TECHNISCHE UNIVERSITÄT WIEN
Vienna University of Technology

Thesis

The effect of local precooling on the predominantly stressed muscles prior to high-performance exercise under normal conditions

under the guidance of

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in cooperation with

EMCOOLS Medical Cooling Systems GmbH

handed in at the

Vienna University of Technology
Faculty of Electrical Engineering and Information Technology

by

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matriculation number: 01429966
studies code: 066 453

Unterschrift Betreuer

Unterschrift Verfasser

Abstract

The effect of local precooling on the predominantly stressed muscles prior to high-performance exercise under normal conditions

by Marko Drndarevic

Whereas humans sustain strict homeostatic range of body core temperature, skin and peripheral muscles may experience some variations in temperature. Recently, many studies were devoted to investigate effect of body precooling on physical performance, especially involving precooling of body core. However, no golden standard exists to study such associations, so protocols differ across a wide variety of ambient temperature, methods of assessment, precooling medium, parameters being evaluated and type of performance. Despite many different variables as well as existing confusing data, there is general strong need to clearly determine the effects of lower limbs precooling on power output during maximal performance of short duration.

This Master's Thesis hereby presents an attempt to evaluate the appropriateness of application of local muscle precooling with cooling packs derived by EMCOOLS through the measurement of power output during “all-out” ergometer test (known as Wingate Anaerobic Test that measures peak and anaerobic power, and anaerobic capacity) at neutral ambient conditions. The secondary goal of present study is to provide recommendations of scientific background enhancing physical performance of skeletal muscles that could be of great value for widely considered athletes and indications of combining precooling with regular professional preparation before sport events. The above aims were achieved by means of research conducted in IMSB Institute in Südstadt from June to September 2017, in which a group of ten volunteers was involved and submitted to standardized Wingate test procedure with and without precooling with precooling packs with continuous measurement of power output and cardiophysiological parameters, such as heart rate, systolic and diastolic blood pressure, body temperature and blood lactate concentration.

The research was basically designed to verify the relevance of hypothesis that precooling intervention of quadriceps applied prior to Wingate test influences power output and associated indices in neutral ambient conditions defined as ± 20°C. The observations reported through the study were in opposition to primarily assumptions. More particularly, precooling intervention significantly impaired power output measured continuously along 30 seconds Wingate test, although anaerobic capacity, anaerobic power, fatigue index, peak and mean power, and hear rate demonstrated tendency towards decline, but not significant.

In summary, precooling significantly decreased power output during anaerobic Wingate test, particularly in neutral ambient conditions. In contrast, such procedure could provide beneficial effect only in hot humid conditions or for performance of long duration. With respect to feasibility, cooling packs derived by EMCOOLS may be regarded as the best practice methods, however some discrepancies within this study highlight further need of investigation towards determination of effects of quadriceps precooling on anaerobic performance of short duration.

Kurzfassung

Der Effekt von lokalem Precooling der hauptbeanspruchten Muskelgruppe vor dem Leistungssport unter Normalbedingungen

von Marko Drndarevic

Die Steuerung der Thermohomöostase sorgt dafür, dass die menschliche Körperkerntemperatur konstant gehalten wird, wohingegen es bei der Haut und der peripheren Muskulatur zu Temperaturschwankungen kommen kann. In zahlreichen Studien wird der Effekt des Precoolings in Bezug auf die Leistungssteigerung untersucht, wobei der Fokus dieser Studien auf die Reduktion der Körperkerntemperatur gelegt wird. Ein Goldstandard diesbezüglich ist nicht vorhanden, wodurch es bei den einzelnen Studien zur Abweichungen in Bezug auf die angewendeten Methoden, das verwendete Kühlmedium, die Umgebungstemperatur, die Art der körperlichen Betätigung sowie die Parameter welche untersucht werden kommt. Ungeachtet der unterschiedlichen Herangehensweisen, ist ein Bedarf zur Untersuchung der Effekte des Precoolings auf die untere Extremität hinsichtlich der Leistung bei maximaler körperlichen Beanspruchung innerhalb eines kurzen Zeitraumes vorhanden.

Diese Masterarbeit beschäftigt sich damit, die Zweckmäßigkeit der lokalen Kühlung der Muskulatur mit Hilfe von bestimmten Kühlpacks des Unternehmens EMCOOLS GmbH während eines Leistungstests zu evaluieren. Als Testverfahren wird ein Wingate Anaerobic Test (Messung der anaeroben Leistungsfähigkeit und anaerobe Kapazität) angewendet, wobei innerhalb kurzer Zeit maximale Leistung erbracht werden muss. Das sekundäre Ziel dieser Arbeit ist es, wissenschaftliche Empfehlungen bezüglich des leistungssteigenden Effekts der vorgekühlten Muskulatur und der Kombination des Precoolings mit entsprechenden Wettkampfvorbereitungen zur Verfügung zu stellen. In Zusammenarbeit mit dem Institut für medizinische und sportwissenschaftliche Beratung (IMSB) sind standardisierte Wingate-Tests mit zehn Probanden durchgeführt worden, wobei pro Proband jeweils ein Wingate-Test mit Precooling und ein Wingate-Test ohne Precooling durchgeführt worden ist. Der

Leistungsoutput sowie kardiophysiologische Parameter wie Herzfrequenz, systolischer und diastolischer Blutdruck, Körpertemperatur und Laktatkonzentration im Blut sind ermittelt worden.

Die Hypothese, dass Precooling der Quadrizepsmuskulatur einen positiven Einfluss auf die Leistung der Probanden während des Wingate-Tests bei einer neutralen Umgebungstemperatur von ± 20°C hat, ist in dieser Arbeit widerlegt worden. Im Gegensatz zu den Erwartungen ist es bei einer maximalen Belastung von 30 Sekunden (Wingate-Test) bei vorgekühlter Muskulatur im Vergleich zu nicht vorgekühlte Muskulatur zu einem Leistungsabfall der Probanden gekommen. Die anaerobe Kapazität, die anaerobe Leistungsfähigkeit, der Ermüdungsindex, die maximale und durchschnittliche Leistung sowie die Herzfrequenz weisen eine fallende, jedoch nicht signifikante, Tendenz bei lokalem Precooling auf.

Zusammenfassend lässt sich sagen, dass Precooling den Leistungsoutput während eines Wingate Anaerobic Test minimiert, insbesondere bei neutralen Umgebungsbedingungen. Ein leistungssteigender Effekt könnte jedoch bei feuchten und heißen Umgebungsbedingungen sowie bei einer längeren Belastungszeit eintreten. Die Kühlpacks der Firma EMCOOLS haben sich bei den Testversuchen als bestmögliche Methode zur lokalen Kühlung erwiesen. Aufgrund einiger Diskrepanzen innerhalb der Studie ist eine weitere Untersuchung der Effekte von lokaler Kühlung des Quadriceps auf die anaerobe Leistungsfähigkeit bei kurzer Belastungszeit von Nöten.

Acknowledgements

Firstly, I would like to express my sincere gratitude to EMCOOLS medical cooling systems GmbH for providing me an opportunity to participate in this study. Special thanks go to Oliver Gindlhumer, Bernhard Fabian and Konstantin Rumler for their guidance and support throught this project.

I would also like to thank my thesis advisor Prof. Eugenijus Kaniusas for his valuable comments and instructions. Besides my advisor, I would like to thank Proj.-Ass. Stefan Kampusch and the rest of my thesis committee.

Futhermore, I would like to thank the IMSB Institute staff for wonderful collaboration, as well as all athletes who participated in this research.

Last but not the least, I would like to thank my family and friends for supporting me spiritually throughout writing this thesis and my life in general.

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Abbreviations

$[La^-]_b$	Concentration of Lactate in Blood
AC	Anaerobic Capacity
ACh	Acetylcholine
ADP	Adenosine 5'-Diphosphate
AMP	Adenosine 5'-Monophosphate
ANOVA	Analysis of Variance
ANS	Autonomic Nervous System
AP	Anaerobic Power
ATP	Adenosine 5'-Triphosphate
AV	Atrioventicular Node
BP	Blood Pressure
BP_{dias}	Blood Pressure Diastolic
BP_{sys}	Blood Pressure Systolic
BPV	Blood Pressure Variability
BT	Body Temperature
Ca^{2+}	Calcium Ions
CNS	Central Nervous System
CoA	Coenzyme A
CP	Creatine Phosphate
CPI	Cardio Performance Indicator test
CV	Coefficient of Variation
d	Distance
Df	Degrees of Freedom
Diff	Difference
ECG	Electrocardiography
F	Force
FAD	Flavin Adenine Dinucleotide
$FADH_2$	Reduced Form of Flavin Adenine Dinucleotide
FFA	Free Fatty Acids
FI	Fatigue Index

GI	Gastrointestinal Tract
GLUT4	Glucose Transporter Type 4
GTP	Guanosine 5'-Triphosphate
H^+	Hydrogen Ions
HLa	Lactic Acid
HR	Heart Rate
HRV	Heart Rate Variability
LA	Left Atria
La^-	Lactate
LT	Lactate Threshold
LV	Left Ventricle
Lwr	Lower 95% Confidence Interval
MCT1	Monocarboxylate Transporter 1
MHC	Myosin Heavy Chain
MP	Mean Power
NADH	Nicotinamide Adenine Dinucleotide
NE	Norepinephrine
OBLA	Onset of Blood Lactate Accumulation test
p	Probability Value
Pi	Phosphate Residues
PP	Peak Power
PWC	Physical Work Capacity test
Ra	Rate of Appearance
RA	Right Atria
RBCs	Red Blood Cells
Rd	Rate of Disappearance
RPM	Rotation Per Minute
RV	Right Ventricle
SA	Sinus Atrial
SD	Standard Deviation
SR	Sarcoplasmic Reticulum
t	Time

TBI	Traumatic Brain Injury
T_C	Core Temperature
TPU	Thermoplastic Polyurethan
T_S	Shell Temperature
Upr	Upper 95% Confidence Interval
VO_{2max}	Maximal Oxygen Uptake
WAnT	Wingate Anaerobic Test

Introduction

To date many studies have been performed in order to evaluate the overall effect of whole-body precooling prior to physical activity, having to say that recent findings indicated significant increase in peak-performance contrary to non-precooled subjects [1]. More specifically, dramatic increase in measured power output has been reported due to body precooling intervention in hot conditions. However the mechanisms underlying these findings remain unclear. Therefore, this project basically aims to investigate overall effect of body precooling on muscle energetics, further perfusion-associated mechanisms that eventually lead to significant improvement in short physical performance of high-energy demand, which are described in greater details in following sections of present Master's Thesis.

With respect to cooperation established between Technische Universität Wien and EMCOOLS, present study targets to evaluate and identify particular effects of local precooling prior to short and maximal power output performance in normal conditions, i.e. 20°C ambient temperature. Moreover, the secondary goal of this Master's Thesis is to provide indications enhancing physical performance of skeletal muscles that could be of great value for widely considered athletes. Therefore, present study involved a group of ten volunteers that submitted to standardized Wingate test procedure with continuous measurement of several cardiophysiological parameters (heart rate, systolic and diastolic blood pressure, tympanic body temperature), blood lactate concentration and physical performance outcome. Briefly, each subject exercised twice at an interval of seven days between attempts, firstly with and secondly without prior precooling of quadriceps muscles with EMCOOLS Flex.Pad™ products. It was expected that results of presented research allow evaluating the outcomes of skeletal muscles precooling in a deliberate manner, contrary to the hitherto described increase in physical performance in thermal conditions.

Theoretical Background

1.1 Muscle Energy Metabolism

Skeletal muscles form a unique human body tissue able to contract, which comprises cells capable of immediate increase in their metabolic rate dependently on the demand of explosive contractions. Due to many different roles that muscles play, three major kinds may be distinguished: smooth, skeletal and cardiac, among which the two latter consumes high portion of available energy. In brief, cardiac muscle cells built up human's heart, a muscular pump that enforces circulation of blood in the body and are a minority of muscle tissue, nevertheless they are of remarkably vivid energy metabolism. In turn, smooth muscle cells line blood vessels as well as respiratory, urinary and gastrointestinal tract, and reproductive system. Through contractions and tonus they control many vital processes such as intestinal motility, urinary functions, blood pressure and flow, and air flow in the respiratory tract. Although smooth muscle cells bear heavy workload, they are characterized by relatively low energy metabolism, whereas high-energy metabolism is a unique feature of skeletal muscles in humans. Specifically, their ability to work in aerobic as well as short-term anaerobic conditions ensures adaptation of energy metabolism in accordance to the intensity of physical activity (prolonged lowered endurance or short-term performance of high-energy). The adaptive characteristics of skeletal muscles metabolism originate from their capacity to adjust utilization of adenosine 5'-triphosphate (ATP) resources, which simultaneously requires adequate compensatory adjustment in blood circulation, cardiac and respiratory capacities [2].

In general all muscles are built up of myofibrils known as threadlike structures. Each muscle cell comprises several myofibrils arranged alternately with thin and thick myofilaments. Differences between metabolic profiles of skeletal muscles arises from the type of fibers they are composed of, thus they can conserve energy or consume high portion for short explosive moments. The major types of fibres that could be distinguished based on myosin heavy chain (MHC) isoforms are type I, IIa, IIx and IIb (fibres IIb are not expressed in humans), among which type I is the slowest, type IIa intermediate and type IIx the fastest [3].

Dependently on the type of contraction different proportions of energy are required to trigger it: continuous vs. short repeated contractions or maximal vs. submaximal endurance, although the muscle metabolism basics are true and common independently on their type. During the contraction, energy demand stems from activity of molecular motors, the myosin heads or cross-bridges as well as ion pumps (sarcoplasmic reticulum (SR) Ca^{2+} pumps driven by ATP, known as Ca^{2+} ATPases) [4]. The main energy resources are stored in cells in form of ATP and creatine phosphate (CP), which may be considered as biochemical capacitors, however the energy is being released from ATP through its breakdown performed by the ATPase enzymes. In skeletal muscle tissue, contractions are followed by cascade of biochemical events: firstly the plasma membrane is depolarized with subsequent initiation of Ca^{2+} release from SR's intracellular storage. Next, Ca^{2+} ions bind to troponin C (filament-associated regulatory protein), thus changing its conformation. Subsequently, changes in shape are transmitted to troponin T, troponin I, tropomyosin and actin, components of other thin filament, causing interaction between actin subunits from thin filament with the adjacent myosin molecules (consisting of thick filaments). Finally, the contraction of muscle is stopped with uptake of Ca^{2+} performed by Ca^{2+} ATPases in SR. In muscle cells, ATP comprises the primary source of energy during the contraction, however the intracellular store is relatively small and with full activation of muscles it becomes depleted within 2 seconds [5]. Therefore, other pathways of energy metabolism must be activated to prevent ATP depletion. Two basic pathways may be distinguished: aerobic and anaerobic, of which the former is usually associated with prolonged submaximal exercise and the latter dominates during physical activity of high-energy demand.

In major, the energy in skeletal muscles is extracted in form of ATP from bonds of the carbohydrates, lipids or proteins (mainly provided with glucose and fatty acids), and is additionally stored in muscle fibres in form of glycogen (from glucose) and triglycerides (from fatty acids). The renewal of ATP resources that are used for skeletal muscles work comes with oxidation of energy sources. ATP molecule consists of one adenosine bounded with three phosphate molecules. The hydrolysis of ATP is accompanied by release of energy with simultaneous production of adenosine 5'-diphosphate (ADP) and is performed by ATPases. Additional energy may be released through hydrolysis of second phosphate bond with simultaneous production of

adenosine 5'-monophosphate (AMP). Each hydrolysis step results in release of 7.3 kcal of energy (Figure 1).

ATP use		$ATP + H_2O \rightarrow ADP + P + H^+ + 7.3\ kcal$
ATP renewal	aerobic	$glucose + 6\ O_2 + 36\ ADP \rightarrow 6\ CO_2 + 6\ H_2O + 36\ ATP$
		$palmitate + 23\ O_2 + 130\ ADP \rightarrow 16\ CO_2 + 16\ H_2O + 130\ ATP$
	anaerobic	$ADP + CP + H^+ \rightarrow ATP + creatine$
		$2\ ADP \rightarrow ATP + AMP$
		$glycogen + 3\ ADP \rightarrow 2\ lactate + 2H^+ + 3\ ATP$

Figure 1: Skeletal muscles energy metabolism based on [6].

As mentioned before, the ATP resources in human body are sufficient only for few seconds of the endurance, however due to several mechanisms the resources are renewed. The resynthesis may be performed from short-term supply via high-energy phosphates like CP, medium-term supply via anaerobic glycolysis and long-term supply via oxidative phosphorylation of glucose and fatty acids. Nevertheless, the ATP resynthesis through oxidation is long and constant process, therefore rapid renewal of ATP resources is done by breakdown of high energy CP in anaerobic conditions. ATP and CP (called hereinafter phosphagen system) in concert are essential for muscle contractions, especially in activities of high-energy demand (i.e. the quick start of sprinters or high jumpers). Noteworthy, equilibrium between the production and hydrolysis of ATP must be achieved for maintenance of aerobic metabolism, where concentrations of ATP and CP become constant (~5 mmol L^{-1} and 30 mmol L^{-1}, respectively). In turn, resynthesis of CP in the recovery phase following muscle contraction is achieved by breakdown of its products (CP, inorganic phosphate) in ATP-dependent manner and the energy required for replenishment of phosphagen system is derived by aerobic metabolism [3, 6].

During prolonged endurance, the main source of ATP is oxidative metabolism of carbohydrates and lipids [7]. The major substrate that participates in aerobic metabolism is glycogen stored in the muscles, however the longer duration of physical activity the higher contribution of extracellular glucose and free fatty acids (FFA) derived from either adipose tissue or triglyceride reserves in ATP production [8, 9]. For

aerobic metabolism, oxygen is carried by bloodstream bound to haemoglobin in erythrocytes to the site of oxidative phosphorylation in active cells and other substrates are transported in plasma.

Oxidative phosphorylation occurs in mitochondria and is considered as great source of energy. As an example, glycolysis in aerobic conditions results in production of 36 moles of ATP, whereas without access to oxygen only 2 moles of ATP are synthesized from 1 mole of glycogen. Figure 2 shows the chain of ATP synthesis in aerobic conditions through glycolysis, the Krebs cycle (also known as citric acid cycle or tricarboxylic acid cycle) and electron transport chain. Briefly, in muscle mitochondria atoms of hydrogen are being obtained from reducing equivalents, which arise via citric acid cycle (oxidative phosphorylation). Subsequently, specialized proteins transfer electrons from atoms of hydrogen to oxygen molecules. Then, the energy is being released and conserved in form of ATP.

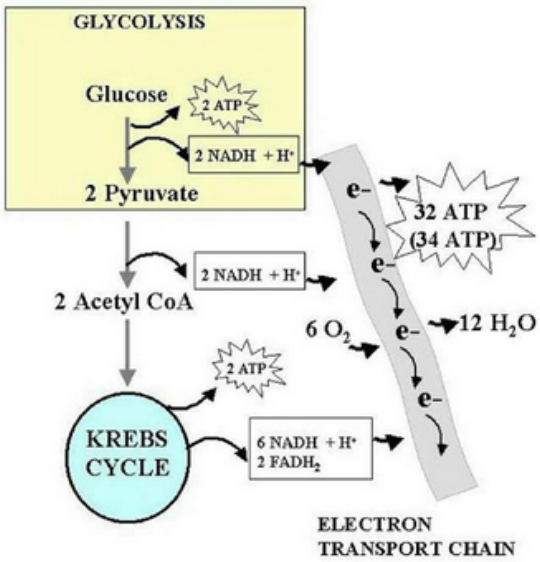

Figure 2: Synthesis of 36 moles of ATP from glucose through glycolysis, the Krebs cycle and electron transport chain (reprinted from [2]).

Within muscle cells the demand of energy determines the rate of oxidative phosphorylation in mitochondria, although availability of substrates determines in turn the level of cellular energy available for the mitochondrial respiration. Therefore, the rate of ATP resynthesis is determined by the rate of its hydrolysis in cellular processes.

Despite mitochondria itself consume high portion of oxygen, they are considered as primary and major source of metabolic energy. Basically, citric acid cycle is essential and final pathway for aerobic metabolism of glucose glycolysis, oxidation of fatty acids and protein catabolism, wherein the two former provide in approximation 95% of acetyl-coenzyme A (acetyl-CoA) molecules required to enter the Krebs cycle followed by its catabolism and release of hydrogen equivalents for generation of ATP from ADP.

As an overview, in the first step of the Krebs cycle acetyl-CoA binds with oxaloacetate and forms citrate molecule. Subsequently, citrate releases from its molecule two atoms of carbons (in form of carbon dioxide, CO_2) generating at the same time one molecule of nicotinamide adenine dinucleotide (NADH). Next, remaining four-carbon molecule participates in series of additional reactions, generating guanosine 5'-triphosphate (GTP) with simultaneous reduction of FAD, an electron equivalent, to $FADH_2$ to produce second molecule of NADH and new molecules of oxaloacetate to start second round of the cycle (Figure 3). Finally, one turn of the Krebs cycle generates 12 ATP molecules [10].

