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# **DOCTORAL THESIS**

**NOVEL INSIGHTS INTO ADIPOSE TISSUE AND  
VASCULAR DYSFUNCTION IN OBESE PATIENTS  
WITH INFLAMMATORY STATUS**

## **ABSTRACT**

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**KEYWORDS:** obesity, low-grade chronic inflammation, visceral adipose tissue, mesenteric arteries, endothelial dysfunction, mitochondrial dysfunction, oxidative stress, monoamine oxidase, vitamin D.

## I. PURPOSE AND OBJECTIVES OF THE RESEARCH

Obesity is considered a pandemic, currently affecting one third of the worlds' population and, together with type II diabetes mellitus, are the major metabolic diseases associated mainly with life-threatening cardiovascular complications. Obesity has been, also, consistently associated with a low vitamin D status, although the pathophysiology of this association is partially elucidated.

In the past decades, *obesity-induced visceral adipose tissue (VAT) dysfunction* has been an active field of research in order to elucidate both the adaptive and maladaptive underlying pathomechanisms. Moreover, *endothelial dysfunction* and *mitochondrial dysfunction* have been also systematically addressed in a plethora of animal and less numerous human studies.

All these three dysfunctions share as common denominators *chronic inflammation* and *oxidative stress* that potentiate each other in long term in a vicious circle. With respect to the latter, the contribution of monoamine oxidase (MAO), a mitochondrial enzyme with 2 isoforms, A and B, to cardiovascular oxidative stress has been reported in the past decades. Thus, an increasing number of recent studies correlated the increased activity and/or expression of one or both MAO isoforms with the development and/or progression of cardiac and vascular disorders. Less information is available in the literature about the role of MAO-induced oxidative stress within the white adipose tissue. Thus, studies regarding MAO expression, regulation and function in adipose tissue remain an open field of research.

The aim of the present doctoral thesis was to provide a thorough characterization of **visceral adipose tissue and vascular dysfunctions, with special emphasis on the role of MAO-related oxidative stress in the setting of obesity in both adults and children**. Moreover, novel therapeutic approaches, such as MAO inhibitors and vitamin D, were investigated in a couple of *in vitro* experimental settings in order to identify viable therapeutic targets able to alleviate both adipose tissue and endothelial dysfunction in the setting of human obesity.

**The research objectives** were as follows:

**1. Assessment of obesity profile: correlations between clinical and laboratory parameters in adults and children.**

**2. Characterization of adipose tissue dysfunction** in tissue samples harvested from adult and pediatric patients with and without obesity, undergoing general surgery: *in vitro* assessment of oxidative stress, MAO expression, and the effects of incubation with a MAO inhibitor and the active vitamin D (calcitriol) on reactive oxygen species (ROS) production, respectively.

**3. Characterization of vascular dysfunction in mesenteric artery branches** harvested from adult patients with and without obesity, undergoing general surgery: *in vitro* assessment of the oxidative stress, MAO expression, and the effects of incubation with a MAO inhibitor and the calcitriol on ROS production, respectively.

**4. Characterisation of mitochondrial dysfunction in visceral adipose tissue** samples harvested from adult and pediatric patients with and without obesity, undergoing general surgery: *in vitro* assessment of mitochondrial respiration and the effects of acute addition of calcitriol.

## II. ASSESSMENT OF OBESITY PROFILE: CORRELATIONS BETWEEN CLINICAL AND LABORATORY PARAMETERS

Patients included in this pilot study were grouped into two groups according to the age: adults (n=30) and children (n=26). Each group was further classified, according to the BMI value, in 2 subgroups, obese and non-obese patients. In the *obese adults*, analysis of preoperative laboratory parameters showed the presence of increased levels of inflammatory markers, with a statistically significant difference for CRP ( $p = 0.02$ , Figure 1 - left) and a tendency for higher ESR. Important, all patients (obese and non-obese) had abnormal serum values for 25(OH)-vitamin D. While non-obese patients presented insufficient levels (<30 ng/ml), obese patients had vitamin D deficiency with average values below 20 ng/ml. Vitamin D showed a significant negative correlation with the BMI values ( $r = -0.476$ ,  $p < 0.05$ ) with a clusterisation tendency becoming evident when the 2 variables were plotted (Figure 1 - right).

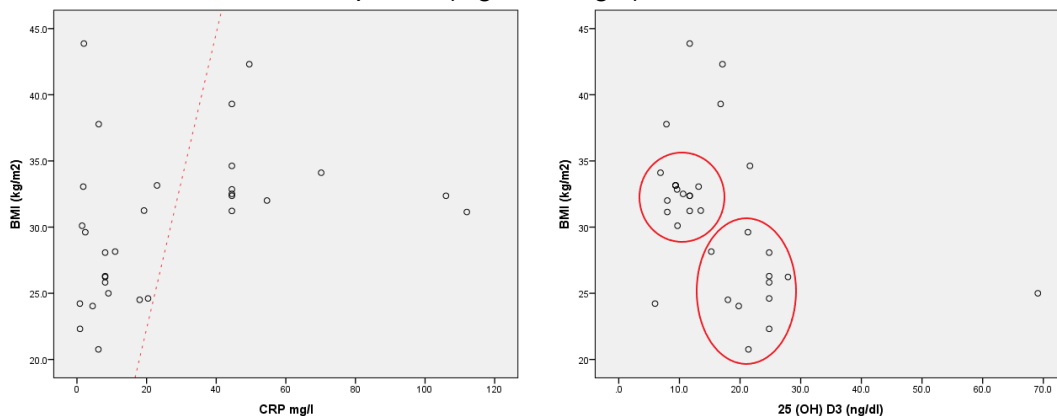


Figure 1. Correlation between BMI values and CRP levels (left) and clusterization of serum vitamin D according to BMI (right), in adult patients.

