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PhD THESIS

**ECHOCARDIOGRAPHIC EVALUATION AND
PHARMACOLOGICAL MODULATION OF THE EPICARDIAL
ADIPOSE TISSUE**

ABSTRACT

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Key words: epicardial adipose tissue, perivascular adipose tissue, coronary artery disease, overweight, obesity, heart failure, echocardiography, prognostic marker, oxidative stress, monoamine oxidase, methylene blue

I. BACKGROUND & RESEARCH OBJECTIVES

Cardiovascular diseases are the leading of mortality due to coronary artery disease (CAD) and morbidity from heart failure (HF). With the ageing of population, the latter syndrome has become a major burden for the health systems worldwide.

The epicardial adipose tissue (EAT) is heart's actual visceral adipose tissue that covers up to 80% of the myocardial surface and has unhindered anatomical continuity with it. In the past decade, EAT, a biologically active organ responsible for the mechanical and thermal protection of the heart and coronary arteries, has been widely recognized as playing a central role in the pathophysiology of cardiac diseases. As such, EAT has been identified as an independent risk factor for the accelerated progression of sub-clinical coronary atherosclerosis. EAT expansion and dysregulation in the setting of CAD are further driven in the presence of metabolic diseases (metabolic syndrome, overweight/obesity and diabetes), which contributes to the phenotype shift of the EAT from a protective type to a pro-inflammatory and pro-oxidant type, thus favoring the progression towards HF.

Non-invasive classic imaging techniques, such as echocardiography (that assess the EAT thickness) and novel more expensive ones, such as magnetic resonance imaging and computed tomography (that also evaluate EAT volume) are currently used for the accurate quantification of EAT and have been correlated with various cardiac and systemic laboratory parameters or the presence of comorbidities.

Incorporating the measurement of EAT into routine cardiovascular risk assessments and diagnostic scores, which could potentially allow for earlier intervention in at-risk populations, is still an unmet goal of the medical community. EAT is currently viewed as a measurable cardiovascular risk factor that adds value to the stratification of cardiovascular risk, *yet few studies assessed its role in patients with CAD-related HF undergoing open heart surgery.*

The perivascular adipose tissue (PVAT) fat depot surrounding arteries and plays critical roles in regulating vascular tone and endothelial function. As EAT, PVAT expresses an inflammatory phenotype in cardiometabolic pathologies.

Besides chronic low-grade inflammation, another important pathomechanism that underlies the malfunction of the epicardial and perivascular adipose pools is *oxidative stress, but the sources of reactive oxygen species (ROS) in the cardiovascular adipose tissues are not completely elucidated.*

In the setting of HF, it had been reported that EAT is an important source of catecholamines and also, of the enzymes (tyrosine hydroxylase and dopamine β -hydroxylase) involved in their synthesis. Catecholamines are the major substrate of monoamine oxidase (MAO), an enzyme with 2 isoforms MAO-A and MAO-B, located at the outer mitochondrial membrane that is responsible for the constant generation of hydrogen peroxide (H_2O_2) as by-product of the deamination reaction of neurotransmitters and biogenic amines. While acknowledged as major ROS source in the brain for more than half century, in the past two decades, increased MAO activity/expression has emerged as an important contributor to oxidative stress in the cardiovascular system. Recently, the enzyme has been reported to be upregulated in the visceral adipose tissue of obese (but not of the lean) individuals. *Whether MAO is present at the level of human EAT and PVAT, contributes to the oxidative stress and can be pharmacologically modulated has not been systematically investigated.*

EAT is also considered nowadays as a modifiable risk factor, which can be pharmacologically targeted with drugs with pleiotropic effects, both old, such as statins and new, as the novel oral antidiabetics that have emerged as disease-modifiers agents in the setting of HF.

Methylene blue (MB), a synthetic compound in use for over 100 years, acts as an antioxidant and an alternative electron carrier able to enhance the energetic metabolism when the mitochondrial electron transport chain is impaired. MB has been previously reported to inhibit MAO in the brain and has beneficial effects, i.e., enhance mitochondrial function and modulate oxidative stress in the hearts of the diabetic rats. *There are no data in the literature regarding the possibility of modulating MAO in the human EAT and PVAT with MB.*

The **present doctoral work** was **double-aimed**: i) to perform a thorough echocardiographic analysis of the epicardial adipose tissue (mainly) in patients with CAD undergoing elective cardiac surgery for a better risk stratification, and ii) to assess the pharmacological modulation of oxidative stress and of MAO expression with MB in the cardiovascular adipose tissue, respectively.

The doctoral work comprised an interdisciplinary collaboration between the university Clinic of Cardiology and Cardiovascular Surgery from the Institute for Cardiovascular Diseases of Timișoara and the Chair of Pathophysiology/Centre for Translational Research and Systems Medicine from the Department of Functional Sciences of “Victor Babes” University of Medicine and Pharmacy from Timișoara, Romania. All the original studies received the ethical approval of the Commission of Scientific Research Ethics of the university and the Commission for Ethics in Research and Development from the Institute for Cardiovascular Diseases of Timișoara, respectively.

