

Biomarker in Screening for Obstructive Sleep Apnea

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Spetses 2019

Obstructive Sleep Apnea (OSA) syndrome

- Recurrent **upper airway obstruction** during sleep with resultant intermittent **hypoxia** and **sleep fragmentation**;
- Non-treated OSA can lead to



daytime sleepiness or fatigue, headaches, nocturia , insomnia

OSA



SNORER



Obstructive Sleep Apnea (OSA) syndrome

- Recurrent **upper airway obstruction** during sleep with resultant intermittent **hypoxia** and **sleep fragmentation**;
- Non-treated OSA can lead to **severe metabolic** and **cardiovascular disorders**.

OSA cardiometabolic Outcomes

Diabetes mellitus type 2, Obesity, Dyslipdemia

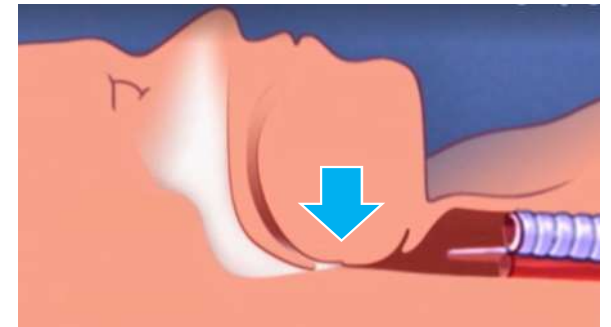
Arterial hypertension, Cardiac arrhythmias

Pulmonary hypertension

Respiratory insufficiency

Stroke

OSA



SNORER



OSA Diagnosis



PSG

Polysomnography

Gold standard for diagnosis

expensive/long waiting lists

delays in diagnosis and treatment

AHI : $\geq 5/h$ (**mild**) $\geq 15/h$ (**moderate**) $\geq 30/h$ (**severe**)
 $< 5/h$ (**Normal Snorer**)

OSA is still underdiagnosed

affecting **22% of men** (range 9-37%) and **17% of women** (range 4-50%) and **3% children** (*Franklin et al , JThoracDis, 2015*).

OSA Treatment



First Line Treatment
mild air pressure to keep the airways open

CPAP or PAP

(Continuous) Positive Airway Pressure

Although **highly efficacious**, the utility of **CPAP** is **limited by poor patient compliance**, and OSA **sequelae** may **not be fully reversed** by CPAP

Biomarkers for Obstructive Sleep Apnea is needed

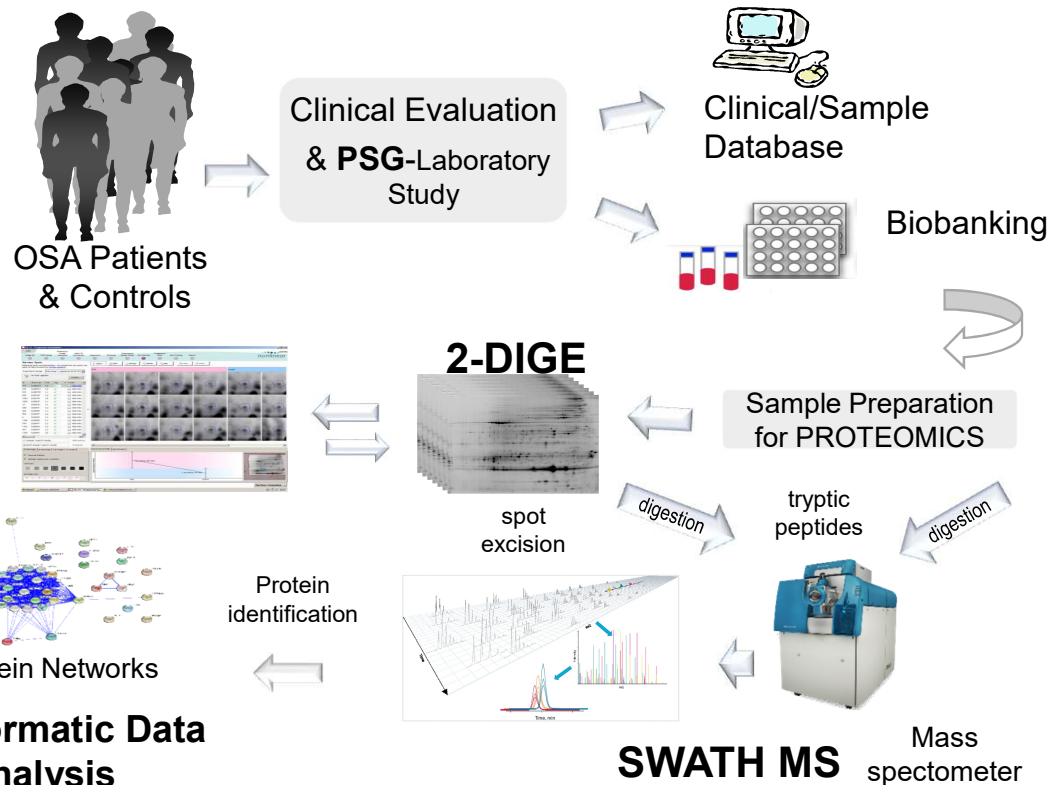


- to **distinguish OSA** from Snorer, facilitating population **screening** and **prevention** of **OSA-associated outcomes**
- to **provide new insights** into pathophysiological aspects of OSA that underlie the increased **cardiovascular and metabolic risk** in general population

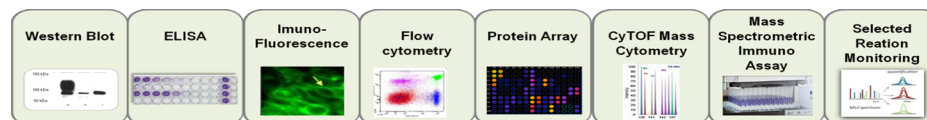


PROTEOMICS

DISCOVERY PHASE



VERIFICATION PHASE



CANDIDATES BIOMARKERS OF OSA

PROTEOMICS STUDY PROPOSED FOR OSA



SPP/PFIZER AWARD 2016



HHS Public Access

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Overview of proteomics studies in obstructive sleep apnea

