VILNIUS UNIVERSITY

VIOLETA BARTUŠKIENĖ

GROWTH PROGRAMMING DURING THE PRENATAL PERIOD: INFLUENCE OF MATERNAL UNDERNUTRITION ON OFFSPRING PHYSICAL STATUS IN RATS (AN EXPERIMENTAL STUDY)

Summary of Doctoral Dissertation
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VILNIAUS UNIVERSITETAS

VIOLETA BARTUŠKIENĖ

AUGIMO PROGRAMAVIMAS PRENATALINIU LAIKOTARPIU: EKSPERIMENTINIS MOTININIŲ ŽIURKIŲ NEPAKANKAMOS MITYBOS IR PALIKUONIŲ FIZINĖS BŪKLĖS SĄSAJŲ TYRIMAS

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ABBREVIATIONS

BMI – body mass index

approx. – approximately

RBC – erythrocytes

HGB – haemoglobin

HCT – haematocrit

MCV – mean corpuscular volume

MCH – mean corpuscular haemoglobin

MCHC – mean corpuscular haemoglobin concentration

RDW – red cell distribution width

PLT – platelets

MPV – mean platelet volume

WBC – leukocytes

LYM – lymphocytes

MONO – monocytes

NEUTR – neutrophils

EOS – eosinophils

HE – haematoxylin and eosin

INTRODUCTION

Factors affecting individual growth and development include diverse environmental and lifestyle aspects. Adverse early life events, especially those occurring during the critical stages of growth, such as embryonic or foetal periods, have been identified as an important risk factor in a number of chronic diseases. Scientific hypotheses even attribute the global pandemic of the chronic diseases to early life events that could have occurred in former generations. In addition, growth and physical status of the offspring is also influenced by the long-term maternal nutritional ecology and environmental resources (*Cameron and Demerath, 2002*). It is therefore necessary to examine the mother's diet before pregnancy.

It is estimated that nutritional deficiency throughout pregnancy can lead to metabolic changes transmitted across several offspring generations. However, there is a lack of studies examining the link between maternal nutrition prior to pregnancy and an overall health status of the child. The existing evidence is scarce and short term. The pre-pregnancy environment is hypothesized to provide metabolic support to alleviate the shortages of nutritional perturbations during pregnancy (*Kuzawa, 2005; Wells, 2010*). Thus, the results of this experiment aim to fully evaluate and generalize the biometric indices and blood composition of the rat offspring as well as their histomorphological profile reflecting the consequences of pre-pregnancy and pregnancy nutrient restriction at different age periods.

The initial pilot study confirmed the long-term effects of food restriction prior to and during pregnancy on body weight and longevity even in the second-generation offspring (*Araminaite et al., 2014*). Recent studies clearly demonstrate that there is a link between the mother's diet during pregnancy and certain diseases in the offspring. Studies describe small birth weight (*Frederick et al., 2008; Pinheiro et al., 2008*), delayed maturation (*van Weissenbruch et al., 2005; Guzman et al., 2006*), earlier reproductive aging (*Khorram et al., 2015*), altered endocrine and nervous regulation of metabolism (*McArdle et al., 2006; Coupe et al., 2010; Reusens et al., 2011; de Oliveira et al., 2012; Garcia et al., 2013*), obesity (*Hoffman et al., 2000; Ozanne and Hales, 2004*), inadequate immune response (*Fulford et al., 2013; Reynolds et al., 2013*), higher risk of non-communicable diseases in adulthood (*Koupil et al., 2007; Torrens et al., 2009; Harrison et al., 2009; Hult et al., 2010*) as well as changes in cognitive function or even circadian rhythms (*Blaise et al., 2007; Gilbert et al., 2010*; *Zhang et al., 2010*) possibly transmittable across several offspring generations. Although widely studied, this subject is rarely examined through a lifetime, so only a glimpse of possible consequences is traced.

Furthermore, there is a lack of studies examining the importance of maternal diet prior to pregnancy on the general health status of the child. This is unfortunate, as the modern culture is excessively propagandized with an extra slim body image and even normal-weight young women choose to diet to live up to the standard. Insufficient maternal weight can also be responsible for the development of metabolic diseases. Furthermore, undernutrition is a worldwide challenge: one in nine people suffer from hunger (795 million), and one in four of the world's children are stunted (*de Onis et al., 2012; FAO,*

2015). As mentioned above, most studies focus on the *in utero* environment, while the period of formation of maternal metabolic capital – the pre-pregnancy period that can be of crucial importance for the development of the foetus, is relatively unexplored. Only a few studies of this kind have been published. Studies associate high weight before pregnancy with obstetric risks, macrosomia, child obesity and related diseases (*Liu et al., 2009; Kahr et al., 2015; Ding et al., 2016*). Meanwhile, underweight mothers are described rarely, but the mentioned can lead to premature birth, low birth weight, cognitive disorders or even premature death of the offspring (*Kalk et al., 2009; Jeric et al., 2013; Fujiwara et al., 2014*). However, the further consequences for the offspring remain in the margins of scientific interest with just a few targeted studies, describing the importance of this period.

In addition, besides insufficiently investigated, this phenomenon is rarely analysed during the course of a lifetime, which means that the knowledge on the potential consequences for the offspring is incomplete and not fully described. Meanwhile, most of the metabolic changes may occur or, on the contrary, normalize as a result of the body's adaptive and compensatory mechanisms, in the second half of life. What is more, studies usually examine only the pregnancy environment, include small samples and the possible metabolic changes are described fragmentary – experimental animals are used to examine just a few indices and a complete metabolic image is not defined. Thus, the observation of metabolic changes after the onset of maturity is meaningful and needed from both scientific and ethical perspectives. In addition, the studies gaining significant results are more likely to be published, so a reliable connection between prenatal well-being and offspring health status in adulthood is difficult to assess. Discrepant research results and the limitations of the hypotheses require a holistic and long-term observation. So, there is a particular need in pregnancy and especially pre-pregnancy nutritional environment research in the context of long-term outcomes such as aging, longevity and the developmental pathways of the subsequent offspring generations.

Therefore, this experiment examines the influence of maternal food restriction and physical status of several rat offspring generations up till spontaneous death and seeks to holistically evaluate and summarize the changes in biometric, histomorphological and blood indices, reflecting the offspring response to nutrient limitation in different age periods.

The goal of the thesis was to evaluate the influence of maternal food restriction prior to pregnancy and throughout pregnancy on variation of physical status (biometric, morphological and metabolic indices) of two rat offspring generations at different periods of ontogenesis.

Objectives:

1. To explore the overall trends of rat offspring body weight dynamics and longevity in the two-offspring-generation pilot study.
2. To examine the dynamics of body weight and additional biometric indices of the first-generation rat offspring.
3. To examine the dynamics of blood composition indices of the first-generation rat offspring.
4. To examine the histomorphological indices of the first-generation rat offspring.

Relevance and scientific novelty of the research

The research aims to holistically evaluate the influence of maternal food restriction on biometric, blood and histomorphological indices as well as the lifespan across several rat offspring generations. This study differs from the others found in the available literature by the period of dietary restriction (the offspring of pre-pregnancy and throughout pregnancy food restricted mothers were observed), observation time (two offspring generations were observed until natural death and physical status evaluated in the longitudinal manner) and complexity (the study involved holistic evaluation of body size and growth, blood composition as well as the histomorphological indices).

As mentioned, unfortunately, studies are mostly investigating nutrition during pregnancy only and are short-term, examine few metabolic indices and involve small samples, thus, the interpretation of the metabolic changes is still fragmentary. In contrast, most of the metabolic changes become evident or normalize in an aged organism – therefore, this experiment investigates the association between the maternal nutritional deficiency and the physical status of several offspring generations in a young, mature and especially aging organism.

Moreover, the period of maternal metabolic capital formation – the pre-pregnancy environment is often underestimated and therefore, is almost non-tested in an experimental context. Furthermore, as mentioned earlier, most studies are carrying out "express research", capturing the effects of early undernutrition on the first-generation offspring only, and for a brief period of time (mostly ranging from 3 to 12 months). Hence, the data on the consequences of the developmental programming in the context of aging is scarce. Thus, the presented results describe the physical status of offspring from both pre-pregnancy and pregnancy food deprived mothers.

MATERIALS AND METHODS

The animal husbandry and experiments on animals were carried out according to the national and European regulations and were approved by the National Animal Care and Use Committee (Permission no. 0211, 2009 and no. G2-20, 2015).

The study was conducted in 2010-2016. The investigation was carried out in two stages:

• The pilot study that involved the overall observation of the rat offspring (N=122) body weight dynamics and longevity across two offspring generations (completed and published). The pilot study revealed the topicality of the study and the long-term outcomes on the physical status of the food restricted mothers' offspring.

• The principal study that involved the comprehensive observation of the physical status (biometric, blood and histomorphological indices) of the first-generation offspring (N=107). The study is being continued with the second-generation offspring.

Dietary conditions

Virgin female *Wistar* rats (the number of maternal rats is presented in the table 1) were divided into 3 groups in respect to nutritional restriction. The rats were fed either a normal (CG – Control group) or a 50% restricted diet: one experimental group was food restricted one month prior to pregnancy only (EG 1) and the other – one month prior to and throughout pregnancy (EG 2). The design of the study is presented in Figure 1. After birth no food deprivation was applied and all offspring rats were fed according to the recommended daily intakes. The litters were culled to 8 pups per litter to standardize the food supply. After weaning the offspring were housed in pairs.

Table 1. *The number of maternal rats. CG – Control group, EG 1 – prior to pregnancy food restricted mothers, EG 2 – prior to pregnancy and throughout pregnancy food restricted mothers.*

Group	Pilot study	Principal study
CG	2	10
EG 1	5	10
EG 2	5	10

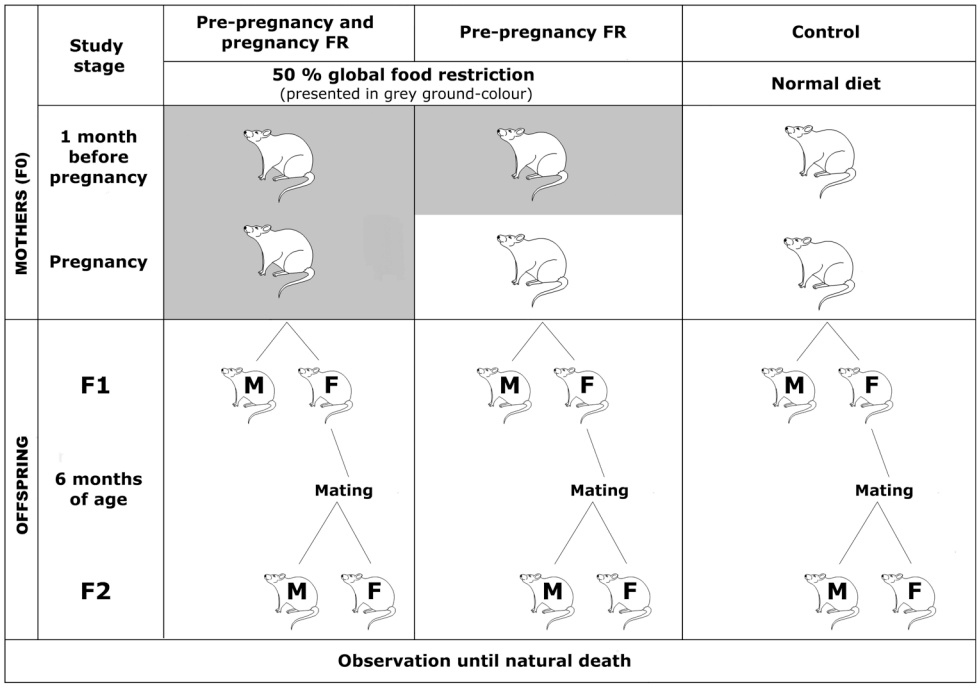

Fig.1. *The design of the principal study. F1 = first-generation offspring, F2 = second-generation offspring; M = males, F = females, FR = food restriction. F0 maternal rats consumed either normal diet (Control group – CG) or were food restricted prior to pregnancy (Pre-pregnancy FR group – EG 1), or prior to pregnancy and in utero (Pre-pregnancy and pregnancy FR group – EG 2). The F0 and F1 females were mated with the proven male breeders outside of the study. The F1 and F2 offspring were observed until natural death.*

Pilot study

The first-generation female offspring were mated at 6 months of age with male breeders from outside the study. First-generation offspring were observed until the 6th month of age, and then F1 females were mated to produce the second-generation offspring (F2). The F1 males were euthanized after 6 months of age, whereas the F0 and F1 female rats were euthanized after weaning of the offspring. The second generation was kept and weighted under the same standardized conditions until natural death. Body weight was measured weekly with calibrated scales starting at one month of age.

Principal study

After summarizing the results of the pilot study, the principal study was started. In the principal study we observed the trends of physical development in the first-generation offspring. The study is being continued with the second-generation offspring. In this study the first-generation offspring were kept and observed until spontaneous death. The first-generation offspring were not eliminated (with an exception of individuals for the

histomorphological comparison), and the offspring were kept to longitudinally evaluate the comprehensive physical status. Body weight and additional biometric indices (body length and circumferences) were measured at relevant periods of ontogenesis (young, mature and aged individuals) (*Lang and Wilson, 1993*). We have also observed blood composition dynamics and histomorphological status of the offspring.

Biometric indices

We assessed the offspring's body weight weekly, while other biometric indices such as body length and circumferences (neck, thoracic, abdominal) were measured at 12, 18 and 24 months of age (N=14/group). The biometric indices were measured in accordance with the standard biometric methodology (*Novelli et al., 2007)*. The body weight and body length were used to determine the body mass index (BMI) = body weight (g) / length^2 (cm^2) and Lee index (Lee index = $\sqrt[3]{}$body weight (g) / length (cm). The ratio of abdominal to thoracic circumference was also calculated.

Blood cell morphology and plasma biochemistry analysis

Blood was collected from the tail vein. Full blood count (N=10/group) was performed at 6, 12, 18 and 24 months of age with veterinary haematology analyser *Exigo EOS Vet (Boule Medical* AB, Sweden). The following indices were tested: RBC (erythrocytes), HGB (haemoglobin), HCT (haematocrit), MCV (mean corpuscular volume), MCH (mean corpuscular haemoglobin), MCHC (mean corpuscular haemoglobin concentration), RDW (red cell distribution width), PLT (platelets), MPV (mean platelet volume), WBC (leukocytes), LYM (lymphocytes), MONO (monocytes), NEUTR (neutrophils), EOS (eosinophils).

Fasting blood samples for biochemical blood analysis (N=6/group) were collected at 12 and 24 months of age. Blood samples were centrifuged, and the serum separated and stored at -20ºC and subsequently analysed with veterinary chemistry analyser *Vetscan VS2* (*Abaxis*, Union City, CA, USA) using diagnostic kits for total protein, albumins, globulins, urea, glucose, cholesterol, alanine transaminase and amylase.

Histomorphological analysis

At the 20th month of age randomly selected rats were sacrificed (N=6/group) for the histomorphological comparison. Tissue samples from the following organs were taken: thyroid gland, liver, pancreas, heart, kidney, ovaries, testes, brain, skeletal muscle and retroperitoneal visceral white adipose tissue. The fixed, processed, paraffin-embedded, and sectioned tissue samples were stained with haematoxylin and eosin (HE). Heart samples were stained in *Gomori's* trichrome solution. The adipocyte morphometry in visceral white adipose tissue was assessed (*Peixoto-Silva et al., 2011; Meena et al., 2014)* using digital images at 100 magnification with CellSens software (Olympus). At least fifty adipocytes per animal were measured.

Data analysis

Data analysis was performed using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY). The body weight data were expressed as z-scores according to the formula: $(x-\mu) / \sigma$, where x – the individual rat's weight; μ – the average weight of the control group rats, σ – the standard deviation of the control group rats' weight. Normality was tested by using *Shapiro-Wilk* normality test as well as evaluating asymmetry, excess and Q-Q diagrams. Differences among the groups were compared using the one-way ANOVA followed by *Bonferroni* post-hoc test; if data was not normally distributed a non-parametric analogue – *Kruskal-Wallis* one-way analysis of variance was used. Furthermore, mixed design ANOVA was used to analyse the body weight data over time. Survivability analysis was performed using the *Kaplan-Meier* estimate followed by the *Tarone-Ware* test. The value of p less than 0,05 was considered statistically significant.

RESULTS

The pilot study

The pilot study aimed to determine the long-term consequences of maternal dietary restriction prior to pregnancy and throughout pregnancy on two offspring generations. The first-generation offspring were kept and observed for six months and eliminated from the study after mating, for many other analogous studies were observing the offspring for a relatively short period of time. The second-generation offspring were observed until spontaneous death. As the first-generation of offspring were not observed throughout all of their postnatal ontogenesis – this fact can be considered a limitation of our exploratory pilot study. Therefore, the principal experiment involved a comprehensive observation of two offspring generations for the entire postnatal ontogenesis.

Weight dynamics of the first-generation offspring

The analysis of variance revealed significantly bigger weight of the first-generation male offspring rats of the EG 2 in comparison to other offspring groups since the 5th month of age (Fig. 2, table 2). We did not find any weight-related differences among all first-generation female offspring groups during the first 6 months of life (Fig. 3, table 2).

