

ACTN3 genotype and modulation of skeletal muscle response to exercise in human subjects

Barbara Norman,^{1,2} Mona Esbjörnsson,^{1,2} Håkan Rundqvist,^{1,2} Ted Österlund,^{1,2} Birgitta Glenmark,³ and Eva Jansson^{1,2}

¹Division of Clinical Physiology, Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden; ²Department of Clinical Physiology, Karolinska University Hospital, Stockholm, Sweden; and ³Department of Clinical Science and Education, Södersjukhuset, Karolinska Institutet, Stockholm, Sweden.

Submitted 7 May 2013; accepted in final form 9 March 2014

Norman B, Esbjörnsson M, Rundqvist H, Österlund T, Glenmark B, Jansson E. *ACTN3* genotype and modulation of skeletal muscle response to exercise in human subjects. *J Appl Physiol* 116: 1197–1203, 2014. First published March 20, 2014; doi:10.1152/japplphysiol.00557.2013.— α -Actinin-3 is a Z-disc protein expressed only in type II muscle fibers. A polymorphism in the *ACTN3* gene (R577X) results in lack of α -actinin-3 in XX genotype. The prevalence of the mutated X-allele is lower among power/sprint oriented athletes compared with controls, indicating that the lack of α -actinin-3 is detrimental in these sports, but a mechanistic link has not been established. Results from *Actn3*-knockout (KO) mouse model suggest that α -actinin-3 may affect muscle mass and muscle glycogen levels. In the present investigation we examined muscle fiber type composition, cross-sectional fiber area (CSA), and muscle glycogen levels at baseline in 143 human subjects with different *ACTN3* genotypes. In addition, hypertrophy signaling and glycogen utilization in response to sprint exercise were studied in a subset of subjects. Glycogen utilization was analyzed in separate pools of type I and type II fibers. No differences in fiber type composition, CSA, or muscle glycogen levels were observed at baseline across the *ACTN3* genotypes. However, the sprint exercise-induced increase in phosphorylation of mTOR and p70S6k was smaller in XX than in RR+RX ($P = 0.03$ and $P = 0.01$, respectively), indicating a less pronounced activation of hypertrophy signaling in XX. Glycogen utilization during sprint exercise varied across *ACTN3* genotypes in type II fibers ($P = 0.03$) but not in type I fibers ($P = 0.38$). The present results are in accordance with findings from the KO mice and reinforce the hypothesis that *ACTN3* genotype-associated differences in muscle mass and glycogen utilization provide a mechanistic explanation for the modulation of human performance by the *ACTN3* genotype.

ACTN3 genotype; exercise; hypertrophy signaling; glycogen; fiber types

α -ACTININ-3 AND α -ACTININ-2 ARE skeletal muscle specific proteins, that act as structural components of the Z-disc where they anchor thin actin filaments from adjacent sarcomeres. α -Actinin-2 is present in all muscle fiber types, whereas α -actinin-3 is confined to fast-twitch (type II) fibers exclusively (37, 50). In addition to their structural role, α -actinins also interact with skeletal muscle proteins with signaling and metabolic functions, and recent studies have indicated a specific role for the α -actinin-3 isoform in these interactions (42, 45, 51).

The α -actinin-3 protein is encoded by the *ACTN3* gene on chromosome 11 (6). A common polymorphism in this gene, R577X (rs1815739), results in a premature stop codon and lack

of α -actinin-3 protein in individuals who are homozygous for the *ACTN3* null allele (XX genotype) (37). This genotype is found in ~16–19% of the general populations of European and Asian origin, and is much less frequent in populations of African origin (53). Deficiency of the α -actinin-3 protein does not result in an evident muscle dysfunction, suggesting that the other isoform, α -actinin-2, largely compensates for its absence. This conclusion is reinforced by observations of overexpression of α -actinin-2 in association with absence of α -actinin-3 in mouse and human muscle (32, 36).

However, there are circumstances in which the presence of α -actinin-3 (RR or RX genotype) is of importance, as exemplified by differences in genotype frequencies between various groups of elite athlete cohorts compared with controls. In several independent studies, the frequency of the R allele has been found to be significantly higher in sprint/power-oriented athletes compared with controls, indicating that the presence of α -actinin-3 provides an advantage for these athletes (4, 29). A large study performed by Ahmetov et al. (2) showed that the presence of α -actinin-3 might also be of advantage for different groups of endurance-oriented athletes, when a power component is involved in their sport.

Although the mechanism underlying the association between the *ACTN3* genotype and athletic performance has not been elucidated, studies on *Actn3* knockout (KO) mice provide valuable information on how the absence of α -actinin-3 affects skeletal muscle properties. These studies have demonstrated that the KO mice have a lower muscle mass as a result of a significant decrease in the size of fast type IIB fibers, which are the fibers that in wild type (WT) mice exhibit the highest levels of expression of α -actinin-3 (31). It has also been reported that the KO mice have muscles with slower contractile properties and higher levels of oxidative enzymes as well as a higher endurance exercise capacity than do WT mice (31). Other characteristics of the KO mice are higher muscle glycogen content, reduced activity of glycogen phosphorylase, and higher glycogen synthase levels (42).

One explanation for the influence of the *ACTN3* genotype on performance may be the involvement of α -actinins in the regulation of muscle mass. An important function of the α -actinins in the Z-disc structure is to act as tension sensors (12, 22, 25). Mechanically induced tension plays an important role in the signaling events that mediate hypertrophy (24). Based on recent observations that a lack of the α -actinin-3 isoform alters the elastic properties of myofibrils (8, 45), it is conceivable that the sensing of mechanical stretch during muscle contraction may be affected by the *ACTN3* genotype. Such differences in mechanical sensing may lead to differences

Address for reprint requests and other correspondence: B. Norman, Clinical Physiology, Karolinska Univ. Hospital, Huddinge, 161 86 Stockholm, Sweden (e-mail: barbara.norman@ki.se).

in hypertrophy signaling and result in *ACTN3* genotype-associated differences in muscle mass.