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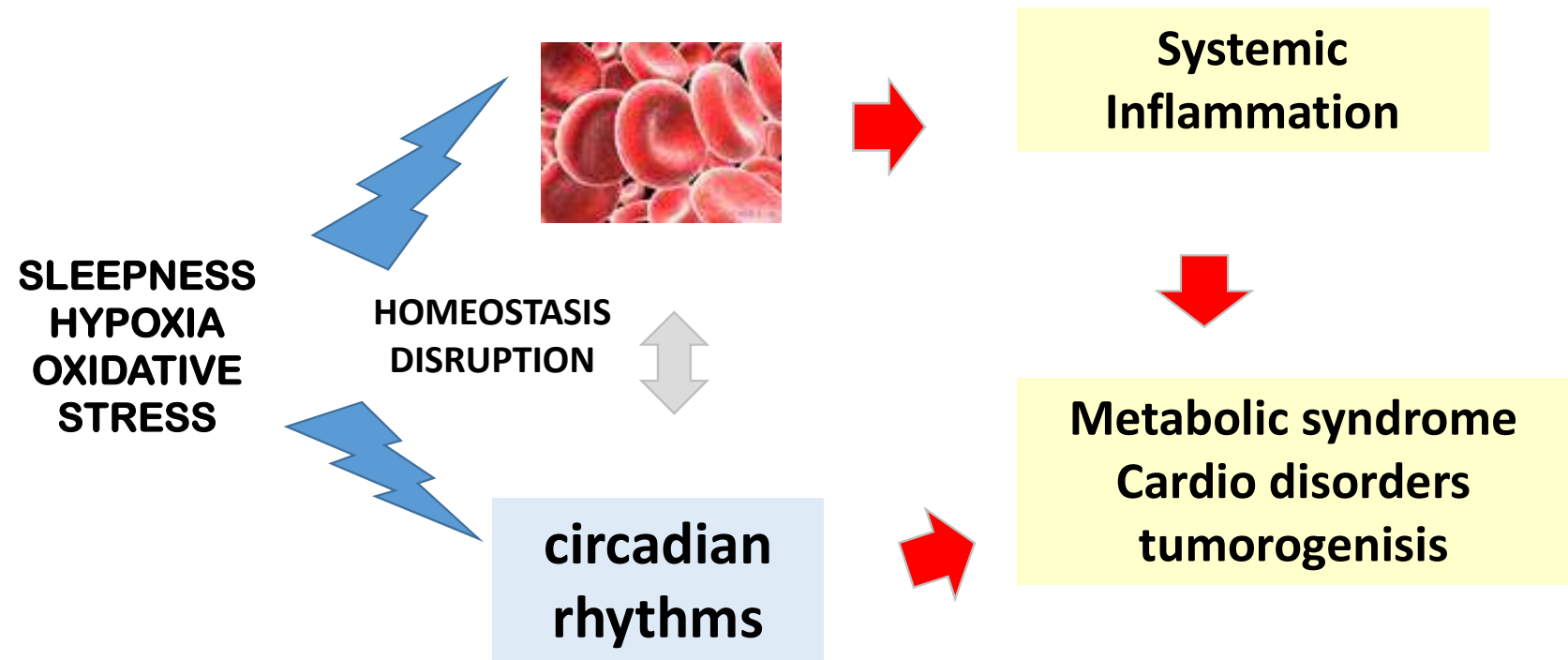
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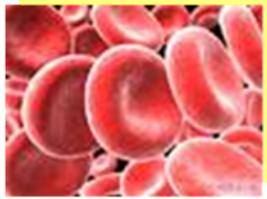
Project funded by Portugal-Havard Medical School Program

SAMPLE to investigate OSA Proteome

Red blood cells



Red Blood Cells



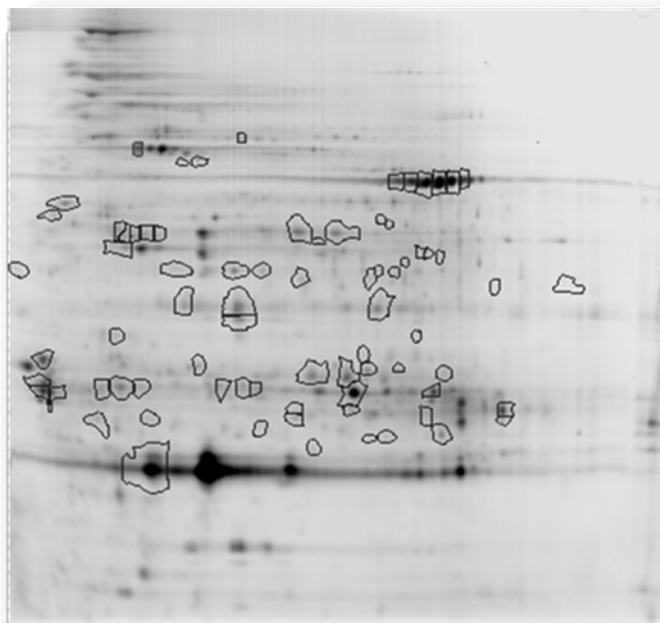
n=104

OSA *vrs* Snorers > EVENING x MORNING

(n=24 patients)

Severe OSA

12.5% SDS-PAGE
pH 3-10



RBC Hemoglobin-depleted
cytoplasmic fraction
2DIGE

76 spots identified differentially
abundant (Anova $p \leq 0.05$)



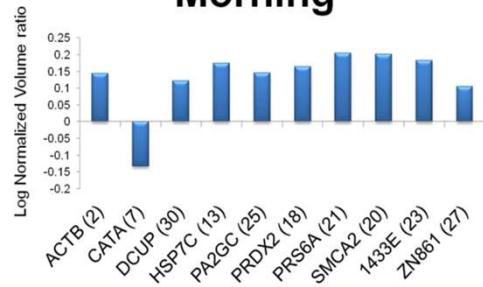
30 spots identified by
MALDITOF, corresponding
21 different proteins



