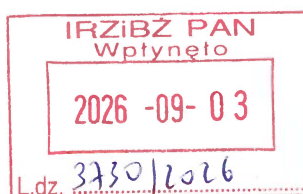




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Poznań, 31.08.2026

Review of PhD thesis

Author: Ranjini Ghosh Dastidar

Thesis title: A Dynamic Regulatory Model of *In Vivo* Vitamin D Responsiveness: From Transcriptomic Discovery to Systems Identification in Human Immune Cells

Scientific supervisor: Professor Carsten Carlberg

General description of the dissertation

The dissertation submitted for evaluation is written in English and comprises 18 chapters: General Introduction, Objectives and Hypotheses, descriptions of four publications, Combined Observations, General Discussion, Conclusions, Implications and Recommendations, List of Figures, List of Tables, List of Publicly Available Data, References, Acknowledgements, Author's Statements, Official Documents, and the appended publications. This is a typical structure of doctoral theses. However, in my opinion chapters 10-18 should not be presented as numbered chapters, similarly to abstracts and abbreviations. Moreover, instead of publication details, a methods chapter could be provided, which could incorporate the reader's guide to the studies in this thesis.

The following publications - three published and one under review - were included in the thesis:

1. Ranjini Ghosh Dastidar, Julia Jaroslawska, Marjo Malinen, Tomi-Pekka Tuomainen, Jyrki K. Virtanen, Igor Bendik, Carsten Carlberg. *In Vivo* Vitamin D Targets Reveal the Upregulation of Focal Adhesion-Related Genes in Primary Immune Cells of Healthy Individuals. *Scientific Reports* (2024) 14:17552 • DOI: 10.1038/s41598-024-68741-9
2. Shareefa A. AlGhamdi, Ranjini Ghosh Dastidar, Maciej Rybinski, Hadeil M. Alsufiani, Sawsan O. Khoja, Nusaibah N. Enaibsi, Safa F. Saif, Carsten Carlberg. Evaluation of the Vitamin D

Response Index in a Saudi Cohort. *Saudi Pharmaceutical Journal* (2024) 32:102137 • DOI: 10.1016/j.jsps.2024.102137

3. Emilia Gospodarska, Ranjini Ghosh Dastidar, Julia Jaroslawska, Maciej Rybinski, Marianna Raczyk, Kornelia Tokarczyk-Malesa, Jerzy Romaszko, Carsten Carlberg. Transcriptomic Profiling of Immune Modulation Induced by Vitamin D₃ in the VitDPAS and VitDHiD Cohort Studies. *Scientific Reports* (2025) 15:17334 • DOI: 10.1038/s41598-025-02495-w
4. Ranjini Ghosh Dastidar, Emilia Gospodarska, Kornelia Tokarczyk-Malesa, Jerzy Romaszko, Elisa Domínguez-Hüttinger & Carsten Carlberg. Dynamic System Identification Defines a Minimal Transcription Factor Control Architecture of Human Endocrine Gene Regulation. Under review

Contribution of the doctoral candidate included: conceptualization, data analysis, visualization and writing of drafts, which indicates her significant role in preparing the articles.

The thesis focuses on the central problem of the lack of response to vitamin D supplementation. An issue of variable response to numerous nutrients or pharmaceuticals is well recognized in scientific literature. However, despite its recognition, the molecular basis of this phenomenon are poorly understood and rarely investigated. For this reason the topic is both highly relevant and scientifically significant and may advance our understanding of interindividual variation in response to vitamin D supplementation.

Comments on the General Introduction:

- This part of the text shows that the author possesses the ability to present scientific ideas with the use of clear language and without overlong descriptions. However, the introduction in this general description, and to some extent introductions in the articles, is written in a rather unusual manner. Typically the introduction includes general background, specific background and knowledge gaps. The introduction in the thesis is mostly focused on explanations to potential reviewers' questions. In this way one of the main issues, which is of lack of response to supplementation in large scale trials is described too briefly, without presentation and comments on these studies. Moreover, some sections could be moved to the description of methods, together with the reader's guide to the studies in this thesis.

- Section 1.1 should include information on dietary sources of vitamin D. Section 1.2: not all biological actions of vitamin D are mediated through VDR.

Comments on Objectives and Hypotheses

The objectives and hypotheses are clearly presented. They are complementary and logically ordered. It should be emphasized that the aims were very broad and ambitious. However, my only comment is that whether variation in transcriptional responses is biologically meaningful has not been tested.

