

**"VICTOR BABEȘ" UNIVERSITY OF MEDICINE AND PHARMACY
FROM TIMIȘOARA
FACULTY OF MEDICINE
DEPARTMENT III FUNCTIONAL SCIENCES**

IONICĂ LOREDANA-NICOLETA



Abstract

**CONTRIBUTIONS TO THE ELUCIDATION OF THE
CARDIOVASCULAR PROTECTIVE EFFECTS OF ANTI-
DIABETIC MEDICATION: FOCUS ON MONOAMINE
OXIDASE**

Scientific Coordinator

PROF. MOZOȘ IOANA-MONICA, MD, PhD

T i m i ș o a r a

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Key words: monoamine oxidase, oxidative stress, animal model, diet-induced obesity, overweight, obesity, heart failure, antidiabetics, metformin, SGLT2 inhibitors, empagliflozin, dapagliflozin

I. BACKGROUND & RESEARCH OBJECTIVES

Globally, cardiovascular diseases continue to be the most prevalent cause of morbidity. In particular, with the ageing of the population, heart failure (HF), has become the greatest challenge for the healthcare systems worldwide. Also, with the overweight/obesity pandemic, the prevalence type 2 diabetes mellitus (T2DM) has dramatically risen and subsequently, the use of the anti-diabetic therapies. Both classic (biguanides, metformin/METF) and novel (sodium-glucose cotransporter-2 inhibitors/SGLT2i, empagliflozin/EMPA and dapagliflozin/DAPA) antidiabetics exert cardiovascular protective effects, independent from their glucose-lowering action. Moreover, the latter class has become the cornerstone therapy for the entire HF ejection fraction spectrum (reduced/HFrEF, mildly reduced/HFmEF and preserved/HFpEF) in the absence of T2DM. In the past decades, the cellular mechanisms underlying cardiovascular protection have been systematically studied and partially elucidated; among them, their antioxidant effect has been mostly addressed.

Oxidative stress is a central pathophysiological mechanism responsible for the occurrence and progression of cardio-metabolic diseases and HF, yet the complex sources of reactive oxygen species (ROS) are far from being fully elucidated. Monoamine oxidase (MAO), with 2 isoforms MAO-A and B, is an enzyme located at the outer mitochondrial membrane whose physiological role consists in the oxidative deamination of neurotransmitters and biogenic amines, with the constant generation of hydrogen peroxide (H_2O_2) as the main ancillary product of the reaction. Over the past decades, there has been a growing interest in unveiling the contribution of MAO-related oxidative stress to the pathogenesis of cardio-metabolic diseases and pharmacological approaches able to mitigate it. Targeting the relevant cellular ROS generators in specific pathological conditions is nowadays considered the realistic approach of mitigating oxidative stress instead of prescribing antioxidant therapy.

The study of the interaction between the antidiabetics and MAO as *constant source of ROS generation* in overweight/obesity is still in its infancy. At the beginning of the PhD only one study reported that both MAO isoforms were overexpressed and contributed to the *cardiac* oxidative stress in a rat model of diet-induced obesity. Acute *ex vivo* incubation of the ventricular samples with METF downregulated MAO expression & mitigated oxidative stress.

No data were available in the literature regarding the effect of METF on MAO-related *vascular* oxidative stress in animal models or humans. Also, information regarding the *interaction of the SGLT2i with MAO in human cardiovascular system* was lacking.

The **aim of the translational research** was to assess the cardiovascular protective effects of METF, EMPA and DAPA with focus on their interaction with MAO in the setting of overweight/obesity. The chosen topic complies with theme no. 2 “*Translational research in the field of oxidative stress (focus on cardiovascular pathology, mitochondrial dysfunction and MAO activity)*” included in the Research Strategy of the university, as well as with the research directions of the Centre for Translational Research and Systems Medicine from Department III Functional Sciences where I was affiliated during my doctoral studies.

The **research objectives** corresponding to the 4 original studies were as follows:

1. Assessment of METF effects on the vasomotor function, MAO expression and oxidative stress in aortas isolated from rats with diet-induced obesity.
2. Assessment of the acute antioxidant effect of METF in atrial samples harvested from overweight non-diabetic patients with HFmEF subjected to elective cardiac surgery.

3. Characterization of METF and EMPA effects on vascular reactivity, MAO expression and oxidative stress in mammary arteries samples harvested from overweight patients with HFmrEF undergoing revascularization therapy.

4. Characterization of EMPA and DAPA effects on MAO expression and oxidative stress in atrial samples harvested from overweight non-diabetic patients with all types of HF subjected to elective cardiac surgery.

II. STUDY 1: CONTRIBUTIONS TO THE ASSESSMENT OF THE METFORMIN EFFECTS ON MAO EXPRESSION, OXIDATIVE STRESS AND VASCULAR REACTIVITY IN A RAT MODEL OF DIET-INDUCED OBESITY

The first study was aimed to investigate METF-MAO interaction in aortic rings isolated from rats with high calorie junk food-induced obesity, an experimental model which mimics unhealthy eating, one of the most common etiology of human obesity. The working hypothesis was that MAO is upregulated after 24 weeks of diet and contributes to the vascular oxidative stress in the obese animals and METF is able to counteract its deleterious effects.

1. METFORMIN MITIGATED MAO-A & B OVEREXPRESSION IN AORTAS ISOLATED FROM THE RATS WITH DIET-INDUCED OBESITY

Both MAO-A and B isoforms were upregulated in aortic rings isolated from obese vs lean rats. Acute *ex vivo* incubation with METF (10 μ M, 12 h) decreased both gene and protein expression in vascular samples from obese rats and had no effect in the control group (Fig.1).