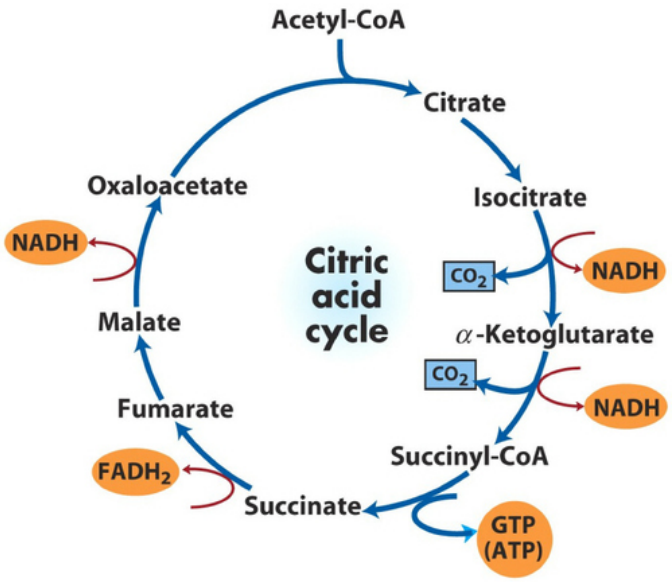

Figure 3: Overview of the Krebs cycle (reprinted from [11]).

As mentioned previously, energy may be acquired from few sources like carbohydrates (glucose and glycogen) and lipids (FFA). Glucose is a sugar derived with bloodstream and its cellular uptake is dependent on exercise duration and intensity, as shown in the Figure 4. Delivery of the glucose accompanied by insulin to the site of contracting

skeletal muscle cells elevates during physical activity, although the increase in glucose uptake is then limited up to 30%, thus other local muscular factors are more significant for the overall metabolism. These factors include heighten sarcolemmal transport of glucose as well as activation of enzymes responsible for glucose metabolism in glycolysis and oxidative phosphorylation [12, 13]. Increased sarcolemmal transport is carried out via facilitated diffusion through glucose transporter type 4 (GLUT4) belonging to the family of glucose transporters expressed mainly in cardiac and skeletal muscle cells [14].

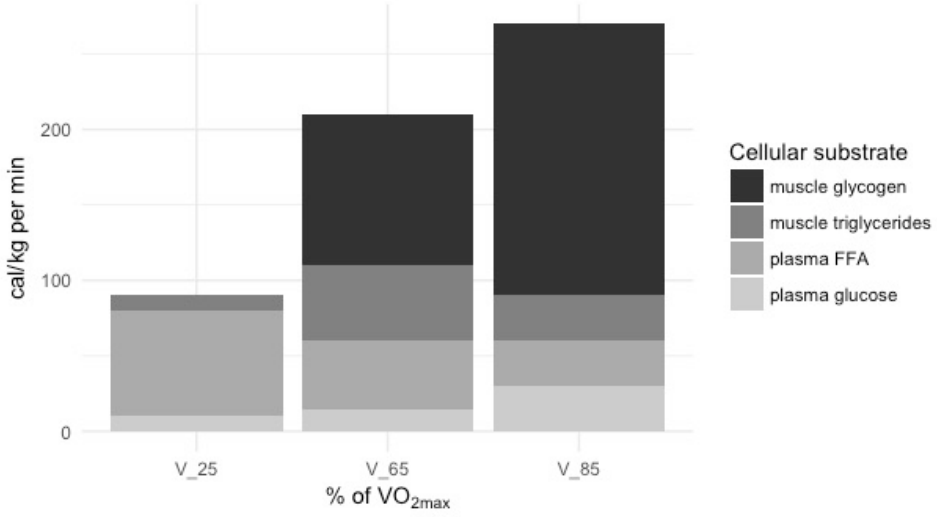

Figure 4: Differential contribution of cellular substrates to energy during physical activity of increasing intensity (reproduced from [15]); VO_{2max} – maxymal oxygen consumption.

Prolonged physical activity of steady intensity causes decreased usage of glycogen and glucose in favour of the lipid oxidation due to reduced glycogen phosphorylase activity (enzyme participating in glycogenolysis) and increased availability of FFA in the bloodstream. Lipid oxidation accompanying physical activity comprises multistep process starting with the hydrolysis of intramuscular triglycerides followed by FFA transport to mitochondria of skeletal muscles for oxidation. Firstly, FFAs are uptaken from sarcoplasm and carried by carnitine combined with fatty acyl-CoA to enter β-oxidation in mitochondria, during which the acyl chain is being successively shortened to finally form acetyl-CoA. For instance, complete oxidation of palmitate

requires for activation 2 ATP molecules and eventually gives a net yield of 129 ATP molecules. Noteworthy, it has been established that availability of FFA does not limit oxidation rate in fasting conditions, however during intense physical activity their availability may become rate limiting [2, 15, 16].

Energy metabolism may also occur in anaerobic conditions due to characteristic feature of glycolysis, in which glucose catabolism may be performed with oxygen (acetyl-CoA as final product) or without (lactate as final product). In skeletal muscles, anaerobic metabolism enables high-energy performance exceeding the levels feasible through aerobic glycolysis. This process involves the incomplete hydrolysis of carbohydrates to lactic acid, which is an end-product accumulating in the cells. Furthermore, lactic acid affects intracellular pH and thus inhibits phosphofructokinase (enzyme phosphorylating fructose 6-phosphate during glycolysis) activity, which in turn could limit the rate of glycolysis. The anaerobic metabolism enables to achieve short performance of high-energy, although increased production of lactic acid may have diverse effect on cells: it has been shown that lactic acid affects function of neuromuscular junctions, damage muscle fibres, cells of connective tissue and blood vessels, but on the other hand stimulates adaptive changes of metabolism. Figure 5 shows changes in muscle energy along with duration of physical activity.

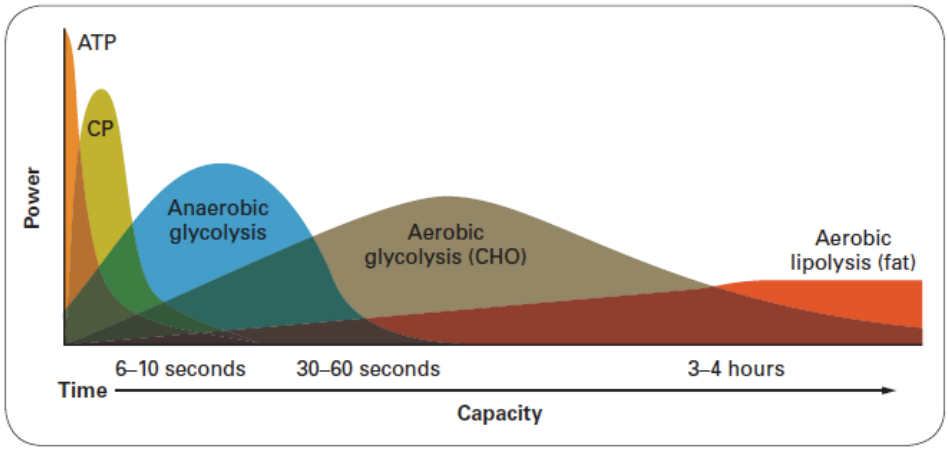

Figure 5: Muscle energy metabolism along with duration of physical activity (reprinted from [17]).

1.2 Differential Contribution of Three Energy Systems

During physical activity humans developed three distinct, but integrated processes that operates in concert in order to satisfy energy demands and prevent its complete degradation through ATP hydrolysis. With intensive physical performance ATP concentration in skeletal muscles is decreased by 30%, however the demand of ATP hydrolysis is significantly elevated. Noteworthy, this phenomenon indicates that ATP synthesis and resynthesis occurs in humans at similar rate. The renewal of ATP storage is possible via phosphorylation process, which may occur in aerobic or anaerobic conditions (oxidative phosphorylation or substrate phosphorylation, respectively). It has been demonstrated that increase in intensity of physical activity results in appearance of deficit phase and acceleration of ATP hydrolysis. Consequently, anaerobic metabolism becomes activated due to insufficient efficiency of oxidative phosphorylation [18]. The highest rate of ATP renewal shows reaction, which uses CP (catalysed by creatine kinase). The maximal velocity of this reaction is achieved in first second of performance followed by significant decrease in concentration of CP. Moreover, the resynthesis of CP takes usually about 2 minutes and occurs at the expense of aerobic metabolism. Several studies showed that physical activity of 35% VO_{2max} causes reduction of CP resources up to 90% of rest state. On the other hand, intensity of exercises raised to 65% VO_{2max} reduces the resources by half, but intensity rate of 90% VO_{2max} uses majority of CP storage [19].

Two first mechanisms incorporate anaerobic system, which may occur in one of two ways: alactic or lactic. Both pathways enable the renewal of ATP at high efficiency rates followed by high-energy output in the muscles, however only limited amount of energy may be released. In particular, this is caused by limited resources of CP with simultaneous accumulation of lactic acid, which together may force decrease in physical activity. The former mechanism involves phosphates of high-energy, the phosphagen system, and the breakdown provides supplies of energy at initial stages of high-energy performance. Moreover, the phosphagen system comprises the quickest option for resynthesis of ATP resources. In greater detail, CP stored in skeletal muscles is a phosphate group donor for ADP to produce ATP. This process does not involve other substrates (carbohydrates, lipids) than CP. The latter lactic mechanism refers to

anaerobic glycolysis: degradation of carbohydrates such as glucose or glycogen stored in the muscles that is processed through pyruvate into lactic acid. When demand of oxygen exceeds respiratory capacities of the body, then conversion of lactate usually occurs. Along with this process, blood-derived glucose or muscle glycogen undergoes a series of chemical reactions to eventually produce ATP and release the energy. In contrast, if sufficient supplies of oxygen are available to satisfy the demand, then pyruvate processed into acetyl-CoA enters the mitochondria and the Krebs cycle for oxidative phosphorylation [20]. The last process, oxidative metabolism, involves oxidation of lipids and carbohydrates, but only in presence of oxygen. This involves the Krebs cycle and electron transport chain, in which pyruvate arising from glycolysis enters mitochondria and forms acetyl-CoA. Subsequently, arising electrons participate in oxidative phosphorylation resulting in formation of water and ATP molecules. On the other hand, acetyl-CoA may be also derived from β-oxidation of FFA in the mitochondria. Despite extremely high amount of ATP produced via aerobic metabolism, this process is limited by the rate of oxidative phosphorylation as well as efficiency of respiratory and cardiovascular system to provide oxygen to the muscles. General characteristics of all three energy systems are shown in Table 1.

Table 1: Characteristics of three energy systems (based on [20, 21]).

System	ATP production rate	Capacity to produce ATP	Substrates used
Phosphagen system (alactic)	Very high	Very low	CP, ATP from storage
Glycolysis (lactic)	High	Low	Blood-derived glucose, Muscle glycogen
Aerobic metabolism	Low	Very high	Blood-derived glucose, Muscle glycogen, Lipids

All together, these systems interact in order to provide high amounts of energy and restore ATP during physical activity of diverse energy demands. In example, Spriet et al. showed that replenishment of ATP from both degradation of CP and lactic glycolysis that lasted less than 10 seconds may reach the range of 6-9 mmol of ATP per kg dry mass^{-1} per sec^{-1} [22]. Furthermore, combination of these two pathways may release the energy reaching even 15 mmol of ATP per kg dry mass^{-1} per sec^{-1} over first few seconds of sprint (half of ATP is derived from CP catabolism) [23]. In contrast, glycolysis-derived ATP resources reach a peak until 5 seconds of exercises followed by steady rate for another several seconds [24]. Despite great energy supplies for performance of high-energy demand that may be increased 100-fold, still the energy released from anaerobic glycolysis appears to be limited and cannot be sustained. The reason could be either inhibition of enzymes participating in glycolysis or no activation of glycolysis process [25]. Gradually arising lactate that decreases cellular pH is likely to reduce activity of several enzymes participating in glycolysis such as phosphorylase or phosphofructokinase and furthermore causes limited reservoir of ATP in muscles [26]. In contrast, aerobic metabolism was thought to have significant contribution during exercise of high-energy demand, nevertheless there are some discrepancies causing general disagreement between scientists and coaches. In example, several studies assessed aerobic contribution to the running events of certain length and found that for 800 m running event of 1-2 minutes duration the aerobic metabolism ranged from 35 to 65%. [27], however more recently another team defined the contribution as 55-65% [28]. Figure 6 shows summary of Spencer's and Gastin's studies that found increase in aerobic energy supply along with accompanying increase in running distance [29]. In turn, Gastin P demonstrated differential anaerobic and aerobic contribution of energy metabolism during physical activity (Table 2). In brief, it was shown that in approximation by 240 seconds of exhaustive exercise contribution of anaerobic metabolism changes from 94% to 21% in favour of aerobic metabolism [30].

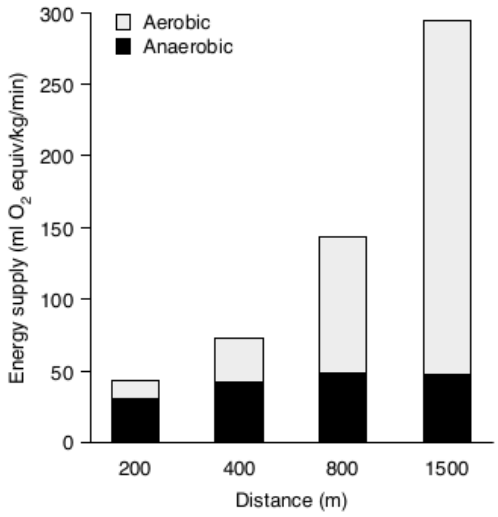

Figure 6: Differential contribution of energy systems to the total energy supply during running event of differential distance (reprinted from [14]).

Table 2: Differential contribution of aerobic vs. anaerobic metabolism during exhaustive exercise (based on [15]).

Duration of physical activity (s)	% contribution	
	Aerobic	Anaerobic
0-10	94	6
0-15	88	12
0-20	82	18
0-30	73	27
0-45	63	37
0-60	55	45
0-75	49	51
0-90	44	56
0-120	37	63
0-180	27	73
0-240	21	79

1.3 Biology of Muscle Fatigue and Factors Affecting Daily Performance

Physical activity of high intensity always eventually leads to muscle fatigue, defined as reduced, but reversal, contractile capacities of skeletal muscles, which is in major part dependent on efficiency of aerobic metabolism. Moreover, the resistance of muscle fibres to fatigue is differential. In particular, slow fibres (oxidative) are more resistant to fatigue in comparison with fast fibres (glycolytic) under normal conditions.

As muscle contraction is complex in its mechanism and controlled by series of events, fatigue may result from impairment of any of these events. The whole process is initialized in the central nervous system (CNS), where activation of α-motoneurons occurs followed by further activation of muscle fibres (together α-motoneurons and target fibres are regarded as the smallest motor unit). First type of fatigue, known as central fatigue, refers to α-motoneurons and motor unit that arises from affected activation of α-motoneurons [31].

Second type of fatigue is known as peripheral fatigue and it refers to contractile capacities of muscle during physical activity. Basically, the whole process of activation of muscle cells is initialized with action potential generated at the neuromuscular junctions and spreading along with the surface of their membranes. This event initializes sequential cascade resulting in opening of calcium ions (Ca^{2+}) channels in the SR, then release of Ca^{2+} ions to the cytosol, Ca^{2+} binding and subsequent changes in the configuration of proteins regulating myofibrils (troponin-tropomyosin protein complex). Next, cross-bridges bind to actin filament, which finally releases contraction of skeletal muscles [32]. In this case peripheral fatigue may stem from production of reduced isometric force, decreased shortening speed, affected relationship between force and velocity as well as delayed muscle relaxation. Moreover, combined reduction in production of force and decreased shortening causes in fact decrease in power output, thus affecting general performance originating from muscle shortening [33].

Another mechanism of muscle fatigue is based on acidosis, which arises due to degradation of glucose in anaerobic conditions, however as several studies showed this phenomenon has little significance on muscle functions such as force production and contractile speed [34]. Additionally, anaerobic metabolism involves in humans

phosphagen system, which acquires energy through breakdown of CP with corresponding release of phosphate residues (Pi). Elevated concentration of Pi within muscle cells has been identified as negative factor affecting contractile capacities of muscles. In more details, it decreases the production of force and cellular sensitivity to Ca^{2+} [35].

Despite intrinsic mechanisms leading to skeletal muscle fatigue, there are two major groups of factors that may accelerate the fatigue occurrence and thus worse the daily physical performance. These factors are environmental (nutrition, temperature, altitude, clothing, quality of sleeping) as well as individual characteristics (age, gender, body built, genetics), which may temporary affect body capacities in terms of physical activity. From the former, one of the most important factors is nutrition, which alterations (underfeeding) may result in depression of immune system, however noteworthy, it may decrease recovery capacities from injury especially in skeletal muscles. Moreover, it has been established that underfeeding of short period may affect physiological response to exercise (i.e. increased heart rate in aerobic work, impaired patient's orthostatic tolerance) as well as muscle fatigue, soreness and deepened muscle weakness after physical activity. In contrast, prolonged underfeeding has been shown as a cause of reduced maximal aerobic and anaerobic powers [36]. Another environmental factor is temperature of both human body and its surroundings. In this case, essential for maintenance of physical performance is ability to remove the heat from skeletal muscles during operation. Noteworthy, as the increase in ambient temperature generally decreases the capacity of transferring heat to the environment, it becomes apparent that physical performance is being temporary impaired. In the study of Goldman RF it has been estimated that for every 1°C of effective temperature, concerned as scale presenting equivalent temperature sensation in differential conditions such as wind speed, relative humidity and dry air, the tolerance of physical work over 4 hours is decreased by 12.35 $kcal \times h^{-1}$ [37]. Several other studies also demonstrated that capacity of physical activity is being reduced due to arising competition for blood flow from the skeletal muscles and from skin, which compete for blood to carry on work or remove the heat, respectively, and thus cause limitation of cardiac output [38, 39]. Remaining factors such as altitude or improper clothing may cause decreased respiratory capacities resulting in insufficient oxygen supplies or difficulties in heat dissipation. However

surprisingly, several studies shown that sleep deprivation does not affect physical performance at any of levels (cardiorespiratory and muscular functions) [40].

1.4 Selection of Relevant Quantities

1.4.1 Blood Lactate Analysis

Lactate (La^-) metabolism involving cell-to-cell lactate shuttle has been established in 1984 by Brooks G and has been so far borne out by multitude of evidence. Briefly, it assumes that formation of La^- followed by its further distribution throughout the organism is likely the major mechanism of indirect metabolism coordination in different cells of different tissues. Moreover, the role of La^- as source of fuel has been highlighted with the finding that over period of physical activity of moderate intensity, the flux rate of La^- in the blood is exceeding blood flux of glucose [41-43].

The concentration of blood lactate ($[La^-]_b$) comprises major parameter measured during exercise test in clinical routine or athlete's performance evaluation. In fact, measurement of $[La^-]_b$ is widely used in diagnostics of coronary artery disease, chronic airway obstruction or chronic renal failure [44], however it has been also recommended as individual indicator of proper intensity of exercise [45]. Nevertheless, interpretation of results derived by $[La^-]_b$ measurement is complicated due to bias of physiology; significant increase in concentration of $[La^-]_b$ may characterize either pathological reaction to physical activity of high intensity or normal response to physical load if the rate at which La^- is removed from the blood vs. it enters the blood has been exceeded through physical performance [46]. In that terms clinicians should be aware of changes of both plasma La^- and $[La^-]_b$ resulting from physical activity at various levels in physiological conditions. Figure 7 shows response of $[La^-]_b$ to exercise testing (progressive, incremental type). It can be observed that during exercise of regular intervals of increasing rate concentration of $[La^-]_b$ primarily increases steadily with following sharp growth along with more intense exercising. More in-depth, the blood concentration of La^- reaches the maximal peak at 15-25 mmol just after initial few minutes of exercise. In contrast, during maximal physical activity of "all-out" kind lasting 60 seconds the peak appears ~3-8 minutes post-exercise, however the

concentration of $[La^-]_b$ remains elevated over next 60 minutes (Figure 8). It should be stated that the above increase presents physiological response to exercise of high intensity rather than pathological alterations, which is very often confusing. In addition, concentration of blood lactate constitutes *quasi* indicator of biochemical balance established between La^- formation and release into the bloodstream by different tissues and simultaneous La^- uptake and removal from the blood by different tissues [9]. Therefore, elevated $[La^-]_b$ observed by physical activity may correspond with increased La^- formation and release from skeletal muscles, decreased uptake and removal of La^- or increase in production and release rate that exceeds uptake and removal rate. Furthermore, regarding light to moderate exertion the concentration of $[La^-]_b$ may only slightly deviate from the level at rest, although the La^- flux may reach repeatedly greater levels than at rest. For illustration, Figure 9 presents schematic La^- appearance, disappearance and overall net $[La^-]_b$ due to progressive exercise test.

