Peer review file

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Comment 1: The work "Metabolomics in Cardiovascular Disease" is presented as a review of the field. It is clear and well written; however, the structure of the manuscript may lead to confusion.

While the beginning and the end of the manuscript focus on metabolomics the core of the manuscript consists in a review of the alterations of myocardial metabolism under different pathologies. The central part of the manuscript is much better than the introduction and conclusions.

Reply 1: We do really thank the reviewer for his general comment and summary of our work. We thoroughly revised our complete manuscript including all of the reviewer´s critiques and suggestions. We rewrote the introduction and conclusion with now focusing not on metabolomics per se, but introduced metabolomics as an important emerging tool for detection of altered metabolic pathways fitting to the rest of the manuscript.

Changes in the text 1: Please see introduction and conclusion.

Important points:

Comment 2: There is a lack of references, for instance the introduction does only contain 2 references in the first paragraph, there are two paragraphs without references. This results in many assertions being apparently unfounded.

Reply 2: We appreciate the reviewer's advice and provided additional 76 references in the hole manuscript, mostly in the part about metabolomic analytic techniques. We highlighted the additional references in the text; please see reference list.

Changes in the text 2: e.g. reference list.

Comment 3: Current research projects section. What is the aim of the authors with this section? It starts with "Several studies recently evaluated……" However, there are no references to the "several" studies. Then it briefly describes the MEMORIAM project, presumably by the same authors of the present review.

Reply 3: We do acknowledge the reviewer's comment. With this section, the authors

wanted to point out an ongoing research project aiming to counterbalance clinically relevant inconsistencies in previous studies investigating myocardial metabolism. We rewrote this section and also rearranged it before the conclusion for a better and more consistent reading flow.
Changes in the text 3: page 22.

Comment 4: Conclusions and future directions section. This section seems to be completely unrelated to the main body of the manuscript. It begins with "Although metabolomic profiling has been proven to be a powerful tool, supporting diagnosis and prognosis of cardiovascular diseases…" However, the main body of the manuscript does not have a word regarding metabolic profiling as a diagnostic tool.
Reply 4: We thank the reviewer for his advice and modified our conclusion section. We now focus on future challenges and possibilities of application of metabolomic analyses in cardiovascular disease and deleted the aspect of metabolomic profiling from the hole manuscript. The main statement of this review should be the demonstration of current knowledge of metabolic alterations during cardiovascular diseases. We now hope that this section completes the other parts of the manuscript adequately, please see page 23.
Changes in the text 4: page 23.

Comment 5: Many of the references cited are reviews/opinion papers/guides, authors should try to use primary sources whenever possible.
Reply 5: We thank the reviewer for his critical remarks and acknowledge that many references are not from primary sources. We optimized our reference list and tried to use as often as possible original research articles on top of the citated review articles, especially in the metabolomic analytic techniques part; please see from page 4 and following as well as updated reference list.
Changes in the text 5: Please see reference list.

Other points:
Comment 6: Introduction, page 2, line 24 "biomarkers". If biomarkers appear in the introduction they also should appear in the body of the manuscript.
Reply 6: This manuscript focuses primarily on metabolic alterations during CVD,

which surely can serve later as possible biomarkers. However, this is not the main statement of this review; additionally, we changed the spin of the introduction, please see page 3, line 24.
Changes in the text 6: page 3.

Comment 7: NMR, page 3, line 21 the low cost of NMR as per sample basis was stated previously by Griffin and cols.
Reply 7: We thank the reviewer for this reference and added this reference at the respective position, please see page 4, line 20.
Changes in the text 7: Advantages of NMR are robust, reproducible results with minimal sample preparation at low costs (26).

Comment 8: NMR, second paragraph, it may be better to use H-nuclei than -atoms. Also, while 2D NMR is able to differentiate between metabolites with similar co-resonances the wording of the manuscript suggests that it should be used preferentially over 1D which is clearly not the case.
Reply 8: We appreciate the reviewer's comments and agree. The manuscript's reading suggested that NMR should be preferably used as 2D. However, this was not our intention. We modified the manuscript, please see page 4 line 20 until page 5 line 2.
Changes in the text 8: Multiple spectral libraries provide detailed information about the metabolite structure for identification, especially using multidimensional NMR including 1D, fast 2D or ultrafast schemes…… H-nuclei exhibit a small range in chemical shift. Therefore, NMR can be used as 2-dimensional NMR to detect and avoid co-resonances of metabolites with similar chemical shifts.

