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Effects of Extreme Physical and Mental Load on Circulating Exerkines in Professional Athletes

Ph.D. Thesis

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List of Abbreviations

Acetyl-CoA: acetyl coenzyme A

AMPK: AMP-activated protein kinase

ANGPTL4: angiopoietin-like 4

ANOVA: analysis of variance

ANP: atrial natriuretic peptide

AP-13: apelin-13

AP-36: apelin-36

APJ: apelin receptor

ATP: adenosine triphosphate

BDNF: brain-derived neurotrophic factor

BNP: B-type natriuretic peptide

cGMP: cyclic guanosine monophosphate

CNS: central nervous system

DAG: diacylglycerol

DBP: diastolic blood pressure

DNA: deoxyribonucleic acid

ELISA: enzyme-linked immunosorbent assay

eNOS: endothelial nitric oxide synthase

ERK1/2: extracellular signal–regulated kinase 1/2

ET-1: endothelin-1

EV: extracellular vesicles

FFA: free fatty acid

FGF-2: fibroblast growth factor 2

GLUT-4: glucose transporter 4

HIIT: high-intensity interval training

HR: heart rate

IGF-1: insulin-like growth factor 1

IL-6: interleukin-6

IP_3: inositol triphosphate

IQR: interquartile range

L-NAME: L-NG-Nitro arginine methyl ester

MLCK: myosin light chain kinase

MET: metabolic equivalent

miRNA: micro ribonucleic acid

mRNA: messenger ribonucleic acid

NO: nitric oxide

NT-proBNP: N-terminal pro–B-type natriuretic peptide

PGC-1α: Peroxisome proliferator-activated receptor-gamma coactivator 1 α

PKC: protein kinase C

PLC: phospholipase C

RAAS: renin-angiotensin-aldosteron system

ROS: reactive oxygen species

RPP: rate pressure product

RQ: respiratory quotient

SBP: systolic blood pressure

SD: standard deviation

SR: sarcoplasmic reticulum

VE: maximum ventilation

VEGF: vascular endothelial growth factor

VLDL: very low-density lipoprotein

VO_2max: maximum oxygen consumption

WAT: white adipose tissue

1. Introduction

1.1 Physical activity and exercise adaptation

Nowadays, the benefits of exercise are widely discussed, and it is common to talk about the healthy benefits of exercise when, in fact, the biologically normal condition would be the exercise-trained state, and physical inactivity should be discussed as abnormal.[1] Physical inactivity is responsible for many disease conditions and chronic diseases.[2]

Physical inactivity shortens lifespan by 5 years.[3] Additionally, a „healthy" lifespan, that is, without long-term disease, is also shorter by 8 years in physically inactive compared to physically active people.[4] Physical exercise is a non-invasive therapeutic solution for improving body weight, metabolic health, and quality of life.[5]

„Exercise is the voluntary activation of skeletal muscle for recreational, sporting, or occupational activities".[1] There are differences in exercise modality, intensity, duration, and many other factors, but the role of the skeletal muscle is unquestionable in all of them.

Muscle contraction requires ATP to support different cellular processes, including Na^+/K^+ ATPase (to maintain sarcolemmal excitability), Ca^{2+} ATPase (to reuptake Ca^{2+} into SR), and myosin ATPase (to generate force with the actin-myosin cross-bridge cycling).[1] Because the stored ATP is relatively low in muscles, different ATP-producing pathways need to be activated depending on the exercise. For short duration, maximum exercise (less than a minute), the muscle-derived creatine phosphate and glycogen is broken down to glucose and used to create ATP.[1] During longer exercise, the ATP is created through other pathways, mainly the hepatic glycogenolysis and gluconeogenesis, and the triglyceride breakdown to FFAs and subsequent release into the circulation.[1] Whether carbohydrates or lipids are used as primary fuels for exercising skeletal muscle depends on the exercise intensity: higher intensity has a preference for carbohydrates, while prolonged exercise with moderate intensity has a preference for fat oxidation.[1]

Several factors have to work in synergy to maximize exercise performance, namely genetic background, mental preparation and toughness, training level, and cardiorespiratory fitness, among others.[6-10] The major hemodynamic challenge of reaching maximum exercise capacity can only be achieved by a highly coordinated effort of the O2 delivery and O2 extraction systems to provide sufficient blood supply to meet the increased demand of skeletal muscles.[11,12] The engine of

cardiorespiratory fitness is the cardiovascular system, which has been the focus of research in exercise physiology for decades.[13]

Oxygen consumption at rest in healthy young individuals is around 3.5 mL/kg/min. The resting O_2 consumption for a 70 kg person is ~250 mL/min. In untrained but otherwise healthy individuals, VO_2max can increase to 10-15 times the resting value, while in elite endurance athletes, VO_2max can exceed 85 mL/kg/min.[14] To reach the highest exercise capacity, many bodily systems need to work in tandem, including the CNS to recruit motor units, the cardiovascular and pulmonary systems to deliver O_2 to working skeletal muscles, and the oxidative pathways to support O_2 consumption by the skeletal muscles.[1] During maximum exercise, the cardiac output can increase 8-fold, and the peak ventilation can increase 40-fold compared to resting values. Furthermore, the blood flow to skeletal muscles can increase 100 times above resting level and account for 80-90% of the cardiac output.[1]

During exercise, the working skeletal muscle undergoes active hyperemia, primarily in small arterioles. Mechanical, neural, and humoral factors are crucial in the vasodilatation leading to hyperemia. These factors include inward rectifying K^+ channels, adenosine, ATP, and ROS.[1] One of the early adaptations of muscle to exercise is the rapid increase in GLUT-4.[15] Redistribution of blood flow also aids increased blood flow to skeletal muscles, and additionally, the increased sympathetic activity helps to offset the fall in systemic vascular resistance caused by the skeletal muscle vasodilatation.[1]

Blood flow to the skin increases first during exercise, as sweating is the main mechanism for heat dissipation. As exercise continues or intensifies, the skin also undergoes vasoconstriction when the redistribution of blood from the splanchnic area or non-working tissues can not meet the increased demand of the skeletal muscles.[1]

The pulmonary system also plays a key role in exercise adaptation. The maintenance of sufficient O_2 levels in the arteries and the removal of CO_2 produced during physical exercise are crucial functions and are achieved by increased ventilation.[1]

The liver also adapts to exercise by lowering its lipid content, reducing VLDL and glucose production, and increasing lipoprotein clearance.[16]

The adaptation of the heart depends on the type of exercise. Dynamic exercises (e.g. running, cycling, swimming) directly affect the autonomic nervous system,[17-20] while isometric exercises

(e.g. strength training) strengthen the heart and skeletal muscles and reduce the overall demand on the circulatory system.[21]

Another adaptation to exercise is the development of physiologic cardiac hypertrophy. The increase in heart mass, left ventricular diameter, end-systolic pressure, and heart rate are all signs of pathologic cardiac hypertrophy, but there is no pathologic evidence, and in professional athletes, physiologic cardiac hypertrophy can be protective.[16] A large single echocardiographic study found that professional athletes have better cardiorespiratory fitness accompanied by increased left ventricular mass that regresses when athletes decondition.[22-24] Additionally, longer follow-up studies of elite athletes show that their life expectancy is longer than the population mean.[25]

Mitochondria are the powerhouses of the cells and play a key role in modulating athletic performance. PGC-1α is a crucial modulator of skeletal muscle mitochondrial biogenesis: a single bout of endurance exercise elevates skeletal muscle PGC-1α levels, resulting in improvements in whole-body VO_2max and better endurance performance.[26,27] Another key player in exercise adaptation is AMPK. Many types of exercise increase AMPK activity, which leads to the phosphorylation of target proteins, including PGC1α and rate-limiting enzymes in energy metabolisms, including acetyl-CoA carboxylase.[28]

The importance of physical exercise in muscle plasticity and renewal has been previously described, too. Muscle cell progenitors (i.e. satellite cells) are stimulated by physical exercise, although the renewal rate is limited in older people.[29,30]

Exercise also facilitates the lipolysis of white adipose tissue (WAT) to provide free fatty acids (FFAs) as fuel.[31]

1.2 Exercise-induced factors – the exerkine concept

An „exercise factor" that is produced during muscle contraction and is able to mediate humoral changes induced by exercise has been searched for many decades.[3] During those decades, several names were used for this factor: „work stimulus," „work factor," or „exercise factor".[32]

Different processes clearly indicated the presence of this „exercise factor"; for example, the increased glucose production by the liver upon physical exercise–induced glucose uptake by muscles, while the body's glucose homeostasis was unaffected.[33] Another clue was the increased release of FFAs by the adipose tissue as a response to exercise. Another example is the „feeling good" feeling after exercise that BDNF, endorphins, serotonin, and dopamine cause.[34] For a long time, however, it wasn't clear how muscle contraction influences these effects. It also took many years to demonstrate that some of the previously thought functions of adipose or muscle tissues are not comprehensive. Besides the storage function for adipose tissue or the contraction function of muscle tissue, these tissues are able to produce and release specific proteins into the bloodstream upon physical exercise.[35]

The idea that contracting skeletal muscles release humoral factors into the bloodstream that act in an endocrine-like way was first proposed by Pedersen et al.[36,37] These factors were termed myokines, and the first myokine identified was IL-6.[38] The fact that IL-6 plasma levels are elevated after physical exercise has been known before the identification of IL-6 as a myokine. Initially, the increase was thought to be an immune-related reaction upon exercise.[39] In 2000, a study reported differential IL-6 responses in working and non-working limb muscles and showed that the working muscle produced significantly higher levels of IL-6 than the non-contracting muscle.[38] When IL-6 has been identified as the first myokine that is produced in the working skeletal muscle and released into the circulation upon physical exercise, the additional finding was that skeletal muscle can produce and release factors capable of influencing metabolic processes.[39]

Since then, hundreds of myokines have been identified, and the concept of contracting skeletal muscles communicating in an endocrine-like manner with other tissues to promote the systemic benefits of exercise has evolved.[40]

Myokines are released from the skeletal muscle in response to physical activity; however, muscle isn't the only tissue releasing peptides, metabolites, DNA, mRNA, miRNA, and other RNA species into the bloodstream upon physical exertion.[40] Due to the skeletal muscle providing almost one-

third of the body mass, the beneficial effects of physical exercise were first attributed to myokines.[41]

As the field of exercise factors expanded and more non-muscle exercise factors were discovered, the term „exerkines" was born. Exerkines are the combination of all humoral factors expressed, produced, and secreted by all exercised or non-exercised tissues to mediate crosstalk between different organs.[40] Another definition describes exerkines as signaling moieties released upon acute exercise and exerting their effects in an autocrine, paracrine, or endocrine manner.[41]

Since then, several peptides have been identified as being secreted by different tissues upon physical exercise (exerkines).[3,36,42-45] Thinking about the thousands of humoral factors released into the circulation by muscle or fat tissues (adipokines), the liver (hepatokines), the brain (neurokines), or the heart (cardiokines), dozens of additional factors will potentially be identified as exerkines.[36] In fact, exerkines can also be neurotransmitters or metabolites, such as catecholamines[46] or lactate.[47]

It became evident that exerkines have emerged as important regulators of exercise adaptation with their local and systemic effects.[3,41,48] Exerkines can act locally, in a paracrine manner. A well-known exercise adaptation using a paracrine factor is the muscle-derived VEGF that helps regulate tissue angiogenesis.[49,50] Additionally, exercise might also improve endothelial function, as the vascular endothelium can act as the recipient of many exerkine-related effects, such as NO-dependent vasodilatation of apelin[51-55] or ET-1-mediated vasoconstriction,[56] all contributing to the overall cardiovascular fitness.

Some exerkines exert their effects on the skeletal muscles themselves (e.g. myostatin) while others (e.g. irisin) have more systemic effects in modulating metabolism or the immune system.[57] Yet others have an important role in improving the endothelial function of the vascular system (e.g. IGF-1, FGF-2).

There are notable differences between acute and chronic exerkine responses. While acute exercise triggers more of a stress-like response,[58] chronic exercise triggers an adaptive response with repeated bouts of exercise.[16] Acute exercise might be initially proinflammatory in nature, but this effect is offset by an anti-inflammatory response.[59,60]

The responses upon acute exercise mediate the maintenance of metabolic homeostasis, assist in shifts in fuel utilization, and balance acute inflammation by anti-inflammatory mediators.[59]

Conversely, chronic exercise responses are associated with long-term metabolic adaptations and anti-inflammatory mechanisms.[61]

Both acute and chronic exercise can alter the profile of exerkines. Exercise-induced adaptations in myokine and adipokine profiles suggest that these secreted proteins may facilitate tissue cross-talk, i.e. tissue-to-tissue communication, to enhance overall metabolic health. The adaptations of skeletal muscle include improved glucose uptake,[62] increased GLUT-4 expression and translocation to the membrane,[63,64] augmented mitochondrial activity,[65] increased fat oxidation,[66] and increased myokine production.[67-69] The adaptations of adipose tissue include increased metabolic activity, improved mitochondrial function, altered adipokine tissue expression, and circulating adipokine levels.[70]

A single bout of acute exercise changed the expression of almost 10 000 analytes in a study, including proteins, lipids, and transcripts.[71] Many factors influence the exerkine response to acute exercise: type and duration of the exercise, fitness and fed-fasting status of the individual, length of the follow-ups, and the timing of sampling.[5,41] The conflicting results about circulating peptide levels after a single bout of exercise might be, in part, due to differences in the type, intensity, and duration of the acute exercise.[72]

Acute high-intensity exercise is associated with higher plasma levels of exerkines, including lactate,[73] irisin,[74] or adiponectin,[75] but for some exerkines, higher intensity results in lower circulating levels.[76] A similar effect can be seen in terms of exercise duration: longer acute exercise led to higher BDNF levels.[77] Regarding irisin, there seems to be a cutoff at 12-16 weeks of chronic exercise duration. When chronic exercise training lasted for less than 12 weeks, irisin levels significantly increased; when the training program was longer than 16 weeks, irisin levels significantly decreased.[78]

Taking high-intensity interval training (HIIT) as an example of how type and intensity of the exercise matter, higher intensity corresponds with higher plasma levels of IL-6, while IL-10 remained unchanged.[79]

There are still unanswered questions about exerkines. One interesting question remaining is how these exerkines are released into the circulation. One hypothesis gaining ground is that the exerkines are contained in extracellular vesicles (EVs).[57] During physical exercise, the action potential through the neuromuscular junction leads to a massive Ca^{2+} efflux from the sarcoplasmic

reticula, which triggers the release of the EVs.[80] The amount and type of exerkines in those vesicles depend on the type of exercise, among other factors (i.e. aerobic vs anaerobic).[45]

Studies in exercise physiology use different methods to search for additional myokines or exerkines. The field of exercise physiology has expanded in recent decades with the introduction of different exercise models.[81-85] For example, apelin[86] and irisin[69] were identified with chronic exercise models, while ANGPTL4[87] was identified with an acute exercise model. Fractalkine also increases upon acute exercise.[88,89] Additionally, IL-6,[38] IL-8,[90] IL-15,[91] BDNF[92] were also identified with an acute exercise model.[16] Another exerkine, fibroblast growth factor 21 (FGF-21), was initially connected to fasting.[93]

The field of exercise-derived factors has been evolving rapidly over the last decade. The number of muscle-derived exerkines exceeded 600 in 2015.[45] The number was over 3000 in 2016.[94]

1.3 Apelin

Apelin was originally isolated from bovine stomach extracts in 1998 and identified as the endogenous ligand of the G-protein–coupled apelin receptor (APJ).[51] The 77 amino acid long preproapelin is cleaved into shorter but functional fragments, including apelin-36 and apelin-13.[95] The shorter fragments show higher affinity for the apelin receptor and higher biological activity.[95] Since its discovery, apelin has been described as a myokine,[86] a cardiokine,[96] and an adipokine,[97] but due to its abundance in the human body, apelin has been proposed as a player in the cross-talk between skeletal muscle and brown-beige adipocytes,[98] regulator of water and food intake, adipocyte differentiation, bone formation,[57] its role has been described in metabolic diseases,[99] and it has been shown to be expressed in cancer tissue,[100] and cardiovascular pathologies.[101]

The apelinergic system plays a crucial regulatory role in the cardiovascular system.[95,102,103] The effects of the peptide are manyfold: apelin stimulates cardiac contractility,[96,104] elicits a blood-pressure-lowering effect in vivo,[51,55] and induces vasodilatation in various vascular beds in vitro.[52-54] The hypotensive and vasorelaxant effects of apelin are NO-dependent[51-54]; however, the positive inotropy is not blunted by L-NAME, a nitric oxide synthase inhibitor,[96] suggesting additional mechanisms of action. In humans, acute apelin administration causes peripheral and coronary vasodilatation and increases cardiac output.[105] Apelin is actively synthesized, among others, in heart muscle cells, endothelial cells, and smooth muscle cells,[106] while APJ is widely expressed in skeletal muscles, the heart, lungs, kidneys, the liver, the adipose tissue, and the brain.[57]

In skeletal muscles, apelin plays a role in muscle regeneration. The apelin receptor is present on muscle stem cells and promotes in vivo proliferation and differentiation of muscle cells.[57]

Contracting skeletal muscles are able to synthesize apelin, and it has also been observed that endurance exercise training upregulates skeletal muscle apelin expression,[86] and exercise-associated skeletal muscle contraction stimulates apelin production by myofibers.[98] The knockout of apelin in the skeletal muscles leads to muscle weakness, lower exercise capacity, and blunted plasma apelin increase in mice.[107]

An additional benefit of apelin is related to its influence on metabolism. In WAT and skeletal muscles, apelin treatment enhances glucose utilization and promotes systemic glucose reduction.[107]

Moreover, apelin acts on muscle metabolism through AMPK-dependent mitochondria biogenesis,[57] and chronic apelin administration increases skeletal muscle mitochondrial function and biogenesis[98] that generally decreases with age.[57]

Apelin has been described as a candidate for participating in cardiac and skeletal muscle adaptation to physical exercise and peak athletic performance.[108]

In 2012, apelin was linked to self-reported physical activity in patients with diabetes.[109] Six years later, the relationship between apelin and physical exercise and the fact that muscle contraction leads to apelin release into the bloodstream was identified.[98] Based on these results, the measurement of apelin after a single bout of exercise has been proposed as an index of exercise achievement.[98]

Several studies have reported the effect of physical exercise on apelin levels[81,83109,110]; however, studies reporting the effects of acute exercise on apelin levels are limited. Furthermore, the contribution of different apelin isoforms to peak athletic performance has not been characterized.

