Investigating molecular mechanisms responsible for the health-promoting effects of regular physical activity

Ph.D. thesis

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1. GENERAL INTRODUCTION

1.1. Literature review

Animal experiments have shown that interventions targeting the core process of aging not only prolong life but prevent age-related diseases also. Most chronic diseases result from an unhealthy lifestyle, so the most effective reduction in morbidity would be interventions from an early age. Research in recent years has shown that regular physical activity (PA) reduce the incidence of cardiovascular disease, malignancies, neurodegenerative conditions, diabetes, osteoporosis, obesity, and premature death.

Our knowledge of the molecular signals caused by exercise and the mechanisms of regular PA preventing or mitigating existing diseases is still very incomplete. However, a better understanding of these biological processes would lead to the development of targeted training programs and pharmacological interventions that "mimic" the positive effects of exercise.

1.2. Combined resistance and aerobic training program

Physical Activity Recommendations (WHO) strongly recommend the inclusion of both aerobic and resistance exercises in training programs to reduce and prevent inactivity-related risk factors, although aerobic and resistance training elicit different biological adaptations. Therefore, special attention needed to the careful planning of combined training programs (CT), for the optimization of the adaptational mechanisms. Although CT has been used in practice for decades, since only few sports are built solely on endurance or resistance exercises, the period of scientific interest is shorter. In reality, however, a combination of strength and endurance is required for optimal performance.

1.2.1. Effect of a combined training program on blood lipids and lipoproteins

Cardiovascular disease is the leading cause of death in developed countries. Both epidemiological and case studies have shown that the LDL-C: HDL-C ratio is a powerful predictor of the risk of coronary heart diseases. It is well known that endurance training improves the lipid profile and reduces the risk of cardiovascular diseases. However, to date, relatively few studies have examined the effects of CT on the fasting lipid profile. Research to date suggests that it may improve dyslipidemia parameters in several populations, which is not surprising considering the positive effects of endurance and resistance training on HDL-C and TG separately. Therefore, it seems reasonable to assume that combination of endurance and strength exercises have additive, positive effects on the improvement of the lipid profile.

1.2.2. Effect of a combined training program on glycemic control

Prevention and treatment of type 2 diabetes and metabolic syndrome, lifestyle changes, including adherence to an appropriate diet and increasing the amount of PA, are considered to be the main non-pharmacological strategies. Because CT combines the benefits of endurance and resistance training, optimizes the metabolic responses of type 2 diabetics. Studies in healthy men have reported greater improvements in fasting glucose after CT than after endurance or resistance training only, suggesting that CT have a significant effect on glucose metabolism even in healthy subjects.

1.2.3. Effect of combined training program on blood pressure and vascular adaptation

High blood pressure is a cardiovascular risk factor associated with changes in the structure and function of the vascular system. Even in the normotensive range, an association exists between the increase in blood pressure and cardiovascular risks, emphasizing that well-suited methods would be needed to prevent and delay the gradual increase in blood pressure throughout life. While endurance exercises have been suggested for antihypertensive treatments, CT is now accepted and also recommended for the prevention and treatment of hypertension. A meta-analysis of 68 studies analyzing the effect of CT on blood pressure showed an overall decrease in systolic blood pressure of 3.2 mmHg and diastolic blood pressure of 2.5 mmHg compared to the untreated control group. However, there is evidence that the sequence of endurance and resistance exercises may influence the degree of vascular adaptation. Okamoto et. al (2007) showed, when CT was initiated with strength exercises, endothelial function improved in young women and men, while no change was observed in the reverse order.

1.2.4. The performance effect of a combined training program

Another meta-analysis has shown that a well-constructed CT program also result in significant VO_2max adaptation. Cardiorespiratory fitness (VO_2max) and muscle strengths are two independent but significant health markers. VO_2max has an inverse association with coronary heart disease, cardiovascular disease, and various cancers. Although the use of different training methods is important, but at the same time, they do not result in the same health-related physiological adaptations. Unfortunately, lack of free time is the major obstacle of regular PA in developed countries, therefore, less time-consuming training methods have recently emerged maximizing the potential health benefits of exercise.

