

Biology Extended Essay

[Physiology]

CHANGES IN CIRCUMFERENCES OF
BODY LIMBS AND HEART RATE
AFTER ANAEROBIC EXERCISES

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1. INTRODUCTION

Why this topic?

The topic is particularly interesting to me as I've been involved in sports throughout my whole life and want to learn more about how the muscles work. I did many different sports including dancing and calisthenics (resistance training). There was a lot of talk about muscle swelling, in slang called: "muscle pump". I wanted to explore if muscles actually get bigger after intense exercise. I remembered that I always felt that my muscles look bigger after exercise. However, I didn't know if it was true. How does it happen? Do muscles swell? I wanted to investigate how the circumference of different limbs of the body changes after different anaerobic exercises to see if what we were seeing wasn't just an illusion.

Background theory

Muscle structure:

According to textbook: *Biology (Campbell, 1993)*¹ skeletal muscles are formed of bundles of long fibers. Each fiber represents one muscle cell and consists of many smaller myofibrils; arranged longitudinally. Fundamental unit of organization in muscle cell is called sarcomere. Sarcomeres are lined up in lines along the myofibril. Myofibrils contain two different myofilaments (protein filaments). Thick myofilaments are composed of the myosin. Thin myofilaments contain two strands of actin, tropomyosin, and troponin proteins. At rest, myosin and actin overlap, but not completely.²

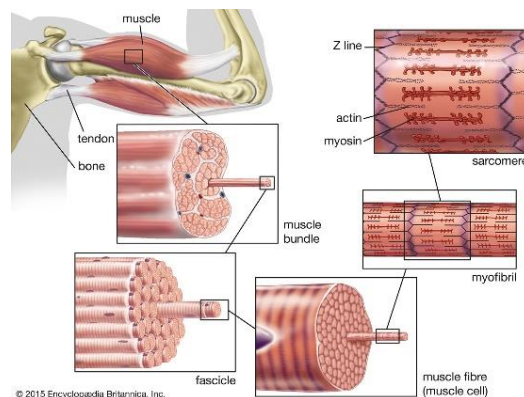


Figure 1: Structure of skeletal muscles (Encyclopædia Britannica, 2015)²

Muscle contraction:

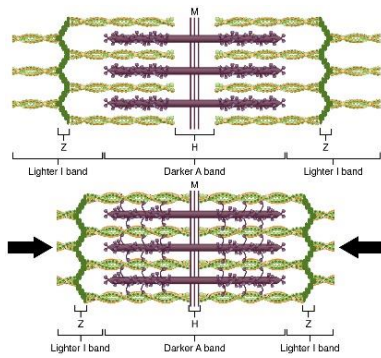


Figure 2: Sliding filament theory (OpenStax, 2013)³

³ When muscles contract, thin and thick filaments slide in opposite directions, passing each other longitudinally, region of overlap increases, and the length of sarcomere becomes shorter (OpenStax, 2013)⁴.

Muscles require ATP in order to contract. ATP is used to form cross-bridges between myosin and actin as well as for transport of calcium ions through active-transport Ca^{2+} pumps in sarcoplasmic reticulum. Calcium ions Ca^{2+} bind to troponin, changing its shape, displacing tropomyosin, and uncovering the binding sites of actin which allows sliding of filaments - muscle contraction.

⁵ The head of myosin can bind with ATP and hydrolyze it into ADP and inorganic phosphate, which produces energy. Energized myosin head can bind with specific site on actin, forming a cross-bridge. This releases energy and myosin attachment changes; it bends inward on itself, pulling the actin towards the centre of sarcomere: power stroke. When new molecule of ATP binds to myosin head, it breaks the bond with actin, and myosin folds back down. Myosin head becomes energized again and binds at a farther along the actin.

For sustained contraction, ATP has to be replaced and regenerated quickly. ATP can generate by different pathways (OpenStax, 2013)⁶. Glycolysis is a non-oxygen-dependent process that breaks glucose to produce pyruvic acid and 2 ATPs. Glucose can arrive from blood glucose or glycogen which is stored in muscles.

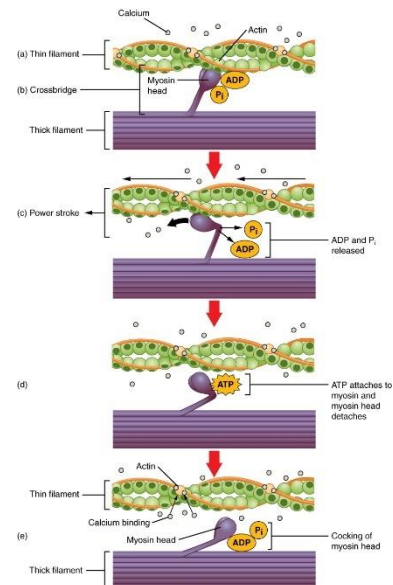


Figure 3: Actin and myosin interaction (OpenStax, 2013)⁵

- When oxygen is available aerobic respiration occurs in mitochondria breaking the glucose with oxygen, producing water, carbon dioxide and ATP. After glycolysis, pyruvic acid is broken down with oxygen. This process is slow and cannot occur without oxygen. It produces 36 ATPs per molecule of glucose.
- During strenuous anaerobic exercise when high amounts of energy are needed, oxygen cannot be supplied sufficiently, oxygen demand becomes greater than oxygen supply. Pyruvic acid converts to lactic acid. This conversion enables regenerating of NADH to NAD⁺, which is needed for glycolysis to continue. The process cannot last for long (1min) but it allows short bursts of high-intensity output. However, glucose isn't utilized very efficiently, producing 2 ATPs per glucose and lactic acid is produced which may contribute to muscle fatigue.

Anaerobic exercises require a quick burst of high energy which are achieved by fast-twitch muscle fibers. Following anaerobic exercises were selected for research: squats, push-ups, calf raises.

Squat involves lowering hips from standing position to the point in which hips are lower than knees and coming back to initial position.

Push-up is performed with prone position of the body with toes and hands on ground and straight back. It involves raising and lowering the body with extending and bending the arms.

Calf raise is performed in standing position, pressing toes to the ground to raise your body by letting your heels of the ground, extending the ankle and standing on your toes.

Different muscle groups are targeted by different exercises. For squats, most active muscles are at thigh region, of which circumference will be measured. During push-ups, exercise dependent on contraction of biceps and triceps, circumference of brachium (upper arm) will be investigated. Calf raises are targeting lower leg muscles, therefore crus (lower leg) will be measured.

Skeletal muscle blood flow

Muscle fibers are surrounded by many capillaries⁷ which are close to the cell to enable smaller diffusion distances for transport of gases: O_2 and CO_2 (Klabunde, 2016)⁸. During exercise, blood flow into the muscles increases; active hyperemia. Because oxygen is needed for aerobic respiration to produce ATP; enable muscle contraction. Cardiac output directed to contracting muscles can increase from 20% to 80%. Skeletal muscle blood flow can increase from 1-4ml/min per 100g to 50-100ml/min per 100g. To help the arterial pressure stay balanced, systemic vascular resistance decreases, resulting in vasodilation. Furthermore, I assumed that can cause circumference increase of the muscle tissue and overall increase in limb circumference.

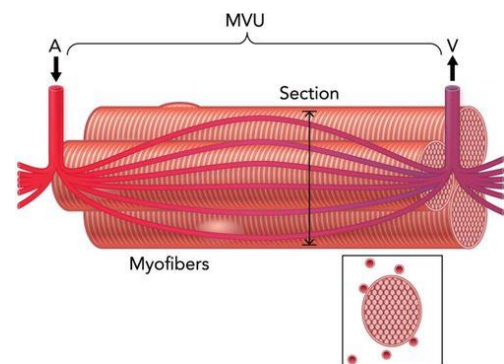


Figure 4: Capillaries surrounding muscle fibers (Latroche, C. 2015)⁷

Do muscle cells swell?

Resistance exercise was shown to cause alternations of intracellular and extracellular water balance (Shoenfeld, 2010)⁹. Muscle cell swelling is highest during exercise that relies on glycolysis, resulting in production of lactate. Lactate causes osmotic changes in skeletal muscles. Especially, fast-twitch fibers. Fast-twitch fibers contain many water channels in sarcolemma, which can facilitate movement of fluid into the cell. Therefore, potentially cause swelling of the cell, increasing its volume.

The study (Sjøgaard, G., Adams, R. P., & Saltin, B. 1985)¹⁰ on six subjects performing one-legged dynamic knee-extensors reported increase of muscle lactate from blood samples of artery and vein

in quadriceps muscle. There was an increase of intracellular amount of water, as well as extracellular. Intracellular potassium concentration decreased from 165mM to 129mM, which affected muscle membrane excitability.

