

Mitochondrial and epigenetic changes in selectively bred rats due to dietary restriction and endurance training

Abstract of the PhD Thesis

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1. Introduction

In the everyday routine one can often meet with individuals whose conditional abilities are superior than the average population. These abilities can be improved in a discrete interval, but the upper limit of the intervals seems to be determined by genetic factors.

A good example of these extremities is the elite sport where athletes can produce almost superhuman performances. These individuals are able to produce extreme results because, in addition to their physical and environmental conditions, the units that make up their body operate with a "code" (DNS) that allows the development of the capabilities required to perform at high level. Determining the upper limit of individual skills is a complex task. Those with moderate abilities may wonder how much and with what means can they improve their conditioning skills? Furthermore, the question is rises where are the intervention points that can be modulated for better results? In the course of our investigations, we worked with a low and high running capacity rat population bred from wild-type rat to model different "talent" and "susceptibility".

In recent years, epigenetic (a hereditary form that is not connected with the base sequence change) has been highlighted, whose results suggest that lifestyle and environmental conditions also affect the modification of the histone component of the chromatin. This in itself does not seem to be so significant, but these changes may affect the expression of certain genes - moreover, can be transmitted to offspring as well. The inherited properties, the network of metabolism, the interaction of gene expression processes and protein functions make the simplest living organisms a very complicated structure. A small part of this complex system is the oxidative metabolic process that can play an important role in physical performance. The central organ of the aerobic metabolism is mitochondria, which functional and quantitative analysis, coupled with epigenetic changes, can bring us closer to understand the physical performance, aging processes or even complex pathomechanisms.

During its development, a living organism experiences many external effect. Two these, that one might most effectively modulate is ones physical activity and diet (regular sports / physical activity, healthy, moderate nutrition). A number of studies have reported that these factors can have the highest positive impact on human health.

In our study we examined mitochondrial biogenesis markers and the epigenetic projection of dietary restriction (DR) on the brain (hippocampus), muscle tissue (gastrocnemius) and male reproductive organ (testis). In the skeletal muscle studies, endurance training was also applied since mitochondrial functions are clearly affected in the skeletal muscle due to exercise training. As an outcome, in addition to the control groups, the effect of nutrition can be compared to the trained skeletal muscle. It was in our interest to demonstrate in two subpopulations possessing different phenotypes the effect of dietary restriction endurance training. Individuals with different abilities often show marked differences, even if the treatment conditions are matched. These observations can also be important for sports professionals, as personalized training programs and nutrition protocols will come to the fore.

2. Aims and objectives

We aimed to determine the epigenetic and mitochondrial changes due to dietary restriction and physical activity on the selectively bred rats based on their running capacity. After the experiment period, we focused on three types of tissue: brain tissue (hippocampus), skeletal muscle tissue (m. gastrocnemius) and male reproductive organ (testis). We asked the question how the different running phenotypes and abilities can be manifested at the epigenetic or mitochondrial signaling. Two populations of the animals were studied: a low running capacity (LCR) and high running capacity population (HCR).

A specific epigenetical modification was investigated by measuring the BDNF gene locus specific acetylation of a 14th amino acid (lysine) of the histone H3 protein of a chromatin. In our research on mitochondrial biogenesis, Sirt1, PGC-1 alpha and related cellular pathways were examined.

In medicine it is long been known that a drug has sometimes different then the "target" effect on individuals. Responders are those where "target" changes is observed (for example, for an antihypertensive drug to reduce blood pressure) and they form the majority of the population. Another smaller group within the population is the "nonresponder" group that does not show any change due to the treatment. In general, the "revers-responder" group with the smallest number of cases produces counter-productive changes (blood pressure increase in the case of the antihypertensive example). In the present animal model, a population is artificially selected rats were used with extremely weak and high performance. It is believed that the rat groups on both sides of the population may react differently to the dietary constraints and to the exercise training. It has been shown that environmental conditions can have a great impact on how the genetic code is read. It is assumed that both the DR and endurance training will induce physiologically positive changes in both examined strains. The magnitude of the effect, however, is expected to be lower than in the LCR group compared to HRC.

