The Relationship between the ACTN3 Genotype and Measures of Stress, Exercise Performance and Body Composition: A Pilot Trial

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ABSTRACT

Antonio J, Knafo S, Ellerbroek A, Vargas L, Silver T, Peacock C, Tartar J. The Relationship between the ACTN3 Genotype and Measures of Stress, Exercise Performance, and Body Composition: A Pilot Trial. JEPonline 2017;20(6):139-153. The purpose of this study was to assess the relationship between the expression of the ACTN3 gene (RR homozygous or RX heterozygous) and measures of stress, performance (i.e., bench press strength and endurance), and body composition. Strength was determined via a 1-RM (one-repetition maximum) for the bench press, and muscular endurance was determined by the number of repetitions to failure that were performed on the bench press at 60% of 1-RM. Body composition was assessed via a dual-energy X-ray absorptiometry (DXA). One hundred and five subjects completed the study ([mean ± SEM]: body weight: R/⁻ group: 73.8 ± 1.7 kg vs. XX group: 66.6 ± 2.8 kg). There were no significant differences between XX and R/⁻ (carriers of ACTN3) for fat mass, lean body mass, % fat or body weight. However, R/⁻ demonstrated significantly greater bone mineral content (mean ± SEM: R/⁻ = 2951 ± 70 g, XX = 2622 ± 73 g) and density (R/⁻ = 1.35 ± 0.02, XX = 1.28 ± 0.03 g/cm²). No differences were found for any of the other parameters. In exercise-trained individuals, carriers of the gene (R/⁻) demonstrated greater bone mineral content and density.

Key Words: Bone, Cortisol, DXA, Gene, Strength
INTRODUCTION

Athletic performance is governed by a host of factors that include but are not necessarily limited to training, diet, supplementation, drugs, and genetics. There is a growing body of work that shows how certain genetic polymorphisms relate to a propensity for high levels of athletic performance (8,11,12,17,26). For instance, one variant of the ACTN3 gene (also known as the “speed gene”) is found in athletes that typically engage in the ‘speed’ sports (e.g., sprinting) (13,27). Specifically, a functional single nucleotide polymorphism (SNP) at codon 577 of the ACTN3 gene (rs1815739) causes arginine (R) to be replaced by a stop codon (X). Carrying two copies of the X allele (XX) prevents any expression of the alpha-actinin-3 protein and, accordingly, XX homozygotes may experience a disadvantage in the speed sports. On the other hand, elite canoe and kayak paddlers that compete at the 1000 meter (m) distance tend to have the XX genotype at a much higher frequency than those who compete at the 200 m distance (18). Prior research has shown that the skeletal muscle actin-binding protein alpha-actinin-3 is absent in 18% of healthy white individuals (26).

Alpha-actinin-3 is the specific protein that in humans is encoded by the ACTN3 gene. This protein is found specifically in type II or fast-twitch skeletal muscle fibers (24). Type II fibers generate much greater power than type I fibers. For instance, ACTN3 577R allele (RR or RX genotype) is associated with performance in Australian elite athletes (26). Conversely, there is no relationship between the ACTN3 genotype and the biceps brachii muscle size (7). In one of the few investigations to utilize the dual-energy-X-ray absorptiometry (DXA) scan to assess body composition, no relationship was found between ACTN3 genotypes and power or body composition-related phenotypes (4).

Thus, the purpose of this exploratory investigation was to determine the relationship between the ACTN3 genotype and measures of muscular strength and endurance in a group of highly competitive athletes or resistance-trained individuals. Furthermore, we assessed the stress response before and after heavy resistance exercise (i.e., a one-repetition maximum (1-RM) bench press strength test followed by the maximal repetitions to failure on the bench press at a weight equal to 60% of the 1-RM).

METHODS

Subjects
One hundred and nine exercise-trained subjects volunteered for this investigation. All subjects either competed in a particular sport (e.g., mixed martial arts fighting, stand-up paddling, distance running, track and field, etc.) or performed heavy resistance training at least 5 d·wk⁻¹. Subjects came to the laboratory on one occasion for testing. In accordance with the Helsinki Declaration, Nova Southeastern University’s Human Subjects Institutional Review Board approved all human subjects procedures. Written informed consent was obtained prior to participation. The order of testing was as follows after the consent form was signed: (a) subjects filled out a demographics form and provided the initial saliva sample (after ~20 min of inactivity) of 1 to 2 mL in a small vial (for lab analysis of cortisol and genotyping); (b) they were scanned via the DXA; (c) 1-RM bench press; (d) bench press repetitions to failure at 60% 1-RM; (e) a visual analogue scale (VAS) was given to each subject pre- and post-exercising testing to determine their self-reported stress level (1 = no
stress; 10 = extreme stress); and (f) the last saliva sample was provided by the subject. All testing took place between 1130 and 1400 hrs.