The **research objectives**, according to the 4 original studies carried out within this doctoral work, are:

1. Echocardiographic evaluation of the EAT thickness as an independent predictor of coronary artery disease severity.
2. Echocardiographic evaluation of the role of the indexed epicardial adipose tissue thickness (EATTi) as prognostic marker in patients with CAD undergoing open-heart surgery in order to improve the risk stratification in surgical patients.
3. Assessment of the modulatory effect of the chronic treatment with statins on the magnitude of local oxidative stress in EAT and PVAT samples harvested from the above mentioned patients and correlations with the echocardiographic parameters.
4. Assessment of the MAO expression and the level of oxidative stress in EAT and PVAT harvested from these patients, as well as the role of methylene blue in modulating these processes.

II. STUDY 1: ECHOCARDIOGRAPHIC ASSESSMENT OF EPICARDIAL ADIPOSE TISSUE THICKNESS AND ITS PREDICTIVE VALUE IN CORONARY ARTERY DISEASE

The first study aimed to analyse the intricate relationship between the epicardial adipose tissue thickness (EATT), oxidative stress in the EAT and the overall complexity of CAD, in order to contribute to the understanding of EAT's role in cardiovascular pathology and surgical outcomes in a pilot group (n = 25) of patients undergoing open-heart surgery. The group was further divided in patients with CAD (n=14) and non-CAD (n=11), respectively. EAT samples were harvested from the anterior wall of the right ventricle immediately after the initiation of cardiopulmonary bypass. Also, PVAT was collected from the peri-aortic and peri-pulmonary artery regions. Oxidative stress was assessed ex vivo using a spectrophotometric method (FOX assay). For each patient, at the time of enrolment, a detailed cardiovascular medical history, physical examination, ECG, biochemical blood analyses, complete echocardiographic examination and coronary angiography interpretation were performed.

The working hypothesis was that oxidative stress is increased in cardiovascular adipose tissues and EATT correlates with the increased oxidative stress in EAT, thereby reflecting the differences in the disease complexity in patients with and without CAD.

1. Increased EATT has been identified as an independent predictor of CAD

Patients in the CAD group (n=14) exhibited a higher mean EATT of 8.15 ± 2.09 mm compared to 5.12 ± 1.8 mm in the non-CAD group (n=11), a statistically significant difference (p value = 0.001) suggesting a potential relationship between increased EAT thickness and the presence of CAD. In terms of left ventricular mass, the CAD group had a lower mean LV mass of 251.51 g compared to 352.94 g in the non-CAD group, reaching statistical significance (p value = 0.012). These results underscore EAT thickness's potential as a valuable diagnostic parameter in cardiac evaluation.

2. Increased EATT was correlated with a higher oxidative stress in EAT

The level of epicardial ROS in the CAD group were notably elevated, averaging 21.4 ± 2.47 nmol H_2O_2 /mg tissue/h, compared to 15.7 ± 1.55 nmol H_2O_2 /mg tissue/h in the non-CAD group. This difference was found to be statistically significant ($p < 0.001$).

Additionally, the previously described echocardiographic assessment revealed a distinct difference in epicardial adipose tissue thickness between the two groups. Regression analysis further demonstrated a statistically significant correlation between EATT and ROS levels in both epicardial adipose tissue ($r=0.75$, $p < 0.001$) and periaortic adipose tissue ($r=0.65$, $p=0.001$).

3. An EATT greater than 6.05 mm was found to effectively identify patients with CAD

In the receiver operating characteristics (ROC) analysis, the parameters of EATT, along with the levels of ROS in both epicardial and periaortic fat, showed substantial diagnostic accuracy in identifying patients with coronary artery disease. Specifically, an EATT threshold greater than 6.05 mm was associated with the diagnosis of CAD, achieving a sensitivity of 86% and a specificity of 73% (Fig.1). The area under the curve (AUC) for this parameter was 0.87, indicating a high level of diagnostic precision. Moreover, the AUC for EAT ROS levels reached 0.99, reflecting an exceptionally high diagnostic capability. These

findings underscored the significant relationship between increased oxidative stress in epicardial and periaortic fat and the presence of CAD, thus confirming the utility of EATT and ROS measurements as important indicators in the clinical assessment of CAD ($p < 0.001$).

