

Załącznik 3  
(Appendix 3)

**SUMMARY OF PROFESSIONAL ACCOMPLISHMENTS AND DESCRIPTION OF THE  
SCIENTIFIC ACHIEVEMENT**

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**SCIENTIFIC DEVELOPMENT:**

**1998 MSc in biology**

University of Białystok, Faculty of Biology and Chemistry, Institute of Biology  
The title of the MSc thesis: The relationship between maximum and basal metabolic rate and mass of internal organs in laboratory mice *Mus musculus*  
supervisor: prof. dr hab. Marek Konarzewski

**2005 PhD in biology**

University of Białystok, Faculty of Biology and Chemistry, Institute of Biology  
The title of the PhD thesis: Energetic costs of mounting of an immune response in laboratory mice  
supervisor: prof. dr hab. Marek Konarzewski

**PROFESSIONAL EXPERIENCE:**

**1998 - 1999** Department of Microbiology, Faculty of Biology and Chemistry, University of Białystok, trainee assistant

**1999 - 2006** Department of Microbiology, Faculty of Biology and Chemistry, University of Białystok, assistant

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**I. SCIENTIFIC ACHIEVEMENTS UNDERLYING THE COMPETITION FOR THE HABILITATED****DOCTOR DEGREE** (according to the article 16 paragraph 2 of the act from 14<sup>th</sup> of March 2003)

A) the title of the scientific achievement:

**The relationship between metabolic rate, immunocompetence and overwinter survival in small mammals**

B) publications being the basis for applying for a habilitated doctor degree:

1. **Książek A.**, Czerniecki J., Konarzewski M. 2009. Phenotypic flexibility of traits related to energy acquisition in mice divergently selected for basal metabolic rate (BMR). *Journal of Experimental Biology* 212: 808-814.  
**IF: 3.320; MSHE score: 35; contribution 60%**
2. **Książek A.**, Konarzewski M. 2012. Effect of dietary restriction on immune response of laboratory mice divergently selected for basal metabolic rate. *Physiological and Biochemical Zoology* 85:51-61.  
**IF: 2.104; MSHE score: 35; contribution 70%**
3. **Książek A.**, Zub K., Szafrńska P. A., Wieczorek M., Konarzewski M. 2014. Immunocompetence and high metabolic rates enhance overwinter survival in the root vole, *Microtus oeconomus*. *Biology Letters* 10, 20140684.  
**IF: 3.089; MSHE score: 30; contribution 50%**
4. **Książek A.**, Konarzewski M. 2016. Heat dissipation does not suppress an immune response in laboratory mice divergently selected for basal metabolic rate (BMR). *Journal of Experimental Biology* 219: 1542-1551.  
**IF: 3.320; MSHE score: 35; contribution 70%**
5. **Książek A.**, Zub K., Szafrńska P. A., Wieczorek M., Konarzewski M. 2017. The nexus of hair corticosterone level, immunocompetence, metabolic rates and overwinter survival in the root vole, *Microtus oeconomus*. *General and Comparative Endocrinology* 250: 46-53.  
**IF: 2.585; MSHE score: 25; contribution 50%**

Total MSHE<sup>1</sup> score for works consisting the scientific achievement is **160**, summarized *Impact Factor*<sup>2</sup> **14.418**, and average participation in publications is **60%**.

<sup>1</sup> According to the announcement of the Ministry of Science and Higher Education from December 12, 2016.

<sup>2</sup> *Impact Factor* score in 2016.

C) discussion of the scientific goals of above works and achieved results

### Introduction

Energy expenditure intended to sustain essential life functions is quantified by measuring metabolic rate (review in Lighton 2008). In my research, I focused on basal metabolic rate (BMR), which, as defined by Schmidt-Nielsen (1997), refers to the minimum rate of energy production needed to sustain the vital function of a non-digested person, who is not physically active and stays in the environment of its thermoneutral zone. At the interspecific level, BMR correlates with other metabolic rate measures (including total energy expenditure and instantaneous, maximum metabolic rate, e.g. White and Kearney 2012), as well as parameters that modulate the most important physiological processes such as the level of hormones (e.g. Li et al. 2010).

In the articles referred to as achievements in habilitation dissertation, I tested the **relationship between BMR and peak metabolic rate (PMR), internal organ phenotypicity, immunocompetence, stress levels, and winter survival at the species level**. There are at least three principal reasons for this approach. Interspecific comparisons give a better chance of concluding on the evolution direction of characteristics being tested, because it occurs at the level of variability within the species (population) and not at the interspecific level. Secondly, based on intra-species comparisons, potential errors in the inference resulting from the influence of phylogeny on the analyzed features are avoided, and these are usually accompanied by interspecies comparisons (Garland et al. 1999). Thirdly, modern technical solutions used in BMR measurement have greatly reduced the scope of measurement errors (Konarzewski et al. 2005) and enabled demonstration of the individual BMR repeatability (review in Nespolo and Franco 2007). This is a strong argument for using the intra-species variability of BMR to test the relationship between it and interpersonal differences such as essential components of energy budgets, metabolic costs of maintaining the internal organs that determines the energy expenditure, metabolic costs of immunocompetence, and finally the relationship of all these traits to survival.