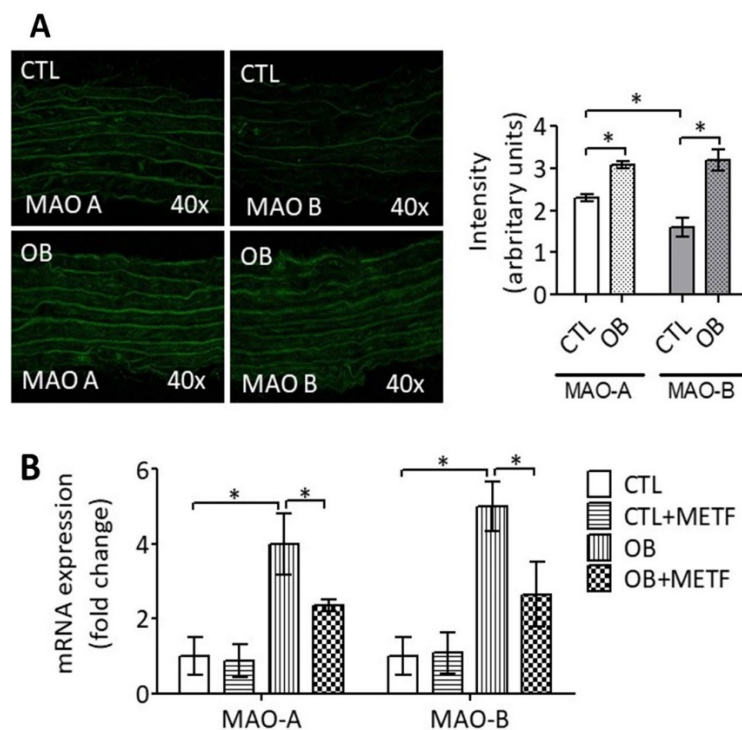


Figure 1. Metformin alleviated MAO-A and B overexpression in aortic rings isolated from obese (OB, n = 8) vs. non-obese (CTL, n = 8) rats. (A) MAO-A and B protein expression. (B) MAO-A and B gene (mRNA) expression (fold change), *p < 0.05

2. METFORMIN DECREASED OXIDATIVE STRESS IN AORTAS ISOLATED FROM RATS WITH DIET-INDUCED OBESITY

Oxidative stress assessed by the dihydroethidium (DHE) stain for the superoxide anion in confocal microscopy (Fig. 2A) and the FOX (Ferrous iron- xylene orange Oxidation) assay for hydrogen peroxide (H_2O_2) in spectrophotometry (Fig. 2B), was significantly higher in aortas from obese as compared to non-obese rats, an effect that was diminished by acute incubation with METF in the former but not in the latter group.

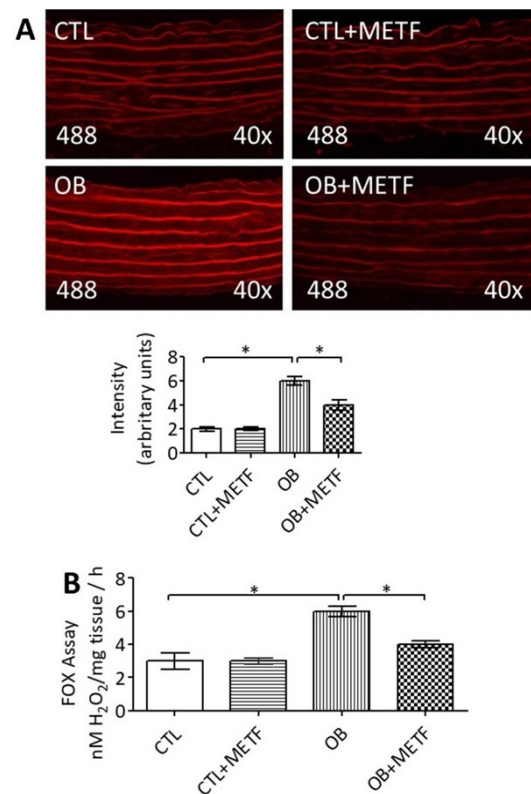


Figure 2. Metformin reduced ROS production in aortas isolated from obese rats (OB, n = 8) vs. non-obese rats (CTL, n = 8). (A) DHE staining, (B) FOX assay, *p < 0.05

3. METFORMIN ALLEVIATED ENDOTHELIAL DYSFUNCTION OF AORTIC RINGS HARVESTED FROM RATS WITH DIET-INDUCED OBESITY

Additional experiments were conducted in order to evaluate the response of aortic rings from obese animals compared to control to phenylephrine (Phe) contraction and acetylcholine (ACh) endothelium-dependent relaxation (EDR). Vascular contractility was elevated and EDR was diminished in vascular samples obtained from obese rats. However, a 12-hour incubation with METF was effective in restoring both vascular contractility and EDR and had no effect on vasomotor function of the aortic rings isolated from the lean animals. (Fig. 3). The beneficial effect of METF can be ascribed to an increased NO bioavailability.

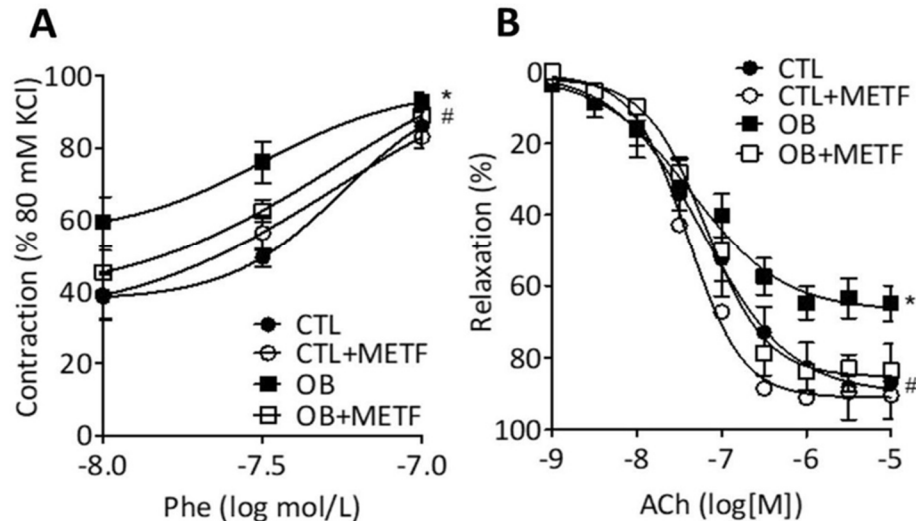


Figure 2. Metformin improves vascular reactivity in aortic rings isolated from diet-induced obese rats (OB, n = 8) vs controls (CTL, n = 8). (A) Phe-induced contractions, (B) Ach-induced endothelium-dependent relaxation.