Another potential explanation for the association between the *ACTN3* genotype and athletic performance is related to differences in fiber type composition between endurance-trained and sprint/power-trained athletes. The latter most often have a high percentage of fast-twitch muscle fibers, which are beneficial for optimal performance in these sports (1, 17, 47). It has been proposed that α -actinin-3 might, through an interaction with the signaling protein calcineurin, promote the formation of fast-twitch fibers (50). The possible involvement of α -actinin-3 in this process may explain the association between the RR-genotype and sprint/power athletic performance. However, in the *Actn3* KO mouse model, no changes in muscle fiber type composition, as defined by the myosin heavy chain isoform have been observed, despite a pronounced shift in the contractile characteristics of fast muscle fibers toward properties of slow oxidative fibers (31). Rather, the demonstrated difference between the *Actn3* KO and WT mice involves metabolic changes such as an increase in the activities of multiple enzymes in the muscles of KO mice, thus promoting oxidative metabolism (18, 31).

Furthermore, a study performed by Quinlan et al. (42) provided evidence that glycogen metabolism might be altered in association with *ACTN3* deficiency. That study showed that several enzymes involved in glycolytic metabolism are upregulated, glycogen phosphorylase (GPh) activity is markedly reduced, and muscle glycogen content is increased in the KO mice (42). On the basis of these findings the authors propose that alterations in GPh activity, in the absence of α -actinin-3, may be an important factor in the association between *ACTN3* genotype and human performance.

Studies of the association between *ACTN3* genotype and properties of skeletal muscle in humans, such as muscle mass, fiber type composition, and metabolic adaptations related to glycogen metabolism, which have been pinpointed as being associated with α -actinin-3 deficiency in the KO mouse model, are still quite scarce and have provided conflicting results (11, 27, 33, 36, 50, 52, 57). It has been estimated that the *ACTN3* polymorphism in humans accounts for only 2–3% of the total variance in baseline muscle performance (9, 34, 35). This implies that the potential *ACTN3* genotype-dependent differences in humans at baseline may be difficult to detect. However, there is evidence that the response to exercise may differ across *ACTN3* genotypes (9, 10, 40, 49). Accordingly, the aim of the present study was to examine the influence of the *ACTN3* genotype on fiber type composition, CSA, and glycogen con-

tent in human subjects at baseline and to examine hypertrophy signaling and glycogen utilization in response to sprint exercise. The hypothesis to be tested was that, although the differences across the three *ACTN3* genotypes at baseline are small and might be difficult to detect, the response to sprint exercise in individuals with the XX genotype is associated with less pronounced hypertrophy signaling and smaller glycogen utilization, thereby promoting adaptations that resemble those found in the *Actn3* KO mice.

MATERIALS AND METHODS

General design

The present investigation is based on retrospective analysis of three different studies performed at our laboratory. Selected muscle characteristics examined in each of these three studies are in the present investigation evaluated with regard to *ACTN3* genotype. *Study I*, is a cross-sectional study and represents a random selection of 27-year-old males and females from a general Swedish population (21). *Study II* (13) and *study III* (14) are experimental studies examining effects of sprint exercise in moderately to well-trained subjects. Muscle fiber dimensions, fiber type composition, and glycogen levels in muscle biopsy samples obtained at rest in these three studies were in the present investigation grouped into one cohort and analyzed with the aim to examine potential influence of the *ACTN3* genotype on these variables. In addition, data from *study II* was used to examine glycogen utilization during sprint exercise across *ACTN3* genotypes. In this unique study, glycogen content was analyzed in separate pools of type I and type II muscle fibers dissected from muscle biopsy samples obtained at rest and immediately after sprint exercise. Moreover, data from *study III* was used to examine muscle hypertrophy signaling in response to sprint exercise across *ACTN3* genotypes by analyzing muscle biopsy samples obtained at rest and 140 min following sprint exercise. The results from the original *study III* (14) are in the present investigation supplemented with data from five additional subjects studied according to the protocols used in *study III*.

Subjects

All subjects participating in the three studies were healthy, young Caucasian males and females. In total, 143 subjects were studied. Number of subjects participating in each study, their *ACTN3* genotype distribution, and the subject characteristics are presented in Table 1. Physical activity level during leisure time was estimated using a questionnaire. The subjects were engaged in various leisure-time sport activities, e.g., resistance exercise, individual or team ball sports, cycling, running, calisthenics, or aerobics. Number of minutes devoted to training per week was used as a measure of training status (Table 2). Subjects in *study I* represent a random selection of Swedish population and consisted of individuals with low as well as medium and high physical activity levels (for details see Ref. 21) and were in

Table 1. Anthropometric characteristics in males and females in studies I, II, and III, across *ACTN3* R577X genotypes

	Study I		Study II		Study III	
	men	women	men	women	men	women
<i>ACTN3</i> genotype distribution	XX = 10 RX = 28 RR = 17	XX = 7 RX = 9 RR = 12	XX = 0 RX = 9 RR = 11	XX = 3 RX = 8 RR = 7	XX = 5 RX = 7 RR = 1	XX = 2 RX = 4 RR = 3
<i>n</i>	55	28	20	18	13	9
Age, yr	27 ± 2	27 ± 2	23 ± 2	23 ± 2	28 ± 3	25 ± 2
Weight, kg	74 ± 9	61 ± 7	75 ± 8	65 ± 8	84 ± 7	67 ± 12
Height, cm	179 ± 6	168 ± 4	178 ± 7	168 ± 6	184 ± 5	171 ± 9
BMI, kg/m ²	23.2 ± 2	21.6 ± 2	23.3 ± 2	23.0 ± 2	24.7 ± 2	22.7 ± 2

Values are means ± SD.