It was a convenient circumstance for my research to become involved in a selective experiment that resulted in two unique lines of experimental mice (*Mus musculus*) characterized by low (L-BMR) or high (H-BMR) metabolic rate (Książek et al. 2004). The starting point for launching the study using this animal model was to evaluate the initial effects of artificial selective pressure. These results were part of my doctor dissertation (Works 3 and 4, Chapter II.A in Appendix 4). At this point, I refer only to those results that were most important from the point of view of the appropriateness of the research described in this chapter. The analyzes have shown that the selection procedure used has led to the disclosure of 30% of the inter-line difference in BMR (at present, this difference is about 50%). Importantly, intra-line differences in BMR have been genetically correlated with those in internal organs weight (small intestine, liver, kidney and heart) directly involved in food assimilation and energy conversion as well as with about 10% difference in consumed food

intake. The analysis also confirmed that BMR is a reproducible and inheritable feature (review in Konarzewski and Książek 2013). There is therefore no better way to test the relationships between the intra-species variability in BMR and the characteristics that affect the adaptation and shaping the life of organisms than the use of animal models with genetically determined inter-line difference in basic energy expenditures on living processes.

Of the five papers identified as the science achievements in the habilitation, three present the results of research I conducted in the laboratory using the model described above. In work No. [1], I demonstrated that high BMR and large mass of metabolically active organs translates into greater reserve capacity and phenotypic plasticity of these organs in response to sudden onset of cold stress (5°C). In work No. [2], I showed that environmental stress in the form of a 30% reduction in the amount of consumed food causes immunosuppression, and even under those circumstances, individuals with high BMR are building a stronger humoral response than those with a low metabolic rate. In work No. [4], I showed that heat transfer in individuals with high BMR and maintaining higher body temperature, was not a factor limiting the amount of energy expenditure devoted to the development of a humorous response, since even under the thermal stress (34 °C), there was no suppression. In summary, the above works show how BMR variability is associated with the ability to compensate both sudden and long-term metabolic stress.

Because laboratory research is conducted under controlled and predictable conditions, it is only possible to suppose how the interdependence of physiological features affects the survival - in addition to the reproduction of the most important parameter of Darwinian adaptation (Stearns 2002). Answers to questions about actual biological significance of metabolic rate variability and related features may be sought only in the field studies involving the monitoring of animals in their natural environment. To meet this challenge, I completed a comprehensive database of not only data on metabolic rate measurements but primarily on the mortality of tundra vole (*Microtus oeconomus*). I carried out the field works in the Biebrza National Park, using the technical facilities offered by the UwB Field Station of the Biology Institute located in the village of Gugny. The information completed during the 3 successive hunting seasons was used to prepare the work No. [3], in which I analyzed the BMR and PMR heterogeneity among individuals (since the individual differences in balancing of the energy budget and maximum energy expenditure are indicated as reasons for decimating the small mammal populations during winter, e.g. Aars and Ims 2002, Ergon et al. 2004, Zub et al. 2014), as well as immunocompetence and the level of parasitic infestation (because parasitic infestation has a significant impact on survival, and development of an immune response against parasites is energetically costly, e.g. Lee and Klasing 2004, Lee 2006, Burthe et al. 2008, Kloch et al. 2012). Next, I investigated whether the correlations between BMR, PMR, immunocompetence and parasitic infestation have a combined effect on winter survival of tundra vole. The results I used in work No. [5] and I tested the effect of corticosterone on the above mentioned features. In addition to cortisol, corticosterone is the main hormone released by rodents in response to environmental stress, which is further attributed to the strong immunomodulating effects (Davis et al. 2008, Demas et al. 2010).

I collected results published in the paper [1] in an experiment conducted using the funds granted by KBN (point 3, chapter II.I in Annex 4). The research described in works No. [2] and [4] was made by means of the use of financial resources from the statutory grant financed by the Ministry of Science and Higher Education. I collected data published in the papers [3] and [5] during the research funded by the Ministry of Science and Higher Education (point 4, chapter II.I in Annex 4).

### Detailed discussion of hypotheses tested in individual papers and results

Książek A., Czerniecki J., Konarzewski M. 2009. Phenotypic flexibility of traits related to energy acquisition in mice divergently selected for basal metabolic rate (BMR). *Journal of Experimental Biology* 212: 808-814 [1]

Phenotypic plasticity is called the body's ability to reverse modulation of features (phenotype) in response to the effects of environmental factors (Roff 2002, Piersma and Drent 2003, Pigliucci 2005). Because the phenotypic plasticity of the characteristics influences on the adaptation of an individual, factors that determine the magnitude of this plastic reaction are now one of the intensively discussed issues of evolutionary biology (Piersma and van Gils 2010, Moczek et al. 2011, Laland et al. 2014, Noble et al. 2014). The phenotypic plasticity of the gastrointestinal tract, and in particular the processes involved in food production and energy production, are indicated as particularly important for adaptation (Karasov and Martinez del Rio 2007, Naya et al. 2008, Karasov et al. 2011, Vidal et al. 2014), including response to sudden and unpredictable metabolic burden. It is hypothesized that phenotypic response to sudden environmental stress is associated with an increase in food consumption and an increase in the rate of energy production dependent on the so-called immediate spare capacity of the digestive system so that the energy obtained can be used to increase the rate of physiological processes in the following step (Karasov and McWilliams 2005). Model proposed by Karasov and McWilliams (2005) suggests that the primary determinant of this reserve capacity of digestive tract (and thus the phenotypic plasticity) is the mass of internal organs directly involved in energy acquisition and processing.