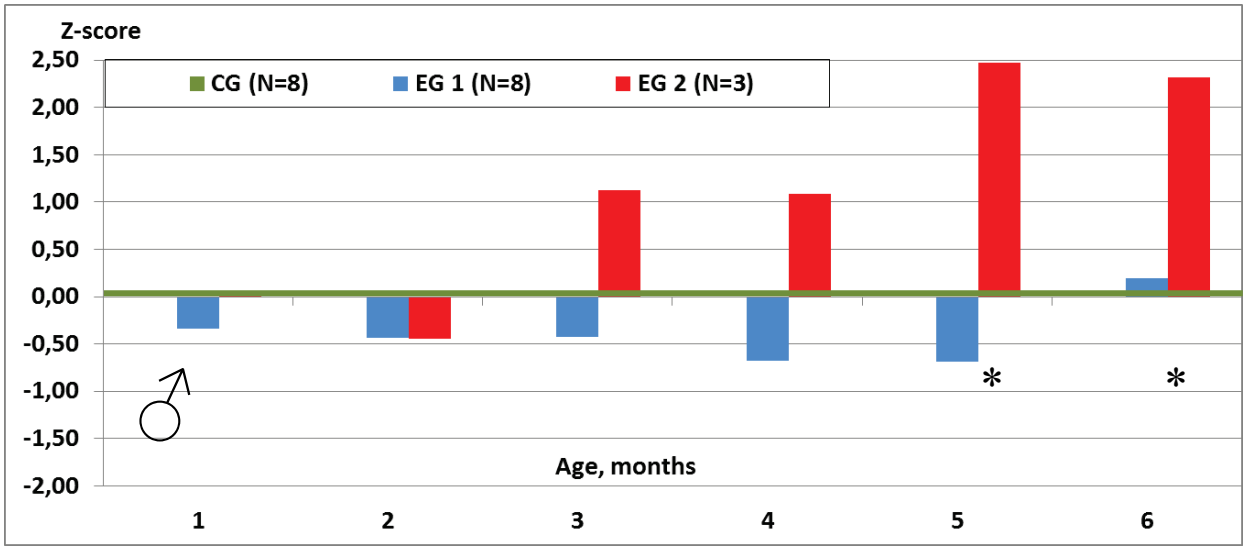

Fig. 2. *Weight dynamics in the first-generation of male offspring rats. Data show z-score means in weight for three experimental rat groups of the first-generation male offspring. The mean z-scores for the food restricted groups (EG 1 – offspring born to pre-pregnancy food restricted mothers, EG 2 – offspring born to pre-pregnancy and pregnancy food restricted mothers; shown in columns) were calculated in relation to the control group's weight. The control group's (CG) mean z-scores for weight in all months are equal to zero (shown as a green line). N – number of offspring. *Indicates statistically significantly different among groups, same sex ($p<0,05$), further statistical information is shown in table 2.*

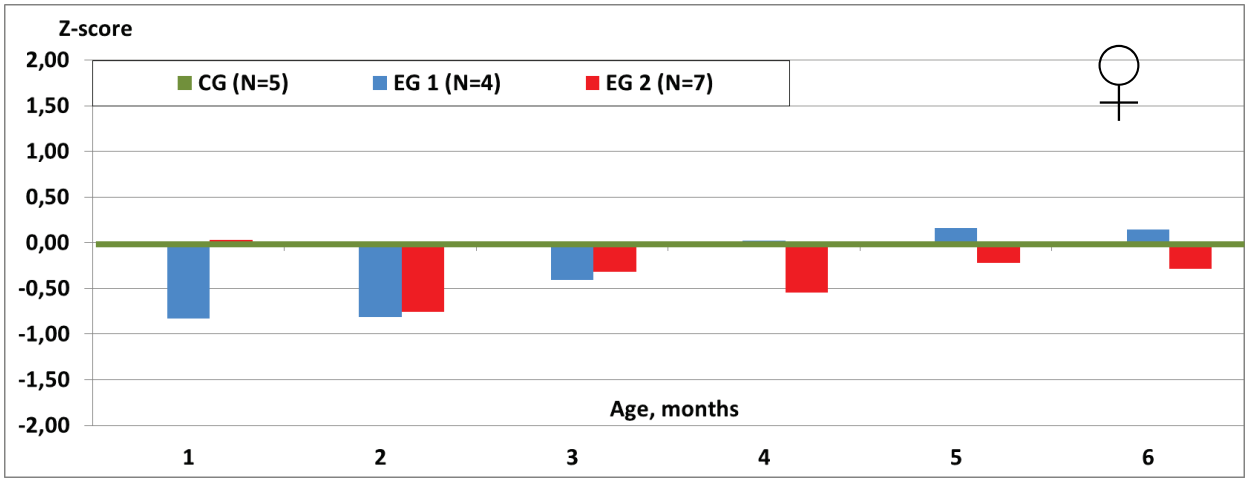

Fig. 3. *Weight dynamics in the first-generation of female offspring rats. Data show z-score means in weight for three experimental rat groups of the first-generation female offspring. The mean z-scores for the food restricted groups (EG 1 – offspring born to pre-pregnancy food restricted mothers, EG 2 – offspring born to pre-pregnancy and pregnancy food restricted mothers; shown in columns) were calculated in relation to the control group's weight. The control group's (CG) mean z-scores for weight in all months are equal to zero (shown as a green line). N – number of offspring.*

Table 2. *Data on the first-generation offspring body weight dynamics.*

Sex	Male offspring				Female offspring			
Age (months)	Mean z-score		F/ χ^2	p	Mean z-score		F/ χ^2	p
	EG 1	EG 2			EG 1	EG 2		
1	-0,33	0,05	0,25	0,78	-0,83	0,03	2,13	0,34[#]
2	-0,43	-0,45	0,38	0,69	-0,81	-0,75	2,52	0,28[#]
3	-0,43	1,13	1,93	0,18	-0,41	-0,32	0,36	0,71
4	-0,67	1,08	2,20	0,14	0,02	-0,54	0,90	0,43
5	-0,69[b]	2,47[ab]	11,75	**<0,01**	0,16	-0,22	0,37	0,70
6	0,20[b]	2,32[ab]	8,67	**<0,01**	0,14	-0,29	0,56	0,59

CG – Control group, EG 1 – offspring born to prior to pregnancy food restricted mothers, EG 2 – offspring born to prior to pregnancy and throughout pregnancy food restricted mothers.

"a" indicates significantly different from the Control group, same sex ($p<0,05$), "b" indicates significantly different from the other food restricted group, same sex ($p<0,05$); [#] indicates that the Kruskal-Wallis test was used, everywhere else – one-way ANOVA followed by Bonferroni post-hoc test was used.

Second-generation offspring

Weight dynamics of the second-generation offspring

Compared to the controls the second-generation EG 2 offspring group had bigger weight in the 1st and the 2nd months of life (Fig. 4, table 3). However, body weight of EG 1 was significantly bigger than that of the control group starting from the 8th up to the 22nd month of life. Besides, the latter offspring group had bigger weight in comparison to EG 2 in the 12th month, also during the 15-20th month of life (Fig. 4, table 3).

There were no differences observed in body weight of the second-generation female offspring rats for almost an entire observation period (Fig. 5, table 3). Female offspring rats from the EG 1 were lighter than those of the control group at 1 month of age only.

Mixed design ANOVA was used to analyse the body weight data over time. For the more accurate interpretation of the results the data for the mixed designed ANOVA was split into intervals based on significant developmental stages of ontogenesis of the laboratory rat (*Lang and Wilson, 1993*): early age (1-4 months), young reproductive age (5-10 months), mature reproductive age (11-18 months) and elderly age (19-23 months). Further analysis was not included as the reduction in sample affected the reliability of the analysis.

The analysis revealed statistically significant effect of food restriction on the growth trajectory of the second-generation offspring ($p<0,05$). The growth trajectory differed among groups ($p<0,001$) and between sexes ($p<0,01$).

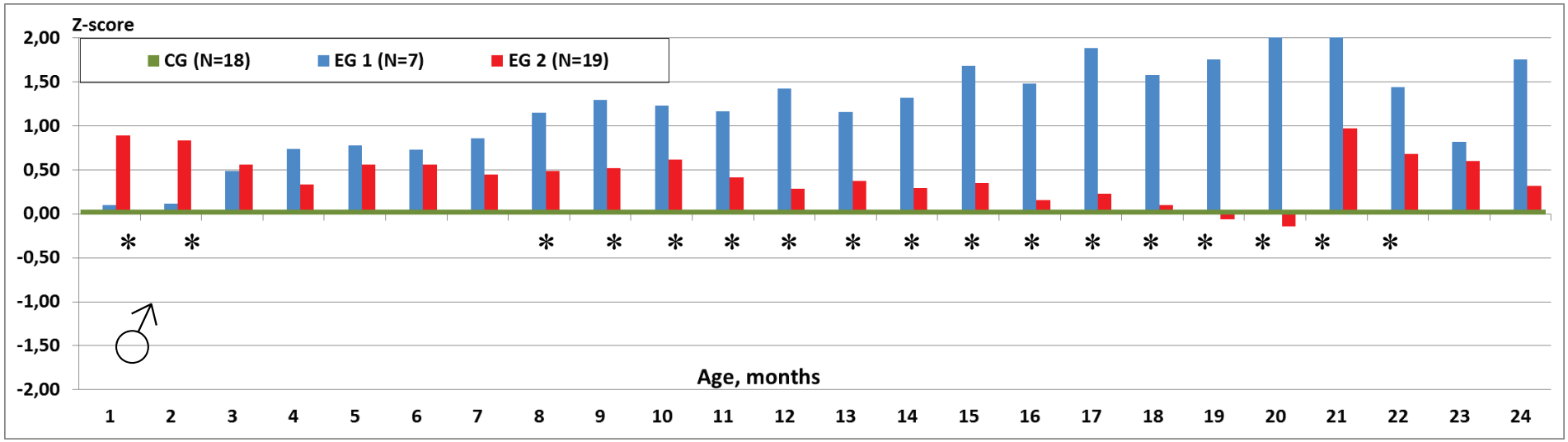

Fig. 4. *Weight dynamics in the second-generation of male offspring rats. Data show z-score means in weight for three experimental rat groups of the first-generation male offspring. The mean z-scores for the food restricted groups (EG 1 – offspring born to pre-pregnancy food restricted mothers, EG 2 – offspring born to pre-pregnancy and pregnancy food restricted mothers; shown in columns) were calculated in relation to the control group's weight. The control group's (CG) mean z-scores for weight in all months are equal to zero (shown as a green line). N – number of offspring. * Indicates statistically significantly different from the Control group, same sex (p<0,05), further statistical information is shown in table 3.*

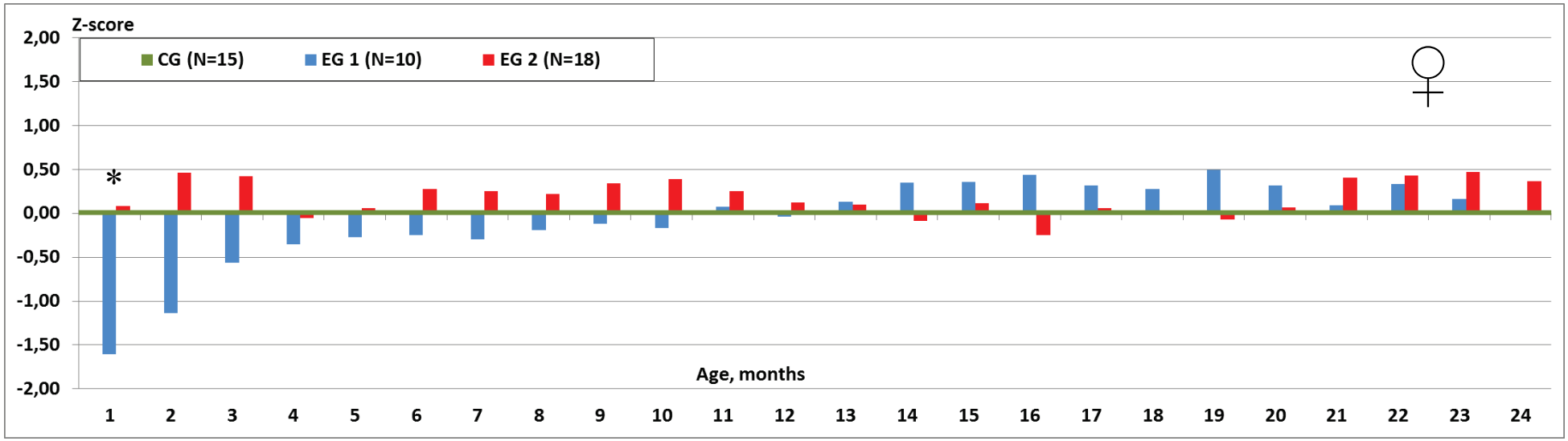

Fig. 5. *Weight dynamics in the second-generation of female offspring rats. Data show z-score means in weight for three experimental rat groups of the first-generation female offspring. The mean z-scores for the food restricted groups (EG 1 – offspring born to pre-pregnancy food restricted mothers, EG 2 – offspring born to pre-pregnancy and pregnancy food restricted mothers; shown in columns) were calculated in relation to the control group's weight. The control group's (CG) mean z-scores for weight in all months are equal to zero (shown as a green line). N – number of offspring. * Indicates statistically significantly different among groups, same sex (p<0,05), further statistical information is shown in table 3.*

Table 3. *Data of the second-generation offspring body weight dynamics.*

Sex	Male offspring				Female offspring			
Age (months)	Mean z-score		F/ χ^2	p	Mean z-score		F/ χ^2	p
	EG 1	EG 2			EG 1	EG 2		
1	0,10	0,89[a]	4,26	**0,02**	-1,61[ab]	0,08[b]	4,32	**0,02**
2	0,12	0,83[a]	4,49	**0,02**	-1,13	0,46	2,69	0,08
3	0,49	0,56	2,12	0,13	-0,57	0,42	1,35	0,27
4	0,73	0,33	2,38	0,10	-0,35	-0,06	0,23	0,79
5	0,78	0,56	5,91	0,05[#]	-0,27	0,05	0,44	0,80[#]
6	0,73	0,56	2,70	0,08	-0,25	0,27	0,91	0,63[#]
7	0,86	0,45	2,54	0,09	-0,30	0,25	1,22	0,54[#]
8	1,15[a]	0,48	3,95	**0,03**	-0,19	0,22	0,63	0,73[#]
9	1,29[a]	0,52	4,70	**0,01**	-0,12	0,34	0,48	0,79[#]
10	1,23[a]	0,61	4,56	**0,02**	-0,17	0,39	0,51	0,61
11	1,16[a]	0,42	3,96	**0,03**	0,08	0,25	0,12	0,89
12	1,42[ab]	0,29[b]	5,45	**0,01**	-0,04	0,12	0,05	0,98[#]
13	1,16[a]	0,37	4,05	**0,02**	0,13	0,10	0,05	0,97[#]
14	1,31[a]	0,29	4,50	**0,02**	0,35	-0,09	0,84	0,66[#]
15	1,68[ab]	0,35[b]	6,10	**<0,01**	0,36	0,11	0,09	0,95[#]
16	1,48[ab]	0,15[b]	4,70	**0,02**	0,43	-0,25	1,97	0,37[#]
17	1,88[ab]	0,23[b]	6,00	**0,01**	0,31	0,06	0,47	0,79[#]
18	1,58[ab]	0,10[b]	5,55	**0,01**	0,28	-0,02	0,09	0,91
19	1,76[ab]	-0,06[b]	5,66	**0,01**	0,49	-0,07	0,46	0,64
20	2,13[ab]	-0,15[b]	4,53	**0,02**	0,32	0,07	0,50	0,78[#]
21	2,08[a]	0,97	6,79	**0,01**	0,09	0,40	0,07	0,96[#]
22	1,44[a]	0,68	4,18	**0,03**	0,33	0,43	0,14	0,93[#]
23	0,82	0,60	1,20	0,33	0,16	0,47	0,22	0,89[#]
24	1,75	0,32	1,32	0,30	0,02	0,36	0,29	0,75

CG – Control group, EG 1 – offspring born to prior to pregnancy food restricted mothers, EG 2 – offspring born to prior to pregnancy and throughout pregnancy food restricted mothers.
"a" indicates significantly different from the Control group, same sex (p<0,05), "b" indicates significantly different from the other food restricted group, same sex (p<0,05); [#] indicates that the Kruskal-Wallis test was used, everywhere else – one-way ANOVA followed by Bonferroni post-hoc test was used.

Survivability analysis of the second-generation offspring

Survival functions differed by gender and experimental group (for males: Fig. 6 and Table 4; for females: Fig. 7 and Table 5).

The second-generation male offspring born to all food restricted "grandmothers" died earlier than the control offspring rats (χ^2=6,23; p=0,04). CG males lived an average of 775 days (approx. 25 months), and the experimental groups – 605 (approx. 20 months) and 715 (approx. 23 months) days for EG 1 and EG 2 respectively. The CG male offspring lifespan significantly differed from the EG 1 (χ^2 = 4,60; p = 0,03), but not from the EG 2 (χ^2 = 1,89; p = 0,17). The longevity between EG 1 and EG 2 did not differ (χ^2 = 3,13; p = 0,08).

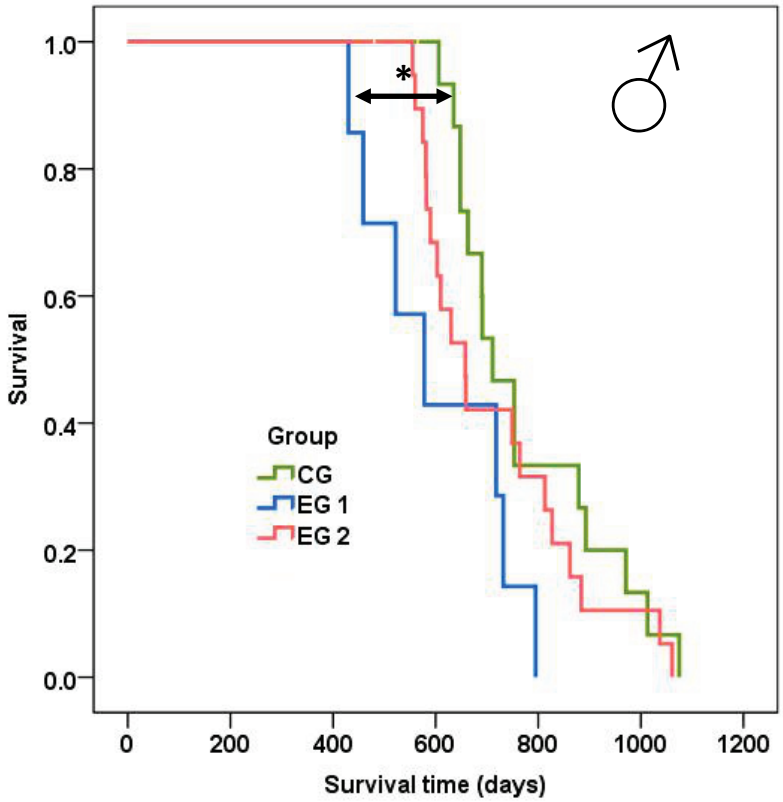

Fig. 6. *Data show survivability indices for three experimental rat groups of the second-generation male offspring. CG – Control group, EG 1 – offspring born to pre-pregnancy food restricted mothers, EG 2 – offspring born to pre-pregnancy and pregnancy food restricted mothers. *Indicates a significant difference between the survival curves (p<0,05).*

***Table 4**. Survivability statistics of the second-generation of male offspring rats.*

Group	Mean				Median			
	Survival (days)	Std. error	95% Confidence Interval		Survival (days)	Std. error	95% Confidence Interval	
			Lower Bound	Upper Bound			Lower Bound	Upper Bound
CG	775,3[a]	39,2	698,5	852,0	711,0	30,4	651,4	770,6
EG 1	604,9[a]	54,5	498,1	711,6	578,0	73,3	434,3	721,7
EG 2	714,8	35,1	646,0	783,6	658,0	35,5	588,3	727,7

CG – Control group, EG 1 – offspring born to pre-pregnancy food restricted mothers, EG 2 – offspring born to pre-pregnancy and pregnancy food restricted mothers. "a" indicates significantly different (p<0,05) from the other group, same sex.