1.3. Regular PA and the immune system

An optimally functioning immune system plays a central role in maintaining health. The immune system does not function in isolation, it is greatly influenced by various external factors, including PA, but the effect of CT on immune processes is less clear. However, several studies have confirmed that a strong association exists between PA levels, immunity, and the incidence of diseases. The effect of lifelong PA on aging of the immune system is largely unknown. Answering this question, a study examined physically active women and men between the ages of 55 and 79 who maintained high levels of PA for most of their lives. Subjects rarely showed physiological changes connected to ageing, such as loss of muscle mass and function (sarcopenia), decreased insulin sensitivity, elevated cholesterol, and hypertension. They also showed little signs of immune sensitivity, mentioning one marker,the decrease in the naive T cell-producing ability of the thymus. Interestingly, the number of naive T cells (RTEs) that recently left the thymus was similar to that in young adults. Another study in healthy men aged 18–61 years (n = 102) also showed a positive correlation between VO_2max and naive T cell counts. Moreover, individuals in the highest VO_2max range also had lower levels of senescent CD28-CD57 + CD4 + and CD8 + T cells. Based on these research findings, PA may counteract the mechanisms associated with aging, improving well-being in old age. However, further human intervention studies are needed to confirm the causal relationships.

2. MAJOR AIMS OF THE STUDY

In my thesis, I present the results and discussion of a 6 months long training program consisting of combined resistance and endurance exercises in two separate chapters.

1. Although studies published to date highlighting the main positive effects of regular exercise and the differences between the inactive and active stages, little attention is paid to interindividual differences. Although the effects detected at the group level may not always well reflect adaptation changes at the level of individuals. Therefore, little is known about the effects of long-term regular exercise, especially in combined training programs, in the individual. Thus, we aimed to investigate **the effect of a 3- and 6 months, personalized, combined training program at individual level, on the physiological, metabolic, and immunological parameters of young, healthy, but physically inactive individuals** using different biostatistical methods.

2. Today, our knowledge of the molecular signals and mechanisms through which regular PA can prevent or alleviate existing diseases is still rather incomplete. Therefore, we aimed to investigate **the effect of 0.5 and at least 25 years of regular exercise on the circulating exosomal microRNA (exomiR) profile and to identify the biological pathways regulated by exomiRs.**

3. Identification of high and low responder individuals adapting to combined training using different biostatistical methods

3.1. INTRODUCTION I.

3.1.1. General overview of the high and low responder phenomenon

In the literature exercise sciences, it is conventional to report only the group mean and standard deviation of exercise or training responses, suggesting that the group average is a good representation of the individual responses induced by a training program. In reality, however, it is more common that participants show a wide range of training responses rather than similar ones. Therefore, the effects detected at the group level do not necessarily show changes at the individual level. This phenomenon can be observed in almost all sports science researches, only very few studies have addressed the problem so far. Based on literature data, we can distinguish high responders and low responders (HiR and LR), HiRs include individuals who show exceptionally high development in one or more examined parameters. At the other end of the range are LRs, individuals showing exceptionally low response to exercise. This phenomenon may be of particular concern, as some individuals cannot enjoy the health benefits of regular exercise to the same extent as others. Therefore, understanding of the HiR and LR phenomenon would provide a deeper insight into the mechanisms of exercise adaptation and exercise-induced prevention in the future. A deeper understanding of non-genetically determining factors of exercise responses would lead to manageable protocols for optimal exercise in the future.
Based on currently available research, the following factors may have a significant impact on the development of interindividual differences in training responses:

3.1.2. Genotype, genetic potential

3.1.3. Factors influencing training adaptation

3.1.4. Peculiarities of the applied training program

3.2. MATERIALS AND METHODS I.

3.2.1. Research participants

We recruited 25 participants (male: n = 3 and female: n = 22) for our lifestyle change group. Participants were healthy but physically inactive young (age: 25 years ± 4.12) students who had not exercised regularly in the 6 months before the program. Participants in the lifestyle change program had 3 workouts per week for 6 months. Our regular exercise program was completed by 20 individuals, based on attending at least 85% of the training sessions. Finally, data from 14 participants (male: n = 2; age: 23±1.98 and female: n = 12; age: 25±3.73) were used for HiR and LR statistical analyzes.

