

**THE COMPARATIVE TRANSCRIPTOME ANALYSIS OF
SHORT- AND LONG-TERM EFFECTS OF ENDURANCE
EXERCISE**

NAME: Alpysova Shakhara
ID NUMBER: 201523697
FACULTY: Department of Sport Management and Rehabilitation
COURSE: Master Research Project / MSMR 701

SUPERVISOR: Syed Ali, Yeltay Rakhmanov

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INTRODUCTION AND PROBLEM STATEMENT

Transcriptomics studies the complete set of RNA and expression levels of mRNAs in a genome, which is widely used in expression profiling. [1] Transcriptomics used for observing the variation in gene expression through comparing two RNA transcripts. It applied to analyze molecular mechanisms that correlates with specific phenotype or in the recognition of biomarkers that vary between two different states. [2] In this research, transcriptome analysis will be used to differentiate how physical exercise affects the expression profile and how the expression differs among people whose frequency of training is higher. Most of the research shows how different types of exercises affect an expression profile, especially which genes are up-or downregulated.

The increased physiological load during change of direction (COD) exercises causes epigenetic changes in skeletal muscle, according to Maasar et al. [3]. These changes have been linked to the evolution of genes involved in muscle adaptations. VEGFA, NR4A3, and NR4A1 expression were shown to be considerably increased 3 hours after COD and straight-line sprint (ST) workouts. (Shakhara, 2021)[20]. The VEGFA for example is involved in the growth of myogenic fibers and protects myogenic cells from apoptosis, minimizing various muscle disorders. [12] This suggests that a specific type of exercise can promote favorable enhancements on specific genes by up-or down-regulation of RNA. According to Maasar et al[3] .s findings, there is a positive association between hypomethylation and gene upregulation, implying that the training involves changes in both DNA and RNA profiles. The genes that were upregulated or downregulated differed between COD and ST exercises. For example, Vissing et al [4] looked at three different training groups: those who got resistance training, endurance training, and a control group. Hundreds of transcripts have been found to be more responsive to one sort of exercise, particularly strength training (Shakhara, 2021)[4][19]. Dickinson et al. conducted a similar experiment in which the response to Aerobic and Resistance exercise was represented by the expression of around 40% more genes at 1 hour post-exercise [6]. In 4h post-exercise samples, however, both aerobic and resistance training triggered a greater number of expressed genes than in 1 h samples [6]. ESRRG

and TNFSRF12A [6], which operate as regulators of muscle mitochondrial activity and oxidative capability, were the genes of particular interest. (Shakhara, 2021). [13][20]

Another study used bioinformatics to look at the impact of chronic and acute resistance exercise (RE) on gene expression in human skeletal muscle and found highly up and down regulated genes [5]. There were 866 up- (270 hypomethylated) and 936 down-regulated genes after a single RE exposure (216 hypermethylated) (Shakhara, 2021). [5][20]

The investigations from several studies prove the expression of several genes for contractile activity upregulated due to physical load. According to research, contractile muscle activity eliminates insulin resistance, thereby playing the role of blocking pro-inflammatory signaling pathways. [14] The goal of Popov's et al [7] investigation was to find genes associated with skeletal muscle contractile activity regardless of fitness level. Some genes linked to genes involved in contractile activity were linked to impacts from acute endurance training [8]. Acute endurance activities resulted in overexpression of transcription factors associated with CREB / ATF / AP1, MYC / MAX, and E2F, all of which are related with skeletal muscle adaptation. (Shakhara, 2021) [8][19].

The majority of studies concentrated on changes in expression profile either from chronic or acute resistance training and very few of them have looked at transcriptome changes within healthy population genome following acute endurance activity. New findings on transcriptome response to physical exercise could help in exercise prescription management and investigate deeply how exactly endurance training is conjugated with favorable changes on the transcriptomic level.

Research questions

Does single endurance training provide enough stimuli for changes in gene expression? How specific training affect the body conditions on molecular level? How short-term effects from acute training in sedentary people will vary from long-term effects from triathletes' samples?

Hypothesis

The acute endurance exercises can serve as a modulator for promoting transcriptomic response.

Rationale

The work sheds light on how acute endurance exercises can act as a modulator for positive gene expression increases. A wide field for future research on molecular pathways involved in endurance training affect is sport as an epigenetic factor. This proposal will examine the potential for short-term changes in the human transcriptome, as well as how these differ from long-term effects. This investigation is significant because it adds to our understanding of how various exercises affect the human body at the molecular level.

