Muscle strength response to strength training is influenced by insulin-like growth factor 1 genotype in older adults

Matthew C. Kostek, Matthew J. Delmonico, Jonathan B. Reichel, Stephen M. Roth, Larry Douglass, Robert E. Ferrell, and Ben F. Hurley

Department of Kinesiology, College of Health and Human Performance, University of Maryland, College Park, Maryland; and Department of Human Genetics, University of Pittsburgh, Pittsburgh, Pennsylvania

Submitted 30 July 2004; accepted in final form 27 December 2004

HUMAN MUSCLE STRENGTH DECLINES at the rate of ~12–14% per decade after the age of ~50 yr (31, 33). This loss of strength with age is due to many factors but is primarily attributed to a loss of muscle mass (21, 42), termed sarcopenia. Because sarcopenia is related to a loss of functional abilities (39), dependency (40), increased risk of falls and fractures (4, 32), and decreased bone mineral density (51), 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 that strength training (ST) is the most effective with the least negative side effects (7, 25). Large increases in strength and muscle mass can result in a relatively short time with ST, but these changes are highly variable (26). For example, in a ST study from our laboratory (26, 30), 65- to 75-yr-old men and women varied in their strength increases from 5 to 59% (5–86 lb.) and muscle volume (MV) increases from 1 to 20% (19–344 ml). This variation in muscle adaptation to training may suggest a genetic influence. Further support for genetic associations comes from twin studies showing that >50% of the variance in baseline strength and lean body mass is heritable (9, 19, 38, 50). Adaptation of muscle to ST has also been shown to be highly heritable (53, 54). 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 (23, 56). Furthermore, exogenous growth hormone administration increases circulating IGF-I levels, which have been shown to increase muscle mass and possibly strength (12, 49). Yet, it seems that circulating levels of IGF-I are not as important for muscle growth as are the isoforms of IGF-I produced by skeletal muscle, which act in an autocrine/paracrine fashion (1, 5, 22). Studies with transgenic mice have shown a direct autocrine/paracrine effect of increased levels of IGF-I expression increasing skeletal muscle mass (17). This same transgenic line of mice, compared with controls, shows reduced age-related losses of skeletal muscle motoneurons and type IIB muscle fibers (35). This preservation of motoneurons and fast-twitch fibers is likely to preserve strength in aging muscle. In addition, Boonen et al. (8) observed an increase in muscle strength in older women after 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) (11). Furthermore, this same marker has been shown to be linked and associated with the change in FFM due to exercise training (11, 52). However, the training modality used in the HERITAGE study (11) 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 cytosine adenine (CA) dinucleotide repeat polymorphism in the promoter region of the IGF1 gene can affect blood levels of IGF-I (20, 29, 36, 44, 46, 58, 59). 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 1,087–1,127 in the human IGF1 DNA sequence Genbank accession number AY260957) has been associated with differences in circulating levels of IGF-I and phenotypes related to IGF-I (20, 29, 36, 44, 46, 58). Although 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 (41), 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 motoneurons, we hypothesize that IGF1 genotype will influence skeletal muscle mass and strength response to ST. Due to the known adverse health consequences of sarcopenia and the reported decline in IGF1 expression in the elderly, we sought to examine this hypothesis in older adults.

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 single-nucleotide polymorphisms (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 before this study (26). All subjects from both cohorts underwent a phone-screening interview, received medical clearance from their primary care physician, and completed a detailed medical history before participating in this study. Only those who had not exercised more than once a week during the 6 mo before 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, which were approved by the Institutional Review Board of the University of Maryland, College Park, were explained, the subjects read and signed a written consent form. 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). The microsatellite was amplified by PCR of genomic DNA using fluorescence-tagged primers. Previously published PCR primers (46) flanking the CA polymorphism were used with the forward primer FAM-labeled 5′-GCTAGGCCAGTGTTAT-TATT-3′ (sense) and 5′-ATGGGAAGGGGCTCACA-GGA-3′ (antisense).