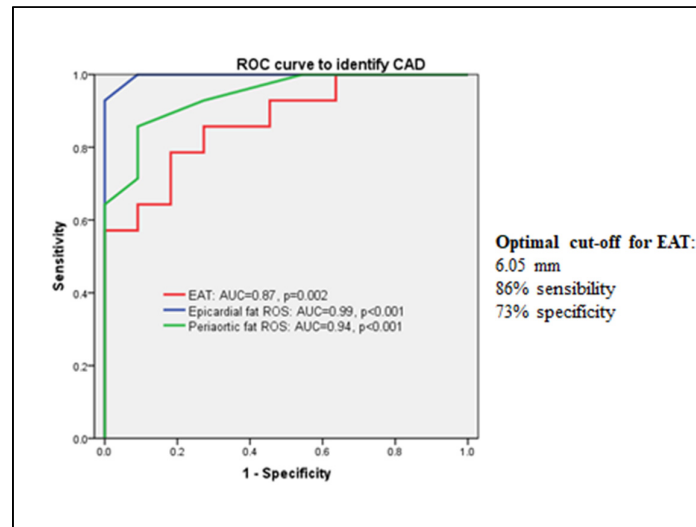


Figure 1 – ROC curves for EATT, ROS in EAT and PVAT to predict coronary artery disease.

4. ROS level was higher in the EAT from CAD vs. non-CAD patients, reinforcing the link between oxidative stress and the CAD severity

Significant differences emerged in ROS levels in epicardial fat among the groups based on their Syntax score: less than 23, between 23 and 32, and greater than 32. The ROS levels increased with higher Syntax scores, measuring at 19.2 for the < 23 group, 20.8 for the 23-32 group, and 21.43 for the > 32 group (p value = 0.002). This trend suggests a potential correlation between increased coronary complexity, as reflected by higher Syntax scores, and augmented oxidative stress in epicardial fat. A similar pattern was observed for ROS levels in periaortic fat, which also rose with increasing Syntax scores: 19.7 in the <23 group, 20.7 in the 23-32 group, and 24.01 in the >32 group (p value = 0.021) – Fig. 2.

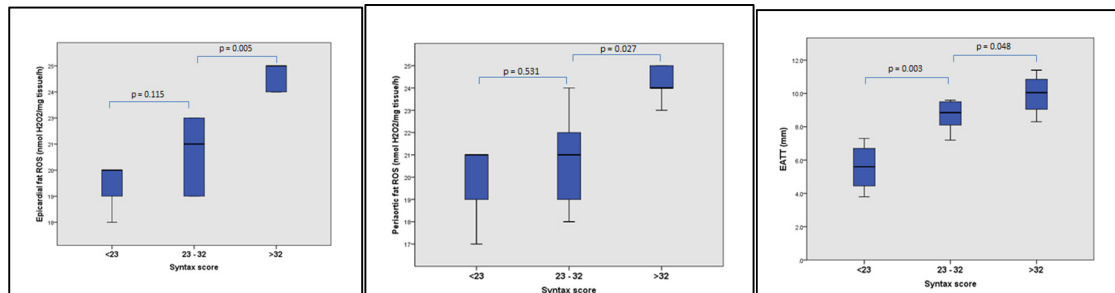


Figure 2 – Boxplot analysis of the relationship between: (a) – Syntax score and epicardial adipose tissue ROS; (b) – Syntax score and periaortic fat ROS; (c) – Syntax score and EATT.

III. STUDY 2: ASSESSMENT OF THE RELATIONSHIP BETWEEN INDEXED EPICARDIAL ADIPOSE TISSUE THICKNESS (EATTi), OXIDATIVE STRESS, AND CAD COMPLEXITY IN OPEN-HEART SURGERY PATIENTS

The second study aimed to explore the relationship between indexed epicardial adipose tissue thickness relative to body surface area (EATTi) and oxidative stress within EAT adipocytes in the context of CAD complexity among patients undergoing open-heart surgery.

1. EATTi significantly correlates with the local EAT oxidative stress, indicating its value in assessing the disease severity in patients undergoing open-heart surgery

The measurement of epicardial adipose tissue using the Iacobelli method and the EATTi exhibited a highly significant correlation with ROS level in EAT (p less than 0.001). This finding underscores that increased thickness of epicardial adipose tissue is directly associated with heightened oxidative stress within this specific adipose depot (Fig. 3).

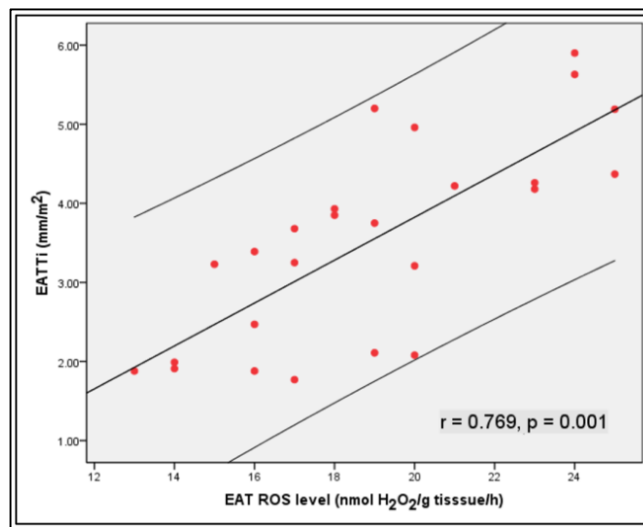


Figure 3 – Scatter plot of the relationship between epicardial adipose tissue thickness indexed to the body surface area (EATTi) and epicardial adipose tissue reactive oxygen species (ROS) levels in patients referred for heart-open surgery

