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Article

Genetic and Physiological Predictors of Bioenergetic Adaptation Skeletal Muscles in Athletes of Cyclic Sports

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Abstract: When performing maximum loads, an important criterion for assessing prospects is the achievement of the best result with the least metabolic changes in the body of athletes, which indicates the possibility of further increasing physical performance. The study of the mechanisms of energy supply and the reaction of body systems when testing performance under special conditions is one of the important conditions for the development of additional biochemical criteria for assessing the prospects of athletes. Purpose: To study genetic and physiological predictors of bioenergetic adaptation of skeletal muscles in athletes of cyclic sports. Methods: 76 athletes of cyclic sports (speed skating, running disciplines in track-and-field) of European origin who lived in the Southern Urals region took part in the study. The average age of the study participants was 22.1 ± 2.5 y.o. Experience in sports was at least 5 years. We used the Step One Real-Time PCR System (Applied Biosystems, USA) device for real-time polymerase chain reaction. The study of bio-energetic indicators of athletes' physical performance was carried out using the bicycle ergometry method (test with maximum load). Biochemical studies were carried out using a Lactate Scout Plus lactometer. Results: Significant differences were found in the ΔLa (%) indicator: in athletes with a dominant homozygous genotype R/R, lactate clearance during a 10-minute rest after performing a bicycle ergometer load is statistically significantly higher than in athletes with a recessive homozygous genotype X/X (20.14±12.74%, versus 11.11±3.12%; $p<0.05$). The major allele C (R) was associated with moderate and high lactate clearance (OR = 2.25 [95% CI: 0.99 – 5.11] and OR = 2.24 [95% CI: 0.91 – 5.51], respectively). At the same time, a statistically significant association was identified between the minor allele T(X) and the homozygous genotype TT (XX) with low lactate clearance (OR = 12.14 [95% CI: 1.30 – 13.55]). High values of lactate clearance indicate the utilization of lactate from peripheral blood and more efficient recovery processes in carriers of the major allele C (R). Conclusions: lactate clearance during a 10-minute rest period after a bicycle ergometer test with maximum load and DNA profiling of the ACTN3 gene rs1815739 can be recommended as significant physiological and genetic predictors of bioenergetic adaptation of skeletal muscles in cyclical sports athletes of Caucasian origin in the Southern Urals.

Keywords: *alpha actinin 3; ACTN3 gene; aerobic energy supply; anaerobic energy supply; cyclic sports; genetic predictor.*

Introduction

It is generally accepted that the nature of adaptive changes in an athlete's body depends on the intensity, direction and duration of physical activity [1], [2]. Regular physical activity improves physical performance, that is, the ability to withstand a given sub-maximal load for a longer period of time or achieve a higher speed of movement over a fixed distance [3]. Increased physical performance is achieved through mechanisms of immediate and long-term adaptation. Both stages of adaptation have a mutual influence on each other. Urgent adaptation is associated with significant biochemical and functional changes during physical activity, which creates the prerequisites for the launch of long-term adaptation mechanisms [4]. In turn, long-term adaptation, increasing the energy potential of the body (mainly due to increased expression of enzymes and proteins involved in metabolic reactions), thereby increases the possibility of urgent adaptation. From this point of view, physical activity of different intensity contributes to the activation of different aspects of metabolism and affects the characteristics of the structure and function of skeletal muscles [1], [2] (Figure 1).