OBJECTIVE AND SPECIFIC AIMS

Objective

The objective of this study is to compare the transcriptome from long-term active triathletes with that of short-term moderately active and sedentary individuals.

Specific Aims

1) Enroll study participants

- a. The approval from ethical committee at Nazarbayev University was obtained, and the informed consents were taken, as blood samples were collected. [3] (Appendix)
- b. International Physical Activity Questionnaire (IPAQ) will be used to recruit participants. [9] [Appendix] Participants will be chosen among volunteers from NU students that have sedentary lifestyle, moderate activity lifestyle and triathletes from Triathlon Federation.
- c. Study participants were recruited: 18 people, which will be aged 20 to 30 years. They were divided into 3 experimental groups: 1) sedentary people who will be trained with endurance

exercises, 2) moderate active group who also will be trained with endurance exercises, and 3) elite triathletes.

d. Inclusion criteria:

- Males
- Age range 20+/-10 years old
- Healthy population

e. Exclusion criteria are:

- Health contraindications which bring potentially risks during endurance training
- Individuals with cardiovascular conditions
- Females.
- Vulnerable groups children, under 18, pregnant women

2) Performing Endurance Training Protocol and Collect blood sample

- a. Participants performed one experimental protocol with regards to participant's state of health. The post-exercise samples for 0h, 4h, and 24h were obtained. [3] (See Table 1.).
- b. Participants performed the endurance training protocol that consist of 1 h pf fast walking on treadmill after 5-minutes of warm-up. [7]
- c. The participants wore pulsometer to follow moderate intensity of exercise, the targeted heart rate maintained in the range of 65-75%. peak of heart rate.[17]

3) Conducting experiment, RNA isolation

- a. After obtaining blood samples the RNA isolation was performed using Trizol extraction protocol [9] and isolated RNA samples will proceed to transcriptome analysis.

4) Data analysis

- a. Two-step ANOVA. The one-way analysis of variance was used to compare the data between all genotypes, but because this study included methylome analysis, two independent variables were used, resulting in a two-way ANOVA. To calculate significant differences,

the Tukey's post-hoc test will be utilized [10]. The differential gene expression analysis and ontology analysis could be done additionally to make a scale analysis.

RESEARCH DESIGN AND METHODS

Study Participants

Participants was chosen among volunteers from Nazarbayev University students that have sedentary lifestyle, regularly trained people, and triathletes. There are 2 main reasons why the student was involved:

1. The access to campus site.
2. The similar range of participants' age

IPAQ Questionnaire was used recruit participants. [9] The International Physical Activity Questionnaires comprises a 27 short-answer questions and administered through the in-person interview. The questionnaire categorizing people to low, moderate, and high active according to the scored metabolic equivalent (rate). The purpose of this questionnaire is to provide comparable data on health-related physical activity.[9]

Sample Size

Participants: 18 men ranging in age from 20 to 10 years old. [3] They'll be split into three groups: 1) sedentary persons who will be trained with endurance exercises, 2) elite triathletes who will be trained with endurance exercises, and 3) moderately active people who will be trained with endurance exercises. The 36 samples were tested because each group had six participants.

Table 1. 3 groups involved within research and samples that will be collected with regards to post-exercise time

	Triathletes (n=6)	Regularly trained (n=6)	Sedentary (n=6)

0-hour sample	+	+	+
4-hour sample	-	+	+
24-hour sample	-	+	+

Exercise

The experimental groups will participate in 1-hour treadmill endurance training. The exercises for this training are similar to a triathlete's endurance program. [10]. Participants will have 5-minutes of warm-up. The participants will wear pulsometer to follow moderate intensity of exercise, the targeted heart rate maintained in the range of 65-75%. Peak of heart rate. [17]

Blood Sample Collection [9]

The blood collection protocol will be done at Nazarbayev University Athletic Centre that located on campus by medical practitioner.