Some studies reported decreased apelin levels,[111-113] others reported increased[86] or unchanged[114] apelin levels in obese individuals.[5] The discrepancies are present in acute exercise, too. Although most acute exercise studies report an increase in myokine levels,[115] data about apelin, especially apelin isoforms, and acute exercise response are missing. In fact, one study in rats showed a decrease in apelin-13 level after a treadmill exercise test.[116] A possible reason for this could be delayed sampling. Since myokine release is a dynamic process, a slight delay in sampling might miss the actual peak of the secreted myokine in the circulation.[115]

Acute endurance exercise increased apelin levels,[81,82,117,118] and acute sprint interval exercise elicited the same effect.[119] Interestingly, other studies found no change in plasma apelin levels after acute exercise.[85]

A meta-analysis found that physical exercise training did not change apelin levels.[120]

Another meta-analysis showed that apelin increased after chronic exercise.[121] Additionally, 4 studies in this meta-analysis reported the change in apelin levels in participants older than 50 and showed the same elevation in plasma apelin levels. Interestingly, the other studies analyzed younger individuals, and only one found increased circulating apelin concentrations. Additional studies in obese individuals reported lower apelin levels after chronic exercise, which could be a result of multiple mechanisms: exercise-induced weight loss,[112,122] or exercise-induced improvements in insulin resistance.[99,113,123] Furthermore, hyperinsulinemia upregulates adipocyte-

derived apelin production, while physical exercise upregulated skeletal muscle-derived apelin production.[116]

1.4 Endothelin-1

Endothelin-1 was first isolated from porcine aortic endothelial cells.[56] The 203 amino acid long preproapelin is the translational product, which is cleaved to big endothelins that are 37-41 amino acids in length. These are biologically inactive and will be further cleaved to mature ET-1.[124] Endothelin-1 is a 21 amino acid peptide synthesized by the endothelial cells and acts locally on the vascular smooth muscle cells. Three isoforms are known: ET-1 and ET-2 (differs in 2 amino acids from ET-1) are strong vasoconstrictors, while ET-3 (differs in 6 amino acids from ET-1) has a potentially weaker effect on the vasculature.[124,125] Interestingly, ET-1 is the only member released from the endothelium, basolaterally, to act on the smooth muscle layer.[124] This also suggests that tissue levels of ET-1 are potentially higher than plasma levels, which might also be due to its short half-life in the blood.[124,126,127]

Pericardial ET-1 levels were significantly higher than respective plasma levels in patients with heart disease, and it can increase up to 200-fold higher in the pericardial fluid than in the plasma of these patients.[176]

Endothelin-1 has many effects on the cardiovascular system, namely coronary vasoconstriction and positive inotropic and chronotropic effect. Additionally, ET-1 has a direct arrhythmogenic effect due to the development of early afterpolarizations[128,129] and might be involved in sudden cardiac death in athletes. Sudden cardiac death caused by ventricular fibrillation or other arrhythmia-related causes is more frequent in professional athletes than in the general population.[130] There are several cases every year of athletes losing their lives unexpectedly, and a high number of these athletes are elite soccer players [https://sportsbrief.com/football/24785-footballers-died-pitch-soccer-players-lost-lives-a-match/]. A wide variety of factors have been identified to play a role in sudden cardiac death (both congenital and acquired heart conditions), but in some cases, the heart has no structural abnormalities.[131,132] Short-term variability in the QT interval was compared in professional soccer players and age-matched sedentary controls, and the study found a higher variability in the soccer players compared to controls.[130] This might lead to repolarization instability, which increases the risk for cardiac arrhythmias, with no underlying heart condition.[130] Interestingly, the fatal event usually does not happen at peak performance but instead in warmup or after training, in a relatively inactive period.[130]

ET-1 also has proliferative activity in vascular smooth muscle cells, thus, it has been implicated to play a role in atherosclerosis.[133] Furthermore, an increased level of ET-1 plays a pathophysiological role in the development of heart failure.[134]

ET-1 elicits its effects through 2 main receptors. The ET_A and ET_B receptors are both G-protein-coupled receptors, and while ET_A mediates vasoconstrictive responses to ET-1,[135] ET_B mediates ET-1 clearance, eNOS and NO production,[136] suggesting that the effect of ET-1 is receptor-dependent and a possible interaction between the vasoconstrictor effect of ET_A and vasodilatator effect of ET_B exists.[137] Furthermore, NO appears to have an antagonistic effect on ET-1 synthesis.[124]

ET_A receptor activation induces PLC activation, which leads to the formation of IP_3 and DAG. IP_3 then binds to specific receptors on the sarcoplasmic reticulum and releases stored Ca^{2+} into the cytoplasm.[124] The increased intracellular calcium level is a key participant in muscle contraction.

Blood flow and shear stress appear to be the most important stimuli for ET-1 synthesis and release, as shear stress receptors on endothelial cells might become activated in response to vasodilatation as a result of increased blood flow. This leads to the release of NO and a reduced production and secretion of ET-1.[138,139] In addition to nitric oxide, natriuretic peptides can also decrease the levels of ET-1 mRNA in endothelial cells.[140-142]

Plasma ET-1 levels were significantly higher in young strength-trained athletes than in endurance-trained athletes.[143] ET-1 levels increase with age, but exercise training could reduce plasma ET-1 levels in older individuals.[133] In another study in healthy young participants, the plasma levels of NO increased, while the plasma levels of ET-1 decreased after an 8-week-long exercise training program. Additionally, there was a negative correlation between plasma NO concentration and ET-1 concentration. The increase in NO and the decrease in ET-1 were maintained for an additional 4 weeks after the cessation of the training program, and after 8 weeks after training cessation, both returned to the baseline measured before the exercise training.[144] Young athletes responded with an increased ET-1 level after a single bout of intense cycle ergometer test. The increase was highest 30 minutes after the exercise cessation, then returned to lower levels at the 60-minute mark.[145]

1.5 NT-proBNP

In 1988, a peptide was isolated from pig brain that caused natriuresis and diuresis similar to ANP. Since it was isolated in brain tissue, the original name was brain natriuretic peptide, which later was changed to B-type natriuretic peptide to better reflect the primary site of BNP synthesis, which is not the brain but the ventricular myocardium.[146]

The translational product of the BNP gene is a 134 amino acid long prepropeptide. After removing the signal peptide, a 108 amino acid long prohormone, proBNP, is produced. This prohormone will be cleaved into 2 fragments in equimolar quantities, NT-proBNP, which is biologically inactive, and BNP, which is biologically active.[147]

N-terminal pro–B-type natriuretic peptide is a member of the natriuretic peptide family consisting of 6 cardiovascular peptides and 3 NP receptors.[147] NT-proBNP may be found outside of the heart, but the main origin of the secreted peptide is the heart. The peptide is the amino terminal pro-segment of BNP. In terms of cardiac expression, atrial NT-proBNP expression is more abundant than ventricular expression; however, due to the greater mass of the ventricles, most of the cardiac NT-proBNP still comes from the ventricles.[147]

The main stimulus for NT-proBNP release from all compartments of the heart is wall stretch.[148,149] Binding of BNPs to their specific receptors leads to the activation of the guanylyl cyclase-cGMP second messenger system, which mediates most of the biological effects of BNPs.[146] The physiological effects of BNP include vasodilatation, natriuresis, and diuresis. The peptide may also downregulate the RAAS and has pro-lusitropic features.[150] Additionally, BNPs cause vascular smooth muscle relaxation leading to arterial and venous dilation.[151,152] Furthermore, BNP has protective effects to prevent the development of heart failure, for instance, vasodilatation, natriuresis, inhibition of the sympathetic nervous system, and the RAAS.[153-155]

Many factors influence the plasma level of NT-proBNP, for instance, age, sex, and body composition.[156] NT-proBNP isn't the sole contributor to circulating BNPs. In fact, several larger or smaller molecules are also circulating in the body; these different fragments are produced after proteolysis, differential cleavage, and other structural changes.[147]

NT-proBNP, as well as other cardiac hormones, is a valuable marker to rule out an existing pathological condition, as its elevated plasma level has diagnostic importance.[148] Automated blood tests to quantify NT-proBNP have been available for more than 20 years.[148]

NT-proBNP measurement is an inexpensive tool for screening for cardiovascular pathologies, including left ventricular hypertrophy, valvular heart disease, atrial fibrillation, or pulmonary hypertension. The measurement of NT-proBNP is at least as useful as the measurement of BNP and, in some comparative studies, even outperforms BNP for population screening.[156] It can also be used as early detection for increased cardiovascular morbidity and mortality risk.[157] The peptide is indirectly proportional to left ventricular ejection fraction and directly proportional to left ventricular mass.[156]

Another reason NT-proBNP is used as a marker for cardiac health is that BNPs, compared to ANPs, show greater increases in disease states.[158,159] While ANPs respond more acutely, BNPs show a better picture of the volume and pressure overload on the heart over a longer period, underlining the suitability of NT-proBNP as a marker of cardiac pathologies.[148]

While one study reported that BNP increases during exercise,[148] in a large systematic review of BNP and NT-proBNP, exercise was one of the factors that decreased NT-proBNP levels.[160]

2 Aims

In the exerkine field, there are conflicting results in the literature about apelin response to exercise. Some studies reported decreased apelin levels, others reported increased levels, while some studies found no change at all. Additionally, most studies did not differentiate between apelin isoforms. The potential involvement of endothelin-1 in sudden cardiac death of young athletes, especially soccer players, renders more research focus on this peptide. Although NT-proBNP has been shown to increase and also to decrease after chronic exercise, it is a good measure of ventricular function since unchanged plasma levels exclude the possibility of cardiac dysfunction. Furthermore, professional athletes are affected by both physical and mental stress during competitive sports.
For this reason, the aim of our research was to analyze the circulating concentration of 4 peptides, namely apelin-13, apelin-36, endothelin-1, and NT-proBNP, upon extreme physical and mental load, with the following aims:

- Characterize plasma level changes upon extreme physical load
- Characterize plasma level changes upon extreme mental load
- Analyze the associations of the peptides with cardiopulmonary exercise parameters
- Compare the peptide response between physical vs mental load

We hypothesized that (1) all of our measured peptides would respond to the extreme physical load; (2) the extreme physical load would lead to a similar peptide response as the extreme mental load; and (3) endothelin-1 and apelins would have opposite responses to both loads.

3 Materials and Methods

3.1 Participants

A total of 58 healthy, normotensive, Hungarian male soccer players (age: 22.9 ± 4.7 years) were included in this study. All participants were of Caucasian origin, self-reported non-smokers, and had no known cardiovascular diseases. Participants, similarly to other competitive athletes, underwent regular medical check-ups, which included a resting blood pressure measurement and a 3-lead ECG.

3.2 Study Protocol

To determine the changes in peptide concentration upon physical load, participants underwent a physical stress test carried out in an exercise physiology laboratory (Department of Health Sciences and Sports Medicine, Hungarian Sports University, Budapest, Hungary). A maximum incremental treadmill running test was implemented (2 min warm-up at 8 km/h speed, which was increased to 10 km/h and then remained constant; elevation was 0% in the first 3 min, and then increased 1.5% after each minute). The treadmill test was performed under standard laboratory conditions. The median temperature was 24.7°C, and the relative humidity was 39.5%. The tests were terminated if a subject was unable to continue (volitional exhaustion).

Participants underwent a mental load protocol at the International Training Centre (Budapest, Hungary) on a separate day to determine the changes in peptide concentration upon mental load. The original protocol was a highly realistic social conflict, which is used, among others, to train police officers who are prepared to work under extreme stress.[161] This protocol was modified to a less complex version. Briefly: after a short briefing, the participants received special protective equipment (face mask, throat protector) and training handguns with ammo, which contained hollow plastic projectiles filled with dyed soap. They then stepped into a room set up as a regular living room of a flat. The room was open from above, and cameras were installed to monitor the actions of the participants. Additionally, heart recording devices were secured to their chests. As the participants were moving into the room, stress triggers, like a stranger appearing in the room, were introduced, and on the apex of the mental stress, a burglar attacked the participants and shot

towards them with the training handguns. The tests were carried out in the presence of a psychologist.

3.3 Blood Sampling and Analysis

Standard phlebotomy was performed by qualified personnel before the load (baseline), immediately after the load (peak), and 30 min into the restitution phase (recovery) in both protocols. The plasma samples were centrifuged (4 °C, 1600 g, 15 min), and the supernatant sera were collected, frozen in liquid nitrogen as soon as possible, and stored at −80 °C until the measurements were performed.

A PowerCube gas analyzer unit supplied by Ganshorn (Niederlauer, Germany) was used to measure peak VO_2 values; the gas analyzer was calibrated before each measurement. An Omron MX2, Cardiosys Human ECG (Experimetria Kft., Budapest, Hungary) was employed for monitoring blood pressure and heart rate. Heart rate and gas exchange parameters were registered continuously during physical stress. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were recorded at three time points during both tests (baseline, peak, and recovery). Lactate concentrations in the physical load were recorded at three time points (baseline, peak, and recovery), and measurements were performed on a Biosen C-line Glucose and Lactate Analyzer (Frank Diagnosztika Kft., Budapest, Hungary).

The peptide ELISA analysis was conducted at the Faculty of Health Sciences, University of Pécs, Pécs, Hungary. Circulating peptide (apelin-13, apelin-36, endothelin-1, and NT-proBNP) concentrations were measured using a Multiskan FC Microplate Photometer (Thermo Fisher Scientific; Waltham, MA, USA). Apelin-13 and apelin-36 (Cusabio; Houston, TX, USA) were measured by a quantitative sandwich assay technique in duplicate. Intra-assay precision was < 8% and < 15%, and inter-assay precision was < 10% and < 15%, respectively. No significant cross-reactivity or interference between human AP-13/AP-36 and analogues was observed. Endothelin-1 (Elabscience; Houston, Texas, USA) was measured by a quantitative sandwich assay in duplicate. The intra-assay precision was 33.60 ± 1.40 pg/mL, and the inter-assay precision was 36.30 ± 1.40 pg/mL. NT-proBNP (Biomedica; Vienna, Austria) was measured by a sandwich immunoassay in duplicate. Intra-assay precision was ≤ 4%, and inter-assay precision was ≤ 7%.

