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

Ph.D. Thesis Booklet

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1. Introduction

Physical activity and exercise adaptation play a crucial role in maintaining overall health, with physical inactivity being linked to various diseases and chronic conditions. Exercise involves the voluntary activation of skeletal muscles and requires ATP for muscle contraction. The choice of energy source (carbohydrates or lipids) depends on exercise intensity. Achieving maximum exercise capacity involves a coordinated effort of various systems, including the cardiovascular and respiratory systems.

During exercise, the working skeletal muscle undergoes active hyperemia, and blood flow to the skin increases for heat dissipation. The pulmonary system plays a key role in maintaining oxygen levels and removing CO_2. The liver, heart, and mitochondria undergo adaptations to support exercise.

The concept of "exerkines" refers to humoral factors released by both exercised and non-exercised tissues, mediating communication between organs. Myokines, the subset released by skeletal muscles, are crucial in exercise adaptation. Exerkines can act locally or systemically, influencing metabolism, inflammation, and endothelial function. Acute and chronic exercise induce different exerkine responses, contributing to metabolic health.

Apelin:

- Apelin, initially discovered in bovine stomach extracts, is a multifunctional peptide involved in cardiovascular regulation, muscle regeneration, and metabolic processes.
- Skeletal muscles synthesize apelin, and exercise increases its expression. Apelin influences glucose utilization, promotes mitochondrial biogenesis, and is linked to physical activity.
- Studies on the effects of acute and chronic exercise on apelin levels have shown conflicting results, with some reporting increases while others decreases.

Endothelin-1:

- Endothelin-1, synthesized by endothelial cells, has diverse effects on the cardiovascular system, including vasoconstriction, positive inotropic effects, and arrhythmogenic potential.
- Exercise can influence plasma ET-1 levels, with potential implications for cardiovascular health. Young athletes may experience an increase in ET-1 levels after intense exercise.
- Short-term variability in the QT interval, influenced by ET-1, may contribute to cardiac arrhythmias in athletes.

NT-proBNP:

- N-terminal pro–B-type natriuretic peptide (NT-proBNP) is released from the heart in response to wall stretch and serves as a valuable marker for cardiovascular pathologies.
- NT-proBNP measurement is a cost-effective screening tool for conditions like left ventricular hypertrophy, valvular heart disease, atrial fibrillation, and pulmonary hypertension.
- While some studies suggest an increase in NT-proBNP during exercise, a systematic review indicates that exercise might decrease NT-proBNP levels.

Understanding the physiological adaptations to exercise, the role of exerkines, and the behavior of specific peptides like apelin, endothelin-1, and NT-proBNP contributes to a comprehensive view of the impact of physical activity on health. The complexities of these responses highlight the need for further research to elucidate the intricate interplay between exercise, biomolecules, and overall well-being in diverse populations.

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 assess peptide concentration changes during physical stress, participants underwent a treadmill running test in a controlled laboratory environment. The test included a 2-minute warm-up at 8 km/h, followed by a constant speed of 10 km/h with a 0% elevation for the first 3 minutes and a 1.5% increase per minute thereafter. Testing conditions were standard with a median temperature of 24.7°C and 39.5% humidity, ending when participants reached volitional exhaustion. For mental stress, a modified realistic social conflict protocol was conducted at the International Training Centre (Budapest, Hungary). Participants, equipped with protective gear and training handguns, faced stress triggers in a simulated living room. A psychologist supervised as scenarios unfolded, culminating in a staged burglary with simulated gunfire. Heart recording devices monitored participants throughout the test.

3.3 Blood Sampling and Analysis

Phlebotomy, conducted by qualified personnel, included baseline, peak, and recovery samples in both protocols. Plasma samples underwent centrifugation, with supernatant sera frozen in liquid nitrogen and stored at −80 °C. PowerCube gas analyzer measured peak VO_2, calibrated before each reading. An Omron MX2 monitored blood pressure and heart rate. Heart rate and gas exchange parameters were registered continuously during physical stress. Systolic and diastolic

blood pressure, and lactate concentrations (only in the physical load) were measured at three points (baseline, peak, and recovery).

Peptide ELISA analysis at the University of Pécs measured apelin-13, apelin-36, endothelin-1, and NT-proBNP concentrations. Apelin-13 and apelin-36 were quantified using a sandwich assay with intra-assay precision < 8% and inter-assay precision < 15%. Endothelin-1 was measured with intra-assay precision of 33.60 ± 1.40 pg/mL and inter-assay precision of 36.30 ± 1.40 pg/mL. NT-proBNP was measured using a sandwich immunoassay with intra-assay precision ≤ 4% and inter-assay precision ≤ 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 O_2/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.

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. 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