Taking blood samples

- i. The vacutainer with participant identification code was prepared
- ii. During sampling the procedure was done sterile conditions.
- iii. The tourniquet was applied above the select venipuncture site
- iv. The site of puncture was cleaned, and the needle was introduced into the vein letting blood into the vacutainer
- v. The tourniquet was released, and the needle removed, to make it safety soft tissue was applied with little pressure to site of puncture

Following the sampling procedure, patients were asked to rest for 5 minutes before being observed to see how they felt. This process was carried out in all three groups during the 0h exercise period, and then repeated with the experimental group in the 4h, and 24h post-exercise periods. Each time, a blood sample of 6 mL was taken.

Materials [9]

- Choloform
- Trizol
- QIAzol® Lysis Reagent (Cat. No. 79306), RNeasy® Mini Kit

Methods

1. Add 750 µl Trizol directly to the maximum of 250 ul whole blood.
2. Shake tube for 15 seconds will speed up lysis process.
3. The 5 minutes of incubation at 56°C will ensure the dissociation of nucleic acids and proteins.

Phase separation

1. Add 200 µl of chloroform
3. Now incubate for 2–3 minutes at 25 C.
4. The centrifugation for 15 min at 12,000 g will provide 2 layers, the top colorless layer is mRNA layer.
5. Remove aqueous layer into the new tube.
6. Introduce RLT (lysis) buffer 700 µl and absolute ethanol 500 ul to the aqueous phase.
7. Pipette this mixture tenderly.

RNA wash & Elution

1. Administer 700 ul of each tube with samples to RNeasy spin column and switch it on.
2. Add 350 µl Buffer RW1 (wash buffer) to every spin column and turn the vacuum on again this done to reach full transfer.
3. Repeat step 2.

4. Administer 500 µl of Buffer RPE (wash buffer) to RNeasy spin column and turn on the vacuum.
5. Repeat step 5.
6. Replace columns in 1.5 ml collection tubes. Introduce 50 ul RNase-free water (elution RNA from membrane) to every spin column membrane. One more time apply high speed centrifugation for 1 minutes.

Using Nanodrop software the concentration and purity of extracted RNA was measured.

(See table 3,4,5)

Data Analysis Strategies

One-way analysis of variance (ANOVA) will be used to compare the records between all genotypes; however, because this study is linked to methylome analysis, two independent variables will be employed, resulting in a two-way ANOVA. To calculate the significant difference, the Tukey's post-hoc test will be utilized [10]. The differential gene expression analysis and ontology analysis could be done additionally to make a scale analysis.

Ethics and Human Subjects Issues

- a) All research subjects participated on voluntary basis
- b) The approval was gotten from Nazarbayev University Institutional Research Ethics Committee. (Appendix)
- c) The informed consent was taken. (Appendix)
- d) Participants were educated about the procedures and about their right to quit the experiment whenever they want.
- e) The procedures within research are considered as risk minimal

RESULTS AND DISCUSSION

To divide participants into three groups according to their activity, the IPAQ was conducted. The results in questionnaire categorizing people to low (LA), moderate (MA), and high

active (EA) based on metabolic equivalent (MET)/minutes, which represents amount of energy needed to perform physical activity. [17] The anthropometric characteristics were measured as well as InBody 777 testing was performed. According to IPAQ scores the range from 0-600 and 600-1500 MET/minutes, for low and for moderate active groups, respectively. The scores that exceed 1500 MET/minutes are for those with high level of activity, in context of this thesis this is the group of triathletes. In this study, the IPAQ answers of participants were analyzed. One of the inconsistencies that appeared during the project is that some participants with sedentary lifestyle had high MET/minutes scores. This could appear due to reliability of the questionnaires. Even though IPAQ is standardized and used valuable tool for quantification physical activity of individuals, it still can introduce errors as the questionnaire score depends on participant's answer. Participants can overestimate or underestimate their activity level and thus the results of the questionnaire can be considered biased. [18] Still IPAQ considered to be standardized tool to measure activity, thus the normalization of the results was done by means of categorizing people by comparing their scores.

Table 2. Anthropometric characteristics, IPAQ scores (MET/minutes) of participants and heart rate during endurance training.

