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Nutritional peculiarities during the prenatal period and physical status of the offspring: a pilot experimental study

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Nutrition *in uterus* has a significant influence on the formation of metabolic phenotype in offspring generations. **The objective** of this study was to investigate maternal nutrition and the changes in body weight at few generations of rat offspring.

Materials and methods. 12 maternal rats and 121 offspring rats were used in the study. Mature female *Wistar* rats were divided into 3 groups with respect to nutritional restriction (one control and two experimental groups). Rats from the first experimental group (1 EG) were 50 percent food-restricted one month prior and during the pregnancy; rats from the second experimental group (2 EG) were 50 percent food-restricted exclusively one month prior to the pregnancy. After weaning all the pups were fed with control diet, weighted weekly, observed and evaluated for the morphological indices of metabolic stress.

Results and conclusions. Maternal nutritional restriction in pre-pregnancy and pregnancy may alter the physical status and behaviour of the offspring: the reactions differ in both sexes; the alterations depend on the time window of exposure. There were no weight-related differences between groups in body weight of female offspring rats during the all time periods of the study. The first generation 1 EG male offsprings were the heaviest; the tendency for a greater than control group weight was also observed at the second generation up till the early reproductive period. The 2nd generation 2 EG male rats were the heaviest and exhibited some evident markers of chronic diseases.

Key words: nutritional deprivation, pregnancy, growth programming, thrifty phenotype

INTRODUCTION

The pathogenesis of chronic diseases depends not only on the environmental factors of the adult individual, but also on the critical moments during his / her growth and development, particularly on the period *in uterus*, also on the conditions before pregnancy (1). Global slimming trends, especially at the post-communistic countries, and prevailing promotion of a slender or even exhausted woman's body often lead to dissatisfaction with one's

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appearance and trigger efforts to lose weight (2). Some human studies and data on animal research showed the link between insufficient nutrition before pregnancy or at the time of pregnancy, and child's susceptibility to various health problems (3–19). The studies often describe various hormonal, cardiovascular, behavioural changes, as well as catch-up growth as a result of prenatal growth retardation. The lack of nutrients *in uterus* causes the formation of "thrifty phenotype", which means that the body is "programmed" to act in a sustainable mode, thus giving an advantage to survive under conditions of nutritional deprivation (20, 21).

Scientific knowledge regarding dietary restriction and further pregnancy outcomes as well as the risk for health is under discussion. In most cases, the studies that provide data on the consequences of prenatal starvation are based on hypothetical theories and retrospective studies. Hence, there is a specific need in prospective studies with laboratory animals to test the above mentioned theories. In addition, this issue is almost unexamined in the context of aging and survivability. Moreover, the prevalence of abnormal metabolic parameters in several offspring generations is purely studied.

The objective of this study was to investigate maternal nutrition (the dietary conditions before and during pregnancy) and the changes in body weight as well as the risk for obesity at few rat offspring generations from birth up till natural death.

MATERIALS AND METHODS

The animals were housed under standard conditions in the Vivarium of the Institute of Biochemistry. All animal procedures were in accordance with the State Food and Veterinary Service (Permit No. 0211).

The cohort of 12 mature female *Wistar* rats was divided into 3 groups with respect to nutritional restriction. The rats were fed either a normal (control group) or restricted diet: one experimental group was food-restricted one month prior to and through the pregnancy period (1 EG) and the other was food-restricted one month prior to pregnancy only (2 EG). Food-restricted rat groups received 50 percent less of the feed eaten in the control group. The maternal rats and the offspring rats were mated at the age of 3–4 months. Body weight was measured weekly with the same calibrated scales starting at the age of 1–1.5 months. Maternal rats were euthanized after weaning. Both offspring generations were kept and weighted under the same standardized conditions. We also evaluated offspring rat's appearance, behaviour (the interest in the environment, fear, aggression, nervousness), and other signs of metabolic stress.

Differences between the groups were compared using the one-way analysis of variance (ANOVA) followed by the LSD test; $p < 0.05$ was considered statistically significant.