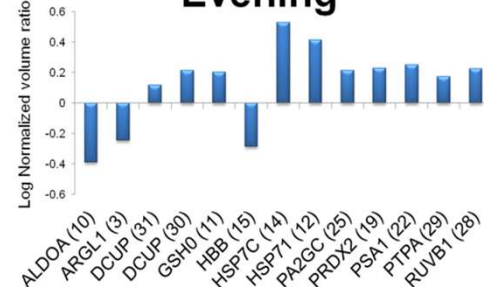
**Existence of Post-translational
Modifications**

OSA x Snorers

Morning

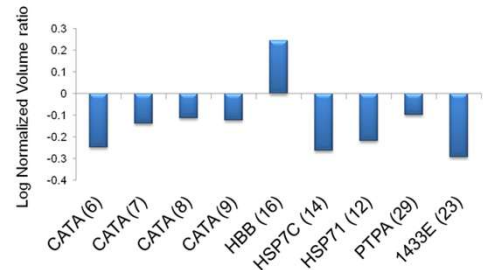


Evening

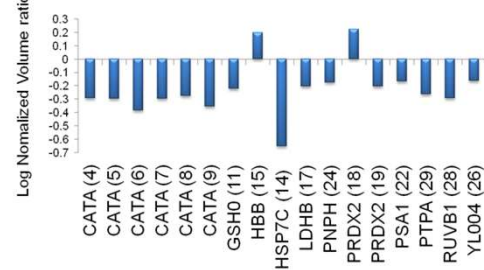


Morning x Evening

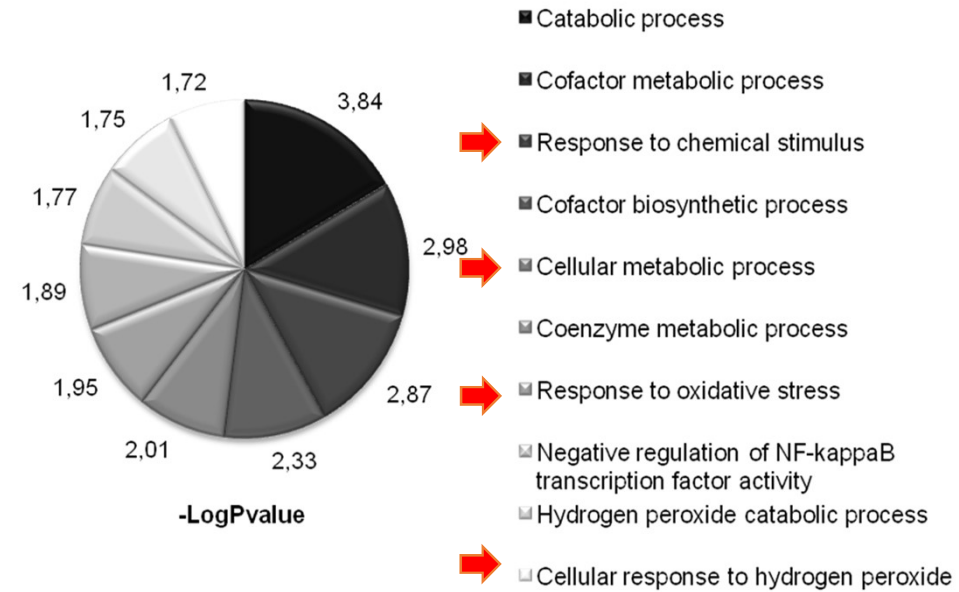
Snorers



OSA

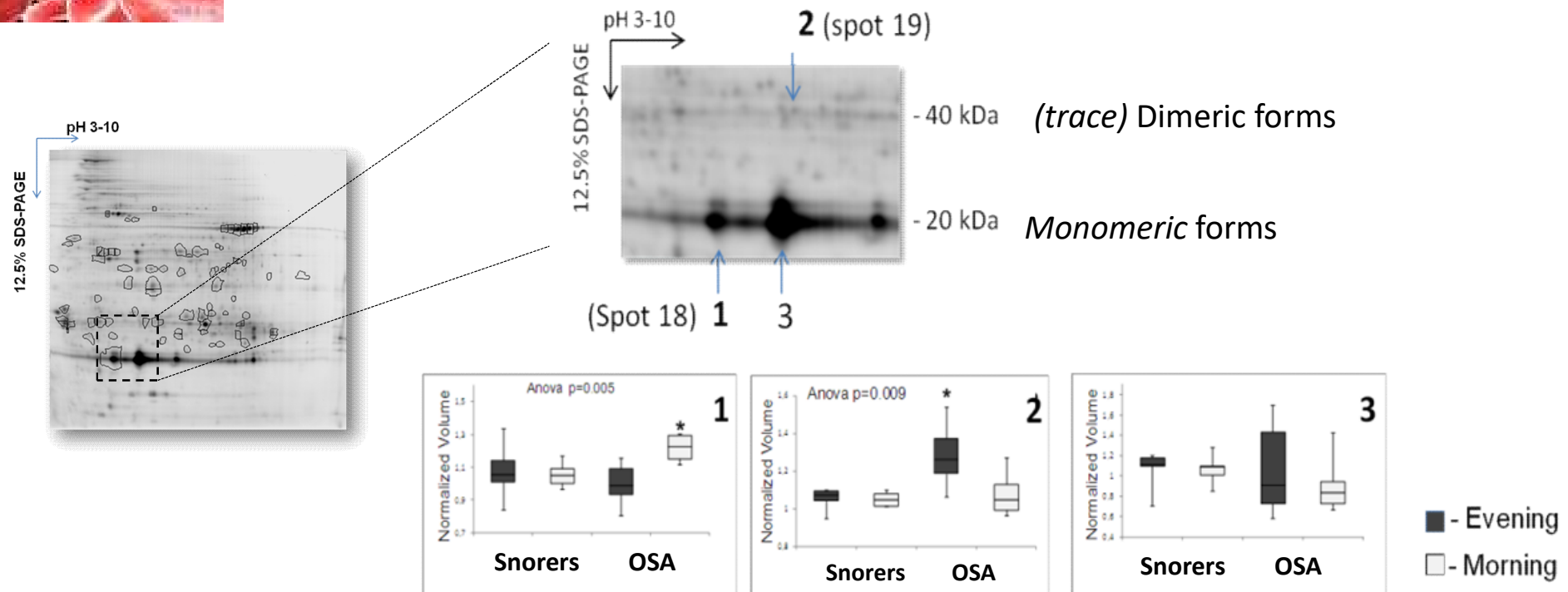


Biological Process





PEROXIREDOXIN-2 PROTEOFORMS



Acidic monomeric (1) and dimeric (2) PRDX2 reported as **oxi/overoxidized forms** were significantly **increased** in **OSA RBC** at **Morning** or **Evening** daytime, respectively.