Our hypotheses can be formulated as follows.

A. In the study of the hippocampus it was assumed:

- a) In the examined groups, the relationship between memory capacity and BDNF level can be detected.
- b) Dietary restriction enhances BDNF production and improves memory in both groups, with epigenetic, histone acetylation modification.
- c) The applied DR treatment has no significant effect on hippocampal mitochondrial biogenesis.

B. In the examination of gastrocnemius muscle it was assumed:

- a) Exercise training do, but the nutrition protocol used does not induce mitochondrial biogenesis in the gastrocnemius muscle.
- b) The weaker performance of LCR animals can be linked metabolic adaptation deficiency.
- c) Endurance training and the dietary restriction will improve the life expectancy of treated groups.

C. In the examination of the male reproductive organ, it was assumed:

- a) Endurance exercise positively influences markers of spermatogenesis in training groups.
- b) Endurance training has effects on mitochondrial biogenesis and oxidative stress markers in testicular tissue.

3. Materials and methods

3.1. Experimental animals

13 weeks old male Wistar rats selectively bred for low and high running capacity were used in our study. Artificial selection took place in the United States (University of Michigan, Ann Arbor, USA) as follows: 186 N: NIH rat rats were run on treadmills with increasing speed. 10%-10% of the worst and the best performing animals were selected. The best performers were mated with peers of similar, and poor performance with the poor performers. High-Capacity Runner: HCR and Low Capacity Runner: LCR groups were identified. LCR animals had a lower resistance to oxidative stress and the LCR line is prone to cardiovascular disease and exhibits symptoms such as visceral fat accumulation, hypertension, dyslipidemia and, last but not least, insulin resistance. Experiential disabilities begin to significantly diverge at the 10th generation. The 22nd generation of LCR and HCR rats were used in our experiments .

In our first experiment 6 control (C) and 6 dietary restricted (DR) animals were used from both lines: HCR-C, HCR-DR, LCR-C, LCR-DR (Figure 1)

In the gastrocnemius experiment, also with $n = 6$, the C, DR and adherent exercising group (T) of both lines were formed: T groups: HCR-C, HCR-DR, HCR-T, LCR-C, LCR-DR, LCR-T.

