The Effect of Several Decades of Physical Exercise on the Content of Extracellular Vesicles Isolated from Human Blood

Abstract of PhD Thesis

Bernadett György

Doctoral School of Sport Sciences
Hungarian University of Sports Science

Supervisor: Dr. Erika Koltai senior research fellow, PhD

Official reviewers: Dr. Éva Martos honorary university professor, CSc
Dr. Ádám Vannay senior research fellow, PhD

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„I was taught that the way of progress is neither swift nor easy.”

Marie Curie

1. Introduction

Extracellular vesicles (EVs) play a key role in intercellular communication and have therefore received increasing attention in health science research. Their functions range from maintaining healthy physiological processes to the development of diseases, and their characteristics vary depending on the cell type, enabling their use in diagnostic and therapeutic applications. The aim of this dissertation is to present two main areas of EV research in human samples: firstly, the methodological aspects of EV isolation and characterization; and secondly, the effects of physical exercise on the molecular composition and physiological roles of EVs.

1.1. General Characteristics and Types of Extracellular Vesicles

EVs are small, membrane-bound particles released by cells into their surrounding environment. These vesicles play an important role in intercellular communication by transporting various biologically active molecules, such as proteins, nucleic acids (including mRNA and microRNA), lipids, and other molecules. They are found in significant quantities in various biological fluids, including blood, saliva, urine, and breast milk.

Based on their biogenesis, EVs can be classified into two main types: exosomes, which originate from the endosomal pathway, and ectosomes, which are directly shed from the plasma membrane. In terms of size, EVs can be categorized as small EVs (sEVs, 50–150 nm), medium EVs (mEVs, 200–800 nm), or large EVs (≥1000 nm).

Nearly all cell types are capable of releasing EVs, and their composition greatly depends on the cell type, its current state, and external environmental influences. Consequently, EVs carry information about the releasing cells, making them particularly valuable for diagnostic purposes and early disease detection.

1.2. The Relationship Between Extracellular Vesicles and Physical Exercise

In recent years, studies have shown that, similar to other cells, skeletal muscle is capable of releasing EVs into the circulation in response to exercise. Frühbeis and colleagues were the first to investigate the levels and properties of small EVs (sEVs) in the bloodstream following exercise. Using cycling and running protocols, they demonstrated that the increased EV levels during exercise reflect the physiological activation of the body. They hypothesized that these vesicles contribute to the regulation of adaptation processes related to physical activity through their role in signal transduction.

In a review study, Safdar and co-authors explored the potential of exosomes released during endurance exercise in the treatment of metabolic diseases. Whitham and colleagues characterized exercise-induced EV proteins using quantitative proteomics and intravital imaging techniques, identifying several novel myokine candidates that enter the circulation via non-classical secretion pathways.

Physical exercise influences both the quantity and composition of extracellular vesicles, suggesting that they may serve as important mediators of the beneficial physiological effects triggered by physical activity.

1.3. The Effects of Long-Term Physical Exercise on the Body and Aging Processes

There is now substantial evidence supporting the significant positive effects of regular physical exercise on the human body, impacting a wide range of physiological systems, including the cardiovascular system, musculoskeletal system, metabolism, and mental health.

Through its plasticity, skeletal muscle responds rapidly to physical activity and plays a key role in metabolic regulation. The molecular adaptations to exercise, including DNA, non-coding RNA, and histone modifications, regulate gene expression via epigenetic mechanisms. It has been shown that higher levels of physical fitness are associated with a reduced incidence of diseases and a slower rate of aging.

1.4. Epigenetics

The concept of epigenetics was introduced by Waddington to connect genetics and developmental biology. According to Riggs and colleagues — a definition now widely accepted — epigenetics studies mitotically and/or meiotically heritable changes in gene function that do not involve alterations in the DNA sequence. This means that genes are turned on or off not through genetic mutations but via other regulatory mechanisms.