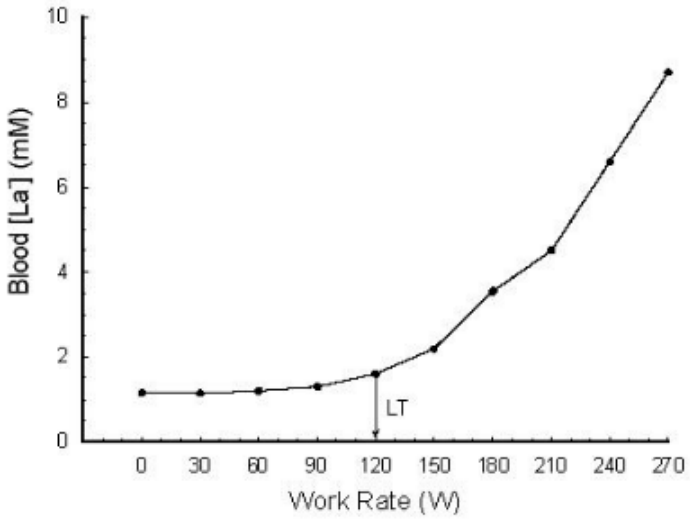

Figure 7: Response in lactate concentration to progressive exercising. In this experiment individuals performed typical incremental exercise test protocol with unloaded pedaling at 60 rpm for 4 minutes on standard indoor ergometer. Subsequently, work rate was increased by 30 W each minute. LT- lactate threshold (reprinted from [47]).

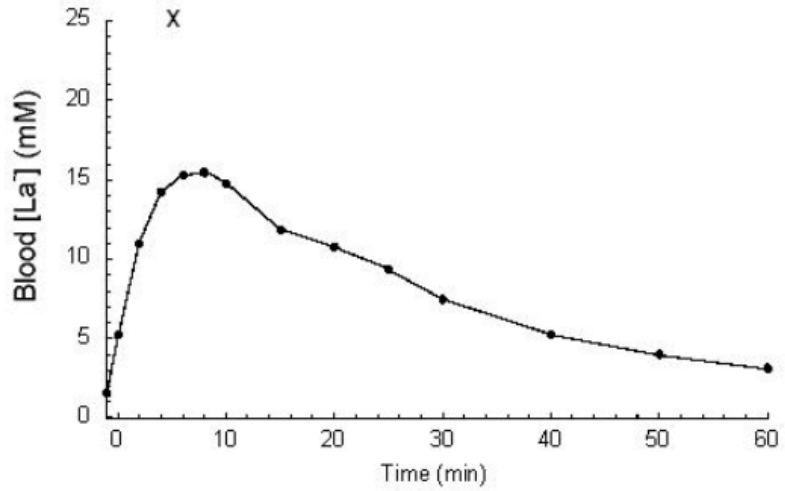

Figure 8: Concentration of $[La^-]_b$ after 60 seconds of maximal exercise of "all-out" type. The $[La^-]_b$ reaches a peak about 3-8 minutes post-exercise (reprinted from [48]).

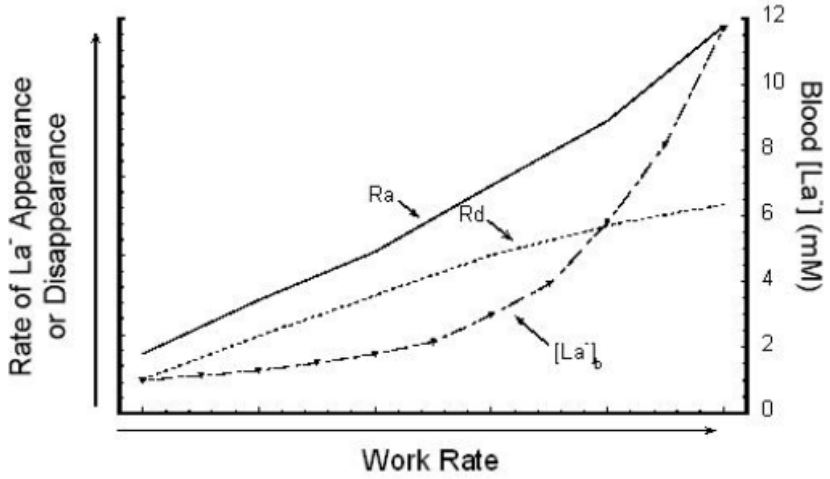

Figure 9: Schematic representation of La^- appearance, disappearance and overall net due to progressive exercise test (reprinted from [49]). R_a-rate of appearance; R_d-rate of disappearance.

Mainly red blood cells (RBCs) use anaerobic metabolism, which from principal leads to formation of La^-, therefore an efficient way of La^- efflux of RBCs is vital. Basically, La^- that is generated by RBCs arises in small amounts, thus likely it could be handled by diffusion performed without presence of any additional La^- carrier. In turn, exertion of high intensity leads to accumulation of La^- and hydrogen ions (H^+) inside of the muscle cells that has been identified as factor affecting physical performance. Therefore, the

transport of La^- across membrane of RBCs is carried out by three distinct pathways of diverse frequency rates that are presented in the Figure 10: 1) non-ionic diffusion of undissociated lactic acid (HLa) (5-12%), 2) the inorganic anion exchange system that utilizes protein known as band 3 (referred as the band 3 system; 5-15%), and 3) monocarboxylate transporter 1 (MCT1) pathway constituting primary pathway of La^- transport across the RBC membranes in humans (75-90%) [50]. Simultaneously with circulation of blood in the body, the gradient of $[La^-]_b$ is being reversed, resulting in influx of La^- from plasma into various tissues with inactive or moderately active skeletal muscle among others. Finally, since plasma concentration of lactate decreases, La^- will leave the RBC. As plasma to RBC lactate gradient elevates with increase in exercise intensity, the plasma levels of lactate increase. Noteworthy, due to dynamic changes in ratio between plasma and RBC it becomes of great significance to know what type of sample is being evaluated; whole blood vs. plasma and RBCs differs in lactate values even if the equilibrium between plasma and RBC has been established. However, the use of automatic analyser may result in errors due to fact that not necessarily it is simply determined what type of measurement has been performed (plasma or whole blood lactate level) [48].

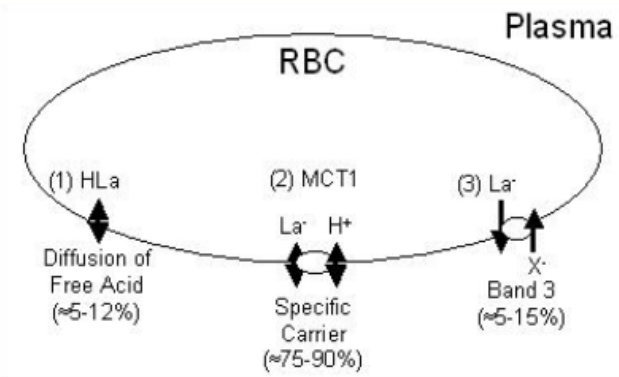

Figure 10: Lactate is being transported across RBC membranes through three distinct pathways (reprinted from [50]).

1.4.2 Heart Rate and Blood Pressure

Physical activity also affects other parameters like heart rate (HR; HR is defined as heartbeat speed measured as quantity of heart contractions per minute) or blood

pressure (BP; BP is defined as pressure that circulating blood exerts on blood vessels; it is divided into systolic and diastolic pressure, which indicate maximum pressure during one heartbeat and minimum pressure in between two heartbeats, respectively) through cardioacceleratory and pressor responses induced by exercises that stem from autonomic reflexes of human body. The level of response from the site of autonomic activity is strongly associated with feedback mechanisms adjusting hemodynamics according to metabolic demands of skeletal muscles at work. The adjustment-related changes involve fluctuations of BP and HR in beat-to-beat mode that are known as blood pressure variability (BPV) and heart rate variability (HRV), respectively [51].

General regulation of cardiovascular system is performed through activity of autonomic nervous system (ANS) consisting of sympathetic and parasympathetic nerves primarily regulated by medulla. More specifically, the nucleus tractus solitarius located in the medulla induces cardiovascular responses for physical stress or emotions. Having regard to presence of sympathetic efferent nerves throughout the atria of heart, the HR is being increased via stimulation of α and β adrenoreceptors. In contrast, parasympathetic nerves that innervate the sinoatrial (SA) and atrioventricular nodes as well as atria of heart act via muscarinic receptors, which causes contrary effects to α and β adrenoreceptors (Figure 11). Other elements of autonomic cardiovascular control involve chemoreceptors, muscle afferents, baroreceptors, hormones and local tissue metabolism. Noteworthy, both sympathetic and parasympathetic systems are active at rest, however fibres of parasympathetic system release acetylocholine (ACh), which in turn retards potential of SA node's pacemaker, hence reduces HR. On the other hand, during physical activity occurs activation of sympathetic system, which causes pupil and bronchiole dilatation, constriction of blood vessels, increased secretion of sweat and renin hormone in kidneys, hence results in increased HR. However on balance, the final effect of ANS on the heart and its functions equals the net balance between the contrary actions of both sympathetic and parasympathetic systems [35].

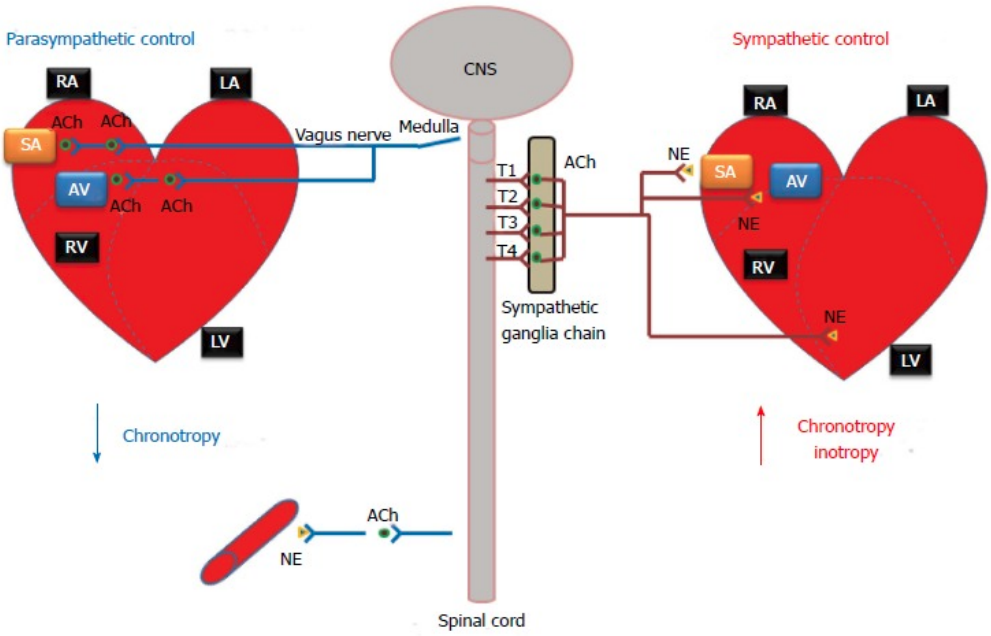

Figure 11: Regulation of heart functions by autonomic nervous system (reprinted from [35]). CNS-central nervous system; RA-right atria; LA-left atria; LV-left ventricle; RV-right ventricle; SA-sinoatrial node ; AV-atrioventricular node; NE-norepinephrine; ACh-acetylocholine.

Long-term adaptation of cardiovascular system to physical activity may be observed through decrease in HR, increase in maximal cardiac output and stroke volume, elevation of oxygen content in blood as well as improvement of general tolerance of exercise and physical performance. In terms of BP, physical activity of gradually increasing intensity may be accompanied by elevation of systolic blood pressure while no changes in diastolic blood pressure may be observed. Additionally, regular physical activity has been shown to decrease pathological BP values due to lowered peripheral resistance and cardiac output. Table 3 shows response in HR and BP to different types of exercise of 10 studied subjects [52].

Table 3: Mean responses in BP and HR to different types of exercise (N=10). Static exercise has been performed at 30% of maximal voluntary contraction (based on [52]).

Variable	Rest	Static exercise	Dynamic exercise at 30% of VO_{2max}	Dynamic exercise at 60% of VO_{2max}
HR (beats/min^{-1})	61.9	84.1	93.7	143.9
Systolic BP (mm Hg)	121.6	166.7	150.8	173.9
Diastolic BP (mm Hg)	76.4	100.8	86.7	97.7
Mean BP (mm Hg)	91.2	122.2	107.7	121.6

1.4.3 Body Temperature

One of the key features of human survival is the ability to sense and regulate the body temperature. The mechanisms of body temperature regulation during rest and physical exercise play important roles in maintaining physiological homeostasis. To protect the athletes from heat injury and to manage the physical performance, it is pivotal to understand the principles of human body thermoregulation. Currently, there are several available methods for measurement of body temperature during the exercise and sport activity; nevertheless accurate assessment still remains challenging.

The body temperature includes core and shell temperature. The core temperature (T_C) is referred to the temperature of thoracic and cranial cavities and temperature of the abdominal region. The shell temperature (T_S) is the temperature of the skin, muscles and subcutaneous tissue, and it is influenced by the environmental conditions and skin blood flow. The core temperature at rest is about 36.8°C in healthy humans and it is regulated by the CNS. Exposure to cold environmental conditions can result in decrease in T_S, whereas T_C may remain relatively constant. In other words, core temperature is endothermic, while the shell temperature is ectothermic [53]. In order to achieve the thermal balance of the organism, ectothermic and endothermic properties of T_S and T_C are synchronised. The normal body temperature value is between 36.5°C and 37.5°C, while the temperature below 35.0°C is considered as hypothermia. The body temperature above 37.5°C and 40.0°C are classified as hyperthermia and hyperpyrexia, respectively [54].

An increase in heat production in the body during the exercise is caused by increased muscular activity due to the inefficiency of the metabolic reactions involved

in providing energy for muscle force development. During the physical activity, most of the heat passes to the body core via the convective flow of venous blood, which returns to the heart. The receptors located in the hypothalamus are responsible for sensing the alterations in body core temperature, which is regulated by several thermoregulation mechanisms, such as radiation, conduction, convection and evaporation. During the high intensity activity, equivalent to about 80-90% of maximal oxygen uptake, heat production can exceed 1000 W, and potentially increase the core body temperature by 1°C every 5-8 minutes, in case of lack of body's heat dissipation mechanisms. Within 15-20 minutes of intensive exercises, the body temperature can reach the dangerous level in hot environment [55]. The body temperature subsequent to exercise also depends on the initial body temperature. Since the large and sudden changes in body temperature during the physical activity have the negative impact on performance, warm-up exercise before the high intensity workout is highly recommended, as well as cool down period afterwards.

Most used non-invasive methods of core temperature measurement, are commonly taken at the sublingual site (oral temperature), the axilla, and the tympanic membrane [55]. Invasive methods for T_C measurement are gastrointestinal tract (GI), rectum and oesophagus. The temperatures measured at these sites are not uniformed, as they represent the local temperature of respective anatomical sites. The choice of the measurement site depends on the purpose of measurement and type of instruments. For sports and exercise purposes, GI tract, oesophagus and rectal T_C measurements are most commonly used, while sublingual, axilla and tympanic methods are mainly used in clinical setting.

Oral temperature measurement is one of the simplest and most commonly used methods in clinical conditions. The sublingual site is easily accessible for temperature measurement and gives the good interpretation of the core temperature. To achieve accurate temperature reading, it requires about 5 minutes steady state. As the reliability of this method can be influenced by breathing rate thus this method should not be regarded as suitable for core temperature measurement in sports and exercise.

The axilla core temperature is measured under the armpit and it is practical, non-invasive and safe. The inaccuracy and instability makes this method as poor choice for research application. Further disadvantages of axillar temperature measurement are

influence of ambient temperature, sweat, humidity and the density of the hair at the axilla, making it unsuitable for measurement of body temperature during sports and exercise.

Tympanic temperature is measured inside the ear, on tympanic membrane, and this method is probably the most precise in T_C measuring, among all non-invasive measurement methods. The blood supply from the internal carotid artery is brought to the tympanic membrane, and this artery also supplies the blood to the hypothalamus, the region in brain where the body temperature is regulated. Tympanic temperature is measured with infrared thermometer and it does not measure the temperature continuously, so this method is not suitable for constant measurement during the exercise. The major advantage of this method is the speed of measurement, which usually takes only few seconds, depending on the instrument type.

One of the most common methods for laboratory research is rectal measuring, which is performed by inserting a thermistor probe or a thermometer about 8 cm past the external anal sphincter. This method for T_C reading is stable and it is not affected by ambient conditions. The invasive nature of rectal temperature measurement can be traumatic and uncomfortable, and the usage of this method may discourage volunteers from participation in the research. The best alternative to the rectal temperature measurement is measuring in the ear canal (tympanic).

The other invasive T_C reading method is oesophagus temperature measurement. It requires inserting a thermistor probe through the oral or nasal passages into the oesophagus. When the probe is inserted, it should be adjusted in order to achieve the highest temperature reading, near the pulmonary artery. This method is often avoided because of its difficulty and discomfort during the research. This method is commonly performed if patients or research volunteers are not averse to the procedure of inserting the probe into the oesophagus.

The most complicated method for core temperature measurement is gastrointestinal method. The temperature is measured by ingesting a telemetric sensor that transmits the temperature wirelessly to an external device. This method is not affected by environmental factors so it is very suitable for temperature monitoring during the exercise, since there is no discomfort to the user and it gives the ability to track the temperature continuously during the sport activity. One of the disadvantages of

gastrointestinal temperature measurement is the difficulty in standardising the location of the sensor in GI tract. Gastrointestinal temperature reading can also be impaired by water and food intake if the sensor is ingested right before the exercise [53].

1.4.4 Ambient Temperature and Humidity

Ambient temperature and humidity can have a significant influence on human physiological responses to physical activity and sport performance. Too high temperatures of environment and elevated relative humidity can cause considerable stress for athletes training and can affect the results during exercise. The effects of ambient temperature and humidity have not been fully elucidated and represent an important question with no definitive answer. However, the environmental conditions are very important in professional sport, and athletes could use this information to prepare better for sport events in different environmental conditions than usual [56].

Currently, several evidence presenting the effect of high environmental temperature on maximal oxygen uptake and sport performance have been provided, and in some studies, it has been proven that elevated ambient temperature can significantly reduce VO_{2max} and impair anaerobic and aerobic performance [57]. Some studies report that the maximal oxygen uptake can significantly increase whit the relative humidity from 40% to 95% at thermoneutral conditions (20°C) in subjects with exercise-induced bronchoconstriction [56].

In the study of Zhao et al. which is described in [56], the Wingate test results under three different conditions with controlled relative humidity showed no significant difference in measurement of peak power, anaerobic capacity, or $[La^-]_b$ maximum levels. On the other hand, high ambient temperature (40°C) can significantly increase maximal lactate levels compared to thermoneutral environment (20°C). Therefore, environmental temperature is extremely important factor affecting the HR during the exercise; higher temperatures result in increased HR, as the blood influx to the skin is enhanced to dissipate the heat and simultaneously maintains the blood flow to the muscles. The BP can also vary in different temperature conditions, since the blood vessels diameter constricts in cold temperatures, causing accelerated HR to push blood

through the veins and arteries. In warmer conditions, both systolic and diastolic readings can increase.

1.5 Anaerobic Test Methods – Wingate Test

Determinant factors in many athletic activities are represented by anaerobic metabolism, speed, strength, maximal power and maximal oxygen uptake. There are two main types of anaerobic tests: 1) tests measuring anaerobic power and 2) anaerobic capacity. Anaerobic power of the athletes can be measured with several subsequent methods, such as monoarticular force-velocity tests, jump tests, staircase tests and cycle-ergometer tests [58]. Maximal anaerobic power, also known as peak power, should be measured with the optimal braking force, depending on the body weight of the subject. The most effective and common cycle-ergometer anaerobic test method for lower body musculature is called Wingate test.

The Wingate test, also known as the Wingate Anaerobic Test (WAnT), was first published in 1974 at the Wingate Institute in Israel. Basically, it was designed to measure anaerobic power of the lower body. However, it can also be used with arm crank devices. The Wingate test is probably the most popular test used for peak anaerobic power, total anaerobic capacity and anaerobic fatigue assessment. The Wingate test requires the subject to pedal a mechanically braked bicycle ergometer, for 30 seconds, at an "all-out" pace. The maximum power, also known as peak power, reached at the achieved maximum cadence. The test procedure should show the associations between test results on the test duration and on the present load. The individual is advised to complete a warm-up before the test, followed by a recovery cool down interval [59]. The output parameters from WAnT are usually represented as Peak Power (PP), Mean Power (MP), Anaerobic Power (AP), Anaerobic Capacity (AC) and Fatigue Index (FI). The whole procedure of Wingate test and output values will be described hereinafter in experimental design chapter.