Female offspring survivability (Fig. 7 and Table 5) did not differ among groups (χ^2=5,91; p=0,05). However, it is evident that female offspring from the food restricted groups had tendencies for shorter survival time. CG females lived an average of 916 days (approx. 30 months), and the experimental groups – 761 (approx. 25 months) and 842 (approx. 28 months) days EG 1 and EG 2 respectively.

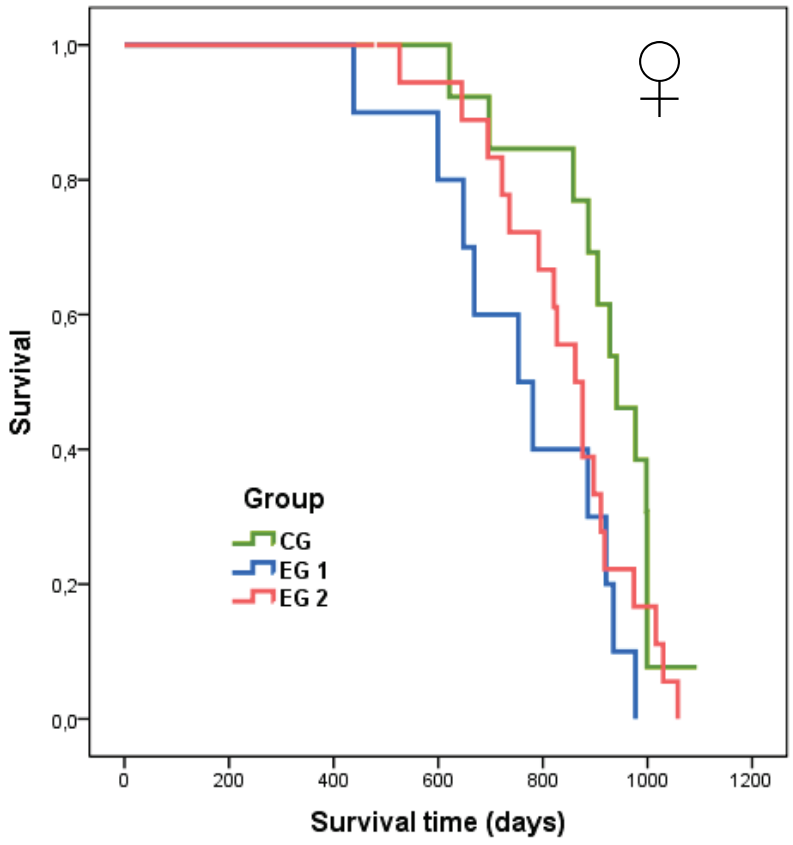

***Fig. 7.** Data show survivability indices for three experimental rat groups of the second-generation female offspring. CG – Control group, EG 1 – offspring born to pre-pregnancy food restricted mothers, EG 2 – offspring born to pre-pregnancy and pregnancy food restricted mothers.*

***Table 5**. Survivability statistics of the second-generation of female offspring rats.*

Group	Mean				Median			
	Survival (days)	Std. error	95% Confidence Interval		Survival (days)	Std. error	95% Confidence Interval	
			Lower Bound	Upper Bound			Lower Bound	Upper Bound
CG	915,8	34,7	847,7	983,8	941,0	43,1	856,4	1025,6
EG 1	760,7	54,8	653,3	868,1	753,0	88,5	579,5	926,5
EG 2	841,9	31,6	779,9	903,8	862,0	34,6	794,1	929,9

CG – Control group, EG 1 – offspring born to pre-pregnancy food restricted mothers, EG 2 – offspring born to pre-pregnancy and pregnancy food restricted mothers.

The principal study

Offspring birth indices

Figures 8, 9 and table 6 show offspring birth indices. Offspring rats from the EG 2 exhibited statistically significantly lower birth weight than other groups (F=48,02; p<0,001). There was no difference in litter sizes between the CG and EG 2. However, mothers from the EG 1 gave birth to significantly fewer offspring than the CG (χ^2=7,38; p=0,03). We have not found difference in the proportion of sexes (female/male) per litter (F=0,50; p=0,95).

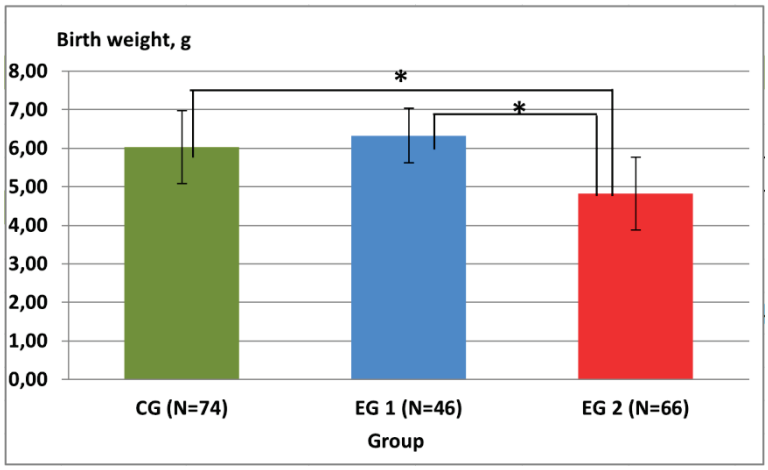

Fig.8. *Birth weight of the first-generation offspring. CG – Control group, EG 1 – offspring born to pre-pregnancy food restricted mothers, EG 2 – offspring born to pre-pregnancy and pregnancy food restricted mothers. Data represent the mean ± SD. *Mean value was significantly different (p<0,05) from the drawn group. N – number of pups.*

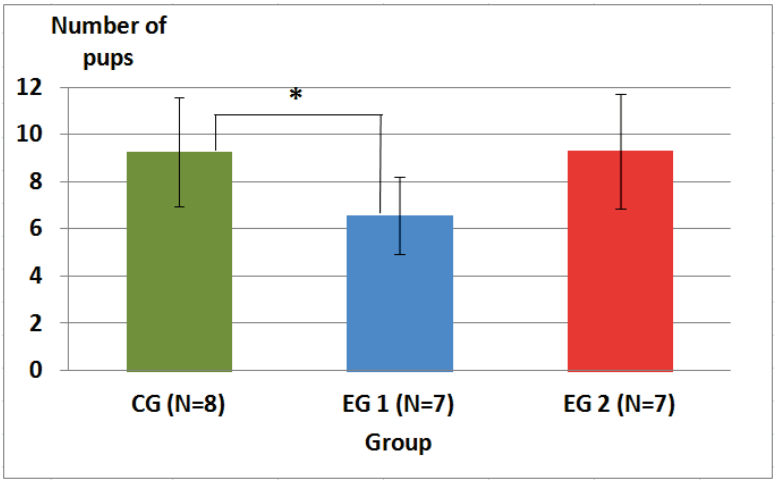

Fig.9. *Litter size of the first-generation offspring. CG – Control group, EG 1 – offspring born to pre-pregnancy food restricted mothers, EG 2 – offspring born to pre-pregnancy and pregnancy food restricted mothers. Data represent the mean ± SD. *Mean value was significantly different (p<0,05) from the drawn group. N – number of maternal rats.*

Table 6. *The birth indices of the first-generation offspring.*

Indice	Group	Mean ± SD	F/ χ^2	p
Birthweight, g	CG	6,03±0,94[a]	48,02	**<0,001**
	1EG	6,33±0,71[b]		
	2EG	4,83±0,95[ab]		
Litter size	CG	9,25±2,32[a]	7,38	**0,03**[#]
	1EG	6,57±1,62[a]		
	2EG	9,29±2,43		
Litter sex distribution (percentage of male pups)	CG	42 %	0,50	0,95
	1EG	46 %		
	2EG	43 %		

CG – Control group, EG 1 – offspring born to pre-pregnancy food restricted mothers, EG 2 – offspring born to pre-pregnancy and pregnancy food restricted mothers. a" indicates significantly different from the Control group, same sex (p<0,05), "b" indicates significantly different from the other food restricted group, same sex (p<0,05); [#]Indicates that the Kruskal-Wallis test was used, everywhere else – one-way ANOVA followed by Bonferroni post-hoc test was used.

Weight dynamics of the first-generation offspring

The study revealed statistically significant weight-related differences in the first-generation male offspring rats (Fig. 10, table 7) born to food restricted mothers in comparison to other offspring groups since the 2nd month of life. The EG 1 males were heavier than the control group during the 2-13th month of life. The EG 2 males, in spite of being born lower in weight, caught up rapidly until weaning, and were significantly heavier than the control group during the 2-6th month of life.

The current study also found weight-related differences among the first-generation female offspring (Fig. 11, table 7). The EG 1 females were statistically significantly heavier than the control group during the 4-6th month of life. No further differences from the control group were observed. However, the female offspring born to the food restricted mothers employed converse growth trajectories. The EG 1 females have been statistically significantly heavier than those of the EG 2 during the 3-12th and the 16-21st month of life.

The mixed design analysis of variance revealed statistically significant effect of food restriction on the growth trajectory of the first-generation offspring ($p<0,05$). The growth trajectory differed among groups ($p<0,01$) and, until the 11th month of age, between sexes ($p<0,01$).

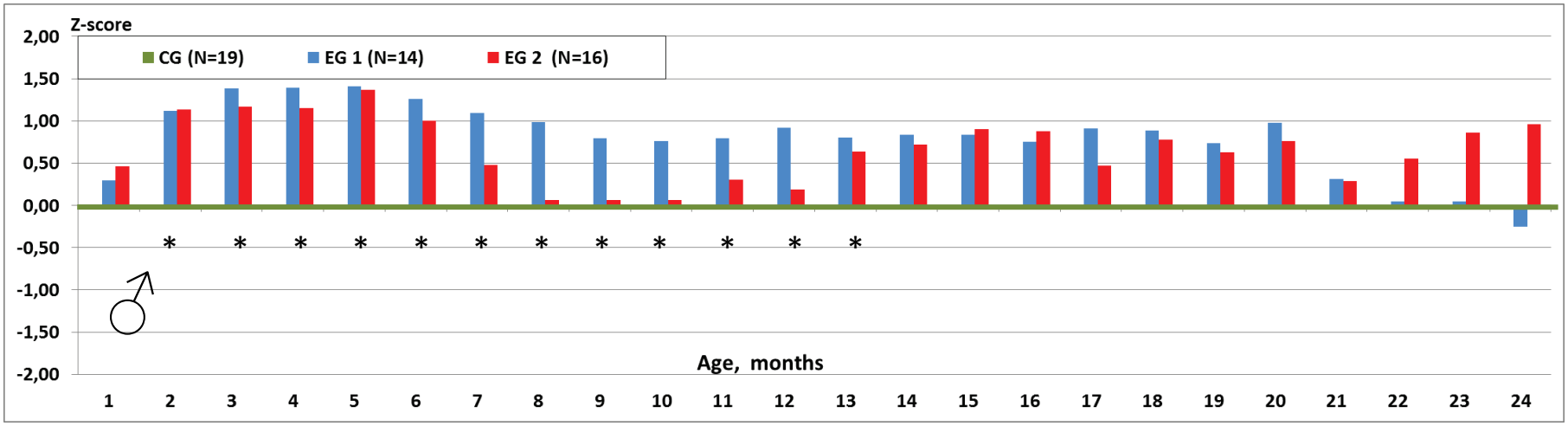

Fig. 10. *Weight dynamics in the first-generation of male offspring rats. Data show z-score means in weight for three experimental rat groups of the first-generation male offspring. The mean z-scores for the food restricted groups (EG 1 – offspring born pre-pregnancy food restricted mothers, EG 2 – offspring born to pre-pregnancy and pregnancy food restricted mothers; shown in columns) were calculated in relation to the control group's weight. The control group's (CG) mean z-scores for weight in all months are equal to zero (shown as a green line). N – number of offspring. *Indicates statistically significantly different from the Control group, same sex ($p<0,05$), further statistical information is shown in table 7.*

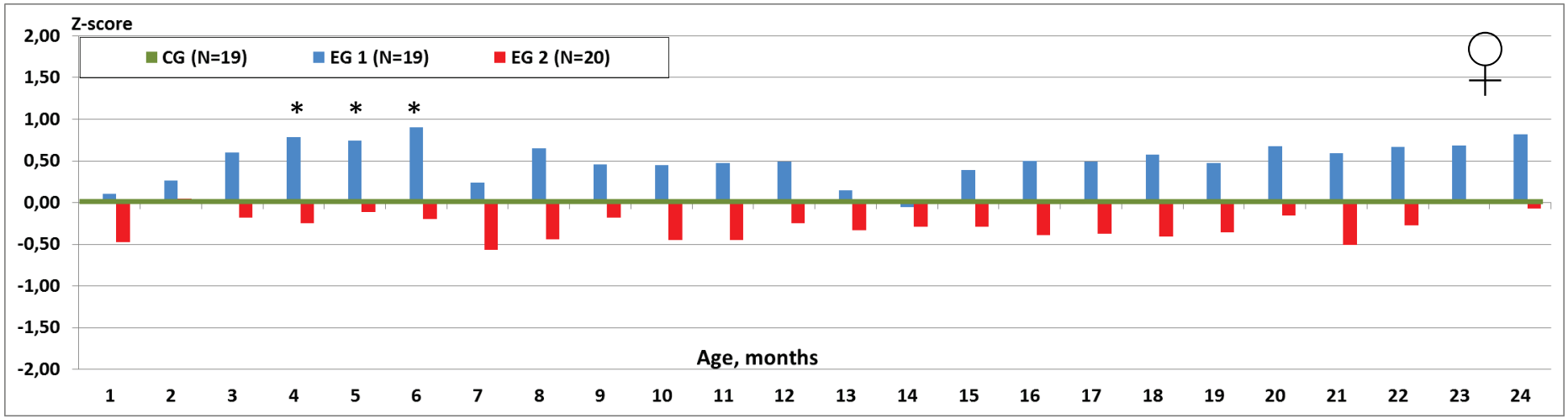

Fig. 11. *Weight dynamics in the first-generation of female offspring rats. Data show z-score means in weight for three experimental rat groups of the first-generation female offspring. The mean z-scores for the food restricted groups (EG 1 – offspring born pre-pregnancy food restricted mothers, EG 2 – offspring born to pre-pregnancy and pregnancy food restricted mothers; shown in columns) were calculated in relation to the control group's weight. The control group's (CG) mean z-scores for weight in all months are equal to zero (shown as a green line). N – number of offspring. * Indicates statistically significantly different from the Control group, same sex ($p<0,05$).*
However the differences between the female offspring from the food restricted groups were statistically significant ($p<0,05$) in almost all periods of life (shown in table 7).

Table 7. *Data of the first-generation offspring body weight dynamics.*

Sex	Male offspring				Female offspring			
Age (months)	Mean z-score		F/ χ^2	p	Mean z-score		F/ χ^2	p
	EG 1	EG 2			EG 1	2EG		
1	0,29	0,47	5,39	0,07#	0,10	-0,48	1,75	0,18
2	1,12a	1,14a	11,50	**<0,01#**	0,27	0,04	0,53	0,59
3	1,38a	1,17a	15,95	**<0,01#**	0,60b	-0,18b	8,53	**0,01#**
4	1,39a	1,15a	13,27	**<0,01#**	0,78ab	-0,25b	11,07	**<0,01#**
5	1,41a	1,37a	16,11	**<0,01#**	0,75ab	-0,12b	11,16	**<0,01#**
6	1,26a	1,00a	14,23	**<0,01#**	0,91ab	-0,19b	13,94	**<0,01#**
7	1,10a	0,48	10,78	**<0,01#**	0,24b	-0,57b	3,78	**0,04**
8	0,99ab	0,07b	9,61	**0,01#**	0,65b	-0,44b	6,42	**<0,01**
9	0,79a	0,06	4,01	**0,02**	0,46b	-0,18b	3,73	**0,03**
10	0,76a	0,06	3,65	**0,03**	0,45b	-0,45b	7,21	**<0,01**
11	0,79a	0,31	3,49	**0,04**	0,48b	-0,45b	16,23	**<0,01#**
12	0,92a	0,19	3,75	**0,03**	0,49b	-0,25b	5,46	**0,01**
13	0,80a	0,64	4,05	**0,02**	0,15	-0,33	1,89	0,16
14	0,84	0,72	2,45	0,10	-0,06	-0,29	0,58	0,56
15	0,83	0,90	3,38	**<0,05**	0,39	-0,29	4,48	0,11#
16	0,75	0,88	4,15	0,13#	0,50b	-0,39b	7,37	**0,03#**
17	0,91	0,47	2,69	0,08	0,50b	-0,37b	8,67	**0,01#**
18	0,89	0,78	3,00	0,06	0,57b	-0,40b	13,25	**<0,01#**
19	0,73	0,63	1,89	0,17	0,48b	-0,36b	9,00	**0,01#**
20	0,98	0,76	2,69	0,08	0,68b	-0,16b	8,99	**0,01#**
21	0,31	0,29	0,32	0,73	0,59b	-0,51b	12,22	**<0,01#**
22	0,04	0,55	0,56	0,58	0,67	-0,27	6,54	**0,04#**
23	0,05	0,86	0,44	0,66	0,69	0,01	3,25	0,05
24	-0,26	0,96	0,60	0,57	0,82	-0,07	2,06	0,15

CG – Control group, EG 1 – offspring born to pre-pregnancy food restricted mothers, EG 2 – offspring born to pre-pregnancy and pregnancy food restricted mothers. "a" indicates significantly different from the Control group, same sex (p<0,05), "b" indicates significantly different from the other food restricted group, same sex (p<0,05); # indicates that the Kruskal-Wallis test was used, everywhere else – one-way ANOVA followed by Bonferroni post-hoc test was used.