Study Participants	MET/minutes	Weight (kg)	Height (cm)	BMI	Body fat percentage (%)	Age	65-75% heart rate (bt/min)
EA/P1	15707	68	178	21,6	8,7	20	*Non-exercised group
EA/P2	9828	67	176	21,8	7,9	21	
EA/P3	16746	72	183	21,5	6,4	21	
EA/P4	9960	67	178	21,2	14,4	20	
EA/P5	19903	66	173	22,1	9,1	23	

MA/P1	4911	70	173	23.5	19	26	126-146
MA/P2	4688	78	180	24.2	20.2	24	127-147
MA/P3	4110	75	178	23.8	21.5	26	126-146
MA/P4	3280	57	171	19.6	10.2	20	130-150
MA/P5	3705	51	181	15.6	5.6	20	131-151
LA/P1	1298	80	188	22,8	17,9	23	128-148
LA/P2	2483	63	178	19,9	17,3	29	124-143
LA/P3	2992	90	175	29,3	33,4	25	126-146
LA/P4	990	97	188	27,7	26,6	28	124-144
LA/P5	996	93	182	28,1	31,5	28	124-144

-EA=Elite Athlete

-MA=Moderate Active

-LA=Low Active

-P=Participant

-MET/minutes=Metabolic equivalent/ minutes

The blood was transported to Nazarbayev University School of Medicine lab, where the RNA extraction took place. The Trizol RNA extraction protocol was used on 15 samples, 5 participants for each group. Using Nanodrop software the Concentration in μg and A_{260}/A_{280} ratio were measured. The next step the transcriptome analysis, which performed by Macrogen labs, for these manipulations the concentration of RNA should be $>1 \mu\text{g}$. The A_{260}/A_{280} ratio defines the purity of extracted RNA, the acceptable purity of RNA should have ratio of 1.7–2.0 [19] The results for A_{260}/A_{280} ratio and RNA concentration presented in the table 3,4 and 5 below in accordance with activity groups.

Table 3 Low active/ Sedentary group Yield and Sample purity for 0-hour, 4 and 24-hours post-exercise

	0-hour sample	4-hour sample	24-hour sample
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Study	Conc.	A ₂₆₀ /A ₂₈₀	Conc.	A ₂₆₀ /A ₂₈₀	Conc.	A ₂₆₀ /A ₂₈₀
Participants						
LA/P1	0.82 µg	1.84	0.96 µg	1.51	0.9 µg	1.77
LA/P2	1.265 µg	1.91	1.665 µg	1.88	1.235 µg	1.94
LA/P3	3.165 µg	1.99	2.615 µg	1.99	1.465 µg	1.94
LA/P4	2.73 µg	1.91	2.225 µg	1.9	2.435 µg	1.94
LA/P5	2.435 µg	1.94	2.415 µg	1.79	1.545 µg	1.9

Table 4 Moderate Active group Yield and Sample purity for 0-hour, 4 and 24- hours post-exercise

Study	0-hour sample		4-hour sample		24-hour sample	
	Conc.	A ₂₆₀ /A ₂₈₀	Conc.	A ₂₆₀ /A ₂₈₀	Conc.	A ₂₆₀ /A ₂₈₀
Participants						
MA/P1	0.87 µg	1.75	1.1 µg	1.89	0.6 µg	1.92
MA/P2	0.65 µg	1.94	1.045 µg	2.02	1.765 µg	1.95
MA/P3	1.025 µg	1.93	1.105 µg	1.95	1.960 µg	1.99
MA/P4	0.725 µg	1.85	0.745 µg	1.85	1.275 µg	1.68
MA/P5	0.435 µg	2.02	0.845 µg	1.84	1.25 µg	1.81

Table 5 Triathlete group Yield and Sample purity for 0-hour

Study participants	0-Hour sample	
	Concentration (µg)	A ₂₆₀ /A ₂₈₀
EA/P1	1.065 µg	1.74
EA/P2	0.575 µg	1.68
EA/P2	0.63 µg	1.89
EA/P4	0.705 µg	1.99
EA/P5	0.995 µg	1.89

One of the crucial steps in achieving purity (A_{260}/A_{280}) And yield was optimization of protocol. During pre-testing period most samples had poor A_{260}/A_{280} ratio, therefore the troubleshooting strategy was conducted. According to et. *Sar. O.*, one way to solve the problem was to run additional elution steps, to eliminate possible phenol contamination from Trizol reagent. [16] The blood samples were carried in conditions, where the degradation of RNA is minimized, such as working with fresh samples, using inverting sample instead of vortex and conducting experiment with autoclaved (sterile/DNase free) equipment. However, despite that the concentration of RNA in sample still was not enough. To achieve wanted yield, the two portions from one blood sample were taken for extraction separately, they were conducted in parallel and then eluted in one column to increase the concentration of RNA.