Because of the genetically determined inter-line differences in food consumption and the weight of the metabolically active organs, above described mouse lines are a convenient object to test this model. **The purpose of the paper was to answer the question whether phenotypic plasticity of internal organs in response to sudden environmental changes is determined by the individual BMR level.** To induce a sudden environmental stress, mice of both selection lines were transferred, without prior acclimation, from an ambient temperature of 23 °C to 5 °C, where they were maintained respectively for 2, 4 or 6 days. I expected that because of having smaller organs (and therefore less reserve capacity), the L-BMR mice would reveal a smaller increase in energy assimilation rate (relative to small organs) in response to the cold stress as compared to high BMR line mice. I also expected that the metabolic burden (measured by the consumption of food or the rate of energy

assimilation by weight of individual organs) forced by the sudden increase in energy demand, would be lower in H-BMR mice because of greater weight of their internal organs. The collected data allowed me to analyze the inter-line differences in food consumption and digestibility, rate of energy assimilation, mass of metabolically active organs (small intestine, liver, kidney and heart) and citrate synthase activity - one of the key enzymes of the Krebs cycle (Schaarschmidt and Jürss 2003, Tripathi and Verma 2004).

In response to exposure to 5 °C, the mice of both selection lines increased food consumption two times in the first 48 hours, with inter-line differences in consumption were constant throughout the experiment - H-BMR mice consumed more than individuals with low BMR. Interestingly, in both selection lines, the increased consumption in the cold corresponded to a decrease in digestibility of food by 25%, on average. However, the decrease in digestibility was, however, higher in low BMR mice. This result demonstrates the lower back-up capacity of internal organs of L-BMR mice to respond to metabolic stress. In addition, at comparable double consumption of food at 5 °C, mice with low BMR had significantly smaller intestines, liver, kidneys and hearts as compared to H-BMR mice. This means that the metabolic burden of internal organs resulting from the increased energy requirements caused by cold stress varied between the selection lines. Even though L-BMR mice 'accelerated' the work of their organs at 5 °C, their metabolic burden was much higher than that observed in high BMR mice. L-BMR mice were close to achieving a negative energy balance. Mice with high metabolic rate, greater body mass and greater back-up capacity of these organs were able to consume and process significantly more food than those with low BMR under the same conditions.

The rate of energy assimilation in the first 2 days of exposure to 5 °C increased in mice from both selection lines, with BMR mice having better coping with energy gain from food. This result corresponds to a faster rate of energy absorption by H-BMR mice observed at 23 °C. This suggests that activity of tissues that build up the reserve performance of individual organs under standard conditions (23 °C) is proportional to the total weight of these organs.

The increase in food consumption at 5 °C corresponded with increased weight of the small intestine, kidney and heart. Organ mass increased in both selection lines, but the magnitude of these lesions in L-BMR and H-BMR mice was similar and proportional to the inter-line differences observed at 23 °C. In addition, high BMR mice were also characterized by elevated citrate synthase in the liver and kidneys observed at 5 °C. Thus, the phenotypic plasticity of both internal organs dimensions and synthase activity was strictly proportional to the size of organs before the effects of metabolic stress, as confirmed by the assumption of the model proposed by Karasov and McWilliams (2005). My findings suggest that BMR variability positively correlates with individual differences in the reserve capacity of metabolically active organs, which increases the functional activity of organs in response to unexpected metabolic stress.

Książek A., Konarzewski M. 2012. Effect of dietary restriction on immune response of laboratory mice divergently selected for basal metabolic rate. *Physiological and Biochemical Zoology* 85:51-61 [2]

While in previous work I have tested the compromises between BMR variability, phenotypic plasticity of internal organs and adaptation to sudden environmental stress, I have been working on compromises that have emerged during the long-term stress in the present study. I focused on energy - a resource for which all physiological functions, including the immune response, compete. I used the protocol to limit the amount of available food (diet restriction, DR) to the animals during the compromise test. Application of the DR protocol at mouse lines, which differ in the amount of energy expenditure needed to maintain the organism, which is additionally correlated with the inter-line differences in metabolically active organ weight and food consumption, has allowed to demonstrate the compromise between immunocompetence and other body functions. **The purpose of the study was to answer the question whether DR induced immunosuppression would result from an energy compromise between the cost of developing a defensive reaction and the cost of maintaining internal organs.** To do this, mice of both selective lines were subjected to a 4-week DR regimen with a 30% daily food intake. After this time, I induced the development of a humoral response by immunizing mice with the KLH antigen. For inter-line DR differences analysis, I collected data on the level of anti-KLH IgM specific antibodies, total number of leukocytes (WBC) and neutrophil counts (N/L), metabolically active and lymphatic organs (lymph nodes, spleen and thymus). I was expecting a selective line dependent energy allocation from the immune response to cover the cost of maintaining the metabolically costly internal organs. I expected immunosuppression to be higher in high BMR mice, because due to the large organs and high energy requirements associated, compromise between them and costs of developing a defensive reaction would be easier to disclose.

The digestive regimen has suppressed the humoral response, but the decrease in antibody production was the same in mice from both selection lines. The reduction of antibody levels was not accompanied by a change in the WBC and N/L ratios. Regardless of the DR effect, high BMR mice were characterized by a higher response to KLH antigen and higher leucocytes count in the blood. Unlike the immunosuppression, the gastrointestinal regime has resulted in a selective line dependent lymphatic organs weight reduction. Spleen and lymph node weight loss was greater at H-BMR mice line and thymus mass decreased in low BMR mice.