Three fundamental mechanisms are primarily studied in epigenetics: DNA methylation, histone modifications, and non-coding RNAs. The rapid advancement of this field has increased interest in technologies capable of mapping epigenetic marks that influence health and disease.

1.5. DNS methylation

DNA methylation is an epigenetic regulatory mechanism that plays a key role in fine-tuning gene expression without altering the hereditary information encoded in the DNA. It is a fundamental epigenetic modification involving the addition of a methyl group to the 5th carbon atom of the cytosine base in DNA, most commonly at regions where cytosine is followed by a guanine base (these are called CpG dinucleotides). DNA methylation is essential for embryonic development and influences processes such as genomic imprinting, X-chromosome inactivation, and the silencing of repetitive DNA sequences.

1.6. Epigenetic Clocks

Aging, as a complex process, is characterized by various changes at cellular, subcellular, and nuclear levels, one of which is epigenetic aging. As the role of epigenetic changes in aging has gained increasing attention, DNA methylation patterns have become a benchmark for determining biological age, currently referred to as epigenetic clocks.

First-generation epigenetic clocks, such as Horvath's pan-tissue clock and Hannum's blood-based clock, estimate biological age. In contrast, second-generation clocks—like DNAmPhenoAge, DNAmGrimAge, and the longitudinally based DunedinPoAm and DunedinPACE—primarily serve to predict mortality risk.

The DNAmFitAge is an epigenetic clock developed as a biomarker of biological age by integrating physical fitness measures such as VO2max and grip strength. This clock was designed to link epigenetics with fitness, paying particular attention to how fitness influences the aging process.

2. Objective

The aim of our research was to investigate the effects of several decades of physical exercise on small extracellular vesicles (sEVs) circulating in human blood. First, we developed and validated a reliable isolation protocol in accordance with MISEV guidelines for subsequent proteomic analyses. We also examined the differences between sEVs isolated from freshly collected and long-term frozen plasma samples. Using the established method, we analyzed the protein content of isolated sEVs by proteomic techniques in samples from two groups with different fitness levels.

With our research findings, we aimed to expand scientific knowledge in a relatively new field studying the impact of sport and physical activity on extracellular vesicles. Exploring this research direction offers opportunities not only for closer integration of exercise physiology, cell biology, and medical sciences but also for identifying new biomarkers and therapeutic targets.

1. Hypotheses Related to Method Development:

1.1: We hypothesize that thorough pre-analytical examination of samples will aid in selecting the most suitable samples for sEV isolation.

1.2: We hypothesize that a 70 nm pore size column isolates sEVs more efficiently and with less contamination for mass spectrometry analysis compared to a 35 nm pore size column.

1.3: We hypothesize that both platelet-free plasma (PFP) prepared from freshly drawn blood and PFP stored frozen for extended periods are suitable for sEV isolation.

2. Hypotheses Regarding the Relationship Between Fitness Level and Small Extracellular Vesicles (sEVs):

2.1: We hypothesize that proteomic analysis of sEV samples will reveal a correlation between the proteins contained within the vesicles and the rate of epigenetic aging.

2.2: We hypothesize that a significant difference in the quantity of sEVs can be detected depending on the fitness level.

2.3: We hypothesize that significant differences will be observed between protein levels and physiological fitness markers according to fitness level.

3. Methods

3.1. Participants

The study was conducted with ethical approval from the Medical Research Council (25167-6/2019/EÜIG) in accordance with the Declaration of Helsinki. Only healthy volunteers who provided written informed consent participated in the research. The main sampling took place at the 2019 Venice Rowing Masters World Championships, followed by recruitment of the control group in Budapest, with a total of 303 participants. Our goal was to form a physically active, trained group and to recruit a control group of inactive, sedentary individuals of the same age group. Participants were categorized based on their physical fitness according to their VO_2max values. Individuals with estimated VO_2max above the 75th percentile for their age group were classified as High-fit (male n = 93, mean age (years) = 60.0 ± 10.6; female n = 91, mean age (years) = 59.0 ± 10.6), while those below this threshold were designated as moderately low-fit (Med-Low-fit: male n = 50, mean age (years) = 63.0±12.4; female n = 62, mean age

(years) = 63.0±12.5). Prior to the main study, a pilot sampling was conducted in Budapest involving 19 healthy subjects.

