ACTN-3 Genotype, Body Composition, Fitness, and $+G_z$ Tolerance in Senior Cadets

Seunghwan Shin; Hyunseok Jee

BACKGROUND: This study aimed to investigate the relationships among *ACTN-3*, body composition, fitness, and $+G_z$ tolerance for senior cadet training development and their safe task performance.

METHODS: The subjects were all senior cadets ($N = 68$) at the Korea Air Force Academy. All cadets are required to pass a physical fitness test (3-km running, sit-ups, push-ups) and body composition test on a semiannual basis. Isokinetic muscle function (strength and endurance), $+G_z$ test ($+6\ G_z \cdot 30\ s^{-1}$), and target gene (*ACTN-3*) were analyzed.

RESULTS: The effects of body composition and physical fitness along with the relationship of the *ACTN-3* genotype to the $+6\ G_z$ test results were determined. Consequently, no significant difference was found concerning the effect of *ACTN-3* on the $+6\ G_z$ test result, body composition, and physical fitness; however, body fat (%) and isokinetic muscle strength (peak torque right leg extension and left leg flexion) showed significance between the pass and failure groups in the $+G_z$ test.

DISCUSSION: The cadets of the Korea Air Force Academy showed dominant fast genetic expression type based on their *ACTN-3* genotype [RR and RX ($N = 51$, 75%) $>$ XX ($N = 17$, 25%)]. Body fat (%) and isokinetic muscle strength (PT R EX, L FL) can be more effective predictors in the $+6\ G_z$ test for cadet training. Another speculation is that more RR- and RX-type-oriented training can promote cadets' G_z tolerance from the isokinetic factors such as high peak torque and low fatigue index.

KEYWORDS: cadet, *ACTN-3*, physical fitness, body composition, $+G_z$ tolerance.

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High performance aircraft, with their capacity for rapid acceleration and sharp rotation, are essential for the functioning of any air force. The skills and physical capacity of the pilots are paramount to the successful operation of these aircraft.[24] The capacity related to the manipulation skills of the pilot is associated with $+G_z$ tolerance, spatial recognition, and many other factors.[5] To become a Korean Air Force pilot, cadets are assessed for $+G_z$ tolerance using the $+G_z$ test ($6\ G \cdot 30\ s^{-1}$). G_z tolerance influences the physical state of pilots because manipulating high-performance aircraft can cause harsh environmental effects.

The *ACTN-3* gene was found to markedly affect the performance of world class elite athletes.[10,21] Alpha-actinins are a family of actin-binding proteins with a role in myofilament contraction, and there are two genes encoding the alpha-actinins: *ACTN-2* (expressed in all muscle fibers) and *ACTN-3* (shown only in type 2 muscle fiber) in humans.[22] *ACTN-3* is composed of 620 amino acid sequences and its genotype is divided into two fast expression types (RR, RX) and one slow expression type (XX), which are related to the oxidative- and glycolytic-oriented exercise types (www.ncbi.nlm.gov/protein/AAH96323.1).[1,22] This dimeric bundling protein causes loose packing that allows myosin molecules to enter, leading to myofilament contraction.[2]

Several studies have reported on G_z tolerance from the medical and physical fitness perspectives.[9,13] A high G_z tolerance is essential for maintaining muscular function and for a fast recovery from fatigue,[18,20] particularly in the 2–3 sorties per day conditions during wartime.[12] We hypothesized that G_z tolerance might be related to motor ability at the genetic level.

In previous studies on G_z tolerance, the subjects were pilots on duty[14,16,19] and they showed no differences in physical fitness, body composition, and other parameters according to the G_z

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test. However, the results of the cadets indicated a significant difference in the isokinetic function of the lower limbs.[15] G_z tolerance is also dependent on the extent of physical fitness and experience level.[11]

No trials have been conducted to determine the genetic effect of G_z tolerance on cadets without experiences of $+G_z$ testing. We thus hypothesized the following: 1) there is a property of G tolerance related to the *ACTN-3* genotype; and 2) there is a relationship between G tolerance and body composition parameters and physical fitness indicators (e.g., cardiovascular endurance, muscle strength, and endurance). Therefore, this study aimed to determine the relationships among *ACTN-3*, body composition, fitness, and G_z tolerance for the effective training and safe task performance of senior cadets and pilots.