4. METFORMIN ELICITED PROTECTIVE EFFECTS IN THE SETTING OF ACUTE VASCULAR STRESS INDUCED BY ANGIOTENSIN II, LIPOPOLYSACCHARIDE & HIGH GLUCOSE

Obesity has been linked to inflammation, high blood pressure, and hyperglycemia when combined within the metabolic syndrome. In order to replicate these settings in an in vitro setting, the acute effect of METF exposure for 12-hour on the rat aortic rings incubated with angiotensin II (AII, 100 nM), lipopolysaccharide (LPS, 1 μ g/mL) or high glucose (GLUC, 400 mg/dL) was evaluated. METF enhanced vascular relaxation, mitigated the oxidative stress and reversed MAO upregulation caused by AII, LPS, and GLUC.

III. STUDY 2. CONTRIBUTIONS TO THE ASSESSMENT OF THE ACUTE ANTIOXIDANT EFFECT OF METFORMIN IN ATRIAL TISSUE HARVESTED FROM OVERWEIGHT, NON-DIABETIC PATIENTS

There is a paucity of data in the literature regarding the acute effect of low, micromolar doses of METF on human samples. A pilot study was conducted in 20 non-diabetic, overweight cardiac patients with a body mass index (BMI) of 27.21 ± 0.96 kg/m² diagnosed with HFmrEF (LVEF = $47.10 \pm 1.59\%$) with indication for elective cardiac surgery.

The working hypothesis of the second study was that METF can alleviate the atrial oxidative stress when acutely applied *ex vivo* (10 μ M, 12 h) on right atrial appendages harvested from non-diabetic, overweight patients diagnosed with HFmrEF, even in the presence of a stressors such as AII, LPS and high GLUC.

1. METFORMIN ACUTELY ALLEVIATED OXIDATIVE STRESS IN HUMAN ATRIAL TISSUE

Oxidative stress was assessed by means of the DHE fluorescent probe (Fig. 3) and FOX assay (Fig.4) for the production of superoxide anion and H₂O₂, respectively. Oxidative stress significantly increased under conditions that imitated the activation of the renin-

angiotensin-aldosterone system (AII), inflammation (LPS), and uncontrolled hyperglycemia (GLUC), respectively. METF significantly mitigated the oxidative stress both stimulated and control (non-stimulated) human atrial samples, suggesting a beneficial effect of the drug in the acute alleviation of the cardiac oxidative stress, irrespective of its severity.

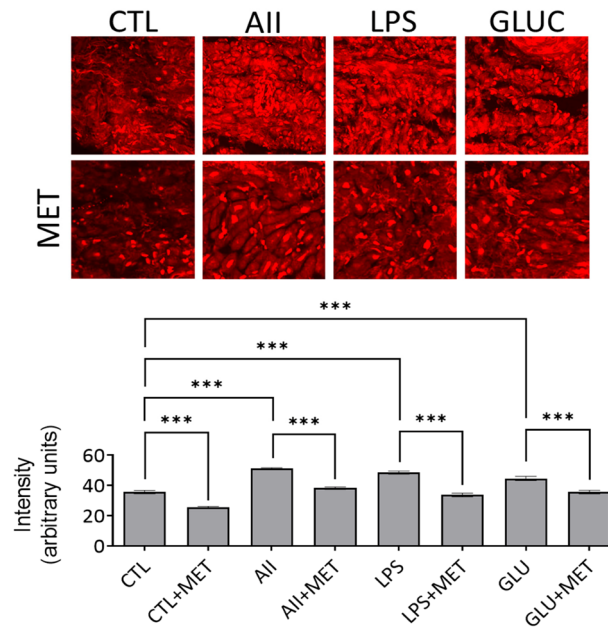


Figure 3. Metformin mitigated oxidative stress assessed by DHE staining in stimulated (AII, LPS, GLUC) and non-stimulated (CTL) atrial samples. n = 20, *** p < 0.001

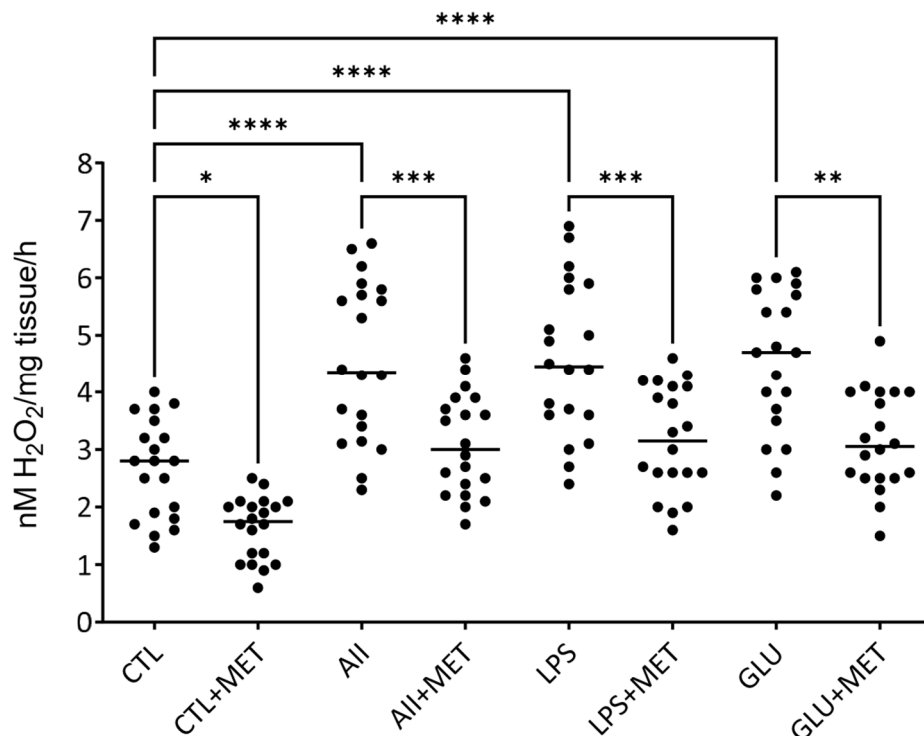


Figure 4. Metformin mitigated oxidative stress assessed by FOX assay in stimulated (AII, LPS, GLUC) and non-stimulated (CTL) atrial samples. n = 20, * p < 0.1, ** p < 0.01, *** p < 0.001, **** p < 0.0001

2. ATRIAL OXIDATIVE STRESS CORRELATED WITH ECHOCARDIOGRAPHIC PARAMETERS

A correlation analysis was conducted to assess the potential association between the the level of oxidative stress (measured by H_2O_2 using the FOX assay) and the echocardiographic parameters. A positive correlation between ROS level and LA diameter, LVED diameter, and RV diameter and a negative correlation between ROS and LV ejection fraction were found.