Limitations

The limitation of this research is that experiment was not completed till the end. The main challenges with finishing the experiment concentrated on restrictions and timeline mismatch. Due to COVID restrictions the access to laboratory work was delayed as well as late arrival of reagents.

CONCLUSION

Overall, acute endurance activities are likely to act as a modulator for gene expression up- or down-regulation. This project intends to shed light on the differences between short-term and long-term effects on gene expression profiles. This experiment demonstrates how a single type of exercise can be used to modulate variations in expression profiles. One of the advantages of this experiment is that it will clarify how specific acute exercises influence the human body on a molecular level, how this fluctuates within 24 hours of exercise, and how long-term exposure differs. This will serve as a foundation for future study into how acute exercise affects epigenetics and how the organism responds to ongoing endurance exercise. This could be used to answer the following questions: can acute exercise give sufficient stimulation for beneficial

enhancements? What effect does single specific training have on bodily conditions at the molecular level, and do upregulations of the following genes result in improved health or athletic performance? Sport could be used as a tool for gene remodeling and to promote a healthy lifestyle in the future.

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Appendix
IPAQ

#	Question	Answer	
1	Do you currently have a job or do any unpaid work outside your home?	yes	No
2	During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, digging, heavy construction, or climbing upstairs as part of your work?	_ days/ week	
3	How much time did you usually spend on one of those days doing vigorous physical activities as part of your work?	____ hours per day ____ minutes per day	
4	Again, think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do moderate physical activities like carrying light loads as part of your work. Please do not include walking.	____ days per week	
5	How much time did you usually spend on one of those days doing moderate physical activities as part of your work?	____ hours per day ____ minutes per day	
6	During the last 7 days, on how many days did you walk for at least 10 minutes at a time as part of your work? Please do not count any walking you did to travel to or from work.	____ days per week	
7	How much time did you usually spend on one of those days walking as part of your work?	____ hours per day ____ minutes per day	
8	During the last 7 days, on how many days did you travel in a motor vehicle like a train, bus, car, or tram?	____ days per week	
9	How much time did you usually spend on one of those days traveling in a train, bus, car, tram, or other kind of motor vehicle? Now think only about the bicycling and walking you might have done to travel to and from work, to do errands, or to go from place to place.	____ hours per day ____ minutes per day	
10	During the last 7 days, on how many days did you bicycle for at least 10 minutes at a time to go from place to place?	____ days per week	
11	How much time did you usually spend on one of those days to bicycle from place to place?	____ hours per day ____ minutes per day	
12	During the last 7 days, on how many days did you walk for at least 10 minutes at a time to go from place to place?	____ days per week	
13	How much time did you usually spend on one of those days walking from place to place?	____ hours per day ____ minutes per day	
14	Think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do vigorous physical activities like	____ days per week	

	heavy lifting, chopping wood, shoveling snow, or digging in the garden or yard?	
15	How much time did you usually spend on one of those days doing vigorous physical activities in the garden or yard?	_____ hours per day _____ minutes per day
16	Again, think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do moderate activities like carrying light loads, sweeping, washing windows, and raking in the garden or yard?	_____ days per week
17	How much time did you usually spend on one of those days doing moderate physical activities in the garden or yard?	_____ hours per day _____ minutes per day
18	Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do moderate activities like carrying light loads, washing windows, scrubbing floors, and sweeping inside your home?	_____ days per week
19	How much time did you usually spend on one of those days doing moderate physical activities inside your home?	_____ hours per day _____ minutes per day
20	Not counting any walking, you have already mentioned, during the last 7 days, on how many days did you walk for at least 10 minutes at a time in your leisure time?	_____ days per week
21	How much time did you usually spend on one of those days walking in your leisure time?	_____ hours per day _____ minutes per day
22	Think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do vigorous physical activities like aerobics, running, fast bicycling, or fast swimming in your leisure time?	_____ days per week
23	How much time did you usually spend on one of those days doing vigorous physical activities in your leisure time?	_____ hours per day _____ minutes per day
24	Again, think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do moderate physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis in your leisure time?	_____ days per week
25	How much time did you usually spend on one of those days doing moderate physical activities in your leisure time?	_____ hours per day _____ minutes per day
26	During the last 7 days, how much time did you usually spend sitting on a weekday?	_____ hours per day _____ minutes per day