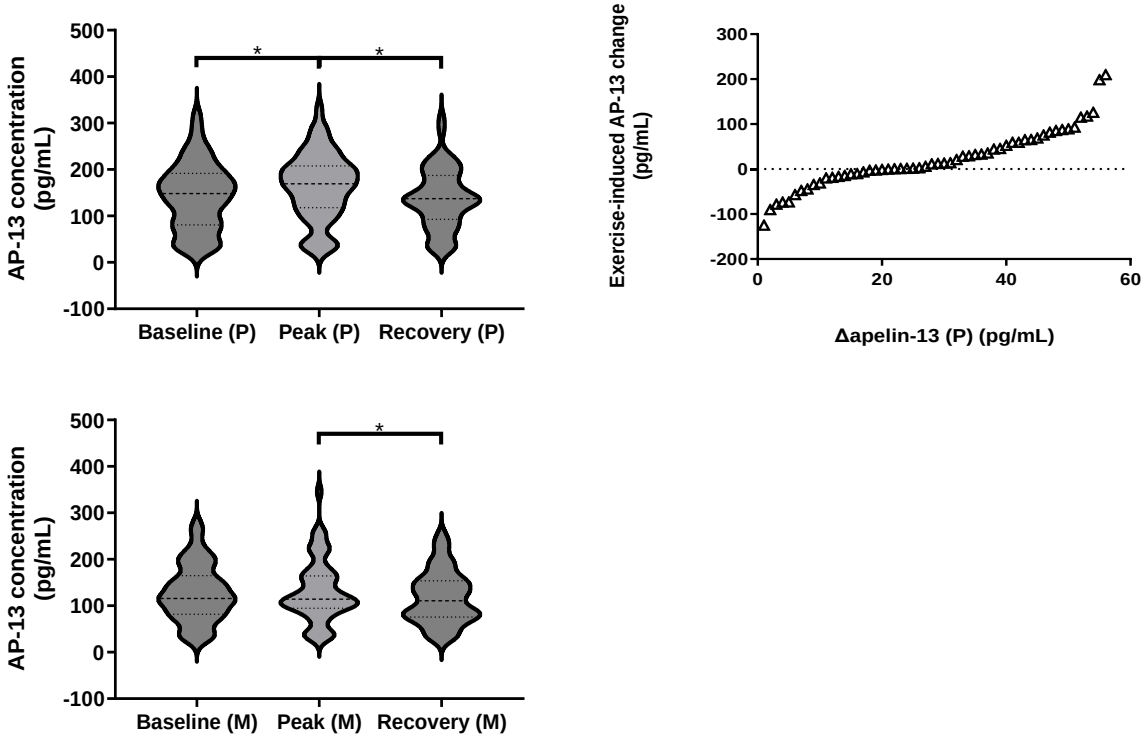


Figure 1. (A) Violin plots comparing the plasma levels of apelin-13 before (baseline), immediately after (peak), and 30 minutes after (recovery) the vita maxima treadmill test. Medians and the 75th and 25th percentiles are shown within the violin plots. Data were analyzed by repeated measures one-way 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.

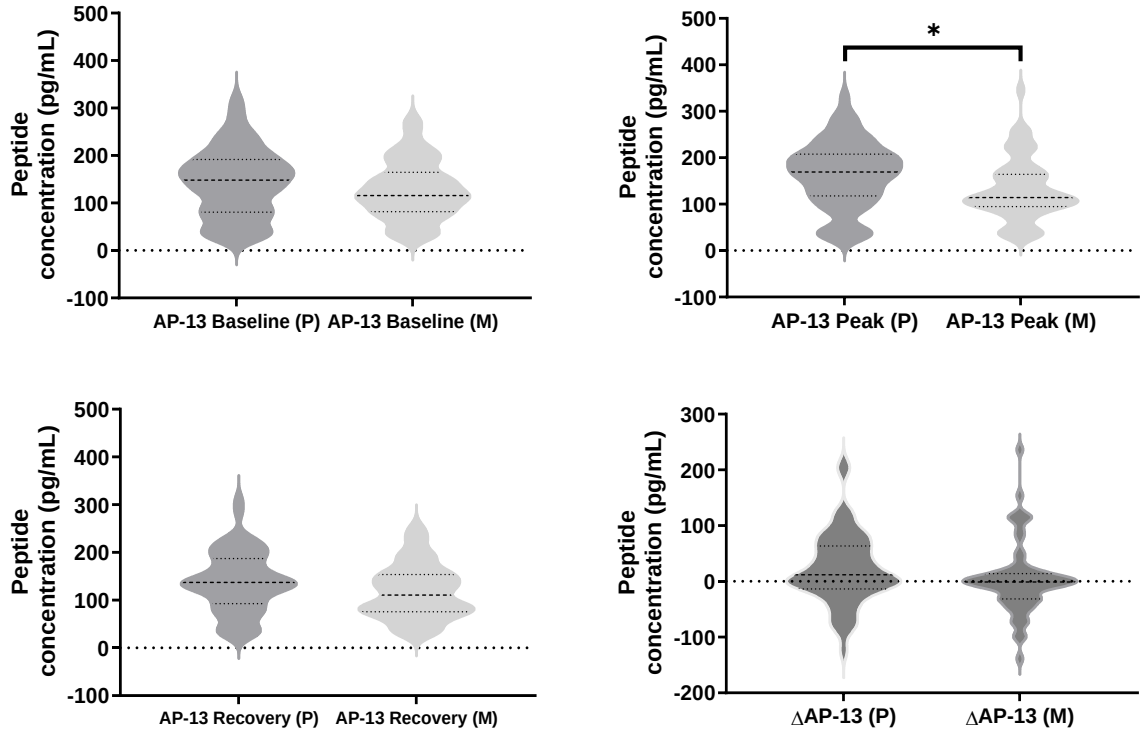
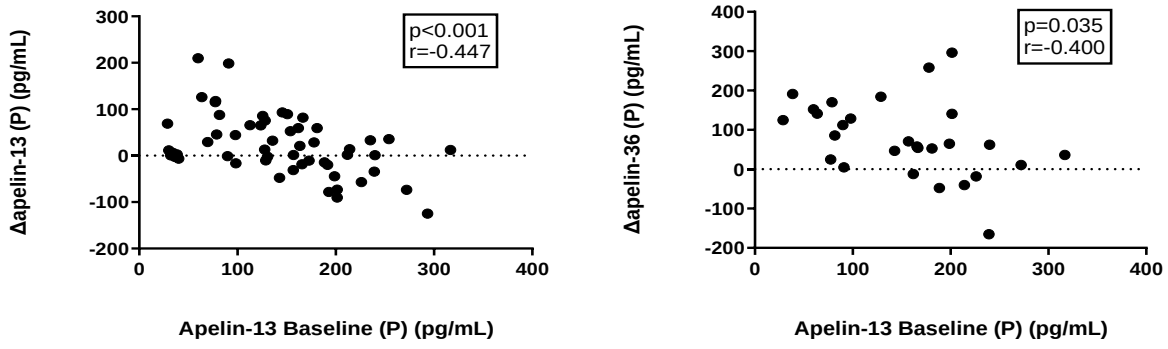


Figure 2. Violin plots comparing the baseline (A), peak (B), recovery (C), and Δapelin-13 (D) levels between the 2 loads. Medians and the 75th and 25th percentiles are shown within the violin plots. Data were analyzed by paired t test (A, C) or Wilcoxon test (B, D) depending on the distribution of data.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

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).