In the *obese children*, increased levels of both inflammatory markers were found, with a statistically significant difference for ESR and CRP ( $p=0.002$  and  $p=0.007$ , respectively); this observation is at variance from the adults that shown a significant difference only for CRP levels (Figure 2). In the pediatric group, vitamin D status showed a similar pattern of impairment as observed in adults, with insufficiency vs. deficiency for normal-weight and obese children ( $p=0.04$ ), respectively. However, at variance from adults, no negative correlation between vitamin D levels and BMI was noted in the obese children. Interestingly, the lipid profile showed normal values for all parameters regardless of weight, except for the HDL-cholesterol that was significantly higher in the subgroup of non-obese children vs the obese one ( $p<0.01$ ).

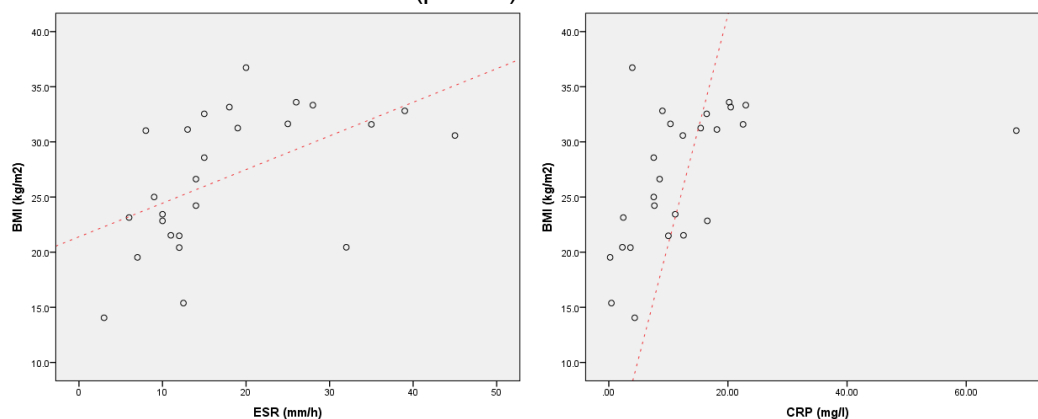


Figure 2. Correlations between BMI value and ESR level (left), and BMI value and CRP level (right), respectively, in pediatric patients.

### III. CONTRIBUTIONS TO THE ASSESSEMENT OF OXIDATIVE STRESS IN THE ADIPOSE TISSUE

In VAT samples of obese adults, a significantly higher level of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) ( $5.74 \pm 0.21$  nM  $\text{H}_2\text{O}_2/\text{h}/\text{mg}$  tissue,  $p < 0.01$ ) was measured using the spectrophotometric FOX assay as compared to the values in lean adults ( $3.09 \pm 0.32$  nM  $\text{H}_2\text{O}_2/\text{h}/\text{mg}$  tissue). Furthermore, 12-hour incubation of tissue samples with calcitriol (100 nM) significantly decreased the level of oxidative stress in the obese patients and had no effect in the non-obese subgroup (Figure 3 A).

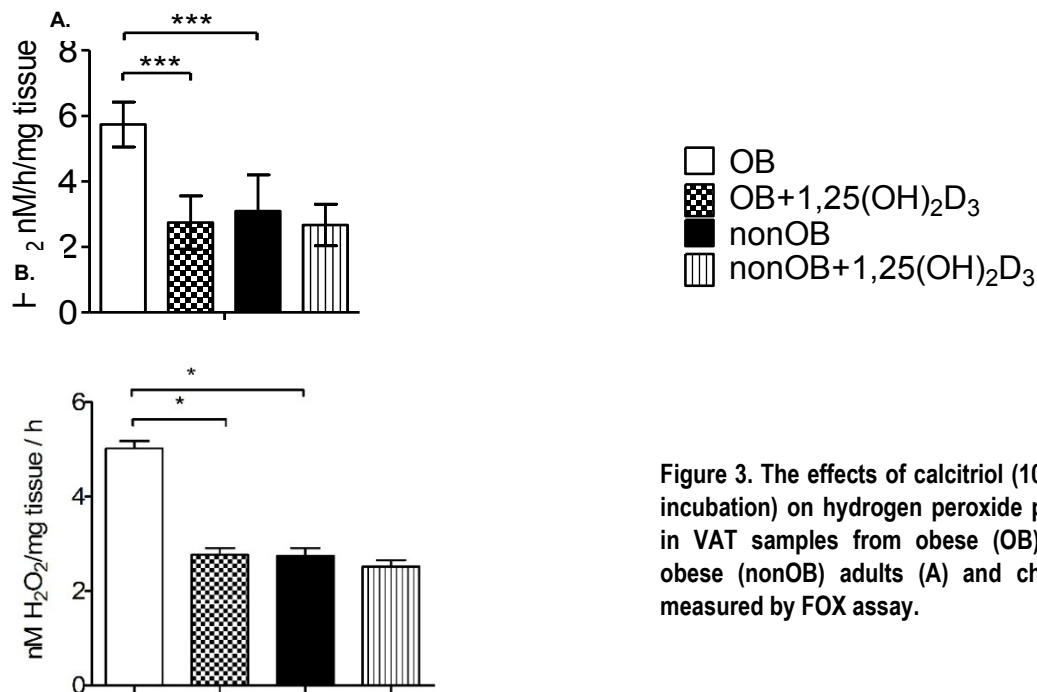


Figure 3. The effects of calcitriol (100 nM, 12h incubation) on hydrogen peroxide production in VAT samples from obese (OB) vs. non-obese (nonOB) adults (A) and children (B) measured by FOX assay.

Similarly, in VAT samples of obese children a mean value of  $5.03 \pm 0.16$  nM  $\text{H}_2\text{O}_2/\text{h}/\text{mg}$  tissue vs  $2.71 \pm 0.18$  nM  $\text{H}_2\text{O}_2/\text{h}/\text{mg}$  tissue in the lean controls ( $p < 0.5$ ) was found. VAT samples of obese children displayed a significant lower degree of oxidative stress following acute incubation with the active form of vitamin D, reaching a mean value close to the one of the control group, namely  $2.78 \pm 0.15$  nM  $\text{H}_2\text{O}_2/\text{h}/\text{mg}$  tissue ( $p < 0.01$ ) - Figure 3 B.

