Response to Reviewer#1

We would like to express our thanks to Reviewer#1 for the careful evaluation of the manuscript and the helpful and constructive suggestions. We have heeded the Reviewer's helpful propositions and prepared a revised version of the manuscript, which includes the alterations suggested by the Reviewer. Please find our response to the comments below.

Reviewer's Comments and Suggestions for Authors:
"In chapter Imaging markers... Transthoracic echocardiography ...It will be usefull to describe not only left atrial changes, but also interatrial septum degeneration and deformation including PFO. Many degenerations of the membranose part of IAS is easy detectible by echocardiography"

Response to Reviewer#1: Thank You for the useful suggestion! Accordingly, we have modified the manuscript (highlighted in yellow, rows 302-316):

"The presence of spontaneous echocardiographic contrast in the left atrium and reduced left atrial appendage blood flow velocity were recognized as markers of atrial cardiomyopathy and associated with an increased risk of thrombus formation [80,91,92]. Furthermore, transthoracic and transesophageal echocardiography can easily detect deformations of the interatrial septum, such as interatrial septal aneurysm (IASA), patent foramen ovale (PFO) or atrial septal pouch (ASP) [93-95]. The IASA is defined as a saccular deformation of the atrial septum, the PFO is a flaplike opening between the atrial septum primum and secundum at the location of the fossa ovalis, and the diverticulum-like ASP occurs when the septum primum and secundum are incompletely fused [93,96]. These deformations are supposed to be associated with thromboembolic events in different mechanisms [93]. Local thrombus formation related to blood stasis within these deformations might represent one mechanism [93]. However, thromboembolism transit through the PFO channel might serve as a further potential mechanism, defined as a paradoxical embolism [93]. Notably, the prevalence of PFO is approximately 25% in the healthy adult population, nonetheless, the risk of embolization in this population is mainly associated with several clinical and morphological factors, such as the length of the PFO channel, the presence of concomitant IASA or prominent Eustachian valve and absence of cardiovascular risk factors [93,97,98].

Advanced echocardiography methods, such as speckle tracking echocardiography represent a sensitive tool to assess atrial function. This technique analyses the unique fingerprint-like natural acoustic marker pattern (or speckle pattern) of grayscale B-mode images of the myocardium, which is tracked consecutively frame-by-frame during the cardiac cycle [99]."

New references:

23. Corban, M.T.; Toya, T.; Ahmad, A.; Lerman, L.O.; Lee, H.C.; Lerman, A. Atrial Fibrillation and Endothelial Dysfunction: A Potential Link? *Mayo Clin Proc* **2021**, *96*, 1609-1621, doi:10.1016/j.mayocp.2020.11.005.