Procedures

Body Composition
Subjects had their height and weight determined using a calibrated scale. Body composition was assessed with a Hologic-WI DXA (Hologic Inc., Danbury CT USA). Quality control calibration procedures were performed on a spine phantom. Subjects wore typical athletic clothing and removed all metal jewelry. They were positioned supine on the DXA within the borders delineated by the scanning table. Each whole body scan took approximately 7 min.

Performance Testing
Performance testing included 1-RM bench press and repetitions to failure (RTF) at 60% of the bench press 1-RM. A certified strength and conditioning specialist conducted all performance tests. All subjects were highly trained (i.e., >1 yr experience at minimum) and were familiar with the performance tests prior to entering the laboratory. In general, each subject performed a movement specific warm up prior to the test (i.e., 3 sets on the bench press at progressively higher submaximal loads). Then, the subjects rested for 2 to 3 min prior to commencing the 1-RM bench press. A maximum of five attempts was used for the 1-RM bench press. Once the subjects achieved their 1-RM, they rested for approximately 2 to 3 min prior to commencing the RTF at 60% of the 1-RM bench press. The maximal number of repetitions was subsequently determined. All subjects were asked to assess their stress level via a visual analogue scale (10 = severe stress, 1 = no stress) before and after the performance tests.

Biomarkers - Cortisol
Subjects provided saliva samples for cortisol quantification and DNA extraction via passive drool through a straw into a 1.5 mL micro-centrifuge tube immediately before and 5 min following the exercise tests. Saliva samples were run in duplicate and quantified via a human melatonin enzyme immunoassay (EIA) kit according to the manufacturer’s instructions (Salimetrics LLC, USA). The samples were immediately read in a BioTek ELx800 plate reader (BioTek Instruments, Inc., USA) at 450 nm with a correction at 630 nm. All samples were within the detection ranges indicated in the cortisol immunoassay kit and the variation of sample readings was within the expected limits. Final concentrations for the biomarkers were generated by interpolation from the standard curve in μg/dL.

Cortisol concentrations were determined at baseline and post-exercise. ACTN3 Genotyping - Genomic DNA was extracted in a QIAcube instrument following the manufacturer’s standard protocol for saliva nucleic acid extraction (QIAGEN, Valencia, CA). After isolation, allelic discrimination for the ACTN3 gene was determined via real-time polymerase chain reaction (PCR) using a TaqMan SNP genotyping assay and fluorogenic probes (Applied Biosystems, CA). Thermal cycling was performed on StepOne Real-Time PCR system (Applied Biosystems, CA). The amplification mix contained the following ingredients: 12.5 μL of PCR master mix (QIAGEN, Valencia, CA), 1.25 μL of TaqMan 20X working stock, 10.25 μL of RNase- and DNase-free water (Sigma), and 1.0 μL of sample DNA, in a total volume of 25 μL per single tube reaction. The PCR conditions were 95°C for 10 min followed by 40 repeated cycles of 95°C for 15 sec and 60°C for 60 sec. Genotypes were determined automatically via
the StepOne software (Applied Biosystems, CA) based on the fluorescence signals. Samples were run in duplicate and in the case of a call discrepancy, samples were rerun.

**Statistical Analyses**

We conducted a series of independent sample *t*-tests to assess the differences, if any, between the R/- and XX groups regarding performance and body composition. Assays for the measures of stress (salivary cortisol and self-reported stress) were analyzed using a repeated measures analysis of variance (ANOVA) where time of measure was the within subject variable and genotype was between subject variable. *Post hoc* analyses were conducted with pairwise comparisons using the Bonferroni correction. The Hardy–Weinberg Exact (HWE) test was used to determine the distribution of allele frequencies. The association of allele status was analyzed using the chi-square test. Furthermore, we ran a Pearson's correlation between strength/endurance versus various physical characteristics. All calculations were conducted using an SPSS statistical package (version 19, SPSS inc., IBM). All reported *p*-values are two-tailed with a priori significance level of P<0.05.