Also, the oxidative stress assessed by means of immune fluorescence (IF) using the dihydroethidium (DHE) probe was significantly higher in the obese vs. non-obese adults with the beneficial effect of the acute incubation with  $1,25(\text{OH})_2\text{D}_3$  (calcitriol) being obvious in the obese (and not in the non-obese) patients (Figure 4 - left). A similar pattern of ROS production and response to acute incubation with calcitriol, respectively was found in the VAT samples harvested from the pediatric group (Figure 4 – right).

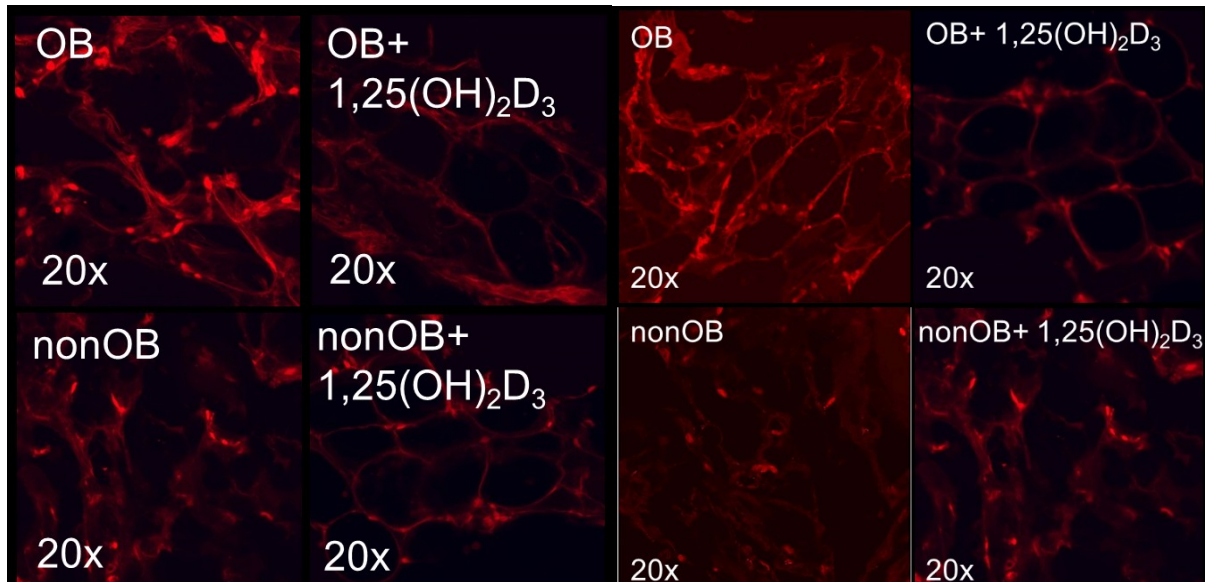


Figure 4. The effects of 1,25(OH)<sub>2</sub>D<sub>3</sub> (100 nM, 12h incubation) on VAT samples of obese (OB) vs. non-obese (nonOB) adults (left) and children (right) assessed in IF (DHE staining).

Another important observation was the correlation between the magnitude of oxidative stress measured in VAT samples with the degree of obesity (and also the inflammatory status). Thus, in adults a strong positive correlation ( $r=0.69$ ,  $p<0.01$ ) was found between the local H<sub>2</sub>O<sub>2</sub> production in adipose tissue (measured by FOX assay) and BMI, with a typical two-cluster pattern (Figure 5 - left). Of note, in children the correlation BMI - ROS production was stronger than in adults ( $r = 0.821$ ,  $p<0.001$ ), suggesting a higher impact of weight increase on oxidative stress at younger age (Figure 5 - right).

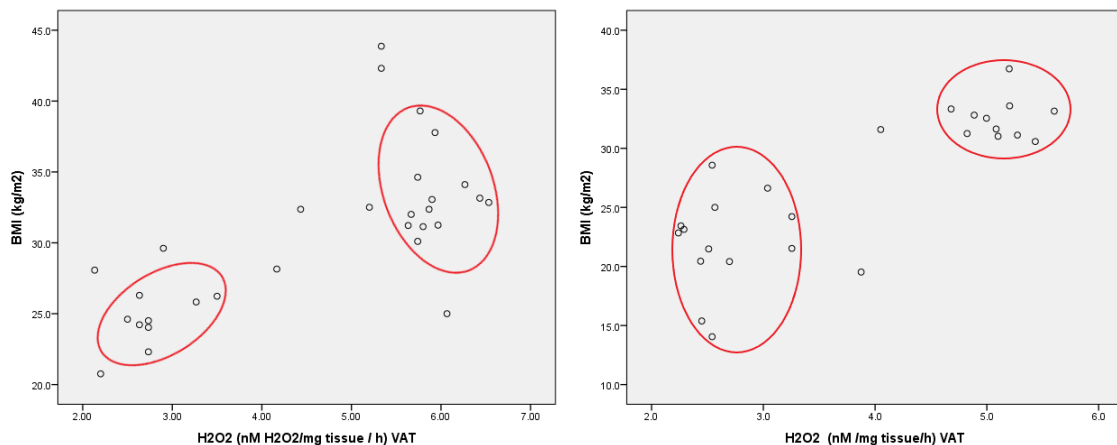


Figure 5. Correlation of BMI and local VAT hydrogen peroxide production in adult (left) and pediatric (right) patients.

Immune histochemistry (IH) studies revealed that both MAO-A and MAO-B isoforms are present in VAT of obese adults, with a clear predominance of the former (Figure 6 – left). qPCR study of mRNA gene expression revealed a significant up-regulation of MAO-A in VAT samples obtained from obese (OB) vs non-OB patients ( $p<0.05$ ) - Figure 6 – right.

