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Review of Łukasz Szczerbiński's doctoral dissertation

The transcriptional signature of dysregulated glucose metabolism in skeletal muscle and its response to three-month exercise intervention in humans

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The doctoral dissertation of Łukasz Szczerbiński concerns an extremely important clinical and social problem which is type 2 diabetes. The rapid increase in the number of diabetic patients is, among others, a consequence of using civilization facilities, leading to a reduction of daily physical activity. Physical slowness is defined by the time spent in motion and the time spent in a sitting position. The body's energy balance in a significant dimension depends on metabolic processes in the muscles. Decreased sensitivity of skeletal muscles to insulin plays an important role in the etiopathogenesis of glucose metabolism disorders expressed by hyperglycaemia.

The subject of the review has a typical layout for doctoral dissertations in the form of a monographic thesis.

The introduction is a comprehensive introduction to the purpose of the work in the text and content. Based on the current literature data, the definition and criteria for the diagnosis of diabetes, the epidemiology of type 2 diabetes and the factors that increase the risk of diabetes mellitus are presented. Also, preventive measures have been described in detail, including the impact of treatment. The types of physical activity and changes taking place in the muscles under the influence of systematic physical training have been described.

The thesis research has been formulated clearly and precisely to justify the overall goal and specific objectives of the study, which was the identification of the transcriptional signature of dysregulated glucose metabolism in skeletal muscle and its response to

three-month exercise intervention in humans, by profiling the transcriptome using next-generation sequencing (NGS). Specifically, this project answers three questions:

1. What is the baseline difference between patients with type 2 diabetes, prediabetes and normoglycemia, both in clinical parameters as well as in the transcriptome?
2. How do subjects with different stages of glucose tolerance clinically respond to the exercise intervention, including body composition, glucose homeostasis and metabolic parameters?
3. What transcriptional mechanisms underline beneficial effects of exercise in skeletal muscles, and how do the transcriptional responses to exercise in skeletal muscle differ in patients with different stages of glucose tolerance?

The research project has the approval of the Bioethics Committee and was carried out by the Helsinki declaration.

This methodological approach is very ambitious. The study was conducted among adults leading a sedentary lifestyle. The selection of patients for the study was deliberate taking into account the inclusion criteria (age: 35-65 years old, BMI: 25-35 kg /m^2, male gender, sedentary lifestyle and possibilities to undertake physical activity, and for the group with type 2 diabetes, additionally HbA1c <6, 5% with only metformin allowed in pharmacotherapy) and exclusion criteria that were cigarette smoking, drug and alcohol consumption, declared highly active lifestyle, chronic medications, except for the use of metformin by patients with type 2 diabetes mellitus and converting enzyme inhibitors due to hypertension and abnormalities found in clinical examination, including chronic neurovascular diabetes complications.

The study included 15 men with normoglycemia, 12 with pre-diabetes, and 8 with type 2 diabetes. The subjects underwent a similar three-month intervention consisting of three training sessions of similar duration, i.e., a 15-minute warm-up, 40 minutes of strength exercises and 30 minutes of moderate intensity endurance exercises. In total, during the intervention, participants held 36 training sessions. Each participant before the intervention underwent the progressive exercise test on a treadmill according to the Balke protocol, which was considered appropriate for the group being examined. VO2max (maximum oxygen absorption capacity) have been measured. Whole body dual-energy X-ray absorptiometry (DXA) scans were performed for body composition analysis. Each participant was tested for an oral glucose tolerance test (OGTT) with blood glucose and insulinemia score at 30, 60 and 120 minutes after consuming 75

grams of glucose. The obtained results allowed to calculate insulin resistance indexes (HOMA-IR, Matsuda index) and beta cell function (HOMA-β). Before and after the intervention, a large lateral thigh muscle biopsy was performed. RNA was isolated from the biopsy material. The mRNA sequencing was performed using the HiSeq 4000 platform. The latest molecular biology techniques were used to evaluate the gene expression profile.

The methodology of the study has been described in detail and in a way that allows reproducing the experiments. The applied methods were selected adequately for the needs of the research using new generation technologies.

The statistical analysis uses tests adequate for the needs of the study.

The results of the study were presented on 62 pages in text, tabular form, and figures. The results were described in an orderly manner, with a logical consequence of the research methods.

In the gene expression profile, there were significant differences between patients with type 2 diabetes and men with normoglycemia and pre-diabetes. Although intervention in the form of physical training proved to be clinically effective in all groups, it did not reduce the differences between pre-intervention groups.

Changes in the gene expression profile were mainly related to those genes that are associated with mitochondrial function.

Studies clearly show that type 2 diabetes is a disease associated with energy destruction and mitochondrial dysfunction. A well-designed and performed study proves that impaired mitochondrial function in skeletal muscle appears in type 2 diabetes. In people at risk of type 2 diabetes with normoglycemia and pre-diabetes in the natural course of the disease, insulin resistance initially affects other organs than muscles.

The results discussed concerning the research of others, and the thesis demonstrates a good understanding of the implications of the work in a broader scientific context.

The conclusions correspond to the stated goal and are justified by the results of the research.

The 436 cited references cover the most important dissertations in the field of a doctoral thesis and confirm, as well as the entire dissertation, the Ph.D. student's great knowledge in the subject.

This doctoral dissertation was written extremely carefully with the preserved logic of information transfer in editorial elegance.

After studying the doctoral dissertation, the content contained in it raised the following questions:

1. Were the Fox and Haskell formula used in the study to determine the maximum heart rate (HRmax) a good choice? Developed in 1971, the most popular and the simplest, but burdened with a large imperfection
2. Which of the elements of the training mainly influenced the improvement of mitochondrial function and were the applied proportions of aerobic and anaerobic exercise optimal in this aspect?
3. In the group of patients with type 2 diabetes, after the intervention, there was a decrease in serum HDL-cholesterol, as in the group with normoglycemia and pre-diabetes, although there was a reduction in body weight, fat loss and lean increase and improvement in VO2max. How to explain it?

In English, the proper name of the Polish Diabetes Association is Diabetes Poland, and not as used in the work of the 'Polish Diabetes Association'.

In summary, the doctoral dissertation submitted for review contains content of great practical importance and constitutes a significant contribution to the existing knowledge on a given topic. The strength of the research is a well-thought-out methodology and the use of innovative research methods. The remarks and doubts contained in the review do not diminish the value of the work but may be useful in preparing the manuscript for publication in scientific journals.

Considering the whole work of the Łukasz Szczerbiński, I claim that the candidate, through his research, analysis and inference, contributes to progress in the field of diabetes research. In this way, it meets the conditions required for doctoral dissertations. The value of the subject taken, the methodology used, and above all, interesting results with important clinical implications make work worthy of distinction.

I am addressing to the High Council of the Faculty of Medicine with the Division of Dentistry and Division of Medical Education in English of Medical University of Bialystok for acceptance of Łukasz Szczerbiński to further stages of Ph.D.

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