The review

of the doctoral dissertation by

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

The aim of the study was to identify the trascriptional signature of dysregulated glucose metabolism in skeletal muscle and its response to three-month exercise intervention in humans by profiling the transcriptome.

The manuscript consists of: 186 pages of text, 436 references. .

The structure of the manuscript is classical and includes: list of abbreviations (page 1-7), Introduction (pages 8-48), Aim of the study (page. 49), Materials and Methods (pages 50-65), Results (pages 66-128), Discussion (pages 129-152), Conclusion (str.153). The manuscript also includes: Table of contents, References, Abstracts in English and Polish, List of Figures and List of Tables.

The subject of the study was chosen very well. Physical activity is nowadays concerned as an integral element of behavioral intervention in diabetes, however it is still under debate what are the short and long term consequences of exercise, what is the sufficient „dose" of exercise.

The study was based on the assessment of the changes of multiple antropometric, clinical and biochemical parameters and skeletal muscle gene profile expression before and after three-month of monitored exercise intervention.

The introduction into the manuscript is extensive. It covers the definition and diagnostics criteria of T2DM, epidemiology and pathogenesis of the disease with particular

focus on the role genetic and environmental factors in the development of T2DM. Finally the methods of primary and secondary prevention of t2DM are being discussed.

The aim of the study is presented In clear way, the study potential, both scientific and clinical, is hard to overestimate.

The studied population consisted of 35 males (15 normoglycemic, 12 prediabetics and 8 T2DM patients). All patients underwent a 3-month intervention composed of mixed trainings: 3 training session per week (85 minutes each) including both aerobic and resistance exercise. The sessions were carefully described with regard to type of exercise and exercise load. Of importance, the trainings were monitored what allowed the author to avoid problems related to low adherence or low persistence.

Of importance, all the patients were also monitored by dietician to assure the lack of diet changes during the observation what could impact study outcomes.

The number of antropometric and biochemical parameters were determined before and after the study, muscle biopsy was taken, RNA was isolated. The analysis of the profile of muscle gene expression was based on advanced technologies, the author used proper methods of statistical analysis.

What is also of importance, the studied group was a very homogenous one. All the patients at baseline were characterized by sedentary lifestyle, the only allowed diabetes-related medication was metformin, HbA1c was below 6.5%, there were no statistically significant differences with regard to BMI among study subgroups.

The study size was May look relatively small, but following factors should be taken under consideration:

- challenging protocol

- time-consuming protocol

- muscle biopsy performed

- being ready to significantly increase physical activity

What I really applaud is the adherence and persistence in studied group with regard to physical activity during whole 3 month follow-up. Avaible data published so far are based

either on animal studies, or, if concern investigation on humans, are based on short time interventions, short follow up, and often are based on younger populations. In this study persistence with regard to physical activity was 100%, moreover, the intervention was succefully introduced among individuals who are of ten reluctant to increase their physical activity: Middle aged obese individuals with sedentary life style. It is worth to underline, that this is exactly the population that should be studied, because the increase of physical activity in such population may be crucial for the efficacy of further treatment and patients long term prognosis. In reviewers opinion this is the most valuable advantage of the whole study.

The results are presented in clear and straightforward way. The presentation of the results includes 33 Tables and 17 Figures. The author quotes 436 references, most of them in the discussion section. It is worth to underline, that the interpretation of the results is very professional. As far as the results are concerned, already the baseline analysis brought interesting results. It was shown that there were differences in gene expression in skeletal muscles between T2DM patients and two remaining groups. Differentially expressed genes related mainly to mitochondrial function.

Exercise intervention was equally effective In All Tyree groups, however the effect of the intervention did not overcome baseline metabolic differences among groups. Changes in genes related to mitochondrial function predominated the transcriptional response to the exercise in all three groups.

The discussion and the choice of references are also very professional. The final conclusions of the study are extremely important for developing our knowledge concerning nature, pathogenesis of T2DM, for exploring the role of physical activity in T2DM prevention and treatment. The major outcomes of the study suggest that mitochondria disfunction play a crucial role in the development of T2DM and physical activity May AT east partially reverse this defect. The lack of differences in skeletal muscle gene profile between normoglycemic and prediabetic subjects may suggest that at early stages of the development of T2DM resistance to insulin may concern other than organs than skeletal muscles.

In summary, the author presented very well designed and conducted study, based on rational assumptions, requiring the usage of advanced technologies, and also requiring lot of labwork. Of note, each of the genes indicated by author as upregulated or downregulated due to physical exercise may lead to the discovery of novel pathways of the pathogenesis of T2DM, be used for the pharmacotherapy of T2DM. The statement by author that one of the

differences between prediabetes and T2DM lies in the differences on organ distribution of insulin resistance is also of great clinical importance. Finally, the outcomes of the study indicate clearly the role of physical activity in the prevention and treatment of T2DM, may have import ant clinical translation.

At the end of my review I would like to rise two issues:

- Patients with T2DM were older that individuals from two other groups-the difference was not statistically significant, however if we take absolute numbers under consideration it was visible (the mean age for nondiabeticas, prediabetics and T2DM patients was 47.66, 47.58 i 54.75 years, respectively). Age is one of the crucial factors influencing mitochondrial function in humans.

- It Gould be of interest per form some short term follow up study concerning changes of skeletal muscle gene expression after cessation of physical exercise (for instance 2, 4, 8 weeks after) however I undestand that some of the patients included into the study may have been reluctant to stop their physical activity completely.

Final conclusions

The general assessment of the doctoral dissertation is highly positive, and I recommend the Faculty of Medicine Council of the Medical University in Bialystok to allow Łukasz Szczerbiński M.D. to proceed for the further steps of the doctorial title conferment procedure. I also strongly believe that the doctoral thesis by Lukasz Szczerbinski M.D. is worth honorable mention.

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