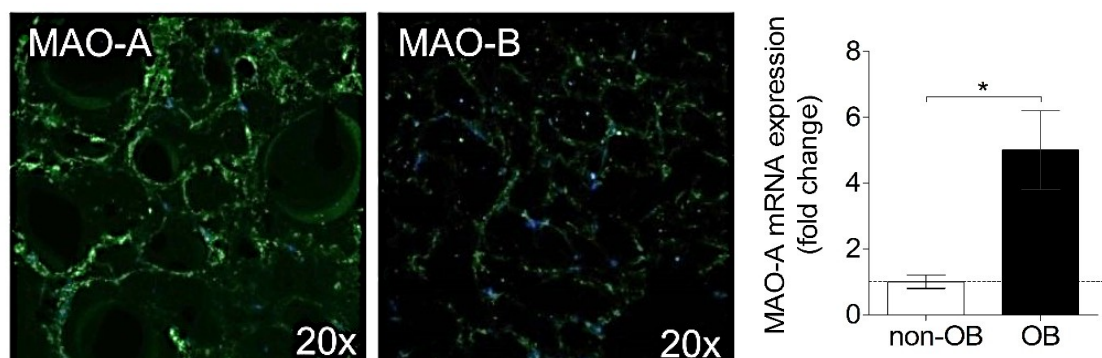


Figure 6. MAO expression in VAT from obese patients in IH, green - anti-MAO-A antibody, blue - DAPI (left). MAO-A gene expression in VAT from OB vs non-OB adult patients in qPCR, \* $p<0.05$  (right).

*Ex vivo* incubation with clorgyline, the irreversible MAO-A inhibitor, significantly decreased hydrogen peroxide production in VAT samples of obese patients ( $p<0.05$ ), with no changes recorded in samples obtained from non-obese patients. Similar results were observed in IF studies following DHE staining, with a significant reduction of oxidative stress in the VAT samples harvested from the obese adults when incubated with clorgyline ( $p<0.05$ ) vs no effect in the non-obese group (Figure 7).

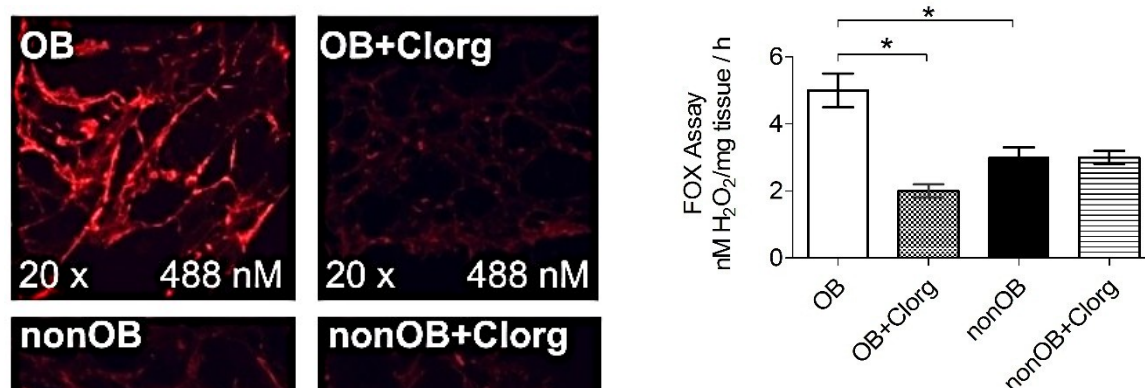


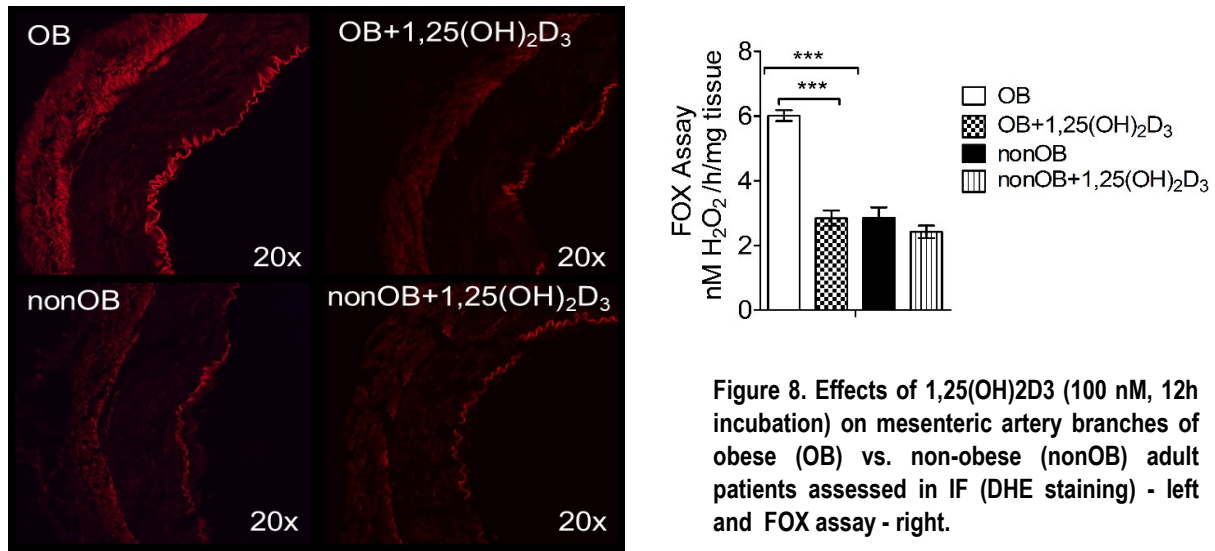
Figure 7. Effects of the MAO-A inhibitor clorgyline (Clorg, 10  $\mu$ M) on ROS generation in VAT samples from obese (OB) vs. non-obese (nonOB) adult patients assessed in IF (DHE staining) - left and by FOX assay - right.