Comment 9: NMR, page 4, line 1, "NMR sensitivity is VERY poor". It is lower than Mass spectrometry but if it were VERY poor nobody would use it, clearly not the case.
Reply 9: We agree with the reviewer and compared NMR to MS. Sensitivity of NMR is poorer that MS, however not very poor, please see page 5 line 5.
Changes in the text 9: However, the sensitivity of NMR assays is poorer compared to MS which relates to the strength of the magnet (7)

Comment 10: Mass spec, page 5 line 7 What is “high molecular mass”? Can small metabolites be separated? Is for example succinate high or low molecular mass?
Reply 10: We apologize for this mistake; it should be called high molecular weight. But also, even smaller metabolites can be separated using LC-MS. We added this point to the manuscript, please see page 6, line 14.
Changes in the text 10: LC is a separation technique often used for involatile and polar compounds with high molecular weight. However, also smaller metabolites can be separated using LC.

Comment 11: Low energy state, page 7, line 15. Please define what authors consider a “low energy state”.
Reply 11: The authors consider a low energy state the failing heart’s challenge by both abnormalities in energy metabolism as well as enhanced oxidative stress, both resulting in decreased energy production and apoptosis (see also Neubauer et al NEJM 2007). We added for clarification a section about the concept of low energy state.
Changes in the text 11: see page 8

Comment 12: Low energy state, page 8, line 2, “Ca2+ is known to ...” please add a reference.
Reply 12: We added a reference to the requested section.
Changes in the text 12: Ca^{2+} is known to be crucial for the cardiac muscle contraction (82, 83).

Comment 13: Fatty acid oxidation, page 8, line 11, please add a reference after the first sentence.
Reply 13: We added a reference to the requested section.
Changes in the text 13: Consequently, after implantation of left ventricular assist device (LVAD) circulating long-chained (L-C) acylcarnitines decreased (88).

Comment 14: Fatty acid oxidation, page 9, line 2 please add a reference after “FA oxidation” or change the sentence to avoid confusion.
Reply 14: We added a reference to the requested section.

Changes in the text 14: Decreased myocardial acylcarnitines might represent the impaired mitochondrial function and subsequent FA oxidation (92-94), which…..

Comment 15: Glucose metabolism, page 9, line 18, it is glucose-6-phosphate. Final e.

Reply 15: We apologize for this mistake and added the final e, please see page 10 line 25.

Changes in the text 15: During glycolysis, glucose is rapidly transformed into glucose-6-phosphate in the cytoplasm, …….

Comment 16: Amino acid metabolism, in the first paragraph it is not clear if metabolic measures are made in the myocardium or blood. Please note that this is a problem that also affects other parts of the manuscript.

Reply 16: We understand that this is a general problem of the manuscript and added also in several other parts the respective source, please see for example page 11, line 21 or page 9, line 1….

Changes in the text 16: …lower circulating levels of essential and nonessential amino acids were found in the plasma, compared to patients…

Comment 17: Ketone body metabolism, page 11, line 12. Please add a reference.

Reply 17: We added a reference to the requested section.

Changes in the text 17: Under physiological conditions ketone bodies play a minor role in the cardiac energy production, but with increasing levels of circulating ketones their contribution to energy production increases (127).

Comment 18: Atherosclerosis, page 13, please add references after "…energy production" (line 5) and …liver" line 14.

Reply 18: We added some references to the requested sections.

Changes in the text 18: Large numbers of patients with clinically diagnosed coronary atherosclerosis present metabolic disorders in the myocardial energy production (19, 21, 132). ….. TMA is then released into the blood stream and converted to TMAO in the liver (135).

Comment 19: Ischemic cardiomyopathy, page 15, line 14, reference 4 is a statement paper. Could the reference be changed to the original data.
Reply 19: We changed the reference to the original data, please see page 16 line 14.
Changes in the text 19: The increase in myocardial glycolysis is directly proportional to the severity and duration of ischemia (1)

Comment 20: Fatty acid metabolism, page 15, From line 17. The authors, in this section try to emphasize the fact that after MI there is a reduction of FA oxidation. This is because b-oxidation, unlike glycolysis, requires oxygen for energy production (glucose yields 2 ATP molecules when transformed to pyruvate).
Reply 20: We agree with the reviewer that this is the basic mechanism for reduced FA oxidation in ischemic myocardium. We made this point clear and added it at the beginning of this section, please see page 16 line 18.
Changes in the text 20: In the ischemic myocardium, FA oxidation rates are decreased in proportion to reduced oxygen supply, because β-oxidation of FA is dependent in oxygen for energy production.

Comment 21: Glucose metabolism, page 16, line 25 please add a reference at the end of the paragraph.
Reply 21: We added a reference to the requested section.
Changes in the text 21: Myocardial lactate concentrations rise with the severity of the ischemic period (158).

Comment 22: Conclusion section, page 21, the conclusions do not fit with the body of the manuscript. For example, in line 11, “Because …. Might influence results”. It has been known that this is the case (se classic work by Kirschenlohr et al, 2006 or more recent work by Lema et al, 2020)
Reply 22: We thank the reviewer for this important aspect and modified the conclusions in line with the message of our manuscript, please see page 23 line 3. (or also comment 4).
Changes in the text 22: see page 23.