2. An EATTi greater than 4.15 mm/m² predicted complex CAD with high sensitivity (80%) and specificity (86%), making it a useful metric for surgical planning and patient management

An EATTi exceeding 4.15 mm/m² can reliably identify individuals with more complex forms of CAD, as evidenced by SYNTAX scores over 22, supported by the observed sensitivity of 80% and specificity of 86% for this EATTi threshold. The intra- and inter-observer reproducibility, assessed in a subset of 15 randomly selected patients, was excellent, with intra-class and inter-class correlation coefficients of 0.911 (95% CI = 0.883–0.939) and 0.895 (95% CI = 0.872–0.918) respectively, affirming the reliability of these measurements in clinical assessments (Fig. 4).

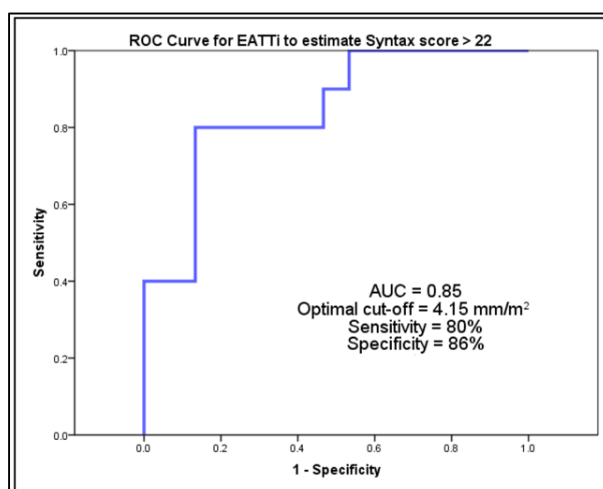


Figure 4 – ROC curves for EATTi to predict coronary artery disease with a SYNTAX score > 22 in surgical patients.

3. Higher EATTi values in patients with both CAD and diabetes, compared to those with CAD alone or with valvular disease, suggesting EATTi's broader applicability in diverse clinical scenarios

Participants were categorized into three subgroups based on their CAD and diabetes mellitus (DM) status: Non-CAD (n = 11), CAD-DM (n = 10), and CAD+DM (n = 4). Analysis of body mass index across these groups did not reveal any statistically significant differences. However, the CAD+DM group exhibited a higher mean SYNTAX I score compared to the CAD-DM group, a difference that was statistically significant. This trend continued with the SYNTAX II scores, where the CAD+DM group also showed higher averages, though these differences did not reach statistical significance.

These results highlight the potential of EATTi as a prognostic marker in the echocardiographic assessment of surgical patients, aiding in the optimization of patient management in cardiac surgery.

This pilot study is the first to demonstrate in international premiere that EATTi significantly correlated with the complexity of CAD, underscoring its potential as a valuable independent predictor for assessing the coronary disease severity.

IV. STUDY 3: ASSESSMENT OF THE ROLE OF CHRONIC STATIN THERAPY IN MODULATING THE OXIDATIVE STRESS IN CARDIOVASCULAR ADIPOSE TISSUE

The third study aimed to assess the level of oxidative stress in EAT and PVAT samples harvested from a pilot group of 25 overweight/obese patients with HF with mildly reduced EF (HFmrEF) who were chronically treated (n=17) or not (n=8) with statins (atorvastatin or rosuvastatin) undergoing cardiac surgery. EAT samples were harvested from the anterior wall of the right ventricle (RV) and PVAT was obtained from the peri-aortic and peri-pulmonary artery regions immediately after the initiation of cardiopulmonary bypass.

To further understand the implications of oxidative stress on cardiac function and the potential benefits of long-term statin therapy, correlations between the magnitude of oxidative stress and the echocardiographic parameters were also searched.

1. Patients chronically treated with statins showed significantly lower levels of ROS in EAT and PVAT vs. those non-treated.

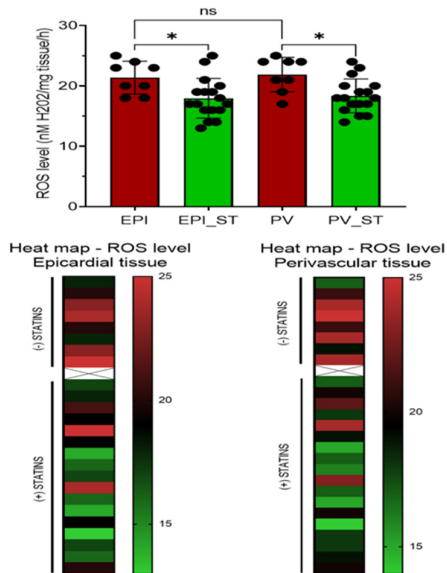


Figure 5 – Level of oxidative stress in epicardial (EPI) and perivascular (PV) adipose tissues.