The Wingate test can also be used in training instances, especially in cyclists. Since this method can increase anaerobic performance, many cycling athletes have taken to using repeated sprint intervals, such as WAnT, as training devices to increase

performance in the final leg of the race. The Wingate tests may be slightly modified versions of the standard test laid out above [60, 61].

1.6 Effect of Local Cooling in the Tissue

The body temperature, both core and shell, can affect the functional properties and performance of skeletal muscles during the exercise. The muscular force generation, contraction and relaxation activities are not independent from alterations in body temperature. It is well known that extreme values of both hypo- and hyperthermia impair muscle function, including contractile and locomotion abilities. Many studies have focused on the influence of temperature range on muscle activity and performance in sport, although providing misleading results and interpretations. From a medical point of view, prolonged or repeated cold exposure can induce fatigue, shivering and vasoconstriction responses, and impair neuromuscular function. In the thermoneutral environment (20°C), during the short-term maximum power output, the temperature of large muscles tends to be slightly higher than that found in resting muscle. Even in moderately cold conditions, the physiological mechanisms of homeothermy are unable to keep limb muscle temperature close to the optimal range. The peripheral parts of the body cool more quickly than the body core when the whole body is exposed to the cold. On the other hand, regarding changes in tissue temperature, the loco-motor speed through the neuromuscular transmission system of both afferent and efferent impulses may be affected [62].

In order to provide better understanding of temperature effects on muscle function, power output and fatigue, it is essential to identify the beyond effects of temperature on the contractile process. There are several steps from volition to power output which may be affected with alterations in temperature. The command chain for muscular contractions and pathway from the CNS to the muscle reaction is shown in Figure 12. Notably, there are several factors that can impair “upstream” or “downstream” communication, although temperature can also affect some of these steps in the pathway [63].

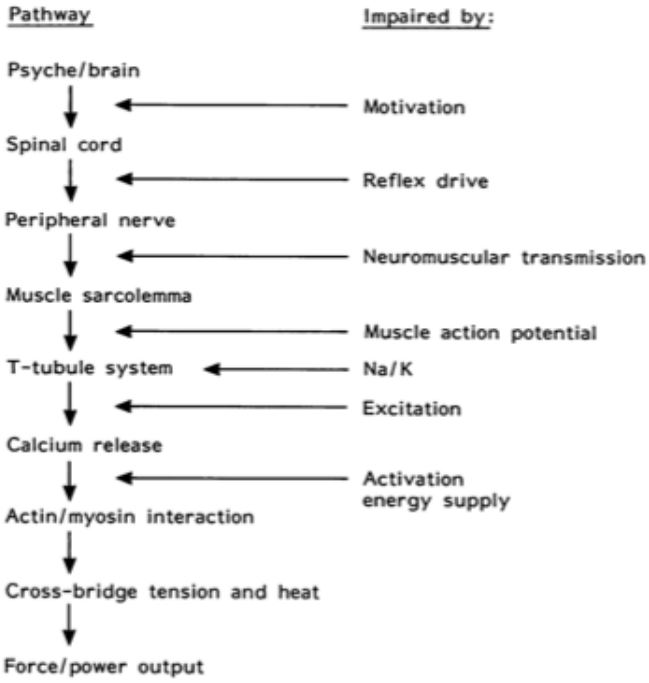

Figure 12: Command chain for muscular contraction (reprinted from [63]).

Local precooling is a popular strategy used for rapid removal of heat from the body immediately prior to exercising. Such technique can be used in order to improve the performance following precooling, since the body has the capacity to store metabolic and environmental heat. In the past three decades, the interest in precooling prior to sport activities was increased and many new techniques were developed. There are several methods for local precooling and they can be classified as external and internal, but there is also combined precooling.

The application of a cold medium or material on the surface of the body is known as external precooling. The most commonly used methods are exposure to cold air, cold water or ice-cold garments or pads. From principle, functioning of these methods relies on heat-transfer of conduction, convection, evaporation and radiation to temporarily achieve thermal equilibrium. During the external precooling, the organism reacts to the cold through cutaneous vasoconstriction, with consequent reduction in skin perfusion. In greater detail, one of the methods of external precooling is cold air exposure (0-5°C) that has been found to boost cycling and running activities, prior to the athletic performance. The effectiveness of mentioned precooling method is highly dependent on the ambient temperature and duration of the exposure. Another alternative

method of precooling is exposure to cold water, which can be performed partially or by whole-body application. The major advantage of water exposure is great heat loss to water, which is four times greater than air at the same temperature. This technique varies according to the duration of exposure, the body surface area that is exposed and the water temperature. The external body precooling can also be performed by wearing various water-perfused garments. The most effective and widely used precooling strategy is application of ice or ice products directly to the skin. Due to the powerful heat transfer capacity, heat from the skin and surrounding tissues is absorbed by ice and through a process called fusion (melting), it is converted to the water. To increase the temperature of water by 1°C, ice requires approximately 80-times more thermal energy, due to the phase change that must occur when water changes from its solid to liquid state. In comparison to water, ice precooling may be achieved at a faster rate, with lower amounts of integument, and to a greater magnitude. Ice-based precooling products offer practical advantages for cooling, since they are highly portable and can be incorporated into a huge range of garments or pads for application on specific body parts. Currently, there is available a wide range of different products based on above principle, in a shape of iced towels, ice vests, cooling vests, phase-change jackets, evaporative cooling garments and gel-based cooling packs [1].

Internal precooling is performed by taking a cold medium into the body. The medium can be injected through the mouth (or nose, in case of breathing) or can be performed by inhalation of cold air and the ingestion of cold fluids or ice. As a precooling strategy for enhancing sport performance, the inhalation of cold air is not typically used. The ingestion of cold fluids or ice is more often used for improving sport performance. The advantage of internal precooling methods is that they can also provide the nutrients (fluids, carbohydrates and electrolytes). However, the internal body precooling is less frequently used then external. Combining of two or more precooling methods can also provide better results in sport performance [1].

The effect of local precooling of lower body parts was the subject of many studies, but the results and opinions are rather diverse. In study of Castle el al. [64], three precooling techniques were used to research the effect during the sprint run in hot (33.7 ± 0.3°C), humid conditions. Twelve male soccer or rugby players completed four cycling intermittent sprint protocols. Each protocol consisted of twenty 2-minutes

periods, each including 10 seconds of rest, 5 seconds of maximal force exercise, and 105 seconds of active recovery. Each of experiments were done in different conditions, without precooling, with precooling via an ice vest, cold water immersion, and ice packs covering the upper legs in randomized order. The peak power output from the ergometer was measured and the results were compared graphically as shown in the Figure 13.

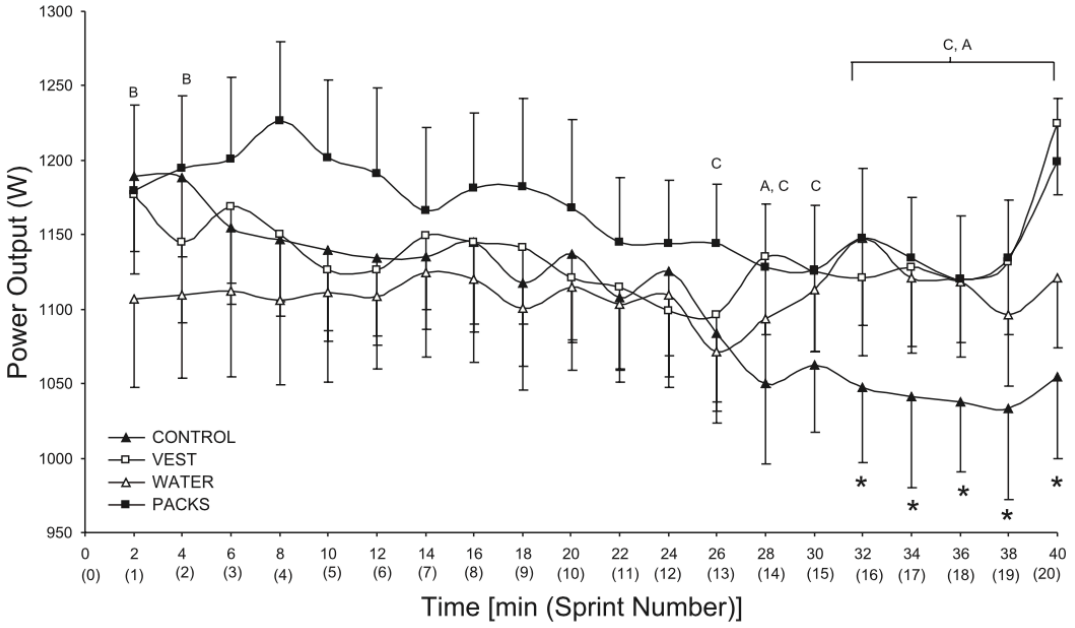

Figure 13: Mean peak power output after 20-minutes exercise in hot, humid conditions, after precooling via vest, water and ice packs (reprinted from [64]). *) p-value < 0.05

More particularly, this study showed significant difference in mean peak power output during the tests with precooling using vest, water and ice packs, compared to test without precooling. As it can be observed in the Figure 13, the highest peak power output was reported when precooling was done with ice packs placed at quadriceps and hamstring muscle group.

One similar research was done by Marsh et al. with thirteen professional cyclists [65]. The subjects were tested in random order, after either 30 minutes of precooling using cold water immersion or under control conditions (no precooling). The protocol consisted of 10-minute warm up, followed by 3 minutes of stretching, and immediately followed by 70 seconds “all-out” power test on a stationary ergometer. As the result,

mean power output for the 70 seconds performance test after precooling was significantly increased (2.7%). The effect of precooling was also noticeable with decreased core, mean body, and upper and lower body skin temperature, and HR was also significantly lower after precooling (Figure 14). In this study no differences in $[La^-]_b$ concentration were detected, however the parameters were measured only three times during the test.

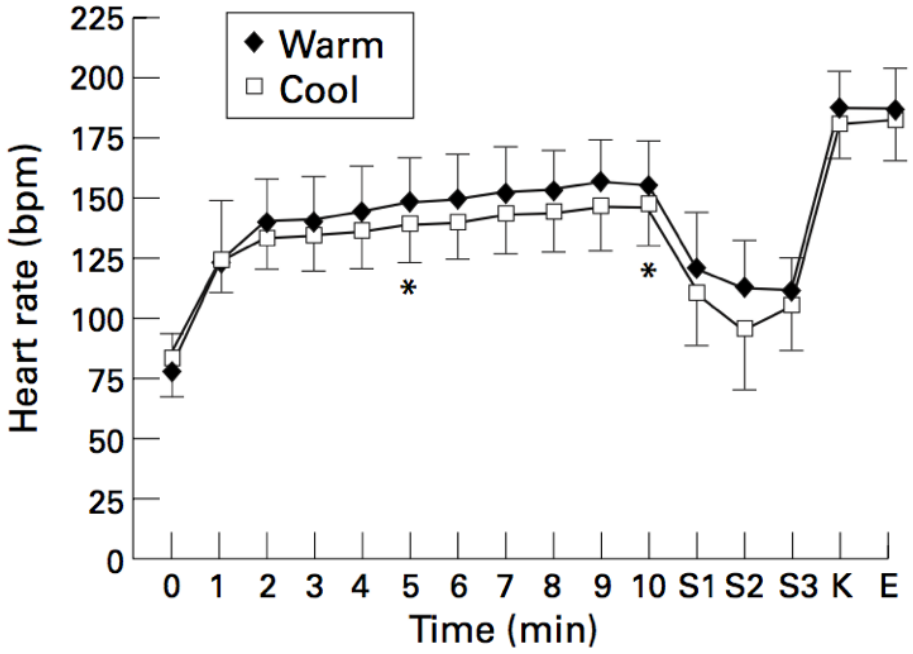

Figure 14: Mean HR through the warm up, stretch period, and performance test for warm and cool conditions (N=13). S1, S2, and S3 indicate first three minutes of the stretch period, and K and E indicate the start and end of the 70 second performance test, *) p-value < 0.05 (reprinted from [65]).

Contrary to previously mentioned studies, Crowley et al. demonstrated different results, the lower peak power output during the test after precooling [63]. In the study, three males were subjected to test, with and without precooling. The subjects were pedalling a mechanically braked Monark cycle ergometer with maximal effort for 30 seconds (Wingate test). The precooling of lower body was done by 11.5 - 12.2°C water for 30 minutes. Table 4 presents lowered average and peak power output during the tests after precooling in all three subjects. The fatigue index was also significantly lower after cold

water precooling than in control conditions, however contrary to other studies the warm up period before the performance has been omitted. Notably, the effect of local precooling in different conditions remains unclear, therefore further investigations should be performed in this area.

Table 4: Effect of cooling on average power output, peak power and fatigue index (based on [63]).

Subject no.	Average power (W/kg)			Peak power (W)			Fatigue Index (W/s)		
	Control* (n=6)	After cold (2 trials)	Difference (2 trials)	Control*	After cold (2 trials)	Difference (2 trials)	Control* (n=6)	After cold (2 trials)	Difference (2 trials)
1	9.42(0.18)	7.37 7.74	2.05 1.68	761(30)	537 629	224 132	13.77(1.32)	9.62 8.38	4.15 5.39
2	8.69(0.23)	5.88 5.31	2.81 3.38	686(17)	419 413	267 273	14.46(0.67)	6.97 10.61	7.49 3.85
3	10.19(0.29)	7.71 7.54	2.48 2.65	950(63)	697 651	253 299	14.29(2.02)	10.35 11.93	3.94 2.36

*Values are mean (SD)

1.7 EMCOOLS Flex.Pad™

Flex.Pad™ is a skin-friendly, non-invasive surface cooling system for temperature management, used in pre- and in-hospital setting, produced by *EMCOOLS Medical Cooling Systems GmbH*. *EMCOOLS* products have been used over ten years in hypothermia or normothermia treatment, however it may be also used in management of fever, hyperthermia, cardiac arrest, myocardial infraction, stroke, sepsis, as well as in local pain relief (cryotherapy) and post-surgical rehabilitation. A *Flex.Pad™* is made from Thermoplastic Polyurethane (TPU), which gets deep-drawn into the shape of a set of energy-cells. The cells are filled with specific material, referred to as *HypoCarbon®,* which is environmental friendly and non-toxic, with excellent heat dissipation. The surface of *Flex.Pad™* is made of medical hydrogel, which is suitable for direct skin contact, skin-friendly and dermatologically tested. A *Flex.Pad™* is 11 mm thick, 205 mm wide, 305 mm long, and weighs 0.55kg, and perforated so it can be divided into

smaller pads [68]. Figure 15 shows two *Flex.Pad™* and its cross-section on patient's skin.

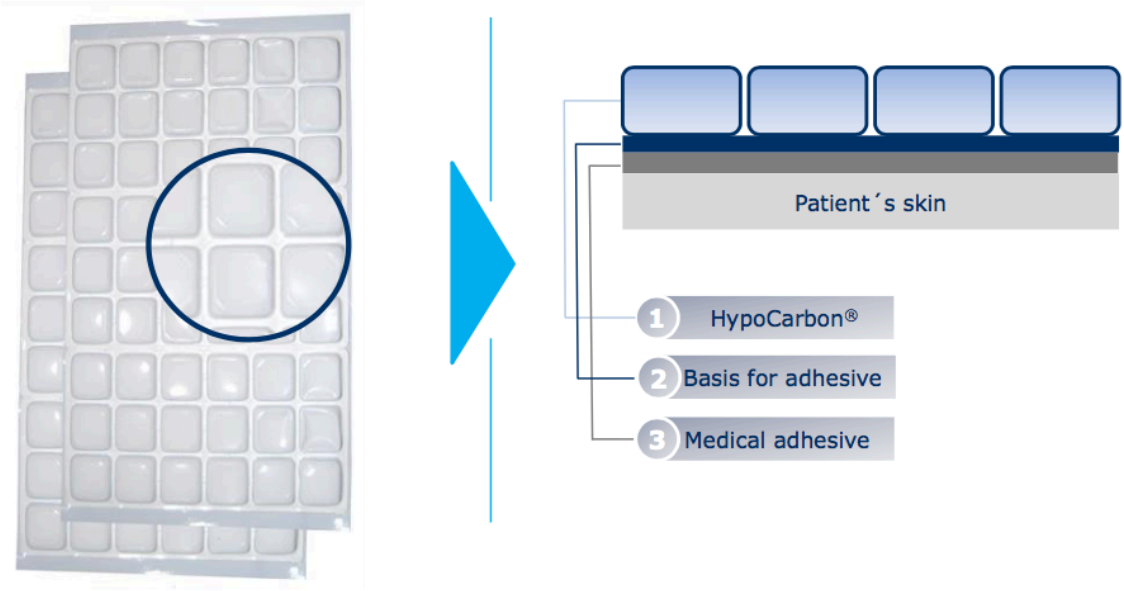

Figure 15: Two EMCOOLS Flex.Pad™ and cross-section of the product (reprinted from [66]).

Prior to application, *Flex.Pad™* should be frozen at -8 to -11°C, which takes 12-48 hours, depending on the freezer. The vacuum package of the product is equipped with the colour indicator, and protects the pad from contamination. When *Flex.Pad™* is ready to use (blue colour indicator), protective foil should be peeled off and placed on the dry skin.

Advanced mobility of EMCOOLS cooling products is provided by *Six.Pack Family* transportation and storage systems, which are characterized by an ideal thermal conductivity, ensuring ready-to-use *EMCOOLS Flex.Pad™* for up to 12 hours. Compared to rubber and polyamides, which have thermal conductivity of λ[1] = 0.16 and λ = 0.25, respectively, *Six.Pack* is made of light and durable materials with thermal conductivity of just λ = 0.019 [66]. Figure 16 shows *Six.Pack Family* systems for transportation and storage, recommended field of application and possible capacity of each product.

[1] λ - thermal conductivity

Figure 16: Six.Pack Family transportation and storage systems (reprinted from [66]).

Previous studies confirmed positive effect of therapy with *HypoCarbon®*-based products in several indications. *HypoCarbon®* provides cooling rates of up to 3.3°C/hour and has thermal conductivity of $\lambda = 35$, which is 15-times better than ice and 58-times better than water [67]. Figure 17 shows the esophageal temperature recorded during the 6 hours fever therapy with two *Flex.Pad™* in an unconscious septic patient. Initial temperature was 39.5°C and target temperature of 36.9°C was reached after 60 minutes with subsequent removal of pads and maintenance of normothermia for at least five hours.

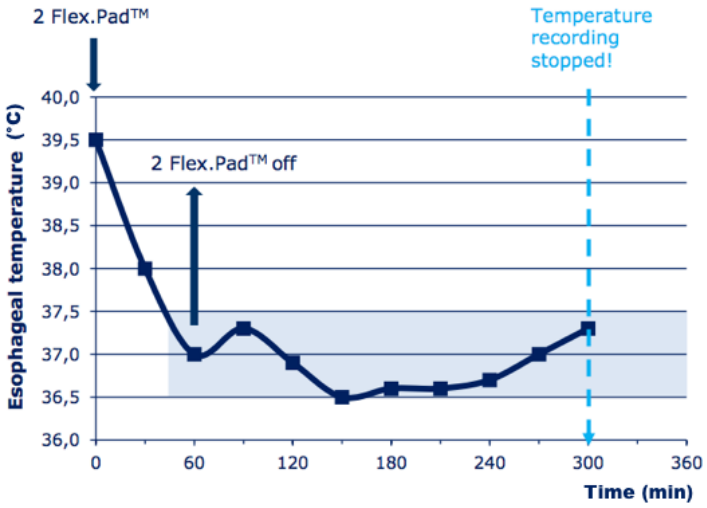

Figure 17: Fever therapy with 2 EMCOOLS Flex.Pad™ in an unconscious septic patient [67]. (Source: Data on file at EMCOOLS. SPCSK Warsaw, Poland)

Another study showed the fever management with 4 *Flex.Pad™* in a patient with severe traumatic brain injury (TBI) and polytrauma. The temperature was measured with an esophageal probe during the 70 minutes of therapy. A target temperature of <37.7°C was reached after 30 minutes, with temperature change of 2.2°C per hour (Figure 18).