The following protocol was used:

3.2.2. 6 months combined training program

3.2.3. Study design

3.2.4. Six-minute walking test (6MWT)

3.2.5. Examination of physical activity pattern with Actigraph device

3.2.6 Heart rate monitoring with Polar Team System

3.2.7. Collection of blood samples

3.2.8. Determination of the absolute copy number of hTREC (T-cell receptor excision circles) using a digital PCR instrument

3.2.9. Separation of high and low-responder individuals

3.2.10. Use of Artificial Neural Networks to evaluate the effectiveness of a lifestyle change program

3.3. RESULTS I.

3.3.1. Physiological parameters

Changes of classical physiological parameters are already well described in the exercise science literature, thus the measurement and evaluation of these parameters (time spent in the sedentary zone, systolic and diastolic blood pressure, 6-minute walking test, and the estimated relVO_2max) helped to validate our training program. Large individual differences were detected for all parameters, so K-means cluster analysis was used to identify two more homogeneous groups. With cluster analysis, it was possible to create significantly separate clusters after both 3 and 6 months of regular exercise.

Time spent in the sedentary (inactive) zone was recorded for 3×7 days with an Actigraph device. Our main goal was to detect interindividual differences independent of the program. Large individual differences allowed the separation of higher (HPA) and lower PA (LPA) clusters. Both at mid-term (3 months) and after 6 months of regular PA, HPA, and LPA clusters were significantly separated ($p<0.001$), while changes at the group level would have hidden the fact of more active and less active individuals.

Although both systolic (SBP) and diastolic (DBP) blood pressure decreased, there was no statistically significant change at the group level, presumably due to large differences between individuals. However, with the aid of cluster analysis we identified significantly separable groups in SBP (0-3: $p<0.001$; 0-6: $p<0.001$) and DBP (0-3: $p<0.001$; 0-6: $p<0.01$).

At the group level, there was a significant improvement in the distance covered in the 6MWT at the end of the program ($p=0.001$). Additionally, while an improvement in proportion of HiRs was observed, the mean of absolute changes was smaller.

Since physical performance correlates well with aerobic capacity, relative VO_2max was also evaluated. As expected, the value of VO_2max increased in HiR individuals. The prevalence of HiRs also increased after 6 months, although a decrease of the mean absolute change was measured, indicating that there was less improvement, more participants performed it.

3.3.2. Glucose and lipid metabolism

Despite interindividual differences, at the group level, there was a significant change in fasting insulin (0-3: $p=0.005$; 0-6: $p=0.026$) and glucose levels (0-6: $p=0.012$) after 3 and 6 months of regular PA. Surprisingly, very few study examined the individual differences in glucose, insulin, HDL, and LDL parameters so far. All four metabolic parameters showed significant segregation of HiR and LR individuals, albeit to varying degrees (glucose: 0-3: $p<0.001$; 0-6:

p<0.01); (insulin: 0-3: p<0.01; 0-6: p<0.001); (HDL: 0-3: p<0.01; 0-6: p<0.01); (LDL: 0-3: p <0.001; 0-6: p<0.001).

3.3.3. Parameters in association with the immune system

Based on literature data, long-term regular, moderate-intensity exercise has a positive effect on the immune system due to chronic low-level stress, also called hormesis. Although large individual differences were detected, at the group level, cortisol levels increase significantly at 6 months (p = 0.000). In CRP values, the difference between HiRs and LRs was greater after 3 months than 6 months indicating by the magnitude of the significance level (0-3: p<0.001; 0-6: p<0.05). Elevated cortisol levels are known to suppress lymphocyte counts. These data are also supported by our results, as at 6 months, in addition to the increase of cortisol levels, there was a significant (p=0.015) decrease in lymphocyte number.

Previous research has reported that regular moderate exercise improves the production of naive T cells in the thymus. Therefore, digital PCR measurements were performed. Our goal was to determine the hTREC copy number, as a by-product of the T cell receptor (TcR) gene rearrangement, produced during the maturation of thymocytes in the thymus. Our data also support that moderate regular exercise enhances thymic function (fresh naive T cell production), as a significant proportion of subjects showed an increase in hTREC copy number.

3.3.4. Artificial neural network (ANN) analysis

Subsequently, ANN analysis was used to determine the correlation patterns between each parameter. One physiological parameter (sedentary state = length of time spent in an inactive state in minutes), one metabolic parameter (insulin), and one molecular immunological parameter (hTREC copy number) showed the strongest correlation with the other analyzed parameters, so these three parameters were called as mastermind parameters.

