF1 gene expression and protein translation will require further study.

The importance of locally produced IGF-I versus the circulatory form in muscle has been exemplified in several studies. As mentioned previously, exogenous administration of GH may lead to an increase in muscle mass, but does seem to affect muscle protein synthesis or consistently affect muscle strength (220). A study by Welle et al. injected a single dose of GH into elderly men and women and reported a significant increase in circulating levels of IGF-I, but no increase in the IGF-I mRNA in skeletal muscle (218). Futhermore, it was demonstrated that liver specific deletion of the IGF1 gene produced mice that lacked the circulating form of IGF-I yet displayed normal growth (192). Thus, suggesting that the circulating endocrine form of IGF-I may not be important for muscle growth or maintenance in humans.

Autocrine/Paracrine role of IGF-I in aging muscle: Circulating levels of GH and IGF-I and muscle expression levels of IGF-I decrease with age (215). The decline

begins in the thirties resulting in a 40 % decrease by the eighties. This decrease in circulating levels of IGF-I specifically is thought to be a causal factor in the decline in muscle function that occurs with aging. Additionally the autocrine/paracrine form of IGF-I produced by muscle is reduced with age, which is adding to or causing the decline in muscle function that occurs with aging.

The IGF1 gene can express multiple isoforms depending on the tissue of origin in addition to the stimulus. Alternative splicing of mRNA derives these isoforms of the IGF1 gene. The predominant circulating form of IGF-I is produced by the liver due to GH stimulation and has been termed IGF-1Eb. The 1Eb isoform is produced utilizing the exon two promoter and therefore does not contain exon one. The muscle expresses two known isoforms of the IGF1 gene when it is subjected to stretch or mechanical stimulation. The first isoform is termed IGF-1Ea (182) and is initiated at the exon 1 promoter similar to the liver form, yet in 1Ea, exon 5 is removed by alternative splicing. Overexpression of this isoform by transgenic mice has shown pronounced muscle hypertrophy (137). Additionally older animals showed signs of protection against the normal loss of muscle mass associated with aging (137). It was concluded that overexpression of this isoform of IGF1 could preserve muscle architecture and the age-independent regenerative capacity of muscle.

The third IGF1 isoform is a splice variant resulting from a novel splice acceptor site in the intron preceding exon 6 and is generated in muscle subjected to stretch and overload (225). This isoform has been termed mechano-growth factor (MGF) or IGF-1Ec due to its expression by mechanical stimulation. Structurally, the MGF mRNA differs from its liver counterpart because of the presence of a 49-base pair insert on the

carboxyl end of the protein, which is derived from exon 5 of the IGF1 gene. It is not glycosylated, therefore, it is expected to have a shorter half-life than the liver IGFs. MGF is thus likely to be designed to act in an autocrine/paracrine, rather than systemic, mode of action. Yang et al. (225) were the first to demonstrate the up regulation of MGF in response to muscle stimulation. In this model, rabbit muscle was subjected to stretch by immobilization and mechanical stimulation both of which produced the response of upregulating the MGF isoform. Further work supported the findings of Yang et al. (124) when it was demonstrated that electrical stimulation and stretch resulted in an even greater increase in MGF and IGF-1Ea than stretch alone. Since then, studies have shown that the MGF isoform of IGF-I can be upregulated in humans in response to ST. Bamman et al. (9) demonstrated an increase of 62% in muscle IGF-I in response to a single bout of eccentric ST while concentric training produced a similar increase it did not reach statistical significance. This is not surprising because it is eccentric exercise that is known to cause more muscle damage than concentric and eccentric is thought to be necessary for muscle growth.

To isolate the IGF-I isoforms, Psilander et al. (156) measured IGF-I mRNA levels in six young men at 1, 2, 6, 24, and 48 hours after a single bout of ST. They found a decrease in IGF-IEa mRNA and no change in IGF-IEbc. The small sample size and inability to distinguish between IGF-IEb and c may explain their null findings. Fiatarone et al. (49) demonstrated a 500% increase in IGF-I expression in frail elders after one bout of ST however they did not isolate isoforms nor did they compare the results to a younger group. To examine the possible age related response, Owino et al. (150) used synergistic muscle ablation to overload muscle in young and old rats. They reported an increase in

IGF-I receptor in the young but not old rats and a dramatic increase in IGF-Ic (MGF) in the young with a small increase in MGF the old rats. The MGF mRNA increase continued for three days in the young rats but plateaued after one day in the old rats. To examine this relationship in humans a study of older (70-82) and younger men (25-36), Hameed et al. (71) measured muscle levels of IGF-1Ea and MGF before and after a bout of high intensity ST. There was no difference between the young and old groups before the ST bout. However, the biopsy taken 2.5 hours after the ST bout revealed an increase in MGF in the younger muscle but no change in the older muscle. No change was observed in the expression levels of IGF-1Ea after the bout of ST in either group. These studies suggest that the older muscle may have reduced capacity to be stimulated by a bout of ST. However, in a more recent study, Hameed et al. (72) demonstrated in 17 elderly men that 5 weeks of ST (24 hours after the last ST bout) would increase muscle MGF ~170% due to the ST, while exogenous supplementation of GH alone showed no effect on MGF. Additionally they showed that ST had no effect on circulating levels of IGF-I 24 hours after the ST bouts.