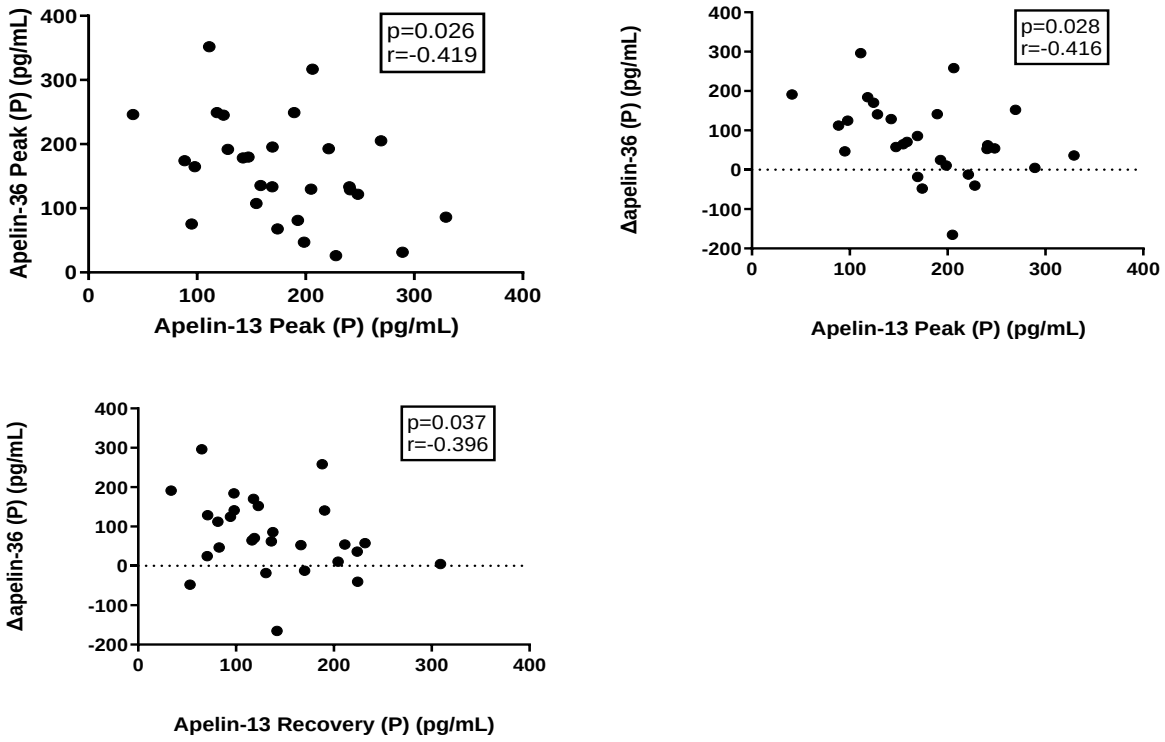
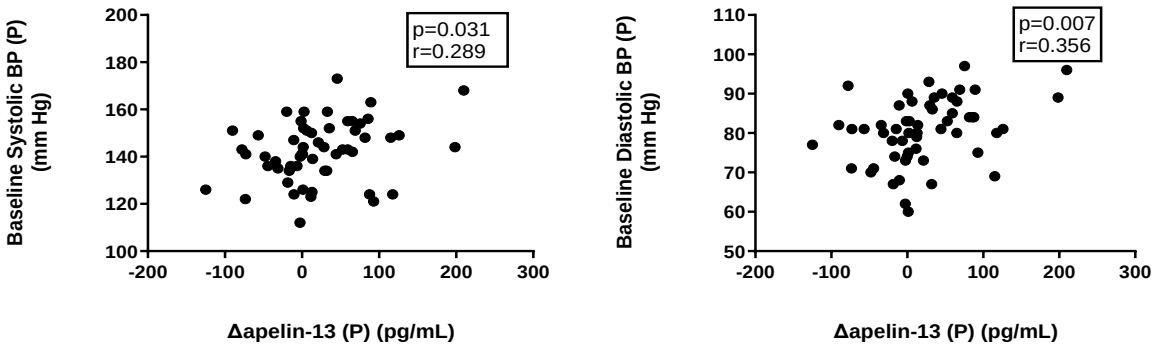


Figure 3. Correlation of apelin-13 baseline and Δapelin-13 (A) and Δapelin-36 (B); apelin-13 peak and apelin-36 peak (C) and Δapelin-36 (D); apelin-13 recovery and Δapelin-36 (E) in the physical load. Data were analyzed by Pearson correlation.

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).



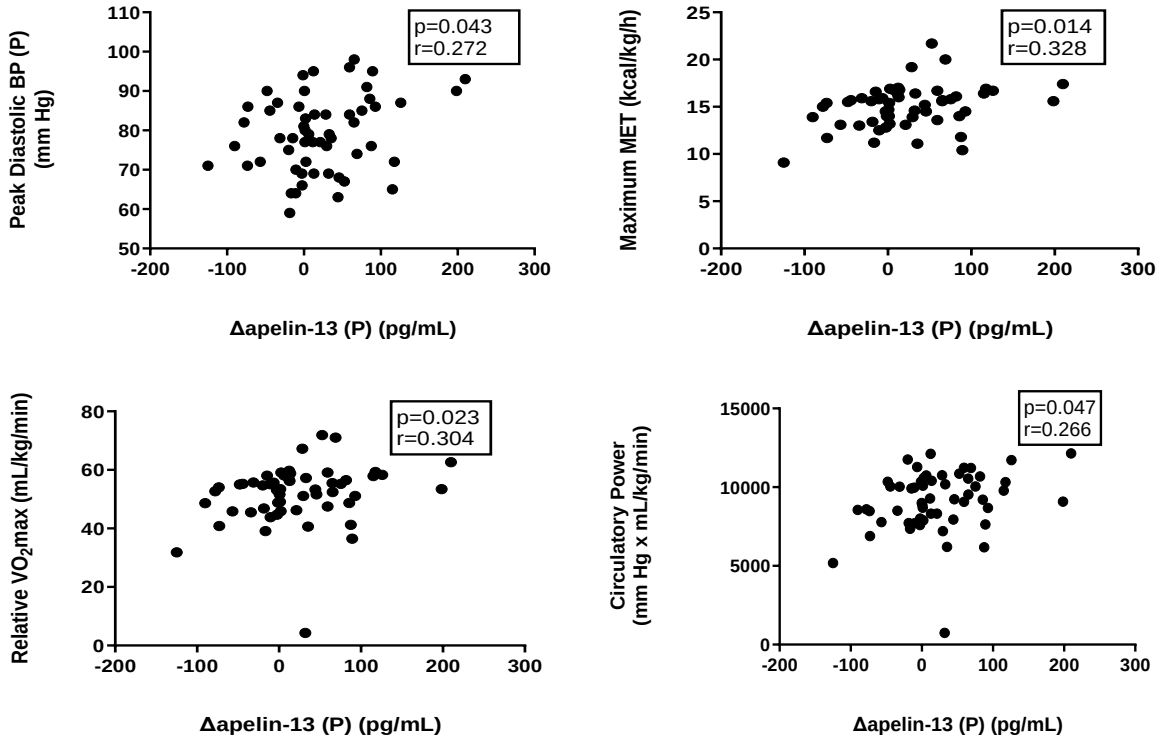
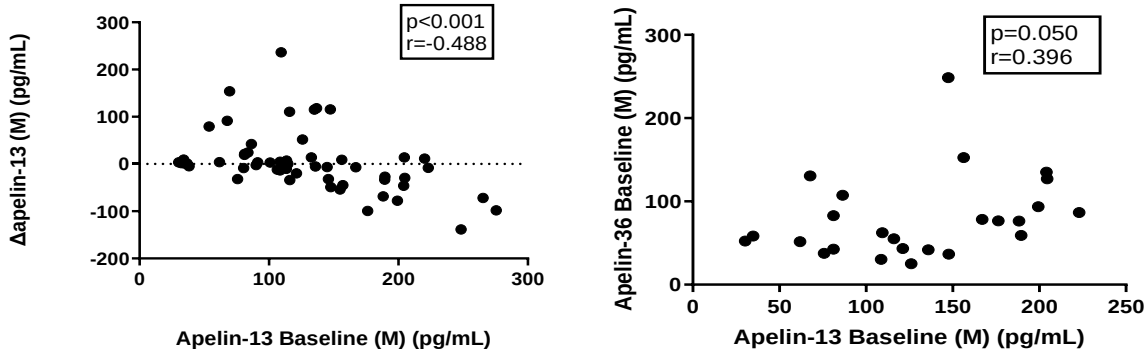