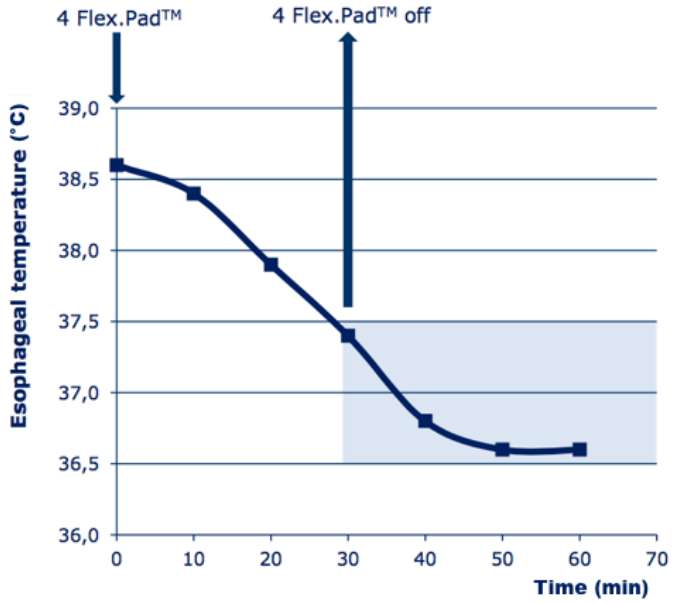

Figure 18: Fever therapy with 4 EMCOOLS Flex.Pad™ in an unconscious patient with severe TBI and refractory fever [67]. (Source: Data on file at EMCOOLS. Farwaniya Hospital, Kuwait)

Effects of fever therapy with four *Flex.Pad™* measured with an axillar temperature probe during 10 hours observation in an awake patient with stroke is shown in the Figure 19. Initial temperature of 38.5°C was decreased to the target temperature of 36.5°C in 60 minutes of therapy and body temperature was normalized for next 9 hours.

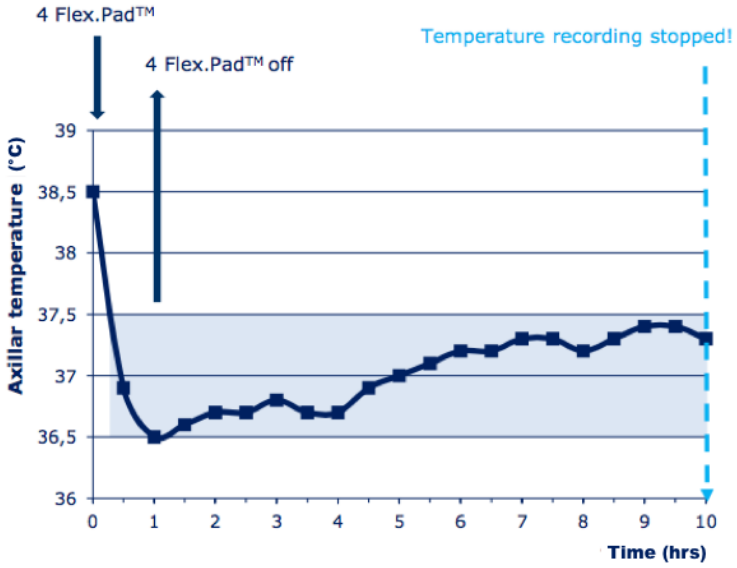

Figure 19: Fever therapy with 4 EMCOOLS Flex.Pad™ in an awake patient with stroke [67]. (Source: Data on file at EMCOOLS. Fakultná Nemocnica Trnava, Slovakia)

Another study demonstrated positive effect of cooling with five *Flex.Pad™* in a patient with a uterine sarcoma; particularly the body core temperature was decreased by 1.5°C in 30 minutes from initial temperature of 39°C to target temperature of 37.5°C (Figure 20).

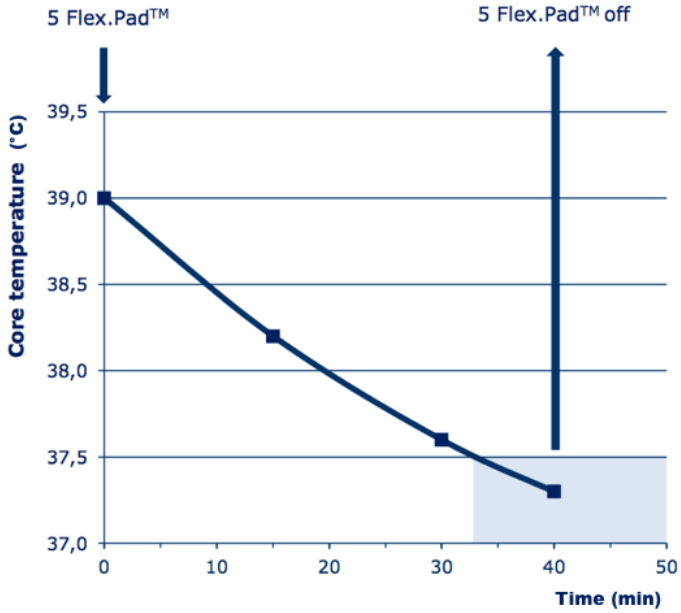

Figure 20: Fever therapy with five EMCOOLS Flex.Pad™ in a patient with an uterine sarcoma [67]. (Source: Data on file at EMCOOLS. Cancer Control Center, Kuwait)

Figure 21 shows therapeutic outcomes of hypothermia (32-34°C) treatment with seven *Flex.Pad™* in an unconscious patient after cardiac arrest. The body core temperature was measured with a vesicular temperature probe during 23 hours and as result we can see that the temperature was decreased by 2.4°C from 36.1°C to target temperature of <34°C in 120 minutes. After two hours, all seven pads were removed and one more pad was added after 17 hours in order to preserve desired core temperature.

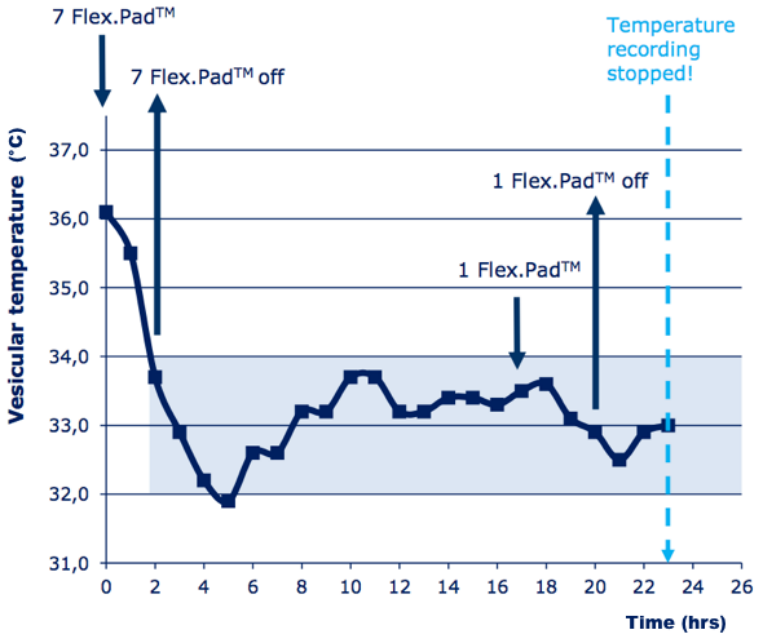

Figure 21: Therapeutic hypothermia with 7 EMCOOLS Flex.Pad™ in an unconscious patient post cardiac arrest [67]. (Source: Data on file at EMCOOLS. Regionshospitalet Viborg, Denmark)

All above studies comprises hard evidence supporting the effectiveness of *EMCOOLS Flex.Pad™* in clinical treatment. However, the application of *EMCOOLS* products during the sports activities, as well as their efficiency, have not been thoroughly investigated yet. However, this argument encourages performing more in-depth research in the field of muscle precooling and its associations with physical performance.

Materials and Methods

2.1 Subjects

Ten healthy, male subjects (mean ± SD; age: 34 ± 11 years (22-56); weight: 75.7 ± 7 kg (62-88); height: 181.8 ± 6.7 cm (170-193)) volunteered to participate in this study. Before initiation of any testing, subjects were fully apprised of the purpose, methods, and potential risks of the study. Each volunteer reserved the right to withdraw at any time without retribution. Prior to submitting to the study, all subjects signed consent form of participation in Wingate test.

Participants were hobby, amateur or professional athletes, older than 18 years, regularly cultivating performance-oriented cycling, no matter what discipline. On the day before each test participants should refrain from sport activities, as well as atypical caffeine consumption. The nutrition of a subject on the day of a test, as well as the 2 preceding days, should be kept in time and in composition. All subjects have completed medical examination before the tests, in order to determine their suitability for high stress exercises including electrocardiography (ECG). The physical characteristics of the subjects are given in Table 5.

Table 5: The physical characteristics of subjects.

Subject	Age	Weight (kg)	Height (cm)
1	45	76.4	184
2	23	68	175
3	29	76	182
4	24	88	182
5	25	78	193
6	56	73	183
7	38	76	182
8	22	62	170
9	42	79	177
10	38	81	190
Mean	34.2	75.74	181.8
(SD)	11.41	7.07	6.73

2.2 Experimental design

The Wingate test was performed twice on group of ten subjects at time interval of seven days, with and without prior precooling of quadriceps muscles, respectively. First group of five participants started with precooling prior to first attempt to Wingate test, whereas second group of another five participants made first attempt without prior precooling. Each test was carried out strictly in accordance to the rules presented in Table 6. Additionally, during the procedure three cardiophysiological parameters such as HR, BP (systolic and diastolic) and tympanic body temperature were being measured according to the protocol shown in Table 6. Moreover, $[La^-]_b$ was measured by collecting blood specimen from earlobe of tested participant at predefined points: prior to and after precooling, at the end of warm-up phase, immediately after Wingate test and subsequently after 1, 3, 5, 7, 10, 15 and 30 minutes after the end of load exercise (Table 7). After the last measurement of $[La^-]_b$ the procedure was considered as completed. The entire experiment was performed at room temperature of 21.2°C ± 0.7°C and humidity of 53.8 % ± 3.1 % (mean ± SD).

Table 6: Protocol of cardiophysiological parameters measurement.

Time (min)	Procedure with precooling	Procedure with no precooling
0	Blood pressure, body temperature	-
	precooling of both quadriceps muscles in sit-down rest position	
10	Blood pressure, body temperature	
	taking an ascending position on ergometer and light warm-up of low cadence and resistance	
15	Blood pressure, body temperature	
	full load exercise according to "Wingate" (flying start)	
15.5	Blood pressure, body temperature	
	slow declining cycling of low cadence and resistance	
20.5	sit-down rest position with no further activity	
	Blood pressure, body temperature	

Table 7: Workflow of the experiment and Wingate test procedure with measurement of lactate.

Time (min)	Procedure with precooling	Procedure with no precooling
0	$[La^-]_b$ measurement	-
	precooling of both quadriceps muscles in sit-down rest position	
10	$[La^-]_b$ measurement	
	taking an ascending position on ergometer and light warm-up of low cadence and resistance	
15	$[La^-]_b$ measurement	
	full load exercise according to "Wingate" (flying start)	
15.5	$[La^-]_b$ measurement	
	slow declining cycling of low cadence and resistance	
16.5	$[La^-]_b$ measurement	
19.5	$[La^-]_b$ measurement	
20.5	sit-down rest position with no further activity	
	$[La^-]_b$ measurement	
22.5	$[La^-]_b$ measurement	
25.5	$[La^-]_b$ measurement	
30.5	$[La^-]_b$ measurement	
45.5	$[La^-]_b$ measurement	

Whole procedure of the Wingate's test has been carried out using ergometer produced by *RBM elektronik-automation GmbH* with *Cyclus2* (Figures 22 and 23) output device. *Cyclus2* enables wide range of performance diagnostic applications including onset of blood lactate accumulation (OBLA), physical work capacity (PWC), maximum cadence, isokinetic maximum strength, cardio performance indicator (CPI) and Wingate tests. Furthermore, through the procedure of the latter test, it allows to measure peak power corresponding with maximal alacticide performance capability of athlete.

In more details, *Cyclus2* output device measures the following parameters during the test:

- peak power (PP) – ideally measured in first 5 seconds interval of the Wingate test and is represented by the Equation 1:

$$PP = \frac{F \times d}{t} \quad (1)$$

where:

(F) – force, the amount of resistance applied to the wheel,

(d) – distance, the number of resolutions multiplied by the distance per evolution,

(t) – time.

The resulting peak power is expressed in watts (W);

- anaerobic power (AP) – simply determined by dividing the peak power by body mass of the subject (W/kg);
- mean power (MP) – the mean value of power during 30 seconds test;
- anaerobic capacity (AC) – mean power divided by the body weight of the subject (W/kg);
- fatigue index (FI) – difference between peak power output and the lowest power output of the successive 5 seconds interval (W/s).

Figure 22: Ergometer used in the experiment produced by RBM elektronik-automation GmbH (taken by author of this thesis).

Figure 23: Cyclus2 output device measuring the Wingate's test results (taken by author of this thesis).

Noteworthy, modified setup of the ergometer was applied with adjusted breaking force estimated as 0.1 kg per 1 kg of subject's body mass [68]. All results were read from the device using *Comsoft PC Software* enabling to connect *Cyclus2* device with PC to read the output data[2].

Each participant used own, adjusted ergometer. Precooling intervention was applied with the use of precooling pads derived by *EMCOOLS* (Figure 24). HR was measured using *Bluetooth Polar H1* sensor connected with *Cyclus2* device (Figure 25). Particularly, H1 sensor comprises of belt-like elastic band that is wrapped around the subject's chest, electrode pad located against skin and transmitter. On balance, sensor records electrical activity of heart in ECG-based manner. Therefore, pad located against skin of user needs sweat to record any electrical signal, which is subsequently sent to transmitter when registered. Noteworthy, this sensor is of high accuracy and has been designed to avoid any potential interference from other devices through transmitting the data in coded 5 kHz transmission form[3]. It consists of two main parts: plastic electrode area placed on a strap and connector. For the purpose of Wingate test the electrode areas on the strap were moistened and the connector was attached. Subsequently, the strap

[2] Complete specification of Cyclus2 may be found at manufacturers website: http://www.cyclus2.com/en/wingate-anaerobic-test.htm
[3] According to manufacturer specification that may be found at Polar website: https://www.polar.com/at-de

was tied around chest of the participant below chest muscles and the moistened electrode areas were firmly against subject's skin.

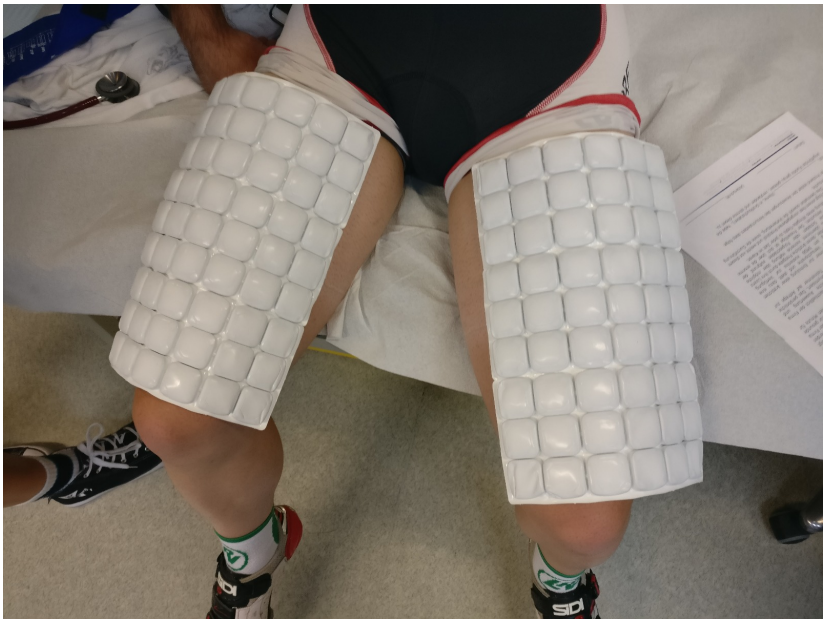

Figure 24: Precooling pads derived by *EMCOOLS* for precooling intervention prior to the Wingate's test (taken by author of this thesis).

Figure 25: Polar H1 Bluetooth sensor that measures HR during the Wingate's procedure (reprinted from manufacturer webpage: www.polar.com).

BP both systolic and diastolic was measured with standard sphygmomanometer and stethoscope according to Korotkov method. Briefly, the air cuff is placed on the shoulder, 2-3 cm above elbow flexion at the height of the heart followed by its inflation

until complete occlusion of the artery (above systolic pressure). Subsequently, the air valve is slowly released. The systolic pressure is recorded when two repetitive clear tones will correspond to two consecutive heart beats and diastolic pressure is recorded as the repeated sounds disappear. The tympanic body temperature was measured with *Braun Thermoscan 4520* (Figure 26). This thermometer measures the infrared heat that is generated by the eardrum and adjacent tissues. For this purpose it is placed in the ear for continuous monitoring of infrared radiation. When the accurate reading was assured, the measurement is finished and displayed. Importantly, the accuracy range for displayed temperature is claimed as ± 0.2°C for 35.5-42°C and ± 0.3°C outside the former range with clinical repeatability of ± 0.14°C [4].

Figure 26: Braun Thermoscan 4520 thermometer for tympanic measurement of body temperature during the Wingate's procedure (taken by author of this thesis).

$[La^-]_b$ measurements were performed using *Biosen* device produced by *EKF Diagnostics* (Figure 27). The *Biosen* device is easy to use and measures lactate and glucose concentration in samples of 20 µl of blood, plasma or serum. The measurement itself is very accurate and reliable due to enzymatic-amperometric method using chip-sensor technology applied. It measures glucose in range of 0.5-50 mmol/L (9-900 mg/dL) and lactate in range of 0.5-40 mmol/L (5-360 mg/dL). The reported imprecision rate is 12 mmol/L that stands for coefficient of variation (CV) lower than 1.5 %. Results

[4] According to manufacturer specification that may be found at Braun website: http://www.service.braun.com/

may be read after 20-45 seconds [69]. Finally, ambient temperature and humidity were measured with *Testo 435-4* produced by *Testo SE & Co. KGaA*.

Figure 27: Biosen device used for measurement of $[La^-]_b$ during the Wingate's test procedure (taken by author of this thesis).

2.3 Statistical Analysis

Firstly, the difference between measurement without and with precooling of all quantitative variables (dependent measurements: anaerobic capacity (AC), anaerobic power (AP), fatigue index (FI), heart rate (HR), peak power (PP), mean power (MP), body temperature (BT), blood pressure systolic and diastolic (BP_{sys} and BP_{dias}, respectively), blood lactate concentration $[La^-]_b$) was tested for normality of distribution with the Shapiro-Wilk test, which assumes following hypotheses:

H_0: the sample comes from population of normal distribution;

H_A: the sample does not come from population of normal distribution.

Parameters, which difference between paired readings complied with the assumptions of normal distribution were tested for significance between performance with and without precooling intervention with two-tailed paired student's t-test. Parameters that did not follow normal distribution according to Shapiro-Wilk test were additionally evaluated using quantile-quantile plots (Q-Q plots). Q-Q plots present probability and serve as graphical method for comparing distributions of samples by plotting quantiles of tested sample against theoretical quantiles of some distribution (normal distribution in this case). Due to small size of group of participants, some of tested parameters might show false deviations from normal distribution, thus Q-Q plot method was used to indicate, in which cases Gaussian distribution would be reasonable approximation despite false null hypothesis in Shapiro-Wilk test. If testing of normality of distribution failed with Q-Q plot either, then non-parametric Wilcoxon test with continuity correction was applied with statistical significance set as $\alpha=0.05$. Following hypotheses were tested:

H_0: precooling intervention does not significantly alter particular parameter;

H_A: precooling intervention alters significantly particular parameter.

Parameters such as BT, BP_{sys}, BP_{dias} and $[La^-]_b$ were tested for significant difference between measurements, separately, at specific time points. Power output, as being measured continuously through 30 seconds (239 readings per test), was tested for significant difference via employment of two-way repeated measures analysis of variance (ANOVA). The ANOVA model was built for power output in dependence of intervention (with or without precooling) and time followed by significant results with *post-hoc* Tukey's test (*a posteriori* Tukey's honestly significant difference test, which

applies to variables of similar number). Multiple regression between parameters like AC, AP, FI, HR, MP and PP under no and with precooling conditions was based on correlation coefficient. Hypotheses' testing for all statistical tests was performed according to the general principle:

if testing probability value (p) > α: there is no basis to reject null hypothesis;

if p ≤ α: the null hypothesis may be rejected and alternative hypothesis may be accepted.

All data were reported as means ± standard deviation (SD) and presented in form of graphs. All analyses were performed in R environment (3.4.1 version), a coding language widely used in biomedical research. More specifically, all graphs were prepared using *ggplot2* package with *ggplot()*, *ggline()* and *ggboxplot()* functions. Statistical analyses were performed with *stats*, *car*, *Hmisc*, *Rmisc*, *ggpubr* and *MASS* packages with *shapiro.test()*, *t.test()*, *wilcox.test()*, *qqnorm()*, *qqline()*, *stat_compare_means()*, *anova()* and *TukeyHSD()* functions. Multiple regression of parameters such as AC, AP, FI, HR, MP and PP under no precooling and with precooling conditions was performed using *GGally* package with *ggpairs()* function.

2.4 Survey for participants of the study

Participants of the study were asked to fill the survey afterwards test with precooling intervention. They responded to open questions regarding usefulness of precooling procedure (is precooling practical and combinable with preparation to any tests) and efficiency of the intervention (were any effects of precooling noticeable/perceptible) as well as questions limited to the scale 1 – 4, where 1 – very good and 4 – very poor, describing features of precooling such as adaptation of pad to surface, suitability of pad size, pad acceptance, cold tolerance, lowered sensitivity of muscles to pain and slowed down fatigue through increased endurance in upper musculature. They were also allowed to enclose any relevant comments.