Peroxiredoxin 2 (PRDX2)

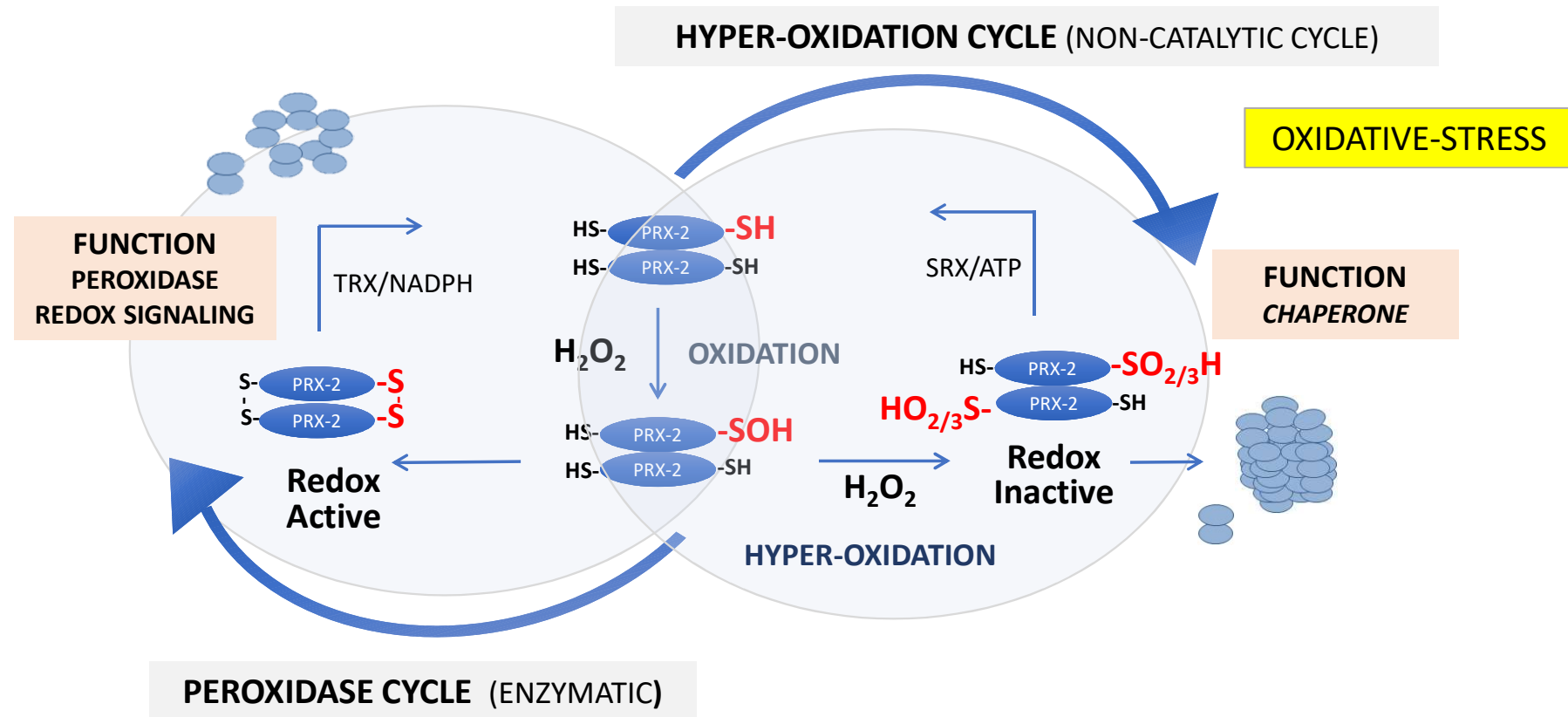
3rd most abundant protein in RBCs

Belongs to the thiol class peroxidases with 2-cysteines (Prdx2-Cys)

