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Association between IGF-2 gene and fat-free mass in response to resistance training

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ABSTRACT

BACKGROUND: It is well established that the human aging process is associated with a significant decline in neuromuscular function and performance. Twin studies demonstrate that genetic factors partially explain the inter-individual variation of fat-free mass (FFM) and muscle strength. PURPOSE: To examine the association between the polymorphism of the gene IGF-2 with phenotypes strength and muscle mass of elderly women pre and post resistance training. METHODS: 76 elderly women participated in this analysis. These volunteers, who should be sedentary for at least 6 months before the study, were assigned four groups in relation to allele GAGG, AA, GAAA and GG. The first analysis compared 60 elderly women with GG and GA allele (GAGG) versus 16 elderly women with AA allele. The second analysis compared 54 elderly women with GA and AA (GAAA) versus 22 elderly women GG allele. The Body mass index, fat free mass, fat mass and Isokinetic Muscle Peak Torque were measured. All volunteers were participating in the Resistance Training program. The DNA was extracted from peripheral venous blood leukocytes using a salting out protocol. The primer for IGF-II gene exon 9 was 5'-GTCCCTGAACCAGCAAAGAG-3' 0.5 µM (0.625 µl) Primer R-5'-TGATGGAAAAGGGAGTGAGG-3' 0.5 µM (0.625 µl), Taq DNA Polimerase enzime 0.5 U (0.1 µl), água milli-Q (3.8 µl) 5 ηg de DNA (3 µl). PCR amplification was performed in a programmable thermal cycler GeneAmp® PCR System 9700. RESULTS: The results of the first analysis GAGG × AA versus the group AA showed the relative increase in knee extensor peak torque relative and also in the fat-free mass and showed a decrease in the fat mass percentage in the GAGG group. The second analysis considered the group GAAA versus group GG showed decreases in the fat-free mass percentage in the GAAA group, also showed the relative increase in the fat-free mass in the GG group and decrease in the fat mass percentage in the GG group. CONCLUSIONS: The results of this study show that 24 weeks of the resistance training improved strength and muscle mass and decreased the fat mass. The results confirm this assertion and suggest the G allele presents more influence over the A allele, in relation to phenotypes strength and muscle mass in elderly women after resistance training.

Keywords: Fat-Free Mass; Fat Mass; Resistance Training; Muscle Strength; IGF-2 Polymorphism; Elderly

1. INTRODUCTION

It is well established that the human aging process, from maturity to senescence, is associated with a significant decline in neuromuscular function and performance [1]. This decline is associated with an increased risk

of falls, hip fractures, and adverse physiological changes, such as glucose intolerance and a loss of bone mineral density [2-5].

Cross-sectional studies have shown significant reductions in muscle mass and strength and alterations in body composition with advancing age [6,7]. Sarcopenia has significant health care cost implications that warrant efforts to understand and counteract this age-related muscle mass and strength decline [8]. On the other hand the resistance training (RT) is well recognized as an effective intervention to increase muscle mass and strength [9]. Strength and muscle mass are increased following resistance training in older adults through a poorly understood series of events that appears to involve the recruitment of satellite cells to support hypertrophy of mature myofibrils [10].

Twin studies demonstrate that genetic factors partially explain the inter-individual variation of fat-free mass (FFM) and muscle strength [11,12]. Devaney *et al.* [13] studied the association of several polymorphisms in the insulin-like growth factor 2 (IGF2) genes in relation to exertional muscle damage of the elbow flexors in 151 young men and women. After a damaging eccentric contraction protocol, loss of isometric strength in response to the damaging exercise protocol was significantly different among genotype groups for multiple polymorphisms in the IGF2 gene region [13].

However, the significant influence of polymorphisms of one such candidate gene, insulin-like growth factor (IGF2), on body composition [14], birth weight, and grip strength in middle age has been reported [15]. It is possible that IGF-II could influence acute regenerative capacity in aging human muscle in a way that the cumulative effect of these diminished responses to exercise or to increasing oxidative stress with age [16] could influence rates of age-associated losses in muscle mass and strength in humans [17].

Thus, the main purpose of the present study was to examine the association between the polymorphism of the gene IGF-2 with phenotypes strength and muscle mass of elderly women pre and post resistance training.

2. METHODS

2.1. Subjects

Volunteers were invited to take part in this investigation by telephone calls and by visits to the University neighborhood centers of attention for this population. After exclusion criteria were applied, a total of 76 elderly women participated in this analysis. These volunteers, who should be sedentary for at least 6 months before the study, were assigned four groups possible in relation to allele GAGG, AA, GAAA and GG. The first analysis compared 60 elderly women (65.3 ± 5.2 years old) with GG and GA allele (GAGG) versus 16 elderly women (66 ± 5.2 years old) with AA allele. The second analysis compare 54 elderly women (65.1 ± 4.8 years old) with GA and AA (GAAA) versus 22 elderly women GG allele (66.5 ± 5.9 years old).