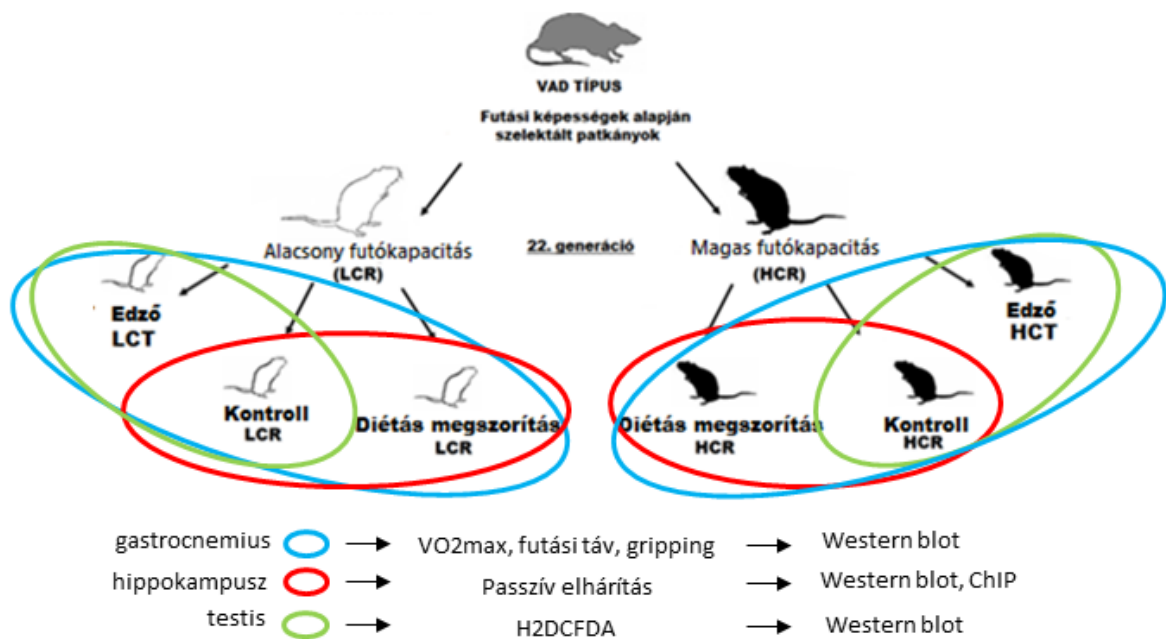


Figure 1.

Grouping of the experimental animals

3.2. Training protocol

The exercising animals are trained at a speed of 60% of their maximal aerobic capacity, on a treadmills developed for small animals. During the first three days of the training period, the animals were accustomed to treadmill running conditions. Subsequently, the animals were trained for 3 weeks with a moderate load at a speed of 10m / min at a 5% slope.

Maximum oxygen uptake was measured by a closed-system spirometry for small animals (Columbus Inst. Columbus, Ohio). The oxygen uptake was recorded with a progressively increasing intensity until: (i) no significant increase in oxygen uptake was achieved by increasing the speed; (ii) until the test animal was unable to maintain its position on the treadmill; and (iii) until the respiratory ratio exceeded Value 1: ($RQ = VCO_2 / VO_2 > 1$). Based on the VO_{2max} data, the exercise intensity was set. The training sessions lasted for 40 to 60 minutes on 5 days of the week for 12 weeks. At the beginning of the training, a 5m / min warm-up phase was added for 5 minutes in each exercise session. The training group (n = 6) was formed from both LRC and HRC. During the VO_{2max} measurement, the running distance was also recorded.

3.3. *Dietary intervention*

In our study every other day fasting (EODF) protocol was applied. This procedure corresponds to a moderate calorie restriction around 20% over the long term. In the following sections our protocol will be referred to as dietary restriction (DR), as this form of construction is not exactly the same as classic calorie restriction (CR). Treatment was started at 13 weeks of age by forming the following groups: High Capacity Runner (HCR-C), High Capacity Runner - Dietary Restricted (HCR-DR) and Low Capacity Runner Control (LCR-C), finally Low Capacity Runner- Dietary Restricted (LCR-DR). $n = 6$ / group (Figure 1). Pellet type rodent chow developed for feeding rats was administered to the animals (LT / r Rodent Nutrition INNOVO Ltd 2100 Gödöllő). Water ad libitum was available for DR animals and food every other day in the morning (CET from 08:00 to 10:00) was completely withdrawn for 24 hours. The treatment lasted 16 weeks. Animal weights were measured on a weekly basis with 5 grams accuracy.

3.4. *Short-term memory measurement*

A passive avoidance test (PAT) is a method for testing the medium and short-term memory of a laboratory animal (mouse, rat). The procedure is based on the properties of the animals meaning that rodents avoid bright open areas and prefer darker areas.

During the acquisition phase animal were placed in the light chamber, after passing through the dark chamber, there was a mild electrical shock on its feet, conditioning it to avoid the dark chamber.

During the test phase animals were placed again in the light chamber and latency spent in the bright chamber measured. If the rat takes a long time in the light, "passive" to normal behavior, one can infer that it has learned that the dark chamber is a "negative environment". The latency time in the bright space has a positive correlation with the ability of animals to remember. The test phase was performed on the next day of the acquisition phase at the same time.

3.5. Tissue Processing

The animals received an appropriate amount of pentobarbital injection (45mg / kg) after the drug exhibited its sedatohipnotic effect, the animals were decapitated, the gastrocnemius muscle and the brain was surgically removed and the hippocampus isolated. The tissues were removed, frozen in liquid nitrogen and stored at -80 ° C.