Table 2. Training volume, fiber-type composition, and cross-sectional fiber area (CSA) across ACTN3 R577X genotypes

	Males				Females			
	all	RR	RX	XX	all	RR	RX	XX
<i>n</i>	83	29	39	15	55	22	21	12
Training volume, min/wk	334 ± 350	437 ± 508	289 ± 238	262 ± 181	289 ± 299	339 ± 357	256 ± 216	251 ± 315
Type I fibers, %	51.4 ± 13	52.8 ± 13	50.6 ± 13	50.7 ± 13	58.8 ± 11	60.4 ± 12	59.0 ± 10	55.4 ± 10
Type IIA fibers, %	32.0 ± 10	32.6 ± 9	31.8 ± 9	31.3 ± 12	29.2 ± 8	28.2 ± 7	28.9 ± 7	31.4 ± 10
Type IIB fibers, %	13.9 ± 10	12.4 ± 10	14.3 ± 9	15.5 ± 11	10.7 ± 7	10.5 ± 7	11.0 ± 8	10.8 ± 6
Type IIC fibers, %	2.8 ± 4	2.2 ± 4	3.3 ± 4	2.6 ± 3	1.2 ± 3	0.8 ± 2	1.1 ± 2	2.3 ± 5
<i>n</i>	67	26	32	9	43	17	17	9
CSA of type I fibers, μm^2	4,564 ± 1,695	4,349 ± 1,048	4,648 ± 2,198	4,888 ± 1,125	3,997 ± 1,056	4,183 ± 879	4,035 ± 1,031	3,574 ± 1,382
CSA of type IIA fibers, μm^2	5,170 ± 1,381	5,358 ± 1,386	4,934 ± 1,363	5,458 ± 1,450	3,656 ± 893	3,907 ± 893	3,506 ± 678	3,463 ± 1,191
CSA of type IIB fibers, μm^2	4,549 ± 1,539	4,660 ± 1,789	4,275 ± 1,405	5,263 ± 1,198	3,073 ± 1,101	3,395 ± 714	2,904 ± 1,270	2,766 ± 1,361
CSA of type IIC fibers, μm^2	4,663 ± 1,028	4,945 ± 534	4,465 ± 1,368	4,679 ± 845	3,234 ± 1,524	3,641 ± 2,555	3,094 ± 1,312	3,170 ± 1,712
Mean fiber area, μm^2	4,813 ± 1,436	4,772 ± 1,014	4,771 ± 1,774	5,086 ± 1,224	3,801 ± 954	4,026 ± 804	3,787 ± 922	3,402 ± 1,222

Values are means \pm SD. No significant differences across the ACTN3 R577X genotypes were detected in either males or females in the ANOVA. CSA was not determined in *study III* and is based on a subgroup of a total sample. Results from *studies I, II*, and *III* are combined and presented subdivided by sex and genotype. Mean fiber area was calculated from the formula: [(type I% + type IIC%) \times mean fiber area type I + type IIA% \times mean fiber area IIA + type IIB% \times mean fiber area of type IIB] $\times 100^{-1}$.

general less homogenous with respect to their training background than the subjects in the other two studies. The subjects in *study II* and *III* ranged from moderately to well-trained, yet were not at an elite or competitive athletic level. All subjects were informed about the experimental procedures, including the retrospectively performed genotyping, and gave their consent to participate. The studies were approved by the Regional Ethical Review Board in Stockholm, Sweden.

Exercise protocols

In *study II*, subjects performed one bout of 30-s sprint exercise (Wingate test) on a mechanically braked cycle ergometer (Cardionics, Bromma, Sweden), and in *study III* the subjects performed three bouts of the 30-s cycle sprints with a 20-min rest between the bouts. The cycle sprints were performed at maximal propelling speed against a resistance of 7.5% of the subject's body mass (5).

Muscle biopsies

Sampling procedure. Muscle samples were obtained from the middle portion of the vastus lateralis muscle using percutaneous needle biopsy technique (7). In *study I* the muscle samples were obtained only at rest. In *study II* and *study III* the samples were obtained at rest and following sprint exercise. The first biopsy sample was obtained randomly in either right or left leg prior to the first sprint. The second muscle sample was obtained from the opposite leg. In *study II* the postexercise biopsies were performed immediately after the 30-s Wingate cycling, and in *study III* the postexercise muscle samples were taken 140 min after the third sprint. The obtained muscle samples were divided into smaller parts for different analyses. A part of muscle sample, intended for histochemical analysis, was frozen in isopentane and cooled to its freezing point by liquid nitrogen. The muscle samples intended for other analysis were immediately frozen in liquid nitrogen. All samples were stored at -80°C until processed.

Histochemical and morphological analysis. Part of the muscle samples obtained at rest in *studies I, II*, and *III* were freeze-sectioned into 10- μm serial transverse sections and analyzed histochemically for fiber types (I, IIA, IIB, and IIC) with a myofibrillar ATPase stain (43). Myosin heavy-chains characteristics, allowing for the denomination of type IIX or IID fibers, the equivalent of the type IIB fibers as assessed by means of the ATPase stain, were not determined. CSA was measured morphologically by planimetry from an NADH-dehydrogenase stained section (38). The relative number of different fiber types, the CSA, and the mean fiber area for the different fiber types for each individual were calculated (for details see Ref. 21).

Single muscle fibers preparation procedures. In *study II* muscle samples were freeze-dried and used for dissection of single fibers. Approximately 100 single-fiber fragments were dissected from each biopsy sample. These were classified histochemically as fiber type I or II (15) and thereafter divided into separate pools of type I and type II. The mean weight of the pools was 40 μg (range 15–75 μg).

Quantitative glycogen analysis. Muscle samples were freeze-dried and dissected free from visible blood and connective tissue and homogenized by hand in a glass homogenizer in 0.1 M phosphate buffer, pH 7.7 with 0.5% BSA. For the determination of glycogen content in *study I* aliquots of the crude muscle homogenate were boiled in 1.5 M HCl for 1 h to break down glycogen to glycosyl units. In *study II* the fiber pools used for glycogen analysis were digested by adding 20 μl of 1M KOH and vigorously mixing and warming the samples for 15 min at 50°C , after which amyloglycosidase was added to break down glycogen to glycosyl units (23). The extracts were neutralized by addition of 0.25 M HCl. Glycogen content was thereafter measured in both types of extracts by analyzing glucose using a fluorometric enzymatic method (28).