27	During the last 7 days, how much time did you usually spend sitting on a weekend day?	_____ hours per day _____ minutes per day
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Written Informed Consent Form (Moderately Active vs Sedentary group)

Introduction. You are invited to participate in a research study entitled The Comparative Transcriptome Analysis on short- and long-term effects of endurance training

Procedures. *The interview will be conducted to collect data about physical activity status. The Questionnaires comprises a 27 short-answer questions and can be administered through either the in-person interview or online interview through zoom platform (depending on epidemiological situation in Nur-Sultan). The purpose of this questionnaire is to provide comparable data on health-related physical activity. This interview will take approximately 10 minutes to complete.*

The experimental groups will receive endurance trainings that consist of 1 hour treadmill.

Blood collection will be conducted in Nazarbayev University Athletic Center by medical partitioner. The blood sample will be taken 3 times before exercise, after 4- and 24- hours post-exercise. Around 6 ml of blood will be taken. This procedure will take approximately 5 minutes to complete.

Risks. The potential risks of participating in this study are:

The intensity of endurance exercise will be controlled by pulsometer to around 110-130 bp/min in order avoid overstraining of body. Also, warm up procedure will be organised for injury prevention.

The blood collection considered to be a minimal risk procedure as it could potentially cause some sense of discomfort.

Benefits. *This study will help to provide answers for following questions Does single-exercise provide enough stimuli for favorable enhancements? How specific training affect the body conditions on molecular level and do the upregulations of the following genes bring to better health state or athletic performance?*

The investigations could result in better understanding how different exercise and time of exposure to them will results in human genome. This could be beneficial in exercise prescription management.

Compensation. No tangible compensation will be given. A copy of the research results will be available at the conclusion of the study.

Confidentiality & Privacy. Any information that is obtained during this study will be kept confidential to the extent permitted by the law. All efforts, within reason, will be made to keep your personal information in your research record confidential but total confidentiality cannot be guaranteed. *A participant's study information will not be released without the written permission of the participant. All study-related information will be stored securely at the study site. All participant information will be stored in locked file cabinets in locked rooms, i.e., access is limited to study staff. All study data collection, process, and administrative forms and other reports will be identified by a coded number to maintain participant confidentiality. All records that contain names or other personal identifiers, such as locator or informed consent forms, will be stored separately from study records identified by a code number. All databases will be secured with password-protected access systems. Forms, lists, logbooks, appointment books, and any other listings that link participant ID numbers to other identifying information will be stored in a separate, locked file in an area with limited access.*

Voluntary Nature of the Study. Participation in this study is strictly voluntary, and if agreement to participation is given, it can be withdrawn at any time without prejudice.

Points of Contact. It is understood that should any questions or comments arise regarding this project, or a research related injury is received, the Principal Investigator, Shakhara Almyssova, +8.700 328 8583, shakhara.almyssova@nu.edu.kz should be contacted. Any other questions or concerns may be addressed to the Nazarbayev University Institutional Research Ethics Committee, resethics@nu.edu.kz.

Statement of Consent.

I

Give my voluntary consent to participate in this study.

The researchers clearly explained to me the background information and objectives of the study and what my participation in this study involves.

I understand that my participation in this study is voluntary. I can at any time and without giving any reasons withdraw my consent, and this will not have any negative consequences for myself .

I understand that the information collected during this study will be treated confidentially.

Signature: _____ Date: _____

Researcher:

Signed _____ Date _____

Nur-Sultan, 18 December 2021

RE: Decision on the project “The comparative transcriptome analysis on short- and long-term effects of endurance exercises” – NOV#05

The integrated student-driven project “**The comparative transcriptome analysis on short- and long-term effects of endurance exercises**” having **Shakhara Alpysova**, as Primary Investigator, and **Prof. Syed Ali**, as Research Advisor, is part of the Master in Sport Medicine and Rehabilitation curriculum.

The above-mentioned student-driven project was evaluated by the NUSOM-IREC (Nazarbayev University School of Medicine – Institutional Research Ethics Committee) as “Expedite” Ethical review.

*This is to inform you that the aforementioned research has been **approved by the NUSOM- IREC as Application NUSOM-IREC-2021-NOV#05** on December 18th, 2021.*

Prof. Alessandro Salustri

Chair of NUSOM-IREC