IV. STUDY 3. CONTRIBUTIONS TO THE ASSESSMENT OF THE PROTECTIVE EFFECTS OF METFORMIN AND EMPAGLIFLOZIN ON MAO AND VASCULAR OXIDATIVE STRESS IN MAMMARY ARTERIES ISOLATED FROM OVERWEIGHT, NON-DIABETIC PATIENTS

Both classic and novel antidiabetics have been reported to provide cardiovascular protection, independent of glucose control, yet few studies compared their effects on human samples. As such, the third study aimed to evaluate the acute *ex vivo* effect of METF and EMPA on vascular reactivity, MAO expression, oxidative stress, in internal mammary artery samples harvested from patients with coronary heart disease who underwent revascularization (CABG). The working hypothesis was that their protective effects will be additive when applied together on vascular samples incubated with AII and high GLUC.

The study was conducted in a pilot group of patients ($n=9$) that were overweight ($\text{BMI} = 26.89 \pm 1.26$), and had HFmrEF ($\text{EF} = 46.87 \pm 2.82\%$).

1. METFORMIN AND EMPAGLIFLOZIN ALLEVIATED ENDOTHELIAL DYSFUNCTION IN HUMAN MAMMARY ARTERY RINGS DURING ACUTE EX VIVO TREATMENT

The effects of METF and/or EMPA on the endothelial-dependent relaxation in response to cumulative doses of ACh was evaluated in mammary arteries rings (Fig. 5).

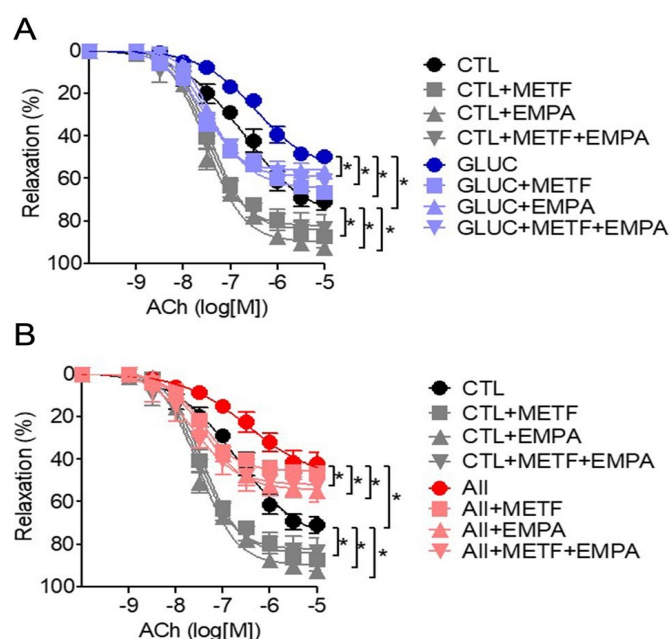


Figure 5. The effects of METF and EMPA (10 μM) on vasomotor function of mammary arteries stimulated or not (CTL). A) High glucose (GLUC, 400 mg/dL) and B) AII (100 nM); $n = 9$, $*p < 0.05$

Acute exposure to either high GLUC (Fig.5A) or AII (Fig. 5B) elicited an important decrease in vascular relaxation to cumulative doses of ACh. METF or EMPA alleviated the EDR both in mammary artery samples stimulated with high GLUC and AII and in control (CTL, non-stimulated) vascular rings. The latter observation is important since it demonstrates that: i) the vascular impairment is already present in overweight (non-obese, non-diabetic) patients with HFmrEF and ii) the occurrence of a reversible endothelial dysfunction of an artery used for revascularization, which can be alleviated by acute exposure to either antidiabetic. Last but not least, no additive effect on vascular reactivity of mammary arteries was found when METF and EMPA were acutely co-administered *ex vivo*.

2. METFORMIN AND EMPAGLIFLOZIN ALLEVIATED OXIDATIVE STRESS IN HUMAN MAMMARY ARTERY SAMPLES DURING ACUTE EX VIVO TREATMENT

Vascular ROS production using the DHE staining (Fig. 6A) and FOX assay (Fig.6B) was significantly increased after acute stimulation with high GLUC or AII.