Western blot. In *study III*, the muscle samples obtained at rest and 140 min following the last of three 30-s Wingate cycling bouts were used for Western blot analysis to study the phosphorylation of Akt^{Ser473}, mTOR^{Ser2448}, p70S6k^{Thr389}, rpS6^{Ser355/236}, and AMPK^{Thr172}. The freeze-dried muscles samples were homogenized in an ice cold lysis buffer using a glass homogenizer. The protein content was determined by a Bio-Rad Bradford protein assay and diluted to a final concentration of 2 $\mu\text{g}/\mu\text{l}$ containing 25% Laemmli buffer. An SDS-PAGE (Criterion precast, 4–20% gradient gels; BioRad) was loaded with 30 μg of protein in each well and was run for 72 min at 200 V. Subsequently, the proteins were transferred to a PVDF membrane during 3 h in ice cold buffer, blocked in TBS containing 5% milk for 1 h at room temperature, and incubated overnight at 4°C in specific primary antibodies purchased from Cell Signaling Technology (Boston, MA): p-Akt^{Ser473} (1:1,000), p-mTOR^{Ser2448} (1:500), p-p70S6k^{Thr389} (1:1,000), p-rpS6^{Ser355/236} (1:1,000), p-AMPK^{Thr172} (1:1,000). The following day the membranes were washed in TBST, incubated in secondary antibody (1:5,000) for 1 h at room temperature, further washed, and finally visualized using enhanced chemiluminescence reagents (Pierce Supersignaling west femto sensitivity substrate, Thermo Fisher Sci, Rockford, IL) and a Molecular Imager ChemiDocTM XRS system. Quantity One version 4.6.3 software (Bio-Rad Laboratories) was used to quantify the resulting blots. For detailed description of the Western blot procedures see Ref. 14.

Genotyping

Genomic DNA was extracted from peripheral blood using the QIAamp DNA extraction kit (Qiagen), from muscle samples by TaqMan Sample-to-snp kit (Applied Biosystems), or from saliva using a self-collection kit from Oragene (DNA Genotek, Ottawa, Ontario, Canada). Genotyping for the identification of the R577X mutation in the *ACTN3* gene was performed by an allelic discrimination assay with fluorogenic probes (Perkin-Elmer ABI Prisma 7700 Sequence Detection system, Applied Biosystems). The primer sequences were ACGATCAGTTCAAGGCAACACT (forward) and ACCCTGGATGCCCCATGATG (reverse), and the TaqMan probe sequences were TCGCTCTCGGTCAGC and CGCTCTCAGTCAGC (polymorphism in bold). The probes were labeled with fluorescent dyes VIC and FAM, respectively.

Statistics

Results are presented as mean \pm SD. Factorial ANOVA with *ACTN3* genotype as independent variable and fiber type composition, CSA and basal glycogen levels as dependent variables was employed to evaluate the potential differences between the *ACTN3* genotypes. ANOVA analysis of fiber type composition and CSA were performed separately for males and females. Multiple regression analysis was applied to evaluate the contribution of *ACTN3* genotype, sex, and training volume as predictors of fiber type composition and CSA. Coefficient of determination (R^2) indicating the percentage of the variance explained by each predictor and the P value is stated in the result section. Due to limited number of subjects with XX genotype in study II, changes in glycogen content in pools of type I or type II fibers across *ACTN3* genotypes were statistically analyzed by Kruskal-Wallis nonparametric test.

RESULTS

Fiber type composition and muscle fiber area

The fiber type composition and the CSA across the *ACTN3* genotypes according to sex are presented in Table 2. No significant differences in the percentage of type I, type IIA, or type IIB fibers were observed across the *ACTN3* genotypes in either males or females (ANOVA, $P > 0.05$). Multiple regression analyses showed that training volume ($P < 0.001$, $R^2 = 0.15$) and sex ($P < 0.001$, $R^2 = 0.10$), but not *ACTN3* genotype ($P = 0.57$, $R^2 = 0.02$) significantly explained the percentage of type I fibers. The percentage of type IIA fibers was dependent on training volume ($P = 0.04$, $R^2 = 0.03$) but was independent of sex ($P = 0.09$, $R^2 = 0.02$) or genotype ($P = 0.68$, $R^2 = 0.01$). Moreover, the percentage of type IIB fibers was significantly explained by training volume ($P < 0.001$, $R^2 = 0.10$) and sex ($P = 0.02$, $R^2 = 0.04$); however, there was no significant contribution of genotype ($P = 0.63$, $R^2 = 0.01$). Finally, the percentage of type IIC fiber was explained by training volume ($P = 0.06$, $R^2 = 0.03$) and sex ($P = 0.01$, $R^2 = 0.05$), with no significant contribution of genotype ($P = 0.52$, $R^2 = 0.02$). Similarly, the CSA of type I, type IIA, type IIB nor type IIC fibers did not differ significantly between the *ACTN3* genotypes in either males or females (ANOVA, $P > 0.05$). A multiple regression analysis showed that the CSA of type I, type IIA, type IIB, and type IIC was significantly explained by sex, whereas the contributions of training volume and genotype were not significant.

Hypertrophy signaling

The activation of the Akt/mTOR signaling pathway, 140 min after three bouts of Wingate cycling, was analyzed in study

III. The phosphorylation of mTOR, p70S6k, rpS6, and Akt was significantly higher after exercise than it was at rest. The relative exercise-induced increase in the phosphorylation of mTOR and p70S6k was significantly smaller in the XX than it was in the RR+RX genotype (ANOVA, $P = 0.03$ and $P = 0.01$, respectively), whereas no significant difference was observed regarding the increase in rpS6 and Akt across genotypes (Fig. 1). AMPK phosphorylation levels 140 min after exercise were not different from the phosphorylation levels observed at rest (data not shown).

Glycogen

Glycogen content in biopsy samples obtained at rest did not differ across the *ACTN3* genotypes (ANOVA, $P > 0.05$). The resting glycogen levels were (mean \pm SD, mmol glycosyl units per kg dry muscle) 641 ± 203 in the RR ($n = 44$), 599 ± 174 in the RX ($n = 50$), and 597 ± 224 in the XX ($n = 18$) genotypes.

In study II, the breakdown of glycogen during exercise was studied by analyzing glycogen content before and immediately after 30 s of Wingate cycling. In this unique study the glycogen level was analyzed in dissected pools of type I and type II fibers. The decrease in glycogen levels with 30-s Wingate cycling in type I and type II fibers, subdivided according to the *ACTN3* genotype, is presented in Fig. 2. The decrease in glycogen content during exercise was significantly different across the *ACTN3* genotypes in type II fibers (Kruskal-Wallis test $P = 0.03$) but not in type I fibers (Kruskal-Wallis test $P = 0.38$).