Standard PCRs were performed on a PTC-100 Thermal Cycler (MJ Research), annealing steps of 1.0 min at 37°C, and elongation steps of 1.0 min at 72°C for 35 cycles were standardized for PCR reactions. All PCR reactions were carried out in a 25–μl volume containing ~20 ng of genomic DNA template: 14.15 ml of dH2O, 2.5 ml of 10 mM PCR buffer, 4.0 ml of 1.25 mM dNTP, 0.375 ml of forward primer (20 μM), 0.375 ml of reverse primer (20 μM), 0.1 ml of Taq DNA polymerase (5 U/ml), and 1.5 ml of 25 mM MgCl2. After 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) and then denatured at 90°C for 2 min and immediately transferred onto ice. Samples were electrophoresed through a capillary on the ABI 3100 DNA sequencer (PE Applied Biosystems). 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 (46). Briefly, use of the forward primer resulted in PCR bands that moved through the gel at the rate of a band 4 bp 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 16–22. 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) (46). 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 under similar conditions as those stated for the microsatellite PCR with the exception of annealing temperatures. All primers were designed based on SNP location and ordered from IDT DNA technologies. Primers and annealing temperatures are listed in Table 1. All PCR amplification checks were performed in 2% agarose gels until optimized.

Restriction enzymes were selected for each SNP by Webcaster 2.0 and purchased from MBI Fermentas. All digests were run according to manufacturers’ recommendations. Restriction enzymes and digestion temperatures are listed in Table 1. Briefly, the 19,779 PCR fragment was digested in a 20-μl dilution of 2 U of enzyme, 2.0 μl of manufacturers’ standard buffer, 2.8 μl of dH2O, and 15 μl of the PCR product. All digests were allowed to proceed overnight and were run on a 2% gel after 24 h, with the exception of 40,395, 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, height was measured to the nearest 0.1 cm using a stadiometer (Harpenden, Holtafn, Wales, UK), and BMI was calculated (kg/m²).

**Dual-energy X-ray absorptiometry.** Body composition was estimated by dual-energy X-ray absorptiometry using the fan-beam technology (model QDR 4500A, Hologic, Waltham, MA). A total body scan was performed at baseline and again 24–48 h 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. Total body FFM, fat mass, and percent 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. The coefficients of variation (CV) for all dual-energy X-ray absorptiometry measurements 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 strength test.** The 1-repetition maximum (1 RM; i.e., the highest resistance at which 1 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 before baseline 1 RM strength testing so that subjects would be familiar with the equipment and proper exercise techniques. After the three familiarization training sessions and before the regular training sessions began, knee extensor 1 RM 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. The knee-extension 1 RM test started with a resistance level estimated to be ~20–30% of each subject’s predicted 1 RM. After ~60 s of rest on 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 1 RM 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 wk of single-leg training were standardized for each subject, including specific seat adjustments, body position, and level of vocal encouragement.

**MV.** 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-wk 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. (57). 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 × 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 software (National Institutes of Health, Bethesda, MD). The quadriceps cross-sectional area 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 was 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 CV was calculated for each investigator based on repeated measures of selected axial sections of one subject on 2 separate days. Average intrainvestigator CV was 1.7 and 2.3% for investigator 1 and 2, respectively. The average interinvestigator CV was 3.8%. Final MV was calculated with the use of a truncated cone formula as reported by Tracy et al. (57) and described by Ross et al. (47). Calculation of muscle quality (MQ) was strength (in N)/MV (in ml). To verify replication of position after training, scout scans were analyzed to verify the ischial tuberosity landmark. If the landmark was found to be >2 cm above or below the most distal point of the ischial tuberosity, subjects’ MV data were not used in any analysis. Additionally, if a quadriceps cross-sectional area was unable to be analyzed due to scan clarity, subjects’ MV data were not used in any analysis.

**Training program.** The training program consisted of unilateral (single leg) training of the knee extensor of the dominant leg three times per week for ~10 wk. 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.

Subjects would warm-up on a bicycle ergometer for ~2 min before each training session. The training consisted of five sets of knee-extension exercise for subjects >75 yr of age and four sets for those >75 yr 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 1 RM strength value. The second set consisted of five repetitions at the current 5 RM value. The 5 RM 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 5 RM value, and then the resistance was lowered just enough to complete one or two more repetitions before muscular fatigue was reached. 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 4 or 5 repetitions at the 5 RM resistance followed by 10 more repetitions for a total of 15 repetitions carried out in the same manner as described above for the 10-repetition set. The fifth set consisted of 4 or 5 repetitions at the 5 RM resistance, followed by 15

---

**Table 1. Sequences of PCR primers used for amplification and sequencing of IGF1 gene SNPs**

<table>
<thead>
<tr>
<th>SNP</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>Annelling Temperature, °C</th>
<th>Restriction Enzyme/Digestion Temperature, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>19779</td>
<td>5'-CAGATTGTGGCCACTAAATG</td>
<td>5'-CTATTAAAGGATGACTGTG</td>
<td>49</td>
<td>EcoRI/37</td>
</tr>
<tr>
<td>40395</td>
<td>5'-AAGTAAAAGAAGCAGTTGTCG</td>
<td>5'-ACGTGCGGAATTGTCGCA</td>
<td>55</td>
<td>Aulr/E37</td>
</tr>
<tr>
<td>82681</td>
<td>5'-AGATACAGGTTCTGGGAAAT</td>
<td>5'-GGTGGAGAGATTGATTGTTT</td>
<td>51</td>
<td>Tail/65</td>
</tr>
</tbody>
</table>