RESULTS

We did not find weight-related differences between 1 EG and 2 EG female offspring rats during the entire study period – both at the first and the second generations of offspring (Figs. 2, 4 respectively). The first generation 1 EG male offspring rats were the heaviest from 3 months of age (Fig. 1); the tendency for a bigger than control group weight was also observed at the 2nd generation up till the early reproductive period (Fig. 3). In the first offspring generation, pre-pregnancy food deprived male rats (2 EG) had similar weight to that of the control group (Fig. 1). However, 2 EG were the heaviest at the second offspring generation starting from the fourth month of age with few minor exceptions (Fig. 3).

DISCUSSION

Nutrition *in uterus* has a significant influence on the formation of metabolic phenotype in offspring generations. Our study did not find weight differences in food deprived female offspring rats. Scarce literature data complements our results (12, 13). It is estimated that female's organism is more adaptive and therefore is able to compensate the stress induced by food restriction (11–15).

Prenatal metabolic stress might also induce "catch up" growth, and the body weight of food deprived offspring rats increases faster than control offspring's weight. Other studies find similar results to those of our study: food deprived male offspring rats weighted more than other control and experimental rats (11–13). In addition, the increase in weight continues even after the catch-up is reached,

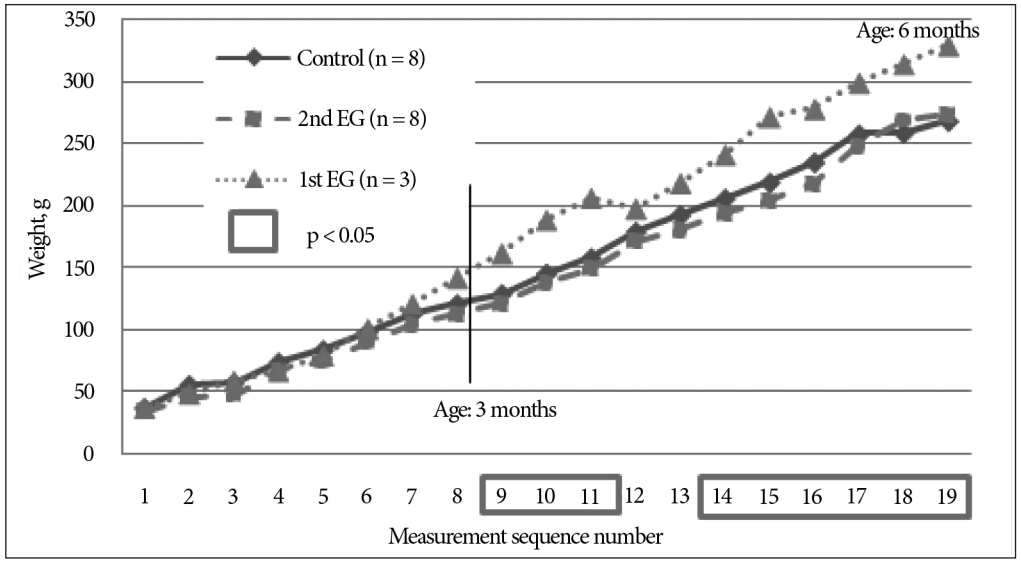

Fig. 1. Weight dynamics in the first generation of male offspring rats

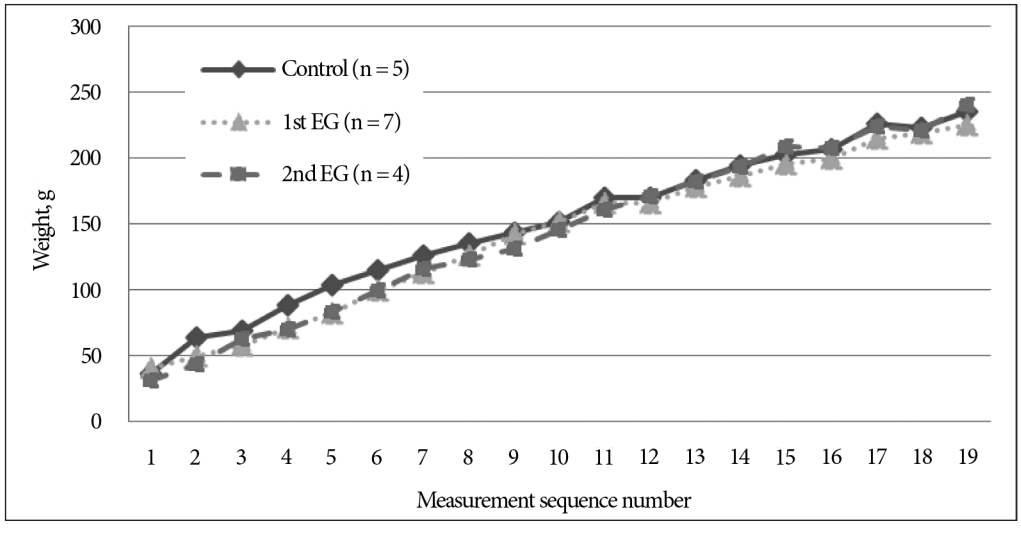

Fig. 2. Weight dynamics in the first generation of female offspring rats

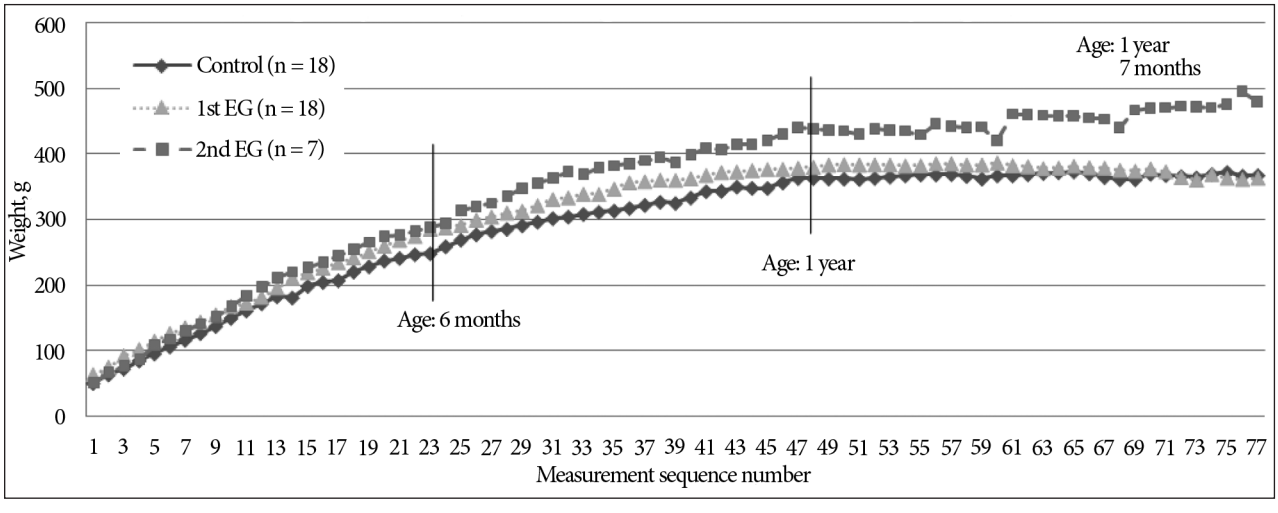