#### IV. CONTRIBUTIONS TO THE ASSESSMENT OF VASCULAR TISSUE DYSFUNCTION

Assessment of the vascular oxidative stress provided results comparable to those obtained for the VAT samples. Thus, ROS production in IF was significantly higher in vascular rings from obese vs. non-obese patients whereas incubation with calcitriol significantly diminished oxidative stress in obese patients, with little difference observed for samples of lean adults (Fig. 8 - left). Hydrogen peroxide levels in vascular samples from obese patients revealed a mean value of  $6.01 \pm 0.17$  nM H<sub>2</sub>O<sub>2</sub>/h/mg tissue in the obese vs  $2.85 \pm 0.33$  nM H<sub>2</sub>O<sub>2</sub>/h/mg tissue in the lean adults ( $p<0.01$ ) - Fig. 8 - right. Twelve-hour



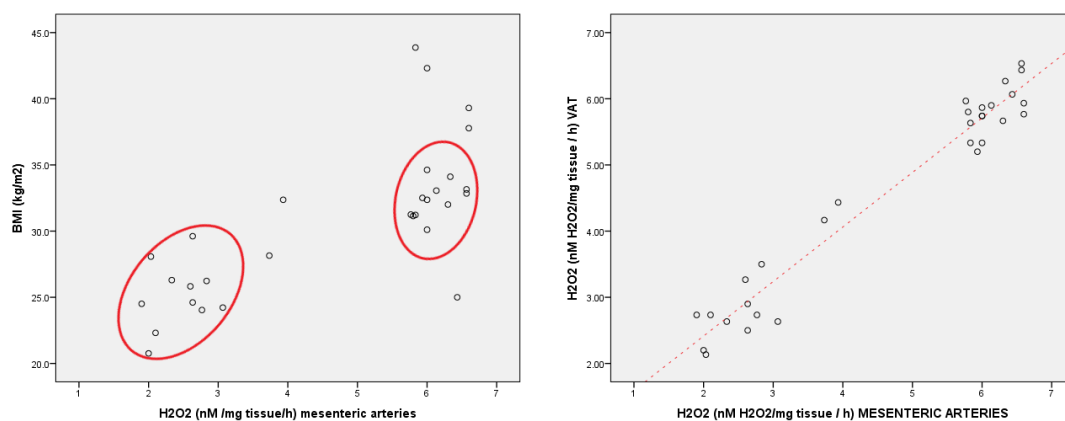
incubation with calcitriol (100 nM) significantly decreased the local oxidative stress in samples of obese individuals.



**Figure 8.** Effects of 1,25(OH)<sub>2</sub>D<sub>3</sub> (100 nM, 12h incubation) on mesenteric artery branches of obese (OB) vs. non-obese (nonOB) adult patients assessed in IF (DHE staining) - left and FOX assay - right.

As reported for the VAT samples, H<sub>2</sub>O<sub>2</sub> production in mesenteric artery branches was significantly correlated with the degree of obesity (and the inflammatory status, data not shown). Accordingly, BMI showed a strong positive correlation ( $r=0.736$ ,  $p<0.01$ ) with the local hydrogen peroxide production (FOX assay) and a similar tendency for clusterization of the low and high values, respectively (Figure 9 - left).

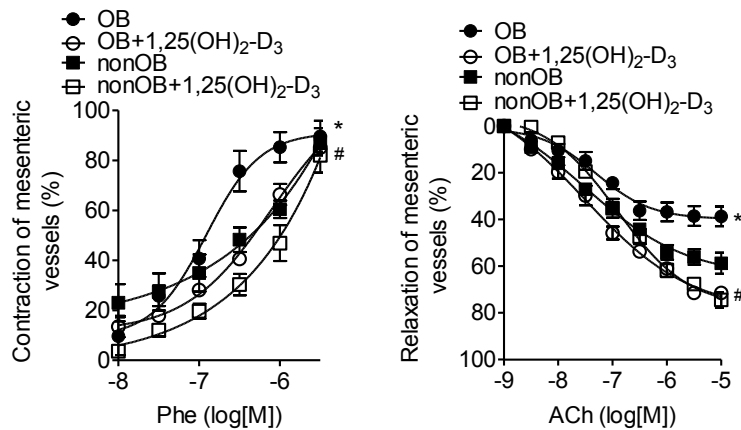
Importantly, the hydrogen peroxide level in vascular samples showed a very strong positive correlation with the one measured in VAT ( $r=0.980$ ,  $p<0.01$ ), suggesting a linearity of local oxidative stress occurring in these 2 tissues in the setting of adult obesity (Figure 9 - right).



**Figure 9.** Correlation between BMI and H<sub>2</sub>O<sub>2</sub> level in mesenteric artery branches of adult patients (left). Correlation between H<sub>2</sub>O<sub>2</sub> production in VAT and vascular samples of adult patients (right).

Assessment of vascular function (organ bath studies) of mesenteric artery branches, revealed a significant higher contractility to cumulative doses of Phe in isolated rings prepared from the obese patients as compared to the lean controls ( $p<0.05$ ). Moreover, measurements of endothelium-dependent relaxation in response to cumulative doses of Ach showed a significant difference between the two subgroups ( $p<0.05$ ), with a decreased relaxation in arteries harvested from the obese patients (Figure 10).

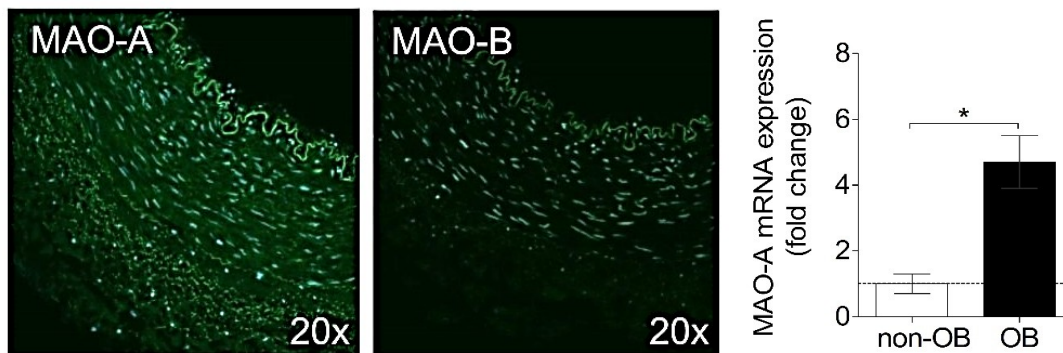




**Figure 10.** Effects of 1,25(OH)<sub>2</sub>D<sub>3</sub> (100 nM, 12h incubation) on phenylephrine-induced contraction and acetylcholine-induced relaxation in mesenteric artery branches of obese (OB) and non-obese (nonOB) adult patients.