Research questions:

1. How does the circumference (measured with sewing tape) [cm] of the thigh and heart rate (measured with pulse oximeter) [BPM] change after: 5, 10, 15, 20 squats repetitions for non-regularly exercising group; and 5, 10, 15, 20 jumping squats repetitions for regularly exercising group?

How does the circumference (measured with sewing tape) [cm] of the upper arm (brachium) and heart rate (measured with pulse oximeter) [BPM] change after: 3, 7, 11, 15 inclined push-ups repetitions for non-regularly exercising group; and 5, 10, 15, 20 push-ups repetitions for regularly exercising group?

How does the circumference (measured with sewing tape) [cm] of the lower leg (crus) and heart rate (measured with pulse oximeter) [BPM] change after increasing repetitions of calf raises; 5, 10, 15, 20 calf raises repetitions for non-regularly exercising group; and 10, 20, 30, 40 elevated calf raises repetitions for regularly exercising group?

2. Does the heart rate (measured with pulse oximeter) [BPM] correlate to the change in circumferences after increasing repetitions of: squats, push-ups, and calf raises? If so, how?
3. Do the stated circumferences and heart rate change more in the regularly exercising group or non-regularly exercising group?

Hypothesis:

1. Circumferences [cm] of the thigh, brachium, crus and heart rate [BPM] increase more after increasing repetitions of: squats, push-ups, calf raises.
2. Heart rate [BPM] increases as the circumferences increase after increasing repetitions of: squats, push-ups, calf raises.
3. Circumferences and heart rate increase more in the non-regularly exercising group than the regularly exercising group.

Variables:

Independent:

- Non-regularly exercising group (N-Group):
 - 6 young males (age: 17 ± 1 year, weight: 70 ± 15 kg) who exercise 1 or less times a week.
- Regularly exercising group (E-Group):
 - 6 young males (age: 17 ± 1 year, weight: 70 ± 15 kg) who exercise at least twice a week.
- Type of exercise:
 - Squats, Jumping Squats
 - Inclined Push-ups, Push-ups
 - Calf raises
- Number of repetitions of different exercises

Dependent:

- Circumferences [$cm \pm 0.1 cm$] of the limbs: brachium, thigh, crus
- Heart rate [*beats per minute*(BPM) $\pm 3 BPM$]

Controls:

1. Same form of the exercise:

All participants were shown how to perform the exercise correctly and were corrected if the form wasn't right. That is important since different movements of bodies between participants could lead to different use of muscles and unreliable measurements of limbs which worked differently between people. Intensity of an exercise should be equal between participants. When doing squats, people had to place their legs in the same width as their shoulders, and go all the way up and down.

2. Same rhythm of the exercise:

Exercise has to be performed with the same speed, all repetitions have to last for equal time among all participants. Therefore, metronome was used that showed participants at which speed the repetition is made. At every click of the metronome participants either went down (push-ups) or up (at the next click). The speed was 60 beats-per-minute.

3. Same warm-up (3minutes):

Warm-up was led by me so that it was the same for every participant. If different warm-ups would be used their ability to complete the exercise could be different and the circumferences could be affected.

4. Time in between sets: 2minutes:

The time between sets is important since more resting time could lead to muscles repairing their damage from the previous set and different results in the end. Therefore, timer of 2min was switched on right after last repetition of exercise. When timer was finished, participant started with next set.

5. Measuring at the same location of limbs (12, 15, 18 cm) before and after:

The participants were measured at the same location of the limbs:

**Described in Procedure, Step 2.*

6. Measuring relaxed limbs:

Circumferences of relaxed limbs were measured. Participants were told to relax their arms, release them next to their body. While measuring leg limbs, they put their weight on the other leg which wasn't being measured and let the foot rest on the ground while measuring. This is important since all limbs have to be measured at same activity. If some participant would deliberately flex their muscles the circumference would be different and incomparable to different levels of flexing the muscle.

7. Temperature of the room:

The temperature of the room can affect vasodilation which can indirectly affect limb circumference. Hence, experiment was conducted in same room where temperature was constant.

8. Same elevation for push-ups and calf raises:

N-Group performed inclined push-ups on footstool placed on the same place so that the elevation was the same – same difficulty. Different elevations differ in strength and force needed to conduct a push-up, therefore it had to remain the same for whole group.

Likewise, E-Group performed the calf raises on first staircase so that the same difficulty within the group is controlled.

9. No exercise on the experiment day:

Participants were told not to exercise on the day as their muscles could already be tired and they wouldn't be able to exercise. Workout beforehand could give them muscle pump, initial measurement of limb would already be affected and might not change same as it would if muscles haven't been active for at least 12 hours.

2. EXPERIMENT:

Equipment:

- Sewing tape
- Fingertip Pulse Oximeter (RoHS 50D)
- Footstool
- Dark-Inked Pen (BIC)
- Timer (Computer)
- Online Metronome (MUSICCA)¹¹



Figure 5: Footstool



Figure 7: Fingertip Pulse Oximeter



Figure 6: Dark-Inked Pen



Figure 5: Sewing tape

Procedure:

1. Metronome and timer on computer were prepared, as well as sewing tape for measuring.
2. Participant arrives and first measurement takes place before any exercise: brachium, thighs, crus. Brachium was measured 18cm proximal to olecranon(elbow joint). Crus was measured 12cm distal to the inferior pole of patella(knee joint). Thigh was measured 15cm proximal to the superior(upper) pole of patella. Both, left and right limbs were measured to gain more sufficient data. Sewing tape was wrapped around the limb and placed firmly on the skin. Initial location of the tape was outlined using black pen to draw a line on the skin, circling the limb at upper edge of sewing tape. *Measurements were recorded in Excel spreadsheet.*



Figure 6: identifying the point of measurement and measuring circumference of brachium



Figure 7: identifying the point of measurement and measuring circumference of crus

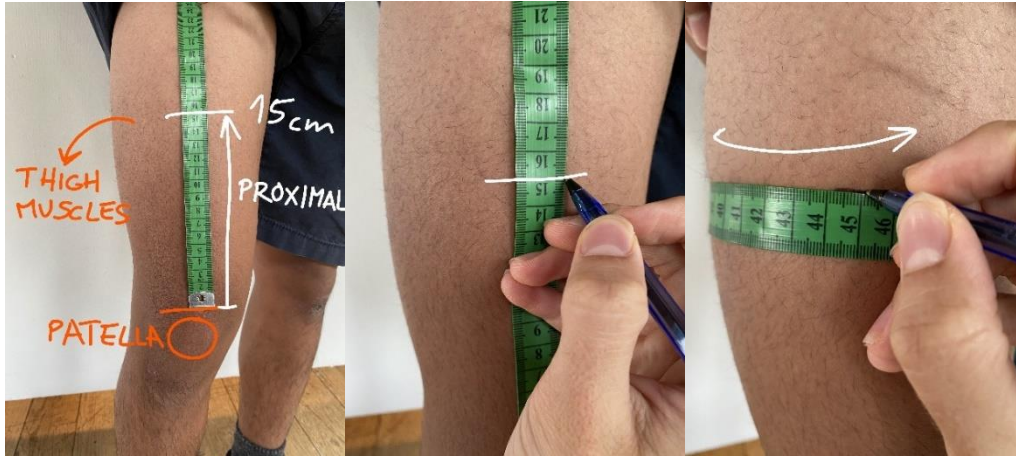


Figure 8: identifying the point of measurement and measuring circumference of thigh

3. 5 minutes timer started for warm-up. With warm-up, possibility of injury is reduced. The review of 9 studies (Fradkin, Gabbe, Cameron, 2006)¹² claims that there is evidence in favour of warm-up reducing injury. We did dynamic movements; 10 circles in each direction (left and right) rotating: head, shoulders, elbows, whole arms, hips, knees, ankles, and wrists. After that followed dynamic stretching to enable body movement in full range of motion, allowing better performance and enhancing muscle power (Opplert, Babault, 2018)¹³: leg swings, walking lunges, spinal rotations, standing to toe touches. Following stretching exercises: quadriceps stretch, knee-to-chest stretch, shoulders stretch, wrist and forearm stretch, calf stretch.
4. Participant from N-Group started with the exercise: squats (sets: 5,10,15,20 repetitions). Heart rate before and after every set was measured. All exercises were done on rhythm of 60 beats-per-minute; one repetition - two beats (first beat-down, second beat-up). Metronome was turned on; participant could hear the beats and squats were performed. There was 2minute timer for break between sets. During that break, thighs were measured with sewing tape on same location that was previously marked by pen. After 2min, same process followed, repetitions increased every next set.



Figure 9: Measuring heart rate with pulse oximeter



Figure 10: one repetition of SQUAT (N-Group)

- Participant moved to next exercise: incline push-ups with legs on floor and hands on footstool. The same process was repeated (4), brachium was measured now. Repetitions: 3,7,11,15.