METHODS

Subjects

The subjects included all senior cadets (pilot-trainee, $N = 68$) of the Korea Air Force Academy. Six cadets who had physical injuries were excluded from this study. Subjects participated voluntarily and signed informed consent forms from the IRB. Their mean age was 22.6 ± 0.75 yr, height was 174.71 ± 4.71 cm, and weight was 69.17 ± 6.1 kg. This study was approved by the institutional review board of the Aerospace Medical Center (ASMC-16-IRB-009).

Equipment

Body composition parameters (muscle mass, body mass index, etc.) were measured by InBody 720 (Biospace, Seoul, Korea). Physical fitness was measured by a push-up device (30 cm high × 1.2 m wide hand bar) and a crunch device (a flat board with a triangle under the knee and a lock-up for foot fix), which were authorized by the Korea Air Force Academy (KAFA) for cadets. The 3-km run was measured with an iPhone 6S (Apple, Cupertino, CA, USA) on an authorized course within KAFA. Lower limb muscle function and G_z tolerance were measured with the Humac norm isokinetic motor performance testing and rehabilitation system (CSMI Solutions, Stoughton, MA, USA) and the ATFS-400 Phoenix platform (ETC, Southampton, PA, USA), respectively. *ACTN-3* gene expression type was determined by DNA sample extraction using the Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Seoul, Korea) and the G-spin Total DNA Extraction Kit (iNtRON Biotechnology, Seoul, Korea). The extracted DNA sample was then analyzed by an authorized agent (Seegene Medical Foundation, https://www.seegenemedical.com).

Procedure

All senior cadets undergo a physical fitness test every April and the cumulative data from those tests were used in this study. The "must pass" standards of the physical fitness tests of the senior cadets, such as the 3-km run (12.5 min), sit-ups (82 per 2 min), and push-ups (72 per 2 min), are strictly maintained and rechecked every 6 mo. The muscle strength specific test for senior cadets is performed by % body weight (kg) with bench press (80%), lat pull down (70%), leg curl (50%), arm curl (35%), and leg press (160%) exercises.

Lower limb muscle function tests (isokinetic strength and endurance) to assess in vivo mechanical function were performed by all subjects. The isokinetic properties during knee flexion and extension were analyzed using constant speeds of $60° \cdot s^{-1}$ [5 repetitions for muscle strength: peak torque (Nm)] and $240° \cdot s^{-1}$ [25 repetitions for muscle endurance: fatigue index (Nm)]. After two practice trials, all subjects had a 1-min break between the tests to minimize the effect of the preceding test.[15] The test values were automatically calculated by corresponding test equipment. The following data were obtained: muscle strength (with peak torque in $60° \cdot s^{-1}$, 5 repetitions), which includes peak torque from the left leg extension (PT L EX), right leg extension (PT R EX), left leg flexion (PT L FL), and right leg flexion (PT R FL); and muscle endurance (with fatigue index in $240° \cdot s^{-1}$, 25 repetitions), which includes fatigue index (FI expressed the power decline divided by the time interval in seconds between peak and minimum power) in the left leg extension (FI L EX), right leg extension (FI R EX), left leg flexion (FI L FL), and right leg flexion (FI R FL).

For the $+G_z$ test (gravity-induced acceleration test), a high-speed cockpit-shaped gondola that generates centrifugal movement, which could induce $1 \text{ G} \cdot s^{-1}$ speed up to 6 G and maintain 6 G for 30 s, was used. A subject who lost consciousness was dropped out at a speed of $6 \text{ G} \cdot 30 \text{ s}^{-1}$. Prior to the $+G_z$ test, an expert explained the correct posture and L-1 respiration method required for the test.[15]

For the target gene (*ACTN-3*) analysis, all subjects were instructed not to participate in any intensive physical activities 1 d before blood collection. Leukocyte genomic DNA was extracted and the concentration of DNA samples was determined using the NanoDrop spectrophotometer (concentration, $\sim 700 \text{ ng} \cdot \mu L^{-1}$) according to the guidelines of the G-spin Total DNA Extraction Kit. The samples were stored at −20°C for further analyses (e.g., gel transfer, Southern blot hybridization) to detect the different expression types (RR, RX, XX) of the target gene *ACTN-3* by an authorized agent.