The regime also reduced the weight of liver, kidneys and heart. Interestingly, mass of the small intestine proved to be significantly higher in DR mice. This reaction suggests an increase in the efficiency of feed digestion when it is supplied in limited quantities. This result corresponds to the observed only in DR and immunized mice, an increase in liver mass - an organ that produces immune proteins, including antibodies. The lack of interaction between DR regime and the selection line suggests that the metabolic response of the small intestine,

liver, and other organs towards DR was similar in both selection lines and corresponded to the absence of intra-line differences in reduction of the DR-induced humoral response.

Contrary to expectations, the immune response in the group of animals subjected to the feed regimen was higher in individuals characterized by high BMR. The obtained result suggests that even if the humoral response against KLH antigen was expensive, as shown by immunosuppression in the DR group, the size of these costs may not have been large enough to reveal a compromise between it and high metabolic costs of large internal organs. This shows, at least in part, that the immune response under conditions of food consumption reduction by 30% is not a priority and that its suppression is adaptive.

Książek A., Konarzewski M. 2016. Heat dissipation does not suppress an immune response in laboratory mice divergently selected for basal metabolic rate (BMR). *Journal of Experimental Biology* 219: 1542-1551 [4]

In this paper I proposed the use of BMR differentiation to test the heat transfer capacity, a potential factor determining the energy expenditure of organisms. Intensively tested recently, the heat dissipation limitation hypothesis (HDL) suggests that animal energy budgets are more limited by the ability to donate the heat generated (review in Speakman and Król 2010), not by the constraints associated with the absorption, processing and allocation of energy. So far, the limited ability to lose heat has only been tested during lactation, i.e. when maternal energy expenditure is the greatest. According to what the HDL hypothesis assumes, during lactation, the consumption of food grows until it is reduced by difficulty of giving back the heat as a byproduct of metabolism (Speakman and Król 2010). The study focused mainly on the manipulation of physical limitations of mothers to give back the heat produced during reproduction (by means of exposure to low or high ambient temperatures or shaving), and limited heat dissipation was assessed only on the basis of parameters determining the parental effort (e.g. the amount of milk produced or the growth rate of the offspring). Results of these experiments proved to be inconsistent and only partly supported the HDL hypothesis (Król and Speakman 2003ab, Król et al. 2007, Wu et al. 2009, Zhao et al. 2010, Yang et al. 2013).

In my approach to HDL testing, I assumed that if the heat transfer capacity really limits the energy expenditure, it should play the same role in performing other physiological functions than reproduction. Secondly, limiting the difficulty of heat transfer should also occur at lower feed rates than during lactation, but provided that the metabolic stressing factor increases the amount of heat produced, accompanied by an increase in body temperature. **The purpose of this study was to test the hypothesis that the reduction of energy expenditure resulting from the difficulty of heat dissipation may also be manifested by the increase in body temperature accompanied by the suppression of functions generating the metabolic heat.** To verify this, mice of low or high BMR lines were immunized with KLH antigen and immediately transferred from 23 °C to 34 °C. Exposure to the heat

lasted for 5 days, i.e. during the period of increasing the energy expenditure associated with the development of a humoral response. Selection for BMR differentiation also resulted in the emergence of inter-line differences that are important from the point of view of the assumptions tested in this work. Since high BMR mice are characterized by a 20-30% increase in the mass of metabolically active organs, they also produce more metabolic heat (Książek et al. 2004), thus maintaining a higher body temperature under standard conditions (23 °C) and in the thermoneutral zone at 30 °C (Gębczyński 2005, Książek and Konarzewski 2016). In contrast, both selection lines do not differ in body weight and have the same thermal conductivity (Gębczyński 2005). The choice of stressors in a form of KLH antigens and 34 °C was not accidental. The response against KLH generates a 20-30% increase in metabolic rate, and thus an increase in the amount of heat produced at mice (Demas et al. 1997). The temperature of 34 °C lies outside the thermoneutral zone for rodents, which means that under such conditions, the heat transfer to the environment is hindered (Król et al. 2003, 2007, Speakman and Król 2010).

I expected that due to the higher energy conversion rate that manifested by a higher body temperature at 23 °C, mice of a high BMR line would be more restricted by giving back the heat at 34 °C than L-BMR individuals. I assumed that if heat transfer limits the energy expenditure, the expenditures of L-BMR mice would equal the energy conversion rate at high BMR mice, and both lines would experience similar overheating. I was also expecting a suppression of the immune response as well as weight reduction of the heat-generating organs to avoid dangerous overheating of the body from the selective line. I expected that immunosuppression would be higher in mice with high BMR because of already high rate of heat production, which could still increase as a result of heat release associated with antibody production. To verify my expectations, I sought for inter-line differences in body weight, humoral response expressed as anti-KLH IgM antibodies and leukocyte counts (WBC and N/L ratio), mass of heat-producing organs (liver, small intestine, kidney and heart) and lymphatic organs (thymus, spleen and lymph nodes).

As expected, immunization with KLH antigen resulted in elevated body temperature, but the observed increase was the same in mice from both selection lines. Independently of the immunization effect, the inter-line differences in body temperature were also maintained at 34 °C, whereas exposure to high ambient temperature alone did not affect the body temperature changes at both selection lines. Metabolic stress at 34 °C did not cause immunosuppression, but the response to KLH antigen was different. Up to 34 °C H-BMR mice maintained a high blood leukocyte count, but their body temperature was identical to that at 23 °C. In contrast, L-BMR mice increased the mass of lymph nodes, which corresponded to the transient increase in their body temperature. Exposure to heat did not result in a reduction in lymphatic organs mass (spleen and thymus), but there was a reduction in peripheral blood leucocytes, decreased weight of liver, small intestine and kidney - organs that generate metabolic heat. The above changes were accompanied by weight reduction. In this way, mice reduced the rate of energy conversion at 34 °C. The analyzes did not confirm the interaction between the selection line and ambient temperature, thus the observed reduction was the

same in both selection lines. Metabolic stress in the form of simultaneous immunization with KLH antigen and exposure to 34 °C did not significantly limit the heat transfer even at high BMR mice and higher body temperature. The results of this study show that at least in relation to the animal model used in this experiment, the heat transfer is not a significant factor limiting the amount of energy expenditure.

