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

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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

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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 content 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
ACTN3 genotype distribution	XX = 10	XX = 7	XX = 0	XX = 3	XX = 5	XX = 2
	RX = 28	RX = 9	RX = 9	RX = 8	RX = 7	RX = 4
	RR = 17	RR = 12	RR = 11	RR = 7	RR = 1	RR = 3
n	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
n	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
n	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 ± 1031	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 ±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%) × mean fiber area type I + type IIA% × mean fiber area IIA + type IIB% × mean fiber area of type IIB)] × 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 μg/μl 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-$rpS6^{Ser235/236}$ (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 ACCCTGGATGCCCATGATG (reverse), and the TaqMan probe sequences were TCGCTCTC**G**GTCAGC and CGCTCTC**A**GTCAGC (polymorphism in bold). The probes were labeled with fluorescent dyes VIC and FAM, respectively.

Statistics

Results are presented as mean ± 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 ± 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

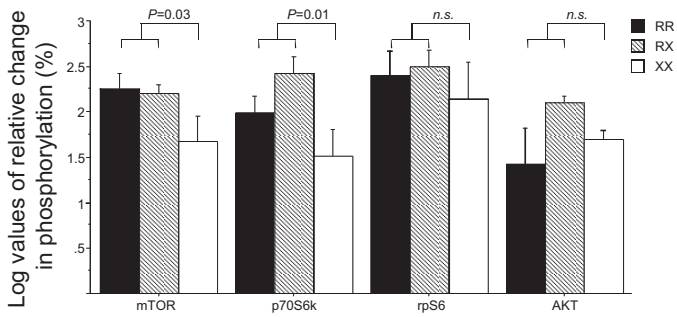

Fig. 1. Percentage change in the phosphorylation of Akt^{Ser473}, $mTOR^{Ser2448}$, $p70S6k^{Thr389}$, and $rpS6^{Ser335/236}$ from baseline to 140 min after sprint exercise in different *ACTN3* genotypes (*study III*); RR ($n = 4$) (dark bars), RX ($n = 7$) (striped bars), and XX ($n = 7$) (white bars).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

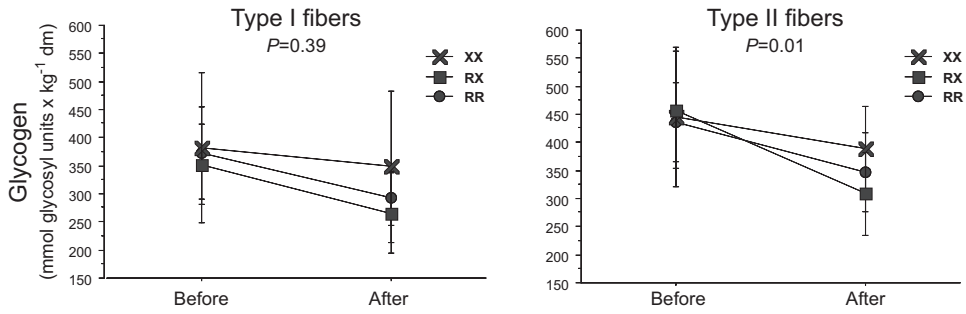

Fig. 2. Glycogen content before and immediately after 30 s of Wingate cycling in pooled type I and type II muscle fibers across different *ACTN3* genotypes (*study II*); RR (n = 18), RX (n = 17), and XX (n = 3). Values are mean ± SD. P values indicate the level of statistical significance for the comparison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

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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.

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