Associated with antioxidant defense

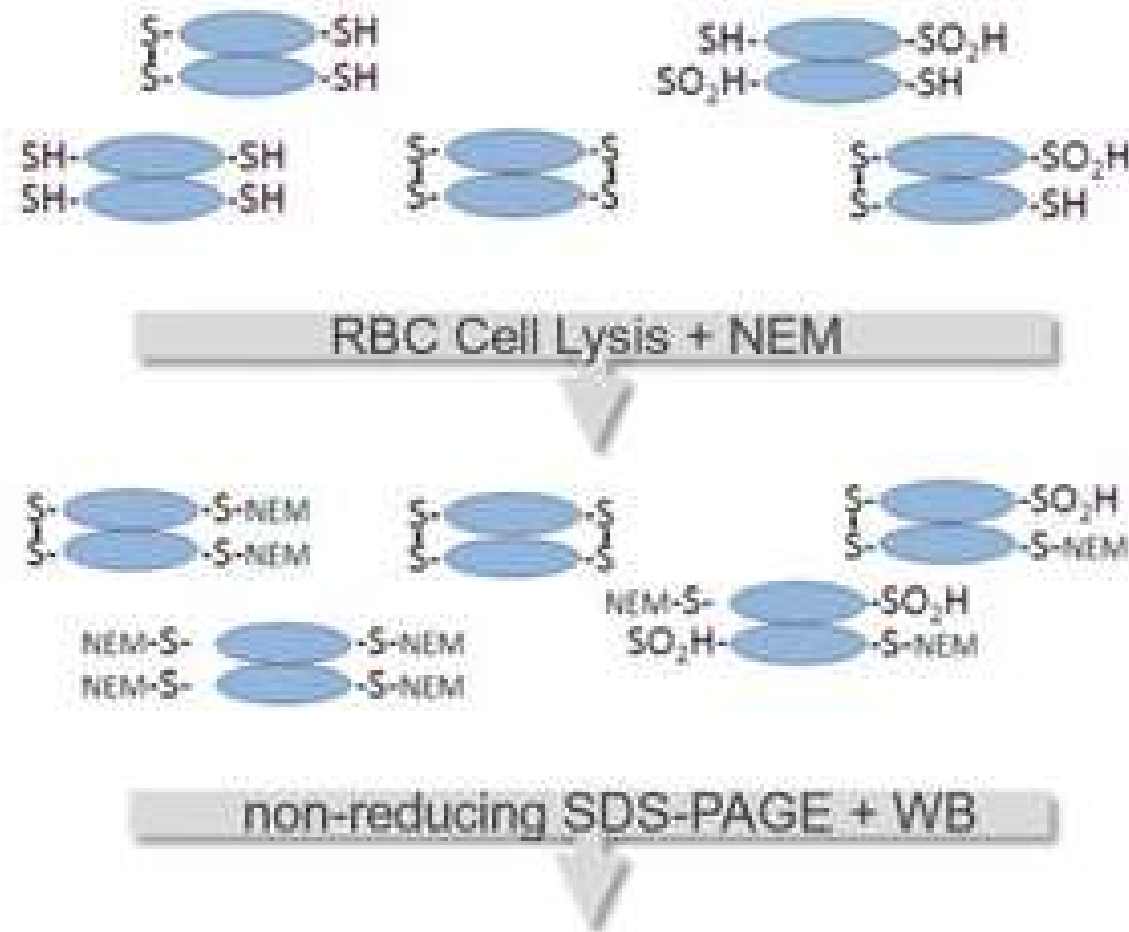
- Degradation of H_2O_2 in water
- Reduction of organic peroxides and peroxynitrite
- Protection RBCs from oxidative stress
- Signalling
- Chaperone Activity
- Role in the physiology of the circadian cycle

PEROXIREDOXIN-2: REDOX/OLIGOMERIC STATES & FUNCTIONS



peroxidation, resolution, recycling

Verification Phase



N-ethylmaleimide (NEM) (alkylating reagent) was added to the samples to prevent exogenous-induced oxidation

Verification Phase

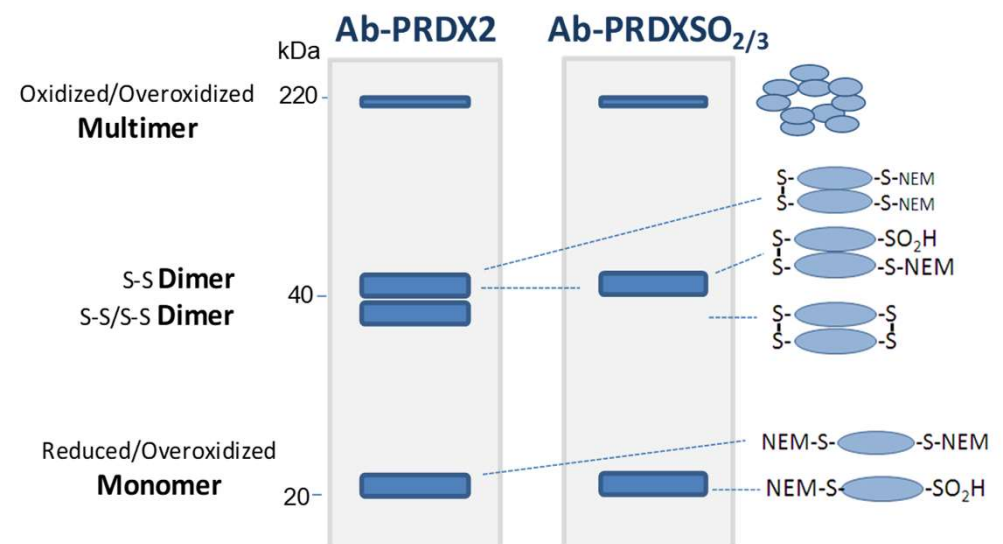
(50 samples ≠, including **PAP after 6 months**)
n= 20 Patients