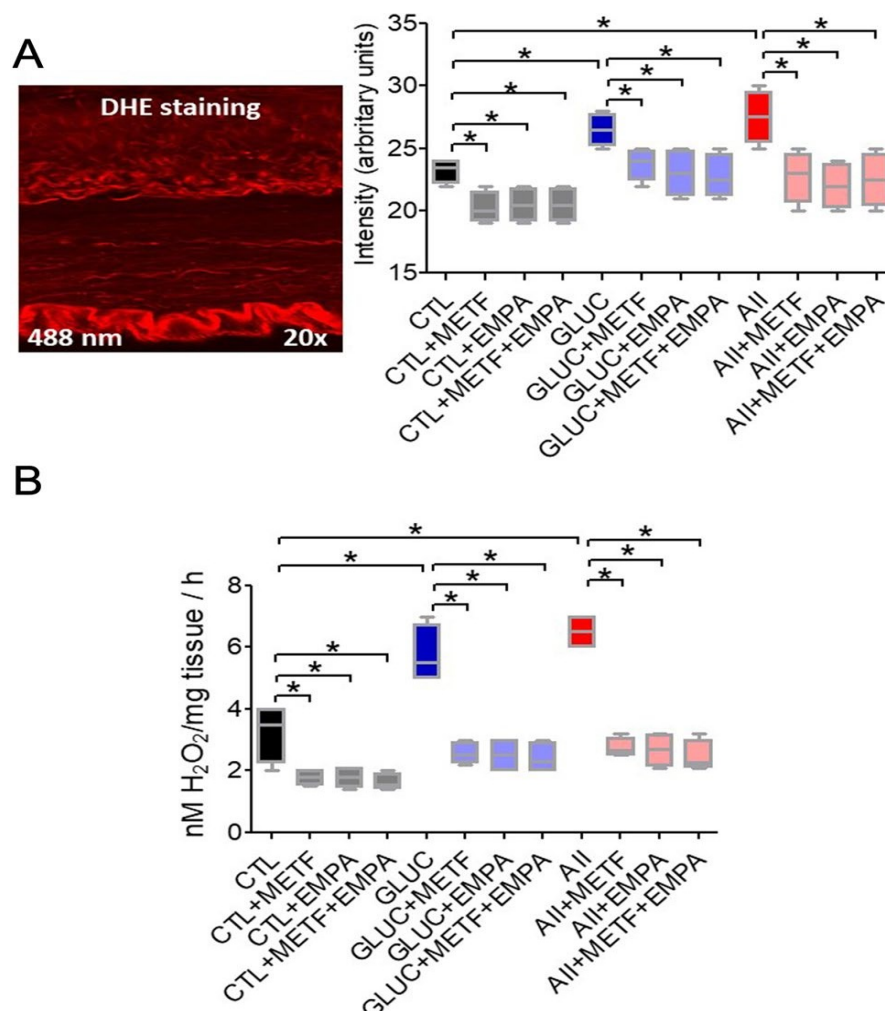


Figure 6. The effects of METF and EMPA on ROS generation in human mammary arteries stimulated with high glucose (GLUC, 400 mg/dL) and angiotensin II (AII, 100 nM). A. DHE staining; B. FOX assay; n = 9, *p < 0.05

Oxidative stress was mitigated by co-incubation with either METF or EMPA, but again there was no cumulative effect when the antidiabetics were applied together. Important, both drugs were independently able to reduce ROS production in CTL (non-stimulated) vessels, indicating that oxidative stress is present (as it was endothelial dysfunction) in mammary arteries used for CABG in overweight, non-diabetic patients with HFmrEF (Fig.6).

3. METFORMIN AND EMPAGLIFLOZIN REDUCED MAO-A AND B OVEREXPRESSION INDUCED BY HIGH GLUCOSE AND AII IN HUMAN MAMMARY ARTERY SAMPLES

MAO protein (immune fluorescence) and gene (qPCR) expression in mammary arteries was assessed. Both MAO-A and B isoforms were equally expressed (Fig. 7A) and were significantly upregulated following GLUC and AII stimulation (Fig. 7B). As for the results on oxidative stress, acute incubation (12 h) with either METF or EMPA mitigated MAO expression not only in GLUC and AII-incubated vessels, but also in CTL vessels (Fig. 7B), while co-treatment with both antidiabetics did not elicit additive protection.

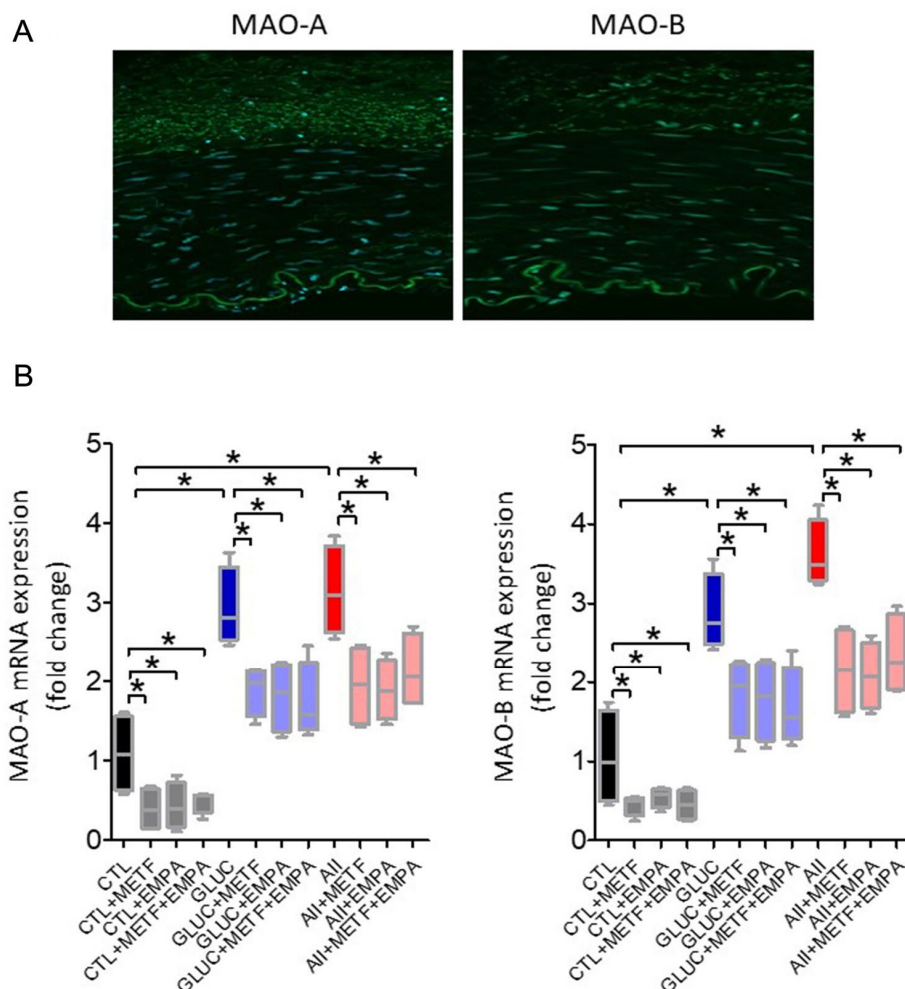


Figure 7. The effect of METF and EMPA on MAO expression in human mammary arteries stimulated with high GLUC (400 mg/dL) and AII (100 nM). A) Protein expression for MAO-A/B; B) Gene/mRNA expression for MAO-A/B; n = 9, *p < 0.05