Figure 11: one repetition of INCLINE PUSHUP (N-Group)

- Calf raises followed and participants had to complete same steps (4), measuring crus. Repetitions: 5,10,15,20.



Figure 12: one repetition of CALF RAISE (N-Group)

- Similar process was repeated by E-Group (1-6). To compare the groups, I decided to adjust the difficulty of the exercise for E-Group, who had to complete harder exercises, meaning that the strength needed is proportionate to their abilities. There was variation in the form and repetitions of exercise. E-Group carried out: Jumping squats with repetitions: 5,10,15,20;

measuring thighs. Push-ups (on the ground) with repetitions: 5,10,15,20; measuring brachium. Elevated calf raises on the stair with repetitions: 10,20,30,40; measuring crus.



Figure 13: JUMPING SQUAT (E-Group)



Figure 14: PUSH-UP (E-group)



Figure 15: CALF RAISE with elevation (E-Group)

Special considerations

SAFETY:

Subjects were separately exercising one by one to prevent potential Covid-19 infections. I washed my hands after measuring every participant.

Exercises could be very demanding for some people. To reduce possibility of injury I led the warm-up before exercise for every participant. I was also asking participants if they are able to do the exercise and how they feel, so that they wouldn't forcefully exceed their limits.

ETHICAL:

I explained participants in detail everything they are expected to do. Making sure subjects will feel comfortable, letting them know that if they are unable to complete the exercise they can stop whenever. They filled in Consent form *[Appendix]*.

3. RESULTS:

Qualitative data:

All participants finished all different repetitions of all the exercises. However, as repetitions increased, the form of performed exercise became worse. Participants were becoming tired, their muscles were in pain, and some didn't follow the rhythm anymore, especially during last set of push-ups. They breathed more heavily after. Subjects were asked how they feel. After push-ups they responded that they feel warmth in their arms and head. They claimed it was harder to do more repetitions and they felt the most pain in their muscles after last set. Some expressed that the feeling of pain was stronger during exercise, and smaller pain was still present afterwards. Furthermore, E-Group had more difficulties in completing jumping squats and push-ups compared to N-Group (normal squats, elevated push-ups). There wasn't a lot of sweating, only one participant said he started to get sweaty after jumping squats. On participant 7 there was redness visible on his arm and neck during and after push-ups. Small red dots after exercise on arm were recognized in two other participants. On participant 11, I saw red spots on the chest as well after push-ups. Some felt sore after, next day.

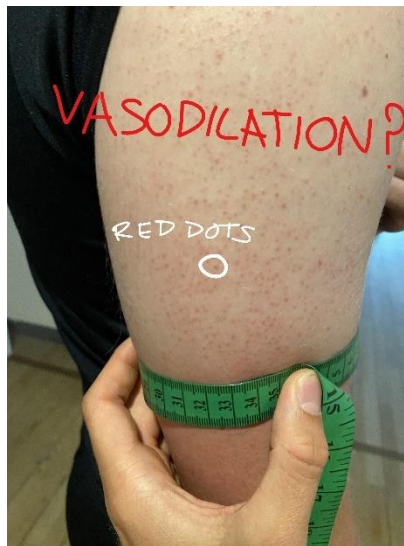


Figure 16: Observation of participant 7;
visible red dots on skin

Quantitative data:

Raw data:

Table 1: Circumferences [$cm \pm 0.1 cm$] of left and right thigh after increasing number of repetitions of squats (0, 5, 10, 15, 20); as well as the heart rate [$BPM \pm 3 BPM$] of participants in non-regularly exercising group before and after every set of squat repetitions

	EXERCISE: SQUATS Repetition number:	CIRCUMFERENCE OF THIGHS [$cm \pm 0.1 cm$]		HEART RATE [$BPM \pm 3 BPM$]	
		Left:	Right:	Before :	After :
PARTICIPANT 1:	0 (Initial)	45	44.9	/	/
	5	45	44.9	83	113
	10	45.2	45	82	127
	15	45.4	45.2	63	135
	20	45.5	45.3	93	141
PARTICIPANT 2:	0 (Initial)	43.2	43.1	/	/
	5	43.5	43.2	67	70
	10	43.5	43.3	69	107
	15	43.6	43.4	55	105
	20	43.6	43.4	70	111
PARTICIPANT 3:	0 (Initial)	44.8	43.6	/	/
	5	44.9	43.8	81	110
	10	45.1	44.3	81	116
	15	45.4	44.4	82	125
	20	45.8	44.7	88	132
PARTICIPANT 4:	0 (Initial)	46.5	46.7	/	/
	5	46.5	46.7	99	107
	10	46.6	46.9	97	115
	15	46.9	47.1	94	129
	20	47	47.2	92	136
PARTICIPANT 5:	0 (Initial)	46.4	47	/	/
	5	46.5	47.2	96	111
	10	46.5	47.5	93	116
	15	46.7	47.7	95	121
	20	47	47.6	92	127
PARTICIPANT 6:	0 (Initial)	46.8	46.4	/	/
	5	46.8	46.4	72	87
	10	46.7	46.6	75	84
	15	46.8	46.7	82	99
	20	46.9	46.8	69	100

Table 2: Circumferences [$cm \pm 0.1 cm$] of left and right thigh after increasing number of repetitions of jumping squats (0, 5, 10, 15, 20); as well as the heart rate [$BPM \pm 3 BPM$] of participants in regularly exercising group before and after every set of jumping squat repetitions

	EXERCISE: JUMPING SQUATS Repetition number:	CIRCUMFERENCE OF THIGHS [$cm \pm 0.1 cm$]		HEART RATE [$BPM \pm 3 BPM$]	
		Left:	Right:	Before :	After :
PARTICIPANT 7:	0 (initial)	54.4	53.4	/	/
	5	54.5	53.6	77	126
	10	55	53.8	81	125
	15	55.1	53.9	87	128
	20	55.6	54.1	85	142
PARTICIPANT 8:	0 (initial)	48.1	48.8	/	/
	5	48.1	49	72	105
	10	48.5	49.4	83	113
	15	48.4	49.5	99	116
	20	48.7	49.5	94	117
PARTICIPANT 9:	0 (initial)	53	53	/	/
	5	53.3	53.1	60	82
	10	53.4	53.3	56	107
	15	53.5	53.4	59	114
	20	53.6	53.7	57	109
PARTICIPANT 10:	0 (initial)	52	52.8	/	/
	5	52.3	53.1	64	99
	10	52.6	53.2	62	114
	15	52.5	53.5	58	128
	20	52.6	53.7	68	134
PARTICIPANT 11:	0 (initial)	48	47.7	/	/
	5	48.1	47.9	73	112
	10	48.1	47.7	72	134
	15	48.5	48.2	89	139
	20	48.8	48.3	107	158
PARTICIPANT 12:	0 (initial)	46.4	46.7	/	/
	5	46.5	47	80	115
	10	46.7	47.3	80	125
	15	46.7	47.4	85	134
	20	47.1	47.5	84	143

Table 3: Circumferences [$cm \pm 0.1 cm$] of left and right brachium after increasing number of repetitions of inclined push-ups (0, 3, 7, 11, 15); as well as the heart rate [$BPM \pm 3 BPM$] of participants in non-regularly exercising group before and after every set of inclined push-up repetitions

	EXERCISE: INCLINED PUSH-UPS Repetition number:	CIRCUMFERENCE OF BRACHIUM [$cm \pm 0.1 cm$]		HEART RATE [$BPM \pm 3 BPM$]	
		Left:	Right:	Before :	After :
PARTICIPANT 1:	0 (Initial)	27.0	27.0	/	/
	3	27.2	27.1	104	117
	7	27.3	27.3	93	109
	11	27.6	27.5	92	123
	15	27.6	27.6	95	130
PARTICIPANT 2:	0 (Initial)	25.8	26.0	/	/
	3	25.6	25.8	66	73
	7	25.7	25.8	74	94
	11	25.9	26.0	68	94
	15	26.2	26.0	72	91
PARTICIPANT 3:	0 (Initial)	25.8	25.7	/	/
	3	26.0	25.9	93	112
	7	26.1	25.7	92	112
	11	26.1	25.9	88	115
	15	26.2	26.1	93	120
PARTICIPANT 4:	0 (Initial)	24.6	24.5	/	/
	3	24.8	24.7	99	106
	7	24.8	24.6	98	115
	11	24.9	24.7	101	131
	15	25.1	24.9	106	135
PARTICIPANT 5:	0 (Initial)	28.1	27.4	/	/
	3	28.0	27.4	89	106
	7	28.1	27.4	90	115
	11	28.3	27.3	94	111
	15	28.5	27.4	91	113
PARTICIPANT 6:	0 (Initial)	25.3	25.8	/	/
	3	25.6	25.9	87	90
	7	25.7	25.9	65	88
	11	25.9	26	83	93
	15	26.2	26	71	90