The effects on vasomotor response following acute incubation with the active vitamin D (100 nM) were further assessed. In the presence of 1,25(OH)<sub>2</sub>D<sub>3</sub> (calcitriol), vascular contractility decreased and endothelium-dependent relaxation significantly increased in samples harvested from the obese adults; no significant changes were obtained in the non-obese group following vitamin D incubation (Figure 10).

Similar to the findings reported in VAT samples, both MAO-A and MAO-B were present in vascular samples of obese adults, MAO-A being the predominant isoform (Figure 11 - left). Analysis of mRNA gene expression of MAO-A in vascular samples showed, as for the VAT samples, an up-regulation in vascular rings of the OB vs non-OB patients (Figure 11 - right).



**Figure 11.** MAO expression in mesenteric artery branches of obese adults in IF: green – anti-MAO-A antibody, blue - DAPI (left). MAO-A gene expression in mesenteric artery branches from OB and nonOB adults in qPCR, \*p<0.05 (right).

Inflammation is a well documented underlying cause for endothelial dysfunction; however its effect on vascular MAO in humans is unknown. *In vitro* stimulation of mesenteric artery branches by incubation with IL-6 (100 ng/ml, 12 h) elicited a significant increase in the MAO-A mRNA at qPCR of both obese children and adults (Figure 12).

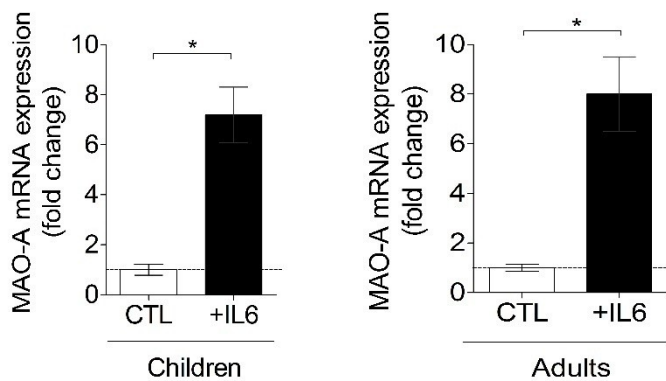


Figure 12. Expression of MAO-A gene in mesenteric artery branches of children (left) and adults (right) following stimulation with IL-6 (100 ng/ml, 12 h).

*Ex vivo* incubation with the irreversible MAO-A inhibitor, clorgyline, was also performed for the vascular samples in order to assess the contribution of MAO to oxidative stress-related dysfunction. Evaluation of hydrogen peroxide production by means of FOX assay showed a significant decrease in vascular oxidative stress in samples harvested from obese (and not for the non-obese) patients (Figure 13 - right). The results were further confirmed by IF studies which also revealed a significant decrease in ROS levels of mesenteric artery branches from obese adults after incubation with clorgyline (Figure 13 - left).

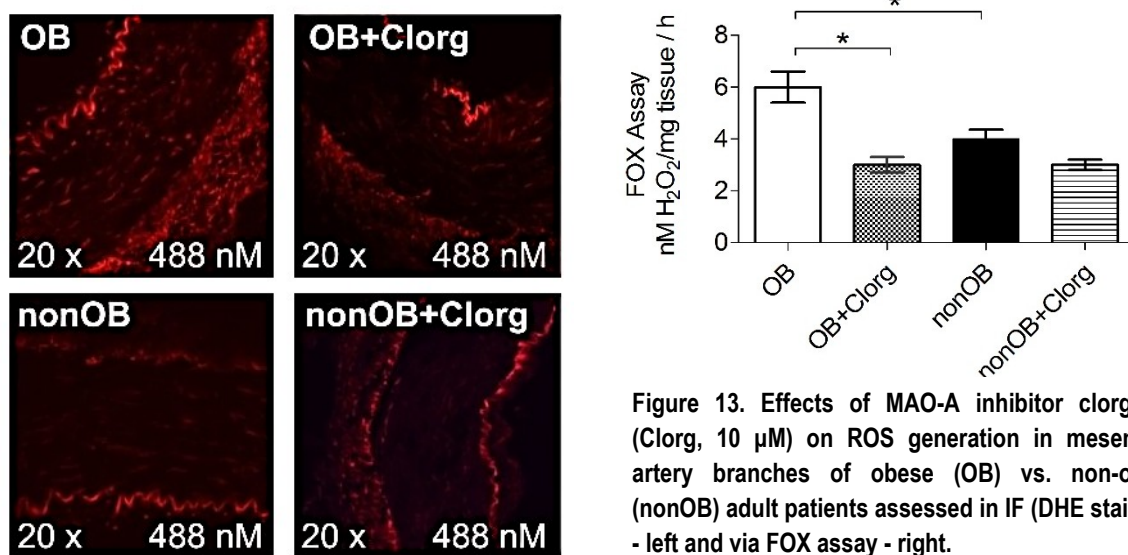


Figure 13. Effects of MAO-A inhibitor clorgyline (Clorg, 10  $\mu$ M) on ROS generation in mesenteric artery branches of obese (OB) vs. non-obese (nonOB) adult patients assessed in IF (DHE staining) - left and via FOX assay - right.

Vascular preparations from the obese patients presented an increased contractility in the presence of cumulative doses of phenylephrine; however, following incubation with clorgyline a significant decrease in the contractile response as compared to the baseline values was observed ( $p < 0.05$ ) - Figure 14 A. Also, the endothelial-dependent relaxation in the presence of cumulative doses of ACh was significantly improved in the arteries of obese patients after incubation with clorgyline ( $p < 0.05$ ). Vascular samples of non-obese individuals displayed minimal changes as compared to baseline measurements after incubation with clorgyline, with no statistical significance (Figure 14 B).