V. STUDY 4. CONTRIBUTIONS TO THE ASSESSMENT OF THE CLASS EFFECT OF EMPAGLIFLOZIN AND DAPAGLIFOZIN ON MAO AND CARDIAC OXIDATIVE STRESS IN ATRIAL TISSUE FROM OVERWEIGHT NON-DIABETIC PATIENTS

The fourth study aimed to assess whether the two widely prescribed SGLT2i, EMPA and DAPA, applied *ex vivo* in clinically relevant concentration decrease MAO expression and alleviate oxidative stress in right atrial appendage samples harvested from overweight, non-diabetic patients with all types of HF subjected to elective cardiac surgery. The working hypothesis was that EMPA and DAPA effects on atrial MAO expression and oxidative stress in human samples are class effects of the SGLT2i.

The pilot study included 24 non-diabetic patients who were overweight (BMI = $28.98 \pm 0.31 \text{ kg/m}^2$) and diagnosed with all types of HF (EF = 43.47 ± 11).

1. EMPAGLIFLOZIN AND DAPAGLIFLOZIN DOSE-DEPENDENTLY MITIGATED OXIDATIVE STRESS IN HUMAN ATRIAL TISSUE

Human atrial samples were acutely incubated (12 h) *ex vivo* with either Ang2 (100 nM) or GLUC (400 mg/dL), in the presence vs absence of 2 doses (1 and 10 μM) of EMPA and dapagliflozin (DAPA). ROS assessment by the DHE staining (Fig. 8) and the FOX assay (Fig. 9) confirmed that Ang2 and GLUC increased oxidative stress. The effect was dose-dependently reduced by co-incubation with either SGLT2i. Both drugs also mitigated ROS production in CLT samples, arguing for the presence of cardiac oxidative stress in overweight, non-diabetic patients.

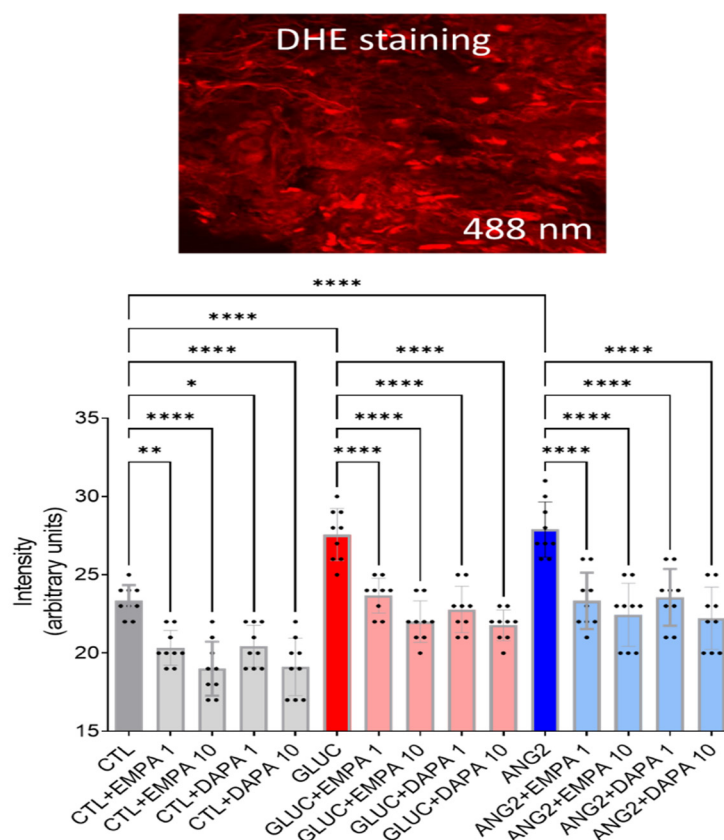


Figure 8. EMPA and DAPA (1, 10 μM) mitigated oxidative stress in human atrial tissue - DHE stain after incubation with Ang2 and GLUC (n=24) *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

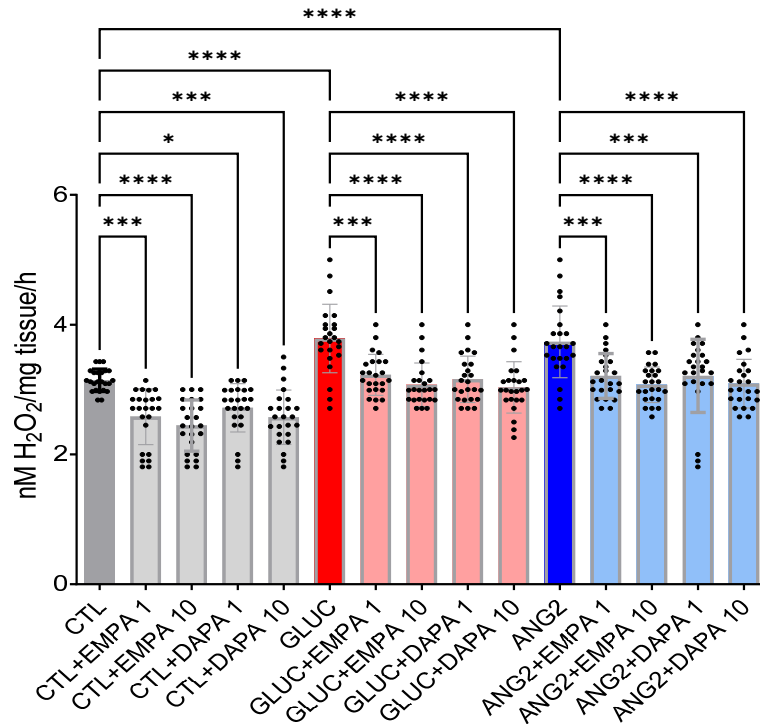


Figure 9. EMPA and DAPA (1, 10 μ M) decreased oxidative stress in human atrial tissue - FOX assay after incubation with Ang2 and GLUC (n=24) *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

In order to dissect the mechanisms underlying the antioxidant effect of SGLT2i we assessed whether EMPA and DAPA may act as *ex vivo* ROS scavengers. Our findings indicate that, as compared to catalase, a classic ROS scavenger, they had a very limited direct antioxidant property. This finding implies that SGLT2i can modulate intracellular ROS sources rather than directly scavenge ROS.