This finding reinforces the pleiotropic effects of statins beyond their lipid-lowering effect confirming their therapeutic benefit in reducing oxidative stress, which is already present within the cardiovascular adipose tissue in overweight/obese patients with HFmrEF (Fig. 5).

2. A positive correlation between ROS level in EAT and the diameter of right ventricle was observed, suggesting that local oxidative stress may impact on cardiac structure

Furthermore, correlation analysis was employed to explore potential relationships between echocardiographic parameters (such as LVEF, left atrial diameter, right ventricular diameter, interventricular septum, left ventricular posterior wall thickness, and left ventricular end-diastolic diameter) and the degree of oxidative stress (measured as H₂O₂ levels by the FOX assay). A significant positive correlation was noted between the ROS levels in the EAT and the diameter of the right ventricle ($r = 0.47$, $p < 0.05$), indicating that an increased oxidative stress may interfere with the RV size (EAT sample was harvested from the anterior wall of the RV). Similar results were also obtained for PVAT (Fig.6).

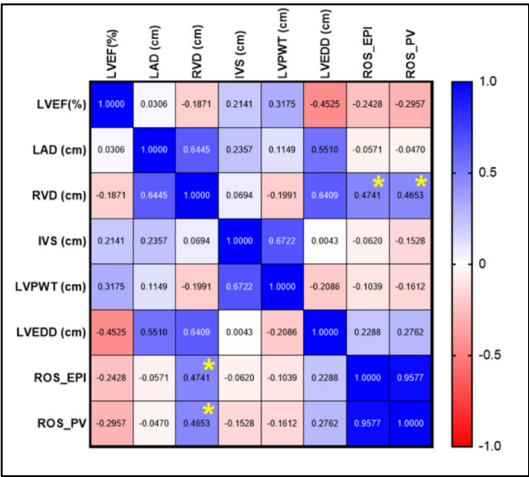


Figure 6 – Correlation matrix between the echocardiographic parameters and oxidative stress.

These results are suggestive for the impact of local oxidative stress on the cardiac structure, further highlighting the complex pathophysiological interactions within the cardiovascular system.

V. STUDY 4: ASSESSMENT OF THE INTERACTION METHYLENE BLUE - MONOAMINE OXIDASE AND OXIDATIVE STRESS IN HUMAN CARDIOVASCULAR ADIPOSE TISSUE

In the fourth study we hypothesized that MAO isoforms are expressed in EAT and PVAT of obese/overweight cardiac patients (n = 25) with HFpEF or HFmrEF with indication of elective cardiac surgery. We also posited that methylene blue (MB) could decrease MAO expression and ROS production, offering a novel therapeutic avenue to alleviate oxidative stress in the setting of cardio-metabolic pathologies.

1. Both MAO-A and MAO-B isoforms were detected in the EAT and PVAT, with MAO-A being the predominant isoform

MAO-A is the predominant isoform in cardiovascular adipose tissues (as reported in the literature for the human myocardium too), and its expression appears to be higher in EAT as compared to PVAT (Fig. 7). This differential expression further highlights the unique metabolic role of the EAT depot and is, most probably, due to the increased concentration of catecholamines (the MAO substrates).

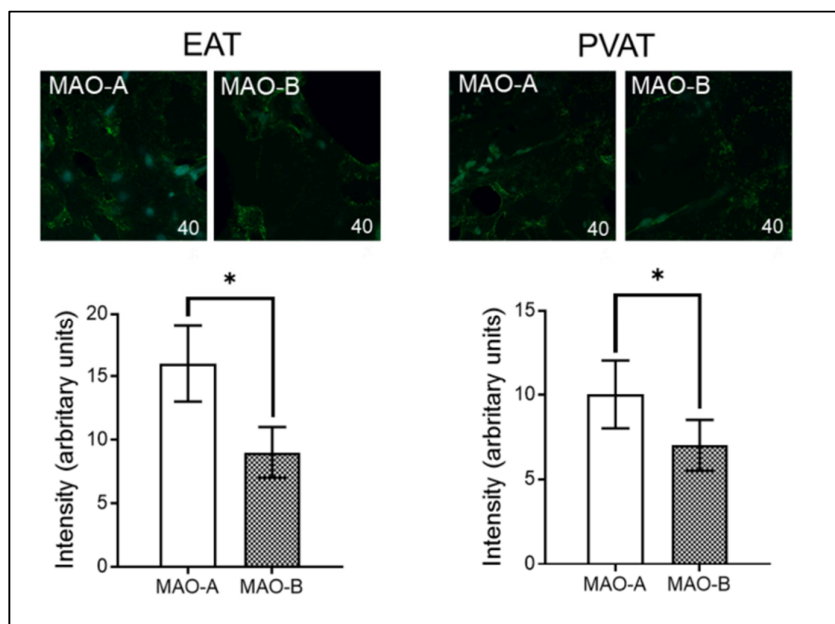


Figure 7 – MAOs' protein expression in human EAT and PVAT samples (immune fluorescence).