Single nucleotide polymorphism (SNP) numbering represents nucleotide position in the human IGF1 DNA sequence. Genbank accession number AY26095.
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 s, respectively. The shortening phase of the exercise took ~2 s, and the lengthening phase took ~3 s. Subjects performed supervised stretching of the knee extensors and hip flexors after each training session.

Statistical analyses. All statistical analyses as described below were performed using SAS software (SAS version 8.2, SAS Institute, 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 postraining) in MV of the untrained leg and the change in MV (pre- to postraining) of the trained leg. For strength, the change in 1 RM value of the trained leg was used as a dependent variable. MQ was calculated as 1 RM/MV, 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 noncarriers 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. The three levels of the predefined groupings of genotype at the IGF1 promoter locus were defined as the fixed effect. Covariates were included in the model when significant. 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 1 RM of the trained leg was the covariate for the analysis of strength). Preplanned comparisons of 192 carriers vs. noncarriers and 192 homozygotes vs. heterozygotes and noncarriers were analyzed for each dependent variable.

The SNPs were analyzed separately due to a smaller sample size because 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 1 or no subjects) were not included in the analysis. One 2 × 2 × 3 reduced model factorial ANOVA for each of the dependent variables was used for the promoter analysis. Post hoc comparisons were made comparing all groups when covaried for sex and age. The 192 homozygotes group was lost before 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 [3 women and 1 man] and three 192 heterozygotes [2 women and 1 man]). Body mass, percent body fat, and FFM for all subjects were included as covariates in the factorial ANOVA as described for the promoter analysis. Post hoc comparisons were made comparing all main effects by least significant difference 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 $\chi^2$ test (degrees of freedom = 1).

Table 2. IGF1 promoter allele and genotype frequency

<table>
<thead>
<tr>
<th>Number of CA Repeats</th>
<th>Total (%)</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;17</td>
<td>1 (&lt;1)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>17</td>
<td>3 (2)</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>18</td>
<td>8 (6)</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>19</td>
<td>82 (62)</td>
<td>38</td>
<td>44</td>
</tr>
<tr>
<td>20</td>
<td>27 (20)</td>
<td>12</td>
<td>15</td>
</tr>
<tr>
<td>21</td>
<td>12 (9)</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>22</td>
<td>1 (&lt;1)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Range</td>
<td>6–22</td>
<td>17–22</td>
<td>6–21</td>
</tr>
</tbody>
</table>

Genotype

| 19/19 (192/192) | 24 (36) | 10 | 14 |
| 19— (192—−)    | 34 (51) | 18 | 16 |
| —/— (Noncarrier)| 9 (13)  | 4  | 5  |

CA, cytosine adenine.

Table 3. IGF1 SNP allele and genotype distribution of all subjects

<table>
<thead>
<tr>
<th>Allele Frequency</th>
<th>Genotype Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>C</td>
</tr>
<tr>
<td>19,779</td>
<td>0.78</td>
</tr>
<tr>
<td>40,395</td>
<td>0.79</td>
</tr>
<tr>
<td>82,686</td>
<td>0.39</td>
</tr>
</tbody>
</table>

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 that employed a similar training and testing protocol. Statistical power for changes in strength a priori was estimated to be $>0.80$ with $\alpha$ set at 0.05 (critical effect size MV = 0.4; 1 RM = 0.45) and accounting for present sample size and genotype distribution. However, MV power was estimated to be ~0.60 due to the smaller training-induced critical effect size.

RESULTS

Genotype. IGF1 promoter allele and genotype frequencies (Table 2) did not differ significantly from Hardy-Weinberg expectations and were similar to those reported previously (36, 46, 58). Likewise, IGF1 SNP allele and genotype frequencies did not differ significantly from Hardy-Weinberg expectations (Table 3).

Subject characteristics. One female subject’s 1 RM value from the 192 homozygote group was lost before 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 [3 women and 1 man] and three 192 heterozygotes [2 women and 1 man]). Body mass, percent body fat, and FFM for all subjects did not change significantly as a result of training (Table 4). 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 5).