Exclusion criteria were as follows: individuals with any metallic implant, those with an artificial pacemaker, those who had been submitted to hip surgery, those who were unable to walk without assistance, those affected by metabolic or endocrine disorder that implies in muscular system, those who presented electrocardiography alterations during the cardio-pulmonary exercise test. There was no statistical difference between groups at baseline.

Each volunteer answered a face-to-face questionnaire addressing medical history, hormone replacement therapy, life style habits and medication use. The study design and procedures were conducted in accordance with the ethical standards of sport and exercise science research [18] and were approved by the University's Ethics Committee under the protocol number 024/2007. Written informed consent was obtained from each participant.

2.2. Anthropometry and Body Composition

Body weight was measured to the nearest 0.1 kg using a calibrated electronic scale with women dressed in a light T-shirt and shorts. Height was determined without shoes to the nearest 0.1 cm using a wall stadiometer, after a voluntary deep inspiration. Body mass index (BMI) was calculated by weight/height ($kg \cdot m^{-2}$). Body composition measurements were conducted at University's Image Laboratory using DXA (DPX-L; Lunar Radiation Corporation, Madison, WI, USA). For the procedure, volunteers laid in the supine position on the center of the DXA scanner. The software provided FFM and fat mass for whole body and specific regions. Appendages were isolated from the trunk and head by using computer-generated lines with subsequent manual adjustment. Regional measurements (arms, legs, and trunk) were determined on the basis of bone landmarks, with vertical boundaries separating the arms from the body at shoulder, and angled boundaries separating the legs from the trunk at the hips. Appendicular FFM (AFFM) was calculated as the sum of both arms and legs FFM. Coefficients of variation of the system were 2.1% and 1.9% for fat mass and FFM, respectively. The equipment was daily calibrated, and all examinations were done by the same trained technician.

2.3. Isokinetic Muscle Peak Torque

Dominant knee extensor isokinetic muscle peak torque was evaluated using the Biodex System 3 dynamometer

(Biodex Medical System, Shirley, NY) and was assumed as a muscle strength index. Before testing, a 5-min warm-up was performed on a stationary cycle ergometer at a comfortable ate and low workload. After a full explanation of the procedures, participants were seated on the dynamometer, which was then carefully adjusted. The rotation axis of the dynamometer arm was oriented with the lateral condyle of participant's dominant femur. Velcro belts were used at the thigh, pelvis, and trunk to avoid any compensatory movement.

Gravity correction was obtained by measuring the torque exerted on the dynamometer with the knee in a relaxed state at full extension. Testing protocol consisted of 3 sets of 4 knee extensor contractions at 60˚/s with 30 s between sets [19,20]. The recorded value was the highest achieved peak torque in Newtons (N) throughout the 3 sets, which was expressed both in absolute values (Nm) and relative to body weight (Nm/kg). Participants were asked to perform the movement with their maximal strength, and verbal encouragements were offered by the examiner during the measurement. Calibration of the equipment was performed according to manufacturer's specifications before every testing session.

Isokinetic testing has become a popular method to assess dynamic muscle strength in both clinical and research settings and is widely used for injury rehabilitation and measurements of muscle torque [20,21]. Furthermore, the isokinetic dynamometer has been broadly used to characterize and/or evaluate older individuals [19, 20].

2.4. Resistance Training Program

All volunteers interested in participating in the RT program underwent physician screening at rest and under cardiopulmonary exercise test conditions. Nine familiarization sessions were performed at the beginning of the training program. Correct lifting techniques were explained and demonstrated to the volunteers, who practiced the exercises with light resistance. Volunteers trained 3 times per week for 24 weeks. Each session lasted around 60 minutes divided into 5 minutes warm-up (at 50% - 60% of maximal heart rate), 50 minutes of resistance exercises, and 5 minutes of stretching exercises performed as cooldown.

The training program involved the following exercises: chest press, lat pulldown, knee extension, hamstrings curl, leg press, hip abduction (all using machines with plates-High on, Righetto Fitness Equipment, Brazil), shoulder abduction (using dumbbells) and orthostatic toe raises. Participants also performed sit-ups and trunk extension. After the familiarization period, participants underwent 1 repetition maximum (1 RM) testing for each of the exercises of the training program, except for trunk extension, toe raises, shoulder abduction, and trunk flexion. This procedure was used to determine the exercise load and was repeated in 4-week intervals during the RT program.