2. Methylene blue reduced MAO expression and oxidative stress in EAT and PVAT samples

MAO gene expression was further quantified using reverse transcription polymerase chain reaction (RT-PCR) in the presence and absence of methylene blue (MB). Acute *ex vivo* incubation with MB (0.1 μ M, 24 h) of EAT and PVAT samples resulted in a significant reduction in the expression of both MAO isoforms in the cardiac adipose tissues (Fig. 8).

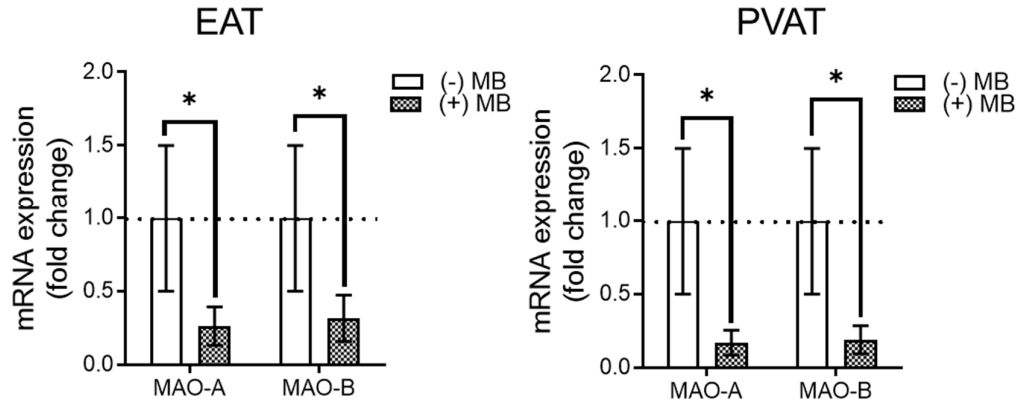


Figure 8 – MAOs' gene expression in human EAT and PVAT in the presence vs absence of MB (RT-PCR).

Assessment of oxidative stress has been performed using the dihydroethidium (DHE) stain (confocal microscopy) – Fig. 9 and FOX assay (spectrophotometry) – Fig. 10 in the presence vs the absence of MB.

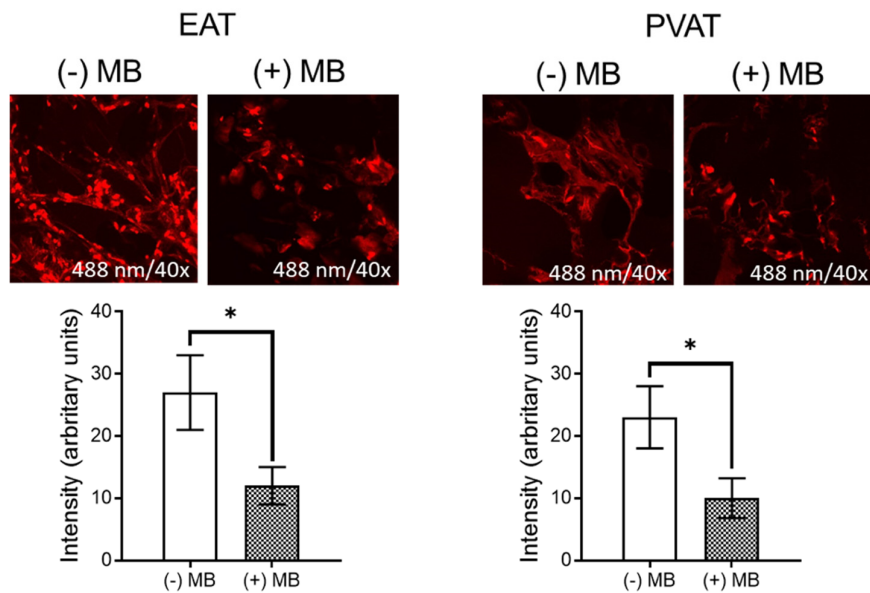


Figure 9 – ROS assessment with DHE stain in the presence vs absence of MB.

A slightly higher level of ROS quantified in immune fluorescence was observed in EAT as compared to PVAT suggesting that the former may be more prone to oxidative stress-related in cardiac patients with HF and metabolic disturbances than PVAT. However, MB equally mitigated ROS production in both EAT and PVAT as assessed by the DHE stain (Fig. 9).