Other biometric indices

In addition to body weight, we have also observed other biometric indices such as body length and neck, thoracic and abdominal circumferences at relevant age periods (12, 18 and 24 months of age representing the rat offspring of reproductive, elderly and old age). We have also calculated certain derived variables (body mass index, *Lee* index, abdominal to thoracic circumference ratio).

The male EG 2 offspring had statistically significantly smaller body length, but larger body mass index, *Lee* index and abdominal to thoracic circumference ratio than the control group's male offspring at 12 months of age (Fig.12-15, table 8). No significant biometric indices-related differences were observed in other age periods in male offspring. However, though statistically insignificant, the same tendencies persisted.

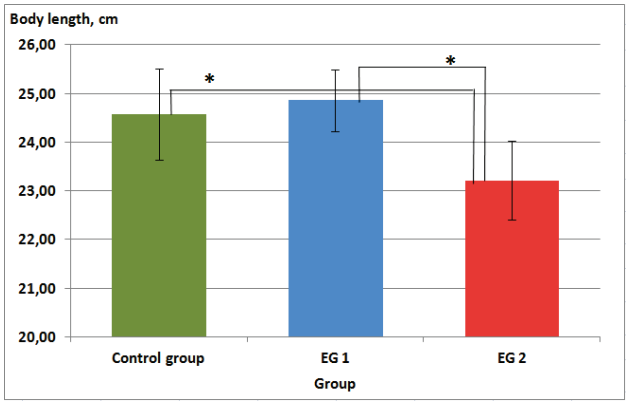

Fig. 12. *Body length of the first-generation male offspring at 12 months of age. CG – Control group, EG 1 – offspring born to pre-pregnancy food restricted mothers, EG 2 – offspring born to pre-pregnancy and pregnancy food restricted mothers. Data represent the mean ± SD. *Mean value was significantly different (p<0,05) from the drawn group.*

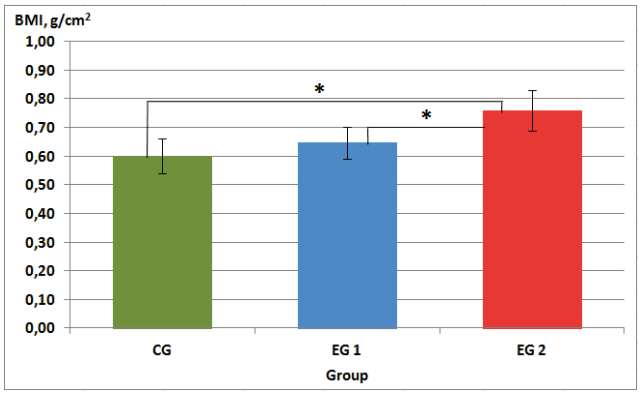

Fig.13. *Body mass index of the first-generation male offspring at 12 months of age. CG – Control group, EG 1 – offspring born to pre-pregnancy food restricted mothers, EG 2 – offspring born to pre-pregnancy and pregnancy food restricted mothers. Data represent the mean ± SD. *Mean value was significantly different (p<0,05) from the drawn group.*

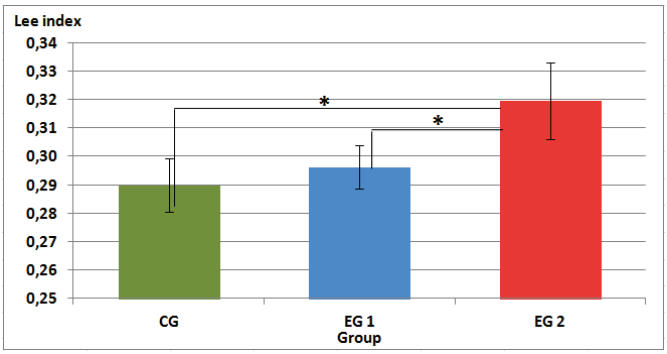

Fig.14. *Lee index of the first-generation male offspring at 12 months of age. CG – Control group, EG 1 – offspring born to pre-pregnancy food restricted mothers, EG 2 – offspring born to pre-pregnancy and pregnancy food restricted mothers. Data represent the mean ± SD. *Mean value was significantly different (p<0,05) from the drawn group.*

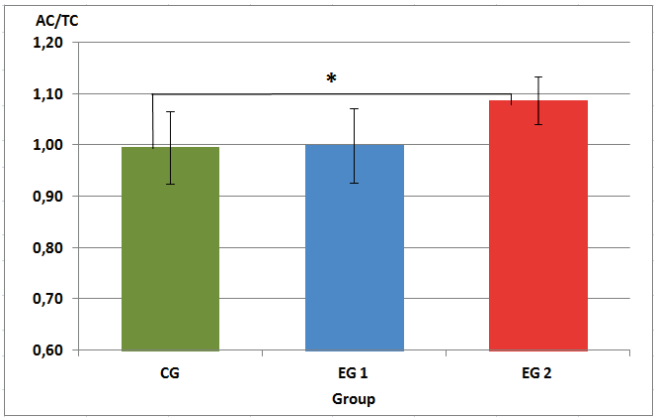

Fig.15. *Abdominal to thoracic circumference ratio of the first-generation male offspring at 12 months of age. CG – Control group, EG 1 – offspring born to pre-pregnancy food restricted mothers, EG 2 – offspring born to pre-pregnancy and pregnancy food restricted mothers. Data represent the mean ± SD. *Mean value was significantly different (p<0,05) from the drawn group.*

***Table 8**. Biometric indices of the first-generation male offspring.*

Indice	Group	Mean ± SD (12 months)	F/ χ^2	p	Mean ± SD (18 months)	F/ χ^2	p	Mean ± SD (24 months)	χ^2	p
Body length, cm	CG	24,57±0,93	10,38	< **0,01**[#]	24,57±0,93	1,06	0,37	24,58±0,38	0,47	0,79[#]
	EG 1	24,86±0,63			24,93±0,79			24,25±1,39		
	EG 2	23,21±0,81			24,29±0,76			24,83±0,76		
Neck circum-ference, cm	CG	10,50±2,10	1,83	0,19	10,07±0,73	0,21	0,82	8,58±0,38	0,62	0,96[#]
	EG 1	10,00±0,41			9,93±0,69			9,08±1,38		
	EG 2	9,21±0,49			9,86±0,45			8,92±0,52		
Thora-cic circum-ference, cm	CG	13,64±1,73	1,55	0,24	13,57±0,93	0,17	0,84	11,17±0,29	1,20	0,55[#]
	EG 1	14,14±1,07			13,32±1,05			11,83±1,26		
	EG 2	13,00±0,58			13,64±1,21			11,92±0,80		
Abdo-minal circum-ference, cm	CG	13,50±1,08	1,31	0,29	14,14±0,94	0,17	0,84	12,08±0,72	2,87	0,24[#]
	EG 1	14,07±0,61			14,43±1,30			11,92±2,50		
	EG 2	14,11±0,57			14,14±0,85			13,62±0,98		
BMI, g/cm^2	CG	0,60±0,06	11,79	< **0,01**	0,76±0,03	0,74	0,49	0,58±0,01	2,40	0,30[#]
	EG 1	0,65±0,06			0,79±0,11			0,62±0,12		
	EG 2	0,76±0,07			0,81±0,08			0,67±0,06		
Lee index	CG	0,29±0,01	15,89	< **0,01**	0,31±0,01	3,30	0,19[#]	0,29±0,01	3,20	0,20[#]
	EG 1	0,30±0,01			0,32±0,02			0,29±0,01		
	EG 2	0,32±0,01			0,32±0,01			0,30±0,01		
Abdo-minal/ Thora-cic circum-ference	CG	1,00±0,07	4,51	**0,03**	1,04±0,06	1,14	0,34	1,08±0,06	3,20	0,20[#]
	EG 1	1,00±0,07			1,08±0,05			1,00±0,12		
	EG 2	1,09±0,05			1,04±0,07			1,14±0,06		

CG – Control group, EG 1 – offspring born to pre-pregnancy food restricted mothers, EG 2 – offspring born to pre-pregnancy and pregnancy food restricted mothers. [#] indicates that the Kruskal-Wallis test was used, everywhere else – one-way ANOVA followed by Bonferroni post-hoc test was used. SD – standard deviation.

As for the female offspring, no significant differences in biometric indices were observed with an exception of the neck circumference at 12 months of age (Fig.16, table 9). The EG 2 female offspring had statistically significantly smaller neck circumference than those from the other groups. Later, no significant differences from CG were observed, however, the EG 2 female offspring demonstrated a tendency for lower values of biometric indices, whereas the EG 1 – the trend for larger biometric indices.

Table 9. *Biometric indices of the first-generation female offspring.*

Indice	Group	Mean ± SD (12 months)	F/ χ^2	p	Mean ± SD (18 months)	F/ χ^2	p	Mean ± SD (24 months)	χ^2	p
Body length, cm	CG	23,29±1,32	2,54	0,28#	23,14±1,49	1,04	0,37	22,42±0,52	0,38	0,83#
	EG 1	22,71±0,99			22,86±0,75			22,67±0,58		
	EG 2	22,14±0,94			22,29±1,04			22,17±1,76		
Neck circum-ference, cm	CG	9,14±0,69	5,35	**0,02**	8,71±0,95	0,27	0,77	7,83±0,52	3,44	0,18#
	EG 1	9,21±0,64			8,71±0,60			8,50±0,66		
	EG 2	8,18±0,66			8,46±0,59			7,58±0,38		
Thora-cic circum-ference, cm	CG	12,36±1,14	1,42	0,27	11,29±0,98	0,15	0,87	11,00±1,00	3,08	0,21#
	EG 1	12,57±0,93			11,32±0,75			11,67±0,76		
	EG 2	11,64±1,14			11,11±0,61			10,42±0,52		
Abdo-minal circum-ference, cm	CG	12,57±1,37	0,55	0,59	12,00±1,08	0,61	0,56	10,92±0,88	4,09	0,13#
	EG 1	12,29±1,41			12,18±0,87			11,75±0,43		
	EG 2	11,89±0,76			11,64±0,80			10,33±1,01		
BMI, g/cm^2	CG	0,55±0,06	2,47	0,29#	0,64±0,07	1,74	0,20	0,59±0,10	3,82	0,15#
	EG 1	0,58±0,04			0,68±0,08			0,66±0,07		
	EG 2	0,59±0,04			0,61±0,05			0,56±0,01		
Lee index	CG	0,29±0,01	2,36	0,12	0,30±0,01	0,75	0,49	0,30±0,02	1,87	0,39#
	EG 1	0,29±0,01			0,31±0,01			0,31±0,01		
	EG 2	0,30±0,01			0,30±0,01			0,29±0,01		
Abdo-minal/ Thora-cic circum-ference	CG	1,02±0,07	0,61	0,55	1,06±0,07	0,49	0,62	0,99±0,01	0,63	0,73#
	EG 1	0,98±0,08			1,08±0,02			1,01±0,04		
	EG 2	1,03±0,11			1,05±0,06			1,00±0,14		

CG – Control group, EG 1 – offspring born to pre-pregnancy food restricted mothers, EG 2 – offspring born to pre-pregnancy and pregnancy food restricted mothers. # indicates that the Kruskal-Wallis test was used, everywhere else – one-way ANOVA followed by Bonferroni post-hoc test was used. SD – standard deviation.

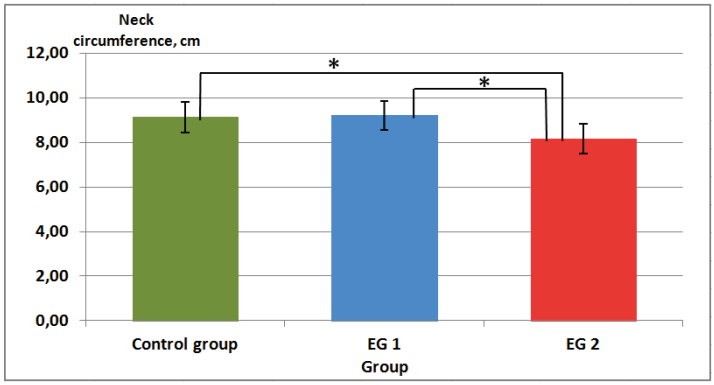

Fig.16. *Neck circumference of the first-generation female offspring at 12 months of age. CG – Control group, EG 1 – offspring born to pre-pregnancy food restricted mothers, EG 2 – offspring born to pre-pregnancy and pregnancy food restricted mothers. Data represent the mean ± SD. *Mean value was significantly different (p<0,05) from the drawn group.*

Blood cell morphology and plasma biochemistry analysis

No significant differences were detected in full blood count analysis of the first-generation offspring (p> 0,05). The indices were within their physiological ranges (data not shown).

Biochemical blood composition was affected by the group of maternal nutrition in a gender specific manner.

The analysis of the male offspring blood biochemical indices (Fig. 17-18, Table 10) showed that the 12-month-old offspring of the EG 1 had lower albumin concentrations than the CG (p <0,05). A similar tendency was also observed in the EG 2 male offspring. Lower blood plasma albumins concentration was also complemented with the tendency for the lower urea concentration in the EG 1 offspring. At the 24 months of age no significant differences among groups were detected, but the EG 1 male offspring had similar blood composition trends to the one-year-old offspring.

Furthermore, at the 12th month of age the fasting glucose concentration in the EG 2 male offspring was significantly lower than that of the EG 1 individuals. However, at the 24th month of age there was a clear, albeit statistically insignificant, upward trend of blood glucose concentration.

The analysis of the female offspring biochemical blood indices (Table 11) did not reveal any statistically significant differences among groups (p> 0,05). Nevertheless, a trend for higher fasting glucose concentration was observed in the female individuals born to food restricted mothers. There was a trend for an increased amylase concentration in the female offspring born to food restricted mothers. Also, cholesterol concentration tended to elevate with age and was higher at 24 months of age compared to 12 months of age.

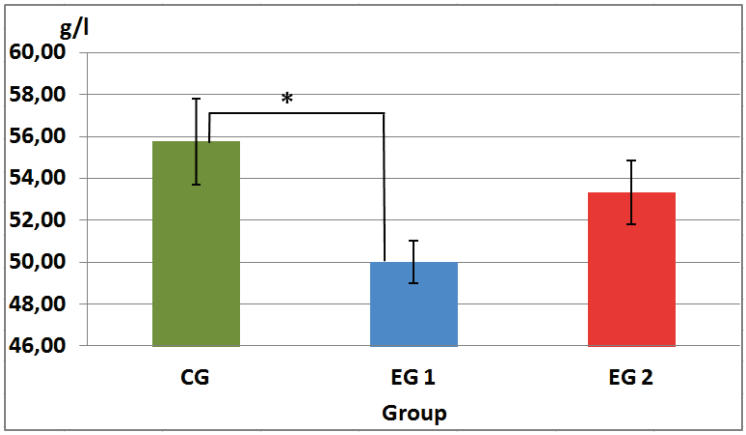

Fig.17. *Albumin concentration of the first-generation male offspring at 12 months of age. CG – Control group, EG 1 – offspring born to pre-pregnancy food restricted mothers, EG 2 – offspring born to pre-pregnancy and pregnancy food restricted mothers. Data represent the mean ± SD. *Mean value was significantly different (p<0,05) from the drawn group.*

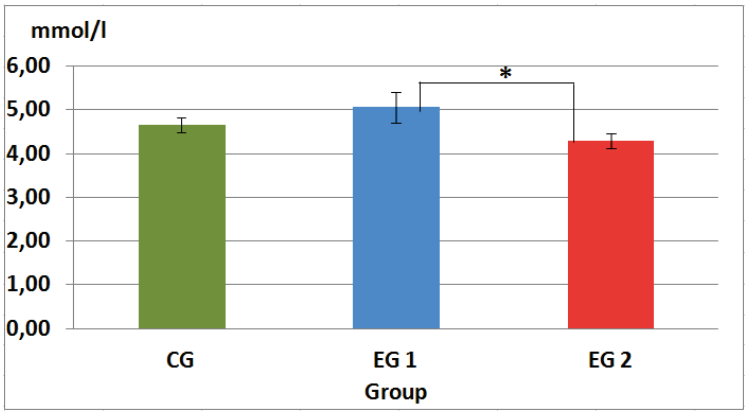

Fig.18. *Fasting glucose concentration of the first-generation male offspring at 12 months of age. CG – Control group, EG 1 – offspring born to pre-pregnancy food restricted mothers, EG 2 – offspring born to pre-pregnancy and pregnancy food restricted mothers. Data represent the mean ± SD. *Mean value was significantly different (p<0,05) from the drawn group.*

Table 10. *Biochemical blood indices of the first-generation male offspring.*

Indice	Group	Mean ± SD (12 months)	χ^2	p	Mean ± SD (24 months)	χ^2	p
Total protein, g/l	CG	81,75±2,22	4,57	0,10	79,67±2,08	0,69	0,71
	EG 1	76,67±3,06			84,33±8,02		
	EG 2	69,67±15,37			82,00±4,36		
Albumins, g/l	CG	55,75±2,06	6,44	**0,04**	43,00±9,64	2,78	0,25
	EG 1	51,00±1,00			37,33±12,74		
	EG 2	53,33±1,53			52,00±5,19		
Globulins, g/l	CG	26,00±3,56	0,07	0,97	36,33±9,24	5,24	0,07
	EG 1	26,00±2,65			47,33±15,04		
	EG 2	26,67±2,52			29,33±1,53		
Urea, mmol/l	CG	4,02±0,96	5,07	0,08	3,63±1,27	1,43	0,49
	EG 1	3,53±0,21			4,80±0,70		
	EG 2	6,33±1,91			4,23±0,65		
Glucose, mmol/l	CG	4,66±0,17	6,62	**0,04**	4,60±0,30	3,52	0,17
	EG 1	5,07±0,35			5,06±1,20		
	EG 2	4,30±0,17			5,73±0,60		
Cholesterol, mmol/l	CG	1,97±0,38	0,69	0,71	2,27±1,02	0,81	0,67
	EG 1	1,80±0,10			2,00±0,56		
	EG 2	2,03±0,40			2,30±0,20		
Alanine transaminase, u/l	CG	48,67±3,79	2,08	0,35	54,33±8,96	1,16	0,56
	EG 1	48,00±15,72			51,67±11,68		
	EG 2	42,33±3,51			46,67±8,51		
Amylase, u/l	CG	718,60±36,56	0,68	0,71	677,67±59,18	1,16	0,56
	EG 1	695,67±90,40			718,33±161,80		
	EG 2	698,33±17,56			780,33±114,61		

CG – Control group, EG 1 – offspring born to pre-pregnancy food restricted mothers, EG 2 – offspring born to pre-pregnancy and pregnancy food restricted mothers. SD – standard deviation. The Kruskal-Wallis analysis of variance was used for comparison.