24. Guazzi, M.; Arena, R. Endothelial dysfunction and pathophysiological correlates in atrial fibrillation. *Heart* **2009**, *95*, 102-106, doi:10.1136/hrt.2007.135277.
25. Takahashi, N.; Ishibashi, Y.; Shimada, T.; Sakane, T.; Ohata, S.; Sugamori, T.; Ohta, Y.; Inoue, S.; Nakamura, K.; Shimizu, H.; et al. Impaired exercise-induced vasodilatation in chronic atrial fibrillation--role of endothelium-derived nitric oxide. *Circ J* **2002**, *66*, 583-588, doi:10.1253/circj.66.583.
26. Zheng, L.H.; Sun, W.; Yao, Y.; Hou, B.B.; Qiao, Y.; Zhang, S. Associations of big endothelin-1 and C-reactive protein in atrial fibrillation. *J Geriatr Cardiol* **2016**, *13*, 465-470, doi:10.11909/j.issn.1671-5411.2016.05.005.
93. Dudkiewicz, D.; Hołda, M.K. Interatrial septum as a possible source of thromboembolic events. *Translational Research in Anatomy* **2022**, *27*, 100190, doi:https://doi.org/10.1016/j.tria.2022.100190.
94. Molnár, A.; Ábrahám, P.; Merkely, B.; Nardai, S. Echocardiographic Evaluation of Atrial Communications before Transcatheter Closure. *J Vis Exp* **2022**, doi:10.3791/61240.
95. Silvestry, F.E.; Cohen, M.S.; Armsby, L.B.; Burkule, N.J.; Fleishman, C.E.; Hijazi, Z.M.; Lang, R.M.; Rome, J.J.; Wang, Y. Guidelines for the Echocardiographic Assessment of Atrial Septal Defect and Patent Foramen Ovale: From the American Society of Echocardiography and Society for Cardiac Angiography and Interventions. *J Am Soc Echocardiogr* **2015**, *28*, 910-958, doi:10.1016/j.echo.2015.05.015.
96. Hołda, M.K.; Koziej, M.; Hołda, J.; Piątek, K.; Tyrak, K.; Chołopiak, W.; Bolechała, F.; Walocha, J.A.; Klimek-Piotrowska, W. Atrial septal pouch - Morphological features and clinical considerations. *Int J Cardiol* **2016**, *220*, 337-342, doi:10.1016/j.ijcard.2016.06.141.
97. Hołda, M.K.; Krawczyk-Ożóg, A.; Koziej, M.; Kołodziejczyk, J.; Sorysz, D.; Szczepanek, E.; Jędras, J.; Dudek, D. Patent Foramen Ovale Channel Morphometric Characteristics Associated with Cryptogenic Stroke: The MorPFO Score. *J Am Soc Echocardiogr* **2021**, *34*, 1285-1293.e1283, doi:10.1016/j.echo.2021.07.016.
98. Kleindorfer, D.O.; Towfighi, A.; Chaturvedi, S.; Cockroft, K.M.; Gutierrez, J.; Lombardi-Hill, D.; Kamel, H.; Kernan, W.N.; Kittner, S.J.; Leira, E.C.; et al. 2021 Guideline for the Prevention of Stroke in Patients With Stroke and Transient Ischemic Attack: A Guideline From the American Heart Association/American Stroke Association. *Stroke* **2021**, *52*, e364-e467, doi:10.1161/str.0000000000000375.
212. Ni, L.; Yuan, C.; Chen, G.; Zhang, C.; Wu, X. SGLT2i: beyond the glucose-lowering effect. *Cardiovasc Diabetol* **2020**, *19*, 98, doi:10.1186/s12933-020-01071-y.
213. McMurray, J.J.V.; Solomon, S.D.; Inzucchi, S.E.; Køber, L.; Kosiborod, M.N.; Martinez, F.A.; Ponikowski, P.; Sabatine, M.S.; Anand, I.S.; Bělohlávek, J.; et al. Dapagliflozin in Patients with Heart Failure and Reduced Ejection Fraction. *N Engl J Med* **2019**, *381*, 1995-2008, doi:10.1056/NEJMoa1911303.
214. Packer, M.; Anker, S.D.; Butler, J.; Filippatos, G.; Pocock, S.J.; Carson, P.; Januzzi, J.; Verma, S.; Tsutsui, H.; Brueckmann, M.; et al. Cardiovascular and Renal Outcomes with Empagliflozin in Heart Failure. *N Engl J Med* **2020**, *383*, 1413-1424, doi:10.1056/NEJMoa2022190.
215. Petrie, M.C.; Verma, S.; Docherty, K.F.; Inzucchi, S.E.; Anand, I.; Belohlávek, J.; Böhm, M.; Chiang, C.E.; Chopra, V.K.; de Boer, R.A.; et al. Effect of Dapagliflozin on Worsening Heart

Failure and Cardiovascular Death in Patients With Heart Failure With and Without Diabetes. *Jama* **2020**, *323*, 1353-1368, doi:10.1001/jama.2020.1906.