Figure 4. Correlation of Δapelin-13 and baseline systolic BP (A), baseline diastolic BP (B), peak diastolic BP (C), maximum MET (D), relative VO2max (E), and circulatory power (F) in the physical load. Data were analyzed by Pearson correlation (A, B, C, D) or Spearman correlation (E, F) depending on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



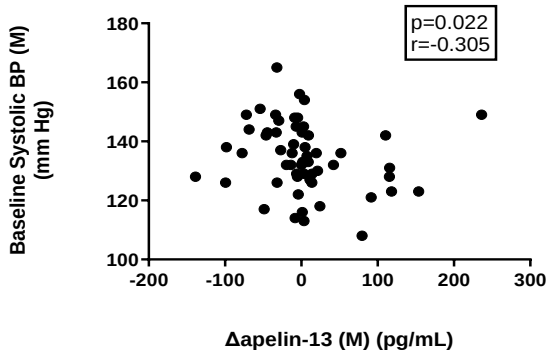


Figure 5. Correlation of apelin-13 baseline and Δapelin-13 (A) and apelin-36 baseline (B); and Δapelin-13 and baseline systolic BP (C) in the mental load. Data were analyzed by Spearman correlation.

4.3 Apelin-36 response to physical and mental load

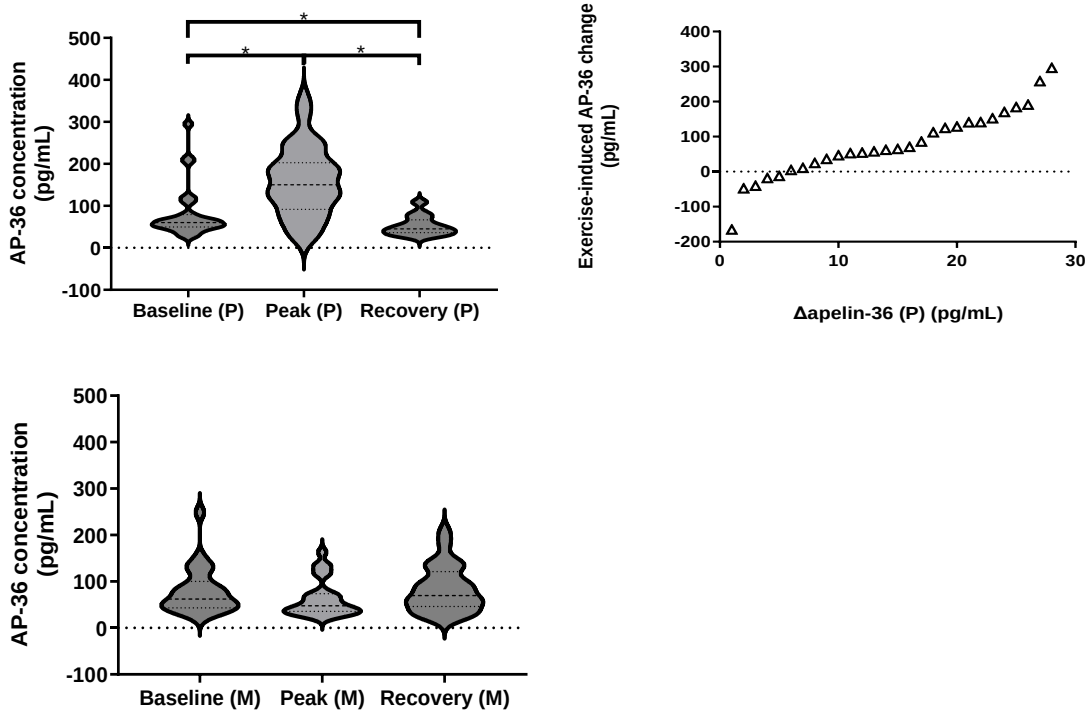


Figure 6. (A) Violin plots comparing the plasma levels of apelin-36 before (baseline), immediately after (peak), and 30 minutes after (recovery) the vita maxima treadmill test. Medians and the 75th and 25th percentiles are shown within the violin plots. Data were analyzed by Friedman test followed by 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.