mild/moderate/severe OSA before/after PAP

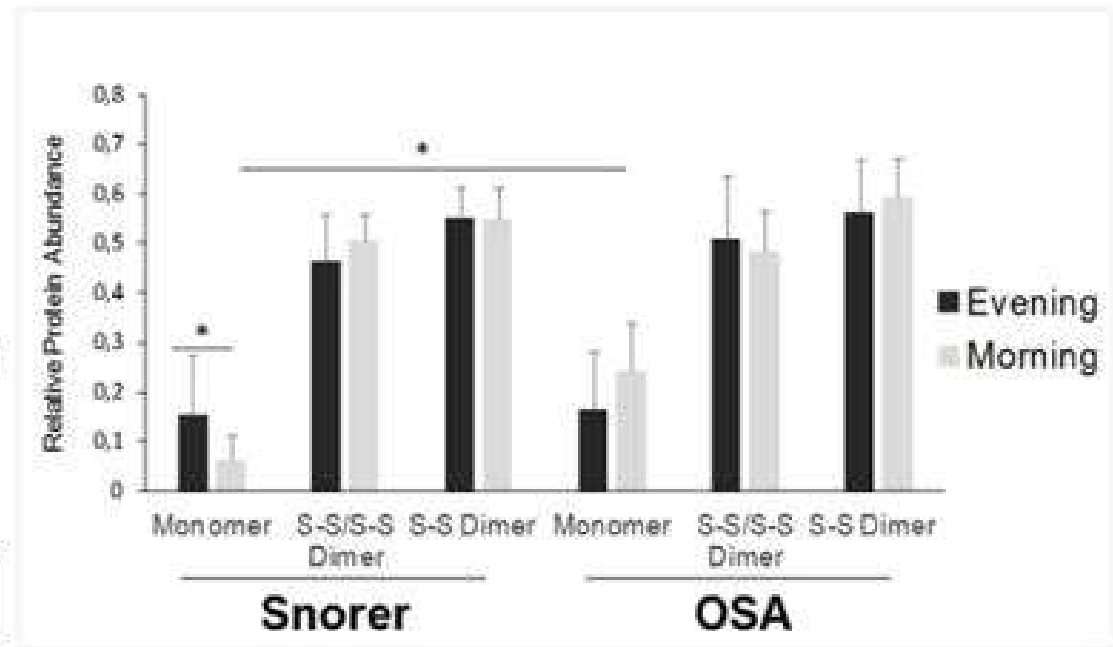
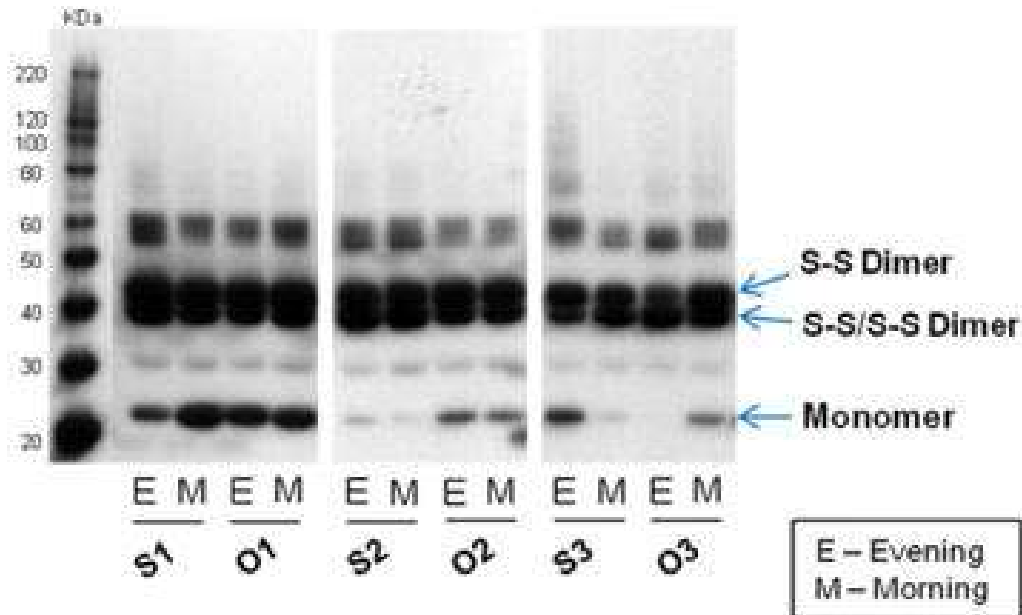
Western Blot NON-REDUCING (no DTT)

S-S cross-links are kept

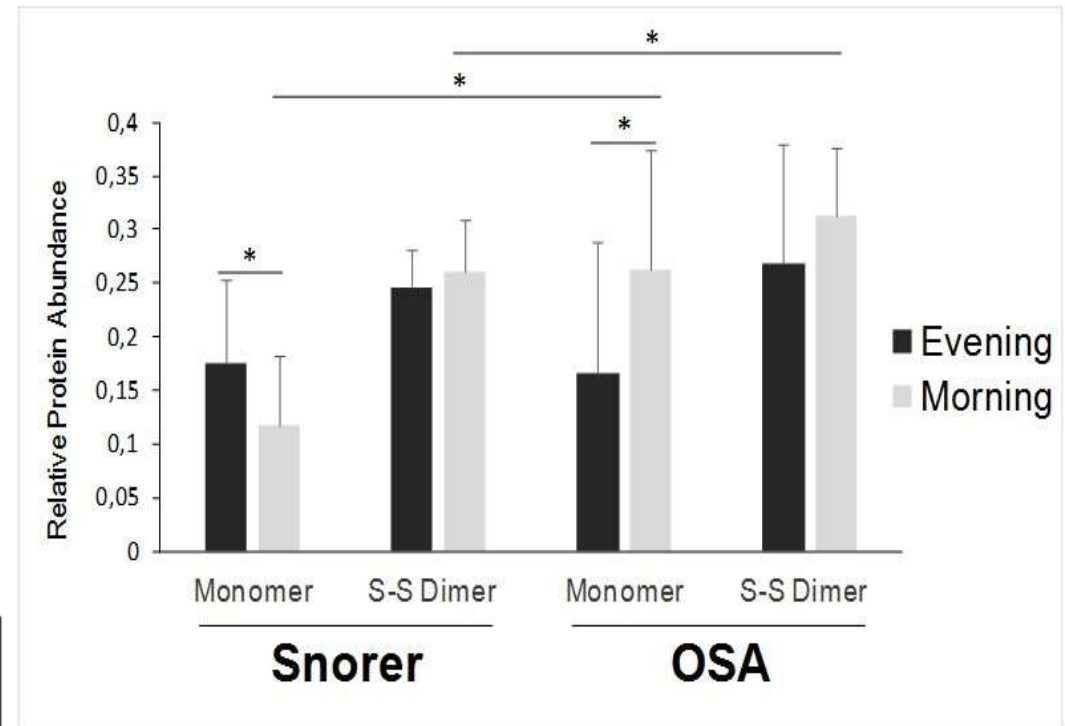
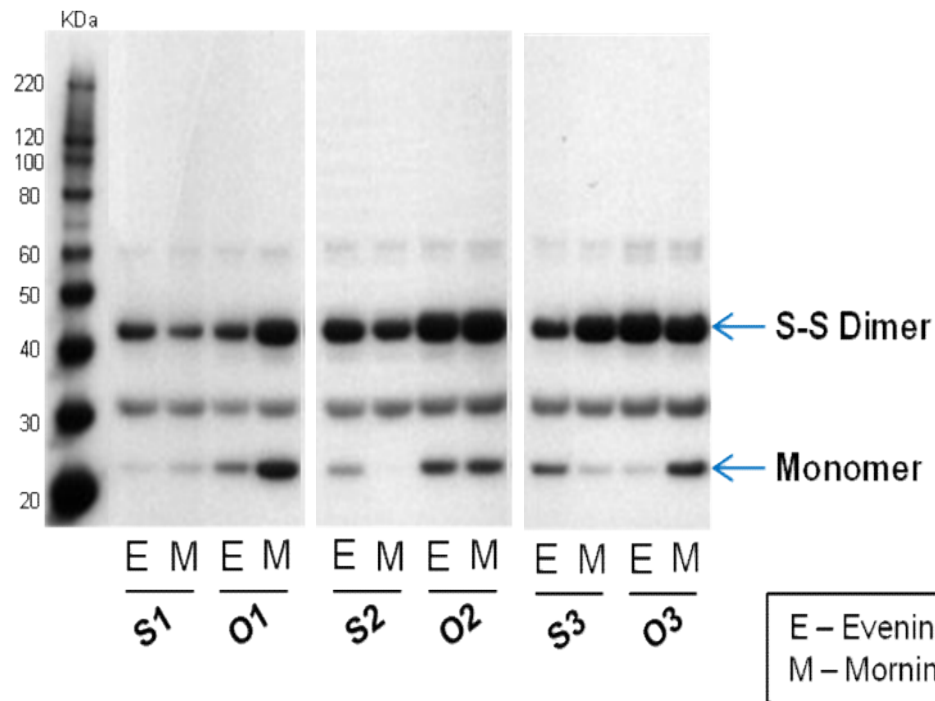