DISCUSSION

The results of the present investigation supported our hypothesis that the *ACTN3* genotype modulates response to exercise, although no differences across the genotypes were detected at baseline. We showed here that the lack of α -actinin-3 in individuals with the XX genotype was associated with less pronounced activation of the signaling events that mediate muscle hypertrophy, which is in agreement with the finding of a decreased size of fast type IIB fibers in the KO mice (31). In addition, our results indicate that the lack of α -actinin-3 in individuals with the XX genotype may constrain glycogen utilization during sprint exercise, thereby promoting the maintenance of high glycogen levels, which is also in

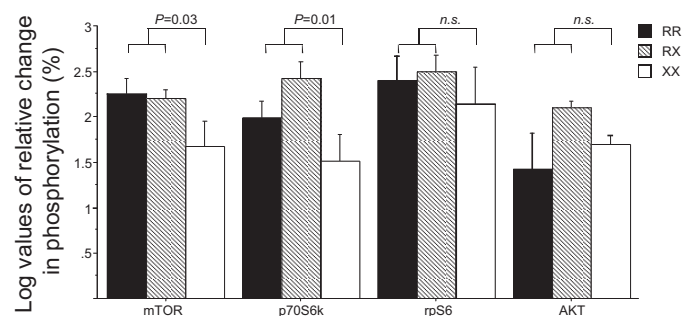


Fig. 1. Percentage change in the phosphorylation of Akt^{Ser473}, mTOR^{Ser2448}, p70S6k^{Thr389}, and rpS6^{Ser335/236} from baseline to 140 min after sprint exercise in different *ACTN3* genotypes (study III); RR ($n = 4$) (dark bars), RX ($n = 7$) (striped bars), and XX ($n = 7$) (white bars). Values are the log of the mean \pm SE. P values indicate the level of statistical significance in ANOVA when pooled values of RR and RX are compared with XX.

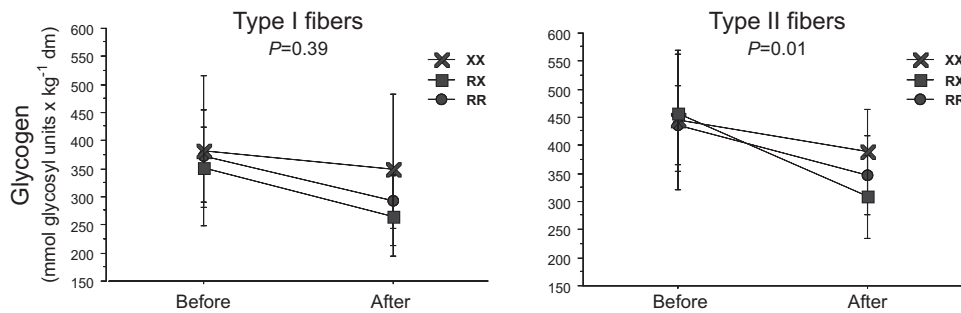


Fig. 2. Glycogen content before and immediately after 30 s of Wingate cycling in pooled type I and type II muscle fibers across different *ACTN3* genotypes (study II); RR ($n = 18$), RX ($n = 17$), and XX ($n = 3$). Values are mean \pm SD. P values indicate the level of statistical significance for the comparison of the change in glycogen levels (delta value) across genotypes (Kruskal-Wallis test).

line with the higher glycogen content observed in the KO mice (42).

Reduced muscle mass has been observed in the KO mice in muscles that normally express α -actinin-3 (31, 44). In humans, lower muscle mass has been observed in older women with α -actinin-3 deficiency (48, 57, 58). A possible mechanism underlying the effect of α -actinin-3 on elite athlete performance may be its potential influence on muscle mass. Mechanically induced tension plays an important role in the signaling events that mediate hypertrophy (24). The findings that α -actinins are involved in sensing tension during muscle contraction and link it with signaling mechanisms (6, 12, 41) may be an explanation of how the lack of α -actinin-3 contributes to variations in muscle mass. The α -actinins form several different complexes at the Z-disc, one of which is a putative mechanosensing complex, in which the α -actinins interact with titin/Tcap/muscle LIM protein (22, 26). Factors that affect the integrity of the complex in response to stretch have been suggested to influence the signaling response (19, 20). The two α -actinin isoforms, α -actinin-2 and α -actinin-3, differ in their interaction with titin (45) and how they affect muscle fiber elasticity (8). Thus it is conceivable that the *ACTN3* genotype impact the stability of the mechanosensing complex, thereby affecting the signaling pathways that lead to hypertrophy. The mTOR pathway is an important signaling pathway in the regulation of muscle mass. Recent studies have provided evidence that mTOR signaling is activated through the enzyme phospholipase D and suggested a model in which mechanical stimuli induce an increase in phosphatidic acid which binds to and activates mTOR (for references see Ref. 24). Interestingly, the interaction between titin and α -actinin is regulated by phospholipids (56). The results of the present study, which showed lower activation of the mTOR signaling pathway in the XX compared with the RR/RX genotype, support our hypothesis that hypertrophy signaling is influenced by the *ACTN3* genotype. This conclusion is also reinforced by the fact that no *ACTN3* genotype-associated differences in Akt phosphorylation were observed in the present study, as it has been shown that activation of the mTOR signaling pathway mediated by mechanical sensing may act independently of Akt/PI3K activation (55). Based on the reasoning presented above, the results of this study support the hypothesis that, in response to exercise, presence of α -actinin-3 promotes the hypertrophy of skeletal muscle.

Furthermore, the findings from *Actn3* KO mice demonstrate that the lack of α -actinin-3 is associated with reduced activity of glycogen phosphorylase and increased activity of glycogen synthase, as well as higher muscle glycogen content (42). The

authors propose that these changes lead to a decreased capacity of muscle to use glycogen as a fuel. However, in agreement with the results of a study by Vincent et al. (52), the present investigation of human subjects did not confirm differences in basal glycogen levels across *ACTN3* genotypes. Nonetheless, our finding that the *ACTN3* genotype had an influence on the sprint exercise-induced decrease in glycogen levels in type II fibers which express α -actinin-3, but not in type I fibers (study II), speaks in favor of the suggested association between the *ACTN3* genotype and glycogen utilization.