2. EMPAGLIFLOZIN AND DAPAGLIFLOZIN DOWNREGULATED MAO EXPRESSION IN HUMAN ATRIAL TISSUE

Both MAO isoforms are expressed in the atrial tissue of overweight patients at protein (Fig. 10, MAO immune fluorescence) and gene (Fig.11, MAO mRNA, qPCR) levels.

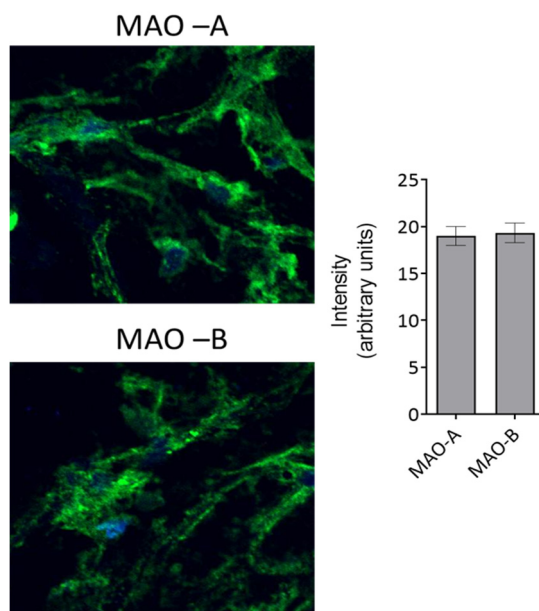


Figure 10. MAO-A and B protein expression in human atrial samples(20x, blue = DAPI, green=MAO-A and B)

Acute incubation with either EMPA or DAPA reduced MAO-A and B gene expression in atrial samples stimulated with Ang 2 and GLUC (Fig. 9), thus confirming the class effect of SGLT2i. As for oxidative stress, the effect was also noted in CTL (non-stimulated) samples, showing that the enzyme is already overexpressed in the atrial tissue of the overweight patients.

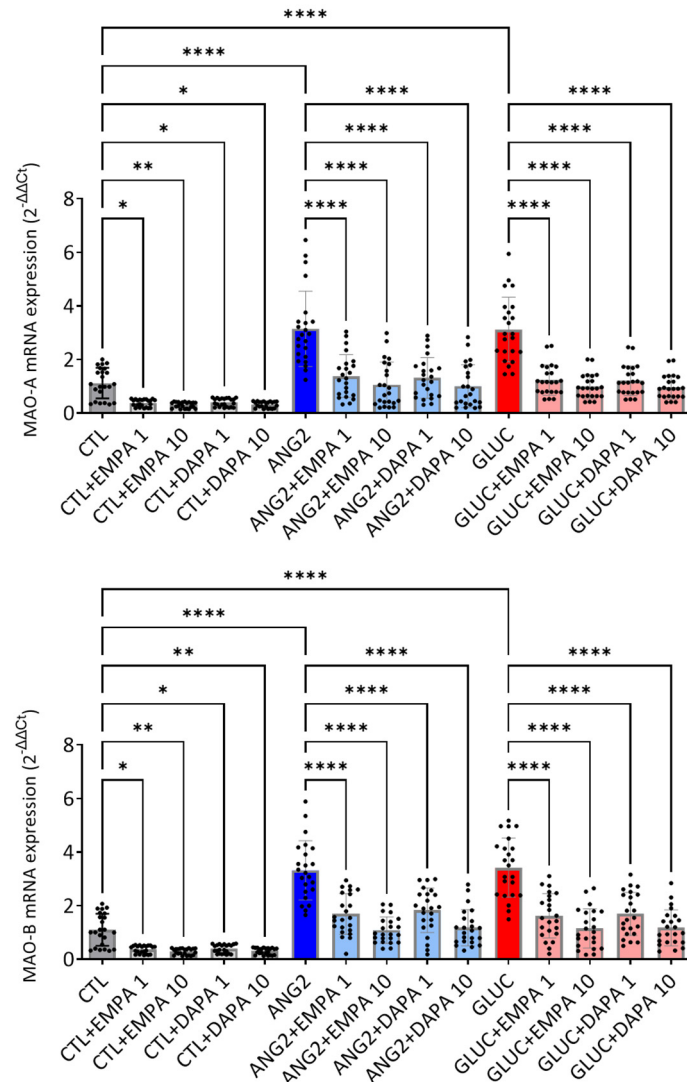


Figure 11. EMPA and DAPA reduced MAO-A and B mRNA gene expression after incubation with Ang2 and GLUC (n=24) *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

3. OXIDATIVE STRESS SIGNIFICANTLY CORRELATED WITH THE ECHOCARDIOGRAPHIC PARAMETERS

A correlation analysis was conducted to search for potential correlations between oxidative stress (H₂O₂ levels using the FOX assay) and the echocardiographic parameters. ROS levels were found to positively correlate with the LA diameter (0.68), LVED diameter (0.56), LVED volume (0.51), and the RV diameter (0.46). Conversely, a negative correlation was seen between ROS and the LVEF, with a correlation coefficient of -0.42.