Results

3.1 Normality of distribution

The difference between measurement with and without precooling was tested for normality of distribution using Shapiro-Wilk test, as recommended for paired dependent variables. Table 8 presents results of testing the normality of distribution with W statistic comprising critical value for assumed probability and number of observations as well as probability value of Shapiro-Wilk test (p-value). Variables such as AC, AP, FI, PP, MP and HR followed Gaussian distribution the same as measurements of $[La^-]_b$, tympanic body temperature and blood pressure at each of time points with exception of 30 minutes after Wingate test in case of $[La^-]_b$, before Wingate test, cool-down and after cool-down in case of diastolic blood pressure.

Table 8: Statistics of testing normality of distribution. Samples, which according to Shapiro-Wilk test do not come from population of normal distribution are bolded.

Variable		W	p-value
Anaerobic capacity		0.9585	0.7686
Anaerobic power		0.91815	0.3418
Fatigue index		0.98934	0.996
Peak power		0.95149	0.6862
Mean power		0.97717	0.9483
Heart rate		0.973	0.9172
Blood lactate	before warm-up	0.95988	0.7845
	before Wingate test	0.93691	0.5192
	before cool-down	0.95752	0.7573
	1 min after Wingate test	0.9223	0.3765
	3 min after Wingate test	0.94543	0.6148
	5 min after Wingate test	0.94666	0.6291
	7 min after Wingate test	0.92496	0.4002
	10 min after Wingate test	0.90658	0.2583
	15 min after Wingate test	0.9699	0.8899
	30 min after Wingate test	**0.81838**	**0.02422**

Body temperature	before warm-up	0.93826	0.5339
	before Wingate test	0.9532	0.7064
	before cool-down	0.91174	0.2932
	after cool-down	0.95942	0.7793
Blood pressure systolic	before warm-up	0.85725	0.07082
	before Wingate test	0.84561	0.05149
	before cool-down	0.90854	0.2711
	after cool-down	0.95067	0.6764
Blood pressure diastolic	before warm-up	0.87845	0.1252
	before Wingate test	**0.76915**	**0.006106**
	before cool-down	**0.82579**	**0.02977**
	after cool-down	**0.63157**	**0.0001319**

Subsequently for samples that did not followed normal distribution according to Shapiro-Wilk test, the Q-Q plots were employed to confirm accurate rejection of null hypothesis. In case of $[La^-]_b$ measurement 30 minutes after Wingate test (Figure 28 A) as well as diastolic blood pressure measurement before Wingate test and cool-down (Figure 28 B and C) normal distribution has been on balance accepted as reasonable approximation. In contrast, measurement of diastolic pressure after cool-down could not be considered as sample complying with assumptions of normal distribution (Figure 28 D).

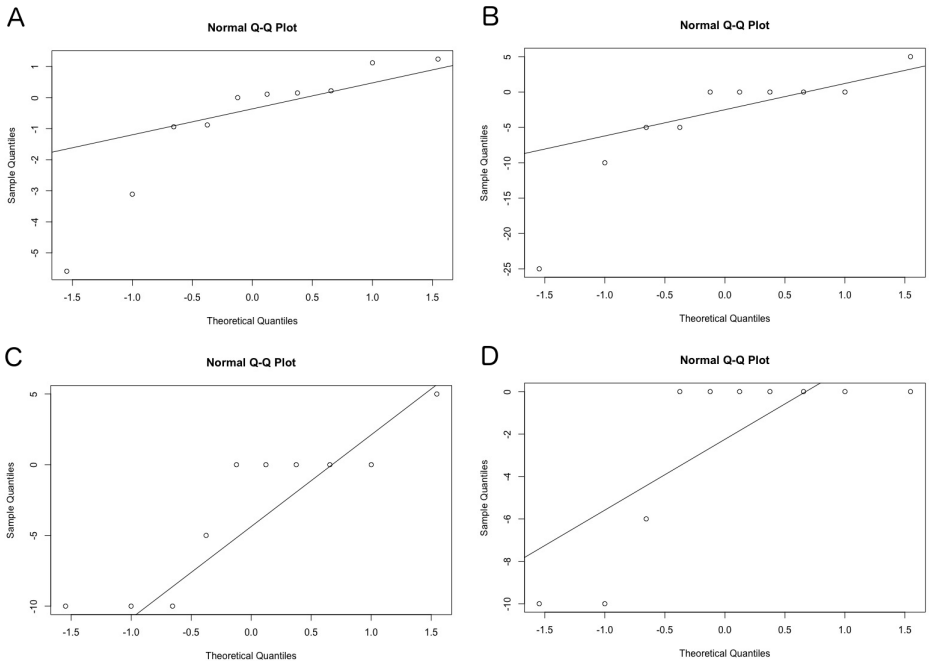

Figure 28: Q-Q plots presenting sample vs. theoretical distribution of quantiles. Samples of A) $[La^-]_b$ measurement 30 minutes after Wingate test; B) diastolic blood pressure measurement before Wingate test, C) before cool-down and D) after cool-down.

3.2 Performance

Analysis of the results using paired student's t-test showed that precooling intervention does not influence overall performance measured as anaerobic capacity, anaerobic power, fatigue index, peak power and mean power in significant manner (Table 9). Nonetheless, with cooled quadriceps, all of above parameters showed tendency towards decline, but did not passed significance threshold (Figures 29-33).

Table 9: Statistics of mean performance parameters by intervention.

Variable	p-value	Mean	
		no precooling	with precooling
Anaerobic capacity	0.12	9.41	9.2
Anaerobic power	0.55	11.74	11.59
Fatigue index	0.91	14.22	14.14
Peak power	0.62	886.19	876.56
Mean power	0.1	709.71	691.73

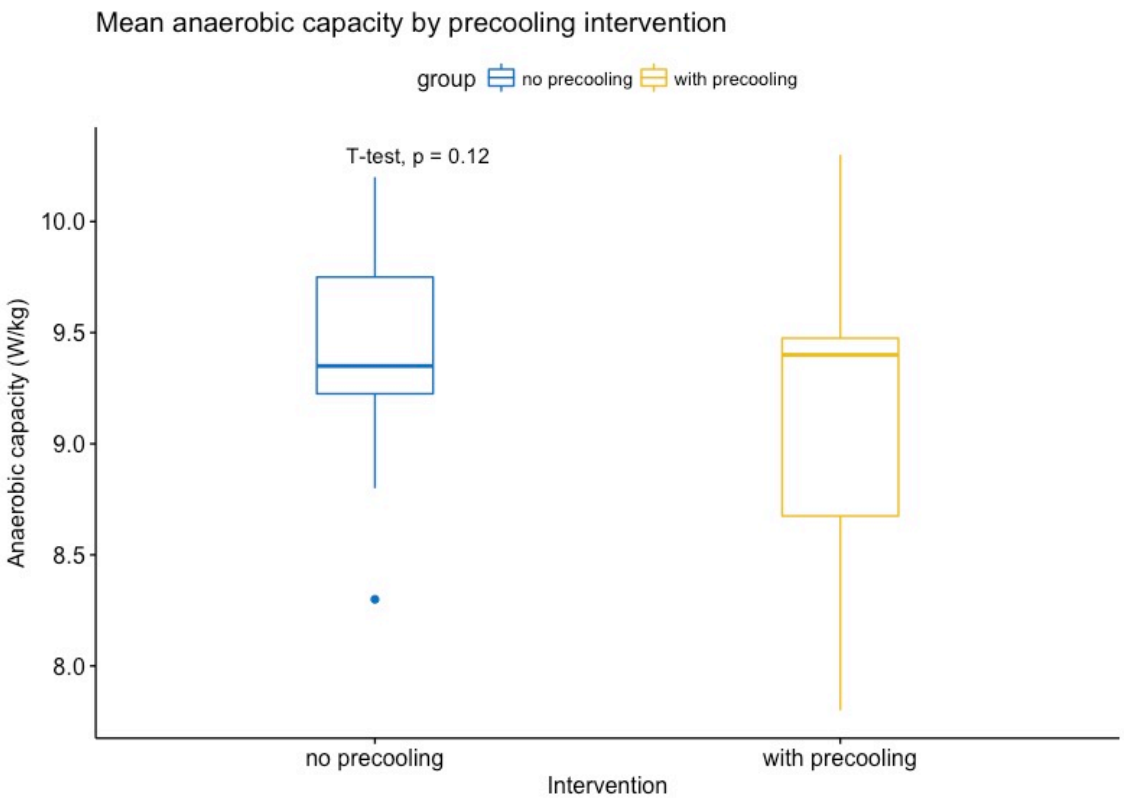

Figure 29: Mean anaerobic capacity by precooling intervention.

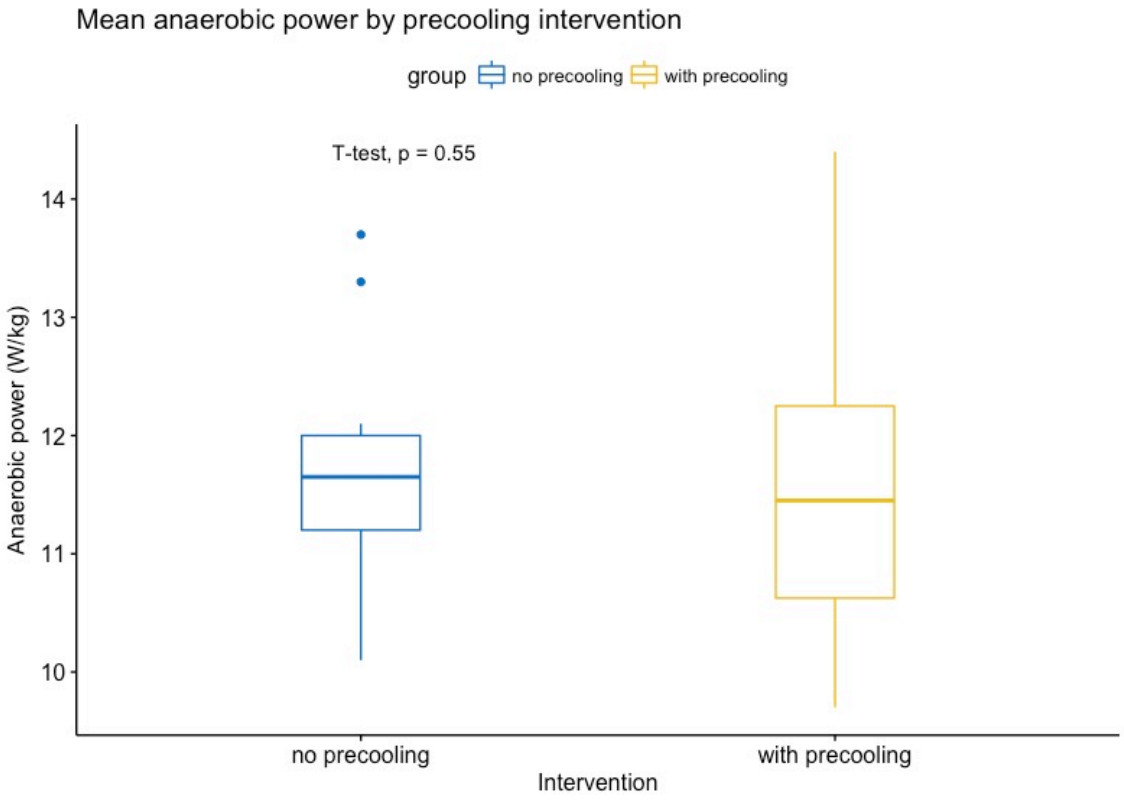

Figure 30: Mean anaerobic power by precooling intervention.

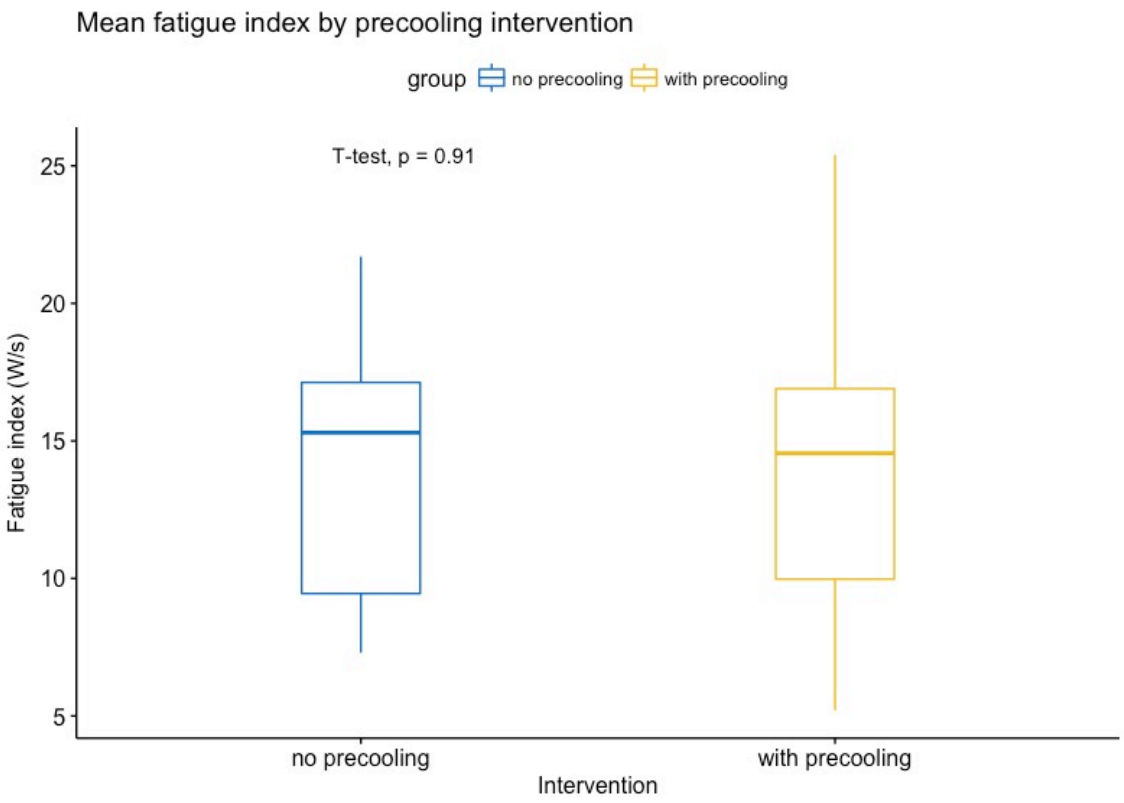

Figure 31: Mean fatigue index by precooling intervention.

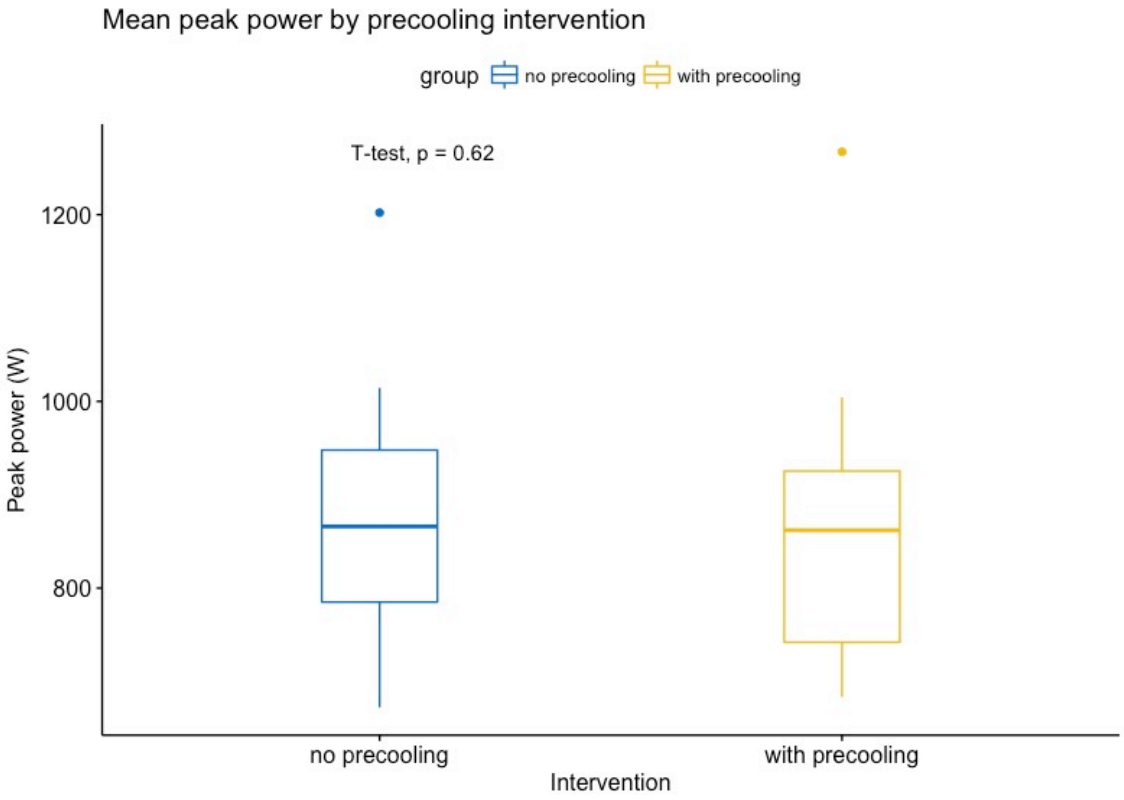

Figure 32: Mean peak power by precooling intervention.

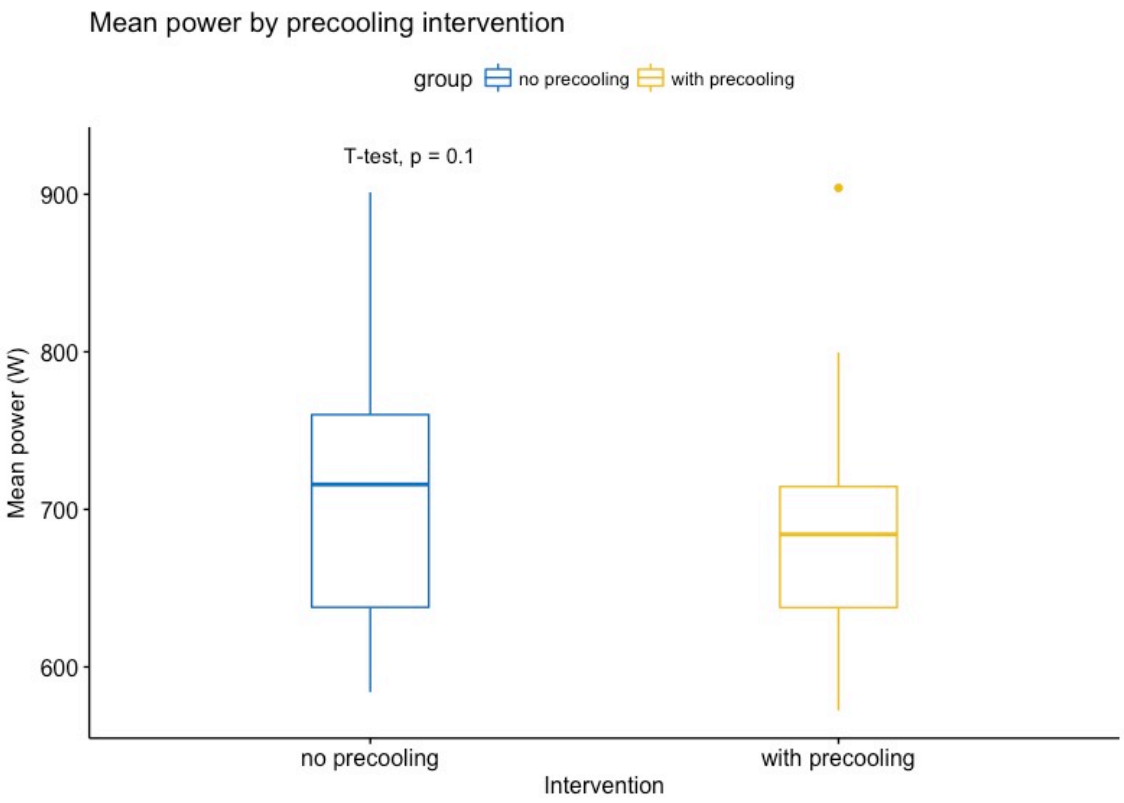

Figure 33: Mean power by precooling intervention.

In contrast, as concluded from ANOVA, precooling intervention significantly lowered power output measured continuously during 30 seconds Wingate test and proved much higher variation between than within groups (F-value=14.158, $p<0.001$). Subsequent *post-hoc* Tukey's honestly significant difference test confirmed the primary finding with difference in power output between tests without and with precooling of 11.99, where power output with precooling averaged 11.99 units lower (Table 10). Importantly, basic assumption of normality of distribution of ANOVA model has been complied as shown in the Figure 34. The mean power output in groups according to time as continuous variable is shown in the Figure 35.

