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**Test hipotezy o ograniczeniach w rozpraszaniu ciepła
u myszy laboratoryjnych o różnym tempie metabolizmu**

Rozprawa doktorska

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Summary

The heat dissipation limitation hypothesis (HDL) suggests that animals' energy budgets are limited by the ability to dissipate body heat to avoid detrimental overheating. According to the life history theory, reproduction is one of the key cycles that constitute a significant burden of animals energy budgets. Therefore, testing of the HDL hypothesis assumptions is primarily based on the manipulation of the temperature gradient between the organism and its environment, mainly in small mammals during the lactation period.

In my work, I propose a new approach to test the HDL hypothesis, based on the incorporation of the energy trade-offs idea. I assumed that, if the HDL hypothesis is correct, additional burden of lactating mother mice with costs of the immune response, should bring dams to upper limits to dissipation of metabolic heat and lead to trade-offs between parental effort and immune response. As an experimental model, I have used laboratory mice selected divergently towards either high and low level of basal metabolic rate (respectively H-BMR and L-BMR line types). This model perfectly fits to the assumptions of the HDL hypothesis due to the interlinear difference in basal metabolism rate at level about 50% and no changes in thermal conduction. Moreover, mice with high basal metabolism rate consume more food, have higher masses of internal organs, maintain higher body temperature and produce more specific antibodies in response to KLH antigen in comparison to the L-BMR line type.

To test the trade-offs between parental effort and an immune response, I have conducted three complementary experiments. In each of them, females of laboratory mice from H-BMR and L-BMR line types for 14 days of lactation period were exposed to two different ambient temperature: 23°C, posing a control environment and 30°C, restricting dams ability to heat dissipation. During lactation, I have controlled dams body mass and body temperature, food consumption, digestibility and the parental effort, measured as a ratio of litter mass growth. At the peak of the lactation (in my experimental model is a period between 12 and 14 days), I have measured the metabolisable energy intake, milk energy output and daily energy expenditures of experimental dams. All mice were sacrificed at 14 day after parturition, when I have collected and weighed their metabolic and lymphatic organs (heart, liver, kidneys, small intestine, brown adipose tissue, thymus, spleen and lymph nodes). Also at 14 day of lactation I have measured the level of anti-KLH IgM antibodies, as a proxy for the immune response.

At the 1st experiment, lactating females were shaved at 6th and 10th day of lactation, to increase their ability to dissipate heat. I have assumed that litters of dams with removed fur, exposed to 30°C will grow faster than litters of unshaved dams, but this realizing effect of

shaving will depend from the line type. Results showed, that in accordance to my assumptions, exposure of mice from H-BMR line type to 30°C reduced their parental effort but realizing effect of shaving to litter mass growth was noticeable only at 23°C. L-BMR line type litters grew faster at 30°C than at control condition, their reproductive effort was higher in comparison to H-BMR line type, even without shaving effect.

At the 2nd experiment, I have tested the reaction of females from both line types to the simultaneous burdening their energetic budgets with the costs of the parental effort and immune response. I have assumed that if the capacity to dissipate body heat is a factor limiting rate of energy expenditures, dams will reveal the trade-offs between both functions and the severity of trade-offs will be dependent from line type. As I have expected, mice from both line types responded divergently to experimental manipulations. Dams with low BMR lactating at both ambient conditions, gave the priority to reproduction and did not start the production of anti-KLH antibodies. Moreover, their litters grew faster compared to dams from H-BMR line type. Despite the higher maintenance costs observed in mice form H-BMR line type, at temperature of 23°C females from this line type have started production of anti-KLH antibodies and their litters grew faster than L-BMR line type litters. At 30°C, mice with high BMR, gave the priority to reproduction in the beginning of lactation, but in the last days of experiment, they have started to reveal the trade-offs, which were manifested by increase of lymphatic organs masses and decreased litter mass growth at the peak of lactation.

The 3rd experiment was designed to test the trade-offs between reproduction and immune response in mice revealed from constraints with heat dissipation (shaving procedure). I have assumed that shaved mice will be able to combine maintaining two energy costly functions. Contrary to my predictions, results showed that mice from different line types, at both temperatures, gave the priority to reproduction and did not build the mechanisms of specific immune response, measured as a specific antibodies level. Moreover, against to my assumptions, litters of shaved mice grew in similar rate as litters of unshaved dams at 2nd Experiment.

The obtained results showed that laboratory mice with high and low basal metabolism rate present a different pattern in response to simultaneous burdening of their energy budgets with costs of reproduction and immune response. Mice with low BMR gave the priority to reproduction at both ambient conditions, whereas dams with high BMR built the trade-offs between both functions in temperature-dependent manner. The above results did not provide the support to assumptions of the HDL hypothesis. Moreover, results suggests that fur as an insulating layer is not a main obstacle in heat dissipation in my experimental model.