Comments on the methods used in the thesis

- The methods are not described after the General Introduction, instead they are presented separately in each article. The thesis is primarily based on data analysis, mostly data obtained from advanced, global gene expression analysis, which is a clear strength of the dissertation. Ranjini Ghosh Dastidar demonstrated the ability of data analysis, interpretation and description. However, although the thesis focuses on data analysis, it should not be presented without a close connection to the way in which these studies were conducted. This in fact determines quality of the data. In my opinion, detailed description of the interventions should be included. Lack of information on the study design, makes it difficult to properly evaluate and interpret the results. Furthermore, characteristics of the participants should also be provided.
- In the ClinicalTrials.gov database VitDmet (NCT01479933) VitDbol (NCT02063334) are described as placebo controlled interventions, whereas VitDHiD (NCT03537027) and VitDPAS (NCT06104111) did not include placebo comparator groups. Therefore in some cases data from placebo groups were available, and even though they were not used in the analyses. Lack of reference groups is the main limitation of this study. This is important for gene expression analysis as transcription profiles may vary for numerous reasons, e.g. season, diet, physiological and health issues, technical variation etc. This issue is especially important for the VitDPAS study, where repeated interventions and longitudinal measurements were applied. Without a placebo group, it is difficult to determine whether observed transcriptional changes are attributable to vitamin D supplementation or they reflect natural biological variation.
- The second potentially disputable aspect concerns using therapeutic doses of vitamin D in healthy people without severe vitamin D deficiency. Although, this approach was explained

in section 1.8 providing pharmacological rationale, the safety of administering such doses to otherwise healthy participants remains important consideration. Furthermore, this also raises a question whether the findings can be extrapolated to a more common situation of long-term supplementation with smaller vitamin D doses.

- The third issue is lack of any physiological, metabolic or health trait, which could be attributed to the observed differences in transcriptional responses. Without that the biological significance of these changes is unknown. Moreover, changes in transcription may not translate into changes in other steps of gene expression or into the different abundance of the final products.
- Statistical methods have not been fully described.

Questions:

- How was potential vitamin D toxicity monitored during the study? Were the volunteers under medical supervision?
- Were participants who were already taking vitamin D supplements prior to enrolment excluded from the study?
- How were changes over time in diet, supplements intake, physical activity monitored and controlled for during the study?
- How can low, medium and high responder be defined?

Comments on chapters 7, 8, 9, 10

- It is relatively common that scientist at the beginning of their career learn analysis of the results fairly quickly. However, a more advanced skill, which is synthesis and integration of knowledge needs usually more time. For this reason, Chapters 7 and 8 demonstrate that the doctoral candidate has successfully developed both these abilities, which should be emphasized.
- In my opinion the effects of vitamin D supplementation should be interpreted within a broader context than only epigenetic memory. There are numerous mechanisms which could contribute to the observed effects. Furthermore, not all main limitations have been identified.

- Ranjini Ghosh Dastidar suggests that supplementation guidelines, which recommend identical doses for all adults within broad age and weight categories, are biologically inadequate. How would the candidate reconcile this perspective with the fact that dietary guidelines, based on intervention studies, are aimed to cover the needs of the vast majority of the general population?
- When it comes to implications of the study for the clinical trial design, I am unsure how could this pre-trial stratification look like.
- In general, the conclusions adequately address the research questions. However, the order of conclusions objectives and hypothesis could be better aligned. Furthermore, the conclusions are properly drawn based on the obtained results, with the important limitation previously mentioned regarding the lack of reference groups, which does not allow to exclude potential random effects. In addition, the numerous exclusions may limit generalizability of the findings, which should be mentioned in the conclusions.

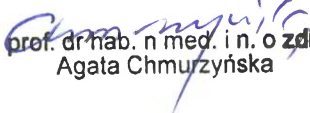
Comments on Article 4

- Overall, the manuscript was difficult to read in its current form.
- The summaries provided after each results section contain statements, which seem to be stronger than justified by the presented data.
- Were adjustments for age, gender or any other baseline parameters applied? If so, please clarify which variables were included, what statistical approach was applied, and how these adjustments were implemented.
- The manuscript states that the causal epigenetic mechanisms underlying this integration remain to be directly demonstrated. Why do you assume epigenetic mechanisms? What about other mechanisms which regulate gene expression?
- The following sentence in the results: *"In contrast, demographic parameters including age, sex, and BMI showed no significant association with response classification."* refers to a very important issue. Please indicate how this analysis was performed, where the detailed results are presented, and provide details of the statistical methods applied.
- Fig 1B and Fig. 2 do not provide information regarding the statistical methods used, and similar details appear to be missing throughout other parts of the manuscript.

- Why do low responders tend to have larger increases in 25(OH)D₃?

Final conclusions

In my opinion, the doctoral dissertation has significant scholarly merit and provides an original solution to the scientific research problem. Moreover, Ranjini Ghosh Dastidar has demonstrated her broad knowledge of the topic at hand. She has also demonstrated her ability to conduct complex research under supervision in order to test scientific hypotheses, and to write well-structured articles. I conclude that the dissertation presented for evaluation meets all the requirements described in the Article 187 of the Act of 20 July 2018 – Law on Higher Education and Science (Journal of Laws of 2024, item 1571, as amended). I therefore recommend the application of Ranjini Ghosh Dastidar to the Scientific Council of the InLife Institute of Animal Reproduction and Food Research for further processing of the PhD procedure.

ZŁOŻA KIEROWNIKA KATEDRY
ŻYWIENIA CZŁOWIEKA I DIETETYKI

prof. dr hab. n med. i n. o zdr.
Agata Chmurzyńska