1 RM strength. The 1 RM strength of the trained leg did not differ significantly at baseline among IGF1 promoter genotype groups when covaried for sex and age. The 192 carriers increased their 1 RM strength (67.3 ± 6.3 N) significantly more than noncarriers (39.8 ± 7.6 N) when covaried for sex and age ($P < 0.05$; Table 6; Fig. 1), but all groups increased significantly with training ($P < 0.01$). There was no significant difference between 192 homozygotes and nonhomozygotes for change in 1 RM. In response to training, SNP analysis of

Table 4. Physical characteristics for all subjects

<table>
<thead>
<tr>
<th></th>
<th>Men (n = 32)</th>
<th>Women (n = 35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>Baseline</td>
<td>After training</td>
</tr>
<tr>
<td>Height, cm</td>
<td>70 (6)</td>
<td>—</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>169 (6)</td>
<td>—</td>
</tr>
<tr>
<td>Fat, %</td>
<td>83.5 (12.0)</td>
<td>84.0 (12.7)</td>
</tr>
<tr>
<td>FFM, kg</td>
<td>29 (5)</td>
<td>29 (4)</td>
</tr>
<tr>
<td>1 RM, N</td>
<td>58.2 (6)</td>
<td>58.5 (7.1)</td>
</tr>
</tbody>
</table>

Values are means with SD in parentheses. FFM, fat-free mass; 1 RM, 1-repetition maximum.
covariance main effects and two-factor interactions revealed no significant differences in the change of 1 RM strength.

**MV and MQ**. MV increased (i.e., increases in trained leg minus changes in untrained leg) in all groups (192 homozygotes, heterozygotes, and noncarriers) in response to training ($P < 0.01$). MV and MQ did not differ significantly at baseline or in response to training among IGF1 promoter genotype groups when covaried for sex and age (Table 6; Fig. 2). However, training-induced increases in MV for 192 carriers (132 ± 12 ml) approached significance for being greater than the increases in noncarriers (94 ± 18 ml; $P = 0.08$). The 192 homozygotes were not significantly different from the heterozygotes and noncarriers 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**

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 associated with MV response to training approached significance.

ST 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 (23), and IGF-I expression levels change as a consequence of ST in older adults (16). The results of this study add to this literature by showing that, in older adults, carriers of the 192 allele at the IGF1 locus have greater strength gains than noncarriers 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 (8, 37). Additionally, muscle stimulation (mechanical, electrical, and stretch) leads to increases in IGF-I protein and overall protein content in skeletal muscle and produces significant increases in muscle size and strength (15, 16, 34, 60). Although a causal relationship between acute or chronic muscle contraction and IGF1 expression has not yet been documented in humans, IGF1 remains one of the strongest candidate genes for ST-induced increases in muscle mass and strength. The increase in IGF1 protein has been reported to be proportional to the increase in muscle strength in elderly adults after ST (16). Thus it seems that increases in IGF1 expression may be causally related to increases in muscle size and strength. In the study by Fiatarone Singh et al. (16), IGF-I protein increased by an average of ~500% in response to ST in the elderly. However, the variation in IGF1 mRNA expression was >500%, suggesting a substantial interindividual variability in response to training.

Indeed, IGF-I blood levels, muscle size, and function are known to be significantly influenced by genes (9, 24, 28, 50, 55). IGF-I blood levels are highly heritable in children, middle-aged, and elderly adults (24, 28). In this regard, a study examining the same CA repeat IGF1 promoter polymorphism as the one in the present study demonstrated a significant age-related decline in circulating IGF1 levels that was associated with this polymorphism (44). Consistent with our findings, this would suggest an

### Table 5. Physical characteristics for subjects by IGF1 promoter genotype

<table>
<thead>
<tr>
<th></th>
<th>192 Homozygotes</th>
<th>192 Heterozygotes</th>
<th>All Other Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>67±1</td>
<td>70±1</td>
<td>68±4</td>
</tr>
<tr>
<td>Height, cm</td>
<td>169±2</td>
<td>167±2</td>
<td>165±3</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>78.6±2.5</td>
<td>75.7±2.0</td>
<td>75.8±5.3</td>
</tr>
<tr>
<td>Fat, %</td>
<td>34±1</td>
<td>34±1</td>
<td>33±2</td>
</tr>
<tr>
<td>FFM, kg</td>
<td>51.8±1.4</td>
<td>50.0±1.0</td>
<td>50.0±2.2</td>
</tr>
<tr>
<td>Male/Female, n</td>
<td>10/14</td>
<td>18/16</td>
<td>4/5</td>
</tr>
</tbody>
</table>

Values are means ± SE. None of the after-training values were significantly different from baseline. Weight, % Fat, and FFM are corrected for mean age (68 yr) and sex (50/50 ratio).