3.4. DISCUSSION I.

6 months regular PA resulted in improvements in several health-related parameters and physical performance, i.e., decreased time spent in the sedentary zone (Δ: -677 min), SBP (Δ: -6.8 mmHg), and DBP (Δ: -1.8 mmHg), fasting glucose (Δ: -1.08 mmol/l), LDL (Δ: -0.02 mmol/l), CRP (Δ: -0.57 mg/l), lymphocyte count (Δ: -0.66), while there was an increase in 6MWD (Δ: +60.7 m), relVO_2max (Δ: 3.7 ml/kg/min), fasting insulin levels (Δ: +23.7 pmol/l), HDL (Δ: +0.15 mmol/l), cortisol (Δ: +180.5 mmol/l) and hTREC (Δ: +3.58 copies/µl). These results are consistent with many meta-analyses showing that CT interventions have positive effects on health at multiple levels, in a time-efficient manner. However, in addition to positive group responses, significant individual differences were also observed. Our results support that although HiR and LR individuals can be distinguished, this classification is only suitable for a single parameter and for a specific training protocol. Consistent with our results, Barbelho et. al (2017) showed that each participant showed improvement in at least one studied variable during a 12-week resistance training program. In addition, for several health-related parameters, we observed a decrease in the number of individuals with adverse reactions over time. In this regard, Ross et. al (2015) have also shown that by gradually increasing training intensity, the frequency of LRs decreases over time until it finally disappears. Individual differences in glucose, cortisol, CRP, lymphocyte count and hTREC parameters have not been previously studied. However, our ANN analysis results suggest that the amount of time spent in the sedentary zone, insulin and hTREC variables may have been important determinants of responses to regular exercise. Therefore, examining these mastermind parameters in more detail with a larger population would be extremely important in the future.

3.5. CONCLUSION I.

Our results confirmed that CT results in several positive improvements in young adults with a previously sedentary lifestyle. The HiR and LR phenomenon described here are not limited to the examined variables, are ubiquitous, and can be observed in a large proportion of sports science research. There is evidence showing the significant heterogeneity in physiological indices of individual risk factors. The presented data suggest that changes in sedentary time, fasting insulin, and hTREC levels may have a significant effect on individual responses to regular exercise, so a detailed examination of these parameters would be extremely important in the future.

4. Effect of short- and long-term physical activity on circulating exosomal miRNA profile

4.1. INTRODUCTION II.

4.1.1. Relationship between exercise and extracellular vesicles

Nowadays, besides myokine theory, more and more research supports the role of extracellular vesicles (EVs), especially exosomes, in intercellular communication during exercise. EVs are membrane-surrounded structures released by most cell types. Based on their origin, size, and protein content, 3 populations are distinguished: exosomes (50–150 nm), microvesicles (150–1000 nm), and apoptotic bodies (800–5000 nm). The population of exosomes is currently well-studied and characterized. Exosomes are released into the extracellular space by the fusion of multivesicular bodies with the plasma membrane. As they bind to the plasma membrane of the recipient cell, their contents RNA, DNA, protein molecules, and metabolites (although their composition also strongly depends on the type of the stimulation) are released into the cytoplasm of the recipient cell, affecting its function. Exosomes also carry endosomal markers (e.g., CD9, CD63, CD81, Tsg101) according to their origin, so they are identified relatively easily. Studies available to date suggest that EVs may be important mediators of systemic exercise adaptation and excellent targets for the exploration of circulating factors underlying exercise-induced health benefits. Among molecules transported by exosomes, miRNAs are small, non-coding RNAs that can be important regulators of gene expression through transcript degradation and inhibition of translation, as well as long-term signaling molecules. They are able to change gene expression under physiological and pathological stress, making them effective in the development of diseases. Given that circulating exosomes reflect the physiological and pathophysiological states of the body and are in the range of ~10^{10} particles/ml, there is a great need to explore the functional effects of exosomes released during exercise. Consequently, further research with different training modalities and durations is needed to a deeper understanding of the role of exosomes and their “cargo”, especially miRNAs, in the prevention of chronic diseases. The importance of this is shown by the National Institute of Health establishing a Common Fund Program to gain a deeper understanding of the molecular transducers behind the health benefits of PA.