Additionally, further associations between all performance parameters were analysed with multiple regression at state without (Figure 36) and with (Figure 37) precooling intervention separately. In greater detail, within both groups all performance parameters showed at least strong, positive, significant correlations. By no precooling simultaneous increase in both parameters has been found for following pairs: FI with PP, AP with PP and FI, AC with PP and AP, MP with PP, FI, AP and AC, whereas with

precooling it has been observed in: HR with PP, FI with PP and HR, AP with PP, HR and FI, AC with PP and AP, MP with PP, HR, FI, AP and AC.

Table 10: Statistics of ANOVA model with following *post-hoc* Tukey's test.

ANOVA				Tukey's HSD				
Variable	Df	F-value	p-value	Variable	Diff	Lwr	Upr	p-value
group	1	14.158	0.00017	with precooling-no precooling	-11.99	-18.24	-5.74	0.00017

Df – degress of freedom; Diff – unit difference between groups; Lwr – lower 95% confidence interval for the difference; Upr – upper 95% confidence interval for the difference

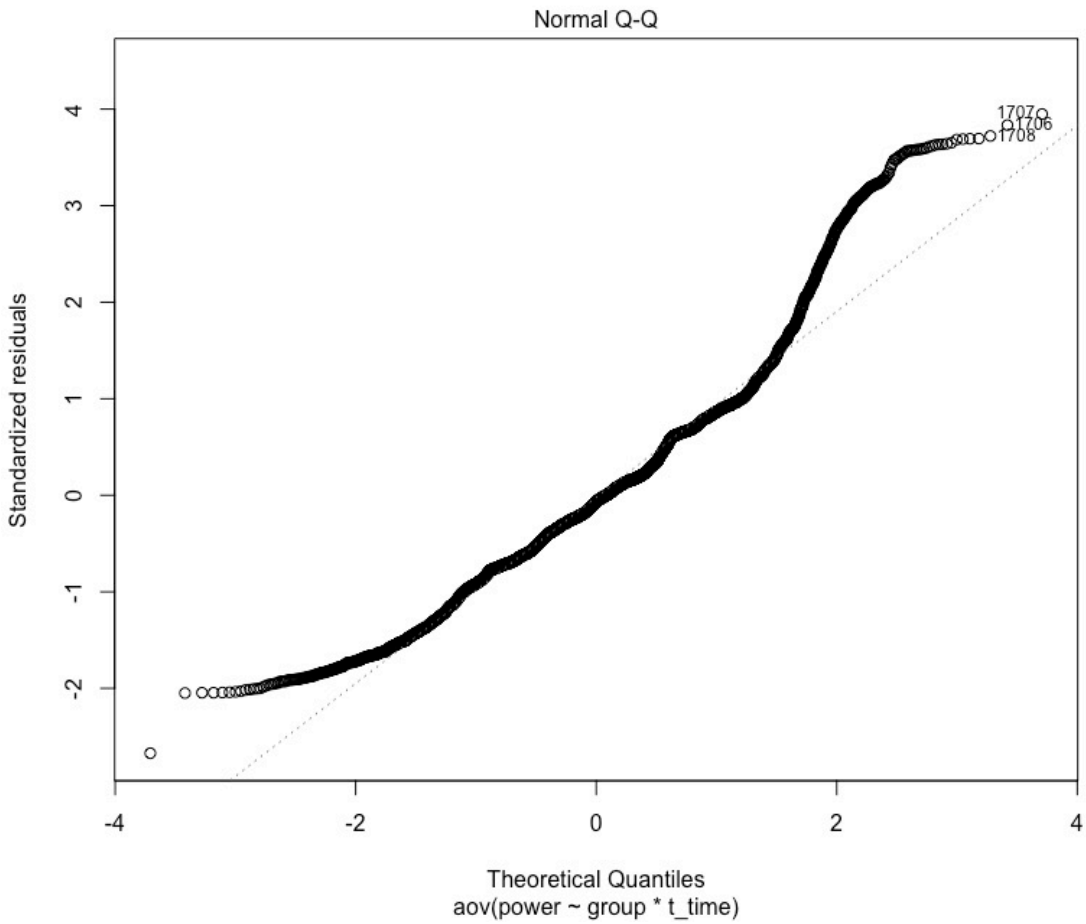

Figure 34: Q-Q plot of residuals distribution of ANOVA model.

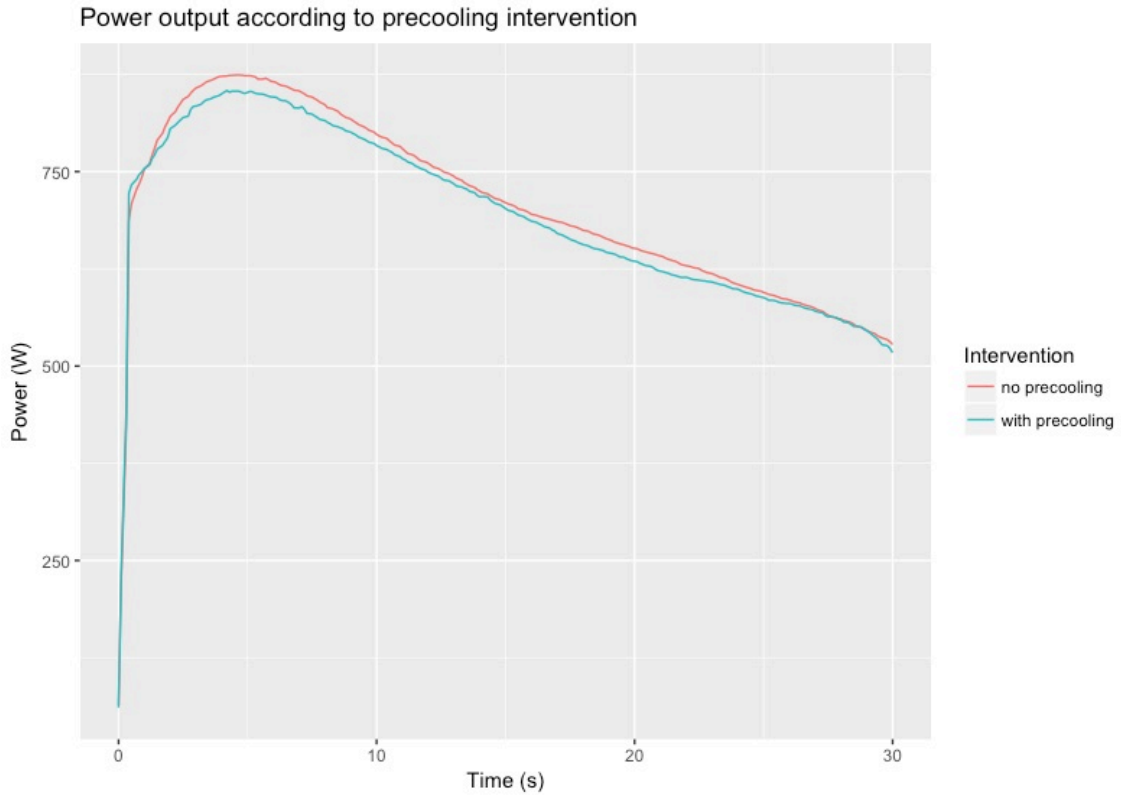

Figure 35: Mean power output by precooling intervention along with 30 seconds Wingate test.

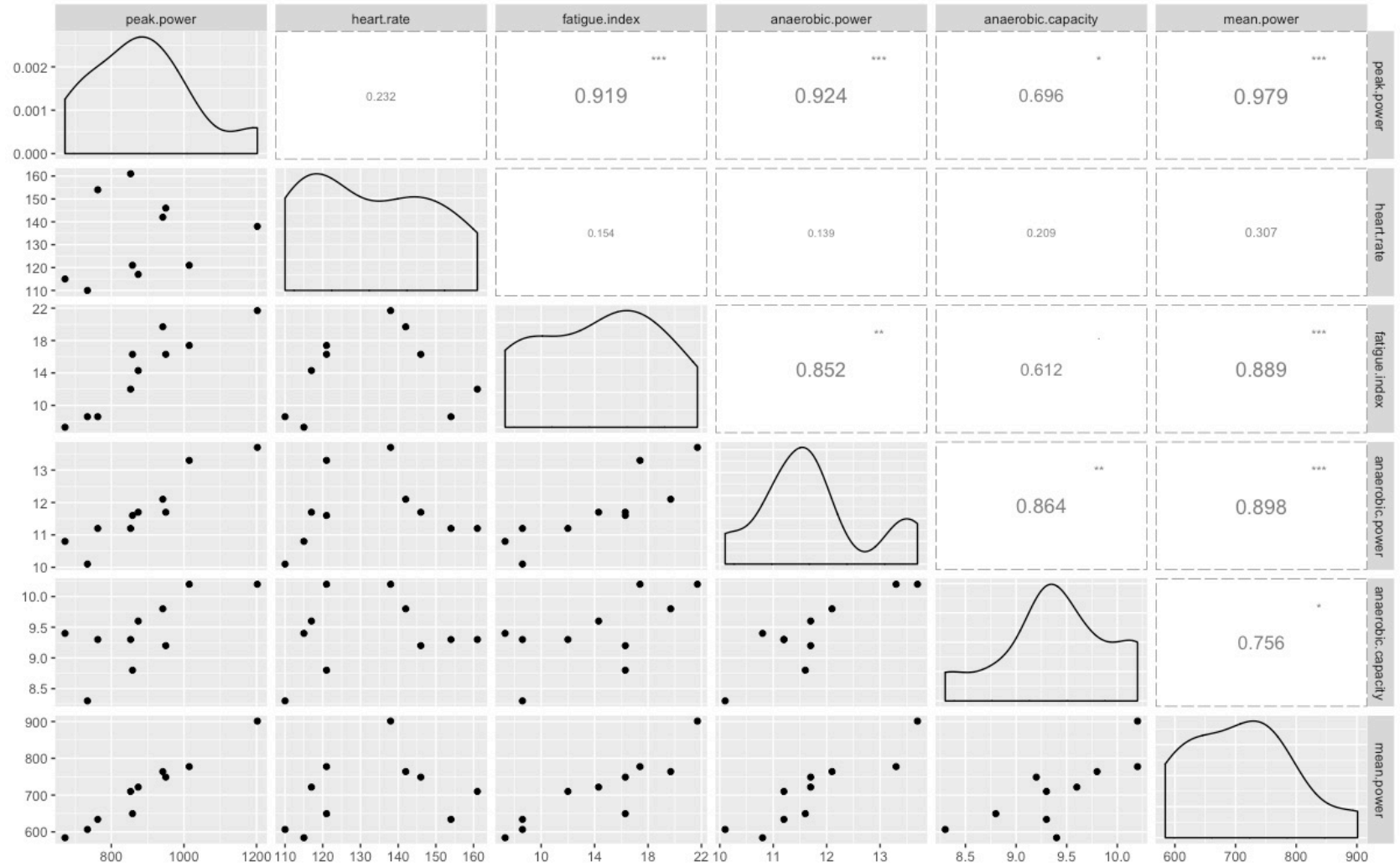

Figure 36: By no precooling, majority of performance parameters show mutual associations. Values represents correlation coefficient with *) p-value<0.05; **) p-value<0.01; ***) p-value<0.001.

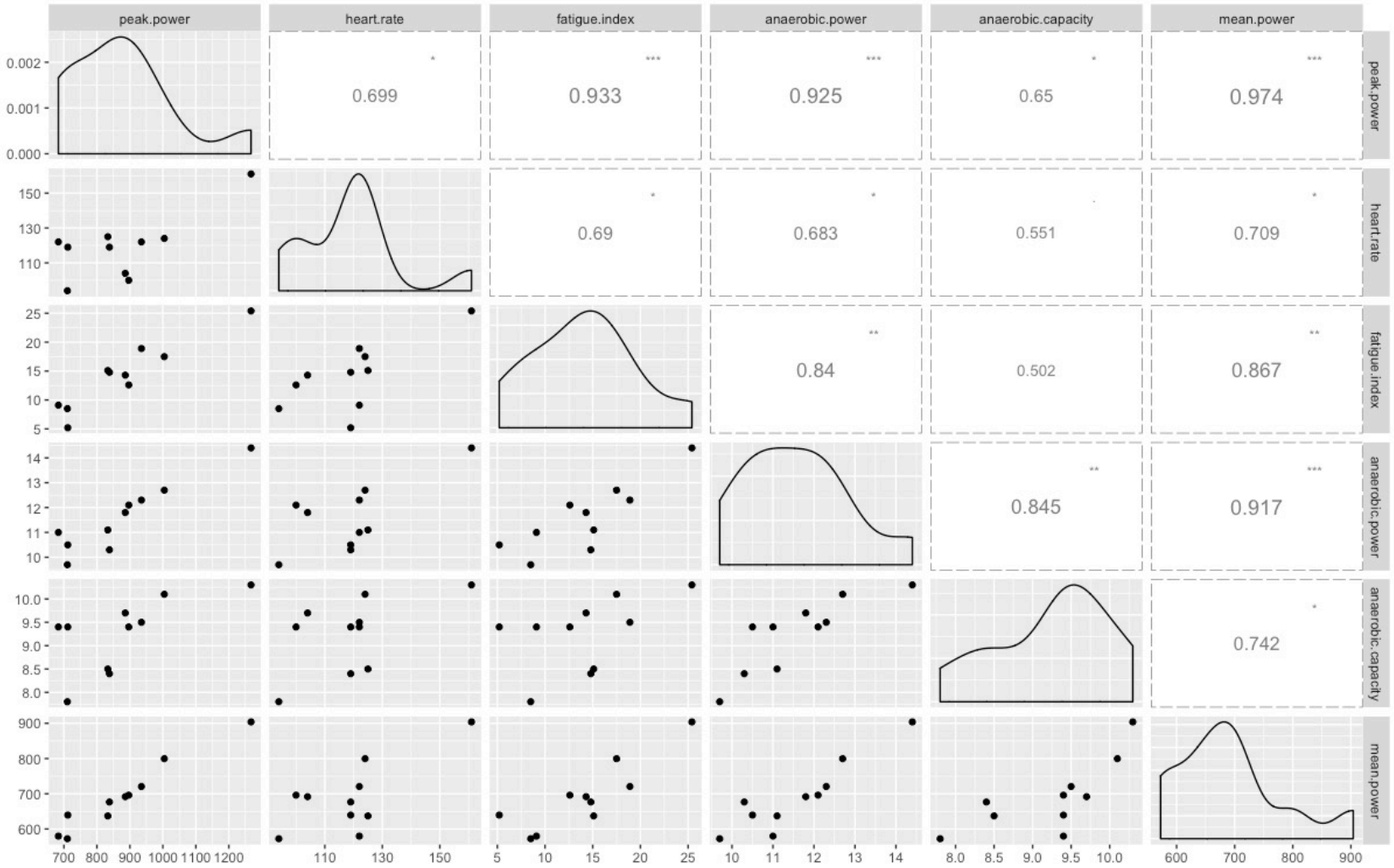

Figure 37: By precooling intervention, majority of performance parameters show mutual associations. Values represents correlation coefficient with *) p-value<0.05; **) p-value<0.01; ***) p-value<0.001.

3.3 Cardiophysiological parameters

Difference between cardiophysiological parameters such as heart rate, tympanic body temperature and systolic blood pressure by intervention without and with precooling were evaluated with paired student's t-test, whereas diastolic blood pressure due to its distribution departing from Gaussian was evaluated with paired Wilcoxon test. By analogy to performance parameters, decrease in heart rate and body temperature with precooling was observed, whereas both systolic and diastolic blood pressure was higher by precooling intervention; although none of the cardiophysiological parameters passed the significance threshold (Table 11). Figures 38 presents mean HR and statistics for the difference between groups and in case of BP (systolic and diastolic), and BT Figures 39-41 show mean value with standard error and statistics between groups at separate time points, respectively.

Table 11: Statistics of mean cardiophysiological parameters by intervention.

Variable		p-value	Mean	
			no precooling	with precooling
Heart rate		0.11	132.5	119
Body temperature	before warm-up	0.94	36.43	36.42
	before Wingate test	0.77	36.49	36.45
	before cool-down	0.92	36.45	36.43
	after cool-down	0.38	36.53	36.39
Blood pressure systolic	before warm-up	0.58	124	121
	before Wingate test	0.3	146	148
	before cool-down	0.07	179	186
	after cool-down	0.83	154	155
Blood pressure diastolic	before warm-up	0.93	74	75
	before Wingate test	0.17	76	80
	before cool-down	0.13	80	83
	after cool-down	0.17	75	78

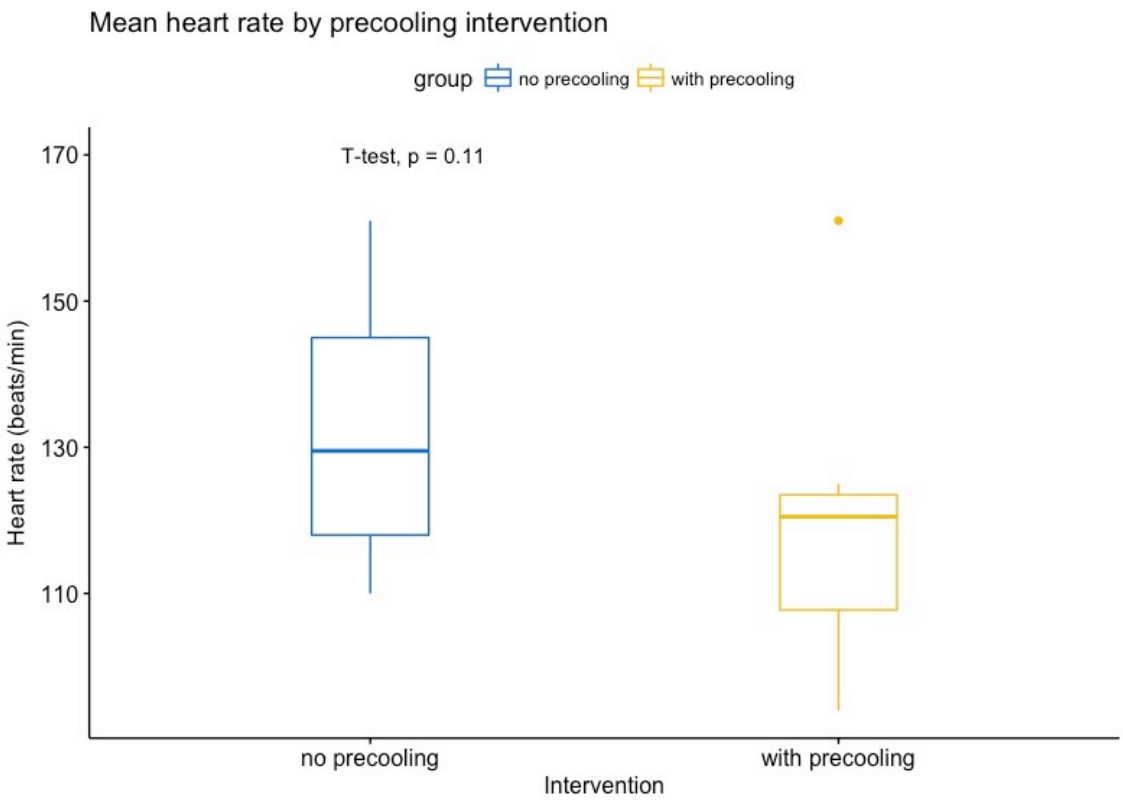

Figure 38: Mean heart rate by precooling intervention.

