



# Blood Oxidative Stress Modulates Alveolar Bone Loss in Chronically Stressed Rats

Micaele Maria Lopes Castro <sup>1</sup>, Priscila Cunha Nascimento <sup>1</sup>, Deiweson Souza-Monteiro <sup>1</sup>, Sávio Monteiro Santos <sup>2</sup>, Mayra Barros Arouck <sup>3</sup>, Vinicius Barreto Garcia <sup>4,5</sup>, Raimundo Fernandes de Araújo, Jr. <sup>4,5,6,7</sup>, Aurigena Antunes de Araujo <sup>2</sup>, Gabriela de Souza Balbinot <sup>8</sup>, Fabrício Mezzomo Collares <sup>8</sup>, Cassiano Kuchenbecker Rosing <sup>9</sup>, Marta Chagas Monteiro <sup>2</sup>, Cristiane Socorro Ferraz Maia <sup>3</sup> and Rafael Rodrigues Lima <sup>1,\*</sup>

<sup>1</sup> Laboratory of Functional and Structural Biology, Institute of Biological Sciences, Federal University of Pará, 66075-900 Belem, PA, Brazil; micaelecastro@hotmail.com (M.M.L.C.); priscilacunha.n28@gmail.com (P.C.N.); deiwesonmonteiro@gmail.com (D.S.-M.)

<sup>2</sup> Laboratory of in vitro Assays, Immunology and Microbiology, Faculty of Pharmacy, Institute of Biological Sciences, Federal University of Pará, 66075-900 Belém, PA, Brazil; saviomontsan@gmail.com (S.M.S.); martachagas2@yahoo.com.br (M.C.M.); auriprinino@gmail.com (A.A.d.A.)

<sup>3</sup> Laboratory of Pharmacology of Inflammation and Behavior, Faculty of Pharmacy, Institute of Health Science, Federal University of Pará, 66075-900 Belém, PA, Brazil; mayraarouckbarros@gmail.com (M.B.A.); crismaia@ufpa.br (C.d.S.F.M.)

<sup>4</sup> Cancer and Inflammation Research Laboratory, Department of Morphology, Federal University of Rio Grande do Norte 59078-970 Natal, RN, Brazil; araujojr@cb.ufrn.br (V.B.G.); araujojr.morfologia@gmail.com (R.F.d.A., Jr.)

<sup>5</sup> Post-Graduation Programme in Health Science, Federal University of Rio Grande do Norte, Natal, RN 59072-970, Brazil

<sup>6</sup> Post-Graduation Programme in Structural and Functional Biology, Federal University of Rio Grande do Norte, Natal, RN 59072-970, Brazil; (araujojr.morfologia@gmail.com)

<sup>7</sup> Department Biophysics and Pharmacology, Post-Graduation Programme in Public Health, Post-Graduation Programme in Pharmaceutical Science, Federal University of Rio Grande do Norte, Natal, RN 59072-970, Brazil

<sup>8</sup> Dental Materials Laboratory, Faculty of Dentistry, Federal University of Rio Grande do Sul, 90040-060 Porto Alegre, Brazil; gabi\_balbinot@hotmail.com (G.d.S.B.); fabricio.collares@ufrgs.br (F.M.C.)

<sup>9</sup> Department of Periodontology, Faculty of Dentistry, Federal University of Rio Grande do Sul, 90040-060 Porto Alegre, Brazil; ckrosing@hotmail.com (C.K.R.)

\* Correspondence: rafalima@ufpa.br or rafaelrodrigueslima@hotmail.com; Tel.: +55 91 3201 7891

This Supplementary Material contains 1 table.



**Table S1.** Quantification data for all analysis carried out presented in mean ( $\pm$ standard error), n= 7 per group.