3.6. Biochemical studies

3.6.1. AcH3-ChIP The chromatin immunoprecipitation process

The procedure is based on the property of the nucleosomal nucleic acid (DNA sequences) to bind histone proteins. These adhesions can be retained even after processing the tissue, and can be extracted from the lysate by Chromatin Immunoprecipitation (ChIP) (Acetyl-Histone H3 Immunoprecipitation Assay Kit (Cat # 17-245 Upstate / Millipore)). ChomLysis buffer was added to the preomogenate and chromatin was sonicated to nucleotide polymers of ~ 1000-4000 base pairs.

3.6.2. AcH3-ChIP-BDNF-qPCR

In order to determine the amount of binding of acetylated histones to the BDNF promoter region, the DNA extract obtained after ChIP was tested by PCR. During the PCR, rat specific BDNF exon IV promoter primer pair was used. In our study (-73 to +14 bp) Fw: TCTATTTTCGAGGCAGAGGAGGTATC, Rv: AATGGGAAAGTGGGTGGGAG, primer was chosen similarly for other high-level publications. (Chen et al., 2003; Gomez-Pinilla et al., 2011)

Chip samples were subjected to quantitative real-time PCR (qPCR) assays. The values obtained in Ch-IP were normalized to the full BDNF exon IV promoter values.

3.6.3. RNA isolation and BDNF mRNA 'real time quantitative PCR' (qRT-PCR)

Isolation was performed with the Nucleospin® RNA II Kit (Macherey-Nagel, Düren, Germany) according to the manufacturer instructions.

For the BDNF mRNA assay cDNA was synthesized using the TetrocDNA Synthesis kit (Cat # BIO-65026 Biotec, Luckenwalde, Germany).

Rat-specific BDNF primer was used to detect BDNF mRNA (Fw: CCATGAAAGAAGCAAACGT, Rv: CTCCAGCAGAAAGAGCAGA). For the PCR reaction Sybr Green detection system was used with EvaGreen® dye (Biotium, Hayward, CA, USA). During amplification equal amount of DNA template, 10µl "ImmoMix™ complete ready-to-use heat-activated 2x" reaction mixture (Biotec GmbH, Luckenwalde, Germany), 1µl 20x EvaGreen dye, 2.5µl 10nMol Fw- Rv mix primer (IBAG, Göttingen, Germany) and distilled water were mixed in a final volume of 20µl. The environmental conditions of amplification were created by Rotor-Gene 6000 thermal cycler (Corbett Life Science / Qiagen, London, UK). After a 10-minute 95 ° C activation phase, 40 cycles of 10sec 95 ° C, 20sec 60 ° C, and 30sec 72 ° C phases were run. After the last cycle we measured the specificity of the measurement by melting point analysis. Reliability was also verified by agarose gel electrophoresis. If only one band was observed after electrophoresis in the ~ 280 bp range, the amplification results were accepted.

3.6.4. BDNF concentration measurement by ELISA technique and Western Blot

The protein content of the samples was determined by Bradford protein assay (BioRad Protein Assay (Dye Reagent Concentrate)). Protein content of the samples was adjusted to the smallest sample concentration (brain: ~ 10mg / ml, muscle: ~ 9.53mg / ml)

To determine BDNF concentration, BDNF ELISA kit (E-Max ImmunoAssay System, Promega, Madison, WI). BDNF concentrations were normalized to the total protein content of the samples.

For Western Blot, after adjusting the same protein concentration, 5% Beta-mercaptoethanol was pipetted to 2x Laemmli Sample Buffer (120mM Tris-HCl pH 6.8, 4%

SDS, 20% Glycerol, 0.01% Bromphenol Blue) to reduce the disulfide bridges and the resulting solution was pipetted 1: 1 and heated at 95 ° C for 5 min. Denatured samples were stored at -80 ° C for later use.