Table 11. *Biochemical blood indices of the first-generation female offspring.*

Indice	Group	Mean ± SD (12 months)	χ^2	p	Mean ± SD (24 months)	χ^2	p
Total protein, g/l	CG	89,60±11,74	1,95	0,38	77,67±5,69	1,70	0,43
	EG 1	92,67±8,66			82,67±2,52		
	EG 2	83,20±5,85			83,33±2,31		
Albumins, g/l	CG	66,25±12,99	0,27	0,87	44,00±20,88	4,86	0,09
	EG 1	67,20±10,69			62,00±2,00		
	EG 2	67,00±10,39			62,00±3,61		
Globulins, g/l	CG	19,50±3,54	3,86	0,15	33,33±19,86	1,19	0,55
	EG 1	26,00±3,61			20,33±1,53		
	EG 2	19,50±0,71			21,33±2,52		
Urea, mmol/l	CG	4,63±0,69	1,85	0,39	4,37±0,97	0,09	0,96
	EG 1	4,02±0,99			4,27±0,58		
	EG 2	4,88±1,40			4,37±0,70		
Glucose, mmol/l	CG	4,38±0,66	3,55	0,17	4,37±0,64	0,43	0,81
	EG 1	5,03±0,62			4,57±0,40		
	EG 2	5,04±0,21			4,63±0,81		
Cholesterol, mmol/l	CG	1,60±0,37	3,53	0,17	2,13±0,68	2,76	0,25
	EG 1	1,97±0,12			2,50±0,92		
	EG 2	1,53±0,12			3,17±0,45		
Alanine transaminase, u/l	CG	41,75±12,28	2,77	0,25	52,33±17,09	0,07	0,97
	EG 1	42,50±5,82			51,00±14,73		
	EG 2	36,40±5,59			52,67±5,69		
Amylase, u/l	CG	680,00±189,55	0,65	0,72	620,67±149,55	5,42	0,07
	EG 1	725,67±98,44			716,67±48,76		
	EG 2	681,60±150,92			849,33±68,88		

CG – Control group, EG 1 – offspring born to pre-pregnancy food restricted mothers, EG 2 – offspring born to pre-pregnancy and pregnancy food restricted mothers. SD – standard deviation. The Kruskal-Wallis analysis of variance was used for comparison.

The biometric and histomorphological analysis of the first-generation offspring

Table 12 summarizes the relative organ weights (organ weight to body weight ratios) of the first-generation offspring. Testicular and ovarian weights are given in absolute values. Such presentation was chosen on the basis of publications summarizing the appropriate usage of relative and absolute organ weights in experiments *(Bailey et al., 2004; Sellers et al., 2007; Nirogi et al., 2014)*.

There were no significant differences in organ weights among groups ($p>0,05$). Nevertheless, we observed a statistically insignificant trend for larger testes in the offspring born to food restricted mothers.

Table 12. *The relative organ weights of the first-generation offspring.*

Organ	Group	Male offspring	χ^2	p	Female offspring	χ^2	p
Thyroid gland	CG	0,0012	5,60	0,06	0,0011	0,36	0,84
	EG 1	0,0011			0,0013		
	EG 2	0,0006			0,0012		
Heart	CG	0,0037	2,93	0,23	0,0042	0,27	0,88
	EG 1	0,0036			0,0044		
	EG 2	0,0032			0,0047		
Pancreas	CG	0,0020	2,50	0,29	0,0015	2,22	0,33
	EG 1	0,0011			0,0018		
	EG 2	0,0033			0,0013		
Brain	CG	0,0044	3,82	0,15	0,0059	3,47	0,18
	EG 1	0,0041			0,0052		
	EG 2	0,0047			0,0054		
Liver	CG	0,0349	4,82	0,09	0,0353	3,20	0,20
	EG 1	0,0304			0,0322		
	EG 2	0,0337			0,0379		
Visceral fat	CG	0,0480	2,52	0,28	0,0686	1,07	0,59
	EG 1	0,0445			0,0587		
	EG 2	0,0323			0,0606		
Spleen	CG	0,0025	3,61	0,17	0,0024	2,22	0,33
	EG 1	0,0020			0,0023		
	EG 2	0,0024			0,0028		
Kidney	CG	0,0039	0,16	0,92	0,0032	0,62	0,73
	EG 1	0,0038			0,0035		
	EG 2	0,0037			0,0033		
Gonad	CG	1,10±0,56	1,11	0,57	0,10±0,07	0,84	0,66
	EG 1	1,44±0,35			0,10±0,04		
	EG 2	1,55±0,22			0,11±0,02		

CG – Control group, EG 1 – offspring born to pre-pregnancy food restricted mothers, EG 2 – offspring born to pre-pregnancy and pregnancy food restricted mothers. The Kruskal-Wallis analysis of variance was used for comparison.

Liver

The histological analysis of the liver (Fig. 19) revealed that the liver of the first-generation male control offspring corresponded to normal histological view: the plates of hepatocytes were well distinguishable as well as were the sinusoidal capillaries, central veins and triads (Fig. 19A). However, the view of the histological slides of food restricted groups offspring (19B and 19C for EG 1 and EG 2 respectively) was different: plates of hepatocytes and sinusoids were less pronounced and the hepatocytes had lipid droplets. The morphologic appearance of the vacuolated hepatocytes was consistent with both macrovesicular and microvesicular fatty change (steatosis) especially in the centrilobular and intermediate zones of the hepatic acinus.

The histological image of the experimental groups of female offspring liver was similar to that of the control group.

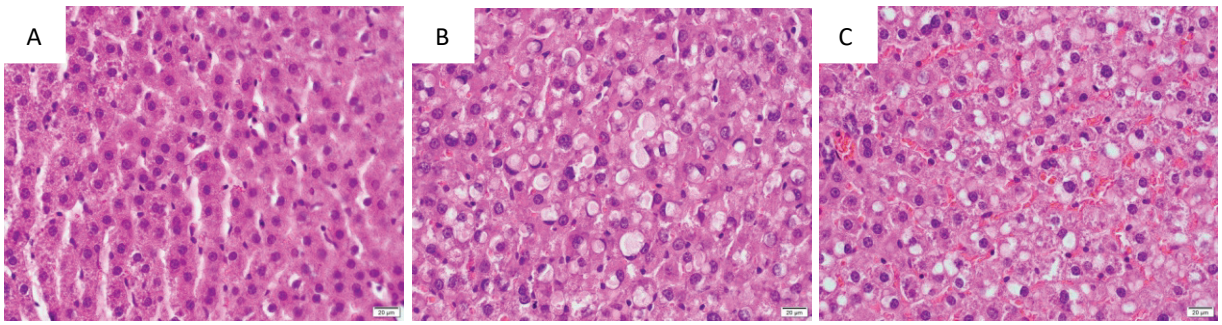

Fig.19. *Photomicrographs of the first-generation male offspring liver at 20 months of age (HE staining, x 400). Images show the liver tissue for three experimental rat groups of the first-generation offspring: A – control group (CG); B – offspring born to pre-pregnancy food restricted mothers (EG 1); C – offspring born to pre-pregnancy and pregnancy food restricted mothers (EG 2). Scale = 20 µm.*

Thyroid gland

Thyroid histological view was characteristic – larger follicles were located in the periphery and the smaller ones were located more centrally. Male CG (Fig. 20A) follicles were lined with cuboidal epithelium, the follicles were filled with colloid, the resorption vacuoles were rare and scarce as well as no hyperplastic epithelial masses among follicles were observed. Among the male offspring of the food restricted groups (Fig. 20B and 20 C for EG 1 and EG 2 respectively) variation in colloid was observed with more condensed colloid in the follicles that was often detached from the follicular wall (marked with brown circle). We have also observed hyperplastic epithelial masses among follicles (marked with a green circle). The EG 2 offspring histology slides (Fig. 20C) revealed less active follicles lined with flattened epithelium (marked with a red circle).

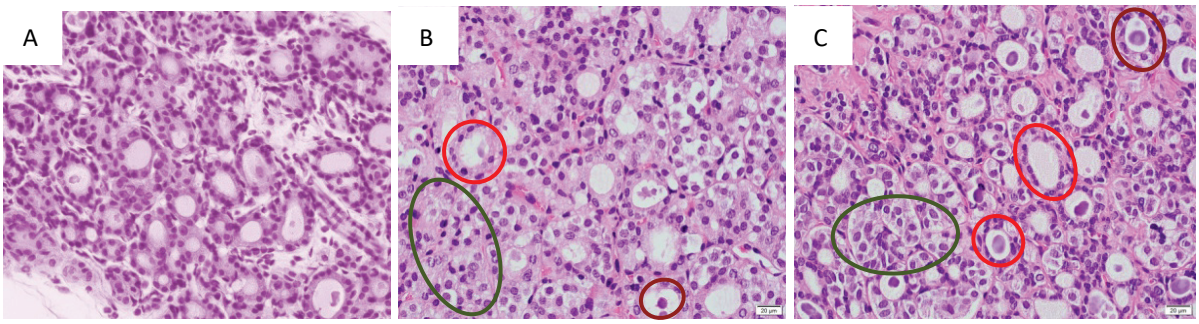

Fig.20. *Photomicrographs of the first-generation male offspring thyroid gland at 20 months of age (HE staining, x 400). Images show the liver tissue for three experimental rat groups of the first-generation offspring: A – control group (CG); B – offspring born to pre-pregnancy food restricted mothers (EG 1); C – offspring born to pre-pregnancy and pregnancy food restricted mothers (EG 2). Scale = 20 µm.*

Female offspring thyroid follicles (Fig. 21) were more active as compared to the male offspring. The follicular cells of the female offspring from the food restricted groups (Fig. 21B, 21C) were lined with higher follicular cells, there was fewer colloid and it was vacuolated (marked with a blue circle). We have also observed hyperplastic epithelial masses among follicles (marked with a green circle). Female offspring of the EG 2 (Fig. 22) had also exhibited large, dilated and degenerated thyroid follicles, abundantly filled with colloid (marked with a yellow circle). Resorption vacuoles in the peripheral parts were also observed.

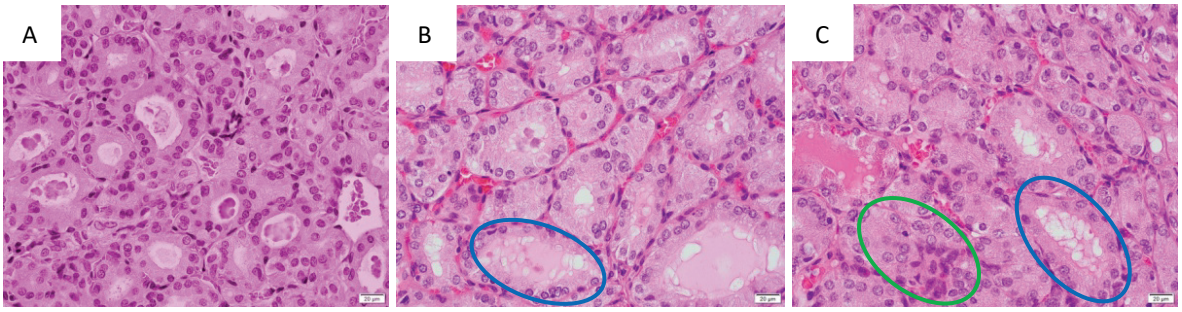

Fig.21. *Photomicrographs of the first-generation female offspring thyroid gland at 20 months of age (HE staining, x 400). Images show the liver tissue for three experimental rat groups of the first-generation offspring: A – control group (CG); B – offspring born to pre-pregnancy food restricted mothers (EG 1); C – offspring born to pre-pregnancy and pregnancy food restricted mothers (EG 2). Scale = 20 µm.*

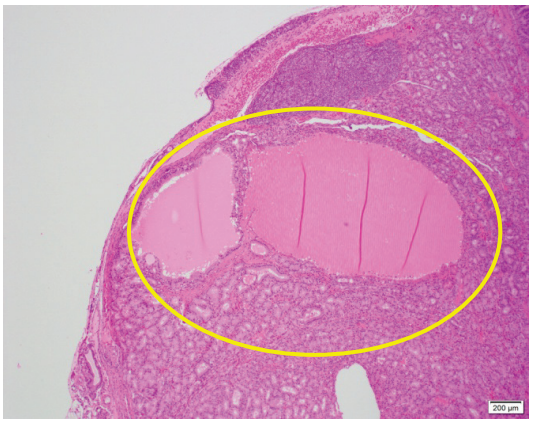

Fig.22. *Photomicrograph of the first-generation female offspring born to pre-pregnancy and pregnancy food restricted mothers (EG 2) thyroid gland at 20 months of age (HE staining, x 100). Scale = 200 µm.*

Pancreas

The analysis of the first-generation male offspring pancreatic tissue (Fig. 23) showed that the endocrine portion of the pancreas (the Langerhans islets) looked similar in all the groups with no abnormalities observed. The exocrine part of the pancreatic tissue appeared normal with no morphological abnormalities found in the lobules, ducts or pancreatic acinar cells. All exocrinocytes were abundantly filled with proenzymes. However, we observed certain differences in the exocrinocytes in relation to the group of maternal nutrient restriction. In the EG 1 (Fig. 23B) we found a slight increase in the amount of lipid droplets, whereas in the EG 2 (Fig. 23C) even more lipid droplets in the cytoplasm of the exocrinocytes were observed.

Meanwhile, an analysis of female histology slides found no differences among groups. The histological view corresponded to control; the lipid inclusions in the cytoplasm of the exocrinocytes were absent or sporadic.

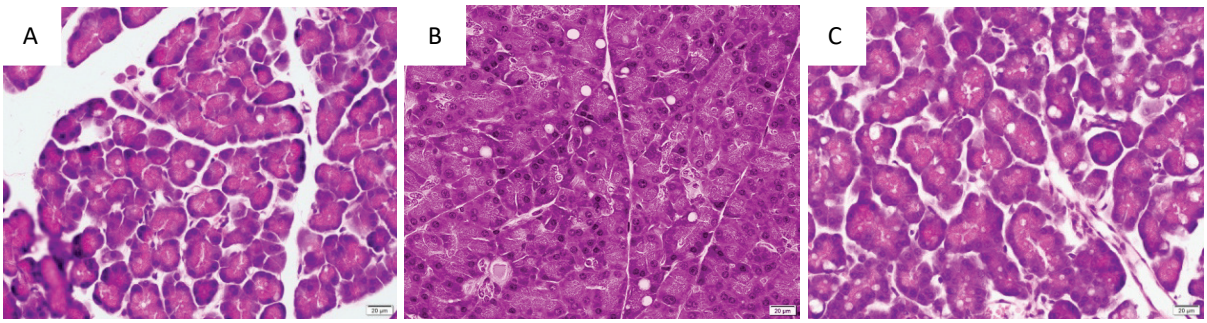

Fig.23. *Photomicrographs of the first-generation male offspring pancreas at 20 months of age (HE staining, x 400). Images show the liver tissue for three experimental rat groups of the first-generation offspring: A – control group (CG); B – offspring born to pre-pregnancy food restricted mothers (EG 1); C – offspring born to pre-pregnancy and pregnancy food restricted mothers (EG 2). Scale = 20 µm.*

Morphometry and histomorphology of the retroperitoneal visceral white adipose tissue adipocytes

A histomorphological analysis revealed that the adipocyte size was affected as a result of maternal undernutrition (Fig. 24, 25). We have observed larger adipocyte size in the food restricted mothers' male offspring: larger mean adipocyte surface area (χ^2 = 124,73; p <0,001), (Fig. 25), mean diameter (χ^2 = 126,66; p<0,001) and maximum diameter (χ^2 = 80,77; p<0,001). The adipocytes from the CG (Fig. 24A) were smaller (mean surface area: 4415,89 ± 1371,01 µm²; mean diameter: 73,39 ± 10,01 µm; maximum diameter: 90,98 ± 17,24 µm) than EG 1 (Fig. 24B) (mean surface area: 6708,21 ± 2002,05 µm², mean diameter: 90,69 ± 13,21 µm; maximum diameter: 109,60 ± 15,27 µm) and EG 2 (Fig. 24C) (mean surface area: 5110,58 ± 1896,78 µm², mean diameter: 78,34 ± 13,89 µm; maximum diameter: 101,08 ± 21,35 µm).
Visceral white adipose tissue adipocytes in the female offspring were not statistically significantly different among groups (p> 0,05).