A

Ab PRDX2

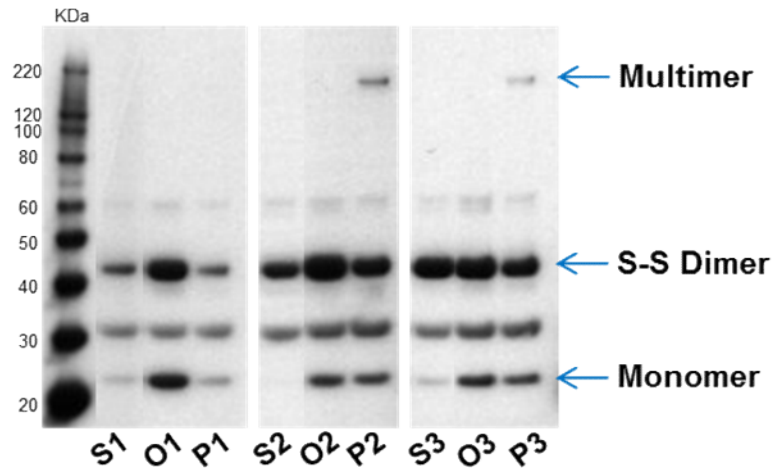


Ab PRDXSO_{2/3}



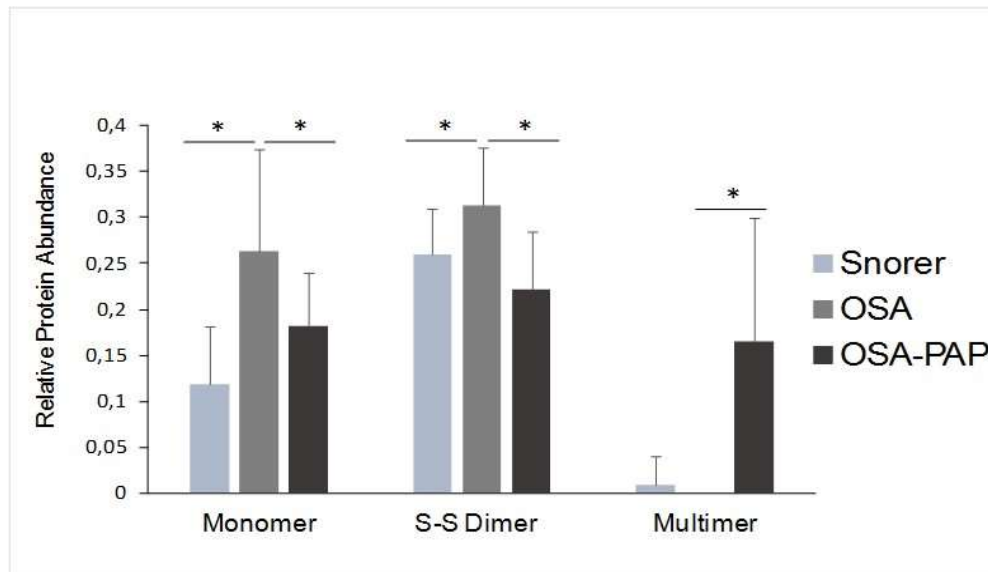
RBC PRDX-2 OVEROXIDATION IS HIGHER IN OSA

PAP TREATMENT EFFECT ON RBC PRDX-2



Ab PRDXSO_{2/3}

Only morning samples



**DECREASED MONOMER/DIMER OXIDATION
&
INCREASED OXIDIZED MULTIMERS > CHAPERONE FUNCTION**

Correlation of redox/oligomeric state of PRDX2 to PSG and metabolic variables under study.

PRDX2	Evening			Morning		
	Correlate	Pearson r value	p value	Correlate	Pearson r value	p value
S-S/S-S dimer	RDI	0.633 [*]	0.049	No significant correlation observed		
	RDI	0.633 [*]	0.049			
	ODI	0.657 [*]	0.039			
	T90%	0.727 [*]	0.017			
SO ₂ monomer	RDI	-0.399	0.081	PSG-arousal index	0.482 [*]	0.031
	RDI	-0.399	0.081			
	ODI	-0.474 [*]	0.035			
	Diurnal oximetry	-0.823 ^{**}	0.000			
S-S/SO ₂ dimer	Diurnal oximetry	-0.467 [*]	0.038	Min O ₂ saturation	-0.586 ^{**}	0.007
				Hb A1C	0.452 [*]	0.045
				Insulin	0.448 [*]	0.048
				HOMA-IR	0.448 [*]	0.048