Measures	Control Group	Experimental Periodontitis	Chronic Stress	Experimental Periodontitis + Chronic Stress	p-value
Rearings (n)	2.5 $\pm$ 0.37 <sup>a</sup>	3 $\pm$ 0.51 <sup>a</sup>	6.35 $\pm$ 0.75 <sup>b</sup>	7.5 $\pm$ 1.44 <sup>b</sup>	
Total time of grooming (s)	36.25 $\pm$ 2.5 <sup>a</sup>	44.8 $\pm$ 1.2 <sup>a</sup>	63.8 $\pm$ 5.46 <sup>b</sup>	69.25 $\pm$ 10.45 <sup>b</sup>	
Open arms entries (%)	47.5 $\pm$ 5.83 <sup>a</sup>	36.55 $\pm$ 6.48 <sup>a</sup>	8.33 $\pm$ 8.33 <sup>b</sup>	4.16 $\pm$ 4.16 <sup>b</sup>	
Time in open arms (%)	6.66 $\pm$ 1.87 <sup>a</sup>	5.6 $\pm$ 1.37 <sup>a</sup>	1.2 $\pm$ 0.79 <sup>b</sup>	0.96 $\pm$ 0.56 <sup>b</sup>	
Alveolar bone-loss area (mm <sup>2</sup> )	1.72 $\pm$ 0.01 <sup>a</sup>	2.53 $\pm$ 0.1 <sup>b</sup>	2.24 $\pm$ 0.1 <sup>b</sup>	3.12 $\pm$ 0.09 <sup>c</sup>	
Distance between the CEJ and ABC (mm)	0.86 $\pm$ 0.01 <sup>a</sup>	1.08 $\pm$ 0.02 <sup>b</sup>	1.04 $\pm$ 0.04 <sup>b</sup>	1.22 $\pm$ 0.03 <sup>c</sup>	
Tb.Th (mm)	0.1 $\pm$ 0.01 <sup>a</sup>	0.11 $\pm$ 0.01 <sup>ab</sup>	0.14 $\pm$ 0.01 <sup>b</sup>	0.19 $\pm$ 0.01 <sup>c</sup>	
Tb.Sp (mm)	0.13 $\pm$ 0.01 <sup>a</sup>	0.21 $\pm$ 0.01 <sup>bc</sup>	0.18 $\pm$ 0.02 <sup>ac</sup>	0.3 $\pm$ 0.02 <sup>d</sup>	
Tb.N (mm)	0.23 $\pm$ 0.01 <sup>a</sup>	0.33 $\pm$ 0.01 <sup>b</sup>	0.32 $\pm$ 0.02 <sup>b</sup>	0.49 $\pm$ 0.02 <sup>c</sup>	
BV/TV (%)	0.39 $\pm$ 0.01 <sup>a</sup>	0.26 $\pm$ 0.02 <sup>b</sup>	0.26 $\pm$ 0.03 <sup>b</sup>	0.2 $\pm$ 0.01 <sup>b</sup>	
IHC score RANK	0 (0-0.25) <sup>a*</sup>	2 (1.75-2.25) <sup>b*</sup>	2 (2-3) <sup>b*</sup>	2 (1.75-2) <sup>b*</sup>	<0.05
IHC score RANKL	0 (0-0) <sup>a*</sup>	0 (0-0.25) <sup>a*</sup>	1 (0-1) <sup>a*</sup>	1 (1-1.25) <sup>a*</sup>	
GSH levels (% of control)	100 $\pm$ 1.95 <sup>a</sup>	79.6 $\pm$ 1.62 <sup>b</sup>	70.13 $\pm$ 2.38 <sup>b</sup>	58.4 $\pm$ 5.37 <sup>c</sup>	
NO concentration (% of control)	100 $\pm$ 7.53 <sup>a</sup>	243 $\pm$ 22.5 <sup>b</sup>	245.7 $\pm$ 25.54 <sup>b</sup>	335 $\pm$ 19.04 <sup>c</sup>	
TBARS levels (% of control)	100 $\pm$ 3.03 <sup>a</sup>	132.7 $\pm$ 3.14 <sup>b</sup>	161.5 $\pm$ 6.13 <sup>c</sup>	180.4 $\pm$ 2.85 <sup>d</sup>	

Similar overwritten letters did not show significant statistical differences (One-way ANOVA and Tukey's post-hoc test); CEJ: cemento-enamel junction; ABC: alveolar bone crest; IHC score: immunohistochemistry score; GSH: glutathione; NO: nitric oxide; TBARS: thiobarbituric acid reactive substance levels; \*results expressed as median and interquartile range (Kruskal-Wallis test).