Książek A., Zub K., Szafrńska P. A., Wieczorek M., Konarzewski M. 2014.  
Immunocompetence and high metabolic rates enhance overwinter survival in the root vole, *Microtus oeconomus*. *Biology Letters* 10, 20140684 [3]

Survival is one of the most important factors shaping the life histories of organisms under natural conditions. The way an animal copes in an environment to survive is made up of many functions, including the effectiveness of the immune system in fighting infections (Råberg et al. 2009). Immunocompetence, understood as the ability to recognize and eliminate pathogens, competes for resources with other metabolically costly functions (Lochmiller and Deerenberg 2000, Råberg et al. 2002, Hegemann et al. 2012). **The purpose of this study was to analyze the inter-individual differentiation of RMR and PMR, immunocompetence and mortality in the natural population of tundra vole** - herbivorous rodent well-known for the effects of pathogens pressure (Telfer et al. 2008, Kloch et al. 2012), as well as individual energy expenditure (Burton et al. 2011, Zub et al. 2014) influencing on the dynamics of this species. Interestingly, so far there is not much research to test the link between different energy expenditure measures (RMR and PMR) and physiological functions (e.g. immunocompetence) and winter survival in natural populations, and those that have undertaken such subjects usually tested each of the mentioned features separately.

I focused on evaluating selected non-specific immunity parameters, because (i) it develops first in response to pathogens, (ii) signals other immune components to action, and (3) its activity contributes to increased energy expenditure and induction of fever (Lee and Klasing 2004, Lee 2006). As a measure of immunocompetence, I have adopted the ratio of neutrophil count to lymphocyte count per 100 leukocytes (N/L), total white blood cell count (WBC) and natural antibody level (NAbs). The assessment of energy expenditure consisted in the measurement of the resting metabolic rate (RMR). Although the RMR includes energy expenditure related to feed digestion (animals are usually not starved before the measurement), this is a very close measure of the BMR. Since the voles live on sediment beds that are periodically flooded by water, the energy expenditure incurred for efficient swimming often in cold water affects their survival. For this reason, I also estimated the maximum instantaneous rate of metabolism (PMR) induced by swimming in the study population. Voles were harvested from a 1 hectare area surrounded by a high, deep-dug fence preventing from predators and prevented voles from migration. Data collection started in the fall (November). After all measurements and bioassays, the marked voles were released at the capture site and re-caught in January and then again in March to monitor their

individual survival. I completed the RMR, PMR and immunocompetence data for 3 consecutive years from 2008 to 2010. As more than half of the surveyed population was affected by the presence of the blood parasite *Babesia* spp. in 2008 (Kloch et al. 2012), I also correlated individual variation in immunocompetence with the level of parasite infestation due to the presence of this protozoa.

Voles with low N/L ratio were also characterized by very high levels of parasite infestation caused by *Babesia* spp. The individuals in this group were characterized by high mortality, especially in the second part of winter (from January to March). This period of winter was best survived by animals, at which I observed an elevated N/L index due to an increase in the number of neutrophils in the blood. Neutrophils are non-specific immunity cells that by phagocytosis can eliminate protozoa (Court et al. 2001, Davis et al. 2008). The increased number of these cells in the blood can therefore be attributed to the lower parasite infestation caused by *Babesia* spp. in voles that survived the second part of winter.

I had expected that in winter, energy expenditure on immunocompetence would compete with other energetically costly physiological functions at this time of the year. I observed that in the first part of winter (November-January) there was a negative correlation between the total number of blood leukocytes (WBC) and the resting metabolic rate (RMR). This means that wintering voles must experience compromises between energy placement into immunocompetence or other functions, such as thermoregulation, the expenses that are reflected by RMR.

In the first part of winter, there was a negative correlation between the N/L ratio and cPMR in studied population. As I mentioned earlier, the increased N/L ratio has helped the voles to control their levels of parasite infestation and increased their chances for survival, but only in the second part of winter. Meanwhile, elevated cPMR positively correlated with early winter survival (Zub et al. 2014). Results of my research suggest that early winter favors individuals that swim well between sedges, but they do less with fighting infections caused by *Babesia* spp. In the second part of winter, when the water freezes and there is no need to invest in swimming in the cold water at this time of year, greater efficacy in controlling infection becomes a factor to increase survival in individuals with high N/L ratio.