Statistical Analysis

All data were expressed as mean ± SD. The Chi-squared test was used for analyzing the results from the $+G_z$ test according to the different expression types of *ACTN-3*. Two-way analysis of variance was used to determine the differences of body composition parameters and physical fitness tests by genotype-dependent *ACTN-3* based on the results of the $+G_z$ test. The Statistical Package for the Social Sciences software version 21 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. A value of $P < 0.05$ was set to indicate a statistically significant difference.

RESULTS

The characteristics of the senior cadets ($N = 68$) of the Korea Air Force Academy are 22.6 ± 0.75 yr, height is 174.71 ± 4.71

cm, and weight is 69.17 ± 6.1 kg. The results of the $+G_z$ test, based on each expression type of *ACTN-3*, are shown in **Table I** [Pass = 31 vs. Failure = 37; 18 RR (9 vs. 9), 33 RX (14 vs. 19), 17 XX (8 vs. 9)]. Results were not significant [$\chi^2(10) = 289$, $P = 0.865$]. For the *ACTN-3* genotype, the fast expression type (RR, RX) was more dominant (75%) than the slow expression type (XX, 25%), and only RRs' pass rate in the $+G_z$ test showed 50%.

To determine the relationship between body composition parameters based on *ACTN-3* genotypes and $+G_z$ test results, we first selected variables such as height, weight, muscle mass, body mass index, and body fat. As shown in Table I, no significant differences were observed in body composition parameters except for body fat (%) on the main effect of $+G_z$ test ($t = 5.438$, $P = 0.023$, df = 1), although no interaction of each expression type with corresponding body fat (%) value was observed in the results of the $+G_z$ test ($F = 2.909$, $P = 0.062$, df = 2). Generally, the pass group showed higher values in body weight (kg), BMI (kg/m^2), and body fat (%).

To assess physical fitness, 3-km running, crunches, and push-ups were performed. No main effects from the *ACTN-3* genotype or interactions were seen on the $+G_z$ test. There seems to be a pattern showing an almost significant difference in crunches based on the main effect by $+G_z$ test ($F = 3.824$, $P = 0.055$, df = 1); however, all variables showed $P > 0.055$, which indicated the least value after performance of crunches in the $+G_z$ test (**Table II**).

In the $60° \cdot s^{-1}$ isokinetic lower limb muscle strength tests, no genotypic difference was shown, but PT L FL ($t = 4.336$, $P = 0.041$, df = 1) and PT R EX ($t = 4.841$, $P = 0.032$, df = 1) showed significances from the main effect on the $+G_z$ test (**Table III**). In the $240° \cdot s^{-1}$ isokinetic lower limb muscle endurance tests, a genotypic difference or a different type of

Table I. $+G_z$ Test Results According to *ACTN-3* Type Based on Body Composition.

					INTERACTION EFFECT			$+G_z$ TEST MAIN EFFECT		
			N	M ± SD	*P*	*F*	df	*P*	*t*	df
Height (cm)	Pass	RR	9	174.77 ± 4.73	0.293	1.251	2	0.819	0.053	1
		RX	14	175.88 ± 5.22						
		XX	8	173.34 ± 3.87						
		Sum	31	174.90 ± 4.74						
	Fail	RR	9	172.33 ± 5.27						
		RX	19	174.99 ± 4.18						
		XX	9	175.83 ± 5.18						
		Sum	37	174.55 ± 4.76						
Weight (kg)	Pass	RR	9	71.90 ± 5.33	0.664	0.412	2	0.100	2.785	1
		RX	14	69.76 ± 6.18						
		XX	8	70.56 ± 7.52						
		Sum	31	70.59 ± 6.18						
	Fail	RR	9	67.29 ± 5.00						
		RX	19	67.53 ± 6.70						
		XX	9	69.61 ± 4.86						
		Sum	37	67.98 ± 5.85						
Muscle mass (kg)	Pass	RR	9	34.34 ± 2.76	0.266	1.352	2	0.546	0.368	1
		RX	14	34.54 ± 3.37						
		XX	8	32.95 ± 2.74						
		Sum	31	34.07 ± 3.03						
	Fail	RR	9	33.23 ± 2.76						
		RX	19	33.05 ± 2.88						
		XX	9	34.22 ± 2.22						
		Sum	37	33.38 ± 2.68						
BMI ($kg \cdot m^{-2}$)	Pass	RR	9	23.53 ± 1.55	0.885	0.122	2	0.093	2.902	1
		RX	14	22.51 ± 1.28						
		XX	8	23.44 ± 1.92						
		Sum	31	23.05 ± 1.57						
	Fail	RR	9	22.64 ± 1.20						
		RX	19	22.05 ± 1.85						
		XX	9	22.59 ± 2.18						
		Sum	37	22.33 ± 1.78						
Body fat (%)	Pass	RR	9	15.93 ± 3.00	0.062	2.909	2	0.023*	5.438	1
		RX	14	12.74 ± 4.07						
		XX	8	16.98 ± 3.66						
		Sum	31	14.76 ± 4.04*						
	Fail	RR	9	13.07 ± 2.11						
		RX	19	13.36 ± 3.57						
		XX	9	13.22 ± 2.69						
		Sum	37	13.25 ± 3.00*						