3.4 Cardiopulmonary Exercise Parameters

In addition to blood pressure, heart rate, and blood lactate, other parameters influenced by the exercise test were measured or calculated. The measured parameters included metabolic equivalent (MET; 1 MET = 3.5 mL O2/kg body weight/minute), peak power output, VO_2max and relative VO_2max (parameters of maximum O_2 consumption), maximum CO_2 production (VCO_2), maximum ventilation (VE), and maximum rate of respiration (number of breath/minute). The calculated parameters included baseline rate pressure product (baseline RPP = baseline systolic BP x baseline HR), peak rate pressure product (peak RPP = peak systolic BP x peak HR), rate pressure product reserve (RPP reserve = peak RPP-baseline RPP), maximum respiratory quotient (RQ = maximum VCO_2/VO_2max), VE/VO_2, VE/VCO_2, circulatory power (VO_2max x SBP), and circulatory stroke work (circulatory power/peak HR).

The following criteria were used to confirm extreme physical load: (1) duration of the activity should be at least 8 min; (2) maximum HR ≥ 160-180 beats per minute, depending on the age of the participants; (3) RQ value ≥ 1.1 at the peak of the load; and (4) lactate concentration at maximum load should be 8 mmol/L or higher.[162]

3.5 Ethics

The study was approved by the National Public Health Center of Hungary (15117–9/2018/EÜIG, 24 May 2018) (Appendix 1). All subjects provided written informed consent prior to participation in the physical and mental load (Appendix 2, 3, 4). The study was conducted in accordance with the World Medical Association Declaration of Helsinki.

3.6 Statistical Analysis

For the statistical analysis, GraphPad Prism (version 10.0.1, GraphPad Software, Boston, MA, USA) and Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA, USA) were used. The Gaussian distribution was tested using the D'Agostino-Pearson omnibus normality test. The results

are presented as mean ± standard deviation (SD) for continuous normally distributed data and median and interquartile range (IQR) for continuous non-normally distributed data. Temporal changes in the normally distributed data due to acute exercise intervention were evaluated by a repeated measures one-way ANOVA test with time as a within-subject factor (baseline, peak, and recovery). To protect against the violation of the sphericity assumption, the Geisser-Greenhouse correction was used. When the main effect was statistically significant, a Tukey's multiple comparisons post hoc test was performed for pairwise comparisons. Temporal changes in non-normally distributed data in response to acute exercise intervention at 3 different time points (baseline, peak, and recovery) were analyzed using a non-parametric Friedman test. Where appropriate, a Dunn's multiple comparisons post hoc test was performed for pairwise comparisons. A paired Student's t-test was used to compare the normally distributed values between the 2 loads, while a Wilcoxon test was used to compare the non-normally distributed values between the 2 loads. Likewise, the correlation was analyzed using either a Pearson correlation for the normally distributed data or a Spearman correlation for the data that did not pass the normality test. The applied statistical tests are detailed in each figure legend. Differences were considered statistically significant at $p < 0.05$.

4 Results

4.1 Peptide and cardiovascular response to physical and mental load

A total of 58 athletes participated in the study. Four peptides (apelin-13, apelin-36, endothelin-1, NT-proBNP) and additionally, cardiovascular, cardiorespiratory, and metabolic parameters were recorded for all participants during the physical load. The same 4 peptides and cardiovascular parameters were recorded during the mental load. Peptide levels (physical and mental), blood pressure (physical and mental), heart rate (physical and mental), and lactate (only physical) concentration were recorded at rest (baseline), at maximum load (peak), and 30 minutes after the maximum load (recovery) (Table 1 and Table 2).

Table 1. Peptide concentration of the athletes at baseline, peak, and recovery time points in both loads.

N=58	Peptide concentration			RM One-Way ANOVA/ Friedman test
Physical load	**Baseline**	**Peak**	**Recovery**	
Apelin-13 (pg/mL)	143 ± 71.5	164 ± 71.2	137 ± 63.3	p = 0.004
Apelin-36 (pg/mL)	60.2 (49.6–79.5)	150 (91.6–203)	45.5 (36.1–66.6)	p < 0.001
Endothelin-1 (pg/mL)	4.38 (2.98-6.93)	5.86 (4.16-7.98)	4.32 (3.16-7.49)	p < 0.001
NT-proBNP (pmol/L)	44.7 (20.6-81.3)	32.7 (21.4-72.5)	41.8 (30.9-71)	p = 0.113
Mental load	**Baseline**	**Peak**	**Recovery**	
Apelin-13 (pg/mL)	116 (82-165)	114 (95-164)	111 (76-154)	p = 0.030
Apelin-36 (pg/mL)	62.4 (43.1–100)	47.6 (35.7-74)	69.5 (46.3–121)	p = 0.147
Endothelin-1 (pg/mL)	4.4 (2.7-7.08)	4.68 (2.92-7.52)	4.88 (3.4-7.57)	p = 0.205
NT-proBNP (pmol/L)	37.5 (22.2-76.5)	38.2 (21.1-77.7)	38.5 (20.3-87.7)	p = 0.966

Variables are expressed as mean ± SD or median (interquartile range, IQR: 25th and 75th percentiles). Data were analyzed by repeated measures one-way ANOVA or Friedman test to compare the changes in peptide concentration across 3 time points.
NT-proBNP, N-terminal pro–B-type natriuretic peptide; RM one-way ANOVA, repeated measures one-way ANOVA.

Acute physical load had a significant effect on apelin-13 (ANOVA F (1.79, 102) = 6.12; p = 0.004), apelin-36 (Friedman statistic: 30.1; p < 0.001), and endothelin-1 (Friedman statistic: 35.5; p < 0.001) level, while the mental load had a significant effect on apelin-13 (Friedman statistic: 7; p = 0.030). NT-proBNP didn't change in the physical (Friedman statistic: 4.36; p = 0.113) or the mental load (Friedman statistic: 0.069; p = 0.966).

Table 2. Cardiovascular and metabolic parameters of athletes at baseline, peak, and recovery time points in both loads.

Physical load	**Baseline**	**Peak**	**Recovery**	RM one-way ANOVA/ Friedman test
Systolic blood pressure (mm Hg)	143 (135-152)	179 (169-188)	127 (120-133)	$p < 0.001$
Diastolic blood pressure (mm Hg)	81 ± 8	79 ± 9	72 ± 7	$p < 0.001$
Heart rate (bpm)	70 (61-80)	187 (184-194)	87 (77-93)	$p < 0.001$
Blood lactate (mmol/L)	0.92 (0.71-1.23)	10.9 (9.55-12.5)	4.35 (3.22-5.79)	$p < 0.001$
Mental load	**Baseline**	**Peak**	**Recovery**	
Systolic blood pressure (mm Hg)	133 ± 12	156 ± 13	130 ± 10	$p < 0.001$
Diastolic blood pressure (mm Hg)	75 ± 8	89 ± 10	74 ± 8	$p < 0.001$
Heart rate (bpm)	71 (63-81)	68 (60-79)	62 (54-73)	$p < 0.001$

Variables are expressed as mean ± SD or median (interquartile range, IQR: 25th and 75th percentiles). Data were analyzed by repeated measures one-way ANOVA or Friedman test to compare the changes in blood pressure, heart rate, and blood lactate (in the physical load) concentration across 3 time points.
RM one-way ANOVA, repeated measures one-way ANOVA.

In the physical load, the Friedman test revealed a significant effect of exercise intervention on systolic blood pressure (Friedman statistic: 124, $p < 0.001$), heart rate (Friedman statistic: 122, $p < 0.001$), and blood lactate (Friedman statistic: 130, $p < 0.001$). Systolic blood pressure increased at peak load compared to baseline and decreased in recovery compared to peak and baseline (Dunn's multiple comparisons test, $p < 0.001$ for all three comparisons). Heart rate increased from baseline to peak load, and, in recovery, it decreased to a level lower than peak but higher than baseline (Dunn's multiple comparisons test, $p < 0.001$ for all three comparisons). Blood lactate concentration increased at peak load compared to baseline and decreased in recovery compared to peak (Dunn's multiple comparisons test, $p < 0.001$ for all three comparisons).

ANOVA revealed a significant effect of exercise intervention on diastolic blood pressure ($F(1.82, 117) = 38.2$; $p < 0.001$). Peak DBP decreased significantly in recovery (Tukey's multiple comparisons test, $p < 0.001$), and recovery DBP was significantly lower than baseline DBP (Tukey's multiple comparisons test, $p < 0.001$).

In the mental load, ANOVA revealed a significant effect of simulation intervention on systolic blood pressure ($F(1.57, 95.6) = 152$; $p < 0.001$). SBP increased at peak load compared to baseline (Tukey's multiple comparisons test, $p < 0.001$) and decreased in recovery compared to peak (Tukey's multiple comparisons test, $p < 0.001$) and baseline (Tukey's multiple comparisons test, $p = 0.003$).

Additionally, ANOVA revealed a significant effect of simulation intervention on diastolic blood pressure ($F(1.59, 96.8) = 160$; $p < 0.001$). DBP increased at peak mental load compared to baseline (Tukey's multiple comparisons test, $p < 0.001$) and decreased significantly in recovery (Tukey's multiple comparisons test, $p < 0.001$).

Regarding heart rate in the mental load, the Friedman test revealed a significant effect of simulation intervention on HR (Friedman statistic: 37.7; $p < 0.001$). Recovery HR was significantly lower than peak HR (Dunn's multiple comparisons test, $p < 0.001$) and baseline HR (Dunn's multiple comparisons test, $p < 0.001$).

4.2 Apelin-13 response to physical and mental load

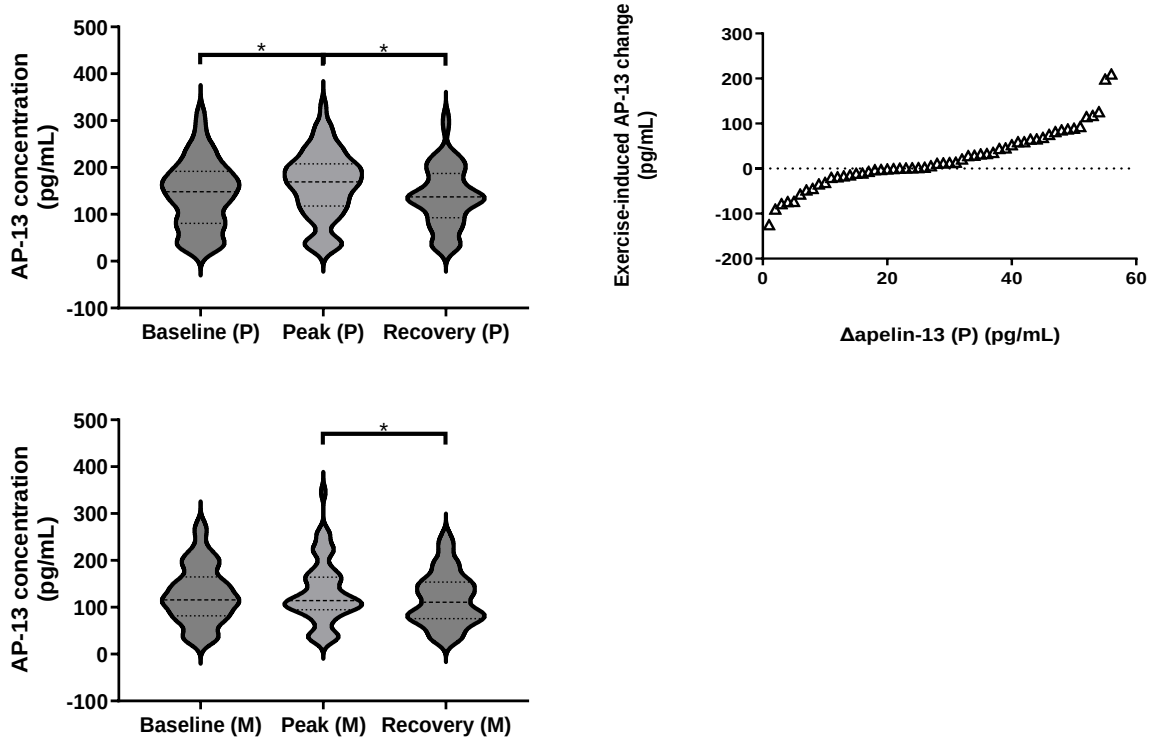


Figure 1. (A) Violin plots comparing the plasma levels of apelin-13 before (baseline), immediately after (peak), and 30 minutes after (recovery) the vita maxima treadmill test. Medians and the 75th and 25th percentiles are shown within the violin plots. Data were analyzed by repeated measures one-way ANOVA followed by Tukey's multiple comparisons test. * $p < 0.05$. (B) Individual apelin-13 responses to the exercise test. Each point represents the change in a participant's apelin-13 level from baseline to maximum load. Baseline values are subtracted from peak values and sorted in ascending order. (C) Violin plots comparing the plasma levels of apelin-13 before (baseline), immediately after (peak), and 30 minutes after (recovery) the extreme mental test. Medians and the 75th and 25th percentiles are shown within the violin plots. Data were analyzed by Friedman test followed by Dunn's multiple comparisons test. * $p < 0.05$.

Apelin-13 levels changed upon both physical and mental load (Table 1). In the physical load (Figure 1A), we found a significant increase at peak compared to baseline ($p = 0.036$) and a significant decrease at recovery compared to peak ($p < 0.001$). In the mental load (Figure 1C), the peak value didn't change compared to baseline but decreased significantly in recovery ($p = 0.042$). Since apelin-13 changed significantly upon physical load, we analyzed the peptide response on an individual level. Figure 1B shows the individual apelin-13 responses. Each point represents the change in a subject's apelin-13 level from baseline to maximum load. Baseline values are subtracted from peak values and sorted in ascending order. The response was heterogeneous, with a mean Δapelin-13 level of 21.9 ± 64.4 pg/mL.

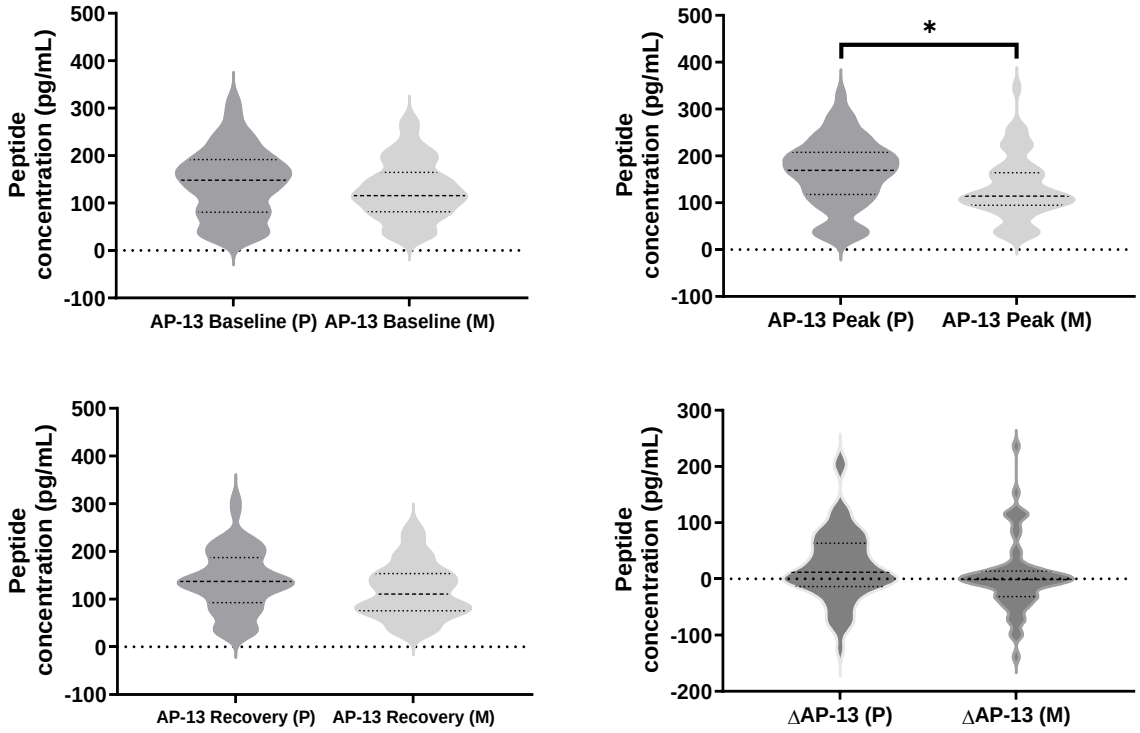


Figure 2. Violin plots comparing the baseline (A), peak (B), recovery (C), and Δapelin-13 (D) levels between the 2 loads. Medians and the 75th and 25th percentiles are shown within the violin plots. Data were analyzed by paired t test (A, C) or Wilcoxon test (B, D) depending on the distribution of data. * $p < 0.05$.