Determination of specific proteins of tissue samples and their relative concentration was determined by Semi-Dry Western Blot technique. The first step of the assay was the separation of protein samples by molecular weight with SDS polyacrylamide gel electrophoresis (SDS-PAGE). Separated proteins were transferred to a PVDF membrane (30kDa <Millipore Immobilon-P pore size: 0.45µm, 30kDa> Millipore Immobilon-PSQ pore size: 0.2µm) using a semi-dry transfer unit (Cleaver Scientific, UK). The membrane was blocked for 2 hours with 5% milk powder TBST or 1% BSA TBST at 4 ° C. Subsequently, membrane was incubated with primary antibody at 4 ° C overnight. After casting the primary antibody, 3x10 minutes of TBST wash cycle occurred. After washing the membrane was incubated rabbit / goat / mouse / rat in secondary antibody for 1.5 hours at 4 ° C. Prior to signal detection, the membrane was treated with SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific, USA) and incubated for 5 minutes. The bands on X-ray film represent the concentration of specific proteins present in the sample after the development. The signal strength was densitometed using imageJ software and quantified by determining the area under the density curve. The density values of the tested proteins were normalized to alpha-tubulin and beta-actin protein levels.

3.6.5. Estimation of the amount of reactive radicals based on 2', 7'-dichlorodihydrofluorocaine diacetate (H2DCFD)

2', 7'-Dichloro-dihydrofluorocaine diacetate (H2DCFDA) fluorescent staining (Invitrogen-Molecular Probes # D399) was used to estimate ROS content in testicular tissue. Staining can estimate tissue's steady state redox status and provides information on tissue oxidative stress levels. Briefly, the staining uses the reduced form of 2', 7'-dichlorofluorescein (DCF) acetate (H2DCFDA), which is not fluorescent at this stage. Concentrated H2DCFDA was diluted to 12.5 mM in ethanol and stored at -80 ° C in the dark. The solution was freshly diluted with potassium phosphate buffer to 125 µM before use. The fluorescent reaction was performed in a 96-well black microplate. Potassium phosphate buffer (pH 7.4) was added to the wells at a volume of 152 mM / well. Then, 8 µl of diluted tissue homogenate and 40 µl of 125 µM dye were added to reach a final dye concentration of 25 µM. Changes in fluorescence intensity were measured every 5 minutes for 30 minutes. During the reaction, the acetate

group of the H2DCFDA molecule is cleaved as a result of oxidation. As a result of the chemical transformation, the deacetylated, oxidized form recovers its fluorescent property, which was detected and quantified at 485/538 nm (excitation / emission). The detected amount was normalized to total protein content

3.7. *Statistical analysis*

For statistical analysis Statistica 13 program was used. After normality analysis (Shapiro-Wilk test), normally distributed variables were analyzed by one-way variance analysis. For variables that did not show normal distribution (gripping test, exon IV acetylation, memory data), Kruskal Wallis ANOVA was used. The significance level was set at $p < 0.05$ and these are indicated in our charts. To determine the memory - BDNF quantity connection, since memory data did not show normal distribution, Spearman's rank correlation coefficient was calculated.

4. Results

4.1. *The physiological and functional results of the animals*

At the beginning of our study, the body weight in the HCR group was significantly less than that in LCR, this difference remained until the end of treatment (LCR-C: 503 ± 72 g, HCR-C: 409 ± 51 g,

Treatments only reduced the body weight in the LCR group (LCR DR 503.57 ± 33.51 : 475.00 ± 27.08 , LCR T 501.25 ± 67.00 : 485.00 ± 66.20). The weight of HCR animals in the control group was not significantly changed (HCR C 409.00 ± 51.41 : 437.00 ± 53.46). At LCR control change did not detected. (LCR C 503.33 ± 72.30 : 515.83 ± 69.31)

The maximum relative oxygen uptake of LCR and HCR animals was different ($p < 0.05$). On average LCRs are has 50 ml and HCR rats has on average 80 ml VO_{2max} . DR did not has a significant impact on VO_{2max} based on our studies. The endurance training has significantly increased the maximum oxygen uptake in both rat lines.