3.2. Exercise Physiology, Anthropometric, and Cognitive Assessments

Prior to sample collection, all participants underwent a standardized battery of tests conducted by the same team of specialists. Anthropometric measurements included body height, body weight, and body mass index (BMI). Physical and cognitive assessments consisted of four tests: hand grip strength measurement, maximal jump test, Chester step test (to estimate VO_2max), and a digit span test to evaluate working memory.

3.3. Blood Sampling, Biochemical, and Haematological Parameter Analysis

Blood samples were collected by trained personnel from the participants' antecubital veins into three types of collection tubes (K2-EDTA, ACD-A, and serum tubes). Some of the samples were analyzed within a few hours (e.g., blood count, biochemical parameters), while others were frozen at –80 °C for later analyses (e.g., sEV isolation, DNA methylation). Blood draws were always performed prior to the exercise test to ensure that acute physical activity did not influence the composition of the biological samples.

3.4. Pre-analytical Examination of Platelet-Free Plasma

To ensure the accuracy of vesicle isolation, pre-analytical assessments were conducted to evaluate the quality of platelet-free plasma (PFP). Samples exhibiting visible haemolysis or lipemia were excluded. Haemolyses, icterus, and lipemia were assessed based on absorbance values measured at specific wavelengths, while platelet and haemoglobin levels were determined using a haematology analyzer. Samples with haemoglobin levels exceeding 100 mg/dL, platelet counts above 5 million cells/L, or inappropriate absorbance values were discarded or subjected to further purification through 0.8 μm filtration, after which platelet counts were recommended to be rechecked.

3.5. Steps for Isolation of Small Extracellular Vesicles

First, samples from the pilot study were isolated to optimize the methodological protocol. Subsequently, 40 samples from High-fit and Med-Low-fit participants were isolated using the validated procedure. For each subject, blood was collected into two ACD-A tubes, and each isolation started from 2.5 ml of platelet-free plasma (PFP).

Frozen samples were thawed at room temperature for 1 hour, then diluted to 5 ml with NaCl-Hepes buffer and tandem filtered. A 0.8 µm syringe filter was used to remove larger particles. Next, samples were centrifuged at 18,000g for 20 minutes to obtain a supernatant enriched in sEVs. The supernatant was further purified using a 0.2 µm filter, followed by concentration to 1000–1500 µl using 100 kDa ultrafiltration. Samples were brought up to 1500 µl with buffer and centrifuged at 10,000g for 10 minutes to remove aggregates.

The 1500 µl samples were applied onto the size exclusion chromatography (SEC) column and eluted with NaCl-Hepes buffer. Fraction collection proceeded as follows: 3 ml discard fraction, then 10 fractions of 0.5 ml each. For the 35 nm column, fractions 1–3 were pooled; for the 70 nm column, fractions 2–4 were pooled. These pooled samples were transferred into ultracentrifuge tubes, topped up with NaCl-Hepes buffer, and centrifuged at 100,000g for 60 minutes. The resulting pellet was resuspended in 15–20 µl buffer and stored at –80 °C for mass spectrometry analysis.

3.6. Methods for Validation of the Presence of Small Extracellular Vesicles

The size distribution and concentration of particles were measured using a ZetaView Z nanoparticle tracking analyzer (Particle Metrix GmbH, Inning am Ammersee, Germany) based on the NTA (Nanoparticle Tracking Analysis) method. To determine the size distribution and concentration of sEVs, various dilutions (50–800×) were prepared from SEC fractions (1–8) and pre-ultracentrifugation pooled fractions. Samples with particle concentrations below 1×10^9 particles/ml in the pooled fractions were excluded from the analysis.