The program followed a progressive intensity, with training loads of 60% of 1 RM in the first 4 weeks, 70% in the following 4 weeks, and 80% in the remaining 16 weeks, with repetitions, respectively, decreasing from 12, 10, and 8. Each exercise was performed in 3 sets with approximately 1-minute rest between sets. Volunteers were asked to breathe comfortably, avoiding the valsalva maneuver. Participants who did not complete at least 75% of the training sessions were excluded from analyses.

2.5. Genotyping

High molecular weight DNA was extracted from peripheral venous blood leukocytes using a salting out protocol [22]. The primer for IGF-II gene exon 9 was 5'-GTCCCTGAACCAGCAAAGAG-3' 0.5 µM (0.625 µl) Primer R-5'-TGATGGAAAAGGGAGTGAGG-3' 0.5 µM (0.625 µl), Taq DNA Polimerase enzime 0.5U (0.1 µl), água milli-Q (3.8 µl) 5 ηg de DNA (3 µl). PCR amplification was performed in a programmable thermal cycler GeneAmp® PCR System 9700 (Perkin Elmer Applied BioSystems, Foster City, CA). The PCR product of 156-bp was mixed with two units Apa I (New England Biolabs, Beverly, MA); two fragments of 102-bp and 67-bp will be present on 4% agarose gel electrophoresis if the product is digestible.

2.6. Statistical Analysis

Exploratory and descriptive statistical procedures were, respectively, used to search for outliers and to calculate means and standard deviation (SD) for all the presented variables. The Kolmogorov-Smirnov test was used to verify data distribution normality.

An exact test was used to verify whether the observed genotype frequencies were in Hardy-Weinberg equilibrium. Individual maximum-likelihood estimation of admixture proportion was carried out based on parental population (European, African and Amerindian) allele frequencies for the 13 genotyped ancestry-informative markers using IAE3CI software.

To analyze difference intra and inter group before and after the RT program, Split-Plot ANOVA was performed. Analyses were considered significant at $p \leq 0.05$ and all statistical procedures were performed using the SPSS 18.0 software (SPSS Inc., Chicago, IL, USA).

3. RESULTS

For the first analysis GAGG × AA, the group GAGG

was composed of 60 volunteers (65.3 ± 5.2 years old) and Group AA was composed of 16 volunteers (66 ± 5.2 years old). The data in **Table 1** shows the relative increase in knee extensor peak torque and also in the fat-free mass and shows a decrease in the fat mass percentage in the GAGG group.

The second analysis considered the group GAAA versus group GG. The group GAAA was composes for the 54 volunteers (65.1 ± 4.8 years old). The group GG was composes for 22 volunteers (66.5 ± 5.9 years old). The data in **Table 2** show a decrease in the fat-free mass percentage in the GAAA group and also showed the relative increase in the fat-free mass in the GG group and decrease in the fat mass percentage in the GG group.

Table 1. Knee extensor peak torque, total fat-free mass, and appendicular fat-free mass before and after the 24 weeks of the study protocol for GAGG × AA.

Variables	Groups			
	GAGG		AA	
	Pre	Post	Pre	Post
KEPT absolute ($N \cdot m^{-1}$)	101.4 ± 19.1	102.9 ± 20.8	115.0 ± 25.3	105.1 ± 19.4
KEPT Relative (Nm·Kg)	146.3 ± 30.1	160.7 ± 31.1*	152.1 ± 19.4	168.8 ± 18.8
FFM (kg)	37.1 ± 6.8	38.3 ± 5.7*	38.3 ± 5.7	38.3 ± 5.5
AFFM (kg)	14.2 ± 3.0	14.1 ± 2.4	14.6 ± 3.1	14.02 ± 1.8
% FM	39.3 ± 6.1	38.2 ± 5.4*	39.8 ± 6.1	39.1 ± 6.3

AFFM = appendicular fat-free mass; FFM = fat-free mass; FM = fat mass; KEPT = knee extensor peak torque; *$p \leq 0.05$ difference between pre and post in the GAGG group.

Table 2. Knee extensor peak torque, total fat-free mass and appendicular fat-free mass before and after the 24 weeks study protocol for GAAA × GG.