**RESULTS**

**ACTN3 Genotype**

One hundred and five trained individuals completed the study (age: 30.2 ± 0.9 yrs; height: 171.3 ± 0.9 cm; data expressed as mean ± SEM). We did not finalize data collection on 4 subjects. The subject population consisted of 21 competitive stand-up paddlers (9 males, 12 females), 15 professional fighters (14 MMA, 1 professional boxer; all males), 18 endurance athletes (world class and collegiate distance runners, world-class and collegiate swimmers and cyclists; 8 males, 10 females), 10 collegiate track and field athletes (5 males, 5 females), and 41 individuals (20 males, 21 females) who regularly performed heavy resistance training. ACTN3 genotype frequencies were as follows: 20.4% XX, 44.4% RX, and 35.2% RR. The HWE test showed that *χ²* = 0.89, *P* = 0.35, suggesting that the population is consistent with Hardy–Weinberg Equilibrium, and confirming that the allele types were randomly sampled. In order to examine the hypothesized strength benefit of carrying at least one R allele, we collapsed across genotypes containing the C nucleotide (R allele carriers) (21). Accordingly, the CC homozygotes (Arg/Arg) and the CT heterozygotes (Arg/X) (n = 86) were compared to the TT homozygotes (X/X) (n = 22). There were no sex differences in genotype. For the purpose of this study, we grouped all R allele carriers (males and females) (i.e., R/- (carriers) vs. XX (does not express the alpha-actinin-3 protein).

**Body Composition**

There were no significant differences between groups for body weight, lean body mass (LBM), fat mass, or % body fat (Table 1 - Data expressed as mean±SEM).

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight (kg)</th>
<th>LBM (kg)</th>
<th>Fat mass (kg)</th>
<th>% Body Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>R/-</td>
<td>73.8 ± 1.7</td>
<td>56.7 ± 1.6</td>
<td>15.9 ± 0.6</td>
<td>21.4 ± 0.7</td>
</tr>
<tr>
<td>XX</td>
<td>66.6 ± 2.8</td>
<td>52.6 ± 3.1</td>
<td>15.6 ± 1.0</td>
<td>22.8 ± 1.7</td>
</tr>
</tbody>
</table>

Data expressed as mean ± SEM. There were no significant differences between the XX and R/- groups.
There was a significant difference (P<0.05) between the XX and R/- groups for bone mineral content (BMC) and bone mineral density (BMD). BMC for the XX and R/- groups were 2622.14 ± 372.14 and 2951.27 ± 642.70 grams, respectively (mean ± SD). BMD for the XX and R/- groups were 1.28 ± 0.13 and 1.35 ± 0.17 g/cm$^2$, respectively (Figure 1 and Figure 2). The mean T-score for the R/- and XX groups 1.7 and 1.4, respectively.

Figure 1. Bone Mineral Content. Data expressed as mean ± SEM. The R/- group was significantly greater than the XX group (P<0.05).

Figure 2. Bone Mineral Density. Data expressed as mean ± SEM. The R/- group was significantly greater than the XX group (P<0.05).
**Stress – Cortisol and Self-Reported**

Repeated measures ANOVA did not show a significant collection time (BL vs. post-bench press) x genotype interaction ($F(1,106) = 0.66$, $P>0.05$) or a main effect of collection time ($F(1,106) = 0.05$, $P>0.05$). However, there was a significant main effect for genotype ($F(1,106) = 4.15$, $P<0.05$). Follow up comparisons showed that the XX genotypes (mean = 0.19, SE = 0.05) had significantly lower cortisol than the R/- group (mean = 0.29, SE = 0.02), $P<0.05$. Combined, these findings suggest that while cortisol levels are higher in the R/- group, cortisol levels in response to a physical challenge are not influenced by ACTN3 genotype (Figure 3).

Perceived stress analyses did not show a significant assessment time (BL vs. post-BP) x genotype interaction ($F(1,106) = 1.85$, $P>0.05$). However, there was a main effect of assessment time ($F(1,106) = 95.20$, $P<0.05$). The post bench press stress measure (mean = 6.00, SE = 0.29) was significantly higher than the baseline measure (mean = 3.44, SE = 0.26), $P<0.05$). There was not a significant main effect for genotype ($F(1,106) = 0.88$, $P>0.05$). These findings indicate that the subjects perceived the bench press task to be stressful, independently of genotype (Figure 4).