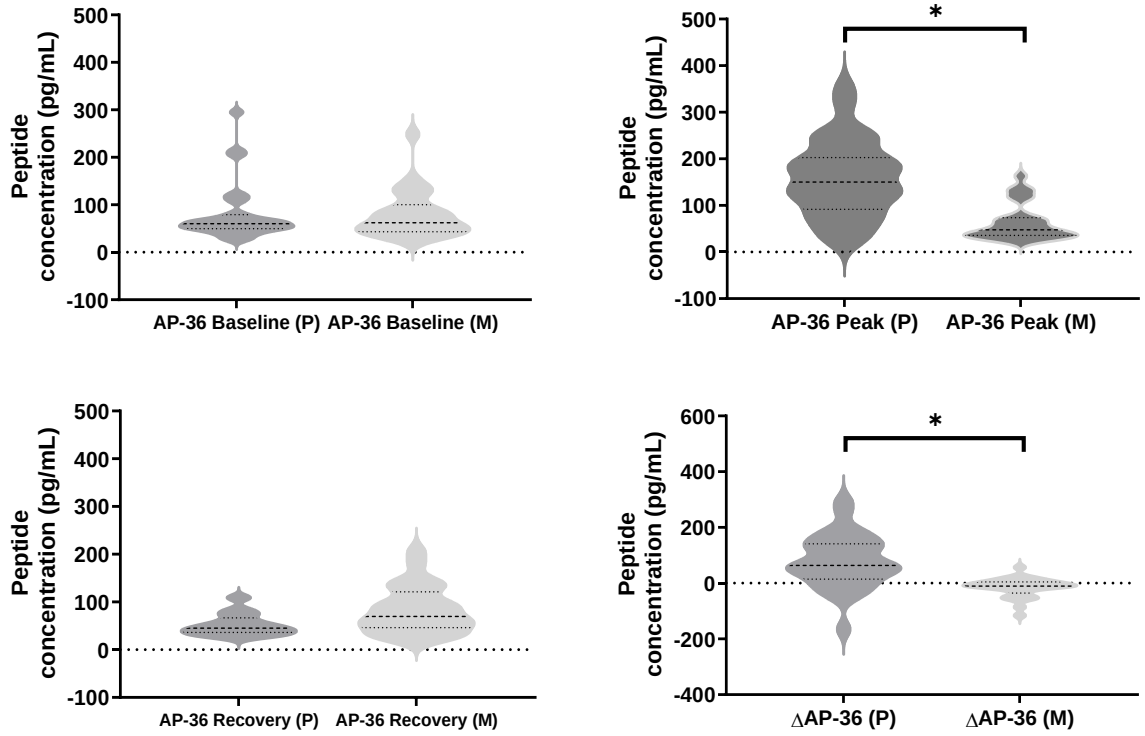


Figure 7. Violin plots comparing the baseline (A), peak (B), recovery (C), and Δapelin-36 (D) levels between the 2 loads. Medians and the 75th and 25th percentiles are shown within the violin plots. Data were analyzed by Wilcoxon test. * $p < 0.05$.

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).

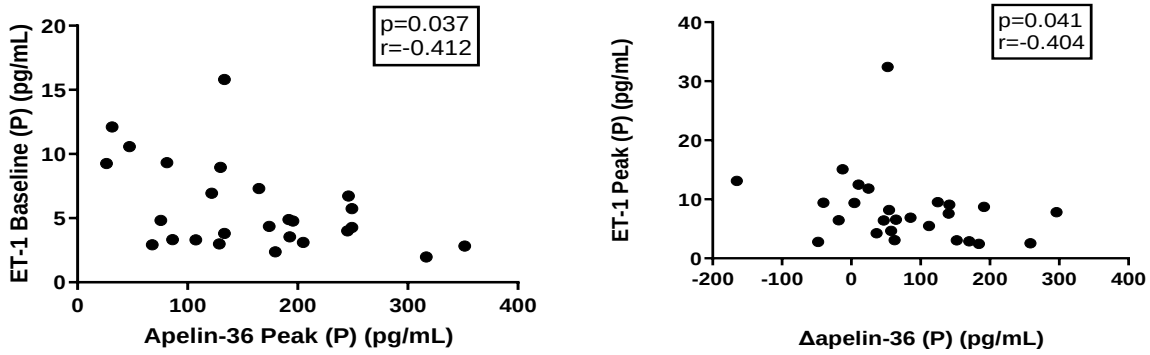


Figure 8. Correlation of apelin-36 peak and ET-1 baseline (A); and Δapelin-36 and ET-1 peak (B) in the physical load. Data were analyzed by Spearman correlation.

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

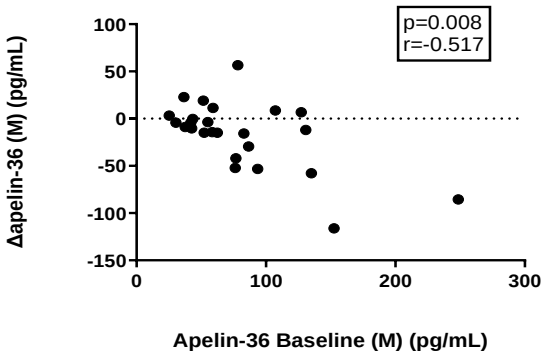


Figure 9. Correlation of apelin-36 baseline and Δapelin-36 in the mental load. Data were analyzed by Spearman correlation.