VI. CONCLUSIONS

1. The experimental model of diet-induced obesity in rats elicited an increased production of reactive oxygen species in aortas from the obese animals as compared to the lean ones.
2. Acute *ex vivo* incubation of isolated aortic samples with metformin alleviated oxidative stress in the obese groups and had no effect in the control group.
3. In the obese animals, vascular contractility was increased, while endothelium-dependent relaxation was reduced as hallmarks of endothelial dysfunction.
4. Metformin, the first line drug of T2DM, alleviated the endothelial dysfunction (reduced contractility and improved relaxation) in aortas isolated from the obese animals and had no effect on vascular reactivity in control vessels.
5. Metformin decreased the L-NAME-induced contraction (the classical NOS inhibitor), indirectly suggesting that an increased NO bioavailability is one possible mechanism of vascular protection.
6. Both MAO-A and MAO-B isoforms were overexpressed in aortic segments isolated from the obese rats (but not in the lean ones) and contributed to vascular oxidative stress.
7. Metformin reduced the MAO expression in aortas isolated from the obese rats with and had no effect on the enzymes responsible for vascular catecholamine generation.
8. In atrial tissue harvested from overweight, non-diabetic cardiac patients with HFmrEF, superoxide anion and H₂O₂ generation were significantly increased by *ex vivo* incubation with angiotensin II, lipopolysaccharide and high glucose.
9. Metformin elicited an antioxidant protective effect in the setting of acute oxidative stress induced by conditions that mimicked the activation of the renin–angiotensin–aldosterone system, acute inflammation, and uncontrolled diabetes as well as against the basal oxidative stress in non-stimulated atrial preparations.
10. The magnitude of basal atrial oxidative stress positively correlated with the LA diameter, LV end-diastolic diameter, and RV diameter and negatively with the LVEF.
11. Acute *ex vivo* stimulation with angiotensin II, lipopolysaccharide and high glucose elicited endothelial dysfunction and increased MAO expression in mammary arteries harvested from overweight, nondiabetic patients with coronary heart disease subjected to bypass grafting.
12. Acute exposure to either metformin or empagliflozin improved vascular reactivity of both untreated and treated vascular samples, with no additive effect.
13. Acute exposure to either antidiabetic drug mitigated both MAO isoforms overexpression; no additive effect was obtained when the drugs were applied together.
14. Acute *ex vivo* stimulation with angiotensin II and high glucose increased oxidative stress in atrial tissue collected from overweight, non-diabetic cardiac patients with all spectrum of HF subjected to elective cardiac surgery.
15. Acute exposure of human cardiac samples to either dapagliflozin and empagliflozin elicited a concentration-dependent antioxidant effect.

16. The antioxidant protective effect of the SGLT2i was also present in the control samples (not stimulated with All or high glucose), demonstrating the occurrence of basal atrial oxidative stress in overweight, nondiabetic patients with HF.
17. Both MAO isoforms, MAO-A and MAO-B, are equally expressed in the human atrial tissue harvested from the overweight failing patients.
18. Acute *ex vivo* exposure to empagliflozin and dapagliflozin reduced MAO-A and MAO-B gene expression, increased in samples stimulated with All or high glucose.
19. SGLT2 inhibitors do not act as ROS scavengers but modulate (several) intracellular ROS sources, such as MAO, as a novel “off-target” class effect.

ORIGINAL CONTRIBUTIONS:

- Characterization of the protective effect of metformin in alleviating endothelial dysfunction and decreasing vascular MAO expression and ROS production in aortic samples isolated from rats with diet-induced obesity;
- Demonstration of the acute antioxidant effect of METF in human atrial tissue collected from overweight, non-diabetic patients subjected to open-heart surgery;
- Demonstration of MAO upregulation upon acute stimulation with All and high-glucose in human mammary arteries and the capability of METF and EMPA to alleviate both oxidative stress and endothelial dysfunction;
- Demonstration of MAO contribution to the human atrial oxidative stress in basal conditions and upon acute stimulation with All and high glucose and the capability of EMPA and DAPA to counteract it, as novel “off-target” class effect of SGLT2i.

FUTURE RESEARCH DIRECTIONS:

- Investigation of the intracellular signaling pathways underlying the metformin – MAO interaction in the setting of overweight/obesity.
- Investigation of the signal transduction mechanism behind the beneficial antioxidant effect of SGLT2 inhibitors in these patients.

VII. SCIENTIFIC PUBLICATIONS

1. **Ionică LN**, Lința AV, Bătrîn AD, Hâncu IM, Lolescu BM, Dănilă MD, Petrescu L, Mozoș IM, Sturza A, Muntean DM. *The Off-Target Cardioprotective Mechanisms of Sodium-Glucose Cotransporter 2 Inhibitors: An Overview. International Journal of Molecular Sciences* **2024**; 25(14):7711. **ISI Journal (IF – 4.9)**
2. **Ionică LN**, Buriman DG, Lința AV, Șoșdean R, Lascu A, Streian CG, Feier HB, Petrescu L, Mozoș IM, Sturza A, Muntean DM. *Empagliflozin and Dapagliflozin Decreased Atrial Monoamine Oxidase Expression and Alleviated Oxidative Stress in Overweight Non-Diabetic Cardiac Patients. Mol Cell Biochem* **2024** Jul 23;doi: 10.1007/s11010-024-05076-z. **ISI Journal (IF – 3.5)**
3. **Ionică LN**, Gaiță L, Bîcă AM, Șoșdean R, Lighezan R, Sima A, Malița D, O. Crețu, O. Burlacu, DM. Muntean, A. Sturza. *Metformin Alleviates Monoamine Oxidase-Related Vascular Oxidative Stress and Endothelial Dysfunction In Rats With Diet-Induced Obesity. Mol Cell Biochem* **2021**; 476(11): 4019-4029. **ISI Journal (IF – 3.8)**
4. Lascu A, **Ionică LN**, Merce AP, Dănilă MD, Petrescu L, Sturza A, Muntean DM, Streian CG. *Metformin Acutely Mitigates Oxidative Stress in Human Atrial Tissue: A Pilot Study in Overweight Non-Diabetic Cardiac Patients. Life* **2022**, 12(12), 2058. doi.org/10.3390/life12122058. **ISI Journal (IF – 3.2)**
5. Lascu A, **Ionică LN**, Buriman DG, Merce AP, Deaconu L, Borza C, Crețu OM, Sturza A, Muntean DM, Feier HB. *Metformin and Empagliflozin Modulate Monoamine Oxidase-related Oxidative Stress and Improve Vascular Function in Human Mammary Arteries. Mol Cell Biochem* **2023**;478(9):1939-1947. doi.10.1007/s11010-022-04633-8. **ISI Journal (IF – 3.5)**