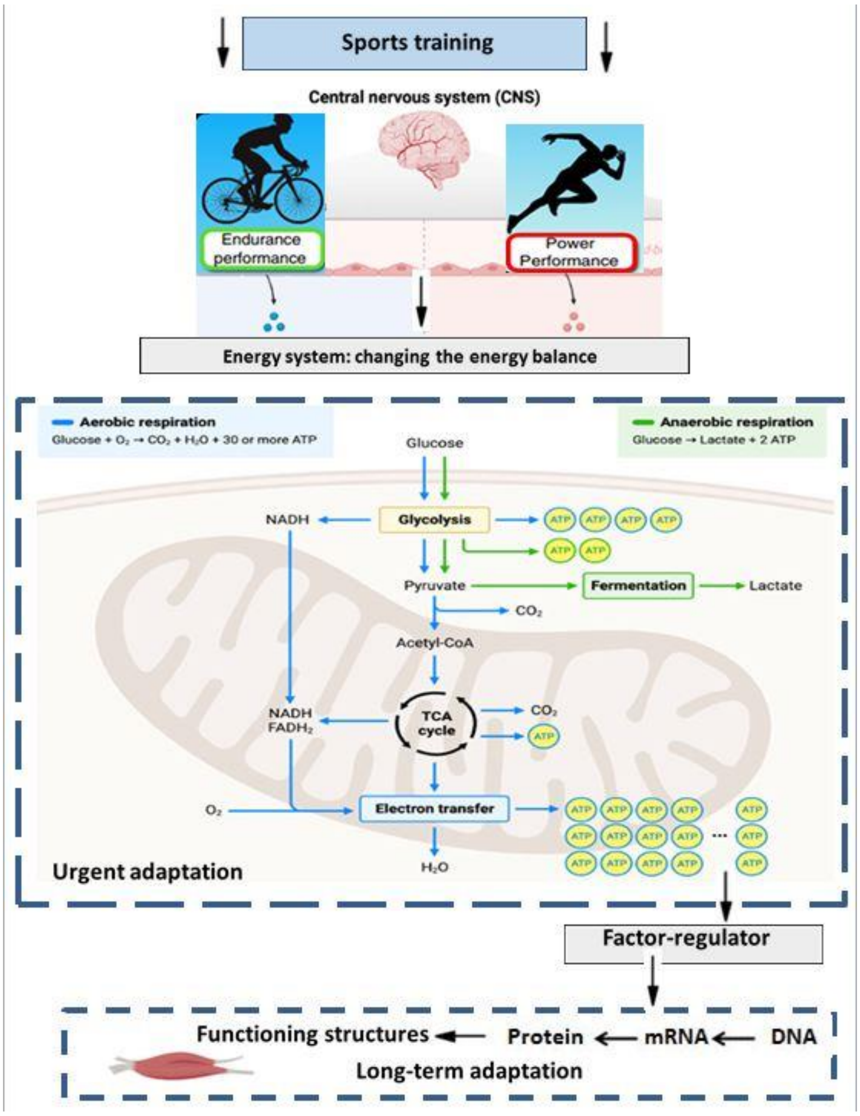

Figure 1. The mechanism of formation of urgent and long-term adaptation of skeletal muscles in athletes to loads with different directions of the training process (endurance and speed/strength).

From a biochemical point of view, predicting the performance of athletes is determined by the high adaptive capabilities of energy supply systems [5]. The aerobic and anaerobic capabilities of the athlete's body are determined by the individual characteristics of the structure of muscle fibers, intramuscular glycogen reserves, the presence and activity of enzyme systems and the characteristics of intracellular regulation of energy transformations in working muscles under conditions of oxygen deficiency. Taking into account the genetic determination of aerobic and anaerobic abilities in athletes, bioenergy indicators can serve as objective criteria for assessing the prospects of athletes [6].

It is known that α-actinin-3 protein is a structural component of skeletal muscle type II-B (fast muscle fibers), which cross-links and stabilizes thin actin filaments on the Z-disc [7]. Its expression and functional activity are controlled by the *ACTN3* gene [8]. Numerous studies have confirmed the association between the C (R) 577 allele of the rs1815739 variant of the *ACTN3* gene and sprinter phenotypes in athletes [7], [8], [9]. Previously, we demonstrated that high athletic performance in sprinters was associated with the homozygous dominant genotype 577 CC (RR) in cyclic sports athletes of Caucasian origin in the Southern Urals. [9]. Of practical interest is the relationship between physiological biomarkers characterizing the individual characteristics of bioenergetic adaptation of skeletal muscles in athletes of cyclic sports and allelic variants of the RS1815739 *ACTN3* gene. However, we have not found studies that would allow a comprehensive assessment of the level of bioenergetic adaptation of skeletal muscles, taking into account such physiological markers as the power of bicycle ergometric load at the level of the aerobic threshold, at the level of the anaerobic threshold, maximum accumulation of lactate at the peak of load, etc.