A total protein content was measured using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific) at 280 nm wavelength, with NaCl-Hepes buffer as the blank. For pilot samples, measurements were done fraction-wise (fractions 1–10), while for other samples pooled fractions were measured. Each 1.5 µl sample was measured in triplicate. The acceptance criterion for both column types was a protein concentration below 0.75 mg/ml.

In accordance with ISEV recommendations, Western blotting was performed to validate the presence of isolated sEVs by detecting specific marker proteins. After ultracentrifugation, the sEV pellet was resuspended in protease inhibitor-containing lysis buffer, and 15 µl of protein lysate was subjected to electrophoresis. Samples were denatured and run on 8–16% Criterion TGX gels in an ice-cooled running bath. Proteins were transferred onto PVDF membranes at 80 V for 3.5 hours, followed by Sypro Ruby staining of the gel. Membranes were blocked with SuperBlock solution, incubated overnight with primary antibodies, then with HRP-conjugated secondary antibodies, and detected using Clarity Max ECL reagent. Images were captured using the ChemiDoc XRS+ system and analyzed with ImageLab software.

We performed visual detection of sEVs using immunotransmission electron microscopy (TEM) following the protocol by Théry et al., with minor modifications. Samples were applied onto formvar-coated nickel grids and fixed with 4% paraformaldehyde (PFA). To reduce nonspecific binding, grids were blocked with 2% sucrose-PBS solution. Subsequently, samples were incubated overnight with the primary antibody, followed by multiple washes and a 1-hour incubation with the secondary antibody. Post-fixation was carried out using 2% glutaraldehyde. Imaging was performed using a JEOL 1011 TEM. The diameters of the EVs were measured using the ImageJ software.

3.7. Mass spectrometry analyses

The vesicle samples were subjected to freeze-thaw cycles, followed by protein precipitation using ethanol. The proteins were then reduced and alkylated in urea. Digestion was performed using Lys-C and trypsin enzymes. The resulting peptides were

purified on a C18 column and analysed using a Bruker Maxis II QTOF mass spectrometer. Data analysis was carried out with Byonic and MaxQuant software, accepting only high-confidence protein identifications.

3.8. Statistical analysis, EV-Track

Statistical analysis of sEVs was performed using GraphPad Prism 9.4.1 software. Variance between groups was assessed with ANOVA, and correlations were analyzed by Pearson's method (or Spearman's method when appropriate) using R version 4.4.1. Normality and homoscedasticity were tested using the Breusch-Pagan and Lilliefors tests. Multiple testing correction was applied at a false discovery rate (FDR) of 0.05. LFQ values were log2-transformed, and only proteins detected in at least 90% of samples were included in the analysis. Protein enrichment analysis was conducted using the PANTHER classification system and Gene Ontology (GO) annotation with Fisher's exact test and FDR correction.

To enhance the transparency and reproducibility of the EV experiments, methodological data were submitted to the EV-TRACK knowledgebase (http://evtrack.org), which collects information on EV biology and methodology.

4. Results

4.1. Results of the Preanalytical Evaluation of Platelet-Free Plasma

Based on the methodological exclusion criteria, 37 platelet-free plasma (PFP) samples were selected for the pilot studies to develop and validate the methodology. For the functional analyses, according to the preanalytical results, 56 High-fit and 10 Med-Low-fit male samples, as well as 50 High-fit and 30 Med-Low-fit female samples met the criteria. Since the sample size in the male group was insufficient, the study continued with only 40 female samples (20 High-fit and 20 Med-Low-fit). The preanalytical results of both the pilot and functional samples were evaluated together (n = 77).

From the 77 samples, 500 µl was used for preanalytical measurements, during which platelet count was measured using a haematology analyzer (average: 1.00 million cells/l before filtration, then 0 million cells/l after filtration) and haemoglobin levels were assessed. Absorbance was measured at multiple wavelengths using an ELISA reader to exclude interference from lipemia, hemolysis, and icterus. Based on the results, all samples were deemed suitable for sEV isolation.