Table 5: Circumferences [$cm \pm 0.1 cm$] of left and right brachium after increasing number of repetitions of push-ups (0, 5, 10, 15, 20); as well as the heart rate [$BPM \pm 3 BPM$] of participants in regularly exercising group before and after every set of push-up repetitions

	EXERCISE: PUSH-UPS Repetition number:	CIRCUMFERENCE OF BRACHIUM [$cm \pm 0.1 cm$]		HEART RATE [$BPM \pm 3 BPM$]	
		Left:	Right:	Before :	After :
PARTICIPANT 7:	0 (Initial)	33.5	32.3	/	/
	5	33.9	32.3	90	133
	10	34.5	32.6	92	135
	15	34.5	32.8	89	157
	20	34.6	33.2	99	165
PARTICIPANT 8:	0 (Initial)	32.5	33.1	/	/
	5	32.7	32.4	69	120
	10	33.3	32.9	90	114
	15	33.3	33.4	96	111
	20	33.3	33.2	88	118
PARTICIPANT 9:	0 (Initial)	28.5	28.5	/	/
	5	29.3	29.1	58	80
	10	29.3	29.2	55	83
	15	29.3	29.1	65	92
	20	29.3	29.3	70	89
PARTICIPANT 10:	0 (Initial)	32.1	32.1	/	/
	5	32.3	32.4	76	90
	10	32.8	32.6	70	102
	15	32.5	32.9	80	105
	20	32.7	32.9	81	111
PARTICIPANT 11:	0 (Initial)	30	30.2	/	/
	5	30	30.2	113	107
	10	29.8	30.3	102	133
	15	29.8	30.3	106	136
	20	30	30.5	107	133
PARTICIPANT 12:	0 (Initial)	29.3	29.1	/	/
	5	29.7	29.5	96	114
	10	29.7	29.5	97	119
	15	29.8	29.5	98	125
	20	29.8	29.6	105	138

Table 5: Circumferences [$cm \pm 0.1 cm$] of left and right crus after increasing number of repetitions of calf raises (0,5,10,15,20); as well as the heart rate [$BPM \pm 3 BPM$] of participants in non-regularly exercising group before and after every set of calf raise repetitions

	EXERCISE:	CIRCUMFERENCE OF CRUS		HEART RATE	
	CALF RAISES	[$cm \pm 0.1 cm$]		[$BPM \pm 3 BPM$]	
	Repetition number:	Left:	Right:	Before :	After :
PARTICIPANT 1:	0 (Initial)	38.9	38.8	/	/
	5	39.1	39.0	109	120
	10	39.2	39.2	95	116
	15	39.4	39.3	98	109
	20	39.5	39.5	97	117
PARTICIPANT 2:	0 (Initial)	34.8	34.3	/	/
	5	35.2	34.6	73	78
	10	35.4	34.7	83	82
	15	35.7	34.8	81	86
	20	35.4	34.8	86	93
PARTICIPANT 3:	0 (Initial)	35.5	35.1	/	/
	5	35.6	35.3	81	93
	10	35.6	35.2	92	105
	15	35.8	35.4	88	103
	20	35.7	35.4	89	105
PARTICIPANT 4:	0 (Initial)	33.6	33.5	/	/
	5	33.9	33.8	110	110
	10	34.1	34.0	92	109
	15	34.2	34.0	105	120
	20	34.3	34.1	104	118
PARTICIPANT 5:	0 (Initial)	33.8	33.5	/	/
	5	34.3	33.9	98	111
	10	34.4	34.1	99	101
	15	34.4	34.2	98	108
	20	34.3	34.3	94	105
PARTICIPANT 6:	0 (Initial)	34.6	35.7	/	/
	5	34.5	35.1	82	87
	10	34.8	34.8	79	82
	15	34.9	34.9	78	88
	20	35.1	34.7	86	87

Table 6: Circumferences [$cm \pm 0.1 cm$] of left and right crus after increasing number of repetitions of calf raises (0,10,20,30,40); as well as the heart rate [$BPM \pm 3 BPM$] of participants in regularly exercising group before and after every set of calf raise repetitions

	EXERCISE:	CIRCUMFERENCE OF CRUS		HEART RATE	
	CALF RAISES	[$cm \pm 0.1 cm$]		[$BPM \pm 3 BPM$]	
	Repetition number:	Left:	Right:	Before :	After :
PARTICIPANT 7:	0 (Initial)	36.3	38.8	/	/
	10	36.6	39.1	111	129
	20	36.6	39.2	105	120
	30	36.8	39.3	95	122
	40	37.1	39.4	100	108
PARTICIPANT 8:	0 (Initial)	36.1	36.6	/	/
	10	36.6	37.4	87	102
	20	36.7	37.4	96	109
	30	37.0	37.5	88	99
	40	37.0	37.5	82	105
PARTICIPANT 9:	0 (Initial)	35.7	35.2	/	/
	10	35.8	35.0	71	86
	20	36.0	35.1	74	84
	30	36.3	35.4	69	94
	40	36.3	35.8	76	93
PARTICIPANT 10:	0 (Initial)	39.8	40.0	/	/
	10	40.2	40.1	88	100
	20	40.3	40.3	82	93
	30	40.7	40.8	77	105
	40	40.9	41.1	83	110
PARTICIPANT 11:	0 (Initial)	35.9	35	/	/
	10	36.1	35.3	106	113
	20	36.3	35.3	113	115
	30	36.4	35.6	110	115
	40	36.6	35.9	106	114
PARTICIPANT 12:	0 (Initial)	35	35.1	/	/
	10	35.1	35.3	109	117
	20	35.2	35.6	109	120
	30	35.4	35.6	105	115
	40	35.6	35.9	105	127

Processed data:

**Information about how Processed data was calculated is in Appendix.*

Processed data tables:

Table 7: Average change in circumference [mm] of thighs (with standard deviation) after increasing number of repetitions of squats (5, 10, 15, 20); as well as the average change in heart rate [BPM] of participants (with standard deviation) in non-regularly exercising group [N-Group] before and after every set of squat repetitions

N-GROUP				
EXERCISE: SQUATS	Average change in circumference of thighs	Standard deviation	Average change in heart rate	Standard deviation
Repetition number:	[mm]		[BPM]	
5	0.8	0.99	17	10.0
10	2.3	1.97	28	12.4
15	4.1	2.02	41	17.7
20	5.3	2.66	41	5.8

Table 8: Average change in circumference [mm] of thighs (with standard deviation) after increasing number of repetitions of jumping squats (5, 10, 15, 20); as well as the average change in heart rate [BPM] of participants (with standard deviation) in regularly exercising group [E-Group] before and after every set of jumping squat repetitions

E-GROUP				
EXERCISE: JUMPING SQUATS	Average change in circumference of thighs	Standard deviation	Average change in heart rate	Standard deviation
Repetition number:	[mm]		[BPM]	
5	1.8	0.99	36	8.0
10	3.9	1.89	47	9.7
15	5.3	1.42	47	16.0
20	7.4	1.66	51	13.6

Table 9: Average change in circumferences [*mm*] of brachium (with standard deviation) after increasing number of repetitions of inclined push-ups (3, 7, 11, 15); as well as the average change in heart rate [*BPM*] of participants (with standard deviation) in non-regularly exercising group [N-Group] before and after every set of inclined push-up repetitions

N-GROUP				
EXERCISE: INCLINED PUSH-UPS	Average change in circumference of brachium	Standard deviation	Average change in heart rate	Standard deviation
Repetition number:	[mm]		[BPM]	
3	0.8	1.62	11	5.8
7	1.2	1.77	20	3.1
11	2.6	2.10	24	7.5
15	4.0	2.42	25	5.8

Table 10: Average change in circumferences [*mm*] of brachium (with standard deviation) after increasing number of repetitions of push-ups (5, 10, 15, 20); as well as the average change in heart rate [*BPM*] of participants (with standard deviation) in regularly exercising group [E-Group] before and after every set of push-up repetitions

E-GROUP				
EXERCISE: PUSH-UPS	Average change in circumference of brachium	Standard deviation	Average change in heart rate	Standard deviation
Repetition number:	[mm]		[BPM]	
5	2.2	3.62	24	18.8
10	4.4	3.73	30	6.8
15	5.0	3.21	32	16.8
20	6.0	3.19	34	15.0

Table 11: Average change in circumferences [*mm*] of crus after increasing number of repetitions of calf raises (5, 10, 15, 20); as well as the average change in heart rate [*BPM*] of participants in non-regularly exercising group [N-Group] before and after every set of calf raise repetitions

N-GROUP				
EXERCISE: CALF RAISES	Average change in circumference of crus	Standard deviation	Average change in heart rate	Standard deviation
Repetition number:	[mm]		[BPM]	
5	1.8	2.79	8	4.7
10	2.8	3.98	9	8.3
15	4.1	4.01	11	3.4
20	4.2	4.56	12	6.2