The purpose of the study is to study the genetic and physiological predictors of bioenergetic adaptation of skeletal muscles in athletes of cyclic sports.

Materials and Methods

Data Collection

The conduct of our study complied with the principles reflected in the Declaration of Helsinki of the World Medical Association (Fortaleza, Brazil, 2013) [10]. All stages of this study were discussed and approved by the local Ethics Committee of the Ural State University of Physical Culture (UralGUFK). All study participants signed voluntary informed consent to participate in this study. The study was conducted on the basis of the Research Institute (Research Institute) of Olympic Sports UralGUFK (Chelyabinsk, Russia) as part of the state assignment of the Ministry of Sports of the Russian Federation (registration number AAAA-A16-116042510005-7 dated April 25, 2016) and was approved by the local ethics committee of the Ural State University of Physical Culture (UralSUPC), Protocol No. 5 dated January 11, 2016.

Our study was an open-label, observational, case-control study. The minimum number of participants in observation groups was calculated using the online calculator "Clin Calc" [11].

Study Participants

This study included 76 Caucasians living in the Southern Urals region, Russia. The study design and general characteristics of the observation groups are presented in Figure 2.

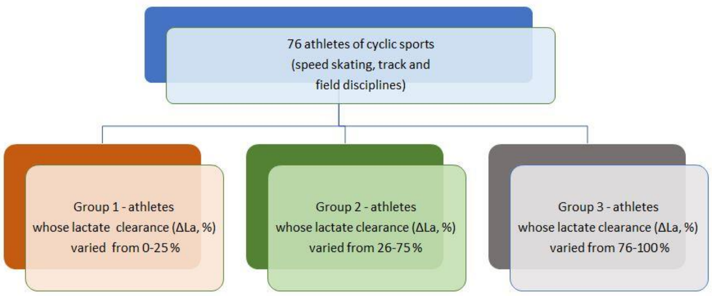

Figure 2. Study design and general characteristics of observation groups.

Criteria for inclusion:

1.residents of the Southern Urals (the city of Chelyabinsk and the Chelyabinsk region, Russia);

2. caucasians;

3. male;

4. age from 17 to 22 years;

5. sports qualification: I adult category, candidate master of sports (CMS), master of sports (MS), master of sports of international class (MSIC);

6. experience in cyclic sports (speed skating, track and field athletics) - 5 years or more;

7. signed voluntary informed consent to participate in this study.

Exclusion criteria from the study: residents of other regions of Russia; not Caucasians; female; age under 17 years; low compliance of study participants with the implementation of the protocol of this study; participation in other studies; current acute and chronic, somatic, neurological, psychiatric, infectious diseases during the period of inclusion in the study or at the stages of the study; alcohol intake during the study period and a history of alcohol abuse; interference of drugs (taking aspirin, energy drinks, ethanol, fructose, sucrose, glucose, terbutaline, tetracosactrine, isoniazid, metformin, prednisolone, nalidixic acid, phenformin; use of propylene glycol as a solvent for injections, use of sodium bicarbonate for intravenous injections; underweight, anemia.

The sample was divided into three groups depending on lactate clearance after bicycle ergometer testing, assessed by the decrease in serum lactate levels (ΔLa, %) immediately after the test and after a 10-minute rest period. Group 1 - athletes whose ΔLa (%) varied from 0-25%; group 2 - athletes whose ΔLa (%) varied from 25-75%; group 3 - athletes whose ΔLa (%) varied from 75-100%.