Książek A., Zub K., Szafrńska P. A., Wieczorek M., Konarzewski M. 2017. The nexuses hair corticosterone level, immunocompetence, metabolic rates and overwinter survival in the root vole, *Microtus oeconomus*. *General and Comparative Endocrinology* 250: 46-53 [5]

Corticosterone is one of the most important hormones of the hypothalamic-pituitary-adrenal axis regulating the physiological and psychological reactions of most vertebrate animals to stress. Previous studies have shown that elevated corticosterone levels in response to long-term stress have immunosuppressive effects (Martin et al. 2005, Demas et al. 2010, Bourgeon et al. 2010, Yadav and Haldar 2014), regulates weight-dependent energy expenditure (Buehler et al. 2012, Dlugosz et al. 2012, Versteegh et al. 2012, Downs et al. 2013) and

consequently also affects survival (Cote et al. 2006, Breuner et al. 2008, MacDougall-Shackleton et al. 2009, Marasco et al. 2015). This work is one of the first to attempt to combine all of these parameters of individual adaptation with the survival of animals in natural populations. In work No. [3] I showed, among other things, that when in 2008 survival of voles in the studied population decreased by 2 times due to infection caused by *Babesia* spp. (Kloch et al. 2012), intensity of the parasite infestation was inversely correlated with the value of N/L index, and individuals that were better at dealing with the infection, were also more likely to survive the second part of winter. **In this work, I wanted to link immunocompetence (expressed by N/L ratio), parasite infestation due to *Babesia* spp., metabolic rate (expressed as RMR and PMR), and winter survival of voles with individual differences in corticosterone levels**, the most important hormone regulating the stress response in rodents (Woolsey et al. 2015, Yu et al. 2015, Jarcho et al. 2016). I want to emphasize that my work is one of the first to test the level of corticosterone in animal hair collected from wild animals remaining in their natural habitat throughout the study period. I sampled hairs from the same individuals that were measured the rate of metabolism and took blood in 2008-2010. Determination of corticosterone in the hair coat becomes an increasingly popular alternative to the evaluation of this hormone in blood, saliva, urine or feces (Koren et al. 2002, Accrosi et al. 2008, Bennet and Hayssen 2010, Galuppi et al. 2013). Hair samples are easier to collect, easy to transport, and above all, hormone levels do not change due to the temporary stress associated with animal harvesting (Yang et al. 1998, Koren et al. 2002). The level of corticosterone in the hair accumulates over a long period of time (weeks or months) with individual experience of an individual, and is therefore a suitable measure of a long-term stress at animals from natural populations.

In the period between November and January, corticosterone levels were positively correlated with body weight, the main determinant of survival in this part of winter among studied vole population (Zub et al. 2014). At the same time, corticosterone levels were negatively correlated with survival at the same time interval. High mortality was observed in those voles that were characterized by high levels of this hormone accumulated in the hair coat.

Analyses have shown that corticosterone was a significant predictor of RMR variability, but this effect disappeared after RMR adjustment for body weight. Considering the aforementioned positive correlation between body weight and corticosterone, as well as positive correlation between body weight and RMR demonstrated in other studies using more individuals in the same population (Zub et al. 2014), I can conclude that the indirect nature of the relationship between corticosterone and RMR levels is due to the very strong body weight association with both stress hormone levels and RMR levels. I have not observed any significant relationship between the level of corticosterone and swimming-induced PMR.

Because long-term stress is believed to be immunosuppressive, I wanted to see if corticosterone deposited in the hair coat could be the predictor of individual differences in immunocompetence. To find out more about the immunomodulatory role of corticosterone, I analyzed its association with N/L ratio and the parasite infestation with *Babesia* spp. in 2008,

when level of survival in the studied population was the highest. If corticosterone is immunosuppressive indeed, I expected a negative correlation between its level and the N/L ratio, which should be different, this time, a positive relationship between corticosterone and the intensity of parasite infestation. Contrary to my expectations, in 2008 there was no correlation between corticosterone levels and N/L ratio, which was negative correlated with corticosterone levels only when the analysis was performed for all voles collected during three years of study (Figure 2 in this study). In addition, in 2008, there was a negative, rather than positive as I expected, relationship between corticosterone and the level of parasite infestation induced by *Babesia* spp. My results suggest that the lack of correlation between corticosterone and N/L ratio was due to small inter-individual N/L values differentiation in 2008, because individuals both with low and high corticosterone levels had comparatively high N/L ratio, which was much higher than in the remaining years (Figure 2). This result does not confirm the immunosuppressive effect of corticosterone. On the other hand, the lack of direct correlation between corticosterone and N/L ratio in 2008 may not be due to a lack of functional association between the hormone levels and immunocompetence, while it may result from diametrically different time scales, in which information on corticosterone levels and blood neutrophils count accumulate - these are weeks or even months in the case of corticosterone accumulating in the hair coat as well as short-term fluctuations in blood counts over days or even hours.

## II. DISCUSSION OF OTHER SCIENTIFIC AND RESEARCH ACHIEVEMENTS

(references in the text correspond to different items in Annex 4)

### A) before obtaining a PhD degree

My interest in science has focused on testing the physiological and evolutionary consequences of inter-individual differentiation of the BMR since I began work at the UWB Biology Institute.

Research conducted for the preparation of the doctoral thesis was of two kinds. Firstly, they concerned the assessment of the use effects of artificial selection pressure on the BMR differentiation (works No.3 and No. 4, chapter II.A in Annex 4). I mentioned some of the most important results in the introduction to chapter I (page 5). At the same time as research on evaluation of selection effects, I also started research on estimating the energy costs of immune response development (works No. 1 and No. 2, chapter II.A in Annex 4). This is how I got involved in the often undertaken attempts to test the energy compromise (trade-off), which is the mechanism that shapes the life histories of organisms (Stearns 2002).

Prior to obtaining the doctoral degree, I published a total of 4 papers, all indexed in the *Journal Citation Reports* database. The dissertation was composed of three articles (points 2-4, chapter II.A in Annex 4); I was the first author in two ones. During this period of my research, I started working with colleagues from the Jagiellonian University (points 1 and 2,

chapter III.Q.1), I also completed several internships to familiarize myself with immunological techniques (points 1-4, chapter III.L). Research on estimating the cost of immune response development at mice differing in BMR was made due to financial resources granted to me by KBN (point 1, chapter II.I).