**Figure 3. Cortisol Data.** Data expressed as mean ± SEM. There was a significant main effect for genotype; the R/- group had significantly higher salivary cortisol than the XX group ($P<0.05$) (a). This effect was not altered by the bench press task (b, c).
Figure 4. **Self-Reported Stress.** Data expressed as mean ± SEM. There was a significant main effect of perceived stress from baseline to the post bench press assessment (P<0.05) (a). There was no influence of ACTN3 genotype on perceived stress (b, c).

**Muscular Strength and Endurance**
There was not a significant genotype effect on muscular strength (assessed via the 1-RM bench press) or endurance (i.e., maximum number of repetitions performed at 60% of the bench press 1-RM) (Figures 5 and 6).

Figure 5. **Muscular Strength.** The solid horizontal line is the mean. Individual data points shown. No differences between groups. 1-RM = one-repetition maximum; kg = kilogram
Figure 6. Muscular Endurance. The solid horizontal line is the mean. Individual data points shown. No differences between groups. 1-RM = one-repetition maximum

Muscular Strength and Endurance – Relationship with Body Composition
There was a significant relationship between 1-RM bench press strength versus body weight, BMD, BMC, LBM, and % body fat but not for fat mass (Figures 7A-7E). There was a moderate relationship (correlation coefficient r) between % body fat versus bench press 1-RM (r=-0.59) and bone mineral density versus bench press 1-RM (r=0.59). There was a strong relationship (correlation) between body weight versus bench press 1-RM (r=0.79), lean body mass versus bench press 1-RM (r=0.84) and bone mineral content and bench press 1-RM (r=0.69). The correlation between muscular endurance and all body composition measures was weak (data not shown).
Figure 7B. Lean Body Mass vs. Strength.

Figure 7C. Percent Body Fat vs. Strength.
Figure 7D. Bone Mineral Content vs. Strength.

Figure 7E. Bone Mineral Density vs. Strength.
DISCUSSION

Body Composition
Our investigation included a wide variety of male and female competitive athletes as well as physically fit individuals who participated in regular resistance training. Twenty-one percent exhibited the XX mutation in the current investigation. This is similar to the work of Yang et al. (26). They reported a frequency of 18% in a subject pool that consisted of controls, sprint athletes, and endurance athletes. It is also similar to the findings of Broos et al. (5) who reported a frequency of 20% for the XX genotype in non-athletic young men (5). In the current study, body weight, LBM, fat mass, and % body fat did not significantly differ between the R/- and XX genotypes. However, there were significant differences in bone mineral content and bone mineral density. The present study is one of the few investigations to assess body composition in a cohort of competitive aerobic and strength-power athletes using the DXA. Thus, our finding of differences in BMC and BMD, though novel, must be further studied to determine if such differences are indeed real. Nonetheless, one investigation found that whole body and thigh non-skeletal lean mass was independent of the ACTN3 genotype in older Caucasian men (14).

In Rugby Union players, investigators found no significant relationship between genotypes or body composition (4). This investigation by Bell et al. (4) in Rugby players is the only study that has assessed body composition in trained athletes using a DXA. In elderly women, the RR genotype was associated with greater bone mineral content and density. But, when the RR and RX genotypes were combined, there was apparently no difference compared to the XX (15). The R/- genotype was no different than the XX in this group of elderly women (15). The greater BMC and BMD in the subjects in the current investigation cannot be explained by greater body weight or LBM inasmuch as no significant differences existed between the XX and R/- groups. One could suggest that despite the statistically significant differences, both groups had significantly higher bone mineral densities compared to the BMD of a normal healthy 30-yr old (i.e., T-Score). For instance, the R/- and XX groups exhibited a T-score average of 1.7 and 1.4, respectively. On average, the subjects in our investigation had bone mineral densities 1.4 to 1.7 standard deviations above the average (16). From a clinical standpoint, the BMD and BMC differences between the two groups are physiologically insignificant.

