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REVIEW

of a PhD thesis by mgr Paulina Samczuk entitled: „*Application of untargeted metabolomics to study metabolic effects of bariatric surgeries*” supervised by dr hab. Michał Ciborowski from the Clinical Research Center of Białystok Medical University and by prof. Coral Barbas from the Centre of Metabolomics and Bioanalysis (CEMBIO) of the University CEU San Pablo

The dissertation addresses the possibility of application of untargeted metabolomics in the analysis of effects on metabolism of bariatric surgery, the only effective method to treat morbid obesity and its complications. The thesis is focused on the differences associated with surgery type and on discerning metabolic determinants of divergent dynamics of the improvement of clinical indices of type 2 diabetes mellitus, a comorbidity of obesity. Recently, a dramatic increase in the number of overweight/obese people has been recorded, also in Poland, and, what is particularly unsettling, also among children. Obesity is no longer considered exclusively as a cosmetic problem since it is a main risk factor for type 2 diabetes and cardiovascular diseases and the incidence of both increases in parallel to obesity. Although its pathogenesis is complex, obesity, as well as other associated disorders, is primary a disturbance in carbohydrate and lipid metabolism. As such, metabolomics, the youngest of “omic” sciences, is the best suited tool for its evaluation. From the public health care point of view, mgr Paulina Samczuk addresses very current and vital issues. In my opinion, the most distinguished advantages of the dissertation are the application of very modern research tools (mass spectrometry combined with advanced bioinformatics), its originality, comprehensiveness, and interdisciplinary approach, especially concerning its interpretative aspect. The Author not only presents “metabolic finger prints” but also gives them a meaning by drawing a net of interactions between metabolites and biochemical pathways or stress responses. Moreover, she proposes and analyses possible cause-and-effect relationships, including the potential effect of digestive tract microbiome, a very hot topic, extensively studied in the context of pathogenesis of a number of civilization diseases.

The dissertation consists of 125 pages of a typescript. It is a collection of three publications – one review and two original papers, in which mgr Pulina Samczuk is the first author and, in two of them, also the corresponding author. Two papers have already been published in journals with an

impact factor (combined IF is 5.716, 60 MNIŚW points) and one is currently under review. Publications are accompanied by structured discussion, consisting of Introduction, Aims and Scopes, Materials and Methods, Results and Discussion, Conclusions and Summary in English and Polish. The thesis is complemented by Table of Contents and List of References and Abbreviations. It is concluded by the statements about the percentage share of individual authors in the publications and their consent to use the work to prepare the dissertation. Leading involvement of mgr Paulina Samczuk - not less than 70% - ought to be stressed, although the respective statements are missing her signature.

Introductory section are written in clear and concise yet comprehensive manner and supported by well chosen references. For this part of the work I have only one polemic note. While discussing causes of obesity, the Author unfairly marginalizes the influence of endocrine disorders. There is particularly close relationship between obesity and hypothyroidism or hyperprolactinemia, both of which have a negative effect on catabolic processes but positive on anabolic ones. Moreover, they contribute to obesity development also indirectly, either *via* inducing hyperinsulinemia and insulin resistance and/or *via* inducing depression. Depression, in turn, is a condition frequently associated with sedentary life-style, low physical activity and improper diet thus behavioral causes of obesity. It is worth remembering that we observe rather rapid increase in the incidence of Hashimoto disease and of thyroid cancer in Poland. Both these diseases are associated with hypothyroidism. Also the incidence of functional hyperprolactinemia is on the rise, and prolactin is a stress hormone just as cortisol is. Non-addressing the endocrine disturbances underlying obesity and promoting a diet not optimal for patients with endocrine disorders as well as a popular belief that a fat-rich diet is a main contributor to weight gain are the key reasons of failure of pharmacotherapy and life-style modifications in the treatment of obesity.

Further, mgr Paulina Samczuk specifies three aims of her thesis, which directly correspond with three articles included in the dissertation. The following chapter, "Materials and Methods", includes a description of criteria applied in literature search for a review paper (data bases, keywords, time period, publication language, species and type of biological material) as well as descriptions of study populations and methodologies of original papers. However, some additional information should be included: 1) blood sample preparation (paper #2); 2) criteria of patients selection (precise inclusion and exclusion criteria) and recruitment time period (in paper #3, the Author declares selection of 20 out of 372 patients); 3) precise criteria for stratifying patients into "slower" and "quicker" remission groups (subjective) – the ones mentioned are inconsistent (it's HOMA-IR in general discussion and HOMA plus some unidentified other parameters in the original paper) and not specific (what were the exact cut-off values for HOMA?); 4) are patients examined in paper #2 and #3 overlapping? In my opinion, using the term „T2DM remission" is inappropriate. T2DM diagnosis is based on detecting hyperglycemia. Thus, by analogy, the disease remission should be marked by normoglycemia. However, high standard deviations accompanying mean concentrations of fasting glucose in both

groups imply that in some patients the remission had not been obtained. Moreover, both studied groups did not differ significantly in terms of glycemia but differ significantly in terms of mean insulin and HOMA-IR levels. As they indeed were separated based on HOMA-IR, they should be referred to as groups with distinct (faster/slower) rate of improving insulin sensitivity.