Looking at the direct comparison of the 3 main time points in the physical and mental load (Figure 2), the apelin-13 level at the peak of the physical load was significantly higher than the apelin-13 level at the peak of the mental load. There was no difference at other time points.

Regarding the association among apelin-13 and other peptides in the physical load, we found negative correlations between apelin-13 baseline levels and Δapelin-13, and Δapelin-36 (Figure 3A, B); apelin-13 peak levels and apelin-36 peak levels, and Δapelin-36 (Figure 3C, D); and apelin-13 recovery levels and Δapelin-36 (Figure 3E).

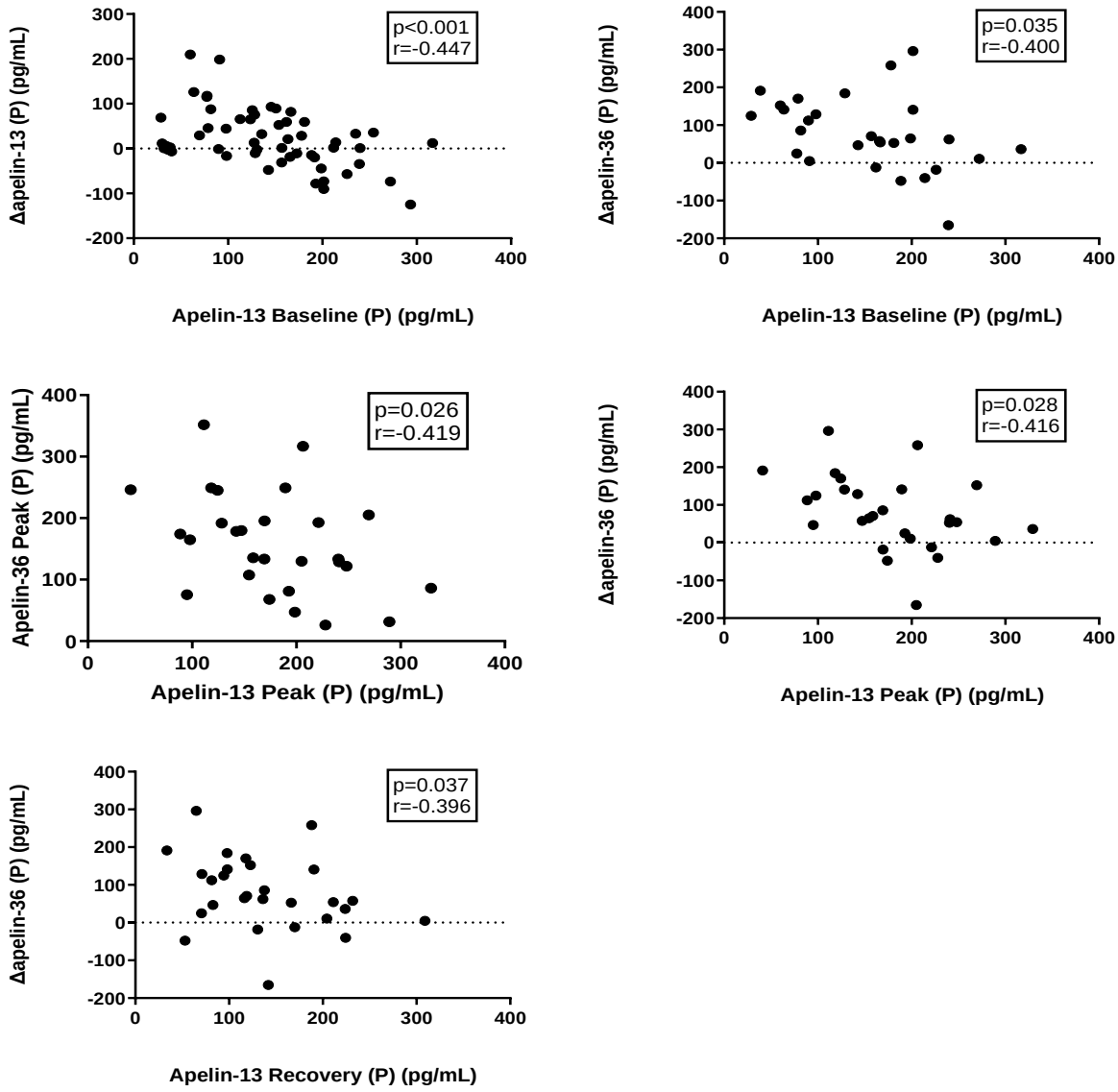


Figure 3. Correlation of apelin-13 baseline and Δapelin-13 (A) and Δapelin-36 (B); apelin-13 peak and apelin-36 peak (C) and Δapelin-36 (D); apelin-13 recovery and Δapelin-36 (E) in the physical load. Data were analyzed by Pearson correlation.

In the physical load, we found a positive correlation between Δapelin-13 and baseline blood pressure, peak diastolic blood pressure, circulatory power, maximum MET, and relative VO_2max (Figure 4).

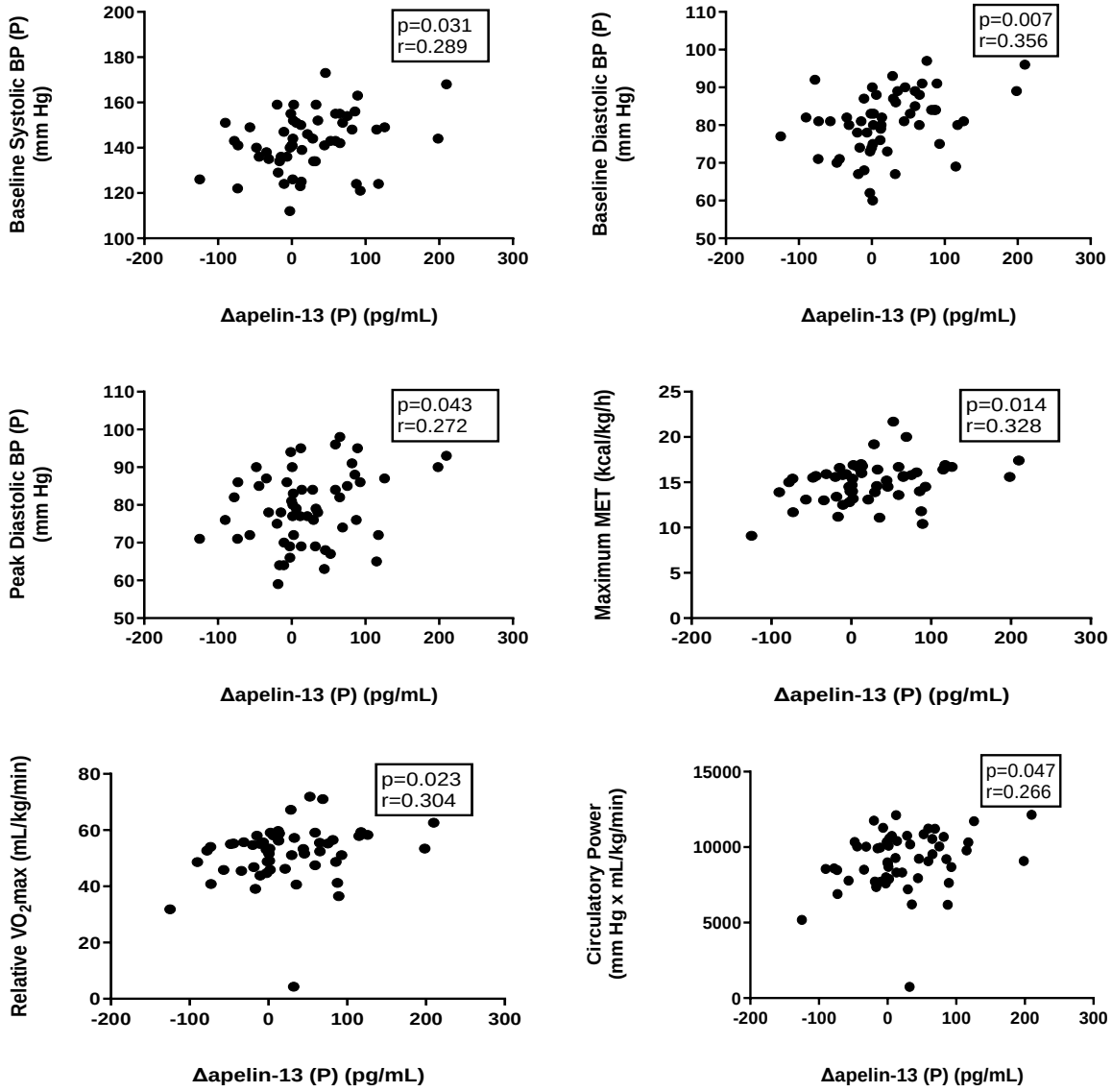


Figure 4. Correlation of Δapelin-13 and baseline systolic BP (A), baseline diastolic BP (B), peak diastolic BP (C), maximum MET (D), relative VO2max (E), and circulatory power (F) in the physical load. Data were analyzed by Pearson correlation (A, B, C, D) or Spearman correlation (E, F) depending on the distribution of data.

In the mental load, we found a positive correlation between apelin-13 baseline and apelin-36 baseline and found a negative correlation between apelin-13 baseline and Δapelin-13; and Δapelin-13 and baseline systolic blood pressure (Figure 5).

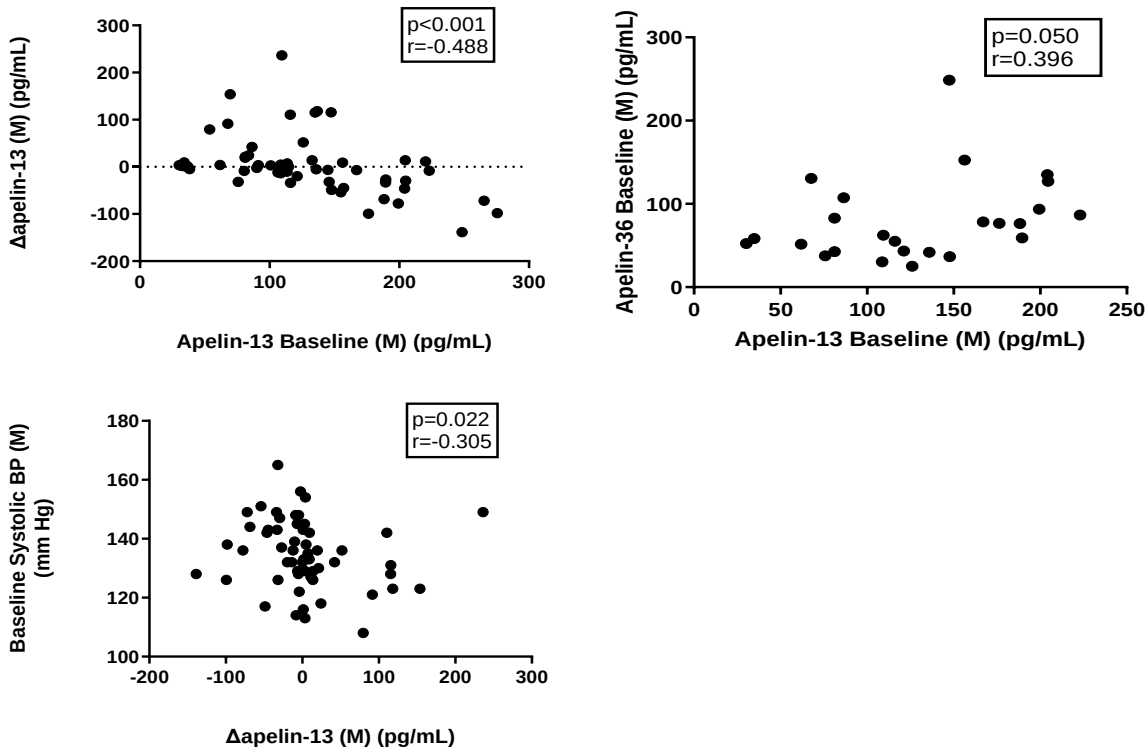


Figure 5. Correlation of apelin-13 baseline and Δapelin-13 (A) and apelin-36 baseline (B); and Δapelin-13 and baseline systolic BP (C) in the mental load. Data were analyzed by Spearman correlation.

4.3 Apelin-36 response to physical and mental load

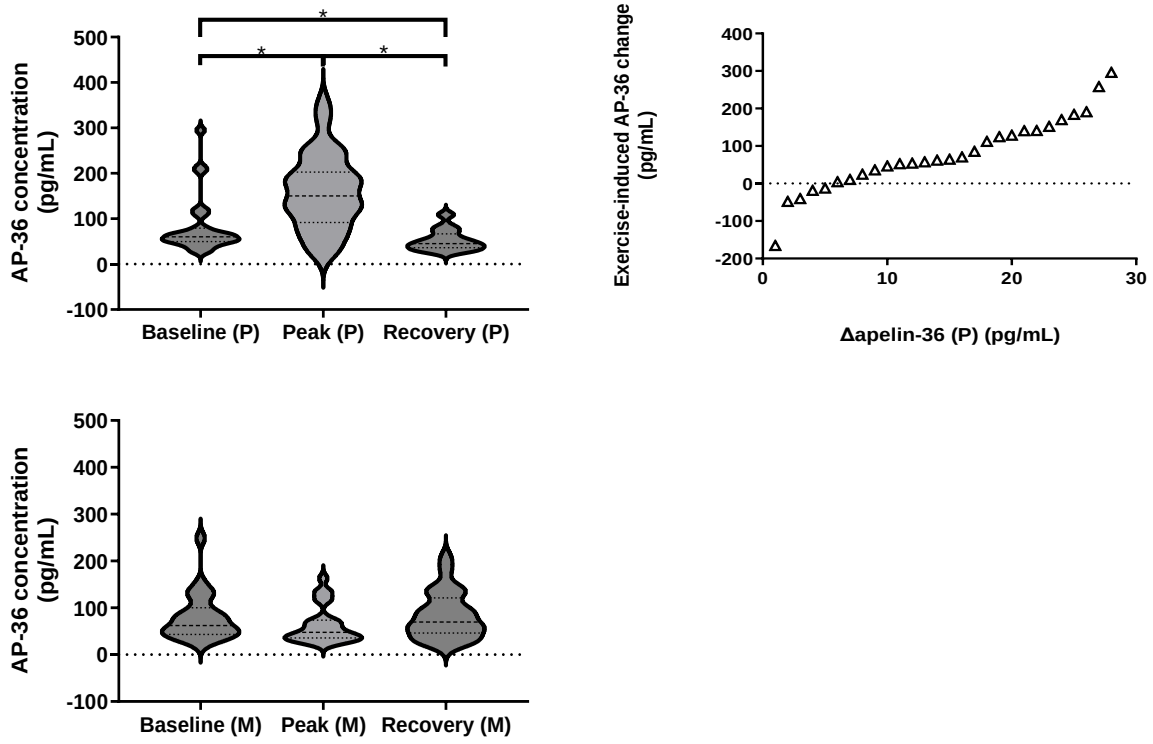


Figure 6. (A) Violin plots comparing the plasma levels of apelin-36 before (baseline), immediately after (peak), and 30 minutes after (recovery) the vita maxima treadmill test. Medians and the 75th and 25th percentiles are shown within the violin plots. Data were analyzed by Friedman test followed by Dunn's multiple comparisons test. * $p < 0.05$. (B) Individual apelin-36 responses to the exercise test. Each point represents the change in a participant's apelin-36 level from baseline to maximum load. Baseline values are subtracted from peak values and sorted in ascending order. (C) Violin plots comparing the plasma levels of apelin-36 before (baseline), immediately after (peak), and 30 minutes after (recovery) the extreme mental test. Medians and the 75th and 25th percentiles are shown within the violin plots. Data were analyzed by Friedman test followed by Dunn's multiple comparisons test.

At the peak of the physical load, apelin-36 levels were significantly higher compared to baseline ($p = 0.001$) and recovery ($p < 0.001$). Additionally, 30 minutes into the recovery phase, apelin-36 level decreased to a significantly lower level than baseline ($p = 0.033$) (Figure 6A). The mental load did not change apelin-36 levels (Figure 6C).