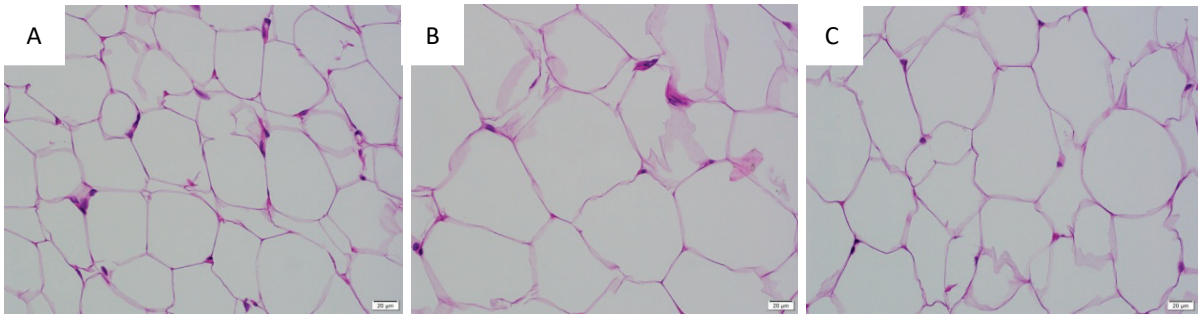

Fig.24. *Photomicrographs of the first-generation male offspring retroperitoneal visceral white adipose tissue at 20 months of age (HE staining, x 400). Images show the liver tissue for three experimental rat groups of the first-generation offspring: A – control group (CG); B – offspring born to pre-pregnancy food restricted mothers (EG 1); C – offspring born to pre-pregnancy and pregnancy food restricted mothers (EG 2). Scale = 20 µm.*

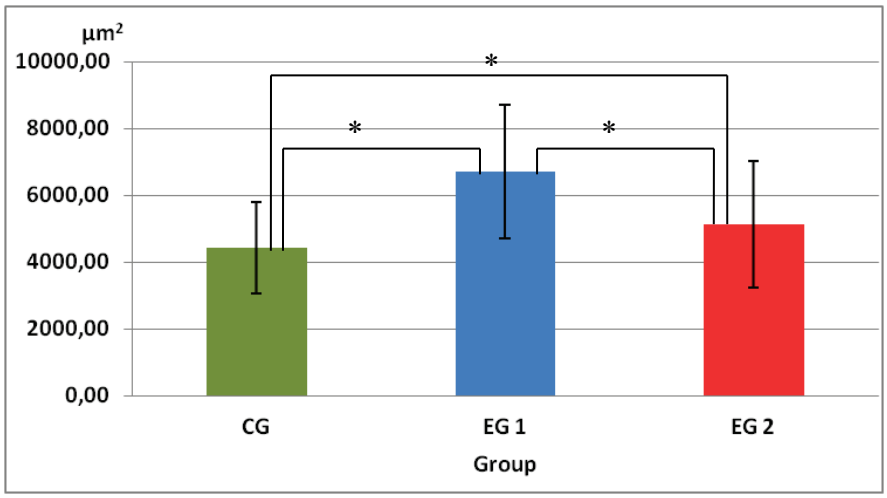

Fig.25. *Surface area of the first-generation male offspring adipocytes at 20 months of age. CG – Control group, EG 1 – offspring born to pre-pregnancy food restricted mothers, EG 2 – offspring born to pre-pregnancy and pregnancy food restricted mothers. Data represent the mean ± SD. *Mean value was significantly different (p<0,05) from the drawn group.*

DISCUSSION OF THE RESULTS

Experimental and epidemiological studies demonstrated a range of metabolic adaptations to ensure conception and childbearing in both human and non-human populations (*Dufour and Sauther, 2002*). It is known that prenatal nutrition is crucially important for subsequent growth and development of the offspring and even mild changes in caloric intake can influence maternal as well as the offspring metabolism towards storage and energetic thriftiness.

Both underweight and overweight before pregnancy can cause similar problems such as inadequate birth weight and subsequent chronic diseases later in life (*Benyshek, 2007; Pinheiro et al., 2008; Yu et al., 2013*). There is a number of studies assessing biometric, hormonal and metabolic changes in several periods of development: pregnancy and lactation. However, the long-term maternal metabolic wellbeing is often overlooked especially in the longitudinal perspective. Hence, the data on the consequences of the developmental programming in the context of aging, longevity and transmittance to future generations is scarce. So, this study examines the physical status for several years of the offspring of both pre-pregnancy and pregnancy food deprived mothers and is still continued on two offspring generations up till spontaneous death.

Birth indices of the first-generation offspring

We have observed lower birth weight in pre-pregnancy and throughout pregnancy food restricted first-generation offspring. This provides clear evidence of the intrauterine growth retardation. Most studies complement our findings (*Garofano et al., 1997; Benyshek et al., 2004, Benyshek et al., 2006; Suzuki et al., 2010; Peixoto-Silva et al., 2011; Ponzio et al., 2012*), however, some did not find such tendencies (*Zambrano et al., 2005; Bellinger et al., 2006; Pinheiro et al., 2008*). Despite low birth weight the litter size and gender distribution of prior to pregnancy and throughout pregnancy food restricted mothers did not differ from the control group. This conclusion is supported by other studies (*Garofano et al., 1997; Zambrano et al., 2005; Bellinger et al., 2006; Pinheiro et al., 2008; Harrison et al., 2009; Suzuki et al., 2010; Peixoto-Silva et al., 2011*).

However, we found another interesting trend. Exclusively prior to pregnancy food restricted mothers gave birth to fewer offspring than the other groups. We have found only one similar study exploring pre-pregnancy protein restriction in rodents, that had also described although statistically insignificant, but a clear trend for smaller litters (*Joshi et al., 2003*). Because the latter group of offspring were born normal-weight, it can be assumed that the metabolic stress that took place for a relatively short period of time determined a proportional allocation of the metabolic resources for a smaller number of individuals. Positive energy balance during the pregnancy period let the offspring to accumulate more nutrients and grow to reach a normal body size. One would assume that this is a compensatory mechanism that enables the compromise of the quality and quantity. A smaller number of offspring would increase the chances of survival and subsequently – the reproductive success. Based on data from rodent experiments, it is known that a large litter size leads to the slower growth in the body by

inhibiting cell division rate and the final cell number in the organs. Contrarily, the smaller litters stimulate the growth and development (*Teicher and Kenny, 1978*).

Meanwhile, if the dietary restriction is continued throughout pregnancy, the compensatory resources are depleted and it becomes impossible to maintain an adequate ratio of the offspring litter size and postnatal physical status. Consequently, the surplus reproduction strategy is implemented – to deliver potentially more offspring than can survive up till the reproductive age in anticipation of favourable postnatal conditions. This paradoxical peculiarity is confirmed by other studies. For example, feeding a low protein diet for a dozen generations of rats still resulted in as much offspring as in the control group (*Galler and Zartarian, 1981*).

Our results showed that sex distribution among groups did not differ. The latter is confirmed by other experiments (*Garofano et al., 1997; Zambrano et al., 2005; Bellinger et al., 2006; Pinheiro et al., 2008; Harrison et al., 2009; Suzuki et al., 2010; Peixoto-Silva et al., 2011*). However, we have also found other results. High in calorie maternal diet can lead to a greater number of male offspring, whereas food restriction during the early stages of gestation can increase the number of female offspring (*Rosenfeld et al., 2003; Fountain et al., 2008; Mathews et al., 2008*). It can be assumed that this is due to the selective prenatal loss of the more sensitive male embryos (*Meikle and Thornton, 1995*).

Peculiarities of offspring body size development in two generations of offspring

The analysis of the offspring growth peculiarities revealed that dietary restriction and gender are important factors for the overall growth of the offspring at both the first and the second offspring generations.

Shortly after weaning, the offspring of prior to pregnancy and throughout pregnancy food restricted mothers caught up and evened (female offspring) or even exceeded the control group in weight after the juvenile age (male offspring). It is sometimes speculated that the catch-up growth might be the cornerstone of many chronic diseases (*Eriksson et al., 1999; Forsen et al., 2000*). However, such reaction may have serious biological considerations. It was found that extremely low birth weight human infants (<750 g), who experienced the catch-up growth, demonstrated a markedly better cognitive development than those who did not catch-up weight (*Claas et al., 2011*). Thus, the neurodevelopment seems to be a priority investment field, with its biological importance way ahead from even the overall metabolic status. After the young reproductive age, no weight differences were found in this group. In spite of balancing the body weight differences, the male offspring exhibited signs of possible chronic diseases such as an enlarged BMI and a tendency towards an increased abdominal circumference predictive of the central fat accumulation. It can be assumed that these offspring could have a higher risk of chronic diseases. Studies describe changes in body mass, insulin sensitivity alterations, adipocyte hypertrophy, cardiovascular defects, leptin resistance and other disorders later in life possibly transmittable across generations (*Zambrano et al., 2005; Benyshek et al., 2006; Pinheiro et al., 2008; Harrison and*

Langley-Evans, 2009; Peixoto-Silva et al., 2011; Ponzio et al., 2012). Interestingly, the offspring from this group were also heavier than the control group at the second-generation for the first months of life. Thus, the trend for an accelerated growth rate is maintained, and, perhaps, transmitted across several offspring generations. As for the female offspring from the same group, they remained tendentiously smaller in size adopting a lower in expenditure energy strategy.

The first-generation male offspring of pre-pregnancy food restricted mothers have continued to be heavier up till the 13th month of age, demonstrating a larger body size strategy that may also carry potential health risks. The female offspring of pre-pregnancy food restricted maternal rats also exhibited a tendency for larger body size throughout ontogenesis and were significantly larger than control group at 4-6 months of age, just around the possible conception time. The latter result is a very clear example of the forethought biological programming directed towards the successful reproduction. It is proposed that the growth of the foetus may be largely affected by factors that have previously determined the mother's size. The deficient maternal metabolic capital before pregnancy could be perceived as the long-term environmental scarcity and induce a thrifty phenotype for several offspring generations.

The majority of other authors did not study nutrition prior to pregnancy and its outlying effects on biometric indices of the second-generation offspring. The present study found that the second-generation male offspring born to pre-pregnancy food deprived "grandmothers" were significantly heavier than those in other groups up till the elderly age. We agree with previous hypotheses that pre-pregnancy undernutrition might exhaust mother's metabolic potential and impair adequate nutritional environment for the offspring (*Wells, 2003; Wells, 2010*). Apparently, mother's body size adjusts to an energy saving regiment as an adaptation for an expected scarce resources environment (*Gluckman et al., 2005; McArdle et al., 2006*). Nevertheless, these conditions change in time of pregnancy since no nutritional deprivation is applied. We presume that mother's organism might be "re-programmed" to live in a nutritionally prosperous environment with a tendency to store energy. Hence, even in the second-generation the male offspring are heavier than those born to the control group.

It is worth to repeatedly discuss the specific growth patterns of the female offspring. Generally, the studies did not find remarkable and long-term differences in the female growth. We found only one study that described larger weight of the prenatally food deprived females (*Bellinger et al., 2006*), whereas in other cases those individuals are even smaller compared to the control group (*Zambrano et al., 2005; Pinheiro et al., 2008*). However, both experimental female groups differed from each other by the choice of a larger (prior to pregnancy food restricted mothers' female offspring) or smaller (prior to and throughout pregnancy food restricted mothers' female offspring) weight gain strategy. It can be assumed that the first group used its metabolic resources in order to ensure greater body size and to optimize the energy balance in case of the potential shortage. Meanwhile, the other experimental group did not have reserves for that, so therefore their body size was reduced. The latter is also evidently illustrated by their smaller neck circumference. In agreement with the previous reports, the present study has not found marked weight differences from the control group in adult second

generation female offspring throughout all the study period (*Benyshek et al., 2004; Zambrano et al., 2005; Pinheiro et al., 2008; Peixoto-Silva et al., 2011*). It is hypothesized, that the female offspring seem to be more adaptive and alleviate some of the damage caused by early undernutrition in previous generations.

The body weight changes are not frequent in moderate caloric restriction models and obesogenic tendencies are usually associated with high-fat postnatal diet or cross-fostering (*Benyshek et al., 2004; Ozanne et al., 2004; Bieswal et al., 2006; Bol et al., 2009*). Similarly, the role of surplus postnatal nutritional environment for the development of obesity is continuously emphasized by developmental anthropologists (*Cameron and Demerath, 2002*). Studies show that feeding the offspring the standard chow may result in lower weight in the first months of life, then the steady catch-up growth and consequently – stable growth along with the control individuals up till the 12^{th} or 18^{th} month of age (*Zambrano et al., 2005; Bellinger et al., 2006*). However, these studies differ from our experiment by the type of dietary restriction (we were limiting the total amount of food rather than its components) and the exposure time (food restriction occurred prior to pregnancy and during pregnancy). Our study shows that even moderate postnatal nutrition can lead to marked differences in the biometric indices of the food restricted groups offspring, especially in males. The described offspring groups still manage to gain weight and exhibit certain morphological and structural alterations up to the cellular level even though raised in relatively well balanced conditions. The changes persist up till the second offspring generation. This shows a long-term influence on the structure and functioning of the vital organs of the developing foetus and could program poor response to surplus nutritional environment.

It is particularly important to note that the biometric changes only hint about the powerful and interconnected infrastructure of the metabolic changes in the offspring associated with maternal dietary restriction. Thus, dietary restriction before conception and during important stages of the early development leads to considerable morphological and physiological changes even in the absence of excess postnatal nutrition. This demonstrates the long-term programming effects on the developing foetus' vital organ structure, function and especially the poor response to excess dietary environment.

One of the unifying results in transgenerational nutrition research is the consequence of malnutrition during lactation. Studies agree that lactation is a crucially important period of development where undernourished offspring can acquire a range of components of metabolic syndrome and some damage caused by undernutrition during gestation can be restored (*Zambrano et al., 2005*). It is argued that moderate protein restriction in the early stages of life (during the lactation period) does not cause obesity or related diseases, but is responsible for underweight and hypoinsulinemia (*Zambrano et al., 2005; Gravena et al., 2007; de Oliveira et al., 2012*). Notwithstanding, with severe restriction, diabetogenic tendencies persist (*Pinheiro et al., 2008*).

Blood composition of the first-generation offspring

In our study, the majority of the morphological and biochemical blood indices compared among the groups were close to the control group levels. Nevertheless, we found a lower albumin concentration in the prior to pregnancy food restricted first-generation male offspring and a similar trend in the other experimental group. The former group has also exhibited a trend for a lower concentration of the protein metabolism product – urea – indicating protein deficiency and possibly perturbations of the protein metabolism and liver function. Serum albumin decline is an informative consequence of maternal nutrient restriction. Unlike fat and carbohydrates, that are stored in the body in the forms of triacylglycerols and glycogen, the proteins do not have marked protein reserves. This means that in conditions of protein deficiency, the albumins will be immediately used for metabolic needs. This peculiarity can be linked to liver histomorphology results that determined hepatocyte dystrophy characterised by the macrovesicular and microvesicular fatty change in the male individuals from the food restricted mothers groups. This means that food restriction *in utero* may have a decisive influence on the development of the liver and protein metabolism in the offspring.

Other authors' results vary and are often concordant. It is agreed that the blood indices largely depend on the period of maternal dietary restriction, its type, offspring strain and age as well as the postnatal nutritional environment. For example, protein restriction prior to pregnancy leads to an increased fasting glucose concentration at the 94th day of age, but no such tendency was observed at the 180th day of age. Interestingly, the cholesterol concentration changed *vice versa* (*Joshi et al., 2003*). Furthermore, some authors demonstrate that dietary restriction during pregnancy may not affect the fasting glucose concentrations of the offspring (*Zambrano et al., 2005; Bieswal et al., 2006; Suzuki et al., 2010; Palou et al., 2015*), while others argue that it might be increased (*Benyshek et al., 2004; Pinheiro et al., 2008; Peixoto-Silva et al., 2011*).

As mentioned earlier, the most evident changes in blood composition are usually found in the high-calorie chow fed individuals. Retrospective studies, analysing populations that underwent *in utero* undernutrition, but did not end up in surplus postnatal environment, often do not observe marked metabolic abnormalities (*Stanner et al., 1997; Moore et al., 2001*). In addition, the concept of the "*metabolically healthy obese*" should also be taken into account. It states that even significantly obese individuals may have perfectly normal blood indices, but it does not necessarily mean an objectively good health status (*Phillips, 2016*). Consequently, the homeostasis of the blood indices is very reliable and stable, so a striking deviation from the physiological norm in adequate postnatal nutritional conditions would signal dramatically significant influence of the prenatal factors on the formation of the offspring phenotype.

Histomorphological indices of the first-generation offspring

We did not find significant differences in organ weights among groups. Other authors usually report no difference (*Bol et al., 2009; Nascimento et al., 2013; Muramatsu-Kato et al., 2015*) or the tendencies for lower (liver, kidney) weight in relation to the control group (*Joshi et al., 2003*). It should be taken into account that other authors are usually

analysing the data from the relatively young (3-9 months) animals, while we investigated elderly individuals.

This experiment also found cellular damage in vital organs important for the metabolic homeostasis. We found lipid droplets in the hepatocytes of the male individuals from the food restricted mothers groups. Our findings are replicated in other studies as well (*Yamada et al., 2011; Nascimento et al., 2013*), especially when offspring are fed high fat chow (*Hyatt et al., 2011; Muramatsu-Kato et al., 2015)*. Interestingly the hepatic and pancreatic enzyme levels remained stable. We did not observe changes in the liver enzyme levels as the concentrations of alanine transaminase remained comparable to that of the control group. Nevertheless, we found protein deficiency in the prior to pregnancy food restricted male offspring, which, together with the hepatocyte fatty change evidently illustrates the potential structural and functional damage of the liver. We have also found the lipid droplets in the exocrinocytes of the pancreas that could lead to pancreatocyte damage (*Matsuda et al., 2014*). Interestingly, the amylase levels were normal. Lipid droplets in the hepatocytes perform the storage function, however, if in excess – the hepatocellular damage might occur (*Willebrords et al., 2015*). The lipid inclusions in the pancreatocytes are also described to lead to the pancreatic degeneration, fibrosis and predisposition to type II diabetes (*Lee et al., 2010; Matsuda et al., 2014*). Scientists attribute these changes to the epigenetic modifications that may lead to metabolic phenotype prone to an encouraged gluconeogenesis and changed lipid metabolism (*Lillycrop et al., 2007; Lillycrop et al., 2008*). Interestingly, hepatocyte steatosis is also replicated in the experimental models of postnatal nutritional restriction (*Makovicky et al., 2011*) or even in women suffering from anorexia (*Rautou et al., 2008*).

The histomorphology analysis of the thyroid gland revealed sexually dimorphic histopathological changes. Food restricted groups of the male offspring were characterized by the epithelial hyperplastic masses between follicles, condensed colloid and the flattened cells lining the follicles in food restricted mothers male offspring. Female offspring thyroid follicles were more active, lined with higher follicular cells, with more and vacuolated colloid in the follicles and also with a larger amount of hyperplastic masses among follicles. Histological data on the *in utero* food restricted offspring thyroid gland is very scarce. Only a few studies described the reduced amount of colloid in the thyroid follicles in the several days old offspring (*Fetoui et al., 2006; Kamel et al., 2012*). Other authors confirm the mentioned findings proposing that maternal dietary restriction may influence the offspring thyroid morphogenesis (*Zeman, 1967; Eguchi et al., 1983*). Offspring can also have lower triiodothyronine (*Anguita et al., 1993; Palou et al., 2015*) and thyroxine (*Martins et al., 2016*) concentrations in the blood plasma. Meanwhile, dietary restriction during the suckling phase may lead to hyperthyroidism (*Lisboa et al., 2012*).