216. Zheng, R.J.; Wang, Y.; Tang, J.N.; Duan, J.Y.; Yuan, M.Y.; Zhang, J.Y. Association of SGLT2 Inhibitors With Risk of Atrial Fibrillation and Stroke in Patients With and Without Type 2 Diabetes: A Systemic Review and Meta-Analysis of Randomized Controlled Trials. *J Cardiovasc Pharmacol* **2022**, *79*, e145-e152, doi:10.1097/fjc.0000000000001183.
217. Lee, T.M.; Chang, N.C.; Lin, S.Z. Dapagliflozin, a selective SGLT2 Inhibitor, attenuated cardiac fibrosis by regulating the macrophage polarization via STAT3 signaling in infarcted rat hearts. *Free Radic Biol Med* **2017**, *104*, 298-310, doi:10.1016/j.freeradbiomed.2017.01.035.
218. Li, C.; Zhang, J.; Xue, M.; Li, X.; Han, F.; Liu, X.; Xu, L.; Lu, Y.; Cheng, Y.; Li, T.; et al. SGLT2 inhibition with empagliflozin attenuates myocardial oxidative stress and fibrosis in diabetic mice heart. *Cardiovasc Diabetol* **2019**, *18*, 15, doi:10.1186/s12933-019-0816-2.
219. Nishinarita, R.; Niwano, S.; Niwano, H.; Nakamura, H.; Saito, D.; Sato, T.; Matsuura, G.; Arakawa, Y.; Kobayashi, S.; Shirakawa, Y.; et al. Canagliflozin Suppresses Atrial Remodeling in a Canine Atrial Fibrillation Model. *J Am Heart Assoc* **2021**, *10*, e017483, doi:10.1161/jaha.119.017483.
220. Sun, H.Y.; Wang, N.P.; Halkos, M.E.; Kerendi, F.; Kin, H.; Wang, R.X.; Guyton, R.A.; Zhao, Z.Q. Involvement of Na+/H+ exchanger in hypoxia/re-oxygenation-induced neonatal rat cardiomyocyte apoptosis. *Eur J Pharmacol* **2004**, *486*, 121-131, doi:10.1016/j.ejphar.2003.12.016.
221. Hatem, S.N.; Sanders, P. Epicardial adipose tissue and atrial fibrillation. *Cardiovasc Res* **2014**, *102*, 205-213, doi:10.1093/cvr/cvu045.
222. Patel, K.H.K.; Hwang, T.; Se Liebers, C.; Ng, F.S. Epicardial adipose tissue as a mediator of cardiac arrhythmias. *Am J Physiol Heart Circ Physiol* **2022**, *322*, H129-h144, doi:10.1152/ajpheart.00565.2021.
223. Gaborit, B.; Ancel, P.; Abdullah, A.E.; Maurice, F.; Abdesselam, I.; Calen, A.; Soghomonian, A.; Houssays, M.; Varlet, I.; Eisinger, M.; et al. Effect of empagliflozin on ectopic fat stores and myocardial energetics in type 2 diabetes: the EMPACEF study. *Cardiovasc Diabetol* **2021**, *20*, 57, doi:10.1186/s12933-021-01237-2.
224. Masson, W.; Lavalle-Cobo, A.; Nogueira, J.P. Effect of SGLT2-Inhibitors on Epicardial Adipose Tissue: A Meta-Analysis. *Cells* **2021**, *10*, doi:10.3390/cells10082150.
225. Yaribeygi, H.; Maleki, M.; Butler, A.E.; Jamialahmadi, T.; Sahebkar, A. Sodium-glucose co-transporter-2 inhibitors and epicardial adiposity. *European Journal of Pharmaceutical Sciences* **2023**, *180*, 106322, doi:https://doi.org/10.1016/j.ejps.2022.106322.
226. Szekeres, Z.; Toth, K.; Szabados, E. The Effects of SGLT2 Inhibitors on Lipid Metabolism. *Metabolites* **2021**, *11*, doi:10.3390/metabo11020087.
227. Llorens-Cebrià, C.; Molina-Van den Bosch, M.; Vergara, A.; Jacobs-Cachá, C.; Soler, M.J. Antioxidant Roles of SGLT2 Inhibitors in the Kidney. *Biomolecules* **2022**, *12*, doi:10.3390/biom12010143.
228. Lavall, D.; Selzer, C.; Schuster, P.; Lenski, M.; Adam, O.; Schäfers, H.J.; Böhm, M.; Laufs, U. The mineralocorticoid receptor promotes fibrotic remodeling in atrial fibrillation. *J Biol Chem* **2014**, *289*, 6656-6668, doi:10.1074/jbc.M113.519256.
229. Mayyas, F.; Alzoubi, K.H.; Van Wagoner, D.R. Impact of aldosterone antagonists on the substrate for atrial fibrillation: aldosterone promotes oxidative stress and atrial

structural/electrical remodeling. *Int J Cardiol* **2013**, *168*, 5135-5142, doi:10.1016/j.ijcard.2013.08.022.
230. Liu, T.; Korantzopoulos, P.; Shao, Q.; Zhang, Z.; Letsas, K.P.; Li, G. Mineralocorticoid receptor antagonists and atrial fibrillation: a meta-analysis. *Europace* **2016**, *18*, 672-678, doi:10.1093/europace/euv366.
231. Neefs, J.; van den Berg, N.W.; Limpens, J.; Berger, W.R.; Boekholdt, S.M.; Sanders, P.; de Groot, J.R. Aldosterone Pathway Blockade to Prevent Atrial Fibrillation: A Systematic Review and Meta-Analysis. *Int J Cardiol* **2017**, *231*, 155-161, doi:10.1016/j.ijcard.2016.12.029.
232. Pretorius, M.; Murray, K.T.; Yu, C.; Byrne, J.G.; Billings, F.T.t.; Petracek, M.R.; Greelish, J.P.; Hoff, S.J.; Ball, S.K.; Mishra, V.; et al. Angiotensin-converting enzyme inhibition or mineralocorticoid receptor blockade do not affect prevalence of atrial fibrillation in patients undergoing cardiac surgery. *Crit Care Med* **2012**, *40*, 2805-2812, doi:10.1097/CCM.0b013e31825b8be2.

Once again, we would like to thank the Reviewer for the insightful comments and suggestions! We believe these resulted in a much-improved manuscript that may be acceptable for publication.

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