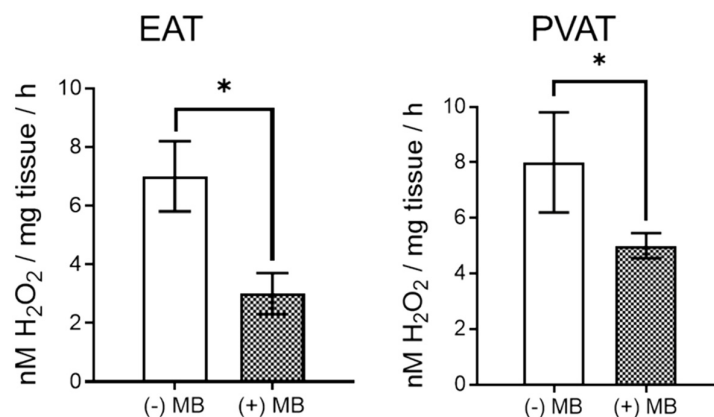


Figure 10 – ROS assessment with FOX assay in the presence vs absence of MB.

The results were recapitulated when the FOX assay was used, i.e., H₂O₂ production was significantly reduced in the presence of MB, both in EAT and PVAT (Fig. 10).

2. *Ex vivo* incubation of EAT and PVAT with serotonin, a MAO-A substrate, led to increased ROS production and MB partially reversed this effect

Besides catecholamines, serotonin (SR) is another widely investigated MAO-A substrate in the heart, but less information is available about its contribution to oxidative stress in the human cardiac adipose tissue. As such, we recapitulated the *ex vivo* experiments of H₂O₂ assessment via the FOX assay in EAT and PVAT samples in the presence vs absence of serotonin, the MAO-A substrate. Addition of serotonin significantly increased H₂O₂ production in both EAT and PVAT, an effect that MB was able to partially mitigate (Fig.11).

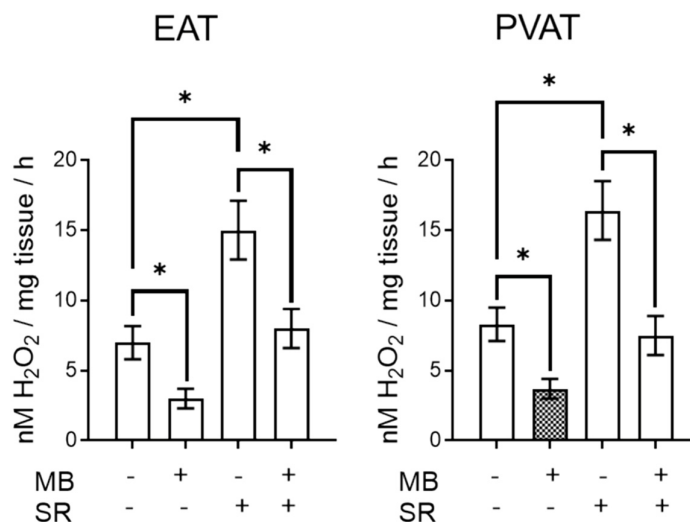


Figure 11 – ROS assessment with FOX assay in the adipose cardiac tissues incubated or not with serotonin (SR) and MB.

The study firstly showed that methylene blue may be beneficial in reducing oxidative stress and MAO expression in the adipose tissues of cardiac patients with HFpEF and HFmrEF and metabolic comorbidities. Further research is needed to confirm these findings in larger groups of patients and also, elucidate the underlying mechanisms.