Values are expressed as means ± SD. M, mean; SD, standard deviation; *N*, number; BMI, body mass index.

* Significant difference between the sum of the pass and fail groups from the $+G_z$ test's main effect.

Table II. G_z+ Test Results According to *ACTN-3* Type Based on Physical Fitness.

					INTERACTION EFFECT			$+G_z$ TEST MAIN EFFECT		
			N	M ± SD	*P*	F	df	*P*	*t*	df
3-km running (seconds)	Pass	RR	9	750.11 ± 46.15	0.451	0.807	2	0.160	2.022	1
		RX	14	741.93 ± 25.22						
		XX	8	728.50 ± 32.07						
		Sum	31	740.84 ± 33.92						
	Fail	RR	9	761.33 ± 38.55						
		RX	19	742.48 ± 38.15						
		XX	9	758.11 ± 49.42						
		Sum	37	750.86 ± 40.97						
Crunches	Pass	RR	9	90.00 ± 10.71	0.989	0.011	2	0.055	3.824	1
		RX	14	84.79 ± 5.87						
		XX	8	85.38 ± 9.64						
		Sum	31	86.45 ± 8.52						
	Fail	RR	9	94.22 ± 7.22						
		RX	19	89.68 ± 10.04						
		XX	9	89.67 ± 9.64						
		Sum	37	90.78 ± 9.33						
Push-ups	Pass	RR	9	81.00 ± 11.83	0.603	0.509	2	0.749	0.103	1
		RX	14	77.79 ± 14.28						
		XX	8	82.50 ± 15.49						
		Sum	31	79.94 ± 13.64						
	Fail	RR	9	79.78 ± 10.05						
		RX	19	82.89 ± 11.35						
		XX	9	81.67 ± 11.57						
		Sum	37	81.84 ± 10.87						

Values are expressed as means ± SD. M, mean; SD, standard deviation; *N*, number.

muscle functional test showed no statistical difference on the $+G_z$ test (**Table IV**).

DISCUSSION

We determined the effect of body composition and physical fitness along with the relationship of the *ACTN-3* genotype to the +6-Gz test in senior cadets. The results showed no significant difference in the effect of *ACTN-3* on the $+G_z$ test; however, percent body fat and muscle strength (PT R EX, PT L FL) showed significant differences ($P < 0.05$) between the pass and failure groups by main effect on the $+G_z$ test.

The Korea Air Force Academy was the first to develop the physical fitness of cadets to improve $+G_z$ tolerance using a strict curriculum. About 48% of the whole physical education curriculum is used for promoting physical fitness; however, almost 50% of the cadets failed in the $+6\text{-}G_z$ 30-s test. The failure rate of gravity-induced loss of consciousness (G-LOC) in other countries such as Australia, Turkey, and Brazil is 8–13%, which is comparatively lower than our data.[3,16,23] These results provide other analytic insights to compensate for the defect of general physical fitness components. Therefore, we focused on investigating at the isokinetic and molecular level, such as the effect of genetic type.