Fig. 3. Weight dynamics in the second generation of male offspring rats

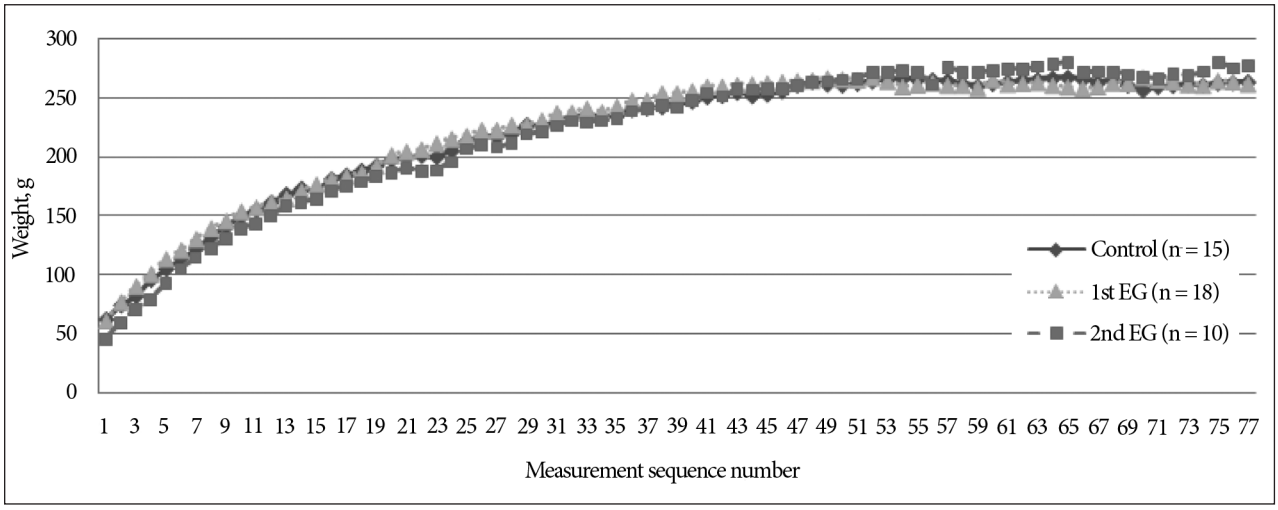

Fig. 4. Weight dynamics in the second generation of female offspring rats

and the subsequent weight increase is due mostly to the fat mass increment (3, 16, 18, 19, 22).

The nutrition exclusively before pregnancy is an almost unexamined topic in the context of offspring health later in the individual's life. It is hypothesized that good physical condition of the mother could partially balance the altered metabolism induced by the nutritional deprivation (23–26). Pre-pregnancy food deprived males were the heaviest as well as demonstrated the majority of the evident external pathology in the second offspring generation. We hypothesize that these offspring rats got the energy saving phenotype from their starved mothers, however, after birth the nutritional conditions for the offspring rats changed, and the "re-programming" might have occurred.

Few animal studies described altered changes in adipose tissue topography, insulin and leptin metabolism even in the case if no weight related differences between groups were observed (3, 11–14, 27–29). Our study did not examine biochemical indices as well as body fat deposition (although the evident central fat distribution was observed in aged male rats from experimental groups in comparison with control ones). Exclusively in the experimental groups, we also followed the morphological indices of the metabolic stress. We have recorded some behavioural disorders such as nervousness, aggressiveness and the opposite – sluggishness, poor spatial orientation and reaction to stimuli. Behavioural pathologies are frequently described in the context of relationship between mother's food deprivation and health risk of her offspring (30–32). In addition, these symptoms were evident at a relatively