It is clear that local expression of IGF-I is induced by ST and it is likely that IGF-I is mediating many of the hypertorphic effects observed in skeletal muscle. Yet as previously mentioned there is significant variability seen in the strength and hypertrophic response of muscle to ST. Additionally, there is dramatic variability seen in the incresase in IGF-I that occurs in response to ST. Hameed et al. (72) demonstrated that in young individuals, the increase of IGF-I in response to ST was 2 – 864%. Furthermore, they demonstrated an average ~500% variation seen in elderly muscle in response to ST (72). Fiatarone et al. (49) reported greater than 500% variation in IGF-I expression in frail

elders following a bout of ST. It would seem likely that genetics could be affecting this response. Indeed, circulating levels of IGF-I have been shown to be almost completely under genetic control in healthy twin children and variability in circulating levels in the elderly has been estimated to be ~63% under genetic control (80,92). To date no studies have examined the heritability of IGF1 muscle expression. An autosomal genome wide search for genes related to fat free mass (FFM) and its changes after exercise training revealed that a polymorphism in the IGF1 promoter (192 allele) displayed significant linkage with changes in FFM (25). Additionally, this same polymorphism displayed association and linkage with baseline FFM and the change in FFM due to exercise training (198). The combined information from the studies of twins and circulating levels of IGF-I and the variable response of IGF1 amongst subjects who have undergone s similar muscular stimulus suggest that variation in IGF-I protein levels are under genetic control. Additionally, the genome-wide scans, linkage, and association studies suggest that a polymorphism near the IGF1 locus is affecting the FFM mass response to training, which is strongly influenced by IGF production. Therefore, it seems likely that the IGF1 promoter polymorphism will be associated with skeletal muscle phenotypes in relation to stimulus known to increase IGF1 expression levels. Numerous studies have examined the role of this polymorphism on circulating levels of IGF-I and various indicators of disease risk yet none have examined muscle related phenotypes.

IGF1 polymorphism: The IGF1 polymorphism examined in the genome wide scan and most association studies is a CA dinucleotide repeat near the promoter region of the IGF1 gene in humans (216). A similar CA repeat near a gene promoter region has been shown to alter gene expression in rats and humans (2,181). Repeat sequences

account for at least 50% of the entire human genome sequence (105). Commonly defined as a perfect or near-perfect sequence repeat of 1 – 5 bases, microsatellites are found in tandem repeat units of 10 – 30 repeats. Alternating purine-pyrimidine nucleotides (i.e. CA) are one type of microsatellite and, other than SNP's, microsatellites are the most variable component of the human genome. Three percent of the human genome consists of microsatellite repeats of which CA dinucleotides are the most common making up 0.5% of the human genome (105). The biologic function of repeat units has not been fully defined but a non-random distribution of repeats exists in the human genome suggesting an evolutionary importance.

A proposed function of some microsatellites is to regulate gene transcription. A disproportionate amount of these repeats are found in the promoter regions of genes in the human genome (189). This along with their known ability to alter gene transcription and the binding affinity of nuclear transcription factors make them areas of likely functional importance. B-DNA is the stable lower energy state of DNA. Under physiological conditions, most DNA in a eukaryotic cell is in right-handed B-DNA form. However, alternating purine-pyrimidine repeats, such as in a microsatellite, have been shown to form left-handed Z-DNA (146,181). Energy input is required to form Z-DNA and this has been shown to come from transcription initiation which negatively supercoils DNA thus stabilizing the Z-DNA conformation (221). Some studies have shown that alternating nucleotides (such as in a microsatellite) may alter gene expression when the repeats are located in the promoter region of a gene. Alternating purine-pyrimidine microsatellites in promoter regions have been reported to be involved in regulation of gene expression (93,140,189). Khashnobish et al. (98) were the first to report in vivo

regulation of gene expression by a CA repeat unit of varying lengths in Podospora anserine. Their results demonstrated that the number of CA repeats positively affects reporter gene expression. In a study by Peters et al. (152), it was shown in vitro that a CA repeat polymorphism commonly found in the human population can affect gene expression depending on length. This was the first study to show that microsatellite length polymorphisms that are commonly found in normal human population can alter gene expression. Furthermore, they demonstrated that the CA repeat element might serve as a binding site for specific nuclear regulatory proteins. Regulation of gene expression by varying lengths of CA repeats in the promoter region, as mentioned, or in introns has been shown to affect gene expression in rats and humans (2,26,181).