Running abilities (running distance) at the start of the experiment showed a near 370% difference between the LCR and the HCR line. In the case of HCR animals, the maximal running distance increased significantly due to training. DR does not seem to have a significant impact on the animal's running ability. (Although increasing trend in the LCR group is observed). Training in the LCR animal population also achieved success in terms of run distance and speed, but the distance and velocity increase not even come close to the development of HCR animals, however relative progression outweighed the HCR group. (HCR + 163%, LCR + 220%)

According to our results we can state that animals of the HCR-DR group exceeded all the other groups in the short-term memory. The absence of the variance suggests that the individuals of this group spent all the 6 minutes in bright chamber. We could also say that they have achieved maximum results on the test.

4.2. Results from hippocampus samples

SIRT1 activity / concentration in many cases increases with calorie restriction / DR in different tissues. According to our results the amount of molecule with histone deacetylase function did not show any significant difference between the groups.

In case of PGC1 alpha transcription cofactor measurement, a significant increase in HCR-DR was observed in comparison to the HCR-C group. This co-activator, as mentioned above, can be related to the activity of SIRT1. Conversely, the nucleic respiratory factor (NRF1) did not show any difference between the groups, although NRF-1 is often referred as a "down-stream" protein of PGC-1 alpha. Levels of PGC1 α increased in the HCR group to DR, but this effect did not seem to be sufficient in the brain to activate NRF-1 signaling.

Measuring COX4 concentration is a widespread method to estimate mitochondrial volume. Hippocampal level of COX4 neither showed any difference in the HCR-LCR populations nor as an outcome DR.

Hippocampal phosphorylation of CREB was associated with a DR increase in both rat lines.

According to our findings, DR can increase BDNF levels in rats with high running capacity. Both Western blots and ELISA measurements are equally confirmed this statement. Quantity based dietary intervention had an effect on cognitive abilities, especially on short-term memory (passive remedial test). Memory and BDNF western blot density values shown a positive correlation by Spearman $R = 0.52$ $p = 0.015$.

Interestingly, total H3 acetylation was only significant in LCR-DR animals. The specific histone H3-BDNF IV. promoter binding however, was higher only in HCR line due to every other day feeding. For LCR individuals only an increased tendency can be observed.

BDNF mRNA results did not follow the trend of co-immunoprecipitation results. For mRNA, only a LRC-DR group (similar to non-specific histone H3 results) was found to have a significantly elevated level.

4.3. Results from gastrocnemius samples

In skeletal muscle samples significantly higher SIRT1 protein levels were observed in HCR-T animals compared to the HCR control group. DR did not affect either SIRT-1 level of in our experimental groups. The acetylation of lysine residues was only increased in the HCR-DR group.

PGC-1 alpha values in parallel with NRF-1 protein levels were significantly elevated in the HCR group. In contrast, the LCR group the only change was in NRF-1 alone, but it did not result in an increase in SDHA mitochondrial protein.

The increase in mitochondrial biogenesis is indicated by the elevated levels of PGC-1 alpha and NRF-1 and of succinate dehydrogenase A (SDHA) subunit in HCR-T animals. According to our measurement, SDHA in LRC animals did not change either by training or by DR.

Akt phosphorylation showed a robust increase in the HCR-T group compared to the control group. In contrast, in LCR animals the activation of Akt signaling processes was not detected.

Another indicator of anabolic processes is the cellular concentration of follistatin in the muscle tissue. For LCR animals, both endurance training and DR induced lower follistatin a level. There was no change in the HCR group. Thus, it is presumed that there was no significant chronic catabolic response to the metabolic stress experienced by the HCR animals. Moreover, Akt phosphorylation data suggest elevated carbohydrate uptake and improved extracellular stimuli integration ability (insulin and growth factor sensitivity). In addition, increased phosphorylation of AMPK protein also confirms our recent view. AMPK is an AMP sensitive molecule and its phosphorylation is an indicator of well-coordinated metabolic responses to energy-deficient conditions.