4.2. MATERIALS AND METHODS II.

4.2.1. Research participants

Participants in the present study were healthy but physically inactive young individuals (n = 14; age: 23±2 years) and trained seniors (n = 11; age: 62±6 years). Participants had no known chronic disease (e.g., metabolic disorder, cardiovascular disease, cancer, etc.). The young group performed moderate-intensity, combined training three times a week for half a year (0.5 year) under the supervision of a personal trainer. Trained, senior subjects have been exercising and playing sports regularly for at least 25 years.

The following protocol was used:

4.2.2. Description of training protocols used

4.2.3. Collection and preparation of blood serum

4.2.4. Isolation of exosomes from serum

4.2.5. Nanoparticle Tracking Analysis (NTA) measurement with Nanosight NS300

4.2.6. Immunofluorescence staining (CD63, CD81)

4.2.7. Transmission Electron Microscopy (TEM)

4.2.8. exomiR expression analysis

4.2.9. Statistical analysis

4.2.10. miRNA target gene and pathway analysis

4.3. RESULTS II.

4.3.1. Anthropometric and physiological parameters

After 6 months of regular exercise, previously inactive young participants showed significant improvements in their VO_2max, glucose and lipid parameters. All of the physiological parameters of the seniors examined in the study were within the normal range, and their VO_2max values were much better than the age-appropriate reference range (relVO_2max: 26–28 ml/kg/min).

4.3.2. Validation of exosomes isolated from serum

Serum-enriched exosomes were first characterized by TEM. TEM analysis showed a characteristic exosomal morphology in the isolated fraction. NTA analysis allowed the determination of size distribution and concentration. The mean diameter of the isolated particles (n = 9) was 143.2±16.43 nm, which is in the size range of the exosomes. Particle concentrations

(n = 9) ranged from 1.97×10^{10} to 3.75×10^{10} particles/ml in each isolate. In addition, labeling of the isolated particles with fluorescent antibodies confirmed the presence of exosome marker proteins. The FM: LSM percentage was 83.87% for CD63 and 76.95% for CD81.

4.3.3. exomiR expression pattern after 0.5 year of regular exercise

Amplification-free Nanostring technology was used to study changes in the expression of serum exomiRs. After analysis and normalization of the raw data, a total of 54 exomiRs were identified. Significant differences in exomiR abundance were observed for several exomiRs (let-7a-5p, $p<0.05$; let-7g-5p, $p<0.05$; miR-130a-3p, FDR<0.05; miR-142 - 3p, $p<0.05$, miR-150-5p, $p<0.05$, miR-15a-5p, $p<0.05$, miR-15b-5p, FDR<0.05, miR-199a-3p , FDR<0.05; miR-199b-3p, FDR<0.05; miR-223-3p, FDR<0.05; miR-23a-3p, FDR<0.05; miR-451a-3p, FDR<0.05; miR-126-3p, $p<0.05$; miR-199-5p, $p<0.05$; miR-21-5p, FDR<0.05; miR-25-3p, $p<0.05$; miR-374a-5p, $p<0.05$) (ArrayExpress accession number: E-MTAB-10067).

4.3.4. ExomiR overlaps between the 0.5 year and 25+ years active groups

Then, we wanted to investigate whether exomiRs expressed differently in the young group after 0.5 year of regular exercise are similarly regulated in the senior group. For this purpose, a hierarchical cluster analysis was performed. The exomiR expression profile of the 0.5 year and 25+-year groups was completely different from the inactive state. However, the young active and the senior groups showed very similar exomiR expression patterns. 12 overlapping exomiRs (let-7a-5p; let-7g-5p; miR-130a-3p; miR-142-3p; miR-150-5p; miR-15a-5p; miR-15b-5p; miR-199a-3p; miR-199b-3p; miR-223-3p; miR-23a-3p and miR-451a-3p) were identified (baseline vs. 0.5 year and baseline vs. 25+ years).

4.3.5. Pathway analysis

To better understand how the 12 identified exomiRs may contribute to the health benefits of exercise, pathway analysis was performed. The KEGG database was used to explore the targeted genes and pathways by each exomiR. A total of 38 significantly targeted KEGG signaling pathways were identified. Most of the targeted mRNAs (148 genes) were located in the “pathways in cancer” pathways. A four of the 12 exomiRs (let-7a-5p; let-7g-5p; miR-15b-5p; miR-23a-3p) targeted 148 genes.