4.2. Characterization of Small Extracellular Vesicles Isolated Using 35 nm and 70 nm Columns

The sEVs were isolated from 31 PFP samples using 35 nm and 70 nm columns. Among these, 21 samples were simultaneously isolated on both columns, 2 samples only on the 35 nm column, and 8 samples only on the 70 nm column. The latter 10 samples were used for mass spectrometry quantification, while 6 samples were used for comparing fresh and frozen samples.

The morphology and size of the sEVs were examined by TEM (n=8) on samples pooled after ultracentrifugation and SEC. The average diameter of vesicles isolated with the 35 nm column was 98.6 nm, while with the 70 nm column it was 70.8 nm. Differences in vesicle shape were attributed to sample preparation methods. Based on TEM and NTA, three particle populations were distinguished: “plasma lipoprotein particles” (“PLP”), sEV, and mEV.

Mass spectrometry showed that the samples from the 70 nm column contained a higher proportion of “vesicular” proteins (12.31% vs. 8.27%), whereas the 35 nm column retained more plasma-derived “PLP” and immunoglobulin proteins. According to ExoCarta and GO annotation, among a list of 130 specific “vesicular” proteins, the 70 nm column showed a significantly higher detection rate, indicating more efficient and purer sEV isolation. Overall, the 70 nm SEC column proved to be a better choice for isolating specific sEVs, while the samples from the 35 nm column was associated with more plasma protein contamination.

4.3. Characterization of Small Extracellular Vesicles Isolated from Fresh and Frozen Platelet-Free Plasma Using the 70 nm Column

sEVs isolated from fresh and frozen plasma samples (stored at –80 °C for more than 2.5 years) were compared using a 70 nm SEC column. TEM, NTA, and NanoDrop measurements showed no differences between the two groups in terms of particle concentration, size, or protein absorbance. Mass spectrometry analysis identified 35 "vesicular" and 279 "non-vesicular" proteins, with no significant differences observed in the proportion of vesicular proteins between the fresh and frozen samples. Our results indicate that frozen plasma samples can also be reliably used for sEV isolation and proteomic analysis.

4.4. The Effect of Fitness Level on Extracellular Vesicles

We conducted comprehensive assessments in the High-fit and Med-Low-fit groups, including exercise physiology, anthropometric, cognitive, and blood tests, as well as self-reported questionnaires to collect lifestyle and health-related data. In the High-fit group, participants reported an average of 20.3 years and 9.1 hours per week of regular physical activity, compared to 15.1 years and 5.9 hours per week in the Med-Low-fit group. Dietary patterns included vegetarian and gluten-free diets, observed primarily in the High-fit group. As the next step in the data analysis, we examined the proteomic profiles of the sEV samples and compared them with the collected clinical parameters.

4.5. Analysis of the Isolated Small Extracellular Vesicles from a Methodological Suitability Perspective

TEM analysis confirmed the presence of EVs with the typical cup-shaped morphology. The total protein concentration measured with NanoDrop was below the exclusion threshold for all samples, and no significant differences were observed between the groups. Using NTA, we assessed the size distribution and concentration of the sEVs, identifying three slightly overlapping particle populations based on size: PLPs, sEVs, and mEVs. The exclusion criterion for particle concentration was 1×10^9 particles/mL. The

average particle concentration was 5.08 × 10^9 particles/mL in the High-fit group and 3.46 × 10^9 particles/mL in the Med-Low-fit group.

The vesicular markers (CD9, CD81, Alix) were analyzed based on mass spectrometry data relative to the total protein intensity. CD81 was detected in 39 samples, CD9 in 27 samples, and Alix in 37 out of 40 samples. The presence of Apolipoprotein A-II indicates that apolipoproteins may co-isolate with the vesicles. The analysis was carried out in accordance with the MISEV2023 guidelines.

The results of the GO enrichment analysis further confirmed the vesicular nature of the samples, highlighting significant enrichment of GO terms associated with vesicular biological processes, molecular functions, and cellular components.