VI. CONCLUSIONS

1. Echocardiographic assessment of the EAT thickness has been identified as an independent predictor of CAD, underscoring the potential role of this echocardiographic marker along with other parameters in elaborating risk scores.
2. Patients with CAD exhibited significantly higher EAT thickness compared to those without CAD, highlighting the relevance of this parameter in distinguishing between various severities of heart disease.
3. An EATT greater than 6.05 mm was found to effectively identify patients with CAD, demonstrating high sensitivity and specificity, thereby supporting its utility for application in the current medical practice.
4. Indexed EAT thickness (EATTi) significantly correlates with the complexity of CAD, indicating for the first time the value of this parameter in assessing the disease severity in patients undergoing open-heart surgery.
5. An EATTi greater than 4.15 mm/m² predicted a complex CAD with high sensitivity (80%) and specificity (86%), indicating for the first time the value of this parameter in assessing the disease severity in patients undergoing open-heart surgery.
6. Higher EATTi values were found in patients with both CAD and diabetes, compared to those with CAD alone or with valvular disease, suggest EATTi's broader applicability in diverse clinical scenarios.
7. Demonstration of an excellent intra- and interobserver reproducibility of EATTi measurements, ensuring their reliability for clinical use.
8. Identification of the potential role of EATTi as a prognostic marker in cardiac surgical assessments, aiding in the optimization of patient management strategies.
9. Higher levels of reactive oxygen species were found in the cardiac adipose tissues of CAD patients as compared to non-CAD patients, reinforcing the link between the oxidative stress magnitude and the occurrence of severe CAD.
10. Elevated EATT is correlated with increased oxidative stress within the epicardial tissue, suggesting a mechanistic link between adipose tissue thickness and the pathogenesis of CAD.
11. Patients chronically treated with statins (atorvastatin and rosuvastatin), showed significantly lower levels of ROS in their epicardial and perivascular adipose tissues compared to those not on statin therapy.
12. A positive correlation between oxidative stress and the diameter of the right ventricle was observed, suggesting its potential impact on the cardiac structure in vicinity.
13. Both MAO-A and MAO-B isoforms were detected in epicardial and perivascular adipose tissues, with MAO-A being the predominant isoform, suggesting that MAO-A is the major contributor to the local oxidative stress in heart failure patients.
14. Methylene blue treatment effectively reduced both MAO expression and oxidative stress in EAT and PVAT samples from heart failure patients undergoing cardiac surgery. Co-incubation with serotonin, a known MAO-A substrate, led to increased ROS production, but methylene blue partially reversed this effect, demonstrating its potential as a therapeutic agent for managing oxidative stress in cardiovascular adipose tissues.
15. Methylene blue may be beneficial in reducing oxidative stress and MAO expression in the adipose tissues of patients with heart failure and metabolic comorbidities.

Original Contributions:

1. Demonstration for the first time that **EATTi significantly correlates with the complexity of CAD**, thus underscoring its potential as a **valuable parameter for assessing the disease severity in patients undergoing open-heart surgery**.
2. Demonstration of **MAO expression, in particular MAO-A and ROS generation are increased in the epicardial and perivascular adipose tissues** harvested from **overweight and obese patients with heart failure with preserved and mildly reduced ejection fraction**.
3. Demonstration for the first time that **MB can mitigate ex vivo the oxidative stress in both types of adipose tissue** involved in the pathophysiology of cardiometabolic diseases

VII. SCIENTIFIC PUBLICATIONS

1. **Braescu Laurentiu**, Gaspar Marinica, Buriman Darius, Aburel Oana Maria, Merce Adrian Petru, Bratosin Felix, Aleksandrovich KS, Alambaram S, Mornos Cristian. *The Role and Implications of Epicardial Fat in Coronary Atherosclerotic Disease*. **Journal of Clinical Medicine**. **2022** Aug 12;11(16):4718. doi: 10.3390/jcm11164718. (IF - 4.9)
2. **Braescu Laurentiu**, Sturza Adrian, Sosdean Raluca, Aburel Oana M., Lazar Mihai Andrei, Muntean Mirela-Danina, Luca Constantin Tudor, Brie Daniel Miron, Feier Horea, Crisan Simina, Mornos Cristian. *Echocardiographic Assessment of Epicardial Adipose Tissue Thickness as Independent Predictor in Coronary Artery*. **Canadian Journal of Physiology and Pharmacology**. **2024** Sept 3 Just-In doi.org/10.1139/cjpp-2024-0188 (IF - 1,7)
3. **Braescu Laurentiu**, Sturza Adrian, Aburel Oana M, Sosdean Raluca, Muntean Mirela-Danina, Luca Constantin Tudor, Brie Daniel Miron, Feier Horea, Crisan Simina, Mornos Cristian. *Assessing the Relationship between Indexed Epicardial Adipose Tissue Thickness, Oxidative Stress in Adipocytes, and Coronary Artery Disease Complexity in Open-Heart Surgery Patients*. **Medicina (Kaunas)**. **2024** Jan 19;60(1):177. doi: 10.3390/medicina60010177. (IF- 2.4)
4. **Braescu Laurentiu**, Maria D. Danila, Karla A.S. Pop, Darius G. Buriman, Silvia Ana Luca, Adrian P. Merce, Oana M. Aburel, Raluca Sosdean, Horea B. Feier, Danina M. Muntean, Adrian Sturza, Cristian Mornos. *Statin Therapy Mitigates Oxidative Stress in Epicardial and Perivascular Adipose Tissue: A Pilot Study in Cardiac Surgery Patients*. **Timisoara Medical Journal**. **2024** (1):3. doi: 10.35995/tmj20240103.
5. Oana-Maria Aburel, **Braescu Laurentiu**, Darius G. Buriman, Adrian P. Merce, Anca M. Bina, Claudia Borza, Cristian Mornos, Adrian Sturza, Danina M. Muntean. *Methylene Blue Reduces Monoamine Oxidase Expression and Oxidative Stress in Human Cardiovascular Adipose Tissue*. **Molecular and Cellular Biochemistry** **2024** Aug. doi: 10.1007/s11010-024-05092-z. (IF - 3.5)