4.4 Endothelin-1 response to physical and mental load

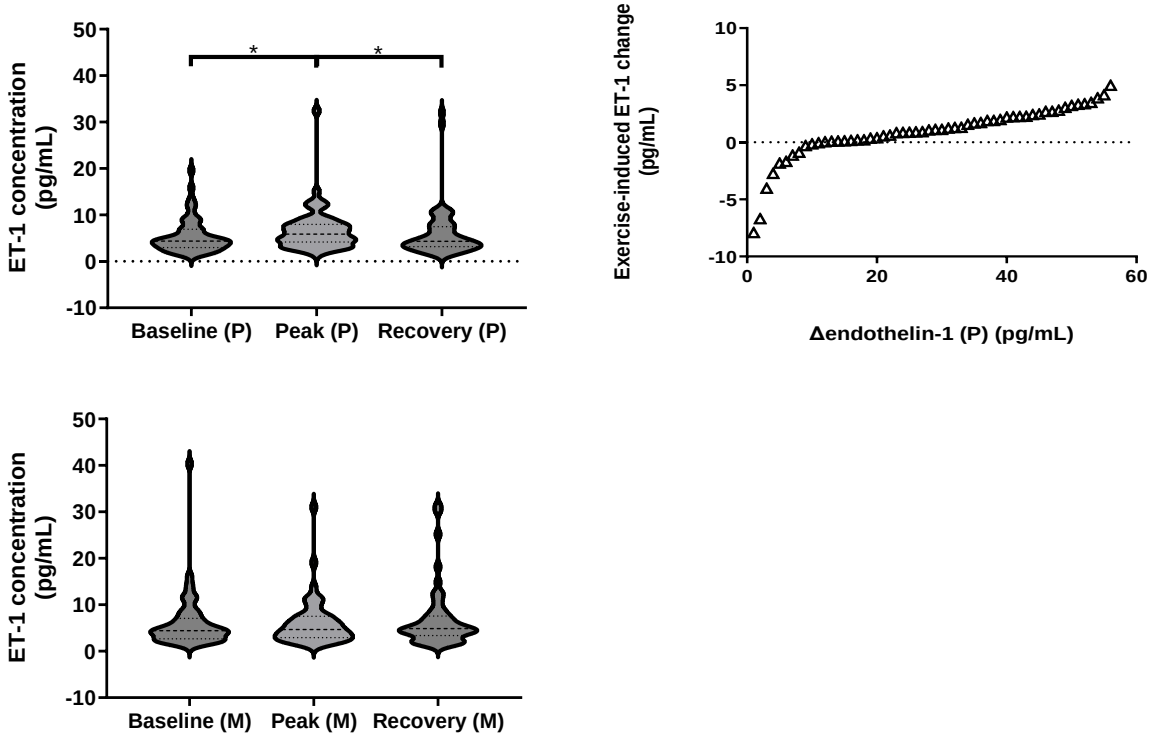


Figure 10. (A) Violin plots comparing the plasma levels of endothelin-1 before (baseline), immediately after (peak), and 30 minutes after (recovery) the vita maxima treadmill test. Medians and the 75th and 25th percentiles are shown within the violin plots. Data were analyzed by Friedman test followed 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.

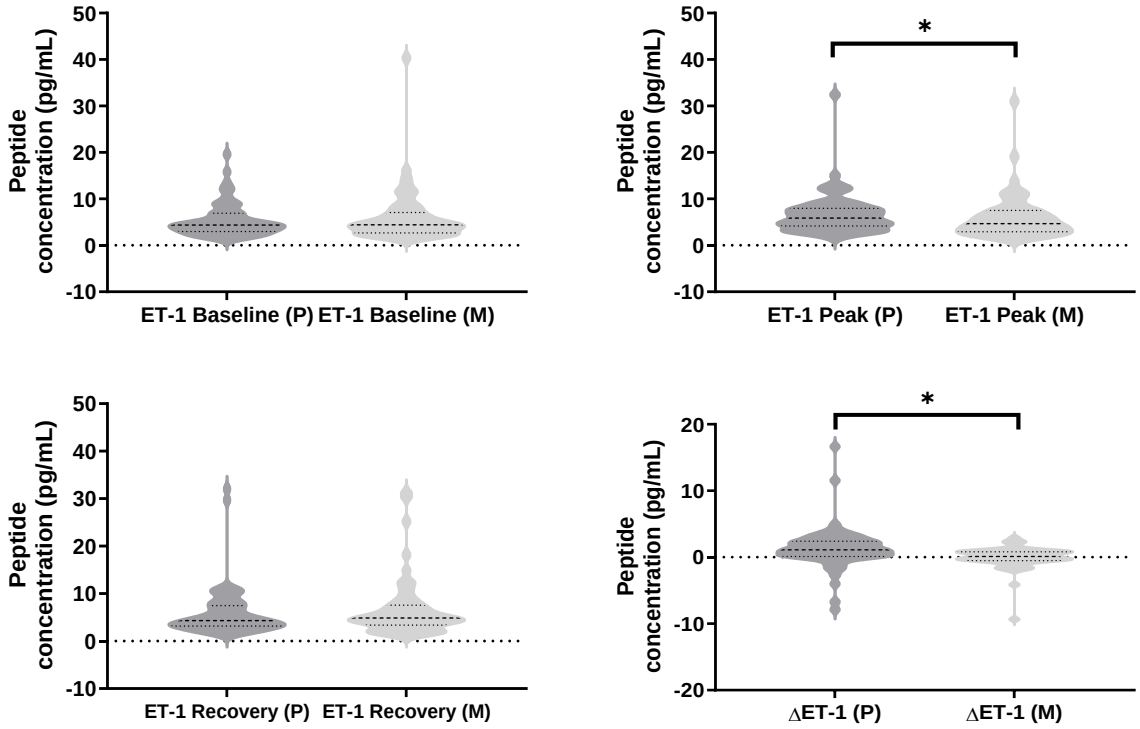


Figure 11. Violin plots comparing the baseline (A), peak (B), recovery (C), and Δendothelin-1 (D) levels between the 2 loads. Medians and the 75th and 25th percentiles are shown within the violin plots. Data were analyzed by Wilcoxon test. * $p < 0.05$.

4.5 NT-proBNP response to physical and mental load

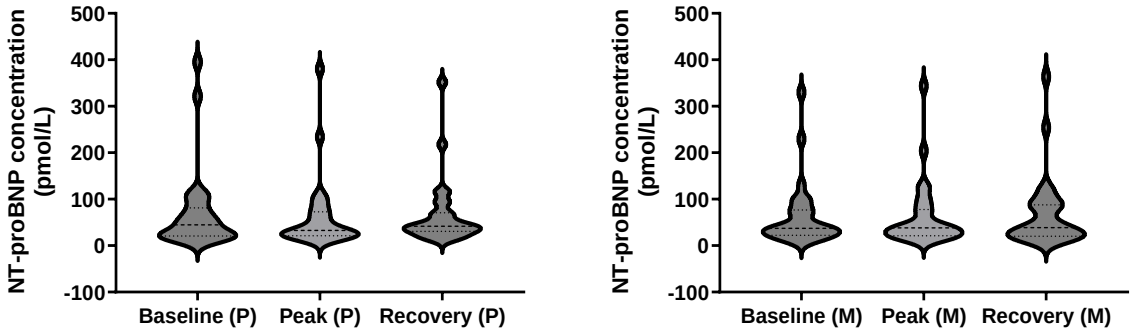


Figure 12. (A) Violin plots comparing the plasma levels of NT-proBNP before (baseline), immediately after (peak), and 30 minutes after (recovery) the vita maxima treadmill test. Medians and the 75th and 25th percentiles are shown within the violin plots. Data were analyzed by Friedman test followed by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