The age distribution of study participants was normal. The average age of participants in the total sample was 22.1 ± 2.5 years. The average age of participants in groups 1, 2 and 3 was not statistically significantly different (22.1 ± 2.4, 22.6 ± 2.5, 21.4 ± 2.7, respectively; $p > 0.05$).

Genetic Analysis

Buccal epithelium (biological sample) was taken from study participants after standard preparation [12] and their signing of voluntary informed consent. After centrifugation, samples were stored in a refrigerator at -18°C. In the course of this study, a biobank of DNA samples was created on the basis of the Olympic Sports Research Institute (Chelyabinsk, Russia). Next, DNA extraction was carried out using a standard protocol [12]. Detection of the major (C or R) and minor (T or X, i.e. stop cadon) alleles of the OHB rs1815739 (C577T) gene ACTN3 (cytogenetic location: 11q13.2; genomic coordinates (GRCh38): 11:66,546,395-66,563,334 chr11: 66,313,866-66,330,805 [13]) was carried out using diagnostic equipment StepOne Real-Time PCR System (Applied Biosystems, Waltham, MA, USA).

Study of Bioenergetic Indicators of Physical Performance

The study of bioenergetic indicators of physical performance of athletes in cyclic sports was carried out using the bicycle ergometry method (test with maximum load). The load in the stepwise bicycle ergometer test was set by pedaling on a LODE "CORIVAL" bicycle ergometer (Groningen, Netherlands). The initial load power was 60 W, and every 2 minutes the load increased by 30 W. Physiological markers of bioenergetic adaptation of skeletal muscles are presented in Table 1.

Table 1. Physiological markers of bioenergetic adaptation of skeletal muscles in athletes of cyclic sports.

Marker	Brief characteristics of the marker
W AP/Mt, W	Load power at the aerobic threshold level (lactate 2 mmol/l), reduced to the athlete's body weight
W AnP/Mt, W	Load power at the level of the anaerobic threshold (lactate 4 mmol/l), reduced to the athlete's body weight
W max/Mt, W	The value of the maximum power at the peak of the bicycle ergometric load, normalized to the athlete's body weight
La max, mmol/l	Lactate concentration in capillary blood at the peak of cycling exercise
La восст, mmol/l	Lactate concentration in capillary blood at 10 minutes of recovery after bicycle ergometer exercise
ΔLa, %	Lactate clearance (dynamics of lactate utilization after performing a bicycle ergometer test)

Laboratory testing

Capillary blood was used as a biological material to perform biochemical studies. Blood was taken from a fingertip in the first half of the day, 4 hours after the morning meal (water intake was allowed). On the eve of the study, participants were advised to avoid increased psycho-emotional and physical stress, including training, drinking alcoholic beverages and smoking. In addition, participants were advised to avoid putting stress on the hand or arm before and during blood collection. No tourniquet was used during blood sampling. Lactate levels were measured in mmol/L (reference values: 0.5 - 2.2 mmol/L). The concentration of lactate in capillary blood was determined before and after performing a bicycle ergometer test with a maximum load to assess metabolic changes caused by the load. Lactate is a product of anaerobic glucose metabolism (glycolysis), during which it is formed from pyruvate under the influence of the enzyme lactate dehydrogenase [14]. With sufficient oxygen supply, pyruvate is metabolized in the mitochondria to water and carbonic acid. Under anaerobic conditions, when there is insufficient oxygen supply, pyruvate is converted to lactate. The main amount of lactate enters the blood from skeletal muscles. Lactate clearance (disappearance from the blood) is mainly associated with metabolism in the liver and kidneys. The absorption of lactate by the liver is a saturable process. Serum lactate concentrations during exercise correlate with the development of muscle fatigue [15].

Biochemical studies were carried out using a Lactate Scout Plus lactometer (EKF DIAGNOSTICS, Germany by DiaSpect Medical GmbH), which has undergone metrological verification and has an appropriate certificate. Reagents - test strips, have a measuring range of 0.5-25 mmol/l, which allows you to measure blood lactate levels in 10 seconds using just 0.2 μl of blood.