Variables	Groups			
	GAAA		GG	
	Pre	Post	Pre	Post
KEPT absolute ($N \cdot m^{-1}$)	103.5 ± 21.2	105.0 ± 20.2	106.3 ± 21.3	99.5 ± 21.0
KEPT relative (Nm·Kg)	149.6 ± 50.0	164.8 ± 30.1	153.4 ± 31.5	148.4 ± 30.6
FFM (kg)	37.5 ± 5.3	37.9 ± 5.3	37.0 ± 9.0	39.1 ± 6.4†
AFFM (kg)	14.2 ± 2.9	14.0 ± 2.1	14.4 ± 3.5	14.5 ± 2.6
% FM	39.8 ± 6.20	38.9 ± 5.7*	38.4 ± 5.9	37.1 ± 5.1†

AFFM = appendicular fat-free mass; FFM = fat-free mass; FM = fat mass; KEPT = knee extensor peak torque; *$p \leq 0.05$ difference between pre and post in the GAAA group; †$p \leq 0.05$ difference between pre and post in the GG group.

4. DISCUSSION

Sarcopenia being described in both elderly men and women [23], has been linked to multiple negative clinical outcomes and thus imposes significant health care cost implications. Resistance training has been pointed as an effective intervention for muscle mass and strength improvements among older adults [24,25]. Exercise training-induced adaptations in general [26] and the adaptations of skeletal muscle to RT in particular [27] are partially determined by genetic factors. However, the identification of specific genes and polymorphisms has yet to emerge. Therefore, it is of particular interest to examine the association of gene polymorphisms and muscular characteristics as well as its interaction with resistance training-induced adaptations in the elderly. Allelic variants in several genes have been examined, but little agreement has been reached on significant contributors [11,28,29]. The main findings of the present study were that polymorphism in the IGF-2 gene was associated with muscle mass and strength response to a 24 weeks RT program. Specifically, the observed data suggest that the G-allele carriers present a greater adaptation to training. These results might help the identification of individuals who are more prone to undergo sarcopenia and benefit from early implementation of individually tailored preventive interventions.

Comparisons between the present findings with current literature are difficult given the nature of the intervention which consists of 24 weeks of progressive RT, following literature recommendations, while most studies conducted a short-term training protocol [30-32]. The observations presented here were based on the three genotypes (*i.e.*, A/A, A/G and GG), as well as combinations of alleles (*i.e.*, G/A + GG vs. AA; and G/A + A/A vs. GG). The findings suggest that individuals carrying the G-allele are more prone to decrease body fat and increase muscle mass and strength when comparing pre and post intervention assessments. The combination of G/A and A/A genotypes also showed body fat decreases, what can be, at least in part, attributed to G-allele predisposition.

The results of fat-free mass and muscle strength are in accordance with previous reports [32]. Schrager *et al.* [32] conducted a 42 years longitudinal study and observed an association between muscle mass and strength with the IGF2 gene. Congruent with the present results, the significant results were driven by greater upper limb isokinetic strength in homozygous GG when compared to homozygous AA. Besides muscle strength, Schrager *et*

al. [32] noted that fat-free mass was also lower for A/A genotype carriers, however, this observation was only significant in women. Roth *et al.* [29] examined the association between a polymorphism in the IGF-2 gene and obesity in 500 healthy men and women with age ranging from 19 to 90 years. These authors report that individuals with the A/A genotype presented higher fat mass when compared to G/G genotype carriers. Although these results are not promptly consistent with the present study, it is possible that the presence of the G-allele might benefit fat mass decrease as results of training, since the G/A + G/G group presented a greater reduction. Probably many genes with small contributions rather than few genes with strong influence are expected to determine the inter-individual differences of muscle-related phenotypes [33,34]. Thus, the present study provides evidence that the IGF-2 is one of the many genetic variants that contribute to FFM in response to RT, though its clinical relevance is unclear.

The present study has some limitations. The sample was composed of Brazilians, which is a population characterized by high admixture. Thus, it can be argued that the association observed in the present study was influenced by genetic ancestry. However, genotyping ancestry-informative single nucleotide polymorphisms and taking the estimated genetic ancestry values as covariates did not alter the observed association with muscle-related phenotypes in our previous report, which included 25% of the same subjects [35]. The number of participants that performed the RT program was relatively small, due to the relatively long period of intervention (24 weeks) and to the characteristics of the training sessions. We attempted to follow literature recommendations for resistance exercise prescription and to mimic its general use in physical activity centers, that is, to address the ecological validity. Also, the participants were limited to women, what removes the well-known influence of sex in genetic association with muscular phenotypes.

5. CONCLUSION

Consistent with literature, it was observed that 24 weeks of resistance training improved muscle mass and strength, and elicited decreases in fat mass. However, the present study provides the evidence of a possible association between the IGF = 2 gene and the response to RT, suggesting that G-allele carriers present a better response when compared to the A-allele. Determination of genetic variants associated with muscular phenotypes in the elderly may be useful in identifying individuals who are more susceptible to lose muscle mass and strength with advancing age. Such knowledge will allow a better understanding of sarcopenia pathophysiology and the development of efficient individualized preventive tools.

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