

Szczecin, 15th July, 2023


The review of the doctoral thesis of Mr. Henryk Kozłowski, M.Sc. entitled “Hyperthermia and Immune Response: exploring the immunoregulatory potential of fever-range temperatures” under the supervision of dr hab. Sylwia Wrotek, prof. NCU from the Department of Department of Immunology, Faculty of Biological and Veterinary Sciences, Nicolaus Copernicus University in Toruń. The review has been prepared due to the vocation of the Biology Discipline Council of the Nicolaus Copernicus University in Toruń dated at 12<sup>th</sup> June 2023, signed by prof. dr hab. Justyna Rogalska.

## I. Basic characteristic of the doctoral thesis

The doctoral thesis of Mr. Henryk Kozłowski, M.Sc. is composed out of three original papers published (or sent to review) in prestigious journals in the field of biology. Here is the list of the papers, with Mr. Henryk Kozłowski, M.Sc. contribution, based on the parts stating the contribution in each paper included in the thesis:

1. **Kozłowski H.M., Sobocińska J., Jędrzejewski T., Maciejewski B., Działuk S., Wrotek S.:** *Fever-range whole body hyperthermia leads to the changes in immune related genes and miRNA machinery in Wistar rats. Int. J. Hyperthermia 2023; 40: 2216899 (IF 3,753, points MEiN 100)*

This paper was under review at the time of collecting the documents, but now it is published. Mr. Henryk Kozłowski, M.Sc. is first and corresponding author of this paper. Moreover, his contribution (as stated in the paper) is also: conceptualization, methodology, software, investigation, resources, data curation, writing the original draft of the manuscript, visualization, project administration. I take this information for granted as true and final, nevertheless I would recommend including the statements from the first Author describing in detail the workload done by him, together with the statements of co-authors with their signatures.


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2. **Kozłowski H.M., Sobocińska J., Jedrzejewski T., Maciejewski B., Działuk A., Wrotek S.:** *Fever-range hyperthermia can affectively switch macrophage polarization towards regulatory phenotype. Sent to the Journal in May 2023*

According to the data available in the documents, the paper was sent to BMC Biology, as far as I know has not been published yet. Mr. Henryk Kozłowski, M.Sc. is the first author of this paper, I have no knowledge on the corresponding author in this paper. Again, the roles of the Author are known by the section contributions, not by the individual statements of the authors, which in my opinion is a pity. Mr. Henryk Kozłowski, M.Sc. claims to take actions in conceptualization, methodology, software, investigation, resources, data curation, writing and preparation of the original draft, visualization, project administration.

3. **Kozłowski H.M., Pawlikowska M., Sobocińska J., Jedrzejewski T., Działuk A., Wrotek S.:** *Distinct Modulatory Effects of Fever-Range Hyperthermia on the Response of Breast Cancer Cells and macrophages to Mistletoe (*Viscum album L.*) Extract. *Pharmaceuticals* 2021, 14, 551 (IF 5,711, points MEiN 100)*

In this paper Mr. Henryk Kozłowski, M.Sc. is first author and the corresponding author together with another author of this paper Mr. Artur Działuk. Mr. Henryk Kozłowski, M. Sc. Claims to take part in conceptualization, methodology, investigation, data curation, writing the original draft of the manuscript, together with editing and review for the final version, visualization and funding acquisition – the paper is sponsored by project “POWR no. 03.05.00-00-Z302/17 “Universitas Copernicana Thoruniensis in Futuro” 2018-2022.

The overall scientific soundness of the papers included in the doctoral dissertation of Mr. Henryk Kozłowski, M.Sc. is high. All papers are published (or sent to review) in well recognized scientific journals from the discipline of biology, listed on JCR list. The subject of the dissertation “*Hyperthermia and Immune Response: exploring the immunoregulatory potential of fever-range temperatures*” is very proper and well-adjusted to the content of the papers. Body temperature increasement is an issue exploit for many years and rising many questions. Numerous studies have been conducted to understand the role and molecular aspects of fever or hyperthermia – a process of raised temperature caused by external factors. Moreover,



the medical application of heat has a long history – hyperthermia might be induced as local, regional or whole-body hyperthermia. Therefore, studies aiming at deepening the knowledge how fever-range hyperthermia (FRH) influences the innate response of the organism is fundamental and crucial.

The thesis consists of three original papers answering the main goals of Mr. Henryk Kozłowski, M.Sc. in this dissertation., which were constructed as follows:

1. To comprehensively investigate how FRH treatment affects hematological profile, and regulatory molecules such as cytokines and miRNA;
2. To accurately identify the FRH-induced macrophage phenotype;
3. To explore the potential of FRH to modify the effects of the TLR-4- dependent stimulators.


The aims are accurate and achievable, but at the same time ambitious and with a deep scientific meaning. It is a pity that in this dissertation on review paper is included, which could give the readers a broader idea of the concept, but the written part of the dissertation fills this gap.

An important advantage of the doctoral dissertation of Mr. Henryk Kozłowski, M.Sc. is also the variety of methods used in the experimental papers introduced in the selection. From in vivo rats' experiments and in vitro experiments with the use of cell lines, flow cytometry, colorimetric assay, blot assays to real time PCR and more.

The overall impression of the doctoral dissertation is very positive, all the stated above arguments confirm the high methodological and substantive preparation of Mr. Henryk Kozłowski, MSc.