B) after obtaining a PhD degree

The main direction of my research work is the study of physiological, ecological and evolutionary effects of inter-individual variability within the BMR. Along with conducting my own research (described in chapter I), I was also engaged in projects implemented by my research group colleagues at my home institution and focused on life-cycle energy issues. Studies, in which I participated, have shown that individuals with high BMR have a mean reduction in the mean number of double bonds in phospholipids of liver cell membranes (point 5, chapter II.A). This dependence turned out to be the opposite of that observed in cross-species comparisons. It is also incompatible with the 'membrane pacemaker' theory that correlates the evolution of high BMR and endothermia with an increase in the number of double bonds in cell membranes (Hulbert 2007). In another work, we have shown that the effect of limiting the amount of food consumed on the rate of metabolism depends on its initial level. Although after the 6-week dietary regimen of delivering 70% of daily requirements, intra-line differences in BMR persisted, however, mice in both selection lines lowered this value, and the BMR was greater in H-BMR line. This is an important observation, because it explains previous difficulties encountered by researchers when testing the effects of dietary regimen, the treatment commonly used in aging studies, and the rate of metabolism (point 6, chapter II.A). The following study showed that in response to the same food regimen, but maintained for 1 month, the inter-line difference in spontaneous motor activity remained at the same level. Contrary to expectations, the weight loss accompanying the regime was the same in both selected lines. H-BMR mice could therefore maintain (genetically superior to L-BMR line mice) spontaneous motor activity even under reduced feed consumption conditions due to diminished BMR (which was still higher than at L-BMR line). This result may indicate a higher plasticity of the energy budget of high BMR mice, which seems to confirm that they were able to maintain higher metabolic rate as compared to L-BMR mice even after a 6-week diet regimen (point 12, chapter II.A). Selected mouse lines have also been used to test the hypothesis of the evolution of endothermia with selection for more effective offspring care (Koteja 2000, Nespolo et al. 2011). It is believed that greater investment in the care of the offspring entails an increase in the cost of reproduction, and one of them may be to raise the level of oxidative stress (Speakman and Garratt 2014). Females of BMR lines actually invest more in young during lactation (Sadowska et al., 2013), but have lower levels of lipid and DNA damage, despite the lack of inter-line differences in antioxidant defense levels. Lactation did not therefore raise the level of damage caused by oxidative stress in H-BMR mice, which means that keeping higher BMR does not necessarily entail the cost of increased oxidative stress during breeding (point 9, chapter II.A). Results of the study

described here have been used to prepare a review of the effects of selective experiments to manipulate the inter-individual differentiation of BMR and other correlated features as well as to discuss the determinants of such differentiation at different levels of biological organization (point 7, chapter II.A).

A long-term project for monitoring the dynamics of population in natural northern vole populations within Biebrza National Park area has given me a great opportunity to examine the plant-herbivore interactions as well as to test the factors that increase the daily energy expenditure of small rodents in their natural habitat. At the same time with my own researches described in works [3] and [5], I also participated in the implementation of scientific projects of colleagues from other research centers. Due to regular animal catch and monitoring of their survival, it was possible to test the hair coat shaving performance as a method to significantly increase the energy expenditure of rodents in the wild. Experimental manipulation of energy expenditure, although successfully tested in nature at birds during reproduction, at mammals is mainly restricted to controlled laboratory conditions. Measurement of daily energy expenditure (DEE) using double-labeled water has shown that voles lacking around 50% of their hair significantly increased their energy expenditure. Interestingly, the likelihood of their re-catching very significantly depended on body weight, but was the same in the shaved and control subjects. In the case of heavier shaved voles, their greater hunting capacity could be due to increased motor activity and prolonged feeding time, which compensated their heat losses caused by experimental hair removal (point 10, chapter II.A). Another issue tested on the same animal population was the relationship between silicon content in sedge tissues and population number. It is assumed that the amount of silicon accumulated in plants is their response to the damage caused by biting. In the population studied, the high density of vole observed at the end of summer caused a remarkable increase in silicon accumulation in the roots and then in the leaves of sedge the following year. During winter, silicon content in plants varied depending on the temperature and thickness of the snow cover, which influenced on the quality of feed available at this time of the year. However, greater mortality of voles observed at the very beginning of winter suggests that the probability of survival did not depend on the quality of feed available in the middle of winter, but rather on the concentration of silicon in sedges in autumn (point 11, chapter II.A).

Free-living voles, although in different from the above population, were also the subject of behavioral research to test the relationship between social status, testosterone levels and odor intensity (point 8, chapter II.A). Social status makes it easier to compete for females and thus increases the reproductive success of males. Although it is generally thought that dominant individuals are more weighty, maintain elevated testosterone levels, and more effectively use odor as an indicator of social position, this relationship is not so universal and at some species in nature it simply does not occur. We observed that the highest level of testosterone remained by males with a subdominant position, the hormone level does not depend on body weight, the intensity of the odor does not correspond to the social position and weight, and the latter is the same in the dominant and subdominant male individuals as

well as those with the lowest status. Studies have shown that in the northern vole population, high body mass, high testosterone levels and odor intensity do not indicate the high social status of males.