Statistical analysis

Database analysis was carried out using ISB SPSS version 22.0 (SPSS Inc, USA). To assess physiological and genetic biomarkers of bioenergetic adaptation of skeletal muscles in athletes of cyclic sports, the odds ratio was calculated (odds ratio 95% confidence interval (CI). Intergroup differences were significant at P-value < 0.05.

Results

Peculiarities of biochemical adaptation in athletes of cyclic sports depending on the carriage of allelic variants of the ACTN3 gene rs1815739

Table 2 presents the results of a study of bioenergetic parameters obtained during bicycle ergometer testing with the participation of athletes in cyclic sports (speed skating, short track, track and field running disciplines).

Table 2. Comparative analysis of indicators of bioenergetic adaptation of skeletal muscles based on the results of bicycle ergometer testing depending on the carriage of allelic variants and genotypes of the rs1815739 *ACTN3* gene

Indicators	R/R (n1 = 28)	R/X (n2 = 35)	X/X (n3 = 12)	p-value
Muscle mass, %	50,00±2,83	49,58±3,71	49,74±3,34	> 0,05
Wap/mt, W/kg	2,05±0,60	2,36±0,49	2,49±0,28	> 0,05
W pano/mt, W/kg	3,29±0,57	3,51±0,58	4,02±0,55	> 0,05
W max/mt, W/kg	3,92±0,68	4,12±0,66	4,62±0,60	> 0,05
La max, mmol/l	10,85±2,95	11,69±3,26	10,68±2,66	> 0,05
ΔLa, %	20,14±12,74	18,69±11,48	11,11±3,12	**< 0,05**

When performing a maximum stepwise increasing bicycle ergometric load to failure, the functional reserves of the body are mobilized, determined by the genetic predisposition and level of training of the athlete. A shift in the pH of the internal environment of the body caused by the products of anaerobic metabolism has a negative impact on physical performance indicators. The magnitude of the change in this indicator (La max, mmol/l) characterizes the degree of metabolic response to high-intensity physical activity or the "metabolic cost of the work performed" and is one of the informative indicators when assessing test results [15].

The results of the study show that no significant intergroup differences were identified for this indicator ($p>0.05$). However, significant differences were found in terms of ΔLa (%): in athletes with the dominant homozygous genotype R/R, lactate clearance during a 10-minute rest after performing a bicycle ergometer load is statistically significantly higher than in athletes with the recessive homozygous genotype X/X (20.14±12.74%, versus 11.11±3.12%; $p<0.05$). High values of lactate clearance indicate the utilization of lactate from peripheral blood and more efficient recovery processes in carriers of the major allele R.

Distribution of genotype and allele frequencies in athletes of cyclic sports with different rates of lactate elimination from peripheral blood

At the next stage of this study, all participants were divided into three groups depending on the clearance of lactate from peripheral blood (Figure 3) by calculating the median (Me) and interquartile range (25th and 75th percentiles).

The distribution of frequencies of major and minor alleles and genotypes of the *ACTN3* gene (C577T, rs1815739) in athletes of cyclic sports was analyzed, regardless of their specialization (short, medium and long distance running), but depending on lactate clearance as a physiological biomarker efficiency of energy supply to skeletal muscles (Table 3).

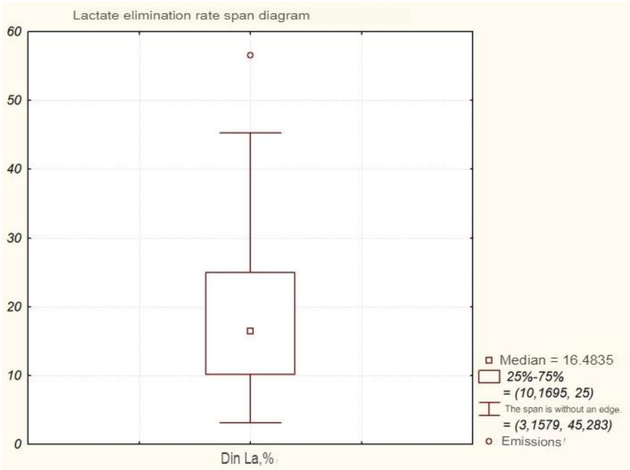

Figure 3. Indicators of lactate clearance in athletes of cyclic sports after performing a bicycle ergometer test (Me, P25, P75).