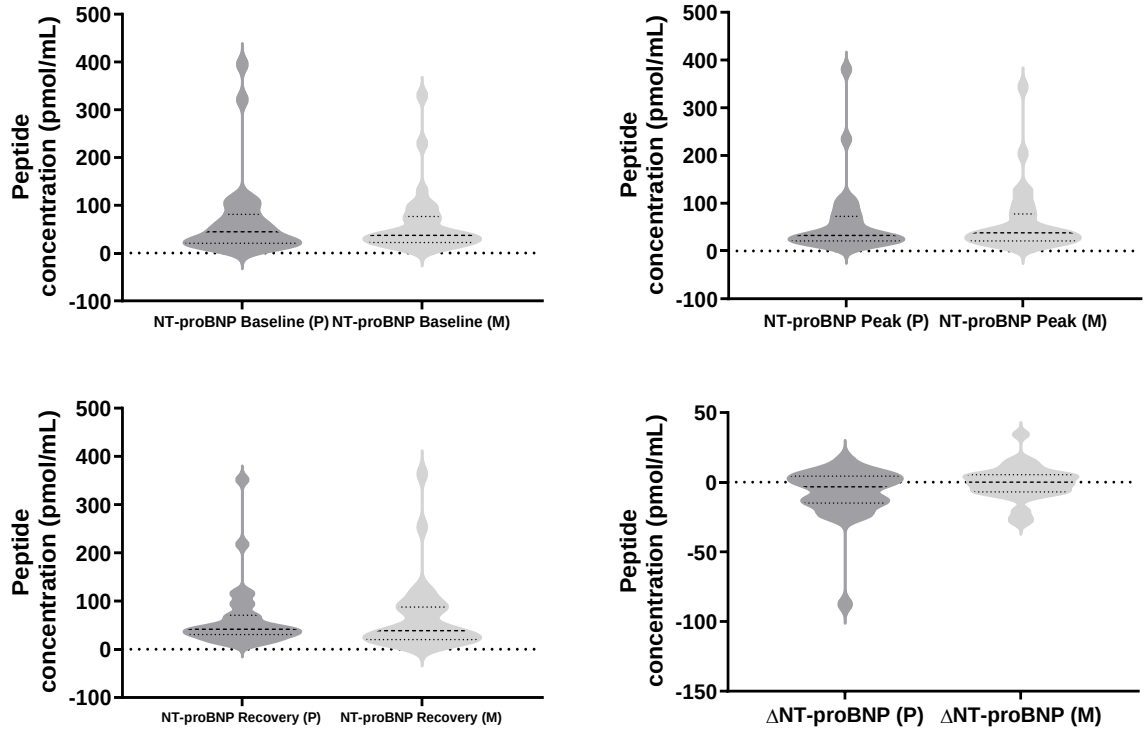


Figure 13. Violin plots comparing the baseline (A), peak (B), recovery (C), and ΔNT-proBNP (D) levels between the 2 loads. Medians and the 75th and 25th percentiles are shown within the violin plots. Data were analyzed by Wilcoxon test.

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).

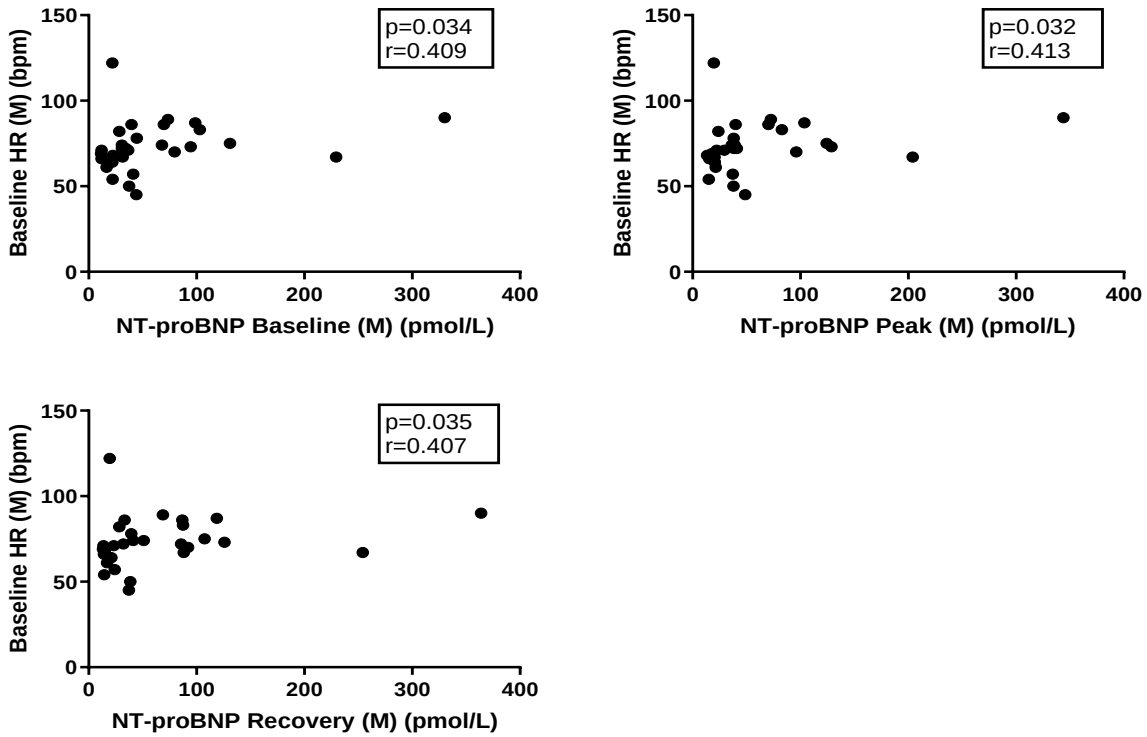


Figure 14. Correlation of baseline HR and NT-proBPN baseline (A), peak (B), and recovery (C) in the mental load. Data were analyzed by Spearman correlation.

5 Discussion

Professional athletes face intense physical and mental loads to achieve the best individual performance. Our study on soccer players explored changes in apelin-13, apelin-36, endothelin-1 (ET-1), and NT-proBNP levels during extreme physical and mental stress, revealing potential roles as exerkines in Hungarian athletes.

Exercise induces exerkine production, influencing exercise adaptations locally and systemically. Skeletal muscle, especially, plays a key role in apelin production. We showed that apelin-13 exhibited a transient increase after a single bout of physical exercise, varying among participants. In contrast, mental stress showed no significant change, evident in the difference between physical and mental peak apelin-13 levels; however, both loads resulted in decreased apelin-13 levels 30 minutes after peak values.

Conflicting literature reports on apelin response to exercise highlight variability due to differing exercise types, intensities or durations. The interindividual variability of apelin-13 responses to

physical exercise might provide an explanation for these controversies about exercise-induced apelin release. Interestingly, higher systolic blood pressure before physical load correlated with a robust apelin-13 response, contrary to mental stress. Baseline apelin-13 levels predicted robust changes in both loads. Apelin isoforms showed an inverse relationship; lower apelin-13 baseline, peak, and recovery levels correlated with higher apelin-36 responses and vice versa.

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. Cardiopulmonary exercise parameters (max MET, relative VO_2max, circulatory power) correlated positively with apelin-13, suggesting its role in peak performance.