4.6. Results of Mass Spectrometric Analysis of Proteins Found in Isolated Small Extracellular Vesicles

Based on our results, we generated a heatmap to visualize the correlations between normalized protein mass spectrometry intensities and physiological markers. No significant differences in protein levels were observed between the High-fit and Med-Low-fit groups, nor were there significant correlations between protein levels and fitness indicators (FDR < 0.05). Correlations with memory function, blood lipid levels, and BMI were also not statistically significant.

Among the proteins found in EVs and the aging clocks, DNAmFitAge age acceleration showed a significant association with 10 proteins ($q < 0.05$), including Annexin 2A ($r = 0.61$), Protein S100A9 ($r = 0.55$), as well as various fibrinogen chains and complement proteins. Other aging markers, such as GrimAge and PhenoAge acceleration, did not show significant correlations.

In our analysis, we examined three protein sets: all proteins detected by mass spectrometry (n = 509) to confirm vesicular origin, proteins present in the High-fit group (n = 24), and proteins significantly associated with DNAmFitAge acceleration (n = 10, FDR < 0.05). Enrichment analysis of all proteins confirmed strong microvesicular association, particularly related to blood microparticles and extracellular vesicles.

GO analysis revealed significant enrichment in immune system processes, coagulation, and cell surface adhesion. The High-fit group had 24 unique proteins, whereas the Med-Low-fit group had only 2. Weekly exercise hours positively correlated with coagulation and complement proteins.

5. Conclusions

5.1. Our key findings and the conclusions

Blood samples were processed within 45 minutes, ensuring that the resulting platelet-free plasma (PFP) samples were suitable for both long-term storage and immediate small extracellular vesicle (sEV) isolation. During preanalytical evaluations, inadequate samples were excluded, highlighting the importance of proper sample preparation, especially in the absence of standardized protocols.

For sEV isolation, we tested size exclusion chromatography using 35 nm and 70 nm IZON qEV columns for isolating sEVs from human plasma. Both columns performed effectively; however, the 70 nm column enabled the isolation of a greater number of "vesicular" proteins. Therefore, the 70 nm column was deemed optimal for proteomic analyses.

No significant differences were observed between sEVs isolated from fresh and from PFP samples stored at −80 °C for over 2.5 years using the 70 nm column. This suggests that properly prepared PFP samples remain stable for at least 2.5 years for the purpose of sEV isolation.

Regarding protein quantity, no significant differences were found between the High-fit and Med-Low-fit groups. Similarly, correlations between protein levels and various physiological markers—including memory function, LDL, HDL, and body mass index (BMI)—did not reach statistical significance (FDR < 0.05). While these associations cannot be entirely ruled out, sample size and biological variability may have limited the detectability of such effects.

We found a correlation between the DNAmFitAge epigenetic clock, which measures epigenetic aging, and the protein levels in ten sEV samples, suggesting that exercise-induced EVs may play a role in regulating epigenetic aging.

No associations were observed between vesicular protein content and either the duration of regular physical exercise or basic dietary habits; however, the number of weekly training hours showed positive correlations with Coagulation Factor V, the C4b-binding protein alpha chain, and Complement C1q subunit B.

Alpha-2-antiplasmin (A2AP), a major inhibitor of fibrinolysis, was present in sEVs from 9 out of 20 samples in the high fitness group, but was not detected in the medium-low fitness group.

The SH3BGRL3 protein was detected only in four samples from the high fitness group and was absent in the medium-low fitness samples.