The contribution of NO to the protective effect of MAO inhibition was further tested by the assessment of vascular sample contractility in the presence of L-NAME, a classic NO synthase inhibitor. Arteries of obese patients incubated with clorgyline showed a significant decrease in contractility to L-NAME ( $p < 0.05$ ) - Figure 14 C.

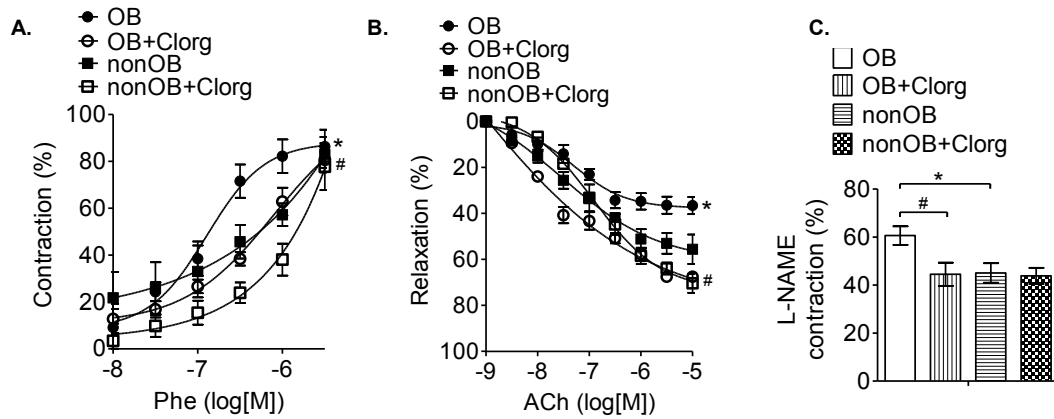


Figure 14. Phenylephrine-induced contraction (A), acetylcholine-induced endothelium-dependent relaxation (B), contraction to L-NAME (C) in mesenteric artery branches obtained from obese (OB) and non-obese (nonOB) adults, incubated or not with MAO-A inhibitor, clorgyline (Clorg, 10  $\mu$ M, 30 min).  
\* $p < 0.05$  OB vs. nonOB, # $p < 0.05$  OB vs. OB+Clorg.

## V. CONTRIBUTIONS TO THE ASSESSMENT OF MITOCHONDRIAL RESPIRATORY DYS/FUNCTION IN ADIPOSE TISSUE

In a subgroup of adult patients, VAT harvested from non-obese ( $n = 8$ ) vs. obese ( $n = 10$ ) patients was evaluated by means of high-resolution respirometry (HRR) to assess the presence of mitochondrial respiratory dysfunction. All respiratory rates: basal (State 2), maximal active (OXPHOS) and maximal uncoupled (ETS) were moderately decreased in the obese adults as compared to the non-obese ones (Table 1).

However, when performing the HRR measurements on VAT samples harvested from a obese ( $n=7$ ) vs non-obese ( $n=9$ ) children, a marked decrease in all respiratory states was found in the setting of infantile obesity, with the most pronounced difference being observed for the basal respiration - a 76% lower rate as compared to controls. (Table 1).

Table 1. Changes in mitochondrial respiratory rates in VAT samples of adult and pediatric patients.

Patients		Basal respiration (State 2)	Active respiration (OXPHOS)	Uncoupled respiration (ETS)
Adults				
	<i>Obese vs control</i>	-21%	-35%	-20%
Children				
	<i>Obese vs control</i>	-76%	-70%	-69%

A difference (albeit non-significant) was also observed between the respiratory rates of obese adults and children. In particular, both active (OXPHOS) and uncoupled (ETS) respiration were slightly lower with 27% and 24% in obese children vs obese adults (Fig. 15). These results suggest that obesity may have a more profound impact on the mitochondrial respiration of adipose tissue in children as compared to adults.

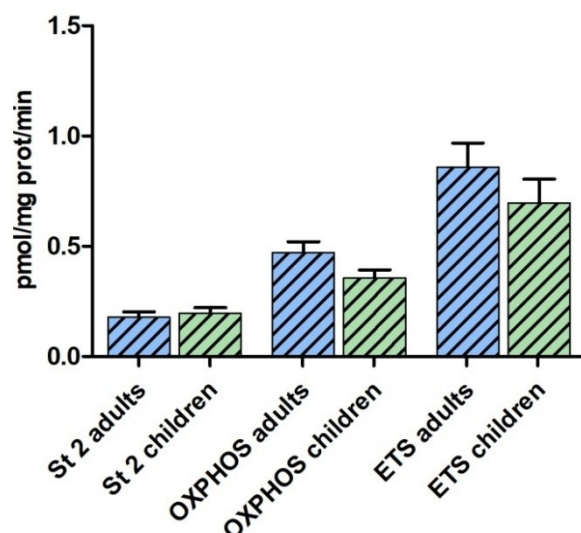


Figure 151. Mitochondrial respiratory states of obese adults vs obese children.

The decrease in mitochondrial respiratory rates observed in obese children as compared to obese adults strongly suggests that early mitochondrial dysfunction represents an unexplored and underestimated event in the pathogenic sequence of obesity that may be considered an attractive therapeutic target in order to prevent the progression and/or treat the obesity-induced complications in later adult life. Further studies are therefore required to provide a more complex characterization of mitochondrial structural and functional impairment in larger patient samples in the setting of pediatric obesity.

With respect of the vitamin D effect at the level of the adipose tissue, two findings are relevant: i) first, vitamin D elicits more important changes in the VAT from non-obese as compared to the obese subjects, regardless the age, and ii) second, the changes of the maximal coupled and uncoupled respiration after acute *in vitro* exposure to the active vitamin D (100 nm) are opposite, i.e. a decrease occurred in adults and an increase in children, respectively; these observations require further investigations.