early age and more common in males than females. We have also observed a number of external indices of stress such as red tears, neck and face oedema, hair loss and others that are considered an important marker for the rodent health risk (33). However, we did not find the similar studies concerning these symptoms. Furthermore, dissection after spontaneous death showed a number of health problems in the experimental groups: tumours, necrotic changes in regulatory organs (heart, liver, lungs, brain) as well as enlargement of hypophysis (an indicator of premature aging). As mentioned, the second generation pre-pregnancy food deprived male rats not only were the heaviest, but also exhibited the majority of the mentioned pathologies. Since the lifespan research with animals is rare, we could not find similar studies regarding the mentioned changes found in autopsy as well as other deleterious effects of epigenetically influenced aging.

Growth programming is a much more complicated process than it was considered up till yet, and frequently its consequences cannot be foreseen. In some cases our study complements other findings, but we have also received a few unexpected results (particularly differences between two experimental groups). The complex epigenetic changes related to nutritional deprivation and their influence on growth and aging remain unclear. The underlying mechanisms as well as the effects of maternal food deprivation in pre-pregnancy and pregnancy should be investigated further.

CONCLUSIONS

Maternal nutritional restriction during and before pregnancy may alter the physical status and behaviour of the offspring: the reactions differ between sexes and depend on the time window of exposure. This pilot study shows the necessity for further investigations of larger samples of the experimental groups.