4.4. DISCUSSION II.

In the present study, we were the first to investigate the effect of short-term (0.5 year) and long-term (at least 25 years) regular exercise on the circulating exomiR profile using an amplification-free Nanostring platform. Most studies to date have focused primarily on the characterization of exosomes released during acute exercise and focused on the characterization of transported cargo using amplification based technologies. The method used in the present study is not only amplification-free, but also sensitive, robust, and easy to reproduce. Our analysis has revealed a significant number of differently expressed exomiRs between groups. A total of 12 exomiRs were detected that were regulated differently ($p < 0.05$) compared to the inactive state in both active groups (0.5 year and 25+ years). KEGG pathway analysis of exomiRs confirmed their important involvement in the regulation of TGF-beta, p53, and mTOR signal transduction pathways. Our observations are supported by the fact that regular PA reduces the risk of cancer. Furthermore, the overall incidence of cancer is significantly lower among athletes than in the average population. To date, the expression of the identified 12 exomiRs have been examined in the context of many chronic diseases, greatly helping us to draw our conclusions. For example, elevated expression of miR-23a, miR-451a, miR-223-3p, and miR-150-5p has already been detected in the sera of several different types of cancer patients (breast, stomach, pancreas, lung carcinoma). Furthermore, recent research has also confirmed that these are transported primarily as exosomal cargo. It is important to note that 5 of the 12 (let-7g-5p; miR-15a-5p, miR-199b-3p, miR-223-3p, miR-23a-3p) were reported by Rani et. al (2017) that their exosomal abundance positively correlated with age and it is well known that the cancers described above occur primarily in older age. Both 0.5 year and lifelong exercise appears to reduce the abundance of age- and cancer-related exomiRs. However, study examining the role of exercise-regulated exomiRs in regulating cancer is still at a relatively early stage, therefore intensive efforts are needed in this area of science.

4.5. CONCLUSION II.

Both short-term (0.5 year) and long-term (over 25 years) exercise significantly altered the serum exosomal miRNA profile, potentially reducing the risk of many cancers, metabolic, and neurodegenerative diseases. The combination of an amplification-free Nanostring platform and bioinformatics analysis has been used to identify miRNAs transported in exosomes. They have been well characterized in the literature earlier and show inverse regulation in chronic diseases upon regular exercise. Their physiological relevance is also supported by a large number of

signaling pathways regulated by miRNAs. In the future, we aim to determine the exact mechanism of action of the identified exomiRs in the context of each chronic disease.

5. SUMMARY OF RESULTS

1. 6 months CT resulted in improvements in several health-related parameters and physical performance in young healthy individuals with a previously sedentary lifestyle.
2. In addition to the positive group responses, we also showed significant individual differences, so examined the responses to CT separately for each studied variable.
3. In contrast to previous studies evaluating and grouping HiR and LR into a single primary variable, we looked at CT responses for each variable separately. Our results support that although HiR and LR individuals can be distinguished, this classification is different for each parameter.
4. We were the first to examine individual differences in glucose, cortisol, CRP, lymphocyte count, and hTREC parameters caused by regular exercise.
5. ANN analysis was used to identify three mastermind parameters (time spent in the sedentary zone, insulin, and hTREC) that may be important determinants of the extent of responses.
6. We have shown that a person who is a HiR in an examined variable might not be a HiR for another variable.
7. For many health-related parameters, we observed a decrease in the number of individuals with adverse reactions.
8. To the best of our knowledge, we were the first to investigate the effect of short-term (0.5 year) and long-term (at least 25 years) regular exercises on the circulating exomiR profile using an amplification-free Nanostring platform.
9. 12 exomiRs were detected, regulated differently ($p < 0.05$) in both active groups (0.5 year and 25+ years) compared to the inactive state.
10. 38 significantly targeted KEGG signaling pathways were identified and most of the targeted mRNAs (148 genes) were in the “pathways in cancer” pathways.
11. The exosome-transported miRNAs identified in this study, based on the literature data, are primarily involved in tumor prevention mechanisms, including tumor suppression, delay of aging processes, induction of apoptosis, and reduction of inflammatory processes.