### Table 6. Muscle volume, 1 RM muscle strength, and muscle quality measurements before and after training by IGF1 192 CA repeat polymorphism

<table>
<thead>
<tr>
<th></th>
<th>192 Homozygotes</th>
<th>192 Heterozygotes</th>
<th>All Other Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trained leg</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscle volume, ml</td>
<td>1.470±33</td>
<td>1.590±37*</td>
<td>1.360±38</td>
</tr>
<tr>
<td>Muscle quality, N/ml ($\times 10^3$)</td>
<td>157±6</td>
<td>187±5*</td>
<td>163±5</td>
</tr>
<tr>
<td>1 RM, N</td>
<td>231±8</td>
<td>298±12*</td>
<td>222±8</td>
</tr>
<tr>
<td><strong>Untrained leg</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscle volume, ml</td>
<td>1.415±48</td>
<td>1.413±48</td>
<td>1.306±40</td>
</tr>
<tr>
<td>Muscle quality, N/ml ($\times 10^3$)</td>
<td>153±8</td>
<td>170±9</td>
<td>163±5</td>
</tr>
<tr>
<td>1 RM, N</td>
<td>217±12</td>
<td>240±11</td>
<td>213±11</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Significantly different from baseline ($P < 0.01$). All values are corrected for mean age (68 yr) and sex (50/50 ratio).
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 (6) and humans (43, 54, 55). Support from human studies come from Reed et al. (43) who observed significant genetic effects for absolute grip strength and adjusted grip strength; 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 (43). Other studies have demonstrated between 31 and 82% of variance in strength is explained by genetic factors (54, 55).

To our knowledge, only two studies have attempted a genome-wide search for genes related to phenotypic measures of muscle (10, 11). In one of these studies, FFM, measured by hydrostatic weighing, showed significant linkage with the IGF1 receptor polymorphism in the Quebec Family Study (10). 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 present study (11). Additionally, linkage and association of baseline FFM and the change in FFM due to exercise training has been demonstrated with the IGF1 promoter polymorphism (52). 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.

The IGF1 promoter polymorphism has been examined in numerous contexts (20, 29, 44, 46). As in the present study, genotyping of the microsatellite is typically separated into three groups: 192 homozygotes, 192 heterozygotes, and noncarriers 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, 48). It has been suggested by King et al. (29a) 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 in phenotypes known to be affected by IGF-I (11, 14, 52).

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 simply a marker for 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, IGF1 expression levels would be needed along with a method of grouping that could account for heterozygotes who carried one copy longer and one copy shorter than 19 repeats. Therefore, in light of the current lack of data on functionality, the conventional grouping of 192 carriers and noncarriers was used as previously reported in the literature.

Rosen et al. (46) first implicated this polymorphism with serum levels of IGF-I and bone mineral density in a study examining older men and women (46). 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 (46). The reports by Frayling et al. (20) and Rietveld et al. (44) concur with Rosen et al.’s (46) findings of decreased blood levels of IGF-I in carriers of the 192 allele. In contrast to Rosen et al.’s findings, subsequent studies have demonstrated that 192 noncarriers have lower IGF-I blood levels and are more likely to be osteoporotic (29, 45). 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 noncarriers of 192. 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 contra-
ceptive use (3, 13, 27, 61). 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 (16, 34). 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 this polymorphism on muscle phenotypes known to be related to IGF1 expression.

Some limitations of the present study are the lack of IGF1 expression data for muscle and IGF1 cellular mediators, the use of only one ethnic group, and the statistical power for MV. The use of one ethnic group was decided on to control for the possible varying effects 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 (13). 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, a-priori, ~0.60, whereas for strength it was >0.80 (post hoc, 0.66 for MV and >0.95 for strength). This is primarily due to the smaller (compared with strength) effect size seen in MV in response to ST. Additionally, the design of the present 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. To asses IGF1 muscle expression and cellular mediators, muscle biopsy samples would be required. However, our focus was to first determine whether IGF1 genotype is related to our muscle phenotypes of interest. Thus, if our results had demonstrated no relationship, having the muscle expression and cellular mediator data might not be very meaningful, or at least difficult to interpret. Yet, the results of the present study do suggest the need for a larger scale investigation and acquiring muscle samples to study gene expression and cellular mediators. 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 MV compared with noncarriers. The results of the present 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.

REFERENCES