4.4. Results from testicular tissue samples

Markers involved in mitochondrial biogenesis showed differences in SIRT1, PGC-1 α and mtTFA proteins. SIRT1 protein was significantly higher in the control high-running capacity group compared to the trained and LCR-C groups. In contrast, PGC-1 α was

significantly increased only in the LCR trained group. Interestingly, mitochondrial transcription factor A (mtTFA) showed a decrease in endurance trained HCR group. The NRF-1 protein showed no significant difference in either group.

Steady state oxidative stress status in low running capacity animals changed favorably as a result of endurance training. In their case, a reduced level of oxidized 2', 7'-dichlorofluorescein was detectable, indicating a lower degree of oxidative stress. Acetylation of P53 increased in LCR animals following exercise, whereas in HCR animals it remained at low levels unchanged.

As regards markers of spermatogenesis, both Odf-1 protein and LDHC protein showed an increase due to endurance training in the LCR group. In addition, in both cases, the difference was significant compared to the HCR-T group, suggesting more effective reproductive function in the LCR group as a result of exercise. In fact, the applied training method in HCR individuals may even impair reproductive potential, suggested by the Odf-1 results.

5. Conclusions

The present study shows that elevated values are likely to be linked to posttranslational modification of histone proteins. It appears that dietary habits also affect the chromatin structure and maybe the gene read mechanisms. Our findings on acetylation of the BDNF IV promoter region and the phosphorylation of the CREB transcription factor (Ser133) reinforce this assumption.

Rats with high running capacity performed significantly better results in our passive avoidance test. These results showed a pattern similar to the BDNF level. According to the neurotrophin hypothesis the survival of neurons promoted by neurotrophins, including BDNF, and is also largely involved in the formation of synaptic network ("competing for the BDNF molecule"). The results of our study suggest that similar mechanisms could probably be the basis of better cognitive abilities in HRC animals.

With the present dietary protocols, we could not detect increased Sirt1 levels in the brain and skeletal muscle, although tendency of elevation in the muscle was observed. Physical activity increased Sirt1 protein concentration only in HCR group skeletal muscle samples. Recently the most widely accepted theory during metabolic stress conditions such as exercise or calorie withdrawal, AMP / ATP or NAD + / NADH rates are altered. The shift leads to the activation of AMP sensitive protein kinase (AMPK). The released growth factors cause phosphorylation of Akt protein. AMPK has a positive effect on the activity of Sirt1 deacetylase (promoted by NAD + level), which results in the deacetylation of the PGC-1 protein in muscle. Deacetylated PGC-1 enhances transcription of genes involved in mitochondrial biogenesis. Such effects only in case of of exercise could be clearly demonstrated here, in skeletal muscle tissue. In the literature, calorie restriction is sometimes associated with contradictory mechanisms in terms of mitochondrial quantity. In addition, data that did not support the central role of Sirt1-induced mitochondrial biogenesis were published.

To clarify the issues, it would be useful to analyze the accumulated information and later conduct using similar measurements as ours, cell line examinations, and bioinformatics methods to unveil obscure details.

The results of the skeletal muscle in the LCR group clearly show signs of malfunctional metabolic adaptation. Whether metabolic markers for energy use: Akt, AMPK or mitochondrial biogenesis: PGC-1 / NRF1 / SDHA is observed the LCR group performs poorly. These properties of LCR animals make their group a population that is susceptible to metabolic disease and they have a weak physical ability. It is important to emphasize, however, that environmental conditions, especially endurance training, can produce significant relative development in the above mentioned population.

In summary, for hippocampal studies the following conclusions can be made about our hypotheses:

A.