In conclusion, in addition to the works indicated as a scientific achievement in the habilitation procedure, after obtaining a PhD degree I published another 8 articles (all indexed in the *JCR* database). These works were created through involvement in research carried out by colleagues from the Institute of Biology UWB, including projects funded by competitions (points 2 and 4, chapter II.I), as well as cooperation with researchers from other scientific units (points 3 and 4, chapter III.Q.1). This stage of my work was particularly valuable to me, because I acquired all the new skills, not only in conducting fieldwork for several years, but also in planning and organization of the technical backbone of such a large project (point 3, chapter III.A.2). The project for monitoring the northern vole populations in Biebrza National Park was also described in the chapter of monographs edited and published by *Trans Humana Editorial* at University of Białystok (section 1, chapter II.D.2).

### III. PLANS FOR THE FUTURE

(references in the text correspond to different items in Annex 4)

In the near future, I intend to continue research on the borderline of physiology, energetics, ecology and evolutionism. In the second half of 2017, I will launch a 3-year project, in which I am going to propose an alternative to the current method of testing, the hypothesis of heat dissipation limitation (HDL). Instead of testing experimental systems, in which mothers did not bear the high cost of maintaining other functions and giving priority to only reproduction, I want to revert to trade-offs concept and to test the circumstances of their emergence in the context of the HDL hypothesis. The main goal of the project is to test the hypothesis, which assumes that if the energy budget of lactating females is limited by difficulty in giving away the heat, then compromise should be made between investing in parental care and the functioning of the immune system. The project was funded by the National Science Center (point 8, chapter II.I). The research will be carried out in international cooperation with Prof. John R. Speakman from the University of Aberdeen, Scotland - the originator of HDL hypothesis (point 5, chapter III.Q.1).

The purpose of another project, in which I have already participated is to study the effect of UCP1 protein on changes in oxidative stress during mammalian thermogenesis (point 7, chapter II.I). Oxidative stress is often referred to as a mechanism that may be at the molecular level responsible for revealing the evolutionary compromises. UCP1 protein allows the production of heat without the synthesis of free radicals, which can result in low oxidative stress during thermogenesis. By examining the activity and role of this protein in the induction of thermogenesis, it will be possible to verify that the higher the rate of metabolism is responsible for the rise in oxidative stress.

Both of the above research problems will be/have been tested using a line of laboratory mice characterized by low or high BMR. The same model was used together with my colleagues from the research team to initiate the pilot experiments exploring the impact of low or high basic metabolism on mitochondrial structure, their respiratory activity, and mTOR protein responsible for the manifestation of inter-individual variability in BMR at molecular level (e.g. Laplante and Sabatini 2009). Results of these pilot studies will influence on the direction of further work.

Recently, I have also collaborated with researchers from other centers (points 3 and 6, chapter III.Q.1), where I have the opportunity to test the impact of the environment on the plasticity of physiological, life and behavioral traits of birds and mammals. I am participating in the implementation of a project on learning about the mechanisms of social evolution at male bats (point 6, chapter II.I) and in the program devoted to the active protection of the wood grouse population and its reintroduction in the Lower Silesian Forests and the Augustów Forest (points 9 and 10, chapter II.I).

### **General summary of scientific achievements**

A detailed list of published research papers, as well as information on didactic work, cooperation with scientific institutes, traineeships and training, as well as work for the home institute, is provided in Appendix 4.

The current academic output consists of a total of 17 publications in JCR journals (including 16 original experimental works and 1 review), 1 chapter in a monograph, and 30 conference reports (6 of which are published in peer-reviewed journals in the Web of Science). The characteristics of the scientific achievements, including the scoring of the publication according to the Ministry of Science and Higher Education as well as the IF coefficient, are presented in Table 1 below.

Of the 13 papers published after obtaining a doctoral degree, 5 experimental papers constitute a scientific achievement in the habilitation procedure. For all 5 publications, I am the first author and correspondent author. The results contained in these papers provide a coherent whole and oscillate around two issues:

- a) physiological and evolutionary trade-offs in a unique world-wide laboratory mouse model selected for low or high metabolic rate (BMR);
- b) inter-individual general (BMR), maximum (PMR) differentiation, non-specific immunity and corticosterone levels, as well as the effect of such differentiation on winter survival of small mammals in natural populations (northern voles).

Web of Science total number of citations: **374**, without self-citations: **338**

Scopus total number of citations: **406**, without self-citations: **370**

Hirsch index according to Web of Science: **h = 10**

Hirsch index according to Scopus: **h = 10**

(updated October 11, 2017) to the communication of MSHE from 12<sup>th</sup> of December 2016, *Impact Factor (IF)* according to 2016).

	BEFORE DOCTORATE			AFTER DOCTORATE			TOTAL		
	number	IF	points MSHE	number	IF	points MSHE	number	IF	points MSHE
Publications other than the scientific achievement	4	14,366	160	8	24,323	270	12	38,689	430
Publications indicated as the scientific achievement	-	-	-	5	14,418	160	5	14,418	160
Chapters in monographs	-	-	-	1	-	-	1	-	-
<b>TOTAL (publications)</b>	<b>4</b>	<b>14,366</b>	<b>160</b>	<b>14</b>	<b>38,741</b>	<b>430</b>	<b>18</b>	<b>53,107</b>	<b>590</b>
Conference abstracts	4	2,382	45	26	11,340	205	30	13,722	250
<b>TOTAL (publications and abstracts)</b>	<b>8</b>	<b>16,748</b>	<b>205</b>	<b>40</b>	<b>50,081</b>	<b>635</b>	<b>48</b>	<b>66,829</b>	<b>840</b>

Białystok, .....du 11.10.2017 o.

Aneta Książek

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