Endothelin-1 (ET-1) levels increased upon physical load but remained unchanged during mental stress. ET-1 and apelin, both potent inotropic agents, demonstrated opposite effects on the vasculature. The interplay between vasoconstrictor (ET-1) and vasodilator (apelin) effects, connected to NO-dependent mechanisms, suggests a fine balance during exercise-induced hyperemia.

NT-proBNP levels remained unchanged upon maximal exercise, aligning with the literature. In mental stress, baseline heart rate correlated with NT-proBNP.

In summary, 3 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

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8 List of Publications

Publications cited in the thesis

Ligetvári, Roland ; Szokodi, István ; Far, Gabriella ; Csöndör, Éva ; Móra, Ákos ; Komka, Zsolt ; Tóth, Miklós ✉ ; Oláh, András ; Ács, Pongrác Apelin as a Potential Regulator of Peak Athletic Performance INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES 24 : 9 Paper: 8195 , 15 p. (2023) **(IF: 5.600)**

Csöndör, Éva ✉ ; Karvaly, Gellért ; Ligetvári, Roland ; Kovács, Krisztián ; Komka, Zsolt ; Móra, Ákos ; Stromájer-Rácz, Tímea ; Oláh, András ; Tóth, Miklós ; Ács, Pongrác Adrenal, Gonadal and Peripherally Steroid Changes in Response to Extreme Physical Stress for Characterizing Load Capacity in Athletes METABOLITES 12 : 2 Paper: 91 , 11 p. (2022) **(IF: 4.100)**

Móra, Ákos ; Komka, Zsolt ; Végh, József ; Farkas, István ; Kocsisné Szilágyi, Gyöngyi ; Bosnyák, Edit ; Szmodis, Márta ; Ligetvári, Roland ; Csöndör, Éva ; Almási, Gábor et al. Comparison of the Cardiovascular Effects of Extreme Psychological and Physical Stress Tests in Male Soccer Players INTERNATIONAL JOURNAL OF ENVIRONMENTAL RESEARCH AND PUBLIC HEALTH 19 : 2 Paper: 715 , 12 p. (2022) **(IF: 4.614)**

Publications and presentations related to the thesis

Ligetvári, R ; Ács, P ; Far, G ; Móra, Á ; Csöndör, É ; Komka, Zs ; Szokodi, I ; Tóth, M ; Stromájer-Rácz, T ; Betlehem, J et al. Differential Apelin Responses To Physical Stress In Elite Hungarian Athletes MEDICINE AND SCIENCE IN SPORTS AND EXERCISE 52 : 5 suppl pp. S558-S558. (2020)

Móra, Ákos ; Komka, Zsolt ; Végh, József ; Farkas, István ; Ligetvári, Roland ; Csöndör, Éva ; Far, Gabriella ; Almási, Gábor ; Ács, Pongrác ; Tóth, Miklós et al. A szívfrekvencia-változékonyság és az Endothelin-1 peptid szintjének változása extrém fizikai terhelés hatására MAGYAR SPORTTUDOMÁNYI SZEMLE 21 : 85 (3) pp. 72-72. , 1 p. (2020)

Ligetvári, R. ; Ács, P. ; Far, G. ; Farkas, I. ; Végh, J. ; Szilágyi, G. ; Csöndör, É. ; Komka, Z. ; Szokodi, I. ; Tóth, M.V. et al. Increased levels of plasma Endothelin-1 (ET-1) in response to acute extreme physical but not to mental stress with preserved left ventricular function in male hungarian

athletes In: Bunc, V.; Tsolakidis, E. (szerk.) 24th Annual Congress of the EUROPEAN COLLEGE OF SPORT SCIENCE - BOOK OF ABSTRACTS Köln, Németország : European College of Sport Science (2019) 847 p. pp. 360-360. , 1 p.

Ligetvári, Roland ; Ács, Pongrác ; Far, Gabriella ; Szilágyi, Gyöngyi ; Csöndör, Éva ; Komka, Zsolt ; Szokodi, István ; Tóth, Miklós Viktor ; Tóth, Miklós ; Stromájer-, Rácz Tímea et al. Különböző Apelin fragmentumok vizsgálata fizikális terhelés hatására magyar férfi kézilabdázókban MAGYAR SPORTTUDOMÁNYI SZEMLE 20 : 5 (82) p. 57 (2019)

Ligetvári, Roland ; Ács, Pongrác ; Far, Gabriella ; Farkas, István ; Végh, József ; Szilágyi, Gyöngyi ; Csöndör, Éva ; Komka, Zsolt ; Szokodi, István ; Tóth, Miklós Viktor et al. Magyar férfi kézilabdázók plazma Apelin-13 szintjének vizsgálata akut fizikális és pszichés terhelés hatására MAGYAR SPORTTUDOMÁNYI SZEMLE 20 : 2 (79) p. 62 (2019)

Roland, Ligetvari ; Pongrac, Acs ; Gabriella, Far ; Eva, Csondor ; Zsolt, Komka ; Istvan, Szokodi ; Miklos, Viktor Toth ; Miklos, Tooth ; Timea, Stromajer-Racz ; Jozsef, Betlehem et al. Increased levels of serum endothelin-1 in response to acute extreme physical stress with preserved left ventricular function in male Hungarian athletes BMC SPORTS SCIENCE MEDICINE AND REHABILITATION 11 : S1 pp. 32-32. , 1 p. (2019)

Ligetvári, Roland ; Ács, Pongrác ; Far, Gabriella ; Farkas, István ; Végh, József ; Szilágyi, Gyöngyi ; Csöndör, Éva ; Komka, Zsolt ; Szokodi, István ; Tóth, Miklós Viktor et al.
Magyar férfi kézilabdázók plazma Endothelin-1 (ET-1) szintjének változása fizikális és pszichés terhelés során
MAGYAR SPORTTUDOMÁNYI SZEMLE 19 : 5 (77) pp. 68-69. , 2 p. (2018)

Ligetvári, Roland ; Ács, Pongrác ; Far, Gabriella ; Farkas, István ; Végh, József ; Szilágyi, Gyöngyi ; Csöndör, Éva ; Komka, Zsolt ; Szokodi, István ; Tóth, Miklós Viktor et al. Fizikális terhelésre adott plazma endothelin-1 (ET-1) szint emelkedés megtartott bal kamrai funkcióval magyar férfi sportolókban MAGYAR SPORTTUDOMÁNYI SZEMLE 19 : 75 pp. 61-61. , 1 p. (2018)