The significant difference in isokinetic peak torque in the right leg extension (PT R EX) suggests that the following situations need to be taken into consideration. Most of the cadets were dextropedal and they usually extend their whole body rather than huddling themselves with extreme tension to avoid G-LOC while undergoing the $+6\text{-}G_z$ test. In this duration, the dextropedal cadets maximally push the pedals with their legs, which possibly increases the muscle strength in the right leg. However, further studies are necessary to identify the significance of the left leg flexion.

Table III. $+G_z$ Test Results According to *ACTN-3* Type Based on the Isokinetic Muscle Strength ($60° \cdot s^{-1}$) of the Lower Limb (Nm) ($+G_z$ Test Main Effect) ($P < 0.05$).

			N	M ± SD (Nm)				*N*	M ± SD (Nm)
PT (L FL)	Pass	RR	9	141.56 ± 24.72	P.T. (R EX)	Pass	RR	9	214.33 ± 26.59
		RX	14	136.21 ± 19.28			RX	14	201.21 ± 32.72
		XX	8	130.50 ± 22.79			XX	8	197.88 ± 23.98
		sum	31	136.29 ± 21.51*			Sum	31	204.16 ± 28.85 *
	Fail	RR	9	116.22 ± 14.69		Fail	RR	9	176.33 ± 27.63
		RX	19	127.47 ± 25.36			RX	19	195.74 ± 35.37
		XX	9	128.56 ± 25.47			XX	9	189.67 ± 28.55
		sum	37	125.00 ± 23.22*			Sum	37	189.54 ± 32.25 *
		df		1			df		1
		t-value		4.436			*t*-value		4.841
		P		0.041			*P*		0.032

Values are expressed as mean ± SD; SD, standard deviation; *N*, number; PT, peak torque; L FL, left leg flexion; R EX, right leg extension.
* Significance is not shown in the interaction effect.

Table IV. $+G_z$ Test Results According to *ACTN-3* Type Based on the Isokinetic Muscle Endurance ($240° \cdot s^{-1}$) of the Lower Limb (Nm) (Interaction Effect) ($P < 0.05$).

			N	**M ± SD (Nm)**				***N***	**M ± SD (Nm)**
FI (L EX)	pass	RR	9	22.67 ± 24.41	FI (R EX)	pass	RR	9	29.56 ± 10.73
		RX	14	31.57 ± 16.67			RX	14	31.64 ± 13.26
		XX	8	27.50 ± 19.81			XX	8	37.38 ± 7.69
		sum	31	27.94 ± 19.63			sum	31	32.52 ± 11.40
	fail	RR	9	34.22 ± 10.21		fail	RR	9	28.11 ± 9.89
		RX	19	33.47 ± 8.98			RX	19	30.79 ± 9.54
		XX	9	33.22 ± 7.97			XX	9	35.22 ± 10.27
		sum	37	33.59 ± 8.81			sum	37	31.22 ± 9.86
	sum	RR	18	28.44 ± 19.10		sum	RR	18	28.83 ± 10.04
		RX	33	32.67 ± 12.61			RX	33	31.15 ± 11.08
		XX	17	30.53 ± 14.57			XX	17	36.24 ± 8.93
		sum	68	31.01 ± 14.91			sum	68	31.80 ± 10.53
		df		2			df		2
		F-value		0.442			F-value		2.305
		P		0.645			*P*		0.108
FI (L FL)	pass	RR	9	19.44 ± 10.51	FI (R FL)	pass	RR	9	31.33 ± 10.71
		RX	14	28.28 ± 9.24			RX	14	26.93 ± 12.56
		XX	8	24.75 ± 13.80			XX	8	28.00 ± 9.19
		sum	31	24.81 ± 11.19			sum	31	28.48 ± 11.06
	fail	RR	9	28.11 ± 8.10		fail	RR	9	22.22 ± 5.91
		RX	19	27.05 ± 7.02			RX	19	30.16 ± 11.71
		XX	9	22.22 ± 7.05			XX	9	26.44 ± 10.99
		sum	37	26.14 ± 7.45			sum	37	27.32 ± 10.68
	sum	RR	18	23.78 ± 10.14		sum	RR	18	26.78 ± 9.61
		RX	33	27.57 ± 7.92			RX	33	28.79 ± 11.99
		XX	17	23.41 ± 10.48			XX	17	27.18 ± 9.90
		sum	68	25.53 ± 9.29			sum	68	27.85 ± 10.79
		df		2			df		2
		F-value		1.65			F-value		0.179
		P		0.200			*P*		0.836

Values are expressed as mean ± SD. *N*, number; M, means; SD, standard deviation; L EX, left leg extension; R EX, right leg extension; L FL, left leg flexion; R FL, right leg flexion; df, degree of freedom; *P*, *P*-value; FI, fatigue index.
* Significance is not shown in all factors' interaction and main effect.