We have not observed harmful fat patterning of increased visceral adipose tissue depots. Results from other studies are discordant: some found it (*Pinheiro et al., 2008; Suzuki et al., 2010; Peixoto-Silva et al., 2011*), while others – did not (*Garofano et al., 1997; Joshi et al., 2003; Bellinger et al., 2006; Thompson et al., 2014*). Although the adipose tissue mass was not enlarged, we have observed an increased adipocyte size in prenatally

restricted male offspring. However, it is generally agreed that for a significant visceral fat accumulation to occur the high-calorie diet is needed (*Cameron and Demerath, 2002*). It should also be noted that we were observing older animals, and with aging a higher proportion of adipose tissue is redistributed towards the periphery and stored there (*Wolden-Hanson, 2010*). The visceral adipose tissue cellular hypertrophy is well described to occur in overweight individuals and is often associated with metabolic illnesses (*Fried et al., 2000*). It is also considered to be more important in this context than the subcutaneous adipose tissue (*Arner et al., 2010; Veilleux et al., 2011*). Adipocyte hypertrophy is a result of an imbalance between fat storage and lipolysis. Studies attribute this phenomenon to a higher transfer of fatty acids to the adipose tissue from the other tissues (*Thompson et al., 2014*).

Survivability indices of the second-generation offspring

The present study has found that the male offspring of pre-pregnancy and pregnancy food restricted mothers have a reduced lifespan compared to those of the control group (*Jennings et al., 1999; Aihie Sayer et al., 2001; Ozanne and Hales, 2004; Langley-Evans et al., 2006*). The comparable data is extremely scarce. Other first-generation studies complement our findings that animals undernourished *in utero* experience catch-up growth in later life and die younger than the control animals, in particular, postnatally overfed individuals – their longevity can be reduced by as much as 50% (*Ozanne and Hales, 2004*). In contrast, those fed normally *in utero*, but exposed to protein restriction postnatally, have an increased longevity (*Jennings et al., 1999; Ozanne and Hales, 2004*). The authors explain that the shorter life span is determined by oxidative stress damage and telomere shortening (*Jennings et al., 1999; Langley-Evans et al., 2006).*

From a biological perspective, foetal growth and development patterns are modelled with a very mathematical logic where one should choose between reproduction and longevity. Only part of the overall metabolic budget can be devoted to growth. If destructive factor is not chronic, a catch-up growth is induced and the body is returned to its own growth path (*Prader et al., 1963*) or even gets insured with trends towards the greater energy storage. However, energy is a limited resource, so the compensatory mechanisms and growth acceleration carry anticipated costs such as an increased risk of chronic diseases and earlier aging (*Cameron and Demerath, 2002*). The argument for these compensatory interventions is balance as the possible damage can be distributed to all organ systems. Without the latter, the “non essential” functions such as growth and reproduction would be sacrificed. Meanwhile, when planning the metabolic compromise in advance it is possible to distribute structural and functional disadvantages to all organ systems in a balanced manner (*Kuzawa, 2005*).

This study is one of the few experimental studies that analysed the influence of maternal undernutrition longitudinally and not only during pregnancy but also prior to pregnancy on the physical status of the growing individual. In addition, the experiment aimed for both the acute and remote changes of the physical status in two offspring generations. Many dietary restriction models describe sexually dimorphic effects for the offspring. It is questionable whether better female offspring physical development indices are

observed because of the optimal response and adaptations of the female sex itself or because of the different maternal investment into different sexes. There is no clear answer to this question and most likely both components are involved and important for the growth and development of the offspring.

Inadequate nutrition at pre-pregnancy and pregnancy can cause both – acute as well as long-term consequences manifesting in the course of ontogenesis. The role of the growth programming in the regulation of growth and aging is a much more complicated process than it was thought up until now and all the aftereffects can often be unstable and unpredictable. Maternal undernutrition prior to pregnancy or throughout pregnancy programs the offspring to act in energy saving regimen at the first and second offspring generations. The organism prepares a wide range of possible adaptations even at the histomorphological level that can manifest in biometric, blood and other metabolic contexts. *In utero* initiated adaptations prepare the progeny for a potential recurrence of the unfavourable nutritional ecology and may be subtle or more pronounced. Unfortunately, these adaptations may be disastrous if the prediction about the postnatal environment is not correct and the offspring appears in the highly metabolically rich environment. Therefore, the greatest damage of the programmed effects of these reactions may become evident in excess energetic conditions.

To summarize, maternal caloric restriction might result in the alteration of the offspring growth, metabolism and tissue development. The effect is sex specific and the consequences are more evident in males. Caloric restriction at pre-pregnancy only and at pre-pregnancy and pregnancy may induce different offspring growth strategies. Changes in body mass and tissue development demonstrate physiological adaptation towards energy storage.

CONCLUSIONS

1. **The pilot study** revealed the differences in offspring growth and longevity that depended on offspring sex, age and the period of maternal food restriction:
   a) The second-generation male offspring born to pre-pregnancy food restricted mothers weighted more than the control group at the reproductive and elderly age. No significant weight differences were observed in the first-generation offspring.
   b) The first-generation male offspring born to pre-pregnancy and pregnancy food restricted mothers weighted more than the control group at young reproductive age, whereas the second-generation offspring were heavier than the control group at puberty.
   c) Female offspring demonstrated balanced growth that did not differ among groups.
   d) The second-generation male offspring born to pre-pregnancy food restricted mothers had shorter lifespan than the control group. Similar tendencies were observed in other food restricted mothers' offspring.
2. **The principal study** revealed the differences in the first-generation offspring growth that depended on offspring sex, age and the period of maternal food restriction:
   a) Maternal undernutrition prior to pregnancy and throughout pregnancy resulted in lower offspring birth weight and further catch-up growth, whereas maternal undernutrition prior to pregnancy resulted in fewer litters.
   b) Prior to pregnancy food restricted mothers' male offspring weighted more than the control group at puberty and reproductive age.
   c) Prior to pregnancy and throughout pregnancy food restricted mothers' male offspring weighted more than the control group at puberty and young reproductive age periods as well as they had enlarged body mass and a tendency towards an increased abdominal circumference predictive of the central fat accumulation.
   d) The female offspring grew similarly to the control group, but maternal dietary restriction induced distinctive offspring growth strategies. Prior to pregnancy food restricted mothers' female offspring gained more weight than other groups at the young reproductive age (period of potential fertilization) and maintained a trend for a bigger weight throughout ontogenesis. Prior to pregnancy and throughout pregnancy food restricted mothers' female offspring employed a more subordinate growth strategy and maintained a trend for a smaller body size throughout ontogenesis.
3. **The principal study revealed** that the majority of the first-generation offspring blood cell morphology and plasma biochemistry analysis indices were within the physiological norm and did not differ from the control group. However, we found a lower blood plasma albumin concentration and a tendency for a lower urea concentration in the male offspring born to pre-pregnancy food restricted mothers at the mature reproductive age. The other food restricted group demonstrated similar albumin concentration trends. The aforementioned changes along with the vesicular steatosis in the hepatocytes may indicate perturbations in the protein metabolism and the liver function.

4. **The principal study revealed** the histomorphological changes in the first-generation offspring born to mothers from the food restricted groups:
   a) The male offspring exhibited tendencies for fat storage in hepatocytes and pancreatocytes while the female offspring did not.
   b) The male offspring exhibited morphological changes in thyroid follicles characteristic to hypofunction, whereas the female offspring – morphological follicular changes with features of degeneration.
   c) The male offspring exhibited hypertrophy of the visceral white adipose tissue cells while the female offspring did not.

   The latter differences in the histomorphological structure may indicate a greater stability of the female offspring in the case of nutritional perturbations.

SUMMARY IN LITHUANIAN

Darbo aktualumas ir reikšmė

Individo sveikatai svarbūs ne tik jo aplinkos ir gyvensenos veiksniai, bet ir ankstyvoji raida, ypač kritiniais augimo tarpsniais, tokiais kaip gemalo ar vaisiaus laikotarpis. Be to, palikuonio augimą ir fizinę būklę lemia ilgalaikė motinos medžiagų apykaitos išteklių būklė, todėl būtina nagrinėti motinos mitybą dar iki nėštumo. Temos aktualumą patvirtino ir atliktas žvalgomasis empirinis tyrimas, kurio rezultatai atskleidė tolimąsias mitybos ribojimo iki nėštumo (vaikingumo) pasekmes net antrojoje palikuonių kartoje.

Pastaruoju metu yra daug duomenų apie motinos mitybos pobūdžio per nėštumą sąsajas su tam tikromis ligomis (pvz., antsvoriu, cukriniu diabetu, širdies ir kraujagyslių ligomis bei kitomis sveikatos problemomis). Tačiau stinga tyrimų, kurie nagrinėtų motinos mitybos iki nėštumo ryšį su bendra vaiko sveikatos būkle. Motinos mitybos iki nėštumo ryšį su antrosios ar dar vėlesnių kartų palikuonių fizine būkle nagrinėja vos kelios studijos. Nustatyta, kad maisto stoka per nėštumą gali lemti kelių palikuonių kartų medžiagų apykaitos pokyčius. Moksliniai tyrimai aprašo įvairias *in utero* mitybos ribojimo pasekmes palikuoniui: mažas gimimo svoris, vėlyvas lytinis brendimas ir ankstesnis reprodukcinis senėjimas, endokrininės ir nervinės medžiagų apykaitos reguliacijos pokyčiai, nutukimas, neadekvatus imuninis atsakas, didesnė rizika sirgti lėtinėmis ligomis suaugus ir kognityvūs ar net cirkadinio rimto pokyčiai, kurie gali būti perduodami ne tik pirmajai palikuonių kartai, bet ir ateities kartoms.

Nepakankamos mitybos problema aktuali visame pasaulyje: 1 iš 9 žmonių (795 milijonai) kenčia badą, o kalbant apie vaikus, šis santykis siekia 1 iš 4. Deja, šis klausimas aktualus ir aukšto pragyvenimo lygio šalyse, kur klesti itin liekno kūno kultas, kai net ir normalaus svorio jaunos moterys dažnai laikosi dietų. Kaip minėta, dauguma tyrimų išskirtinį dėmesį teikia *in utero* aplinkai, o motinos metabolinio potencialo raidos laikotarpis – sąlygos ir fizinė būklė iki nėštumo – mažai tyrinėjami. Atliktos vos kelios tokio pobūdžio studijos. Tyrimai sieja didelį svorį iki nėštumo su nėštumo rizikomis, makrosomija, vaiko antsvoriu ir susijusiomis ligomis. Nepakankamas motinos svoris aprašomas retai, bet gali lemti priešlaikinį gimdymą, mažą naujagimio gimimo svorį ar būti ankstyvos jo mirties priežastimi. Tolesnės pasekmės palikuoniui lieka mokslinio intereso užribyje – yra vos keletas tikslinių tyrimų, aprašančių šio laikotarpio svarbą.

Be to, nors ir pakankamai tyrinėjamas, šis reiškinys retai analizuojamas viso gyvenimo metu, vadinasi, galimos pasekmės palikuoniui yra aprašomos neišsamiai ir nepakankamai. Didžioji dalis medžiagų apykaitos pokyčių gali išryškėti arba, atvirkščiai, organizmo adaptaciniams ir kompensaciniams mechanizmams veikiant, normalizuotis antrojoje gyvenimo pusėje. Reikia pažymėti, kad esami tyrimai dažniausiai nagrinėja tik nėštumo sąlygų įtaką, atliekami surinkus mažas tiriamųjų imtis ir galimus medžiagų apykaitos pokyčius tiria fragmentiškai – eksperimentiniai gyvūnai sunaudojami vos keletui žymenų ištirti ir nesudaromas išsamus medžiagų apykaitos pokyčių vaizdas. Vadinasi, brandaus amžiaus individų medžiagų apykaitos pokyčius stebėti tikslinga moksline ir etine prasme. Be to, reikšmingų rezultatų gaunančios studijos yra

publikuojamos dažniau, todėl patikimą ryšį tarp prenatalinės gerovės ir sveikatos būklės suaugus įvertinti sunku.

Skirtingi atliktų tyrimų rezultatai ir hipotezių nepakankamumas reikalauja holistinio ir ilgalaikio fizinės būklės ir medžiagų apykaitos pokyčių įvertinimo. Todėl šiuo tyrimu buvo siekiama nustatyti motinos mitybos stokos ir kelių kartų palikuonių fizinės būklės sąsajas iki spontaninės žiurkių palikuonių mirties ir visapusiškai įvertinti bei apibendrinti biometrinius žiurkių palikuonių augimo, morfologinius bei kraujo rodiklių pokyčius, atspindinčius maisto medžiagų ribojimo pasekmių raišką skirtingais amžiaus tarpsniais.

Darbo naujumas

Tyrimu siekiame kompleksiškai ištirti motinos mitybos lemtus kelių kartų palikuonių biometrinius, kraujo sudėties, histomorfologinius ir gyvenimo trukmės pokyčius. Šis tyrimas skiriasi nuo kitų mitybos ribojimo laikotarpiu (buvo ištirti motinų, kurioms iki vaikingumo ir jo metu ribotas maistas, palikuonys), trukme (stebėtos dviejų palikuonių kartų žiurkės iki natūralios mirties) ir kompleksiškumu (vertinti kūno biometriniai rodikliai, kraujo sudėtis, organų morfologiniai rodikliai).

Darbo tikslas ir uždaviniai

Tikslas: įvertinti nepakankamos motinos mitybos iki vaikingumo bei jo metu ir dviejų kartų palikuonių fizinės būklės (biometrinių, morfologinių ir medžiagų apykaitos rodiklių) kitimų ryšį skirtingais ontogenezės laikotarpiais.

Uždaviniai:

1. Nustatyti bendrąsias žiurkių palikuonių kūno svorio ir gyvenimo trukmės tendencijas dviejų palikuonių kartų žvalgomuoju tyrimu.
2. Išnagrinėti kūno svorio ir papildomų pirmosios kartos žiurkių palikuonių kūno biometrinių rodiklių kitimo tendencijas.
3. Išnagrinėti pirmosios kartos žiurkių palikuonių kraujo sudėties rodiklių kitimo tendencijas.
4. Išnagrinėti pirmosios kartos žiurkių palikuonių histomorfologinių rodiklių pokyčius.

Tyrimo medžiaga ir metodai

Tyrimas buvo atliktas 2010-2016 metais. Jis vyko dviem etapais:

• Žvalgomasis dviejų kartų palikuonių (N = 122) svorio pokyčių ir išgyvenamumo stebėjimas (baigtas ir publikuotas). Žvalgomasis tyrimas parodė temos aktualumą ir tolimąsias pasekmes palikuonių fizinei būklei.
• Pagrindinis išsamus pirmosios kartos palikuonių (N = 107) fizinės būklės stebėjimas (darbas tęsiamas toliau su antrosios kartos palikuoniais).

Žvalgomuoju tyrimu siekėme nustatyti bendrąsias svorio ir gyvenimo trukmės tendencijas, būdingas mitybos ribojimą patyrusių motinų palikuonims, o pagrindiniame tyrime išsamiai stebėjome pirmosios kartos palikuonis ir detalizavome biometrinius, kraujo sudėties ir histomorfologinius fizinės būklės pokyčius. Tiek žvalgomajame, tiek pagrindiniame tyrime motininėms patelėms taikėme 50 proc. mitybos ribojimą tik iki vaikingumo (nėštumo) arba iki vaikingumo ir per vaikingumą. Tuo pačiu metu buvo augintos ir kontrolinės grupės patelės, tačiau jų mityba nebuvo ribota. Skirtingų grupių žiurkės buvo augintos šiomis sąlygomis:

1. I eksperimentinės grupės (1 EG) žiurkėms buvo 50 proc. ribotas maistas 1 mėnesį iki vaikingumo.
2. II eksperimentinės grupės (2 EG) žiurkėms buvo 50 proc. ribotas maistas 1 mėnesį iki vaikingumo ir visą vaikingumo laikotarpį.
3. Kontrolinė grupė (KG) buvo maitinama, atsižvelgiant į pašaro gamintojų rekomendacijas.

Palikuonių mityba buvo įprastinė.

Visos procedūros buvo suderintos su Valstybine maisto ir veterinarijos inspekcija ir gautas leidimas atlikti eksperimentus su gyvūnais (Nr. 0211, 2009 ir Nr. G2-20, 2015).

Stebėjome šiuos palikuonių rodiklius:

- Žvalgomajame tyrime: kūno svorio ir gyvenimo trukmės tendencijas.
- Pagrindiniame tyrime:
  - *Biometrinius rodiklius*: kūno svorį (kartą per savaitę), kūno ilgį ir apimtis (12, 18, 24 mėnesiais: reprodukcinio, pagyvenusio ir senyvo palikuonims);
  - *Kraujo rodiklius*: atlikome bendrąjį kraujo tyrimą (N=10/tyrimo etapui): 6, 12, 18, 24 gyvenimo mėnesiais, ir biocheminį kraujo tyrimą (N=6/tyrimo etapui): 12 ir 24 gyvenimo mėnesiais (albuminai, alanintransaminazė, amilazė, šlapalas, gliukozė, bendras baltymas, globulinai, cholesterolis);
  - *Histomorfologinius rodiklius*: dvidešimtąjį gyvenimo mėnesį buvo imami vidaus organai (N=6/grupės): skydliaukė, kepenys, kasa, širdis, inkstai, kiaušidės, sėklidės, smegenys, ruožuotasis raumeninis audinys, retroperitoninis visceralinis baltasis riebalinis audinys.