Table 3. Distribution of allele and genotype frequencies depending on the carriage of the rs1815739 variant of the *ACTN3* gene in athletes of cyclic sports depending on lactate clearance after a bicycle ergometer test with maximum load.

Alleles, genotypes	ΔLa, % Percentile 0-25% (n1=20)	ΔLa, % Percentile 26-75% (n2=38)	ΔLa, % Percentile 76-100% (n3=18)	**p-value**
C (R)	20 (52.6%)	50 (71.4%)	24 (70.6%)	< 0.05
T (X)	18 (47.4%)	20 (28.6%)	10 (29.4%)	< 0.05
CC (RR)	6 (31.6%)	16 (45.7%)	8 (47.1%)	> 0.05
CT (RX)	8 (42.1%)	18 (51.4%)	8 (47.1%)	> 0.05
TT (XX)	5 (26.3%)	1 (2.9%)	1 (5.8%)	< 0.05

The frequency of the major allele C (R) was statistically significantly lower in athletes of group 1 (low lactate clearance values) compared to group 2 (average lactate clearance values) and group 3 (high lactate clearance values), p1-2 < 0.05, p1-3 < 0.05. In addition, the frequency of the homozygous TT (XX) genotype was statistically significantly higher in group 1 compared to groups 2 and 3 (p1-2 < 0.05, p1-3 < 0.05).

To study the odds ratio between lactate clearance and carriage of minor or major alleles and genotypes of the rs1815739 gene of *ACTN3*, we used two models: multiplicative (to estimate the frequency of alleles) and additive (to estimate the frequency of genotypes). The results obtained are presented in Table 4. When studying the carriage of rs1815739 gene ACTN3, it was found that the data obtained are consistent with the HWE law: in group 1 – $\chi 2$ = 0.23 (p-value = 0.63); group 2 – $\chi 2$ = 1.62 (p-value = 0.22) and group 3 – $\chi 2$ = 0.41 (p-value = 0.85).

The major allele C (R) was associated with moderate and high lactate clearance (OR = 2.25 [95% CI: 0.99 – 5.11] and OR = 2.24 [95% CI: 0.91 – 5.51], respectively). At the same time, a statistically significant association was identified between the minor allele T(X) and the homozygous genotype TT (XX) with low clearance (OR = 12.14 [95% CI: 1.30 – 13.55]) (Table 4).

We found statistically significant differences in the frequency of the T (X) allele between athletes in cyclic sports depending on lactate clearance. Thus, the carriage frequency of the minor allele T (X) rs1815739 of the *ACTN3* gene was 47.4%, 28.6% and 29.4%

in groups 1, 2 and 3, respectively (p-value < 0.05), and the frequency of the recessive homozygous genotype was 26.3%, 2.9% and 5.8%, respectively (p < 0.05)

Table 4. Odds ratio between lactate clearance and carriage of rs1815739 alleles and genotypes of the *ACTN3* gene in athletes of cyclic sports.

Alleles, genotypes	χ^2	**p-*value***	**OR**	**95% CI**
		Group 1 vs. Group 2		
C (R)	3.82	0.05*	2.25	0.99-5.11
T (X)			0.44	0.20-1.01
CC (RR)			0.55	0.17-1.77
CT (RX)	6.93	0.04*	0.69	0.22-2.12
TT (XX)			12.14	1.30-13.55
		Group 2 vs. Group 3		
C (R)	0.01	0.93	0.96	0.39-2.37
T (X)			1.04	0.42-2.57
CC (RR)			1.06	0.33-3.37
CT (RX)	0.32	0.85	0.84	0.26-2.68
TT (XX)			2.13	0.12-36.18
		Group 1 vs. Group 3		
C (R)	3.15	0.08	2.24	0.91-5.51
T (X)			0.45	0.18-1.10
CC (RR)			3.41	0.92-12.62
CT (RX)	3.77	0.05	0.54	0.16-1.83
TT (XX)			0.30	0.03-2.81