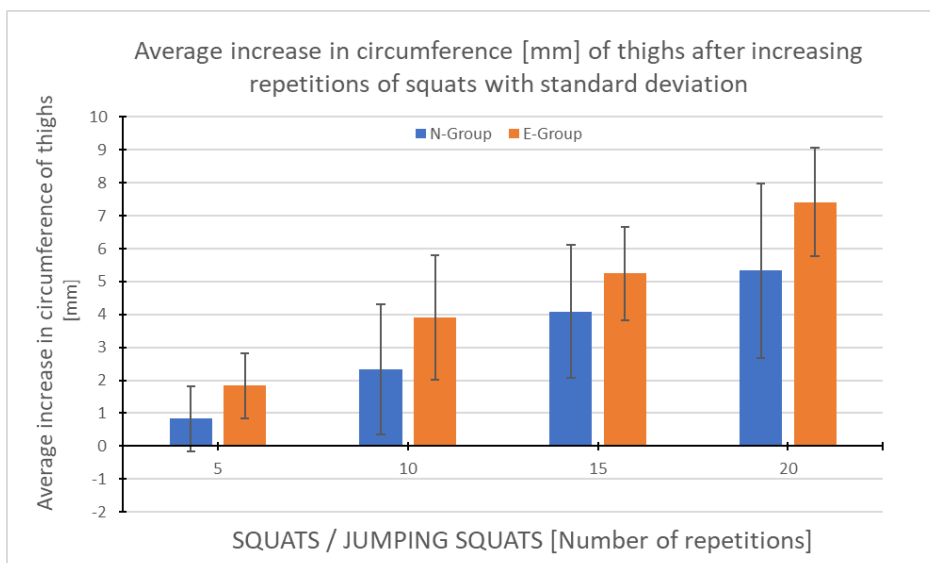
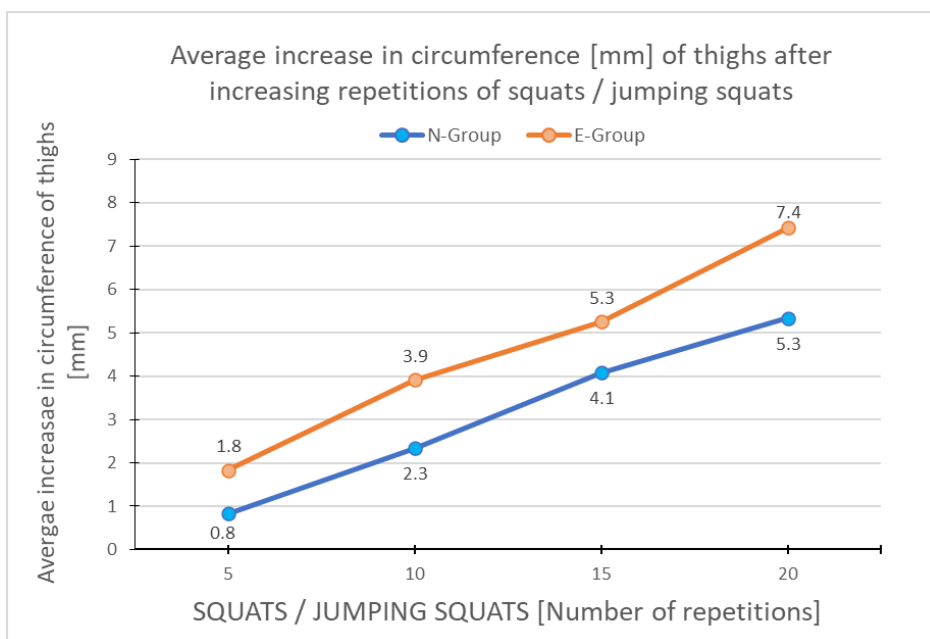
Table 12: Average change in circumferences [*mm*] of crus after increasing number of repetitions of calf raises (10, 20, 30, 40); as well as the average change in heart rate [*BPM*] of participants in regularly exercising group [E-Group] before and after every set of calf raise repetitions

E-GROUP				
EXERCISE: CALF RAISES	Average change in circumference of crus	Standard deviation	Average change in heart rate	Standard deviation
Repetition number:	[mm]		[BPM]	
10	2.6	2.36	13	3.9
20	3.8	2.13	10	4.1
30	6.1	2.14	18	9.2
40	8.0	1.78	18	7.3

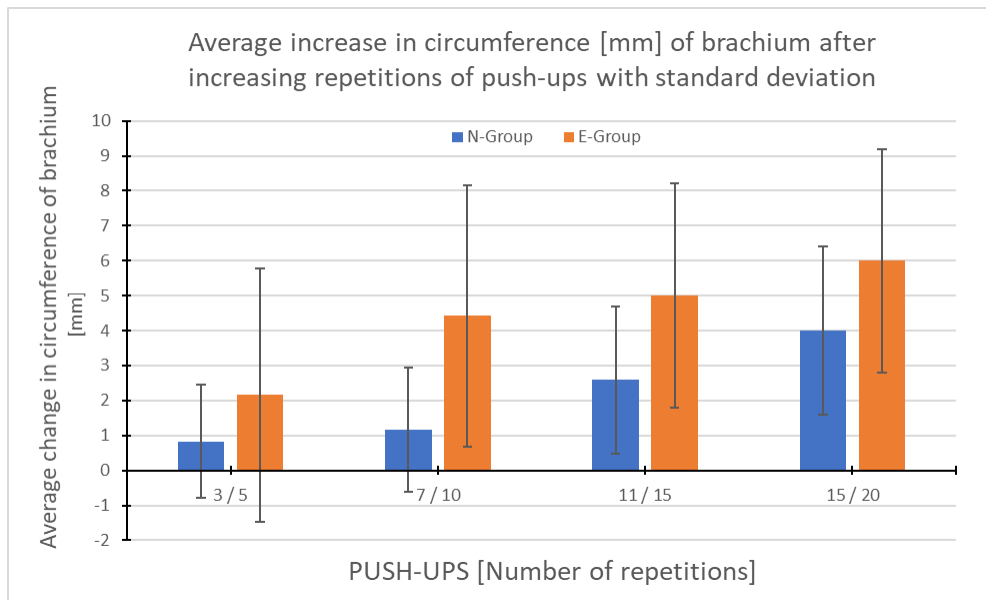
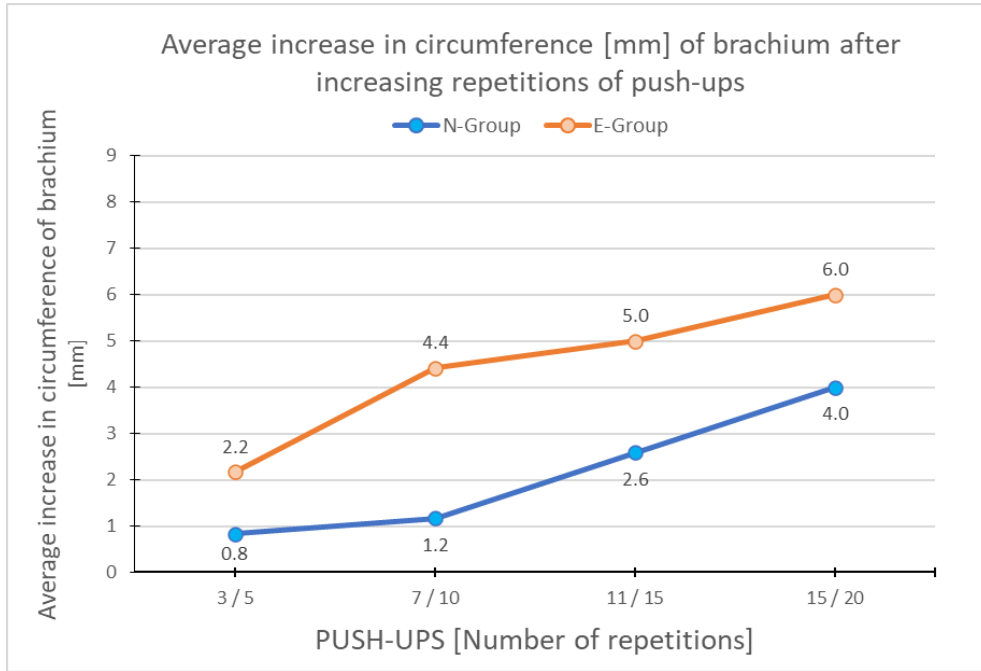
Graphs:

**Since the average changes were all positive, I decided that graphs will be shown as “average increase in circumference...” To show the data more accurately, the bar graph with standard deviation was made separately since standard deviations would otherwise overlap each other and would be harder to recognize.*