Since apelin-36 changed significantly upon physical load, we analyzed the peptide response on an individual level. Figure 6B shows the individual apelin-36 responses. The response was heterogeneous, with a median Δapelin-36 level of 63.5 pg/mL (IQR, 14.2-141).

Looking at the direct comparison of the 3 main time points in the physical and mental load (Figure 7), the apelin-36 level at the peak of the physical load was significantly higher than the apelin-36 level at the peak of the mental load. Furthermore, Δapelin-36 was also higher in the physical load than the mental load. There was no difference at other time points.

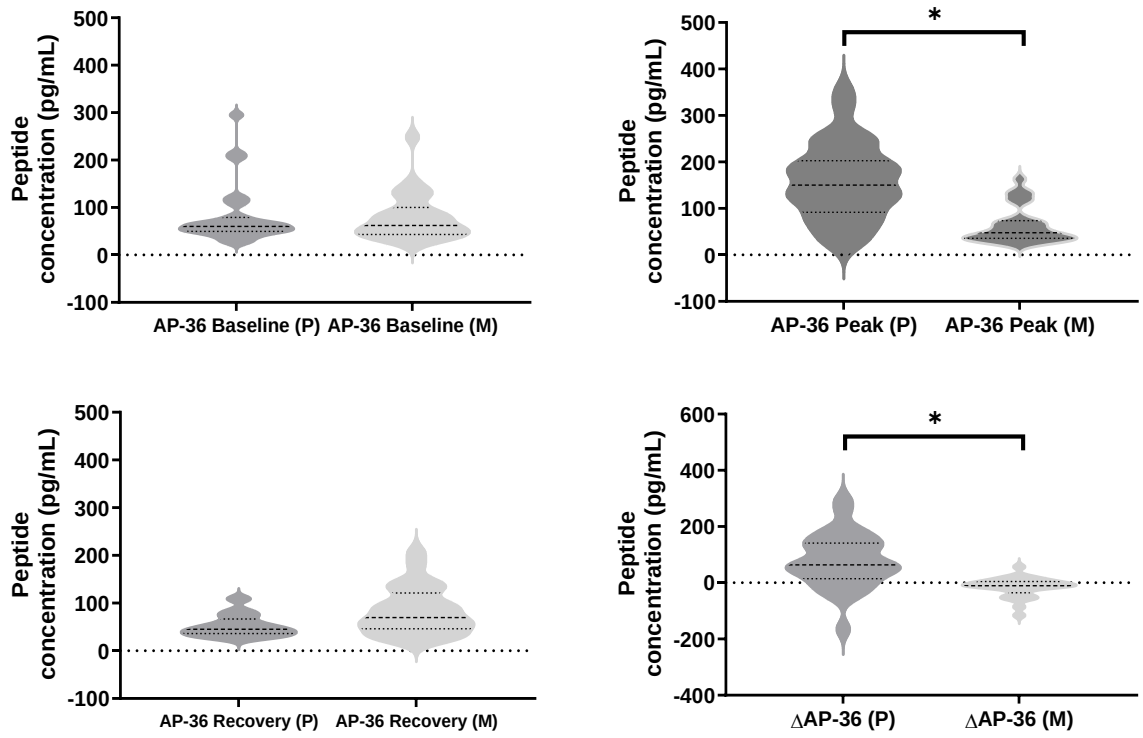


Figure 7. Violin plots comparing the baseline (A), peak (B), recovery (C), and Δapelin-36 (D) levels between the 2 loads. Medians and the 75th and 25th percentiles are shown within the violin plots. Data were analyzed by Wilcoxon test. * $p < 0.05$.

In the physical load, we found a negative correlation between apelin-36 peak and endothelin-1 baseline; and Δapelin-36 and endothelin-1 peak (Figure 8).

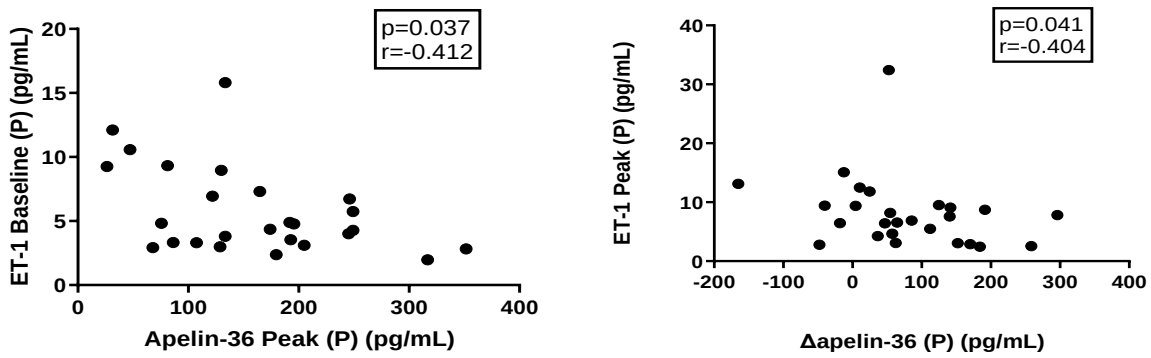


Figure 8. Correlation of apelin-36 peak and ET-1 baseline (A); and Δapelin-36 and ET-1 peak (B) in the physical load. Data were analyzed by Spearman correlation.

In the mental load, apelin-36 baseline negatively correlated with Δapelin-36 (Figure 9).

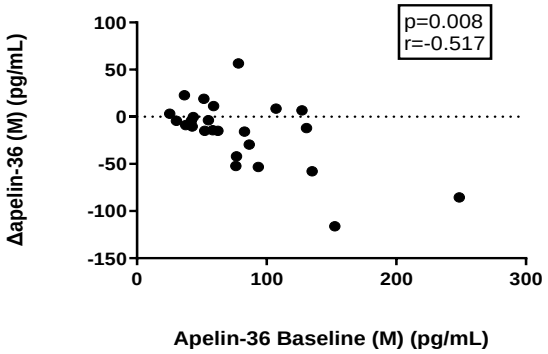


Figure 9. Correlation of apelin-36 baseline and Δapelin-36 in the mental load. Data were analyzed by Spearman correlation.

4.4 Endothelin-1 response to physical and mental load

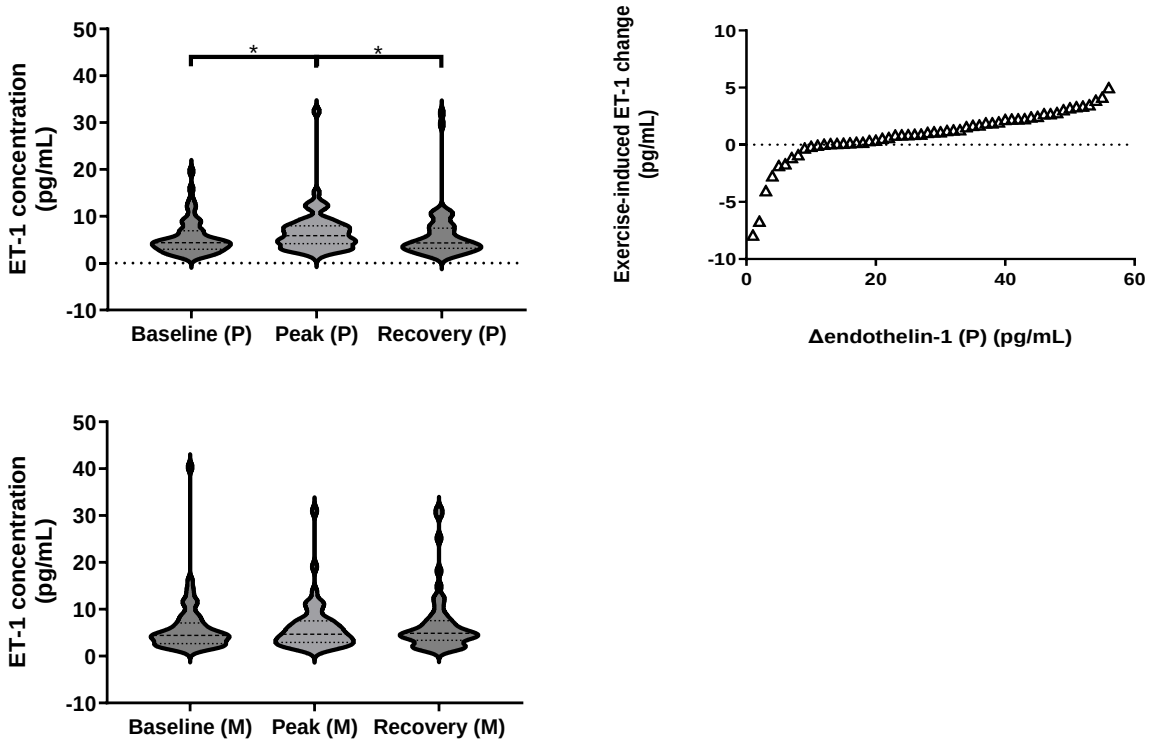


Figure 10. (A) Violin plots comparing the plasma levels of endothelin-1 before (baseline), immediately after (peak), and 30 minutes after (recovery) the vita maxima treadmill test. Medians and the 75th and 25th percentiles are shown within the violin plots. Data were analyzed by Friedman test followed by Dunn's multiple comparisons test. * $p < 0.05$. (B) Individual endothelin-1 responses to the exercise test. Each point represents the change in a participant's endothelin-1 level from baseline to maximum load. Baseline values are subtracted from peak values and sorted in ascending order. (C) Violin plots comparing the plasma levels of endothelin-1 before (baseline), immediately after (peak), and 30 minutes after (recovery) the extreme mental test. Medians and the 75th and 25th percentiles are shown within the violin plots. Data were analyzed by Friedman test followed by Dunn's multiple comparisons test.

In the physical load, we found a significant increase in endothelin-1 levels at peak compared to baseline ($p < 0.001$) and a significant decrease at recovery compared to peak ($p < 0.001$) (Figure 10A). ET-1 did not change in the mental load (Figure 10C).

Since ET-1 changed significantly upon physical load, we analyzed the peptide response on an individual level. Figure 10B shows the individual ET-1 responses. The response was heterogeneous, with a median Δendothelin-1 level of 1.13 pg/mL (IQR, 0.12-2.43).

Looking at the direct comparison of the 3 main time points in the physical and mental load (Figure 11), the endothelin-1 level at the peak of the physical load was significantly higher than the endothelin-1 level at the peak of the mental load. Furthermore, Δendothelin-1 was also higher in the physical load than in the mental load. There was no difference at other time points.

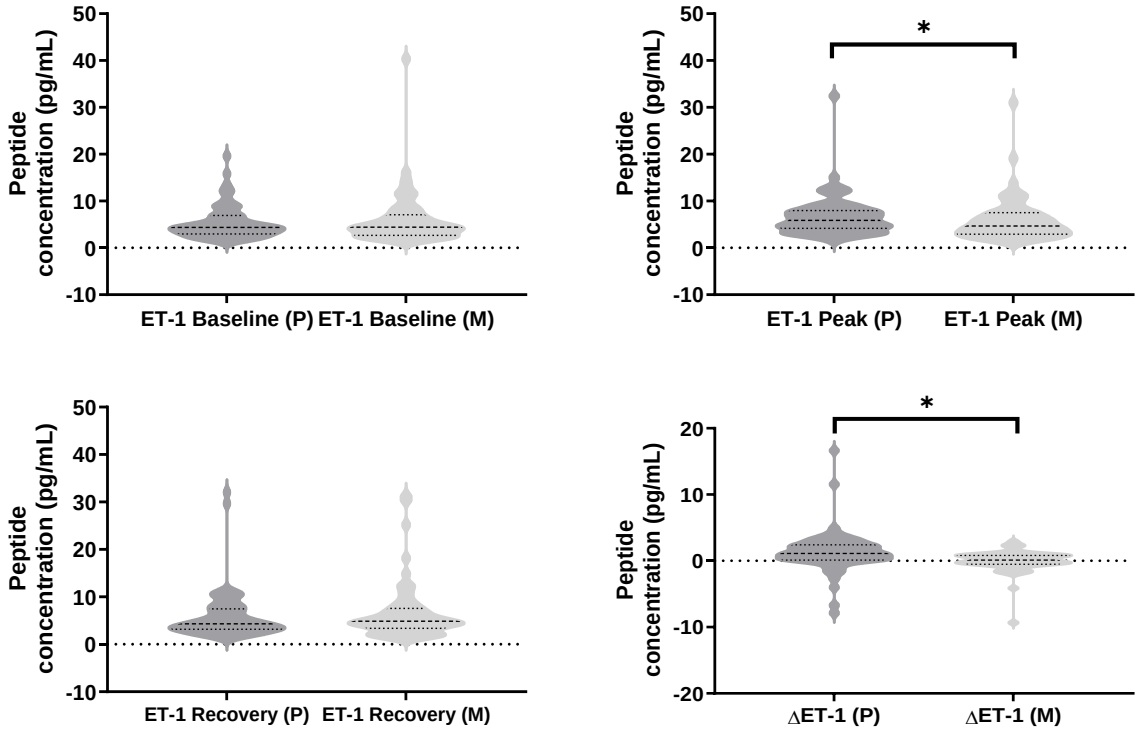


Figure 11. Violin plots comparing the baseline (A), peak (B), recovery (C), and Δendothelin-1 (D) levels between the 2 loads. Medians and the 75th and 25th percentiles are shown within the violin plots. Data were analyzed by Wilcoxon test. * $p < 0.05$.

4.5 NT-proBNP response to physical and mental load

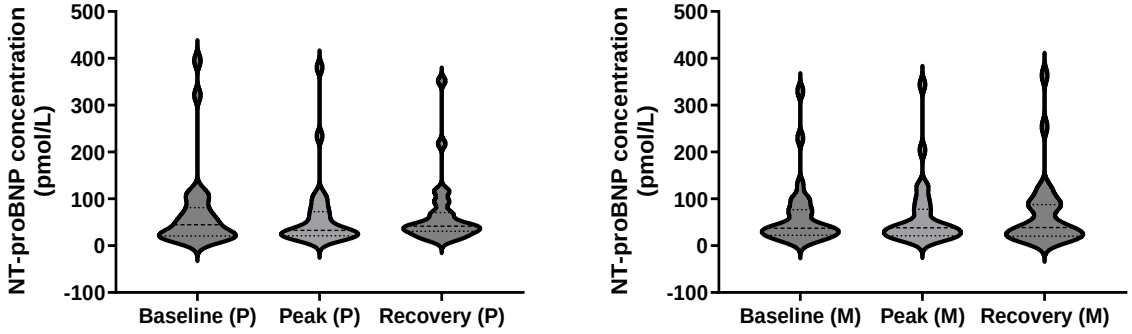
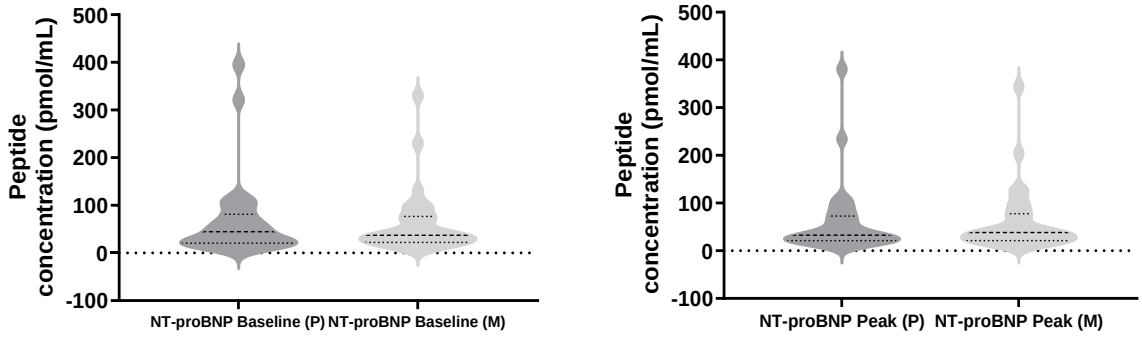


Figure 12. (A) Violin plots comparing the plasma levels of NT-proBNP before (baseline), immediately after (peak), and 30 minutes after (recovery) the vita maxima treadmill test. Medians and the 75th and 25th percentiles are shown within the violin plots. Data were analyzed by Friedman test followed by Dunn's multiple comparisons test. (B) Violin plots comparing the plasma levels of NT-proBNP before (baseline), immediately after (peak), and 30 minutes after (recovery) the extreme mental test. Medians and the 75th and 25th percentiles are shown within the violin plots. Data were analyzed by Friedman test followed by Dunn's multiple comparisons test.