Statistinė duomenų analizė atlikta naudojant programinį paketą IBM SPSS 21. Skirtingų grupių palikuonių rodikliams palyginti taikyta vienfaktorinė dispersinė analizė, naudojant *Bonferroni post-hoc* kriterijų. Jeigu empiriniai duomenys netenkino normalumo prielaidų, buvo taikytas neparametrinis dispersinės analizės analogas – *Kruskalo ir Walliso* testas.
Siekiant nustatyti, ar palikuonių svorio skirtumai priklauso nuo lyties ir motinos maisto ribojimo, taip pat norint įvertinti minėtų veiksnių įtaką augimo trajektorijai taikytas mišraus dizaino dispersinės analizės metodas.
Išgyvenamumo analizei naudotas *Kaplano ir Meierio* kreivių metodas, palyginimui taikant *Tarone ir Ware* kriterijų.
Pasirinktas statistinio reikšmingumo lygmuo $p < 0,05$.

Pagrindiniai rezultatai

Žvalgomojo tyrimo rezultatai

Penktąjį ir šeštąjį palikuonių gyvenimo mėnesį nustatyta, kad 2 EG palikuonys buvo sunkesni už kitų grupių individus ($p < 0,05$). Moteriškosios lyties palikuonys augo panašiai, statistiškai reikšmingų svorio skirtumų nenustatyta ($p > 0,05$)

Mišraus dizaino dispersine analize nustatėme kad motinos mitybos ribojimas statistiškai reikšmingai lėmė antrosios kartos palikuonių augimo trajektoriją ($p < 0,05$), kuri skyrėsi tarp grupių ($p < 0,001$) ir tarp lyčių ($p < 0,01$). Analizuojant antrosios kartos palikuonių vidutinį svorį pamėnesiui, nustatyta, kad pirmaisiais dviem gyvenimo mėnesiais vyriškos lyties 2EG palikuonys buvo statistiškai reikšmingai sunkesni už KG ($p < 0,05$). Tuo tarpu, 1EG vyriškos lyties palikuonys svėrė statistiškai reikšmingai daugiau nei KG 8–22 gyvenimo mėnesiais ($p < 0,05$).

Analizuojant moteriškosios lyties palikuonis, reikšmingų svorio skirtumų nenustatyta. Vis dėlto pirmąjį gyvenimo mėnesį antrosios kartos 1EG moteriškosios lyties palikuonės buvo mažesnio svorio nei kitų grupių patelės ($p < 0,05$).

Nustatyti gyvenimo trukmės skirtumai tarp vyriškos lyties palikuonių grupių. Maisto ribojimą patyrusių motinų antrosios kartos vyriškos lyties palikuonys gyvena trumpiau nei kontrolinės grupės motinų palikuonys ($p = 0,04$). Kontrolinės grupės vyriškosios lyties palikuonių gyvenimo trukmė statistiškai reikšmingai skyrėsi nuo 1EG ($p = 0,03$), tačiau nesiskyrė nuo 2EG ($p = 0,17$).
Moteriškos lyties palikuonių išgyvenamumas, statistiškai reikšmingai nesiskyrė ($p = 0,05$), vis dėlto matyti, kad maisto ribojimą patyrusių motinų antrosios kartos moteriškosios lyties palikuonims būdinga trumpesnio išgyvenimo tendencija.

Pagrindinio tyrimo rezultatai

2EG motinų palikuonys gimė mažesni nei kitų grupių ($p < 0,001$). KG ir 2EG motinoms gimusių palikuonių skaičius nesiskyrė, tačiau buvo statistiškai reikšmingai mažesnis 1EG grupės ($p = 0,03$). Palikuonių pasiskirstymas pagal lytį nesiskyrė ($p = 0,95$).

Mišraus dizaino dispersine analize nustatėme kad motinos mitybos ribojimas statistiškai reikšmingai lėmė pirmosios kartos palikuonių augimo trajektoriją ($p < 0,05$), kuri skyrėsi tarp grupių ($p < 0,01$) ir, iki 11-ojo gyvenimo mėnesio, tarp lyčių ($p < 0,01$).

2EG vyriškosios lyties palikuonys atsigriebė svorį ir įgijo svorio pranašumą, palyginti su kontrolinės grupės svoriu, 2–6 gyvenimo mėnesį, o 1EG palikuonys svėrė statistiškai reikšmingai daugiau nei KG 2–13 gyvenimo mėnesį ($p < 0,05$).
Analizuojant moteriškosios lyties palikuonis matyti, kad 1EG grupės palikuonys svėrė statistiškai reikšmingai daugiau už KG 4–6 gyvenimo mėnesį ($p < 0,05$). Daugiau reikšmingų skirtumų, palyginti su KG, nenustatyta, tačiau eksperimentinių grupių patelių

augimo kreivės skyrėsi tarpusavyje. 1EG patelės buvo statistiškai reikšmingai sunkesnės už 2EG pateles 3–12 ir 16–21 gyvenimo mėnesį ($p < 0,05$).

Vertinant kūno dydį nustatyta, kad 12 mėnesių amžiaus vyriškosios lyties 2EG palikuonys buvo trumpesni, tačiau turėjo didesnius kūno masės ir *Lee* indeksus bei pilvo / krūtinės apimčių santykį, palyginti su kitomis grupėmis ($p < 0,05$).
12 mėnesių amžiaus moteriškosios lyties 2EG palikuonys turėjo statistiškai reikšmingai mažesnę kaklo apimtį nei kitų grupių individai ($p < 0,05$).

Analizuojant vyriškosios lyties palikuonių biocheminius kraujo rodiklius nustatyta, kad 12 mėnesių amžiaus 1EG palikuonių albuminų koncentracija kraujyje buvo mažesnė už KG ($p < 0,05$). Panaši albuminų koncentracijos tendencija buvo ir 2EG palikuonių kraujyje. Taip pat pastebėtos mažesnės šlapalo koncentracijos tendencijos 1EG palikuonių grupėje.
Analizuojant moteriškosios lyties palikuonių biocheminius kraujo rodiklius, matyti, kad patelių kraujo sudėtis statistiškai reikšmingai nesiskyrė ($p > 0,05$).

Organų svoriai tarp grupių statistiškai reikšmingai nesiskyrė ($p > 0,05$). Tačiau skyrėsi jų histomorfologinė struktūra.

Analizuojant kepenų histologinius mikropreparatus, nustatyta, kad eksperimentinių grupių vyriškosios lyties individams būdinga mišri mikropūslelinė ir makropūslelinė riebalinė distrofija, būdingesnė pericentrinėms ir tarpinėms acinusų zonoms, taip pat neryškūs hepatocitų lapeliai, mažiau išreikšti sinusiniai kapiliarai. Eksperimentinių grupių moteriškosios lyties palikuonių kepenų histologinis vaizdas buvo panašus į kontrolinės grupės.

Analizuojant skydliaukės mikropreparatus nustatyta, kad vyriškosios lyties eksperimentinių grupių palikuonių skydliaukėse matyti hiperplastinių masių tarp folikulų, kondensuotesnis koloidas, dažnai atkibęs nuo folikulo sienelės. 2EG palikuonių preparatuose folikulai dažniau iškloti žemesnėmis, suplokštėjusiomis folikulinėmis ląstelėmis. Moteriškos lyties eksperimentinių grupių palikuonėms būdingos aukštesnės folikulinės ląstelės, koloido nedaug ir jis gausiau vakuolizuotas. Tarp folikulų matyti hiperplastinių masių. Taip pat moteriškosios lyties 2EG palikuonims būdingi degeneravę folikulai, gausiai pripildyti koloido su rezorbcinėmis vakuolėmis periferinėse dalyse.

Išanalizavus pirmosios kartos eksperimentinių grupių vyriškos lyties palikuonių kasos preparatus, 1EG grupės palikuonims nustatytas nedidelis riebalinių intarpų kiekio padidėjimas, o 2EG grupės – aptikta dar daugiau ir dar didesnių riebalinių intarpų egzokrinocitų citoplazmoje. Išanalizavus moteriškosios lyties histologinius preparatus buvo nustatyta, kad vaizdas atitinka kontrolę, riebalinių intarpų egzokrinocitų citoplazmoje nėra (arba jie pavieniai).
Analizuojant vyriškosios lyties palikuonių riebalinio audinio preparatus, 1EG ir 2EG grupių preparatuose stebimi išsiplėtę adipocitai, statistiškai reikšmingai skiriasi jų paviršiaus plotas ($p < 0,001$), vidutinis skersmuo ($p < 0,001$) ir maksimalus skersmuo ($p < 0,001$). Moteriškos lyties palikuonių visceralinio baltojo riebalinio audinio adipocitų morfologiniai rodikliai statistiškai reikšmingai nesiskyrė ($p > 0,05$).

Išvados

1. **Žvalgomasis tyrimas** atskleidė palikuonių augimo ir gyvenimo trukmės skirtumus. Jie priklauso nuo lyties, amžiaus ir motinos mitybos ribojimo laikotarpio:
   a) Iki vaikingumo maisto ribotai gavusių motinų antrosios kartos vyriškosios lyties palikuonys svėrė daugiau, palyginti su kontroline grupe, reprodukcinio ir pagyvenusio amžiaus laikotarpiais. Pirmosios palikuonių kartos kūno svoris buvo artimas kontrolinės grupės svoriui.
   b) Iki vaikingumo ir jo metu maisto ribotai gavusių motinų pirmosios kartos vyriškosios lyties palikuonys svėrė daugiau, palyginti su kontroline grupe, jauno reprodukcinio amžiaus laikotarpiu, o antrosios kartos palikuonys – lytinės brandos laikotarpiu.
   c) Moteriškosios lyties palikuonių augimas buvo harmoningas ir tarp grupių nesiskyrė.
   d) Iki vaikingumo maisto ribotai gavusių motinų antrosios kartos vyriškosios lyties palikuonių gyvenimo trukmė buvo trumpesnė. Panašios tendencijos būdingos ir kitiems maisto ribotai gavusių motinų palikuonims.
2. **Pagrindinis tyrimas** atskleidė biometrinius pirmosios kartos palikuonių augimo skirtumus. Jie priklauso nuo lyties, amžiaus ir motinos mitybos ribojimo laikotarpio:
   a) Motinos mitybos ribojimas iki vaikingumo ir jo metu lėmė mažesnį palikuonių gimimo svorį ir tolesnį atsigriebimo augimą, o motinos mitybos ribojimas iki vaikingumo lėmė mažesnį palikuonių skaičių.
   b) Iki vaikingumo maisto ribotai gavusių motinų vyriškosios lyties palikuonys svėrė daugiau, palyginti su kontroline grupe, lytinės brandos ir reprodukcinio amžiaus laikotarpiais.
   c) Iki vaikingumo ir jo metu maisto ribotai gavusių motinų vyriškosios lyties palikuonys svėrė daugiau, palyginti su kontroline grupe, lytinės brandos ir jauno reprodukcinio amžiaus laikotarpiais, taip pat jiems buvo būdingos didesnių antsvorio ir centrinio riebalų kaupimo rodiklių tendencijos.
   d) Moteriškosios lyties palikuonys augo panašiai, palyginti su kontrolinės grupės palikuoniais, tačiau buvo nustatyta motinos mitybos ribojimą patyrusių palikuonių augimo strategijos pokyčių. Iki vaikingumo maisto ribotai gavusių motinų palikuonės priaugo daugiau svorio nei kitų grupių palikuonės jauname reprodukciniame amžiuje (potencialaus apvaisinimo laikotarpiu) ir išlaikė tendencingai didesnį svorį vykstant ontogenezei. Ribojant motinos mitybą iki vaikingumo ir jo metu, išlaikomos mažesnio palikuonių kūno dydžio tendencijos.
3. Nustatyta, kad pirmosios kartos palikuonių kraujo morfologiniai ir biocheminiai rodikliai buvo fiziologinės normos ribose ir artimi kontrolinės grupės rodikliams. Vis dėlto nustatėme mažesnę albuminų koncentraciją ir mažesnės šlapalo koncentracijos tendencijas iki vaikingumo maisto ribotai gavusių motinų brandaus reprodukcinio amžiaus vyriškosios lyties palikuonims. Panašios albuminų koncentracijos tendencijos buvo būdingos ir per vaikingumą maisto ribotai gavusių motinų palikuonims. Šių rodiklių pokyčiai kartu su riebaline hepatocitų distrofija gali rodyti baltymų apykaitos ir kepenų funkcijos nuokrypius.
4. Nustatyti pirmosios kartos palikuonių histomorfologiniai pokyčiai maisto ribojimą patyrusių motinų palikuonių grupėse:

a) Vyriškosios lyties palikuonims nustatytos riebalinių intarpų kaupimo kepenų ir kasos ląstelėse tendencijos. Moteriškosios lyties palikuonių kasos ir kepenų histomorfologiniai rodikliai buvo artimi kontrolinės grupės rodikliams.
b) Vyriškosios lyties palikuonims nustatyti hipofunkcijai būdingi morfologiniai skydliaukės folikulų pokyčiai, o moteriškosios lyties palikuonėms – morfologiniai folikulų pokyčiai su degeneracijos požymiais.
c) Vyriškosios lyties palikuonims nustatyta visceralinio baltojo riebalinio audinio adipocitų hipertrofija. Moteriškosios lyties palikuonių adipocitų morfometriniai rodikliai buvo artimi kontrolinės grupės rodikliams.

Pastarieji histomorfologinės struktūros skirtumai gali rodyti didesnį moteriškosios lyties palikuonių stabilumą mitybos sutrikdymo atveju.

PUBLICATIONS

Articles on present research results:

1. **Araminaitė V,** Žalgevičienė V, Šimkūnaitė-Rizgelienė R, Stukas R, Kaminskas A, Tutkuvienė J. Maternal caloric restriction prior to pregnancy increases the body weight of the second generation male offspring and shortens their longevity in rats. Tohoku J Exp Med. 2014;234(1):41-50 (ISI *Web of Science,* IF = 1,351).
2. **Araminaitė V,** Žalgevičienė V, Šimkūnaitė-Rizgelienė R, Bukelskienė V, Tutkuvienė J. Nutritional peculiarities during the prenatal period and physical status of the offspring: a pilot experimental study. Acta Medica Lituanica 2013;20(1):13-18 (*Index Copernicus*).

Other articles:

Stankevič J, Audickaitė A, **Araminaitė V,** Šimčikas V, Žalgevičienė V, Tutkuvienė J. Šungitu apdoroto vandens įtaka žiurkių embrionų raidai („*The influence of Shungite saturated water on rat embryo development"*). Laboratorinė medicina 2013;15(3):131-136 (*Index Copernicus*).

Conference presentations on present research results:

1. **Araminaitė V**, Tutkuvienė J. Mitybos ypatumų prenataliniu laikotarpiu sąsajos su palikuonių fizine būkle: žvalgomasis eksperimentinis tyrimas („*Nutritional peculiarities during the prenatal period and physical status of the offspring: a pilot experimental study"*). Bioateitis: gamtos ir gyvybės mokslų perspektyvos („*Biofuture: the perspectives of natural and life sciences"*): December 5th, 2012, Lithuanian Academy of Sciences, Vilnius, Lithuania: abstract book. p. 13-14 (oral presentation).
2. **Araminaitė V,** Tutkuvienė J. Tracing maternal undernutrition: physical status and longevity of the second generation offspring. Morphological sciences in the experimental and clinical medicine: Baltic Morphology VII scientific conference, November 7th-9th, 2013, Riga, Latvia: abstract book. p. 15 (oral presentation).
3. **Araminaitė V,** Žalgevičienė V, Tutkuvienė J. Maternal undernutrition and its consequences for the early development of the offspring. Evoliucinė medicina: sveikatos sampratos ir ligų suvokimo perspektyvos („*Evolutionary medicine: perspectives in understanding health and disease*"). May 27-30th, 2014, Vilnius University, Vilnius, Lietuva: abstract book. p. 45 (oral presentation).

4. **Bartuškienė V**, Čepulienė R, Šimkūnaitė-Rizgelienė R, Žalgevičienė V, Tutkuvienė J. Thrifty boys: growth programming following maternal undernutrition. The 8th Baltic morphology scientific conference: interdisciplinary nature of contemporary morphology. November 12-14th, 2015, Vilnius, Lithuania: abstract book. p. 39 (best PhD student oral presentation).
5. **Bartuškienė V,** Čepulienė R, Šimkūnaitė-Rizgelienė R, Žalgevičienė V, Tutkuvienė J. From cells to organism: offspring growth peculiarities following in uterus undernutrition. 3-ioji tarptautinė konferencija „Evoliucinė medicina: šiuolaikinių sveikatos problemų evoliuciniai mechanizmai ir dėsningumai (*The 3rd international conference "Evolutionary medicine: pre-existing mechanisms and patterns of current health issues"*). June 14-19th, 2016, Vilnius, Lithuania: abstract book. p. 50 (best PhD student oral presentation).
6. **Bartuškienė V,** Čepulienė R, Šimkūnaitė-Rizgelienė R, Žalgevičienė V, Tutkuvienė J. The effect of maternal undernutrition on the offspring growth trajectory and adipocyte profile. 20th Congress of the European Anthropological Association "European Anthropology in a Changing World: from Culture to Global Biology". August 24-28th, 2016, Zagreb, Croatia: abstract book. p. 41 (best student oral presentation).

Other conference presentations:

1. **Araminaitė V,** Šimčikas V, Žalgevičienė V, Tutkuvienė J. Skirtingos mineralizacijos vandens įtaka žiurkių palikuonių fizinei būklei *(„The influence of low mineralization drinking water on physical status of the rat embryo").* Bioateitis: gamtos ir gyvybės mokslų perspektyvos („*Biofuture: the perspectives of natural and life sciences"*): December 11th, 2013, Lithuanian Academy of Sciences, Vilnius, Lithuania: abstract book. p. 13 (oral presentation).
2. **Araminaitė V,** Stankevič J, Audickaitė A, Šimčikas V, Žalgevičienė V, Tutkuvienė J. The influence of Shungite supplemented water on physical status of the rat embryo. Morphological sciences in the experimental and clinical medicine: Baltic Morphology VII scientific conference, November 7-9th, 2013, Riga, Latvia: abstract book. p. 67 (oral presentation).

Awards for the dissemination of present research results:

1. The winner of the *Famelab* science communication competition in Lithuania and the participant of the world *Famelab* semi-finals and finals in Cheltenham Science festival in the United Kingdom (2013).
2. The premium from the Lithuanian Research council for academic achievements (2015).
3. Award from European anthropological association (EAA) for the best student presentation at the 20th EAA Congress (2016).

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