- a) In the examined groups, the relationship between memory capacity and BDNF level can be detected.
 - TRUE
- b) Dietary restriction enhances BDNF production and improves memory in both groups, with epigenetic, histone acetylation modification.
 - PARTLY TRUE, given that this effect is not observed in LCR animals and locally the histone acetylation increase is only significant in HCR animals
- c) The applied DR treatment has no significant effect on hippocampal mitochondrial biogenesis.
 - TRUE

Based on our results, the following conclusions can be made about our hypotheses for gastrocnemius muscle study:

B.

- a) Exercise training do, but the nutrition protocol used does not induce mitochondrial biogenesis in the gastrocnemius muscle.
 - Partially TRUE, since mitochondrial biogenesis in LCR is a can not be seen

b) The weaker performance of LCR animals can be linked metabolic adaptation deficiency.

- TRUE.

c) Endurance training and the dietary restriction will improve the life expectancy of treated groups.

- TRUE, but predominantly for endurance training

C.

Based on our results, we can make the following conclusions about our hypotheses after conducting research on male rat reproductive organ:

a) Endurance exercise has a positive effect on spermatogenesis markers in training groups.

Partly TRUE. No clear positive change was found in the LCR group. The applied training what based on relative abilities, in the HCR group may even negatively affect spermatogenesis (LDHC results).

b) Endurance training affects mitochondrial biogenesis and markers of oxidative stress in testicular tissue.

- In part, it is TRUE, since in LCR animals, a decrease in oxidative stress markers can be detected as a result of endurance training, but no clear effect on mitochondrial biogenesis was found.

Finally, we would like to draw attention to genetic and epigenetic studies in the field of sport science. In the selection and in the designing of personalized nutrition and training formulas, the genetic background plays a major role. More over the environmental influences and epigenetic modifications (DNA methylation, histone acetylation, activation of transcription factors, just to name a few examples) are of enormous importance. In sports science, however, its significance is not yet well appreciated. "Sport genetics" is mostly exhausted in the search for certain declarative; predestine genetic variants, gene constellations, with little attention to the "reading comprehension" of the genetic code. Genetic control is far more complex than we would have thought 20-30 years ago. The importance of post-transcriptional, posttranslational (especially epigenetic) modifications and nucleic acid interference phenomena, that have been in the focus of genetic research in recent years, should make it clear to the sport professional or scientist that he or she can not ignore

these new knowledge. Whether one needs to build a workout plan or to design a dietary regimen, knowing and understanding the above-mentioned processes of regulation, can bring sport performance enhancement or overall performance to a next level. If you like to "educate an even healthier person". Health is a difficult concept, but it is an understandable need of all responsible human being.

6. List of own publications

6.1 Publications related to the dissertation

Torma F, Bori Z, Koltai E, Felszeghy K, Vacz G, Koch L, Britton S, Boldogh I, Radak Z. (2014) Eating habits modulate short term memory and epigenetical regulation of brain derived neurotrophic factor in hippocampus of low- and high running capacity rats. *Brain Res Bull*, 107: 54-60.

Torma F, Koltai E, Nagy E, Ziaaldini MM, Posa A, Koch L, Britton S, Boldogh I, Radak Z. (2014) Exercise Increases Markers of Spermatogenesis in Rats Selectively Bred for Low Running Capacity. *PLoS One*, 9: e114075

6.2. Independent publications

Radak Z, Torma F, Berkes I, Goto S, Mimura T, Posa A, Balogh L, Boldogh I, Suzuki K, Higuchi M, Koltai E. (2018) Exercise effects on physiological function during aging. *Free Radic Biol Med*, 132: 33-41

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Blood flow restriction in human skeletal muscle during rest periods after high-load resistance training down-regulates miR 206 and induces Pax7 (in press)Torma F., Gombos Z., Fridvalszki M. Langmar G. Tarca Zs. Merkely B., Naito H., Ichinoseki-Sekine N., Takeda M. Murlasits Zs., Osvath P., Radak Zs. *JSHS*