The level of NT-proBNP did not change upon either load (Figure 12).

Looking at the direct comparison of the 3 main time points in the physical and mental load (Figure 13), there was no difference at any time point.



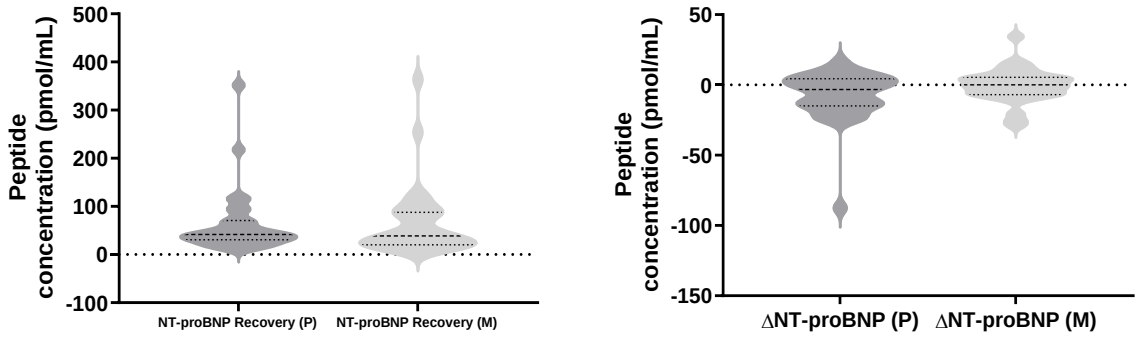


Figure 13. Violin plots comparing the baseline (A), peak (B), recovery (C), and ΔNT-proBNP (D) levels between the 2 loads. Medians and the 75th and 25th percentiles are shown within the violin plots. Data were analyzed by Wilcoxon test.

In the mental load, we found a positive correlation between baseline HR and NT-proBNP baseline, NT-proBNP peak, and NT-proBNP recovery (Figure 14).

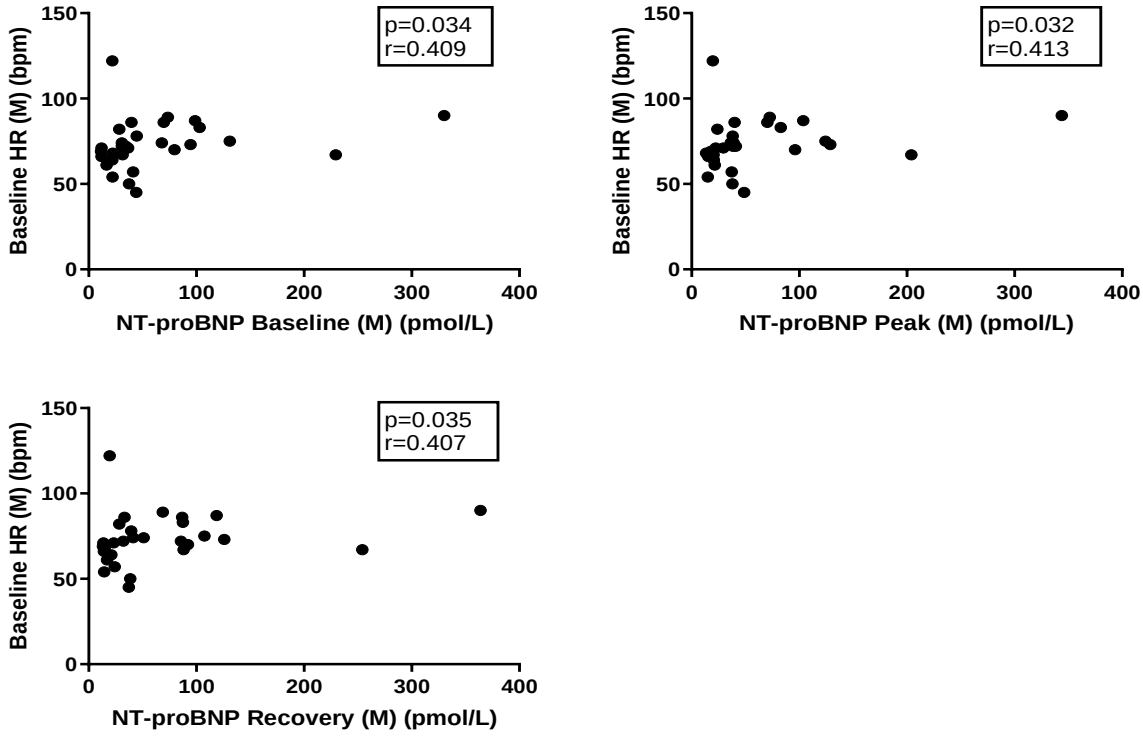


Figure 14. Correlation of baseline HR and NT-proBPN baseline (A), peak (B), and recovery (C) in the mental load. Data were analyzed by Spearman correlation.

5 Discussion

Professional athletes undergo intense physical and mental loads during regular training and in the actual games. Both parts are necessary to achieve the best individual performance, and their synergistic effects are crucial in reaching peak performance. For this reason, we analyzed a group of professional soccer players in an extreme physical and an extreme mental model and described changes in circulating apelin-13, apelin-36, endothelin-1, and NT-proBNP levels at different time points during both loads and their potential role as exerkines in Hungarian professional athletes.

Physical exercise has a profound effect on many bodily tissues, and many of these tissues are able to secrete exercise-induced factors upon acute or chronic exercise.[3,36,42-45] These factors are called exerkines, and their local and systemic roles in regulating exercise adaptation have become evident over the last decade.[3,41,48]

Skeletal muscle appears to have the main role in exercise-induced apelin production, though several tissues might contribute to the overall change in plasma levels. We showed what we believe for the first time, that apelin-13 shows a transient and heterogenous increase in response to a single bout of maximum exercise test in professional soccer players. The majority of the participants responded with elevated plasma apelin-13 levels, while a smaller proportion responded with reduced plasma apelin-13 levels. This was only true for the physical load, plasma apelin-13 did not change significantly upon the mental load, which is also evident from the difference between physical peak and mental peak apelin-13 levels; however, 30 minutes after both loads, the level of apelin-13 decreased below the respective peak values.

There are conflicting results in the literature about the apelin response to acute or chronic exercise. Some studies reported decreased apelin levels,[111,112,113] others reported increased[86] or unchanged[114] apelin levels upon physical exercise. These conflicting results are most likely due to the differences in the type, intensity, or duration of the acute exercise.[72] For example, a single bout of cycling sprint exercise increased apelin levels,[83] 200 meters and 400 meters swimming also increased apelin levels,[82] a marathon race reduced apelin levels,[110] while a 50-meter swimming exercise or a treadmill running bout in healthy individuals did not influence apelin levels.[82,85] The interindividual variability of apelin-13 responses to physical exercise might provide an explanation for these controversies about exercise-induced apelin release.

Interestingly, a higher systolic blood pressure before the physical load resulted in a more robust apelin-13 response, while the opposite was true in the mental load. In contrast, the baseline apelin-13 level was a good estimate of how either load would affect apelin-13; in both the physical and mental load, a lower baseline level resulted in a more robust change in apelin-13.

Our research provides valuable insights into the interplay between different apelin isoforms. Preproapelin is 77 amino acids long and is cleaved into shorter fragments, including both apelin-36 and apelin-13. It is also clear from the literature that the shorter fragments have higher biological activity.[95] In general, there was an inverse relationship between the 2 fragments. Thus, participants with lower baseline, peak, and recovery apelin-13 levels had higher apelin-36 responses to physical exercise. We also showed the inverse relationship of the two fragments on the peak of the physical load, where a lower apelin-13 peak correlated with a higher apelin-36 peak. In contrast, when we compared the baseline levels of the 2 peptides in the mental load, we found a direct relationship: higher apelin-13 baseline levels correlated with higher apelin-36 baseline levels in the mental load.

In some comparisons, apelin-13 and apelin-36 showed similar responses. For instance, the heterogeneity of the apelin response was observed for both peptides. Another similarity between the 2 isoforms is that both apelin-36 and apelin-13 were higher on the peak of the physical load than on the peak of the mental load. Interestingly, however, we found a connection between endothelin-1 and apelin-36 but not between endothelin-1 and apelin-13.

In the mental load, apelin-36 baseline level was a good estimate of the apelin-36 response: a lower baseline level resulted in a more robust change in apelin-36. This was observed for apelin-13 baseline level in mental load, too.

The metabolic equivalent (MET) can be used to represent the intensity of the exercise based on the basic metabolic rate.[163] Another parameter to describe aerobic capacity and cardiorespiratory fitness is relative VO_2max.[164] Yet another parameter to assess the pumping capacity of the heart is circulatory power, which is the product of VO_2max by peak systolic arterial pressure.[165] In our sample population, all 3 of these cardiopulmonary exercise–related parameters showed a positive connection with apelin-13, but not with apelin-36. This might suggest the role of apelin-13 in peak performance.[108] Additionally, the higher the apelin-13 response to physical exercise, the higher the diastolic pressure. Again, this was only true for apelin-13, not apelin-36. Apelin fragments were initially thought to be cleaved sequentially from proapelin, meaning that apelin-36 is the first

cleavage product, and then it is cut into smaller fragments. Later it was reported that the enzyme furin could directly cut apelin-13 from preapelin without producing longer fragments. These different isoforms show differential receptor affinity and biological activity,[95] which was also shown in our research. Apelin-13 was closely connected to cardiopulmonary exercise test-related parameters, while apelin-36 was not. This suggests that the 2 isoforms might play different roles during acute exercise.

Studies show that a 1 MET increase in exercise capacity means ~10-25% reduction in mortality.[166,167] At the peak of the cardiopulmonary exercise test, the soccer players reached a maximum MET four times higher than the resting MET. The maximum circulatory power was higher in the soccer players than previously reported values in age-matched, healthy individuals.[168] In professional athletes, there is a close relationship between physical performance, whole-body O_2 consumption, and the pumping capability of the heart during maximal cardiopulmonary exercise testing. This intimate connection was reinforced by the strong association of relative VO_2max with max MET and circulatory power. Relative VO_2max values for elite soccer players were reported between 59.2 and 66.6 mL/kg/min, which is comparable to the relative VO_2max values in our participants.[169] Soccer is a team sport where individual players need a combination of endurance, strength, sprinting, and jumping skills for shorter periods, meaning that aerobic and anaerobic demands are both present.[84] However, information on plasma apelin levels in soccer players is scarce in the literature. In one study of a Serie A team, throughout a season, apelin showed fluctuations, but no association has been reported between the fluctuating apelin levels and performance.[170]

Apelin has a potent vasodilator and positive inotropic effect, which is a rare combination among endogenous agents. The vasodilator effect is NO-dependent, while the inotropy is NO-independent.[96] Increased myofilament Ca^{2+} sensivitity[104,106] and the activation of PKC, ERK1/2, and MLCK[171] pathways might all be involved in the latter. The blood flow to the contracting skeletal muscles during intense dynamic exercise can increase up to 100-fold,[11] which can only be accommodated by a significant increase in cardiac output. An increase in heart rate, ventricular work, and myocardial contractility, all determinants of the cardiac output, leads to an increased myocardial O_2 demand, which can be matched by the elevated coronary blood flow,[172] potentially mediated by apelin-dependent NO production in response to exercise.[54,55] Furthermore, apelin may

balance oxygen demand and supply in the heart and regulate skeletal muscle performance via exercise-induced hyperemia.[107]

We analyzed endothelin-1 response to physical and mental load. ET-1 is one of the most potent endogenous inotropic agents.[56] Besides the positive inotropy, the peptide has many effects on the cardiovascular system, coronary vasoconstriction, and positive chronotropy, among others.[124] It has been reported that the tissue levels of ET-1 are potentially higher than the circulating levels. For instance, the pericardial ET-1 levels can increase up to 200-fold higher than the plasma levels in certain pathologies.[124,126,127,128] Similarly to apelin-36, ET-1 increased upon physical load but did not change upon mental load. Consequently, the peak ET-1 level in the physical load was significantly higher than the peak ET-1 level in the mental load. ET-1 and apelin have opposite effects on the vasculature, yet both are potent inotropic agents. ET-1 elicits its vasoconstrictor effect by binding to the ET_A receptor; however, ET-1 binding to the ET_B receptor mediates ET-1 clearance, eNOS and NO production.[137] Additionally, the vasorelaxant effects of apelin are also mediated by the eNOS-NO systems.[51-55] Both peptides are sensitive to changes in shear stress, and the active hyperemia during exercise might influence their secretion.[41,138,139] Altogether, these findings suggest a fine interplay between the vasoconstrictor and vasodilator effects of ET-1 and apelin and their close connection to the NO-dependent mechanisms mediating skeletal muscles. Furthermore, the production of these vasoactive peptides can be significantly and reciprocally promoted by acute exercise. Of note, the increased apelin or ET-1 plasma concentration might be merely a spillover of the locally produced amount.[135]

Our results align with other studies in the literature stating that NT-proBNP levels did not change upon maximal exercise load.[173] However, prolonged strenuous exercise (e.g. marathon running) increased NT-proBNP levels.[174] Interestingly, in the mental load, the baseline heart rate positively correlated with all 3 NT-proBNP time points. While cardiac ANPs are stored in secretory granules, BNPs are not stored to the same extent, meaning that the increased secretion and, subsequently, the elevated plasma level may require more time than it would for ANP.[148] NT-proBNP is a good measure of ventricular function since unchanged plasma levels exclude the possibility of cardiac dysfunction.

Professional athletes are affected by both physical and mental stress during competitive sports.[175] Three of the analyzed 4 peptides increased, on average, upon extreme physical load, while only apelin-13 changed upon extreme mental load. Additionally, our research showed that apelin-13 is

an exerkine associated with cardiopulmonary exercise–derived parameters (max MET, relative VO_2max, circulatory power), i.e. athletic performance.

6 Conclusion, summary of novel findings

We measured circulating peptide responses upon an extreme physical and an extreme mental load in Hungarian professional soccer players. Apelin-13, apelin-36, and endothelin-1 all responded to the extreme physical, while only apelin-13 responded to the mental load. NT-proBNP did not change in either load indicating an intact left ventricular function in our sample population. Additionally, apelin-13 correlated with measures of physical performance, whole-body oxygen consumption, and the pumping capability of the heart.

In conclusion, our research provided several novel findings to the exerkine field:

- Apelin-13, apelin-36, and endothelin-1 all showed a transient and heterogenous increase in response to a single bout of maximum exercise test in professional soccer players
- An inverse relationship exists between apelin-13 and apelin-36 response upon extreme physical load
- Apelin-13, but not apelin-36, showed an intimate relationship with performance-related cardiopulmonary exercise parameters
- An inverse relationship exists between endothelin-1 and apelin-36, but not apelin-13, upon extreme physical load
- In the mental load, the baseline levels of apelin-13 and apelin-36 were good predictors of the response of the mental load

7 Limitations

The interest in exerkines over the last two decades has grown rapidly, shifting from analyzing singular changes of one peptide to exerkine profiling. Our study focused on several exerkines, but the origin of these factors was not identified in our research. Indeed, analyzing several exerkines simultaneously with “omics” platforms and documenting the interaction among these factors is the next step in the exerkine evolution, which was beyond the scope of the current research.

Additionally, many factors influence the exerkine response upon acute exercise: the type and duration of the exercise, fitness, or timing of sampling. We analyzed 4 peptides in professional soccer players after a vita maxima treadmill test. Additional research with a different type (anaerobic vs resistance) or duration (e.g. HIIT) of exercise, and in different sports disciplines, is necessary to clear the conflicting results in exerkine response.