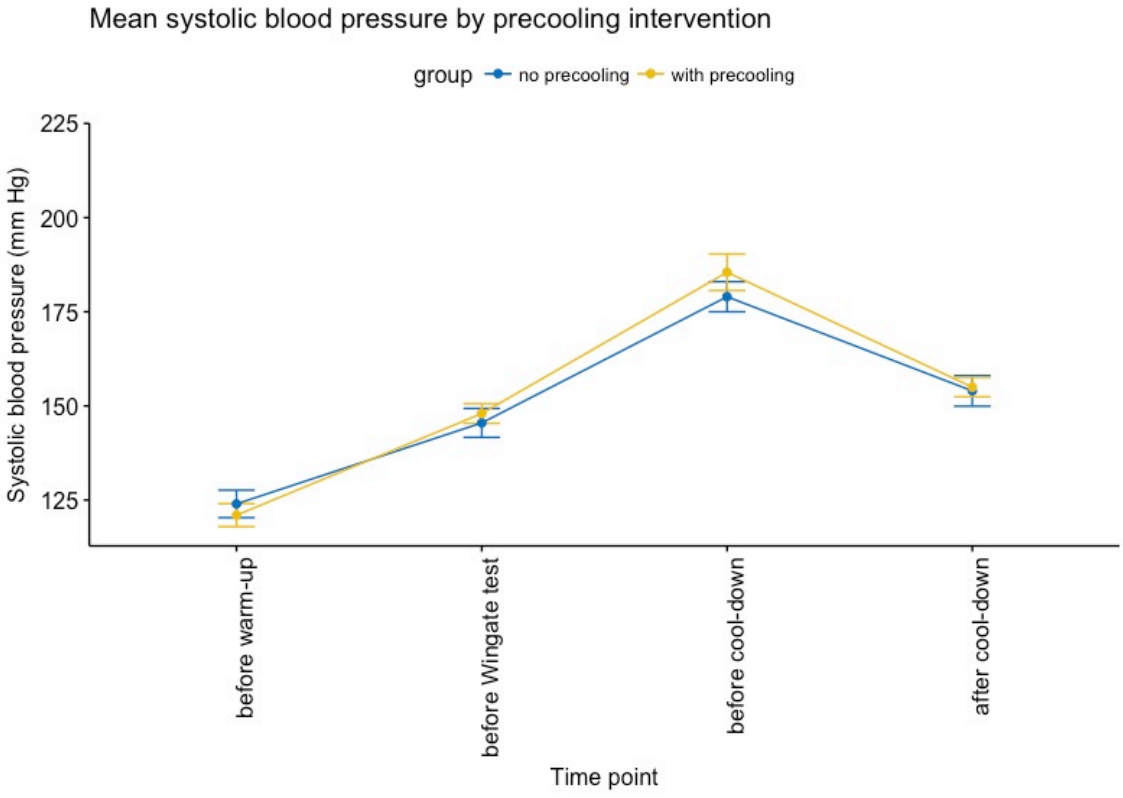

Figure 39: Mean systolic blood pressure by intervention.

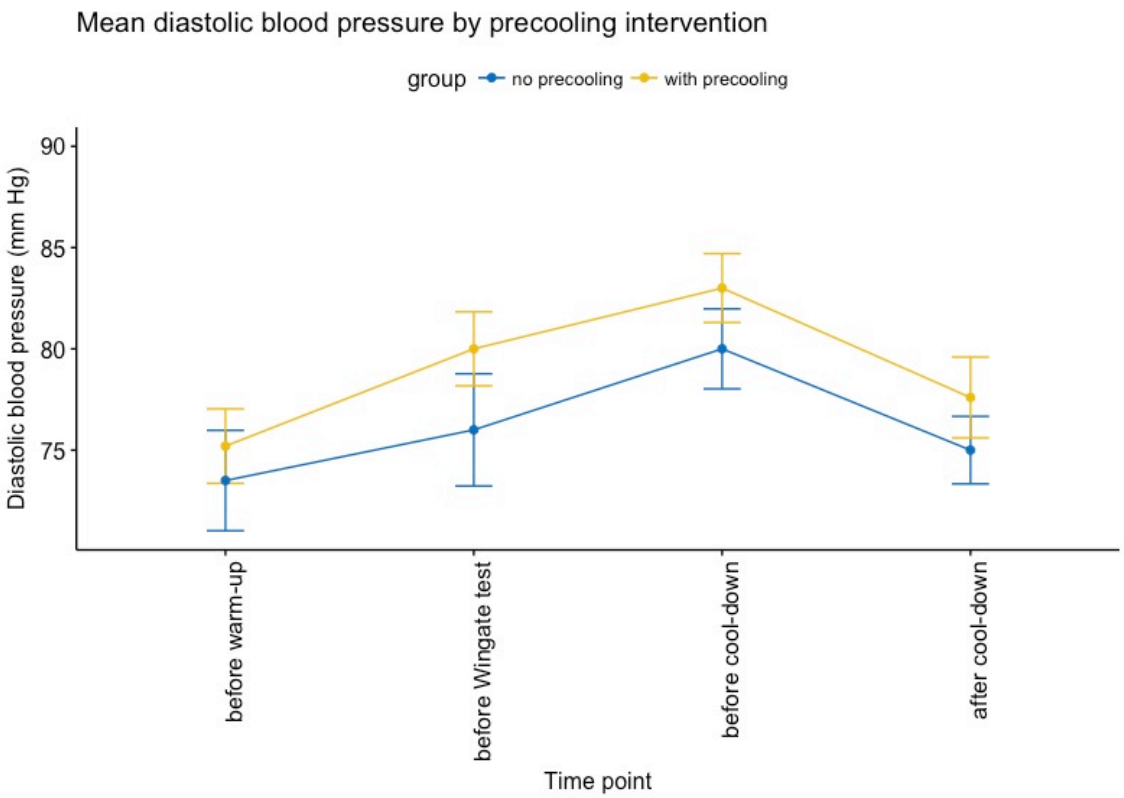

Figure 40: Mean diastolic blood pressure by intervention.

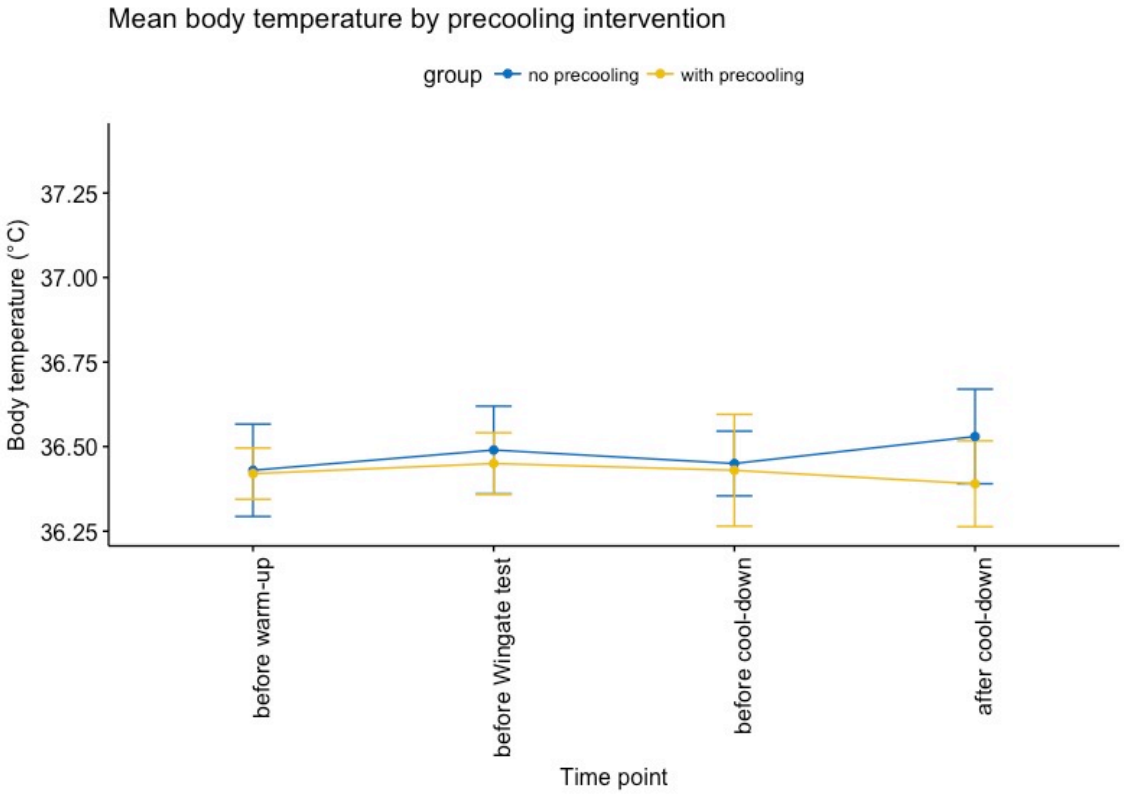

Figure 41: Mean tympanic body temperature by intervention.

3.4 Blood lactate concentration

The differences between concentration of blood lactate within group of no and with precooling intervention was analysed with paired student's t-test at each of time points separately. At different points, the ratio of means was altered in distinct direction. More specifically, the concentration of blood lactate was higher without precooling before warm-up, before cool-down, 1, 3, 5 and 7 minutes after Wingate test. In contrast, the concentration of blood lactate was higher with precooling before Wingate test and 10, 15, and 30 minutes after Wingate test (Figure 42). Table 12 presents detailed statistics of analysis.

Table 12: Statistics of mean blood lactate concentration by intervention. The significant findings are bolded ($p<0.05$).

	Variable	p-value	Mean	
			no precooling	with precooling
Blood lactate	**before warm-up**	**0.022**	**1.011**	**0.863**
	before Wingate test	0.541	0.958	1.045
	before cool-down	0.183	3.957	2.98
	1 min after Wingate test	0.678	7.927	7.725
	3 min after Wingate test	0.981	9.879	9.867
	5 min after Wingate test	0.851	10.112	10.005
	7 min after Wingate test	0.847	9.582	9.429
	10 min after Wingate test	0.957	8.785	8.822
	15 min after Wingate test	0.136	7.08	7.841
	30 min after Wingate test	0.276	3.03	4.068

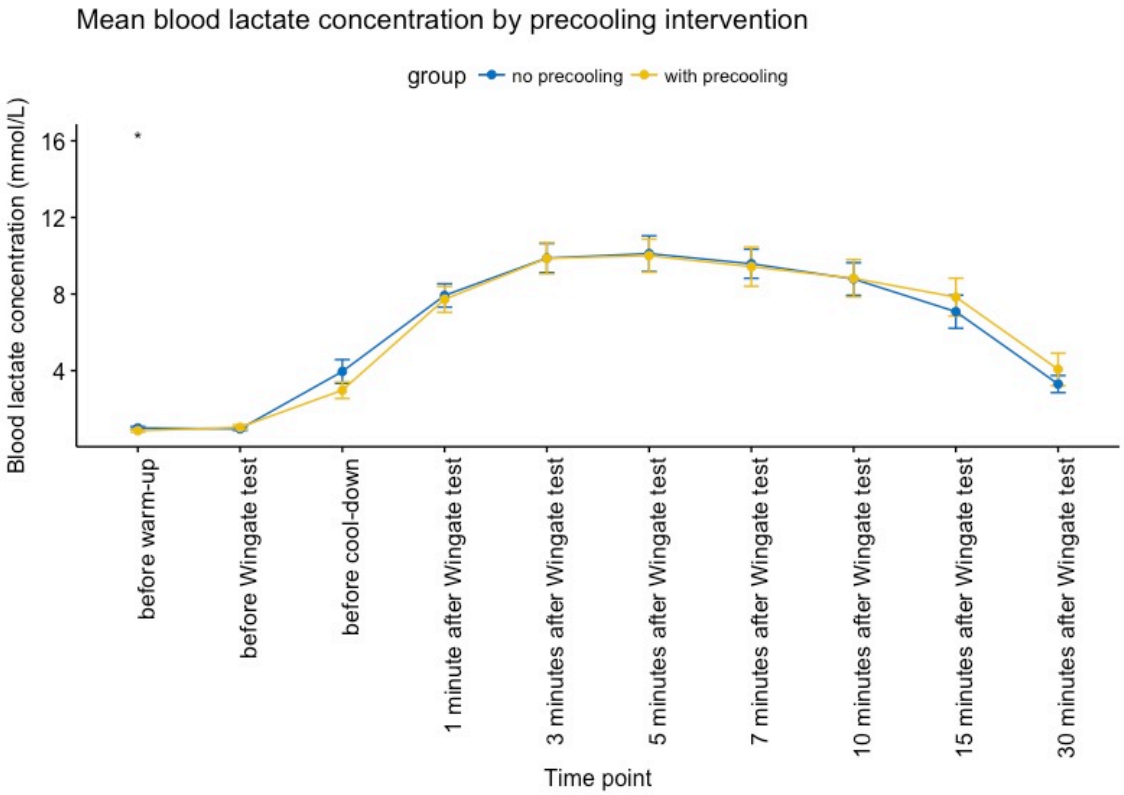

Figure 42: Mean blood lactate concentration by precooling intervention at each time point. *) p-value<0.05.

3.5 The survey – subjective evaluation by participants

Nine of ten study participants filled the survey subsequently to Wingate test with precooling. In general, they found precooling as practicable, mostly combinable with tests and suitable rather for professional athletes. Moreover, in majority of cases participants subjectively considered precooling intervention as improving their performance. However, they also identified several disadvantages and potential defects of precooling, i.e. difficulties in combination of precooling with preparation with long distances tests (mountain bike disciplines), too short warm-up phase to prepare muscles before performance (too cold muscles), no perceptible differences in performance with and without precooling as well as skin injuries caused by precooling pads visible few days subsequently to procedure. Furthermore, they evaluated few, general features of precooling pads. Table 13 summarizes the results of survey.

Table 13: Summary of survey results (N=9).

Feature	very good (1)	good (2)	rather poor (3)*
Pad adaptation to surface	66.7 %	33.3 %	-
Suitability of pad size	66.7 %	33.3 %	-
Pad acceptance	22.2 %	33.3 %	44.5 %
Cold tolerance	66.7 %	22.2 %	11.1 %
Lowered sensitivity of muscles to pain	44.5 %	33.3 %	22.2 %
Increased endurance of upper musculature	11.1 %	77.8 %	11.1 %

*In the table, the last option of response (4 – very poor) was omitted, because none of participants marked this response.

According to participants' answers, precooling pads were considered in major as very good in terms of their adaptation to surface (66.7 % of answers), suitability of pad size (66.7 % of answers) and tolerance of cold (66.7 %). Additionally, seven out of nine participants stated increase in endurance of upper musculature as correct. However, pads were mostly not acceptable (44.5 % of answers) and did not yielded in lowered sensitivity of muscles to pain (22.2 % of answers). Importantly, few comments referred to skin injuries caused probably by excessive precooling properties of pads.

Discussion

The aim of present study was examination of effects of precooling intervention that was designed to target local muscle precooling with ice pads for 30 minutes before Wingate test compared with a control condition of no precooling on power performance. It was hypothesized that precooling intervention would improve supramaximal cycling performance measured as power output, anaerobic capacity, anaerobic power, fatigue index, peak power and cardiophysiological parameters of ten individuals during Wingate test.

By means of external precooling, this intervention is defined as the application of any cold material to the body surface in form of, mainly, ice pads, cold water or cold air exposure. In addition, the precooling procedure may be defined as rapid removal of body heat before exercise to create a larger capacity of heat storage. In order to maintain thermobalance, human body reacts to cold through cutaneous vasoconstriction followed by further reductions in skin perfusion. In fact, ice-products have been considered as very efficient precooling strategy during preparation of athletes.

Considering the evidence that muscle power output becomes reduced by CNS to protect body from heat stroke with elevation of body core temperature, it is apparent that the precooling-induced heat buffer should allow athletes to perform more work before reaching the critical limit for body core temperature [70]. Contrary to the above, the major findings of present study indicated that precooling lowered power output (both, peak and average) and had negative effect on further parameters associated with performance such as AC, AP and FI, although none of them has reached the significance threshold; the strongest decrease was found in AC and MP, which both were on the verge of statistical significance ($p=0.12$ and $p=0.1$, respectively). Noteworthy, power output measured continuously throughout 30 seconds of Wingate test showed significant decline with precooling of quadriceps, which remains in compliance with previous findings. As reported in the literature, Marsch D and Sleivert G proposed that exercise performance of high-intensity and short duration might be improved through increase in blood volume caused by precooling-forced peripheral vasoconstriction as well as following increased muscle blood flow and availability of

oxygen [65]. On the other hand another study, however, has proved that reduction in muscle temperature affects mechanical power output of muscle fibers at lower temperatures and thus decreases performance of high-intensity [71]. However surprisingly to assumed hypothesis, the hereby demonstrated research complied with the latter findings and confirmed that precooling of lower limbs worse the supramaximal performance of short duration as well as stayed in agreement with results of other studies. More specifically, Crowley et al. reported in their research significant impairment in PP during Wingate test subsequently to precooling of lower limbs. It was also found that having reached decreased PP, the precooled muscles were losing power less quickly and therefore showed decreased FI in precooled individuals. Nevertheless, precooling affected muscles so that they could not achieve as much cumulated work during the Wingate test in comparison as they could when warmed and that effect could be noticed through correlation of all above parameters that has been determined [63]. In turn, Sleivert et al. demonstrated that precooling of torso slightly increased mean power output, although precooling of torso combined with legs significantly reduced power (peak and average). Furthermore, they established that short warm-up before actual performance ameliorated reported declines caused by precooling, hence it could be concluded that precooling leads to impaired contractile function of muscles and anaerobic metabolism [72].

Furthermore, blood lactate concentration fluctuated along points during exercise procedure and was significantly lowered with precooling before warm-up. The reason underlying these fluctuations with advantage of higher blood lactate levels under no precooling remains elusive. In theory, during supramaximal exercise, if a higher blood volume leads to enhanced availability of blood to the muscles, it would improve oxygen delivery leading to increased contribution of aerobic system to energy supply for any generated power output. Additionally, this mechanism may also increase the clearance of lactate from the muscles, as metabolic by-product, thus with elevated lactate clearance from muscles one could expect greater lactate levels in blood. Surprisingly, the findings of present study indicated worsened lactate metabolism with precooling, which may be due to several factors: 1) increase in blood flow to the muscles under load may heighten the contribution of aerobic system to supply energy and simultaneously decrease accumulation of blood lactate; 2) higher blood flow may enhance lactate

removal via increased pH gradients, although raise in the absolute levels of blood lactate could be transient since lactate is being rapidly oxidised [65]. Noteworthy, 15 minutes after Wingate test the ratio of blood lactate reversed with higher levels found with precooling intervention. This alteration may be explained with increase in temperature of muscles caused by 30 seconds of maximal performance during Wingate test *per se* and following cool-down through next 15 minutes in form of declining cycling of low cadence.

Finally, other analysed parameters such as HR, BT, BP (both, systolic and diastolic) were not affected by precooling intervention in significant manner. In greater detail, HR showed the strongest difference, as it decreased with precooling ($p=0.11$). According to Wegmann's meta-analysis, such decrease in HR may be considered as symptom of reduction in thermal stress within muscles. According to their findings, in 23 out of 27 analysed studies the reduction in HR after precooling intervention was noticed possibly resulting from implications of Q10 rule stating that velocity of biological and chemical processes increase with temperature as well as of baroflexes due to altered skin temperature, greater tension in veins leading to enhanced venous return or improved utilization of oxygen in the blood influenced by delay in the opening of the arteriovenous anastomoses. The reduction in HR may be also accounted for improved central supplies of blood and larger stroke volume due to reduced core temperature of the body and sympathetic activation. Blood pressure demonstrated general rising trend with precooling, which was more observable in diastolic than systolic BP, nevertheless the differences were not significant at any point of testing procedure. Tympanic BT was slightly lowered, however not significant with precooling, with the biggest difference at point after cool-down (-0.14°C) [73].

On the contrary, several studies proved relevant impact of precooling on performance, however, their effects were assessed within different ambient conditions (hot humid vs. neutral conditions) and thus demonstrated distinct results [1, 64, 74]. In greater detail, precooling was found as improving power output only if the associated thermal stress was large enough so that arises from exercise of long duration and/or high humid ambient temperature. Notably, it has been also estimated that potential impairment of muscle functions caused by precooling lasts not longer than 20 minutes, therefore any performance longer than that time may benefit from favourable effect of

precooling. Nevertheless, for duration exceeding 60 minutes of performance the precooling effect seemed to decline, which could be explained with somehow limited duration of cooling effect. Apparently, the effects of heat on exercise depends from principle on environmental conditions such as temperature, relative humidity as well as individual features of ability to regulate body core temperature and on the intensity of exercise. Whereas maximum muscle contraction during performance of high-intensity seems to be not affected by heat, prolonged duration of exercise could be impaired by local hyperthermia. Therefore in the heat, contribution of CNS to fatigue could be of large extent than at neutral ambient conditions [73]. Taking into consideration all above evidence, precooling intervention should be rather recommended for physical performance of long duration or exercise of short duration at hot humid conditions.

Conclusions

The aim of this Master's Thesis was the determination of effect of precooling intervention on high-intensity maximal physical performance in neutral ambient conditions including evaluation of output power and cardiophysiological parameters of ten study participants. Noteworthy, the findings of the research were far distinct from primarily assumed hypothesis of beneficial effect of precooling on power output; although given the small body of available literature on local precooling for high-intensity physical performance of short duration, this study could be of great value reporting potential detrimental impact on forms of exercise that require high-power outputs.

Results obtained throughout the whole study lead to establishment of unequivocally significant influence of precooling of lower limbs, thus providing strong indications of potential incorporation of such procedure into professional use in athletes. Notably, due to small size of studied group majority of parameters did not passed significance threshold, nonetheless it allowed concluding that precooling intervention does not apply to performance of high intensity due to transient impairment of contractile functions of muscles at neutral ambient conditions an thus lowered power output and anaerobic capacity. In turn, such procedure could be of great practical value if applied at hot and humid conditions or for performance of long duration regarding the improvement of individual's heat storage capacity. Noteworthy, some inconsistencies or small population sample emphasize strong need of further investigation into the appropriateness of precooling procedure and metabolic alterations associated with exercise of particular type.

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