6. FUTURE PLANS

As genetic factors handle 30–50% of response heterogeneity according to the literature, this suggests that other physiological factors are likely to play a role in diverse responses to PA. However, because of the increasing incidence of illness among young people and adolescents, there is an increasing urgency to understand the impact of each prevention strategy, especially for those who respond well (HiR) to a particular exercise program. Using ANN analysis, we identified three potential mastermind parameters (amount of time spent in the sedentary zone, insulin, and hTREC) that might be important determinants of the extent response to an exercise program. However, it would be important to have a deeper understanding of the contribution of these three parameters in physiological responses. Intensive research is also underway to explain the exact nature of factors released into the circulation by skeletal muscle. Future studies identifying the nature of PA-induced miRNA expression may reveal new mechanisms of tissue communication. exomiRs may also offer the opportunity to customize exercise programs as a prognostic marker. It has now become apparent that several pathways with redundant roles coordinate training adaptation responses. Therefore, studies focusing on a specific molecular target may not be sufficient to fully explain the complex nature of dynamic training responses. Thus, the focus of future research should be understanding biological pathways and their relations to different training responses.

7. LIST OF PUBLICATIONS

7.1 Articles related to this thesis

Garai, K., Adam, Z., Herczeg, R., Katai, E., Nagy, T., Pal, Sz., Gyenesei, A., Pongracz, E. J., Wilhelm, M., Kvell, K. Artificial Neural Network Correlation and Biostatistics Evaluation of Physiological and Molecular Parameters in Healthy Young Individuals Performing Regular Exercise. Frontiers in Physiology 10, (2019). **IF: 3.367**

Garai, K., Adam, Z., Herczeg, R., Banfai, K., Gyebrovszki, A., Gyenesei, A., Pongracz, E. J., Wilhelm, M., Kvell, K. Physical Activity as a Preventive Lifestyle Intervention Acts Through Specific Exosomal miRNA Species — Evidence From Human Short- and Long-Term Pilot Studies. Frontiers in Physiology 12, 1–13 (2021). **IF: 4.566**

7.2 Articles not related to this thesis

Boda, F., Banfai, K., **Garai, K.,** Curticapean, A., Berta, L., Sipos, E., Kvell, K. Effect of Vipera ammodytes ammodytes Snake Venom on the Human Cytokine Network. Toxins, 1– 10. (2018). **IF:3.895**

Banfai, K., **Garai, K.,** Ernszt, D., Pongracz, E. J., Kvell, K. Transgenic exosomes for thymus regeneration. Frontiers in Immunology 10, 1–9 (2019). **IF:5.085**
Banfai, K., Ernszt, D., Pap, A., Bai, P., **Garai, K.,** Djeda, B., Pongracz, E. J, Kvell, K. " Beige " Cross Talk Between the Immune System and Metabolism. Frontiers in Endocrinology 10, 1–16 (2019). **IF:3.644**

Kovács, N. P., Almási, A., **Garai, K.,** Kuzma, M., Vancea, Sz., Fischer, E., Perjesi, P. Investigation of intestinal elimination and biliary excretion of ibuprofen in control and hyperglycemic rats. Canadian Journal of Physiology and Pharmacology, 1-34. (2019). **IF:1.946**

Boda, F., Banfai, K., **Garai, K.,** Kovacs, B., Almasi, A., Scheffer, D., Sinkler, R., Csonka, R., Czomboly, T., Kvell, K. Effect of Bitis gabonica and Dendroaspis angusticeps snake venoms on apoptosis-related genes in human thymic epithelial cells. Journal of Venomous Animals and Toxins including Tropical Diseases, 1-16. (2020). **IF:2.831**

Csenki Z., Garai E., Faisal Z., Csepregi R., **Garai K.,** Sipos DK., Szabó I., Kőszegi T., Czéh Á., Czömpöly T., Kvell K., Poór M. The individual and combined effects of ochratoxin A with citrinin and their metabolites (ochratoxin B, ochratoxin C, and dihydrocitrinone) on 2D/3D cell cultures, and zebrafish embryo models. Food and chemical toxicology, 158 (2021). **IF:6.023**

Cumulative impact factor related to my thesis: 7.933

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Cumulative impact factor**: 31.357**

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Oral presentations related to this thesis:

Garai, K., Adam, Z., Herczeg, R., Banfai, K., Gyebrovszki, A., Gyenesei, A., Pongracz, E. J.,Wilhelm, M., Kvell, K. Serum exosomal miRNA pattern of an active lifestyle, its correlation with anthropometric parameters and role in chronic disease prevention. 11th ISCTICO-HUPHAR- IUPHAR-Coference, Pécs, 2021.