Stress Response
The stressor imposed on the subjects in the current study was a strength test (i.e., 1-RM bench press). Subsequently, the subjects performed the maximal number of repetitions on the bench press at a weight equal to 60% of the 1-RM. There were no differences in either strength or muscular endurance between the groups, thus the actual physical stress imposed was similar for both groups. The subjects in the present study exhibited similar physical fitness levels as assessed by the bench press test compared to the subjects in the Escalante et al. (10) study. Most of our female subjects exhibited bench press strength ~75 kg or less; whereas, our male subjects were typically over 75 kg with some exhibiting maximal strength as high as 175 kg. Our subjects were highly trained and were quite familiar with the exercise tests. What is interesting is that there was an overall genotype difference in salivary cortisol (R/- greater than XX), but no relationship between the physical stress task and genotype on cortisol levels.
A previous study of 37 professional soccer players showed that XX genotypes had lower cortisol levels after intense circuit training (20). This study did not look at baseline cortisol measures, so it is uncertain if the R allele carriers had higher levels of cortisol at baseline. In addition, the intensity of the exercise in this study was greater, involved cardiovascular activities, and was longer in duration (~45 min). Consequently, it would be interesting for future work to investigate the possibility that while R allele carriers have higher cortisol levels at rest, XX ACTN3 genotypes have higher levels after a high intensity workout. This is an intriguing possibility given that muscle damage is associated with increased cortisol levels (19).

The finding that there was no main effect change in cortisol pre- versus post-exercise is in contrast with prior work showing an acute elevation in cortisol after high-intensity interval training in healthy trained men (9), a 164-km road cycling event in highly trained cyclists (25), and following an exhaustive endurance exercise session (3). It is entirely possible that the bench press 1-RM and repetitions to failure task did not serve as a sufficient stressor to induce a rise in cortisol. Perhaps a higher volume of resistance-training exercises would be needed to elicit a significant hormonal response. It is clear that exhaustive endurance exercise can indeed promote a stress response vis a vis cortisol (3,25). Alternatively, cortisol can in fact decrease immediately after resistance exercise followed by a significant elevation 30 min later (22). Thus, it may be possible that waiting an additional 30 min could have resulted in a different (i.e., higher) result in terms of cortisol concentrations. Moreover, the self-reported stress (via a VAS) was not different between groups. When all genotypes were pooled, self-reported stress did indeed increase significantly. Nonetheless, this was not reflected in the salivary cortisol measures.

**ACTN3 and Strength/Endurance**

Our investigation found a statistically significant relationship between all measures of body composition (except fat mass) versus both muscular strength and endurance. However, the correlations tended to be quite weak in predicting muscular endurance. With regards to muscular strength, LBM (r = 0.84), body weight (r = 0.79), BMC (r = 0.69), BMD (r = 0.59), and % body fat (r = -0.59) demonstrated a fairly strong linear relationship. It would make sense that LBM would be the best predictor of skeletal muscle strength since large muscle cross-sectional areas can produce more force, in general, than smaller muscle cross-sectional areas. For instance, the size of the intrinsic hand muscles positively correlate with handgrip strength (1), and there is a strong relationship between forearm-ulna muscle thickness and handgrip strength (2). Trappe et al. (23) showed that calf muscle strength and size was positively correlated as well. Certainly, as LBM increases, body weight will also increase whereas % body fat will likely decrease. In fact, this may be why body weight and % body fat are so closely related to muscular strength while it is apparent that gains in fat mass weight alone are unrelated to gains to strength.

Other investigations have found a relationship between bone health and strength. Cipriani et al. (6) found a significant correlation between BMD and maximal voluntary contractions. Min et al. (15) is one of the few investigations to examine the role of the ACTN3 genotype and BMD and BMC. They reported that at least with the RR genotype (i.e., homozygous for the ACTN3 gene), their subjects tended to have higher BMD in the trunk, pelvis, and spine when compared to the RX genotype. However, they also showed no differences between the RR and RX versus XX genotype for segmental or total BMD. It is not clear why differences would exist between the RR and RX genotypes but not between the RR/RX and the XX genotypes.
Nonetheless, this investigation was done on elderly women, which is in contrast to the findings in the present study on highly trained athletes.

**CONCLUSIONS**

The findings in this study demonstrate that the presence of the ACTN3 577R allele does not confer a performance advantage with regards to muscle strength or endurance as assessed by the 1-RM bench press and repetitions to failure exercise tests. Additionally, there were no differences in the stress response to exercise as assessed by a VAS and salivary cortisol. No differences existed in body composition between the R/- and XX groups with the exception of bone mineral content and density. It is unclear why these differences exist, given that body weight and lean body mass were similar between groups. Furthermore, while we found a very strong relationship between lean body mass and muscular strength, none of the anthropometric data correlated well with muscular endurance. Based on the results from the present study, the use of ACTN3 gene as a marker for strength or muscular endurance is not warranted. However, it would be noteworthy to determine if this gene could serve as a useful tool for predicting bone mineral density.

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**REFERENCES**


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