Finally, the current data suggest that the *ACTN3* genotype does not play a significant role in determining muscle fiber type composition or fiber dimensions at baseline in moderately to well-trained subjects. Using our relatively large cohort consisting of males and females with variable training backgrounds, we showed that training status and sex, but not *ACTN3* genotype, had a significant effect on fiber type composition. The present results were consistent with the results of an earlier study from our laboratory, which demonstrated that fiber type composition is independent of *ACTN3*-genotype (36). Our results were, however, in contrast with those of a study by Ahmetov et al. (3) who reported a significantly higher proportion of type I fibers in subjects with the XX genotype. The cohort investigated by Ahmetov et al. consisted of a group of physically active males merged with a group of male and female athletes. Our results were also in contrast with those of a study by Vincent et al. (50) who reported that the percentage surface area occupied by type IIX fibers and the number of type IIX fibers (which are equivalent to type IIB fibers) were greater in individuals with the RR- compared with those with the XX genotype. However, a subsequent study by Vincent et al. (52) did not confirm their previous findings of an association between *ACTN3* and fiber type proportions. The potential effect of *ACTN3* genotype on fiber type composition is probably small and the discrepancies observed between studies may depend on the extent to which other factors known to influence fiber type composition, such as training background and sex, varied within each study. Nonetheless, studies that used the KO mouse model suggest that adaptation of the α -actinin-3 deficient muscle occurs via signaling pathways that are involved in the regulation of the size and metabolic properties of muscle fibers, rather than their myosin heavy chain composition (18, 31).

In summary, reports from several independent studies indicate that the presence of the α -actinin-3 protein provides an advantage for power- and strength-oriented athletes (4, 16, 30, 39, 54). The mechanism via which the *ACTN3* genotype may influence performance is unclear and only a few studies aimed

at elucidating this question at the cellular level in humans have been published (8, 46, 51, 52). To our knowledge, the present study is the first that demonstrated *ACTN3* genotype-associated differences in the activation of the mTOR signaling pathway in human skeletal muscle. The present data showing the effects of *ACTN3* genotype on glycogen utilization during sprint exercise only in type II fibers, which normally express α -actinin-3, and not in type I fibers, are also novel, but should be considered as preliminary because of the limited number of subjects with XX genotype that were identified and could be included in the analyses. In conclusion, the present findings, reinforce the proposal based on findings from the KO mouse model that *ACTN3* genotype-associated differences in hypertrophy signaling and glycogen handling, provide a mechanistic explanation of how *ACTN3* genotype may influence human physical performance (31, 42).

GRANTS

This study was supported by grants from the Swedish National Center for Research in Sports, and by the Magnus Bergvall Foundation.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: B.N. and E.J. conception and design of research; B.N., M.E., H.C.R., T.x., B.G., and E.J. performed experiments; B.N. and M.E. analyzed data; B.N. and E.J. interpreted results of experiments; B.N. and M.E. prepared figures; B.N., M.E., H.C.R., T.x., and E.J. drafted manuscript; B.N., M.E., H.C.R., and E.J. edited and revised manuscript; B.N., M.E., H.C.R., T.x., B.G., and E.J. approved final version of manuscript.