## VI. CONCLUSIONS

1. Obesity is strongly associated with an inflammatory status in both children and adults; the BMI positively correlates with the serum level of C reactive protein (CRP) and the erythrocyte sedimentation rate (ESR) in children, and CRP in adults, respectively.
2. In obese children significant lower values for HDL cholesterol were found as compared to the non-obese ones.
3. Vitamin D deficiency was the hallmark of both obese adults and children, whereas the non-obese patients were vitamin D insufficient.
4. Vitamin D levels presented a strong negative correlation to the BMI value in adults, finding that was not recapitulated in children.

5. Oxidative stress is significantly increased in visceral adipose tissue and mesenteric artery branches from obese patients vs lean individuals, as assessed by both spectrophotometry and confocal microscopy.
6. The local production of hydrogen peroxide in adipose tissue and vascular samples showed a strong positive correlation with the BMI values in both adults and children; two individual clusters that associated low levels of H<sub>2</sub>O<sub>2</sub> with low values of BMI and high levels of H<sub>2</sub>O<sub>2</sub> with increased values of BMI, respectively were evident in both tissues.
7. A very strong positive correlation was found between the H<sub>2</sub>O<sub>2</sub> levels in VAT and mesenteric artery branches samples, suggesting that the degree of oxidative stress in adipose tissue parallels the one from the abdominal vascular bed.
8. Vitamin D mitigated ROS production in both adipose tissue and vascular samples in obese patients, and had little effect on samples from non-obese individuals.
9. *Ex vivo* incubation with the active vitamin D (in submicromolar concentrations) significantly improved endothelial-dependent relaxation and decreased contractility in vascular samples from obese adults.
10. The antioxidant effect of vitamin D is not mediated via a scavenger effect in these tissues.
11. MAO-A is the predominant isoform identified in both adipose tissue and vessels in the setting of obesity, as shown in confocal microscopy.
12. The obese patients demonstrated a significantly higher mRNA level of MAO-A at qPCR as compared to the lean adults.
13. Acute *ex vivo* exposure to IL-6 significantly increased the mRNA expression of MAO-A isoform in mesenteric artery branches harvested from adult and pediatric patients.
14. Acute *ex vivo* inhibition of MAO-A with clorgyline significantly mitigated the oxidative stress in both adipose tissue and mesenteric arteries from the obese patients.
15. Acute *ex vivo* inhibition of MAO-A with clorgyline significantly alleviated the endothelial dysfunction of mesenteric artery branches from the obese adult patients.
16. Mitochondrial respiration is reduced in visceral adipose tissue samples of obese adults and children.
17. Mitochondrial respiratory rates are higher at baseline (up to 70%) in normal-weight children as compared to the normal-weight adults; these values are lower (up to 25%) in obese children vs obese adults.
18. Acute *ex vivo* exposure of adipose tissue to the active vitamin D elicited more important changes of mitochondrial respiration in the non-obese individuals vs the obese patients, regardless the age.
19. A dichotomic effect was found for vitamin D after acute exposure on the adipose tissue respiration, i.e. a decrease occurred in adults and an increase in children, respectively.

## VII. ORIGINAL CONTRIBUTIONS

- Acute *ex vivo* administration of active vitamin D mitigates oxidative stress in visceral adipose tissue harvested from obese adults and children.
- Acute *ex vivo* administration of active vitamin D reduces oxidative stress and improves vascular reactivity of mesenteric artery branches isolated from obese adults.
- Obesity and inflammation are associated with increased MAO-A isoform expression in both visceral adipose tissue and vascular samples of obese adults and children.
- MAO-A is a source of ROS in visceral adipose tissue and mesenteric vasculature in the setting of low-grade chronic inflammation and obesity.
- Acute *ex vivo* MAO-A inhibition decreases ROS generation in both visceral adipose tissue and mesenteric vasculature of obese adults.
- Acute *ex vivo* MAO-A inhibition improves vascular reactivity of mesenteric artery branches isolated from obese adults.
- Mitochondrial respiration is decreased in visceral adipose tissue of obese patients, particularly in children.
- Acute *ex vivo* exposure to the active vitamin D elicited opposite changes on adipose tissue respiration, namely a decrease in adults and an increase in children, respectively.

## VIII. FUTURE RESEARCH DIRECTIONS

- Elucidate the mechanisms underlying the antioxidant effect of vitamin D.
- Investigate whether the vitamin D deficiency in the setting of obesity interferes with the postoperative recovery, particularly in relation to bacterial infections and organ failure.
- Investigate the occurrence of an increased MAO expression in adipose tissue of other non-communicable disease, in particular in diabetes mellitus.
- Confirm the beneficial effects following the *in vivo* reversible MAO-A inhibition on adipose tissue and endothelial dysfunction in a pilot clinical trial.
- Further characterize the mitochondrial respiratory dysfunction observed in adipose tissue of obese adults and children, along with the exploration of other mitochondrial abnormalities (impaired calcium retention, autophagy etc).
- Further characterize the effects of *ex vivo* vitamin D effects on mitochondrial function in the setting of obesity.

## IX. SCIENTIFIC PUBLICATIONS

1. **Ionică M.**, Aburel O.M., Văduva A., Petruș A., Rațiu S., Olariu S., Sturza A, Muntean D.M. **Vitamin D Alleviates Oxidative Stress in Adipose Tissue and Mesenteric Vessels from Obese Patients with Subclinical Inflammation.** *Can J Physiol Pharmacol.* 2020; 98(2):85-92. doi: 10.1139/cjpp-2019-0340 (IF=2.041).
2. Sturza A., Olariu S., **Ionică M.**, Duicu O.M., Văduva A., Boia E., Muntean D.M., Popoiu C. **Monoamine Oxidase is a Source of Oxidative Stress in Obese Patients with Chronic Inflammation.***Can J Physiol Pharmacol.* 2019; 97(9):844-849. doi: 10.1139/cjpp-2019-0028 (IF=2.041).
3. Sturza A., Popoiu C.M., **Ionică M.**, Duicu O.M., Olariu S., Muntean D.M., Boia E.S. **Monoamine Oxidase-Related Vascular Oxidative Stress in Diseases Associated with Inflammatory Burden.** *Oxid Med Cell Longev.* 2019; 8954201. doi: 10.1155/2019/8954201 (IF=4.868).