We suggest that the training program developed and used by the Korea Air Force Academy is effective since no difference was found in the physical fitness test.[7] Further studies are warranted to confirm whether 14% body fat is the correct level for determining success in the $+G_z$ test. Our results were consistent with those of Park et al., which showed that subjects with 14% body fat passed the $+G_z$ test (the % body fat of the current failed group and that reported by Park et al. were 15% and 12%, respectively).[15] Combined with the significant results of % body fat on the $+G_z$ test in this study (Table I), BMI might need to be considered further as a selection indicator because the value of BMI does not precisely and directly indicate the % body fat (there is no significance shown).

Although no statistical difference was observed in the FI (muscle endurance),[6] the RR-type-dominant cadets tended to show lower FI values (L/R EX, R FL) than those with the XX type. The senior cadets who passed the $+G_z$ test had lower FI values (L EX/FL) than the failed cadets. This probably indicates the higher maximal performance capability and repeatability of the tasks of senior cadets.

Moreover, the results suggest that RR-type-oriented training (such as eccentric contraction)[8] should be designed and developed to lower the FI for promoting $+G_z$ tolerance of senior cadets. On the basis of these findings, the cadet training policy of the Korea Air Force Academy must be reassessed for improving anaerobic fitness.

The dominant fast expression type (RR and RX) is more advantageous than the slow expression type (XX) in terms of $+G_z$ tolerance. However, there were several cases in which fast-expression-type dominant cadets easily lost consciousness. Therefore, other factors, such as psychological factors, need to be considered for cadets' G_z tolerance improvement.

In conclusion, this study aimed to determine the relationships among *ACTN-3*, $+G_z$ tolerance, body composition, and physical fitness for designing and developing cadet training contents and their safe mission performance as pilots. Our novel findings based on these study aims were as follows:

1. The senior cadets of the Korea Air Force Academy showed the dominant fast expression type based on their *ACTN-3* genotype (RR and RX > XX).

The *ACTN-3* gene was found to affect the required high performance level in world class elite athletes,[10,22] but not in the senior cadets of the Korea Air Force Academy, who only tried to pass the $+G_z$ test (i.e., their success in the $+G_z$ test did not require an extremely high performance level or high muscle function). However, in our results, only RRs' pass rates in the $+G_z$ test showed 50%.

From only these results, *ACTN-3* is difficult to justify as the representative indicator in spite of the RR type's results. But our other data [presented at the 89th AsMA Scientific Annual Meeting; Abstract #123; AMHP 2018; 89(3):202] with the pilots ($N = 57$) of the F-15/16 showed the RR type's superior $+G_z$ tolerance statistically. The muscle of humans usually predominantly consists of slow fiber[4] and there have been controversies about the inconsistent matching of isotypes of muscle, indicating molecules such as myosin heavy chain, myosin light chain, troponin, and tropomyosin.[17] Thus, further studies are needed to validate the relationship between the current *ACTN-3* results and other dominant muscle types indicating proteins (e.g., myosin heavy chain isoforms). We also suggested that other possibilities (e.g., increasing subject numbers, using other sensitive measures of factors such as blood pressure, etc.) might affect the different results of this study.

2. No effect of the *ACTN-3* genotype was found. This may be because the exercise intensity of the +6 G_z test is not highly affected by *ACTN-3*. However, in the +G_z test, the pass and fail groups showed significance in terms of body fat (%; P = 0.023) and the possibility of performing crunches (P = 0.055) using the *t*-test. Therefore, the training program of the Korea Air Force Academy may be effective.
3. Another speculation is that more RR-type- and RX-type-based muscle function training can promote +G_z tolerance (muscle strength shown in PT R EX, PT L FL) and can lower the fatigue (FI L/R EX). Future studies are necessary to investigate training contents and evaluate the effectiveness of anaerobic fitness.

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