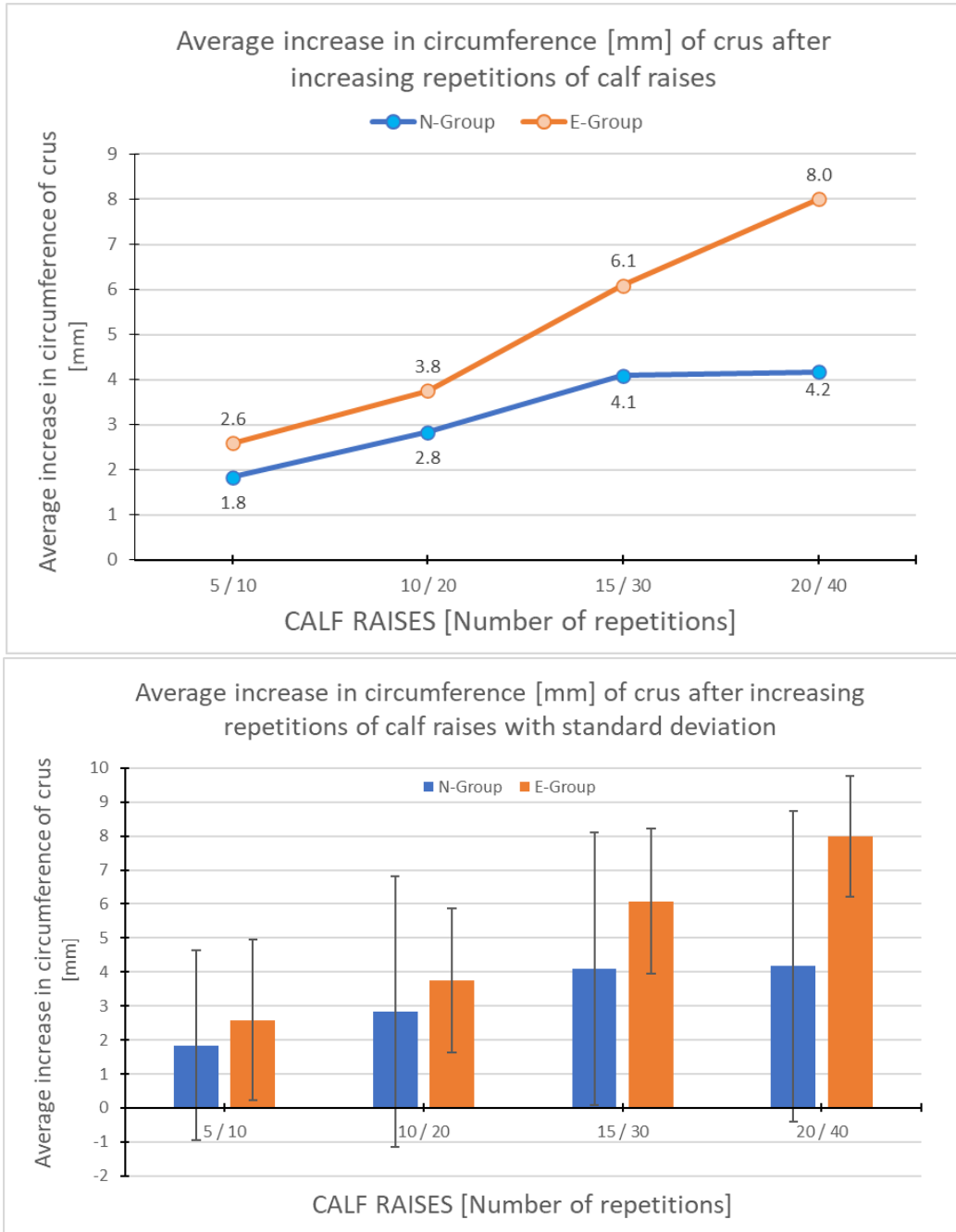
Graph 1: Average increase in circumference [mm] of thighs after increasing number of repetitions of squats (5, 10, 15, 20) in non-regularly exercising group [N-Group]; as well as average increase in circumference [mm] of thighs after increasing number of repetitions of jumping squats (5, 10, 15, 20) in regularly exercising group [E-Group]



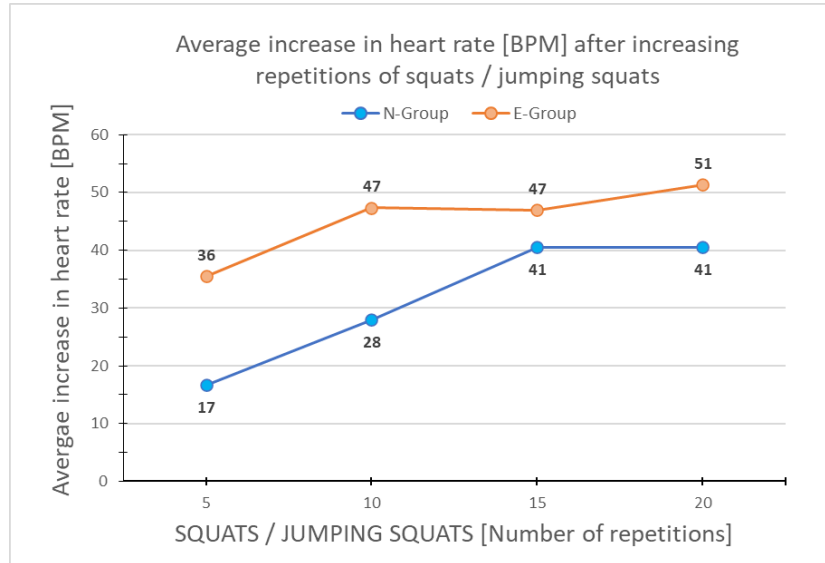
Graph 2: Average increase in circumference [mm] of brachium after increasing number of repetitions of inclined push-ups (3, 7, 11, 15) in non-regularly exercising group [N-Group]; as well as average increase in circumference [mm] of brachium after increasing number of repetitions of push-ups (5, 10, 15, 20) in regularly exercising group [E-Group]



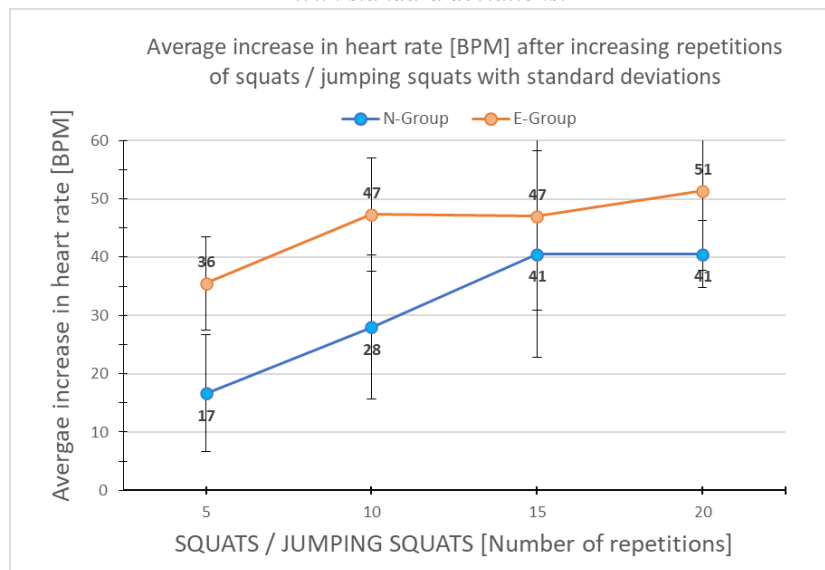
Graph 3: Average increase in circumference [mm] of crus after increasing number of repetitions of calf raises (5, 10, 15, 20) in non-regularly exercising group [N-Group]; as well as average increase in circumference [mm] of crus after increasing number of repetitions of calf raises (10, 20, 30, 40) in regularly exercising group [E-Group]



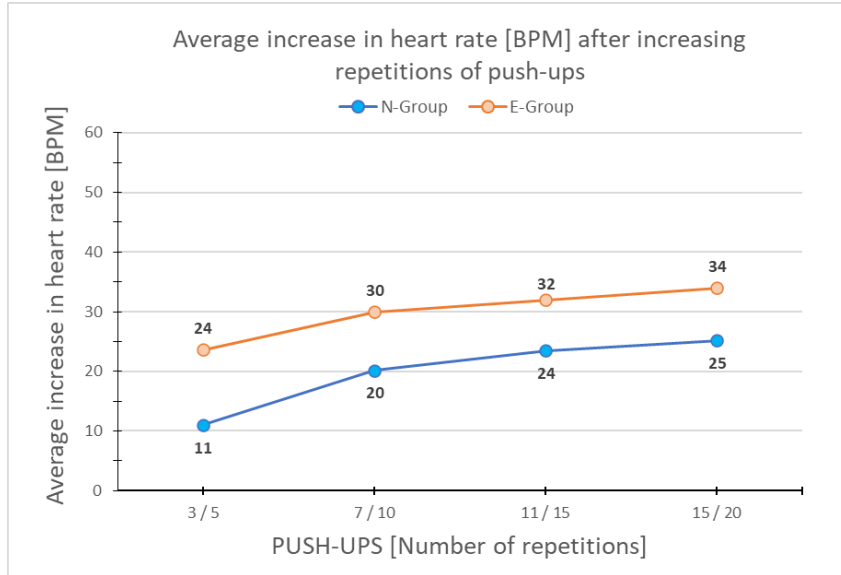
Graph 1: Average change in circumference [mm] of thighs after increasing number of repetitions of squats (5, 10, 15, 20) in non-regularly exercising group [N-Group]; as well as average change in circumference [mm] of thighs after increasing number of repetitions of jumping squats (5, 10, 15, 20) in regularly exercising group [E-Group]