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References

1. Benyshek DC. The developmental origins of obesity and related health disorders – prenatal and perinatal factors. Coll Antropol. 2007; 31(1): 11–7.
2. Tutkuvienė J, Sakalauskaitė E. Kūno įvaizdis: veiksniai ir sąsajos su fizine būkle. Laboratorinė medicina. 2009; 11(4): 215–22.
3. Suzuki M, Shibanuma M, Kimura S. Effect of severe maternal dietary restriction on growth and intra-abdominal adipose tissue weights in offspring rats. J Nutr Sci Vitaminol (Tokyo). 2010; 56(5): 293–8.
4. Prentice AM, Moore SE. Early programming of adult diseases in resource poor countries. Arch Dis Child. 2005; 90: 429–32.
5. Smith PK, Bogin B, Varela-Silva MI, Loucky J. Economic and anthropological assessments of the health of children in Maya immigrant families in the US. Econ Hum Biol. 2003; 1(2): 145–60.
6. Popkin BM, Udry JR. Adolescent obesity increases significantly in second and third generation U. S. immigrants: the National Longitudinal Study of Adolescent Health. J Nutr. 1998; 128(4): 701–6.
7. Gordon-Larsen P, Harris KM, Ward DS, Popkin BM. National Longitudinal Study of Adolescent Health. Acculturation and overweight-related behaviors among Hispanic immigrants to the US: the National Longitudinal Study of Adolescent Health. Soc Sci Med. 2003; 57(11): 2023–34.
8. Kaplan MS, Huguet N, Newsom JT, McFarland BH. The association between length of residence and obesity among Hispanic immigrants. Am J Prev Med. 2004; 27(4): 323–6.
9. Bibbins-Domingo K, Coxson P, Pletcher MJ, Lightwood J, Goldman L. Adolescent overweight and future adult coronary heart disease. N Engl J Med. 2007; 357: 2371–9.
10. Thornburg KL, O'Tierney PF, Louey S. Review: The placenta is a programming agent for cardiovascular disease. Placenta. 2010; 31 Suppl: S54–59. Epub 2010 Feb 9.
11. Zambrano E, Martínez-Samayoa PM, Bautista CJ, Deás M, Guillén L, Rodríguez-González GL, et al. Sex differences in transgenerational alterations of growth and metabolism in progeny (F_2) of female offspring (F_1) of rats fed a low protein diet during pregnancy and lactation. J Physiol. 2005; 566: 225–36.
12. Pinheiro AR, Salvucci ID, Aguila MB, Mandarim-de-Lacerda CA. Protein restriction during gestation and / or lactation causes adverse transgenerational effects on biometry and glucose metabolism in F1 and F2 progenies of rats. Clin Sci (Lond). 2008; 114(5): 381–92.
13. Benyshek DC, Johnston CS, Martin JF. Post-natal diet determines insulin resistance in fetally malnourished, low birthweight rats (F1) but diet does not modify the insulin resistance of their offspring (F2). Life Sci. 2004; 74(24): 3033–41.
14. Benyshek DC, Johnston CS, Martin JF. Glucose metabolism is altered in the adequately-nourished grand-offspring (F3 generation) of rats malnourished during gestation and perinatal life. Diabetologia. 2006; 49(5): 1117–9.
15. Stewart RJ, Preece RF, Sheppard HG. Twelve generations of marginal protein deficiency. Br J Nutr. 1975; 33(2): 233–53.
16. Yura S, Itoh H, Sagawa N, Yamamoto H, Masuzaki H, Nakao K, et al. Role of premature leptin surge in obesity resulting from intrauterine undernutrition. Cell Metab. 2005; 1(6): 371–7.
17. Belkacemi L, Nelson DM, Desai M, Ross MG. Maternal undernutrition influences placental-fetal development. Biol Reprod. 2010; 83(3): 325–31.
18. Alexe DM, Syridou G, Petridou ET. Determinants of early life leptin levels and later life degenerative outcomes. Clin Med Res. 2006; 4(4): 326–35.
19. Lim K, Armitage JA, Stefanidis A, Oldfield BJ, Black MJ. Intrauterine growth restriction in the absence of postnatal 'Catch-Up' growth leads to improved whole body insulin sensitivity in rat offspring. Pediatr Res. 2011 Jun 23. Epub ahead of print.
20. Gluckman PD, Hanson MA, Spencer HG. Predictive adaptive responses and human evolution. Trends Ecol Evol. 2005; 20(10): 527–33.