Discussion

In the present study, lactate clearance was examined as an important physiological predictor of bioenergetic adaptation of skeletal muscles in athletes of cyclic sports. Lactic acid formed as a result of glycolysis in the skeletal muscles of an athlete after performing bicycle ergometer testing with a maximum load dissociates into hydrogen ions and compounds with sodium or potassium ions and forms a salt (sodium lactate or potassium lactate, respectively). The metabolic pathway is then carried out in the Cori cycle with the participation of the enzyme lactate dehydrogenase, which converts lactate into pyruvic acid, followed by its oxidation in the mitochondria to carbon dioxide and water in skeletal muscles. The other part of the lactate enters the systemic circulation through the blood capillaries and is delivered to hepatocytes, where it is included in metabolic reactions leading to the synthesis of glycogen. Excessive amounts of lactate are excreted from the body through urine and sweat [15]. Glycogen in the liver is used by the body to restore bioenergy reserves in skeletal muscles. We hypothesized that low lactate clearance, occurring both during and after a maximal load bicycle ergometer test, is an important physiological predictor of lactic acid accumulation in skeletal muscle fibers, which is the main cause of skeletal muscle acidosis. In this case, the main damaging agent is hydrogen ions, the source of which is the hydrolysis of ATP. The process of ATP hydrolysis causes the accumulation of hydrogen ions and a shift in the pH of the sarcolemma of muscle fibers to the acidic side, which is associated with rapid fatigue during intense physical activity due to the parallel formation of lactate in skeletal muscles, which is the end product of anaerobic glycolysis. Low lactate clearance leads to an increase in osmotic pressure, resulting in water entering the muscle fibers, swelling of the muscle fibers with subsequent compression of muscle pain receptors [16], [17].

Some authors express the opinion [18] that the accumulation of lactate in skeletal muscles underlies the development of their mechanical tension with subsequent myofibrillar hypertrophy and an increase in strength, which is typical for sprinters. This effect is achieved due to the fact that the metabolites cause a cascade of mechanotransduction in most muscle fibers and increase muscle activation.

Lactate is a key biomarker of metabolic stress and one of the most studied metabolites during exercise. High lactate concentration affects the expression of regulators of muscle differentiation [19]. The authors suggest that skeletal muscle may sense changes in extracellular lactate. A study by Hashimoto T. et al. [20] demonstrated that 20 mM lactate induced the expression of rat L6 myotubes, lactate-related genes. Ohno Y. et al. [21] found that 20 mM lactate was able to induce anabolic signaling and hypertrophy in C2C12 cells, possibly in a GPR81-dependent manner. This suggests that extracellular lactate may initiate signaling events through membrane-bound receptors in skeletal muscle.

In individuals with average lactate clearance, it is eliminated within 30-90 minutes of rest, however, if after physical activity you perform a light aerobic activity for 10-15 minutes (for example, pedaling on a bicycle ergometer), then lactate is removed faster. This explains why, when developing the design of our study, lactate clearance in the observed athletes of cyclic sports was studied immediately after the end of the load and after 10 minutes of rest.

Consequently, the low clearance of lactate that we have shown, associated with a slowdown in its removal from skeletal muscles during the rest period after performing a bicycle ergometer test with maximum power, explains why long-distance athletes need to continue physical activity at a low or moderate pace to ensure accelerated removal of lactate from the muscles. fibers and preservation of bioenergetic reserves of skeletal muscles. On the contrary, sprinters showed a high lactate clearance after performing a bicycle ergometer test during the rest period. It can be assumed that the high lactate clearance in carriers of the major C (R) allele, widely represented among sprinters, represents the adaptive ability of skeletal muscles to perform physical activity under conditions of high metabolic stress.