## **II. Description of the papers included into the doctoral dissertation**

As stated before, the dissertation is a compilation of the published (or sent to review) papers and has been prepared with a large dose of diligence. The Author has included an abstract in Polish and English, which is a good summary of the most important data included in the papers. Unluckily, I do miss the statements of the Authors, by which one could have an overall information about the process of preparation of these papers.



**II.1. Paper 1:** *Kozłowski H.M., Sobocińska J., Jędrzejewski T., Maciejewski B., Działuk S., Wrotek S.: Fever-range whole body hyperthermia leads to the changes in immune related genes and miRNA machinery in Wistar rats. Int. J. Hyperthermia 2023; 40: 2216899*


In this paper it is shown, that FRH in addition to being a therapy, is also used as a surrogate of fever in experimental settings. Setting these experimental conditions is also an achievement of Mr. Henryk Kozłowski, as he claims to set up the best model for work with FRH in Wistar rats. As result is its claimed that FRH triggers overexpression of G-CSF in rats' liver and upregulates the IFN- $\gamma$ , resulting in the overall increase of proinflammatory profile in the organism. Moreover, the whole body FRH decreases the level of miRNA-155. Mr. Henryk Kozłowski, M. Sc. Concludes that FRH may modify the expression of regulatory molecules – cytokines and miRNA, which is an important finding in the whole dissertation.

**II.2. Paper 2:** *Kozłowski H.M., Sobocińska J., Jędrzejewski T., Maciejewski B., Działuk A., Wrotek S.: Fever-range hyperthermia can affectively switch macrophage polarization towards regulatory phenotype. Sent to the Journal in May 2023*

This is in my opinion a very novel idea and the most important achievement in the dissertation of Mr. Henryk Kozłowski, M.Sc. as he showed that FRH-treated macrophages display increased expression of surface CD163 and arginase-1 and lower levels of CD80, iNOS and nitric oxide, which proves that during FRH treatment macrophages polarize towards M2 subtype, and to be more specific M2b polarization. This is a significant finding confirming that elevated body temperature may have a role in resolving inflammation (worth stating that M2b macrophages express high levels of TLR4, that allow intermediately the above conclusion).

**II.3. Paper 3:** *Kozłowski H.M., Pawlikowska M., Sobocińska J., Jędrzejewski T., Działuk A., Wrotek S.: Distinct Modulatory Effects of Fever-Range Hyperthermia on the Response of Breast Cancer Cells and macrophages to Mistletoe (*Viscum album L.*) Extract. Pharmaceuticals 2021, 14, 551*

In this paper a natural trend was implicated, that is a response of FRH to breast cancer cells and macrophages to the extract of mistletoe (ME). The administration of ME did not affect the cell cycle distribution in macrophages, but it reduces the viability of these cells. Simultaneously,



the administration of ME did not generate ROS in macrophages, but the increase of the temperature from 39 °C to 41 °C provoke a twofold increase in ROS levels and increase in the expression of inflammatory cytokines IL-6 and IL-1 $\beta$ , as well as COX-2 mRNA. As a result, one may conclude that FRH at 41 °C may elicit a stronger pro-inflammatory response.

Mr Henryk Kozłowski, M.Sc. concluded the following, from the papers being his doctoral dissertation:

1. FRH as a medical treatment has potential for achieving various therapeutic effects at various levels – gene expression, proteins, and whole organism. Moreover, FRH may also be beneficial in promoting recovery by maintaining the immune homeostatis;
2. Febrile increase in body temperature is a crucial factor in the resolution of inflammation, preventing chronic inflammation by inducing regulatory subset of macrophages;
3. FRH is a potent immunomodulator that can change the effects of other stimulators, therefore the method may serve as a potentially immunomodulationg.

In my opinion, the conclusions are drawn correctly with a huge sense of scientific maturity. The studies of Mr. Henryk Kozłowski, M.Sc. are of a biological importance and applicational (medically) character. The results constitute an important step forward understanding the role of body temperature and its immunomodulatory effect.

### **III. Final remarks and conclusion**

The doctoral dissertation of Mr. Henryk Kozłowski, M.Sc. entitled "*Hyperthermia and Immune Response: exploring the immunoregulatory potential of fever-range temperatures*" is an original and complete selection of published (and sent to review) papers in well-recognized journals. The scientific soundness of the papers included in the selection is high. The conclusions constitute an important new element of biological knowledge in the field.

Bearing in mind all the advantages of the doctoral dissertation presented to me for evaluation, in particular:

- accurate verification of the research problem and the specific type of gap that the research fills;
- well-established knowledge of the research;
- properly planned and performed research experiments presented in the papers, with a wide range of used methods;

- correct confrontation and argumentation, as well as interpretation of the obtained results and skillful discussion;
- the appropriate language, specific for the subject of the research, the coherence of the texts;
- the ability to correctly draw conclusions,

I confirm that the doctoral dissertation of Mr. Henryk Kozłowski, MSc., written under the supervision of dr hab. Sylwia Wrotek, prof. NCU, complies with the conditions set out in the Act of 20 June, 2018 Law on higher education and science (Art.187 on the doctoral thesis) and my opinion on the given dissertation is **positive**. Moreover, Mr. Henryk Kozłowski, M.Sc. has fulfilled the requirements stated in the Act of 20 June, 2018 Law on higher education and science (Art. 186 paragraph 1 point 5) and should be admitted to defend his doctoral dissertation.

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