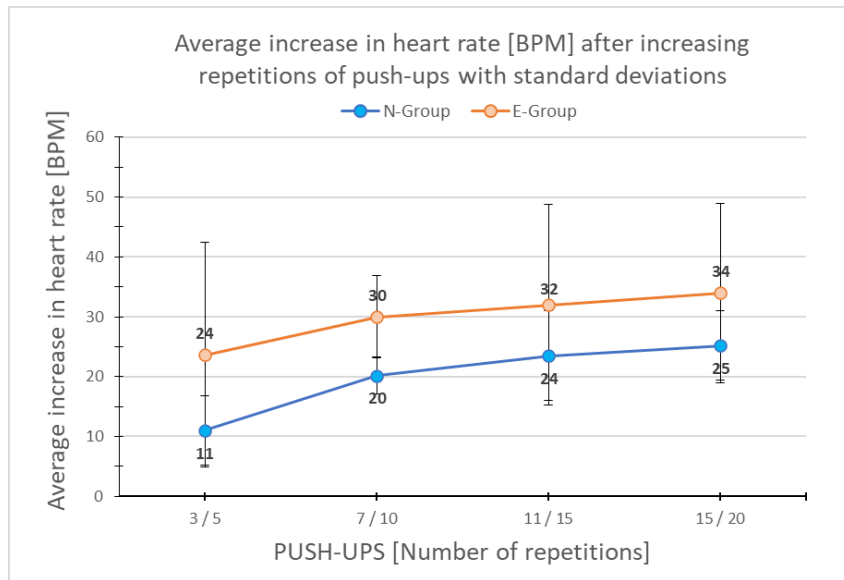
**With standard deviations:*



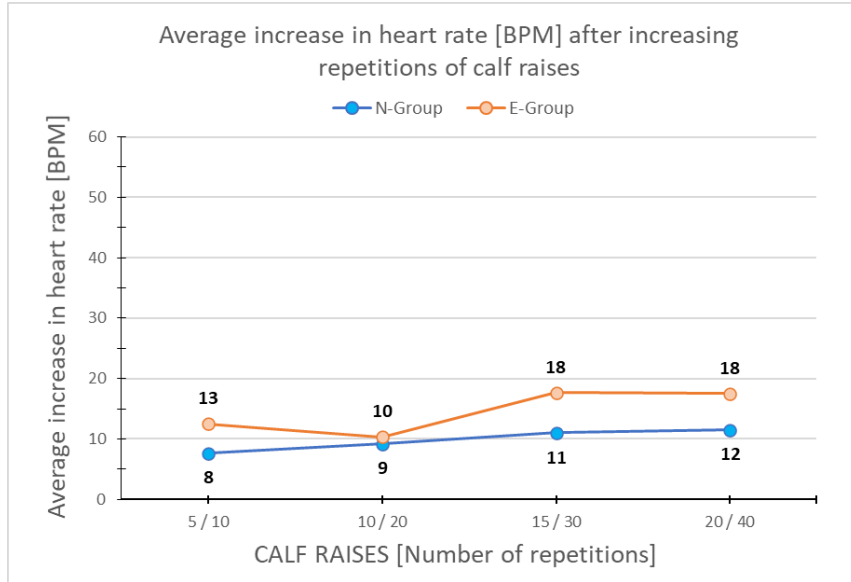
Graph 2: Average increase in heart rate [BPM] after increasing number of repetitions of inclined push-ups (3, 7, 11, 15) in non-regularly exercising group [N-Group]; as well as average increase in heart rate [BPM] after increasing number of repetitions of push-ups (5, 10, 15, 20) in regularly exercising group [E-Group]



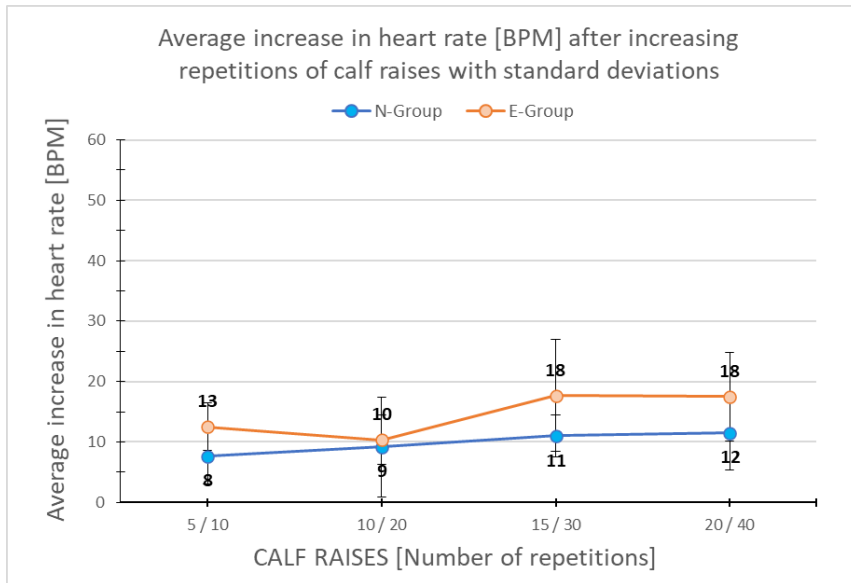
**With standard deviations:*



Graph 3: Average increase in heart rate [BPM] after increasing number of repetitions of calf raises (5, 10, 15, 20) in non-regularly exercising group [N-Group]; as well as average increase in heart rate [BPM] of brachium after increasing number of repetitions of calf raises (10, 20, 30, 40) in regularly exercising group [E-Group]



**With standard deviations:*



4. DISCUSSION:

ANALYSIS:

Circumference:

Average circumference of all the limbs measured changed after repetitions in all exercises, circumference increased. Average circumference of body limbs increased as the number of repetitions increased. Paired T-Tests were made to prove that there is significant increase from initial measurement.

Table 13: Paired T-Test (with p-values) between average circumference of: thighs (after squats), brachium (after push-ups), crus (after calf raises) after increasing repetitions (sets: 1., 2., 3., 4.) for non-regularly exercising group [N-Group]:

N-Group	P-VALUES		
	Circumference of thigh after squats	Circumference of brachium after push-ups	Circumference of crus after calf raises
Between sets:			
1. and 2.	0.007	0.305	0.060
2. and 3.	0.000	0.001	0.000
3. and 4.	0.011	0.001	0.857

Table 14: Paired T-Test (with p-values) between average circumference of: thighs (after squats), brachium (after push-ups), crus (after calf raises) after increasing repetitions (sets: 1., 2., 3., 4.) for regularly exercising group [E-Group]:

E-Group	P-VALUES		
	Circumference of thigh after squats	Circumference of brachium after push-ups	Circumference of crus after calf raises
Between sets:			
1. and 2.	0.003	0.015	0.001
2. and 3.	0.028	0.339	0.000
3. and 4.	0.000	0.046	0.001

Green cells ($p\text{-value} < 0.05$) show that there is significant difference between initial circumference and circumference after certain set of exercise. To re-assure the difference was in circumference of crus in N-Group (*Table 13*), I made T-Test between 1. and 4. set, $p < 0.05$ ($p = 0.0116$).

Graphs show that circumferences increased more in E-Group than N-group. However, due to high standard deviation and uncertainty, two-sample T-Test was conducted to compare values (increase in circumference) of E-Group and N-Group for every specific set for every exercise.

Table 15: Two-sample T-Test (with p-values) between non-regularly exercising group [N-Group] and regularly exercising group [E-Group] comparing change in limb circumferences (thigh, brachium, crus) after every set of every exercise:

Compared set number	P-VALUES		
	Circumference of thigh after squats	Circumference of brachium after push-ups	Circumference of crus after calf raises
1	0.03	0.28	0.50
2	0.07	0.02	0.51
3	0.13	0.05	0.16
4	0.04	0.11	0.02

Majority of p-values are greater than 0.05; there isn't significant difference (red-coloured cells) between change in circumference of limbs between N-Group and E-Group. However, $p < 0.05$ in last set of squats and calf raises, which means that circumferences increased more in E-group.