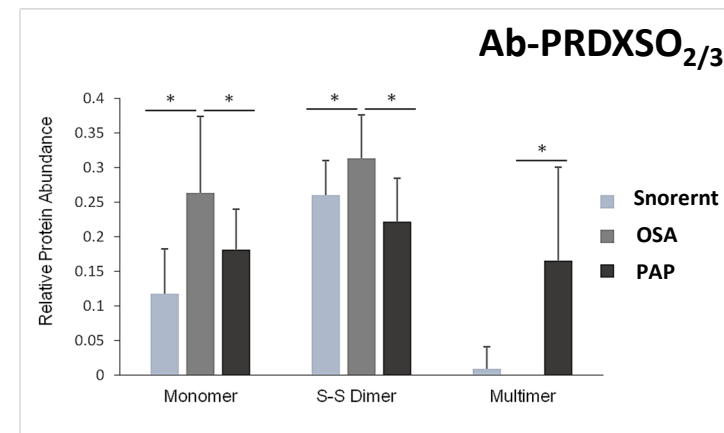
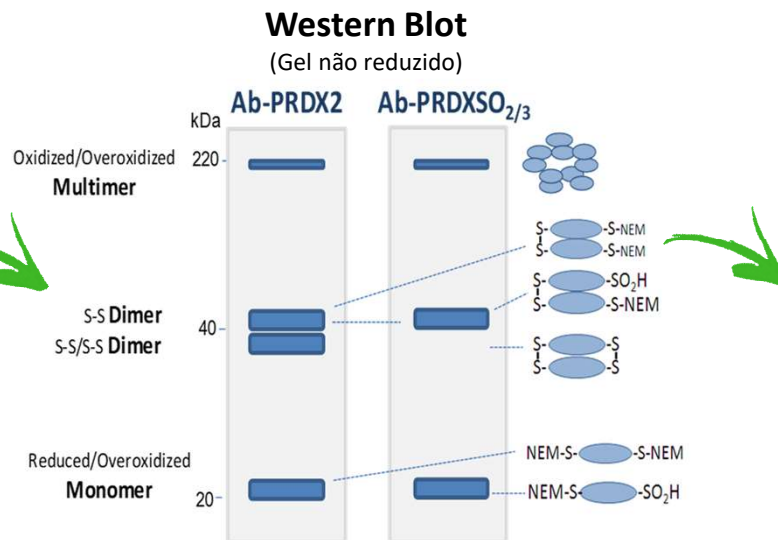
RDI, respiratory disturbance index; ODI, oxygen desaturation index; T90%, the time spent with saturation < 90%; PSG, polysomnography; Min O₂ saturation, minimum oxygen saturation HbA1C, glycated hemoglobin; HOMA-IR, homeostatic model.

^{**} Correlation is significant at the 0.01 level (2-tailed).

^{*} Correlation is significant at the 0.05 level (2-tailed).

Verification Phase

N= 20 Patients
(50 samples \neq , including PAP
after 6 months)



PRDXSO _{2/3}	Morning	Evening
Monomer SO _{2/3}	-	RDI (-0,399)
Dimer SO _{2/3}	Hb A1C (0,452) Insulin (0,448)	-



Data Article

Evening and morning alterations in Ob
Sleep Apnea red blood cell proteome

Amélia Feliciano^{a,b,1}, Fátima Vaz^{b,c,1},
Cristina Valentim-Coelho^b, Vukosava M. Torres^{b,c},
Vesna Prosinecki^b, Bruno M. Alexandre^b, Andreia
Catarina Almeida-Marques^b, Ana S. Carvalho^d,
Rune Matthiesen^d, Atul Malhotra^e, Paula Pinto^{a,f},
Cristina Bárbara^{a,f}, Deborah Penque^{b,c,g}

PRDX2 – Candidate Biomarker for OSA

- OSA Severity
- PAP Treatment Monitoring

Hematological evaluation in males with obstructive
sleep apnea before and after positive airway pressure

A. Feliciano^{a,b,*,}, R. Linhas^c, R. Marçôa^c, A. Cysneiros^a, C. Martinho^d, R.P. Reis^{a,b},
D. Penque^f, P. Pinto^{b,h,i}, C. Bárbara^{d,h,i}



with

P. Reis^{b,d}, D. Penque^g,



Proteomics of OSA RBCs after PAP treatment

DATA NOT PUBLISHED

Study Workflow

Biobank



Snorers (control)

Severe OSA

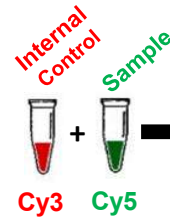
PAP (after 6 months)

Depletion
+
Desalting

Red Blood Cells

N=30

(morning samples)

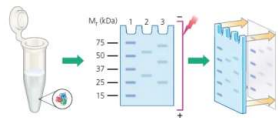


Snorers

OSA

PAP

2D-DIGE Mini Gel



Validation

(Western Blot)

New Cohort N= 59

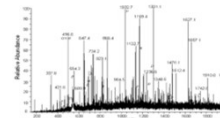
Snorers (Control)

OSA (Mild/ Moderate/ Severe)

PAP (after 6 months/>4hours/night)



**Pathway
Analysis**



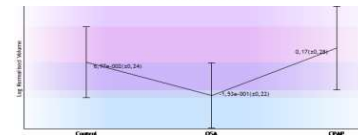
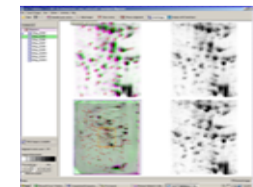
MALDI-TOF/TOF



Peptides

Mass Spectrometry

Progenesis SameSpot v.4.5



**Image
Analysis**

Conclusion

- OSA induces alterations in the redox/oligomeric state of GAPDH as in PRDX2 that can be reverted/modulated by PAP treatment.
- PAP-induced PRDXSO_{2/3} multimeric forms with chaperone protective function were positively associated with GAPDHSO₃ tetramers, which may indicate latter's involvement in cell protection.
- The clinical significant of all these findings needs further validation and investigation.



Further Work

PTMs of GAPDH and PRDX2 oligoforms are under investigation

RBC & Plasma proteome in OSA by SWATH-MS are also in progress

Multidisciplinary Team

Laboratório de Proteómica (INSA-Ricardo Jorge)

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