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Viktor, Sándor ; Roland, Ligetvári ; Csilla, Fenyvesiné Páger ; Lilla, Makszin ; Anikó, Kilár ; Ágnes, Dörnyei ; Béla, Kocsis ; Ferenc, Kilár Novel Results with Capillary Electrophoresis Coupled to Mass Spectrometry In: [sn] (szerk.) 16th CEEPUS Symposium and Summer School on Bioanalysis : book of abstracts Warsaw, Lengyelország : University of Warsaw (2016) 87 p. p. 2

10 Appendices

Appendix 1

PÉCSI TUDOMÁNYEGYETEM
2018 MÁJ 24.
PTE/42310/2018

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EMBERI ERŐFORRÁSOK MINISZTÉRIUMA

ORSZÁGOS TISZTIFŐORVOSI FELADATOKÉRT FELELŐS HELYETTES ÁLLAMTITKÁRSÁG
EGÉSZSÉGÜGYI IGAZGATÁSI FŐOSZTÁLY

Iktatószám: 15117-9/2018/EÜIG

Hiv. szám: -
Ügyintéző: Dr. Sajtos János
Telefon: +36 1 476 1100/2124
Melléklet: -

Tárgy: emberen végzett orvostudományi kutatás engedélyezése

HATÁROZAT

A „GINOP-2.3.2-15-2016-00047 Analitikai és diagnosztikai kutatási kiválóság központ az egészség és a sportteljesítmény szolgálatában" című emberen végzett orvostudományi kutatást a **Pécsi Tudományegyetem Egészségtudományi Kar Fizioterápiás és Sporttudományi Intézet** (7623 Pécs, Rét u. 4.) képviseletében **Prof. Dr. Tóth Miklós** kutatásvezetőként **előterjesztett kérelmére az alábbiak szerint**

engedélyezem:

- kutatás azonosító: „*GINOP-2.3.2-15-2016-00047 Analitikai és diagnosztikai kutatási kiválóság központ az egészség és a sportteljesítmény szolgálatában*"
- a kutatás anyagi fedezetét biztosító intézmény: GINOP-2.3.2-15-2016-00047 pályázat
- a kutatásban résztvevő intézmény
  - Pécsi Tudományegyetem Egészségtudományi Kar (7621 Pécs, Vörösmarty u. 4.)
  - Pécsi Tudományegyetem Természettudományi Kar (7624 Pécs, Ifjúság útja 6.)
  - Pécsi Tudományegyetem Általános Orvostudományi Kar Szentágothai János Kutatóközpont (7624 Pécs, Ifjúság útja 20.)
  - Pécsi Tudományegyetem Klinikai Központ Orvosi Genetikai Intézet (7624 Pécs, Szigeti út 12.)
  - Debreceni Egyetem Sporttudományi Koordinációs Intézet (4032 Debrecen, Egyetem tér 1.)
  - Szegedi Tudományegyetem Szent-Györgyi Albert Klinikai Központ I. sz. Belgyógyászati Klinika (6720 Szeged, Korányi fasor 8-10.)
- a kutatásvezető neve: Prof. Dr. Tóth Miklós
- a kutatás várható időtartama: jelen határozat véglegessé válásától számított 2 év 11 hónap
- a kutatásba bevonni tervezett résztvevők
  - Pécsi Tudományegyetem Egészségtudományi Kar: 325 fő, mindkét nem, 12-25 év között
  - Pécsi Tudományegyetem Természettudományi Kar: 500-1000 fő, mindkét nem, 10-30 év között
  - Debreceni Egyetem Sporttudományi Koordinációs Intézet: 650 fő, mindkét nem, 14-18 év között
  - Szegedi Tudományegyetem Szent-Györgyi Albert Klinikai Központ I. sz. Belgyógyászati Klinika: 150 fő, mindkét nem, 18-40 év között

A jelen eljárásban szakhatóságként megkeresett Egészségügyi Tudományos Tanács Tudományos és Kutatásetikai Bizottsága (1054 Budapest, Alkotmány u. 25., elnök: dr. Schaff Zsuzsa egyetemi tanár, a továbbiakban: ETT TUKEB) 25229-5/2018/EKU ügyiratszámú állásfoglalásának rendelkező részében kimondta:

Cím: 1097 Budapest Albert Flórián út 2-6. Tel: + 36 1 476 1100, Fax: + 36 1 476 6401
e-mail: tisztifoorvos@emmi.gov.hu

„Az országos tisztifőorvos nevében az EMMI Országos Tisztifőorvosi Feladatokért Felelős Helyettes Államtitkárság Egészségügyi Igazgatási Főosztálya (a továbbiakban: országos tisztifőorvos) a(z) Pécsi Tudományegyetem Egészségtudományi Kar Fizioterápiás és Sporttudományi Intézet (7623 Pécs, Rét u. 4.) képviseletében Dr. Tóth Miklós kutatásvezető (továbbiakban: Kérelmező) által kezdeményezett „Analitikai és diagnosztikai kutatási kiválóság központ az egészség és a sportteljesítmény szolgálatában" című, beavatkozással járó kutatás engedélyezésére indult eljárásban felkérte az Egészségügyi Tudományos Tanács Tudományos és Kutatásetikai Bizottságát (ETT TUKEB) szakhatósági állásfoglalás kialakítására.

Az ETT TUKEB a kutatás engedélyezése iránti kérelmet megvizsgálta és a következő, testületi véleményen alapuló szakhatósági állásfoglalást hozta:
Az engedély iránti kérelmet az ETT TUKEB szakmai és etikai szempontból megfelelőnek találta, ezért a kutatás engedélyezéséhez hozzájárul.

Jelen szakhatósági állásfoglalás ellen önálló fellebbezésnek nincs helye, az csak az országos tisztifőorvos eljárást befejező döntése elleni jogorvoslat keretében támadható meg."

Jelen határozatom véglegessé válásával elrendelem az engedélyezett kutatás hatósági nyilvántartásba vételét.

Jelen engedélyem címzettje működése során köteles a kutatási tevékenységhez szükséges személyi és tárgyi feltételeket folyamatosan biztosítani, valamint a további, a tevékenységre előírt speciális jogszabályi előírásokat betartani.
Felhívom a figyelmét, hogy az előírtak be nem tartása esetén egészségügyi bírság kiszabására van lehetőség.

Kérelmező a beadványában úgy nyilatkozott, hogy nem kereskedelmi kutatást kíván végezni, ennek megfelelően eljárási költséget nem állapítok meg.

Jelen határozattal szemben jogszabálysértésre hivatkozással közigazgatási per indítható a közlést követő naptól számított 30 napon belül a Fővárosi Közigazgatási és Munkaügyi Bírósághoz címzett, de Hivatalomhoz benyújtott keresetlevéllel. A közigazgatási per illetéke 30.000,- Ft, amely vonatkozásában a feleket jövedelmi és vagyoni viszonyaikra tekintet nélkül illetékfeljegyzési jog illeti meg.

INDOKOLÁS

Az Emberi Erőforrások Minisztériuma Országos Tisztifőorvosi Feladatokért Felelős Helyettes Államtitkársághoz (a továbbiakban: EMMI OTFHÁT) 2018. március 21-én érkezett beadványában Prof. Dr. Tóth Miklós a Pécsi Tudományegyetem Egészségtudományi Kar Fizioterápiás és Sporttudományi Intézet (7623 Pécs, Rét u. 4.) képviseletében „GINOP-2.3.2-15 - 2016-00047 Analitikai és diagnosztikai kutatási kiválóság központ az egészség és a sportteljesítmény szolgálatában" című emberen végzett orvostudományi kutatás engedélyezését kérelmezte az EMMI OTFHÁT-tól, amely alapján 2018. március 22. napján közigazgatási hatósági eljárás indult.

A kérelmet megvizsgáltam és megállapítottam, hogy az hiányos, érdemben nem bírálható el. Kérelmezőt hiánypótlásra szólítottam fel 2018. április 13-én kelt 15117-3/2018/EÜIG ikt. számú végzésben, melyet a Kérelmező 2018. április 25-én teljesített.

A tervezett kutatás rövid leírása:
„Projektünk célja egy európai színvonalú, sport- és extrém igénybevétel analízisén alapuló, a fizikai teljesítmény növelésének lehetőségét kutató kiválósági központ létrehozása. A műhely munkájának alapja egy olyan tudásbank létrehozása, amely nemzedékek számára lehetővé teszi az egészséges teljesítményfokozás követését és megvalósítását. Ennek érdekében laboratóriumi körülmények között és pályatesztek során elvégzett teljesítménydiagnosztikai mérések eredményeit, valamint a sportteljesítményhez kapcsolódó edzés hatására változó fiziológiai és biomechanikai paramétereket nagy pontossággal és megbízhatóan, meghatározott időszakonként kívánjuk rögzíteni. A projekt során biztosítjuk az elvárt minőségű biológiai mintát (pl. vér, vizelet és nyál). A minták feldolgozásából nyert proteomikai és metabolomikai információk és a sportolók teljesítménye közötti kapcsolatokat nagy pontosságú mérések elemzésével határozzuk meg. A genetikai, epigenetikai információk és az edzésmódszerek adatainak összehangolásával lehetőség nyílik a teljesítményfokozására, a tudományos metaanalízisek és felmérő vizsgálatok segítségével sportág- és sportoló-specifikus módszerek kidolgozására. Kutatásaink három fókuszpont körül koncentrálódnak:

1) Sportolók teljesítményének multifaktoriális non-invazív diagnosztizálása új mérési megközelítésre alapozott multifaktoriális teljesítménydiagnosztikai protokoll kifejlesztése, amely alkalmas iskolások, amatőr és professzionális sportolók fizikális és mentális vizsgálatára a „Virtual reality" (VR), „Augmented reality" (AR), „Eye tracking" (ET), Thermokamera (ThC), 3D szkennelés (3D Sc) eszközök alkalmazásával. A cél a tömeggyártott csúcstechnológia (VR, AR, 3D Sc) felhasználásával olcsó, sportegészségügyi centrumokon kívül is alkalmazható, általános antropometriai és sportág-specifikus sportegészségügyi-központú vizsgálatok elvégzése. A szkenneléssel előállított adatok alapján, megfelelő algoritmusok segítségével objektív elemzés adható a csontozat és vázizom felépítéséről, állapotáról és eltéréseiről.
2) Finomanalitikai kutatások: Kutatásaink célja et- és nem élsportolók teljesítőképességének diagnosztikája, apró eltérések kiszűrése, illetve kardiovaszkuláris változások és sportadaptáció vizsgálata kontrollált edzés hatására. Ennek keretében egy, a sportoló terhelhetőséget egyértelműen meghatározó általános protokollt kívánunk kidolgozni. Másodlagos célunk a sportoló sport-adaptációjának meghatározása, vagyis a sporttevékenység által leginkább igénybe vett szervek (szív, tüdő, izomzat, ízületek, pszichés státusz) alkalmazkodásának számszerű meghatározása és változásainak longitudinális követése. A finomanalitikai mérések közvetlen célja a biológiai mintákban (vér, nyál, vizelet, stb.) detektálható ismert és ismeretlen stressz-es más biomarkerek azonosítása és mennyiségi meghatározása, ezzel együtt a már meglévő műszeres analitikai eszközpark továbbfejlesztése. A finomanalitikai vizsgálatokat a terhelés-élettani vizsgálatokkal párhuzamosan kívánjuk elvégezni. A terhelés alatt fülcimpából, vagy ujjbegyből vett kevert kapilláris vérből határozzuk meg a laktát koncentrációt. A tömegspektrométeres analízis nem csak vénás, hanem - ahol ezt a mérés elve megengedi - szűrőpapíron beszárított kevert kapilláris vérből is kifejlesztésre kerül. Így a 18 év alatti sportolók is nagy számban vizsgálhatók, esetükben ugyanis a vénás vérminta vétel nehézségekbe ütközik.
3) Teljesítménynövelés, valamint a szervi és szöveti regeneráció lehetőségeinek vizsgálata molekuláris biológiai mechanizmusok feltárásával. Alapkutatásainkat az újonnan kifejlesztett kísérletes edzéstervek végrehajtása során a sportolok edzettség és teljesítményszintjének állandó biomechanikai, fiziológiai és pszichés monitorozásával párhuzamosan tervezzük megvalósítani. Minimális vér, vizelet, ill. nyálminták levételével a molekuláris paraméterek meghatározása (mRNA expressziós profil, miRNA expressziós profil, szekretált peptidek analízise) a Szentágothai János Kutatóközpontban történik majd. A kapott eredményeket a Funcionális Genomikai Kutatócsoport bevonásával kívánjuk elemezni. Kutatási eredményeink várhatóan az alábbi területeken kerülhetnek hasznosításra: sportolok (fegyveres testületi tagok) teljesítményének folyamatos monitorozása, illetve optimalizálása, a teljesítményfokozás finomhangolása, részben tömeggyártott csúcstechnológia felhasználásával (3D teljes testszkenner), részben minimális mintamennyiségekből közvetlenül az edzés során és helyszínén alkalmazható mini-tesztekkel; a kardiológiai betegek párhuzamos szűrésével a pathológiás versus sportolói szívizom-hipertrophia vizsgálata, megelőzési módszertanának kidolgozása, mindehhez fiatalkori szűrési stratégia kifejlesztése; kutatási eredményeink felhasználása hozzájárulhat a magyar sport, illetve parasport minél eredményesebb szerepléséhez a világversenyeken, illetve a reménybeli hazai olimpián; eredményeink új, eddig meg nem ismert celluláris és szervi regenerációt segítő molekuláris mechanizmusok megértéséhez és felfedezéséhez vezethetnek; kutatási projektünk számos új hazai és külföldi kollaboráció kialakítására ad lehetőséget, elősegítve ezzel fiatal kutatók foglalkoztatását és nemzetközi szinvonalú képzését a kontinensek között."

- kutatás tervezett időtartama: 2 év 11 hónap
- Témavezető neve: Prof. Dr. Tóth Miklós
- Támogató/szponzor neve, címe: GINOP-2.3.2-15-2016-00047 pályázat
- kutatóhelyek felsorolása:
    - Pécsi Tudományegyetem Egészségtudományi Kar (7621 Pécs, Vörösmarty u. 4.)
    - Pécsi Tudományegyetem Természettudományi Kar (7624 Pécs, Ifjúság útja 6.)
    - Pécsi Tudományegyetem Általános Orvostudományi Kar Szentágothai János Kutatóközpont (7624 Pécs, Ifjúság útja 20.)
    - Pécsi Tudományegyetem Klinikai Központ Orvosi Genetikai Intézet (7624 Pécs, Szigeti út 12.)
    - Debreceni Egyetem Sporttudományi Koordinációs Intézet (4032 Debrecen, Egyetem tér 1.)
    - Szegedi Tudományegyetem Szent-Györgyi Albert Klinikai Központ I. sz. Belgyógyászati Klinika (6720 Szeged, Korányi fasor 8-10.)

A kérelemről és a kutatási tervről megállapítottam, hogy annak tartalma megfelel *az emberen végzett orvostudományi kutatások, az emberi felhasználásra kerülő vizsgálati készítmények klinikai vizsgálata, valamint az emberen történő alkalmazásra szolgáló, klinikai vizsgálatra szánt orvostechnikai eszközök klinikai vizsgálata engedélyezési eljárásának szabályairól szóló 235/2009. (X.20.) Korm. rendelet* (továbbiakban: Kormányrendelet) 3/A. § (1) és (3) bekezdésében foglaltaknak, továbbá a beadvány tartalmazza a Kormányrendelet 3/A. § (2) bekezdése szerinti mellékleteket.

Az ETT TUKEB szakhatósági eljárása és állásfoglalása elsősorban az egészségügyről szóló 1997. évi CLIV. törvény (továbbiakban: Eütv.) 158.§ (3) bekezdésén, az Eütv. 159 § (6) bekezdés a) pontján, az 531/2017. (XII. 29.) Korm. rendelet 1. melléklet 2. pontjában foglalt „Egészségügyi ügyek" táblázat B: 10. mezője rendelkezésein, a 235/2009. (X. 20.) Korm. rend. 7. §, valamint az általános közigazgatási rendtartásról szóló 2016. évi CL. törvény (továbbiakban: Ákr.) 55. § (1)-(2) bekezdésein, az Ákr. 81. § (1) és (4) bekezdésein alapul.
Az ETT TUKEB hatáskörét és illetékességét az 531/2017. (XII. 29.) Korm. rendelet 1. § (1) bekezdése, az 531/2017. (XII. 29.) Korm. rendelet 1. melléklet 2. pontjában foglalt "Egészségügyi ügyek" táblázat D:10. mezője rendelkezése, valamint a 235/2009. (X. 20.) Korm. rendelet 2. § e) pontja állapította meg.
A fellebbezésre az Ákr. 55. § (4) bekezdése vonatkozik."