The results are presented as numerous tables, graphs, diagrams, and schemes, which makes the comprehension and analysis of such vast and complex scientific evidence possible. However, from the reviewer's obligation, I must point that clarity of some tables and figures would be substantially improved by supplementing them with legends (Fig.1, p.70, Fig.3, p.77) and data dispersion measures (Fig.1, p.70) and by making them self-explanatory (most of tables and figures have unexplained abbreviations). Also, unifying the way a potential link between metabolite and a pathway/process is presented (e.g. by stating direction of the association and avoiding redundant entries – Tab.1, p.44; Tab.2, p.45) would help. The tables presenting data on fold change are confusing and impeding proper interpretation. It has not been stated whether the results present “before surgery-to-after surgery ratios” or the other way around. While the latter form would be more intuitive, the caption of these tables states otherwise („0 vs 1 month”, Tab. 3, p.72-73 and Tab.4, p.74). The issue is further complicated by the fact that FC result exceeding one, obtained for uric acid, is still interpreted in general discussion as a postoperative drop. Also, some of phrases used in the paper are uncommon and need explanation („biochemical depression” - ?, Tab.1, p.44) or some phenomena seem to be incorrectly named („inflammation retirement” - resolution? Fig.2, p.46 and Fig.4, p.91; „time of T2DM lasting” – T2DM duration? Tab. 1, p.70). Correlation results, in turn, would be more comprehensible if presented in a form of a table (p.74-75). The dissertation is overall well written; however, it requires some language correction, particularly the parts which are not published, as well as some editing since a lot of abbreviations are unexplained and/or used inconsistently and small and capital letters in Tables are used in rather chaotic manner.

In all discussions, mgr Paulina Samczuk efficiently compiles the results of her own with the data from the literature, showing her expertise in the area. As a reviewer, I am particularly impressed by the ease with which she analyzes complex metabolomic data, efficiently picking out significant differences and giving them meaning by combining altered metabolites with appropriate biochemical pathways, which they might affect. This proficiency testifies to a very good knowledge of biochemistry and scientific maturity of mgr Paulina Samczuk. Still, some errors have been made: methionine, alanine and lysine are not aromatic aminoacids (p.34) and lactate is not a direct intermediate of Krebs cycle (p.37). In some instances, addressing the observed controversies is missing. For example, the Author reported an increase in postoperative uric acid. This finding is counterintuitive as uric acid is known to induce hiperinsulinemia and insulin resistance. Still, both fasting insulin and HOMA-IR decreased following surgery. Also, the observed postoperative rise does not corroborate findings of others (ref. 31: Oberbach et al.). However, the Author does not address this controversy. Moreover, in results section (p.43, &3.1) and discussion (p.90) there is an incorrect

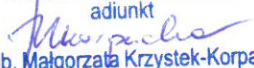
information on postoperative drop of uric acid, contradicting the results presented in tables (Tab.S1, p.53 and Tab.3, p.72). During public defense of doctoral thesis I would also like to ask mgr Paulina Samczuk to address the discrepancy concerning lactic acid results obtained with LC-MS (Tab.3, p.72) and GC-MS (Tab.4, p.74). In discussion to paper #3 (p.79), the Author points at citrate association with insulin secretion without, however, addressing the fact that the citrate increased and insulin secretion rather decreased following surgery and the known effect of citrate on insulin secretion ought to be stimulatory. The reviewed dissertation is concluded by five concise statements, directly resulting from the obtained results and therefore justified.

In summary, the study conducted by mgr Paulina Szymczak is innovative, the work has been well planned and well executed, and its results are important from both scientific and public health care point of view. I would like to strongly encourage the Author to continue her research and seek confirmation of her PhD's thesis on a larger population. It would allow for a better match of patients and facilitate application of a wider panel of statistical tests and thus validate the results obtained in pilot studies reported here. I would like to emphasize that the remarks mentioned in the review do not undermine the correctness of the assumptions of the work or its substantive value, nor do they undermine my very positive reception.

I hereby declare that the dissertation entitled: „*Application of untargeted metabolomics to study metabolic effects of bariatric surgeries*” by mgr Paulina Samczuk meets all conditions listed in art. 13 & 1 of Law on Academic Degrees and Academic Title, and Degrees and Title in the Field of Art from 14 of March, 2003 (Dz. U. Nr 65, poz. 595, z późn. zm.). That is why I apply to the Medical Faculty Council with the Division of Dentistry and the Division of Teaching in English at the Medical University of Białystok to accept the doctoral dissertation by Paulina Samczuk and proceed with further stages of the doctoral thesis. Let me also submit a motion to the Faculty Council for the distinction of the dissertation due to its novelty and high substantive value.

Wrocław, 06.11.2018

Sincerely

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adiunkt

dr hab. Małgorzata Krzystek-Korpacka