REFERENCES

- Aagaard P, Andersen JL. Correlation between contractile strength and myosin heavy chain isoform composition in human skeletal muscle. *Med Sci Sports Exerc* 30: 1217–1222, 1998.
- Ahmetov II, Druzhevskaya AM, Astratenkova IV, Popov DV, Vinogradova OL, Rogozkin VA. The ACTN3 R577X polymorphism in Russian endurance athletes. *Br J Sports Med* 2008.
- Ahmetov II, Druzhevskaya AM, Lyubaeva EV, Popov DV, Vinogradova OL, Williams AG. The dependence of preferred competitive racing distance on muscle fibre type composition and ACTN3 genotype in speed skaters. *Exp Physiol* 96: 1302–1310, 2011.
- Alfred T, Ben-Shlomo Y, Cooper R, Hardy R, Cooper C, Deary IJ, Gunnell D, Harris SE, Kumari M, Martin RM, Moran CN, Pitsiladis YP, Ring SM, Sayer AA, Smith GD, Starr JM, Kuh D, Day IN. ACTN3 genotype, athletic status, and life course physical capability: meta-analysis of the published literature and findings from nine studies. *Hum Mutat* 32: 1008–1018, 2011.
- Bar-Or O. The Wingate anaerobic test. An update on methodology, reliability and validity. *Sports Med* 4: 381–394, 1987.
- Beggs AH, Byers TJ, Knoll JH, Boyce FM, Bruns GA, Kunkel LM. Cloning and characterization of two human skeletal muscle alpha-actinin genes located on chromosomes 1 and 11. *J Biol Chem* 267: 9281–9288, 1992.
- Bergström J. Muscle electrolytes in man. *Scand J Clin Lab Invest Suppl* 68: 1–100, 1962.
- Broos S, Malisoux L, Theisen D, Francaux M, Deldicque L, Thomis MA. Role of alpha-actinin-3 in contractile properties of human single muscle fibers: a case series study in paraplegics. *PloS One* 7: e49281, 2012.
- Clarkson PM, Devaney JM, Gordish-Dressman H, Thompson PD, Hubal MJ, Urso M, Price TB, Angelopoulos TJ, Gordon PM, Moyna NM, Pescatello LS, Visich PS, Zoeller RF, Seip RL, Hoffman EP. ACTN3 genotype is associated with increases in muscle strength in response to resistance training in women. *J Appl Physiol* 99: 154–163, 2005.
- Delmonico MJ, Kostek MC, Doldo NA, Hand BD, Walsh S, Conway JM, Carignan CR, Roth SM, Hurley BF. Alpha-actinin-3 (ACTN3) R577X polymorphism influences knee extensor peak power response to strength training in older men and women. *J Gerontol A Biol Sci Med Sci* 62: 206–212, 2007.
- Delmonico MJ, Zmuda JM, Taylor BC, Cauley JA, Harris TB, Manini TM, Schwartz A, Li R, Roth SM, Hurley BF, Bauer DC, Ferrell RE, Newman AB, Health ABC, MrOS Research Groups. Association of the ACTN3 genotype and physical functioning with age in older adults. *J Gerontol A Biol Sci Med Sci* 63: 1227–1234, 2008.
- Epstein ND, Davis JS. Sensing stretch is fundamental. *Cell* 112: 147–150, 2003.
- Eshjornsson-Liljedahl M, Sundberg CJ, Norman B, Jansson E. Metabolic response in type I and type II muscle fibers during a 30-s cycle sprint in men and women. *J Appl Physiol* 87: 1326–1332, 1999.
- Eshjornsson M, Rundqvist HC, Mascher H, Osterlund T, Rooyackers O, Blomstrand E, Jansson E. Sprint exercise enhances skeletal muscle p70S6k phosphorylation and more so in females than in males. *Acta Physiol (Oxf)* 205: 411–422, 2011.
- Essen B, Jansson E, Henriksson J, Taylor AW, Saltin B. Metabolic characteristics of fibre types in human skeletal muscle. *Acta Physiol Scand* 95: 153–165, 1975.
- Eynon N, Duarte JA, Oliveira J, Sagiv M, Yamin C, Meckel Y, Goldhammer E. ACTN3 R577X polymorphism and Israeli top-level athletes. *Int J Sports Med* 30: 695–698, 2009.
- Fry AC, Schilling BK, Staron RS, Hagerman FC, Hikida RS, Thrush JT. Muscle fiber characteristics and performance correlates of male Olympic-style weightlifters. *J Strength Cond Res* 17: 746–754, 2003.
- Garton FC, Seto JT, Quinlan KG, Yang N, Houweling PJ, North KN. α -Actinin-3 deficiency alters muscle adaptation in response to denervation and immobilization. *Hum Mol Genet* 2013.
- Gehrmlich K, Geier C, Osterziel KJ, Van der Ven PF, Furst DO. Decreased interactions of mutant muscle LIM protein (MLP) with N-RAP and alpha-actinin and their implication for hypertrophic cardiomyopathy. *Cell Tissue Res* 317: 129–136, 2004.
- Geier C, Perrot A, Ozelik C, Binner P, Counsell D, Hoffmann K, Pilz B, Martiniak Y, Gehrmlich K, van der Ven PF, Furst DO, Vornwald A, von Hodenberg E, Nurnberg P, Scheffold T, Dietz R, Osterziel KJ. Mutations in the human muscle LIM protein gene in families with hypertrophic cardiomyopathy. *Circulation* 107: 1390–1395, 2003.
- Glenmark B, Hedberg G, Jansson E. Changes in muscle fibre type from adolescence to adulthood in women and men. *Acta Physiol Scand* 146: 251–259, 1992.
- Hampton CM, Taylor DW, Taylor KA. Novel structures for alpha-actinin: F-actin interactions and their implications for actin-membrane attachment and tension sensing in the cytoskeleton. *J Mol Biol* 368: 92–104, 2007.
- Harris RC, Essen B, Hultman E. Glycogen phosphorylase activity in biopsy samples and single muscle fibres of musculus quadriceps femoris of man at rest. *Scand J Clin Lab Invest* 36: 521–526, 1976.
- Hornberger TA. Mechanotransduction and the regulation of mTORC1 signaling in skeletal muscle. *Int J Biochem Cell Biol* 43: 1267–1276, 2011.
- Knoll R, Buyandelger B, Lab M. The sarcomeric Z-disc and Z-discopathies. *J Biomed Biotechnol* 2011: 569628, 2011.
- Knoll R, Hoshijima M, Hoffman HM, Person V, Lorenzen-Schmidt I, Bang ML, Hayashi T, Shiga N, Yasukawa H, Schaper W, McKenna W, Yokoyama M, Schork NJ, Omens JH, McCulloch AD, Kimura A, Gregorio CC, Poller W, Schaper J, Schultheiss HP, Chien KR. The cardiac mechanical stretch sensor machinery involves a Z disc complex that is defective in a subset of human dilated cardiomyopathy. *Cell* 111: 943–955, 2002.
- Lima RM, Leite TK, Pereira RW, Rabelo HT, Roth SM, Oliveira RJ. ACE and ACTN3 genotypes in older women: muscular phenotypes. *Int J Sports Med* 32: 66–72, 2011.
- Lowry OH, Passonneau JV. A flexible system of enzymatic analysis. New York: Academic Press, 1972.
- Ma F, Yang Y, Li X, Zhou F, Gao C, Li M, Gao L. The association of sport performance with ACE and ACTN3 genetic polymorphisms: a systematic review and meta-analysis. *PloS One* 8: e54685, 2013.
- MacArthur DG, North KN. A gene for speed? The evolution and function of alpha-actinin-3. *Bioessays* 26: 786–795, 2004.
- MacArthur DG, Seto JT, Chan S, Quinlan KG, Raftery JM, Turner N, Nicholson MD, Kee AJ, Hardeman EC, Gunning PW, Cooney GJ, Head SI, Yang N, North KN. An Actn3 knockout mouse provides