Comparison between the groups

I hypothesized that circumferences are going to increase more in N-Group, however the results show differently. I assume that E-Group had to complete a lot more difficult exercises and the proportions weren't equal compared to N-Group. If N-Group had more repetitions, I assume their circumferences will still increase more due to worse efficiency of mitochondria in N-Group and slower aerobic respiration, leading to more anaerobic respiration and accumulation of lactate. Therefore, an improvement for experiment could be having slightly easier exercises for E-Group.

Heart rate:

On average, heart rate increased as number of repetitions increase in both groups after all exercises. Average heart rate increases more in E-Group. However, due to high standard deviation and uncertainty, two-sample T-Test was conducted to compare values (increase in heart rate) between E-Group and N-Group after every specific set for every exercise.

Table 16: Two-sample T-Test (with p-values) between non-regularly exercising group [N-Group] and regularly exercising group [E-Group] comparing change in heart rate after every set of every exercise:

Compared set number	P-VALUES		
	Heart rate after squats	Heart rate after push-ups	Heart rate after calf raises
1	0.01	0.20	0.11
2	0.02	0.02	0.78
3	0.56	0.34	0.18
4	0.15	0.26	0.19

Majority of p-values are greater than 0.05 which means there isn't significant difference between change in heart rate of the two groups.

It seems that circumferences increase with heart rate increasing in squats and push-ups. Therefore, we could assume that circumference of limbs increases because heart rate increases to supply oxygen and nutrients to working muscles and enable ATP synthesis. That could lead to vasodilation in veins and arteries. Vascular resistance decreases and cardiac output increases. Leading to an overall increase in circumference. For calf raises it is questionable that the heart rate has a role in circumference (*discussed in evaluation*).

Why did the limb circumference actually increase?

Method involved measuring circumferences of whole limb, meaning that many factors could affect it. Hence, we need to evaluate what played main role in increasing circumference. Due to limited equipment, I was unable to see what was happening in muscle cells directly and had to think from the outside how we can explain limb circumference increase using obtained results.

Vasodilation

Vasodilation of veins could play a factor in increasing circumference since heart rate increases, cardiac output increases, and blood vessels widen. However, smooth muscle cells in middle layer of arteries have to relax to allow vasodilation and decreased total peripheral resistance (*Wikipedia, 2021*)¹⁴. Does the outer circumference of the arteries actually increase during vasodilation? Or do the smooth muscles in the middle layer of arteries and arterioles shrink to increase the volume inside arteries? If second is true, overall volume of the limb wouldn't increase because of vasodilation.

Also, vasodilation to dissipate heat occurs when exercise raises body temperature (*Gleeson, 1998*)¹⁵. To release additional heat and contain homeostasis our body responds by thermoregulatory vasodilation (*Charkoudian, 2003*)¹⁶, which increases skin blood flow to dissipate heat. I saw that by red dots on skin of arms after participant concluded push-ups.

Heart rate

Interestingly, HR changed less after calf raises (more static exercise) than push-ups and squats. Majority of force is dependent on calf muscles and HR doesn't increase more after more repetitions. However, circumference of crus increases more than proportionately to HR increase. When comparing crus circumference increase to brachium and thigh increase, we can see that crus increased the most in E-Group: 8mm. The p-value of 4th set of calf raises is showing significant difference between E-Group and N-Group. Furthermore, p-value of the same set for heart rate doesn't show difference between the groups. This shows that HR wasn't different from the groups,

but circumferences were, which could mean that circumference isn't depended only on heart rate. That observation could help us explain why limb circumference increases. Is it really solely because of HR increase, cardiac output increase; leading to vasodilation? Although there was small HR increase, circumferences increased similarly to other exercises which were accompanied with higher HR. These observations suggest that muscle cells might increase in volume because of contraction processes.

ATP deficit?

According to sliding filament theory, ATP is required to break attachment of myosin heads to actin-binding sites: cross-bridge. That could indicate that without enough ATP, actin molecules could stay attached to myosin heads. In absence of ATP cross-bridges stay in place. I wondered if muscles can stay contracted without sufficient amount of ATP that occurs during anaerobic exercise. I was thinking further; after doing an intense set of push-ups, my muscles still feel a bit contracted. I have a feeling that my arms aren't fully extended, sensing a force of contraction, pulling in the elbow. That could show how deficit in oxygen can lead to anaerobic production of ATP, and lack of ATP to fully break and recover all the myosin-actin attachments, leading to shorter sarcomeres after exercise. Then, I asked myself: "If sarcomeres are shorter, does it really mean that muscle cell will have a greater width or volume, and therefore greater circumference of the limb?".

pH

Lactic acid produced in anaerobic respiration lowers cellular pH which alters functionality of enzymes that are needed to produce energy from glucose. At lower pH it will be harder to produce ATP and muscles to contract (*Robb, 2021*)¹⁷. I assume that pH could also change functionalities of transport enzymes which could result in different membrane potential and assuming; increased volume of the cell.

Lactic acid draws water?

The article (*Capritto, 2021*)¹⁸ explains that lactic acid builds up in muscles and draws water into them, making muscle cell to swell. However, the article doesn't include any scientific experimentation, data or results, and is questionable. However, as mentioned in background theory, there has been proved higher presence of water in muscles after exercise.

EVALUATION:

Reliability of the data:

There were uncertainties involved in measuring with sewing tape. Tape could be placed very firmly on the skin or just loosely which could lead to different measurements, bias, and uncertainty that has to be taken into consideration.

Pulse oximeter sometimes didn't detect the finger immediately after it was placed in. Therefore, measurement was sometimes recorded 5-10 seconds after putting in the finger. While, heart rate was already starting to decrease. Measurement might have been inaccurate sometimes, leading to underestimated HR. Using better and faster Heart-Rate-Meter would be improvement.

Inaccuracies in experiment:

Time duration of rest wasn't enough. Heart rate didn't decrease to its rest level in 2minutes; therefore, changes in heart rate were smaller than they could've been from resting value. Hence, although exercise repetition increased, heart rate didn't increase proportionately since HR was already high before that set; HR was still influenced by previous set. If rest time was longer, HR could recover and differences would be more accurate. Squats, push-ups, and calf raises were tested right after each other. HR might have been high already because of previous exercise. For example when starting push-ups, heart rate was still unrecovered from squats. Time in between different exercises could also be higher to gain more reliable results.

Uncontrolled factors:

There were uncontrolled factors that could affect the results. Participants completed experiment at different times of the day. Their meals were at different times; nutrition could affect their biological process happening in their body; food digestion, and availability of glucose for muscle contraction. Therefore, their exercise performance may differ.

Everyone's body is different. Participant 2 reported about unequal lengths of his legs. Participant 7 had a leg-injury before, as well as participant 10.

Participants might respond differently to different exercises. Some might be stronger in specific muscles and others in different ones. Therefore, exercises allocated might not suit everyone's ability at the same level. Difficult to decide the repetitions and type of exercise that would suit all.

5. CONCLUSION

Circumferences of thigh, brachium, and crus increase after increasing repetitions of: squats, push-ups, and calf raises. Heart rate increases after increasing repetitions of: squats, push-ups, calf raises. Heart rate corresponds to the increase in circumference; as the circumference increase, heart rate increases. However, not in all cases. After calf raises, heart rate increases relatively little, while the circumference increases relatively high.

Main reason for increased limb circumference remains unclear. Increased heart rate and vasodilation of vessels in muscles might play a role in increasing the circumference of body limbs. Other reasons could lead to increase of limb circumference: different pH value, lack of ATP, lactic acid build-up, drawing water inside cells, muscle cell swelling.

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Appendix

*Processed Data:

Change in circumference was calculated by subtracting circumferences after every set of repetitions [N] with initial measurement before any exercise:

$$\text{Change} = \text{circumference after } N \text{ repetitions} - \text{initial measurement of circumference}$$

Average was calculated by adding all the changes from participants (left- and right-side limb) of the same group performing same number of repetitions and dividing the sum by number of limbs measured (number of participants \times 2).

Change in heart rate was calculated by subtracting measurement after certain set of repetitions with heart rate before that particular set. Average was made of the participants of the same group.

To obtain the dispersion of data relative to its average, standard deviation of the population was calculated using Excel Spreadsheet. Formula for standard deviation is:

$$S = \sqrt{\frac{\sum(x - \bar{x})}{N}}$$

*Consent Form:

Biology EE Experiment - Consent Form

I hereby confirm that I have voluntarily and willingly agreed to participate in this experiment with no force or manipulation of any sort. I am fully aware of what I am participating in and I am fully aware of the risks that come with it. I am aware of my rights in the experiment; e.g., right to withdraw.

*Required

What is your name? *

Your answer _____

What is your age? *

Your answer _____

Do you consent to the experiment? *

Yes

No