21. McArdle HJ, Andersen HS, Jones H, Gambling L. Fetal programming: causes and consequences as revealed by studies of dietary manipulation in rats – a review. Placenta. 2006; 27 Suppl A: S56–60. Epub 2006 Mar 13.
22. Padmavathi IJ, Rao KR, Venu L, Ganeshan M, Kumar KA, Rao ChN, et al. Chronic maternal dietary chromium restriction modulates visceral adiposity: probable underlying mechanisms. Diabetes. 2010; 59(1): 98–104.
23. Wells JC. Maternal capital and the metabolic ghetto: An evolutionary perspective on the transgenerational basis of health inequalities. Am J Hum Biol. 2010; 22(1): 1–17.
24. Ellison PT. Energetics and reproductive effort. Am J Hum Biol. 2003; 15(3): 342–51.
25. Wells JC. The thrifty phenotype hypothesis: thrifty offspring or thrifty mother? J Theor Biol. 2003; 221(1): 143–61.
26. Wells JC. Flaws in the theory of predictive adaptive responses. Trends Endocrinol Metab. 2007; 18(9): 331–7.
27. Bellinger L, Sculley DV, Langley-Evans SC. Exposure to undernutrition in fetal life determines fat distribution, locomotor activity and food intake in ageing rats. Int J Obes (Lond). 2006; 30(5): 729–38.
28. Zamenhof S, van Marthens E. Effects of prenatal and chronic undernutrition on aging and survival in rats. J Nutr. 1982; 112(5): 972–7.
29. Law CM, Barker DJ, Osmond C, Fall CH, Simmonds SJ. Early growth and abdominal fatness in adult life. J Epidemiol Community Health. 1992; 46(3): 184–6.
30. Zhang Y, Li N, Yang Z. Perinatal food restriction impaired spatial learning and memory behavior and decreased the density of nitric oxide synthase neurons in the hippocampus of adult male rat offspring. Toxicol Lett. 2010; 193(2): 167–72.
31. Blaise SA, Nédélec E, Schroeder H, Alberto JM, Bossenmeyer-Pourié C, Guéant JL, et al. Gestational vitamin B deficiency leads to homocysteine-associated brain apoptosis and alters neurobehavioral development in rats. Am J Pathol. 2007; 170(2): 667–79.
32. Durán P, Galler JR, Cintra L, Tonkiss J. Prenatal malnutrition and sleep states in adult rats: effects of restraint stress. Physiol Behav. 2006; 89(2): 156–63.
33. Šimkevičienė V, Rukšėnas O. Laboratorinių gyvūnų mokslo pagrindai. Vilnius: Vilniaus universiteto leidykla; 2001. 237 p.

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MITYBOS YPATUMŲ PRENATALINIU LAIKOTARPIU SĄSAJOS SU PALIKUONIŲ FIZINE BŪKLE: ŽVALGOMASIS EKSPERIMENTINIS TYRIMAS

Santrauka

Nepakankama mityba iki nėštumo ar per nėštumą gali keisti postnatalinį augimą, lemti palikuonio medžiagų apykaitos pokyčius, antsvorį ar lėtines neinfekcines ligas jam suaugus. Ši studija – viena iš nedaugelio eksperimentinių tyrimų, analizavusių sąlygų ne tik per nėštumą, tačiau ir iki nėštumo įtaką augančio organizmo fizinei būklei. Be to, palikuonių sveikata stebėta iki pat spontaninės mirties, todėl galima įvertinti medžiagų apykaitos pokyčius organizmo senėjimo kontekste. Žvalgomajame tyrime tirta 12 motininių žiurkių patelių ir 121 jų palikuonis. Nustatyta, kad motinos mitybos ribojimas per nėštumą ar iki nėštumo gali keisti postnatalinį augimą priklausomai nuo lyties ir mitybos ribojimo ekspozicijos laikotarpio. Tarp mitybos ribojimą patyrusių žiurkių palikuonių patelių svorio pokyčių nenustatyta, o vyriškos lyties palikuonių eksperimentinėse grupėse stebėti augimo ir sveikatos pakitimai. Iki nėštumo ir per nėštumą maisto medžiagų ribojimą patyrusių žiurkių vyriškos lyties palikuonys buvo reikšmingai didesnio svorio už pirmos ir antros palikuonių kartos kontrolinę grupę. Reikšmingai daugiau už kitas grupes svėrė iki nėštumo mitybos ribojimą patyrusių antros palikuonių kartos žiurkių vyriškos lyties palikuonys, jų gyvenimo trukmė buvo trumpesnė, ryškesnė lėtinių neinfekcinių ligų simptomatika. Planuojama studiją tęsti didinant eksperimentinių grupių ir tiriamųjų žiurkių skaičių, taikant įvairesnes mitybos sąlygas iki nėštumo ar po nėštumo.

Raktažodžiai: nepakankama mityba, nėštumas, augimo programavimas, taupusis fenotipas