- mechanistic insights into the association between alpha-actinin-3 deficiency and human athletic performance. *Hum Mol Genet* 17: 1076–1086, 2008.
32. Macarthur DG, Seto JT, Raftery JM, Quinlan KG, Huttley GA, Hook JW, Lemckert FA, Kee AJ, Edwards MR, Berman Y, Hardeman EC, Gunning PW, Eastal S, Yang N, North KN. Loss of ACTN3 gene function alters mouse muscle metabolism and shows evidence of positive selection in humans. *Nat Genet* 39: 1261–1265, 2007.
 33. McCauley T, Mastana SS, Folland JP. ACE I/D and ACTN3 R/X polymorphisms and muscle function and muscularity of older Caucasian men. *Eur J Appl Physiol* 109: 269–277, 2010.
 34. Moran CN, Yang N, Bailey ME, Tsiokanos A, Jamurtas A, MacArthur DG, North K, Pitsiladis YP, Wilson RH. Association analysis of the ACTN3 R577X polymorphism and complex quantitative body composition and performance phenotypes in adolescent Greeks. *Eur J Hum Genet* 15: 88–93, 2007.
 35. Niemi AK, Majamaa K. Mitochondrial DNA and ACTN3 genotypes in Finnish elite endurance and sprint athletes. *Eur J Hum Genet* 13: 965–969, 2005.
 36. Norman B, Esbjornsson M, Rundqvist H, Osterlund T, von Walden F, Tesch PA. Strength, power, fiber types, and mRNA expression in trained men and women with different ACTN3 R577X genotypes. *J Appl Physiol* 106: 959–965, 2009.
 37. North KN, Yang N, Wattanasirichaigoon D, Mills M, Eastal S, Beggs AH. A common nonsense mutation results in alpha-actinin-3 deficiency in the general population. *Nat Genet* 21: 353–354, 1999.
 38. Novikoff AB, Shin WY, Drucker J. Mitochondrial localization of oxidative enzymes: staining results with two tetrazolium salts. *J Biophys Biochem Cytol* 9: 47–61, 1961.
 39. Papadimitriou ID, Papadopoulos C, Kouvatsi A, Triantaphyllidis C. The ACTN3 gene in elite greek track and field athletes. *Int J Sports Med* 2007.
 40. Pereira A, Costa AM, Izquierdo M, Silva AJ, Bastos E, Marques MC. ACE I/D and ACTN3 R/X polymorphisms as potential factors in modulating exercise-related phenotypes in older women in response to a muscle power training stimuli. *Age* 35: 1949–1959, 2012.
 41. Pyle WG, Solaro RJ. At the crossroads of myocardial signaling: the role of Z-disks in intracellular signaling and cardiac function. *Circ Res* 94: 296–305, 2004.
 42. Quinlan KG, Seto JT, Turner N, Vandebrouck A, Floetenmeyer M, Macarthur DG, Raftery JM, Lek M, Yang N, Parton RG, Cooney GJ, North KN. Alpha-actinin-3 deficiency results in reduced glycogen phosphorylase activity and altered calcium handling in skeletal muscle. *Hum Mol Genet* 19: 1335–1346, 2010.
 43. Schantz P, Billeter R, Henriksson J, Jansson E. Training-induced increase in myofibrillar ATPase intermediate fibers in human skeletal muscle. *Muscle Nerve* 5: 628–636, 1982.
 44. Seto JT, Chan S, Turner N, MacArthur DG, Raftery JM, Berman YD, Quinlan KG, Cooney GJ, Head S, Yang N, North KN. The effect of alpha-actinin-3 deficiency on muscle aging. *Exp Gerontol* 46: 292–302, 2011.
 45. Seto JT, Lek M, Quinlan KG, Houweling PJ, Zheng XF, Garton F, MacArthur DG, Raftery JM, Garvey SM, Hauser MA, Yang N, Head SI, North KN. Deficiency of alpha-actinin-3 is associated with increased susceptibility to contraction-induced damage and skeletal muscle remodeling. *Hum Mol Genet* 20: 2914–2927, 2011.
 46. Seto JT, Quinlan KG, Lek M, Zheng XF, Garton F, Macarthur DG, Hogarth MW, Houweling PJ, Gregorevic P, Turner N, Cooney GJ, Yang N, North KN. ACTN3 genotype influences muscle performance through the regulation of calcineurin signaling. *J Clin Invest* 123: 4255–4263, 2013.
 47. Tesch PA, Thorsson A, Kaiser P. Muscle capillary supply and fiber type characteristics in weight and power lifters. *J Appl Physiol* 56: 35–38, 1984.
 48. Walsh S, Liu D, Metter EJ, Ferrucci L, Roth SM. ACTN3 genotype is associated with muscle phenotypes in women across the adult age span. *J Appl Physiol* 105: 1486–1491, 2008.
 49. Venckunas T, Skurvydas A, Brazaitis M, Kamandulis S, Snieckus A, Moran CN. Human alpha-actinin-3 genotype association with exercise-induced muscle damage and the repeated-bout effect. *Appl Physiol Nutr Metab* 37: 1038–1046, 2012.
 50. Vincent B, De Bock K, Ramaekers M, Van den Eede E, Van Leemputte M, Hespel PJ, Thomis M. The ACTN3 (R577X) genotype is associated with fiber type distribution. *Physiol Genomics* 32: 58–63, 2007.
 51. Vincent B, Windelinckx A, Nielsens H, Ramaekers M, Van Leemputte M, Hespel P, Thomis MA. Protective role of alpha-actinin-3 in the response to an acute eccentric exercise bout. *J Appl Physiol* 109: 564–573, 2010.
 52. Vincent B, Windelinckx A, Van Proeyen K, Masschelein E, Nielsens H, Ramaekers M, Van Leemputte M, Hespel P, Thomis M. Alpha-actinin-3 deficiency does not significantly alter oxidative enzyme activity in fast human muscle fibres. *Acta Physiol (Oxf)* 204: 555–561, 2012.
 53. Yang N, Garton F, North alpha-Actinin-3 Performance K. *Med Sport Sci* 54: 88–101, 2009.
 54. Yang N, MacArthur DG, Gulbin JP, Hahn AG, Beggs AH, Eastal S, North K. ACTN3 genotype is associated with human elite athletic performance. *Am J Hum Genet* 73: 627–631, 2003.
 55. You JS, Frey JW, Hornberger TA. Mechanical stimulation induces mTOR signaling via an ERK-independent mechanism: implications for a direct activation of mTOR by phosphatidic acid. *PloS One* 7: e47258, 2012.
 56. Young P, Gautel M. The interaction of titin and alpha-actinin is controlled by a phospholipid-regulated intramolecular pseudoligand mechanism. *EMBO J* 19: 6331–6340, 2000.
 57. Zempo H, Tanabe K, Murakami H, Iemitsu M, Maeda S, Kuno S. ACTN3 polymorphism affects thigh muscle area. *Int J Sports Med* 31: 138–142, 2010.
 58. Zempo H, Tanabe K, Murakami H, Iemitsu M, Maeda S, Kuno S. Age differences in the relation between ACTN3 R577X polymorphism and thigh muscle cross-sectional area in women. *Genet Test Mol Biomarkers* 15: 639–643, 2011.