The microsatellite polymorphism in the IGF1 gene is typically between 16 and 22 CA repeats and is commonly referred to by the base pair length of the amplified DNA fragment (e.g. 192 bp). The 192 allele (19 CAs at nucleotide position 1087-1127 in the human IGF-1 DNA sequence Genbank accession number AY260957) in the IGF1 promoter polymorphism has been examined in numerous contexts. Genotyping of the microsatellite is typically separated into three groups, 192 homozygotes, 192 heterozygotes, and non-carriers of the 192 polymorphism. Whether the 192 polymorphism is causally related to changes in IGF1 function is not known; yet, the 192 polymorphism is the most prevalent allele in the majority of the populations studied to date. Although the 192 allele is not proven to be functional, it has proven to be a potential marker for disease related phenotypes and possibly IGF-I expression levels as discussed below. Its variation in the human population and demonstrated linkage with

skeletal muscle related phenotypes make it an ideal candidate polymorphism for directly examining its association with well-defined skeletal muscle phenotypes.

Rosen et al. (177) first implicated this polymorphism with serum levels of IGF-I and BMD in a study examining older men and women (178). It was reported that 192 homozygotes had lower blood levels of IGF-I and in a group of older men, 192 homozygotes made up a disproportionately large number of those with idiopathic osteoporosis (178). The reports by Frayling et al. (58) and Rietveld et al. (169) concur with Rosen's findings of decreased blood levels of IGF-I in carriers of the 192 polymorphism. In a cross-sectional study of young men and women, Frayling et al. (58) reported that 192 carriers had decreased blood levels of IGF-I, although no other associations were found. The study by Rietveld et al. (169) of older men and women, reported an age- related decline in blood levels of IGF-I in 192 homozygotes. In contrast to Rosen's findings, consequent studies have demonstrated an association of this polymorphism with osteoporosis but have concluded that 192 *non*-carriers have lower IGF-1 blood levels and are more likely to be osteoporotic (99,171,214). A longitudinal study by Missmer et al., (132) in middle-aged and older men and women reported a lower level of IGF-I in control subjects who were non-carriers of the 192 allele. This study concluded that 192 carriers would have the highest circulating levels of IGF-I. Similarly, Vaessen et al. (214) concluded that non-carriers of 192 again, had decreased blood levels of IGF-I and an increased risk for type II diabetes and myocardial infarction. Further support for 192 carriers having the highest circulating levels of IGF-I come from a study in Korean women; Kim et al. (100) reported the 192 homozygotes as having the highest blood levels of IGF-I.

Some reports have concluded that the 192 allele has no effect on blood levels of IGF-I or only has an effect when combined with oral contraceptive use (89,230). Yet both of these studies found an association with a measured phenotype, breast cancer, or the interaction of blood levels, oral contraceptive use, and the 192 allele. There have been two additional reports of the 192 allele showing no association with IGF-1 blood levels or any measured phenotype (3,34). The study by Delellis et al., (34) examined 250 adult women for association of the IGF-I microsatellite and breast cancer risk while the report from Allen examined older and younger men for association of blood levels of IGF-I and the IGF-I microsatellite polymorphism. It was concluded by these studies that the 192 polymorphism is unlikely to be associated with IGF1 function. Although conclusive determination of the effect of the 192 polymorphism from the aforementioned studies is difficult, it seems likely that the IGF1 192 polymorphism may affect skeletal muscle related phenotypes related to IGF1 expression being that both of the studies that have examined FFM reported a positive association.

If the 192 allele itself is not functional it would at least seem to be a valid marker for phenotypes related to IGF-I expression. It is possible that the 192 polymorphism is in linkage disequilibrium with a functional polymorphism in the IGF1 gene. Irrespective of the functionality there is yet to be a study examining the relationship of this polymorphism with the physiological phenotype most directly under the control of IGF-I, skeletal muscle. A prospective intervention study is needed to address this issue. The ideal intervention would be one known to stimulate both IGF-I expression and muscle hypertrophy. It is known that the elderly suffer various health consequences from the loss of skeletal muscle function. Furthermore, this loss can be offset or reversed through

a ST program. Yet, there is significant inter-individual variability in the response to ST.

With the heritability of skeletal muscle phenotypes and IGF-I, and the known relationship

between the two, an association study examining skeletal muscle phenotypes with a gene

related to IGF-I function could be extremely informative. From the public health

perspective, the greatest predictive benefit is derived from polymorphisms that are

prevalent in the population. The understanding of rare alleles (< 10% of the population)

involved in complex phenotypes are unlikely to provide significant public health benefits.

While the understanding of common polymorphisms would impact the greatest number

of people. These relationships should be addressed in the planning of future studies.

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