Garai, K., Adam, Z., Temesfoi, V., Wilhelm, M., Kvell, K. The potential use of urine-derived exosomes and microvesicles as a non-invasive tool in sport medicine diagnosis. Interdisciplinary Doctoral Conference, Pécs, 2019.

Ádám, Z., **Garai, K.,** Gyebrovszki, Á., Wilhelm, M., The changes of HRV due to a 6-month long physical activity program in healthy young sedentary women. ECSS, 2019.

Garai, K., Banfai, K., Herczeg, R., Adam, Z., Gyebrovszki, A., Gyenesei, A., Pongracz, E. J., Wilhelm, M., Kvell, K. The potentiol of exercie-derived exosomes to treat chronic diseases. V. International Cholnoky Symposium, Pécs, 2019.

Garai, K., Banfai, K., Gyebrovszki, A., Adam, Z., Katai, E., Nagy, T., Pongracz, E. J., Wilhelm, M., Kvell, K. Exercise alleviates senescence-related parameters in healthy, young individuals. 4th International Cholnoky Symposium, Pécs, 2018.

Garai, K., Wilhelm, M., Kvell, K. Will different levels of physical activity influence the status of the immune system? International Multidisciplinary Conference, Miskolc, 2017

Poster presentations related to this thesis:

Wilhelm, M., Gyebrovszki, A., **Garai, K.,** Adam, Z., Horvath-Szalai, Z., Kvell, K. Heath related fitness and health promotion of young Hungarian adults. EUPHA, 2020.

Garai, K., Gyebrovszki, A., Katai, E., Nagy, T., Pongracz, E. J., Kvell, K., Wilhelm, M. Correlation of exosomal miRNA- and anthropometric profile of an active lifestyle. ISEV 2018, Barcelona, May, 2018.

Garai, K., Banfai, K., Herczeg, R., Adam, Z., Gyebrovszki, A., Katai, E., Nagy, T., Pongracz, E. J., Wilhelm, M., Kvell, K. Circulating exosomal miRNA profile of lifestyle intervention. 47th Annual Meeting of the Hungarian Society for Immunology, Bükfürdő, 2018.

Gyebrovszki, A., **Garai, K.,** Kvell, K., Adam, Z., Wilhelm, M., Benefits of lifestyle-changes in psycho-immuno-and physical functions of university students, FENS 2017.

Oral and poster presentations not related to this thesis:

Banfai, K., Ernszt, D., Pap, A., Bai, P., **Garai, K.,** Belharazem, D., Pongracz JE., Kvell, K. Beige hints of thymic adipose involution. 5th International Cholnoky Symposium. Pécs, 2019.

Banfai, K., **Garai, K.,** Ernszt, D., Pongracz, E. J., Kvell, K., Role of exosomes in thymic regeneration. 47th Annual Meeting of the Hungarian Society for Immunology. Bükfürdő, 2018.

Banfai, K., Ernszt, D., **Garai, K.,** Pongracz, E. J., Kvell, K.Benefit of exosomes in artificially engineered 3D thymus cultures. 4th International Interdisciplinary 3D Conference, Pécs, 2018.

Banfai, K., **Garai, K.,** Ernszt, D., Pongracz, E. J., Kvell, K., Significance of Wnt4-exosomes in thymic senescence. 17th International Summer School on Biocomplexity, Chania, Kréta, 2018.

Banfai, K., **Garai, K.,** Ernszt, D., Pongracz, E. J., Kvell, K., Role of Wnt4 exosomes in thymic ageing. ISEV 2018, Barcelona, 2018.

Miskei, J., **Garai, K.,** Banfai, K., Papp, E., Torok, Zs., Kvell, K., Sarosi, V., Pongracz, E. J. Exosomes contain Wnt signals that regulate vascularization in lung cancer. ISEV 2018, Barcelona, 2018.

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