A benyújtott kérelem, és a csatolt dokumentumok, valamint az ETT TUKEB szakhatósági állásfoglalása és Hivatalom rendelkezésére álló iratok és adatbázis alapján megállapítottam, hogy

- a kutatást végző a személyi és tárgyi feltételeknek megfelel,
- kérelmező rendelkezik a kutatáshoz szükséges, az Eütv. 164. § (2) bekezdése szerinti felelősségbiztosítási szerződéssel,
- a kutatásban résztvevők személyes adatainak kezelése, valamint az azok megismerésére jogosultak köre megfelel a kutatás követelményeinek,
- a tervezett kutatás az Eütv.-ben meghatározott feltételeknek megfelel.

Fentiekre tekintettel a kérelmező beadványában meghatározott emberen végzett orvostudományi kutatást a Kormányrendelet 3. § (1) bekezdésben alapján a rendelkező részben foglaltak szerint engedélyeztem, egyúttal rendelkezve a kutatás hatósági nyilvántartásba vételéről.

Tekintettel arra, hogy jelen eljárás a fentiekben részletezettek szerint díjmentes, egyéb eljárási költség pedig nem merült fel, az eljárási költség viselésére vonatkozó döntést mellőztem.
Engedélyes figyelmét az alábbiakra hívom fel:

- A Kormányrendelet 9. § (3) bekezdése értelmében a kérelmező köteles jelen határozatról értesíteni az illetékes intézményi etikai bizottságot (továbbiakban: IKEB), a kutatást vezető intézmény vezetőjét és a kutatásvezetőt.
- A Kormányrendelet 11. § (1) bekezdése alapján a kutatási engedély véglegessé válását követően a kérelmező a kutatási tervet módosíthatja, ezt a kérelmező köteles bejelenteni Hivatalomnak.
- A Kormányrendelet 11. § (2) bekezdése értelmében a kutatási terv lényeges módosítása esetén a kérelmezőnek az engedély módosítását kell kérelmeznie az engedélyezőnél. A kutatási terv lényeges módosításának minősül különösen, ha
  a) a módosítás hatással lehet a kutatásban résztvevők biztonságára,
  b) a módosítás megváltoztathatja a kutatás elvégzését alátámasztó tudományos dokumentumok értelmezését,
  c) a módosítás a kutatók részére készített ismertetőt érinti,
  d) az addigi kutatási eredmények az írásos tájékoztató módosítását teszik szükségessé,
  e) a kutatásba új kutatási helyszín kerül bevonásra, vagy
  f) a kutatás vezetőjének személye változik.
- A Kormányrendelet 14. § (1) bekezdése szerint: Ha a kérelmező kívánja a kutatást annak befejezése előtt felfüggeszteni vagy megszüntetni, erről az indokok felsorolásával legkésőbb a felfüggesztéssel vagy megszüntetéssel egyidejűleg értesíti az engedélyezőt, az etikai bizottságot, a szakértőt, és több központban végzett kutatás esetén valamennyi érintett magyarországi kutatóhelyet.
- A Kormányrendelet 14. § (2) bekezdése értelmében a kutató a kutatást köteles haladéktalanul felfüggeszteni és a kutatásvezetőt értesíteni, ha azt tapasztalja, hogy a kutatás folytatása a résztvevő alanyok életét vagy egészségét sérti vagy veszélyezteti. A kutatásvezető erről értesíti a kérelmezőt és az engedélyezőt annak érdekében, hogy szükség esetén az engedélyező a 13. § (1)-(2) bekezdésében foglaltak alapján járjon el.
- Az EüM rendelet 20. §-a alapján a kutatás vezetője, jelentési kötelezettsége keretében a kutatás megkezdésétől kezdve minden második év végén, valamint a kutatás befejezését követő 15 napon belül köteles jelentést küldeni Hivatalomnak, az ETT TUKEB-nek és a kutatást végző intézményben működő IKEB-nek. A jelentésben be kell számolni a kutatás tapasztalatairól, a ténylegesen bevont betegek számáról, valamint külön-külön az előfordult nem kívánatos eseményekről, és a súlyos nem kívánatos eseményekről. A kutatás akkor tekinthető befejezettnek, ha minden beteg – kutatási terv szerinti – utolsó észlelése megtörtént.
- A fenti bejelentési, adatszolgáltatási kötelezettségek elmulasztása esetén *az egészségügyi hatósági és igazgatási tevékenységről szóló 1991. évi XI. törvény* (a továbbiakban: Ehi.) 13/A § (1a) bekezdése alapján *egészségügyi bírság* kiszabásának van helye.

Az Ehi. 13/A § (1b) bekezdése értelmében továbbá egészségügyi bírság kiszabásának van helye akkor is, ha jelen engedély előírásait engedélyes nem tartja be.
Az egészségügyi bírság összege az Ehi. 13/A § (5) bekezdése szerint harmincezer forinttól ötmillió forintig terjedhet.

Jelen határozatomat az Eütv. 159. § (6) bekezdésében, a Kormányrendelet 3. § (1) bekezdésében, a fővárosi és megyei kormányhivatal, valamint a járási (fővárosi kerületi) hivatal népegészségügyi feladatai ellátásáról, továbbá az egészségügyi államigazgatási szerv kijelöléséről szóló 385/2016. (XII. 2.) Korm. rendelet (továbbiakban: KR.) 8. § (1) bekezdés bc) pontjában meghatározott hatáskörömben eljárva adtam ki.
Illetékességemet a KR. 13. § (3) bekezdése határozza meg.

A határozat annak közlésével egyidejűleg az Ákr. 82. § (1) bekezdésének rendelkezése alapján végleges.

Határozatom ellen a fellebbezés lehetőségét az Ákr. 116. § (1)-(2) bekezdése zárja ki. A határozattal szembeni közigazgatási per indításának az Ákr. 114. § (1) bekezdése alapján van helye. A Fővárosi Közigazgatási és Munkaügyi Bíróság hatáskörét és illetékességét a közigazgatási perrendtartásról szóló 2017. évi I. törvény (Kp.) 12. § (1) bekezdése és az Eütv. 158. § (5) bekezdése határozza meg. A keresetlevél benyújtásának helyéről és idejéről a Kp. 39. § (1) bekezdése szerint adtam tájékoztatást.
Az illeték mértékét az illetékekről szóló 1990. évi XCIII. törvény (továbbiakban: Itv.) 45/A. § (1) bekezdése határozza meg. Az illetékfeljegyzési jogról az Itv. 62. § (1) bekezdés h) pontja rendelkezik.

Budapest, 2018. május 14.

Az Emberi Erőforrások Minisztériuma Szervezeti és Működési Szabályzatáról szóló 33/2014. (IX. 16.) EMMI utasítás alapján az országos tisztifőorvos nevében eljárva:

Dr. Paphalmi Rita
főosztályvezető

Kapják:

1. Prof. Dr. Tóth Miklós, Pécsi Tudományegyetem Egészségtudományi Kar Fizioterápiás és Sporttudományi Intézet, 7623 Pécs, Rét u. 4. tothmik1@hotmail.com (TV)
2. Egészségügyi Tudományos Tanács Tudományos és Kutatásetikai Bizottság, levelezési cím: 1051 Budapest, Széchenyi István tér 7-8. (TV)
3. Nemzeti Egészségbiztosítási Alapkezelő, 1139 Budapest, Váci út 73/A. (TV)
4. Irattár

Appendix 2

VIZSGÁLATI TÁJÉKOZTATÓ

Tisztelt Uram!

A tudomány fejlődésével a betegségek vizsgálatában és kezelésében egyre újabb molekulák szerepének vizsgálata válik szükségessé. A kutatásaink nagymértékben elősegíthetik a stressz egészséges és különböző kórállapotokban a keringésre gyakorolt hatásaival kapcsolatos ismereteink bővülését. Eredményeink közelebb vihetnek a sportolói stressz feldolgozás sajátosságainak megismeréséhez, továbbá nagy szerepet tölthet be a sportolói rosszindulatú, akár végzetes ritmuszavarokra, sportolói hirtelen szívhalálra való hajlam felismerésében.

Az eddig még e téren ismeretlen szerepű molekulák működésének pontosabb megismerése tudományos vizsgálatok által lehetséges. Az ebben való közreműködésre kérjük fel Önt.

A vizsgálat címe: *„A pszichés illetve a fizikális stressz szerepének vizsgálata sportolóknál"*

Vizsgálatunk két helyszínen zajlik egymást követő különböző napokon.
Az **első vizsgálat** során az ergometriai laborban (futószalaggal / kerékpárral) fizikai megterhelésnek tesszük ki, és az élettani paraméterek (pulzus szám, vérnyomás, vér oxigén telítettsége, EKG) mellett speciális, a stressz kialakulásában és a keringés szabályozásában részt vevő molekulák szintjét mérjük az Ön vérében (Adrenalin, Noradrenalin, Dopamin, Cortisol, Angiotenzin-II, Endothelin-1, Oxytocin, tesztoszteron, ACTH, ADP, IL-6, IL-10, teljes oxidatív kapacitás, Hsp70 ill. Hsp 60). A vizsgálat során több alkalommal (terhelés előtt illetve terhelés után 0, 30, 60 és 120 perccel) veszünk kb. 18 ml vért Öntől. A vérvételhez vénaszúrásra van szükség hagyományos egyszer használatos vérvételi tűvel. A vérvételi időpontokkal megegyezően fülcimpából kapillárisvércsepp-mintát is veszünk szűrőpapírra. A levett mintákon biokémiai, laboratóriumi méréseket végzünk. A vizsgálatok alacsony kockázatúak (szövődmény lehetőségek: a bőr helyi irritációja, vénagyulladás, kis mértékű vérzés, véraláfutás).
A második vizsgálati napon egy speciális Lélektaktikai házban a valóságot jól mintázó virtuális valóságban hajtatunk végre nem mindennapi, de bármikor előfordulható szituációs feladatokat. Ennek során fokozott pszichés stressz keletkezik a szervezetben, mely közben a fizikális terhelés során is mért élettani mutatókat monitorozzuk.
A vizsgálatok során több alkalommal (terhelés előtt nyugalomban, terhelés után a megnyugvási szakaszban) hagyományos módszerrel 12 elvezetéses EKG felvételeket készítünk. Ez a vizsgálat beavatkozással vagy fájdalommal illetve szövődménnyel nem jár.

A számos betegen tervezett vizsgálat reméljük, közelebb visz a stressz hatásának pontosabb megismeréséhez, így hatékonyabb tréningmódszer alakítható ki. A kutatás eredményei által az egészség megőrzésében, a sérülések elkerülésében is jobb lehetőségekhez jut a sportoló és az edzője egyaránt. Az általunk tervezett kutatás eredménye egészségesebb, hatékonyabb versenyző lesz.

Kérjük ezért Önt, járuljon ahhoz hozzá, hogy az Önből eltávolított vérmintákon kizárólag tudományos (nem kereskedelmi célú!) vizsgálatokat végezhessük. Így ez az Ön érdekeit semmiben nem sérti, azaz semmiféle beavatkozást nem igényel, a feldolgozás névtelenül történik, viszont elősegítheti az orvostudomány fejlődését.

Köszönettel az együttműködésért,

Prof. Dr. Tóth Miklós
tanszékvezető

Dr. Komka Zsolt
laborvezető

Appendix 3

1. Vizsgálati beleegyező nyilatkozat – SE-TSK

A vizsgálat címe: *„A pszichés illetve a fizikális stressz szerepének vizsgálata sportolóknál"*

A vizsgálat azonosító száma: _____

Alulírott (nyomtatott betükkel): _____

anyja neve: _____

születési hely: _____

TAJ szám: _____

lakcím: _____

Önként vállalkozom a Semmelweis Egyetem Testnevelési és Sporttudományi Karának Egészségtudományi és Sportorvosi Tanszékén (1123 Budapest, Alkotás u. 44.) folytatott vizsgálatban való részvételre. A szóbeli tájékoztatás során módomban állt kérdéseket feltenni. Elolvastam az Írásos vizsgálati tájékoztatót (mellékelve), és megértettem azt. A vizsgálatokkal kapcsolatban felmerült kérdéseimre kielégítő választ kaptam. Tudomásul veszem, hogy a vizsgálat adatai anonim (név nélkül) módon tudományos feldolgozásra bocsátják. Tájékoztattak továbbá arról is, hogy a vizsgálat során a további részvételemet bármikor megtagadhatom, ezt akár szóban is közölhetem, és ebből a későbbiekben semmilyen hátrányom nem származik. Az Írásos betegtájékoztató egy példányát átvettem.

Budapest, 201 . hó nap

_____
A vizsgált személy aláírása

Alulírott (nyomtatott betükkel): _____

beosztás: _____

munkakör: _____

munkahely: _____

ismertettem a tervezett klinikai vizsgálat célját, lényegét, valamint részletesen elmagyaráztam, hogy milyen beavatkozásokra kerül sor.

Budapest, 201__ . _____hó ____ nap

_____
A tájékoztatást adó személy aláírása

Appendix 4

2. Vizsgálati beleegyező nyilatkozat – BM NOK

A vizsgálat címe: *„A pszichés, illetve a fizikális stressz szerepének vizsgálata sportolóknál"*

A vizsgálat azonosító száma: _____

Alulírott (nyomtatott betűkkel): _____

anyja neve: _____

születési hely: _____

TAJ szám: _____

lakcím: _____

Önként vállalkozom a Belügyminisztérium, Nemzetközi Oktatási Központ Lélektaktikai Házában (1126 Bp., Böszörményi út 21.) folytatott vizsgálatban való részvételre. A szóbeli tájékoztatás során módomban állt kérdéseket feltenni. Elolvastam az Írásos vizsgálati tájékoztatót (mellékelve), és megértettem azt. A vizsgálatokkal kapcsolatban felmerült kérdéseimre kielégítő választ kaptam. Tudomásul veszem, hogy a vizsgálat adatai anonim (név nélkül) módon tudományos feldolgozásra bocsátják. Tájékoztattak továbbá arról is, hogy a vizsgálat során a további részvételemet bármikor megtagadhatom, ezt akár szóban is közölhetem, és ebből a későbbiekben semmilyen hátrányom nem származik. Az Írásos betegtájékoztató egy példányát átvettem.

Budapest, 201 . _____ hó_____nap

_____
A vizsgált személy aláírása

Alulírott (nyomtatott betűkkel): _____

beosztás: _____

munkakör: _____

munkahely: _____

ismertettem a tervezett klinikai vizsgálat célját, lényegét, valamint részletesen elmagyaráztam, hogy milyen beavatkozásokra kerül sor.

Budapest, 201__ . _____ hó_____nap

_____
A tájékoztatást adó személy aláírása

Appendix 5

7. sz. melléklet

DOKTORI ÉRTEKEZÉS BENYÚJTÁSA ÉS NYILATKOZAT A DOLGOZAT EREDETISÉGÉRŐL

Alulírott

név: Ligetvári Roland

születési név: Ligetvári Roland

anyja neve: Polgár Bernadett

születési hely, idő: Tatabánya, 1989.08.15

Effects of Extreme Physical and Mental Load on Circulating Exerkines in Professional Athletes című doktori értekezésemet a mai napon benyújtom a(z)

Pécsi Tudományegyetem Egészségtudományi Doktori Iskola 7. Program/PR-7 (Sport és Egészségtudomány), S-26 (A kardiorespiratórikus rendszer szabályozása megnövekedett fizikális és pszichés terhelés alatt) Programjához/témacsoportjához

Témavezető(k) neve: Prof Dr. Ács Pongrác, Prof. Dr. Tóth Miklós

Egyúttal nyilatkozom, hogy jelen eljárás során benyújtott doktori értekezésemet
- korábban más doktori iskolába (sem hazai, sem külföldi egyetemen) nem nyújtottam be,
- fokozatszerzési eljárásra jelentkezésemet két éven belül nem utasították el,
- az elmúlt két esztendőben nem volt sikertelen doktori eljárásom,
- öt éven belül doktori fokozatom visszavonására nem került sor,
- értekezésem önálló munka, más szellemi alkotását sajátomként nem mutattam be, az irodalmi hivatkozások egyértelműek és teljesek, az értekezés elkészítésénél hamis vagy hamisított adatokat nem használtam.

Továbbá nyilatkozom, hogy hozzájárulok a doktori értekezésem DOI azonosító igényléséhez.

Dátum: Pécs, 2024.01.11.

Ligetvári Roland
doktorváramányos aláírása

témavezető aláírása

Tóth Miklós
társtémavezető aláírása

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