Based on our results, the following conclusions can be drawn regarding the hypotheses established during the development of the methodology:

We hypothesize that thorough pre-analytical examination of samples will aid in selecting the most suitable samples for sEV isolation. **TRUE**

We hypothesize that a 70 nm pore size column isolates sEVs more efficiently and with less contamination for mass spectrometry analysis compared to a 35 nm pore size column. **TRUE**

We hypothesize that both platelet-free plasma (PFP) prepared from freshly drawn blood and PFP stored frozen for extended periods are suitable for sEV isolation. **TRUE**

Based on our results, the following conclusions can be drawn regarding our hypotheses on the relationship between fitness level and small extracellular vesicles (sEVs):

We hypothesize that proteomic analysis of sEV samples will reveal a correlation between the proteins contained within the vesicles and the rate of epigenetic aging. **TRUE**

We hypothesize that a significant difference in the quantity of sEVs can be detected depending on the fitness level. **NOT TRUE**

We hypothesize that significant differences will be observed between protein levels and physiological fitness markers according to fitness level. **NOT TRUE**

5.2. Summary

Recreational physical activities are becoming increasingly popular nowadays, and the beneficial effects of regular exercise on health are often emphasized. Research continuously investigates the role of physical activity in disease prevention and the slowing of aging processes. While it is known that regular exercise positively impacts health, the underlying mechanisms are not yet fully understood. One possible mediator could be extracellular vesicles, which circulate freely in the bloodstream and transport nucleic acids, proteins, and lipids encapsulated in their membranes, potentially contributing to the systemic effects of exercise.

In our research, we aimed to investigate the proteomic differences in small-sized extracellular vesicle-containing samples isolated from individuals of high and moderately low fitness levels. Through this, we hoped to expand the current knowledge on the aforementioned topic. Our first goal was to develop a suitable methodology to isolate high-quality and sufficient quantities of small extracellular vesicles from the blood samples of the subjects.

In our study, we isolated small extracellular vesicles from the blood plasma of healthy subjects using the protocol we developed. We then performed proteomic analysis of the isolated samples and evaluated the data through software.

In conclusion, we found that the proteomic content of extracellular vesicles from groups of different fitness levels correlates with DNAmFitAge acceleration, indicating exercise-induced modulation of epigenetic aging. The specific protein content of these vesicles plays a role in inflammation, immune system regulation, metabolism, and cellular regeneration. Our results suggest that physical condition and DNA methylation based aging modulation are interconnected, and the preventive effects of exercise may be partly mediated by extracellular vesicles.

6. List of own publications

List of publications related to the dissertation:

György B, Szatmári R, Ditrói T, Torma F, Pálóczi K, Balbisi M, Visnovitz T, Koltai E, Nagy P, Buzás EI, Horvath S, Radák Z. (2025) The protein cargo of extracellular vesicles correlates with the epigenetic aging clock of exercise sensitive DNAmFitAge. Biogerontology, 26:35.

György B, Pálóczi K, Balbisi M, Turiák L, Drahos L, Visnovitz T, Koltai E, Radák Z. (2024) Effect of the 35 nm and 70 nm Size Exclusion Chromatography (SEC) Column and Plasma Storage Time on Separated Extracellular Vesicles. Curr Issues Mol Biol, 46:4337-4357.

List of publications independent of the dissertation:

Jokai M, Torma F, McGreevy KM, Koltai E, Bori Z, Babszki G, Bakonyi P, Gombos Z, **Gyorgy B**, Aczel D, Toth L, Osvath P, Fridvalszky M, Teglas T, Posa A, Kujach S, Olek R, Kawamura T, Seki Y, Suzuki K, Tanisawa K, Goto S, Kerepesi C, Boldogh I, Ba X, Davies KJA, Horvath S, Radak Z. (2023) DNA methylation clock DNAmFitAge shows regular exercise is associated with slower aging and systemic adaptation. Geroscience, 45:2805-2817.

Aczel D, **Gyorgy B**, Bakonyi P, BukhAri R, Pinho R, Boldogh I, Yaodong G, Radak Z. (2022) The Systemic Effects of Exercise on the Systemic Effects of Alzheimer's Disease. Antioxidants (Basel), 11:1028.

György B, Torma F, Babszky G, Jókai M, Gombos Z, Bakonyi P, Búzás E, Pálóczi K, Szabó T, Radak Z, Koltai E. (2021) Time frame of the extracellular vesicles release after high intensity exercise. MAGYAR SPORTTUDOMÁNYI SZEMLE, 22:77-81.

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