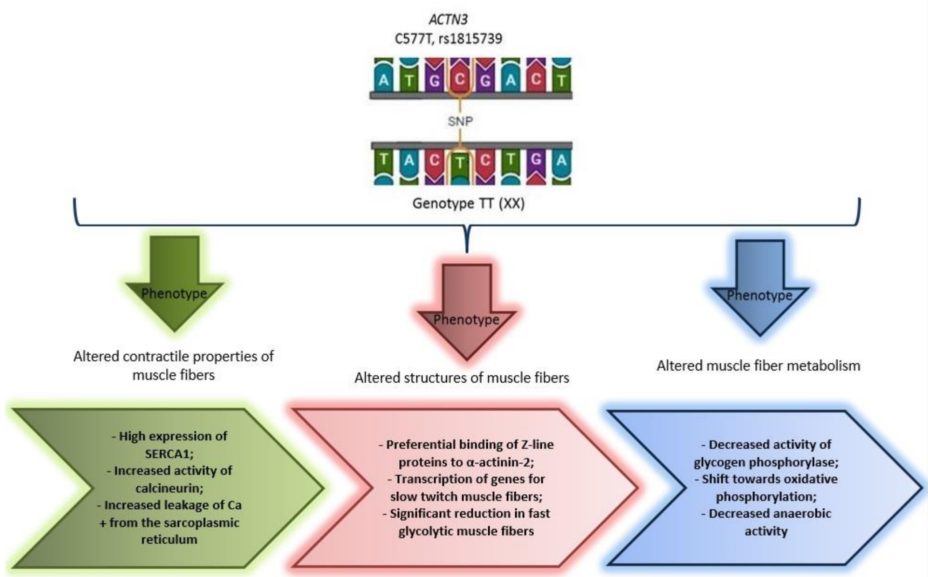

Figure 3. The effect of genetically determined dysfunction of the actinin binding protein of type II fast muscle fibers associated with the single nucleotide variant rs1815739 of the *ACTN3* gene [9].

Thus, our hypothesis, formulated based on the results of the first stage of this study (association of the minor allele T (X) and the homozygous recessive genotype TT (XX) of the rs1815739 variant of the ACTN3 gene with long-distance running (stayers), published earlier (Figure 4) [9]), confirmed by the results of the present stage of the study (association of the minor allele T (X) and the homozygous recessive genotype TT (XX) of the rs1815739 variant of the ACTN3 gene with low lactate clearance during a 10-minute rest period after a bicycle ergometer test with maximum load) among athletes of European origin living in the Southern Urals (Russia).

Conclusions

Our study demonstrated that the minor allele T (X) and the homozygous recessive genotype TT (XX) of the rs1815739 variant of the *ACTN3* gene are statistically significantly associated with low lactate clearance during a 10-minute rest period after performing a bicycle ergometer test with a maximum load, which is most typical for stayers. For athletes of this specialization, it is important to continue to perform moderate-intensity physical activity to ensure accelerated removal of lactate from skeletal muscles to ensure the preservation of their optimal bioenergy reserve and high athletic achievements.

Thus, lactate clearance during a 10-minute rest period after a bicycle ergometer test with maximum load and DNA profiling of the *ACTN3* gene rs1815739 in athletes of cyclic sports can be recommended as significant physiological and genetic predictors of bioenergetic adaptation of skeletal muscles.

Author Contributions: Conceptualization, N.A.S.; methodology, E.V.L.; investigation, O.V.B.; data curation, E.V.L.; writing—original draft preparation, O.V.B. and V.V.T.; visualisation, O.V.B. and V.V.T.; writing—review and editing, N.A.S.; project administration, N.A.S. and E.V.L. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of the Ural State University of Physical Culture (UralSUPC), Protocol No. 5 dated January 11, 2016

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

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Conflicts of Interest: The authors declare no conflict of interest.

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