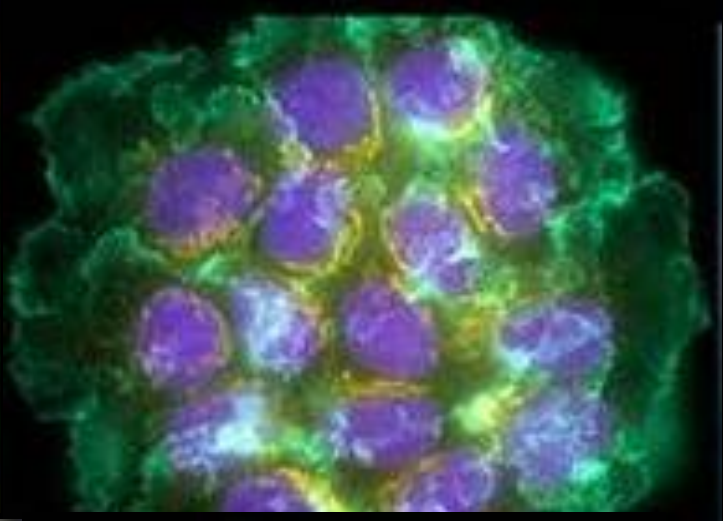
# V Congreso de la Rama de Especies Reactivas del Oxígeno en Biología y Medicina de la SOCIEDAD MEXICANA DE BIOQUÍMICA

**18-21 marzo, 2015. Hacienda San José Vista Hermosa, Morelos.**

# PROGRAMA



**V MEETING OF THE FREE RADICALS AND OXIDATIVE STRESS BRANCH OF THE MEXICAN BIOCHEMICAL SOCIETY**

**March 18-21, 2015**
**Hacienda San José Vista Hermosa, Puente de Ixtla, Morelos**

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## VI INTERNATIONAL WORKSHOP ON COMPARATIVE ASPECTS OF OXIDATIVE STRESS IN BIOLOGICAL SYSTEMS

P R O G R A M

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| **WEDNESDAY, MARCH 18TH, 2015** | |
| 15:00-17:30 | Registration desk open |
| 17:30-18:00 | Opening remarks |
| 18:00-19:00 | **Inaugural Lecture**<br>"Control of breast cancer cell bioenergetics by nitric oxide"<br>**Dr. Neil. Hogg.** Key-Note Speaker<br>Medical College of Wisconsin. Milwaukee, WI.<br>President Society of Free Radicals in Biology and Medicine SFRBM |
| 19:00-20:00 | Ice-breaker |
| 20:00-22:00 | Dinner |
| **THURSDAY, MARCH 19TH, 2015** | |
| 07:00-08:45 | Workshop: Early Morning graduate course – day one<br>"¿Qué es un radical libre?"<br>**Dr. Mina Königsberg**<br>Universidad Autónoma Metropolitana-Iztapalapa. México |
| 08:00-13:00 | Registration desk open |
| 08:45-10:00 | Breakfast |
| | **Topic: Free radicals in Medicine** |
| 10:00-11:00 | **Alejandro Silva-Palacios**<br>tBHQ/Nrf2 confers neuroprotection to old rats against an oxidant treatment with 3-nitropropionic acid.<br>Universidad Autónoma Metropolitana, Iztapalapa<br><br>**Adriana Guadalupe Pérez-Ruiz**<br>Effect antiproliferative of (-)-epicatechin and its relationship with reactive oxygen species in breast cancer cell.<br>Escuela Superior de Medicina - IPN<br><br>**Laura Guerrero-Medrano**<br>Undesaired toxicity of cyclophosphamide associated with increased oxidative stress in cancer-free organs.<br>Bioquímica, CINVESTAV - IPN |



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| 11:00-12:00 | Invited Plenary Lecture<br>**Dr. Francisco Laurindo Martins**<br>"Protein disulfide isomerases: novel roles in redox signaling and homeostasis"<br>Instituto del Corazón, Universidad de Sao Paulo, Brazil |
| 12:00-12:15 | Break |
| | **Symposium:**<br>**Free radicals and antioxidants in microorganisms** |
| 12:15-12:45 | **Dr. Mario Pedraza Reyes**<br>"Consequences of faithful or error-prone processing of ROS-promoted DNA damage in *Bacillus subtilis.*"<br>University of Guanajuato, Guanajuato, México |
| 12:45-13:15 | **Dr. Alejandro de las Peñas**<br>"Oxidative stress response, adherence and resistance to azoles in the opportunistic fungal pathogen *Candida glabrata*."<br>Instituto Potosino de Investigación Científica y Tecnológica, San Luis Potosí, México |
| 13:15-13:45 | **Dr. Javier Barrios González**<br>"In search for the environmental stimuli that induce higher lovastatin production in solid-state fermentation: ROS regulate the pathway genes"<br>Universidad Autónoma Metropolitana-Iztapalapa. México |
| 13:45-16:00 | Lunch |
| 16:00-17:00 | **Fabiola Jaimes-Miranda**<br>The role of Sim35 during aging and oxidative stress in the yeast *Saccharomyces cerevisiae.*<br>Instituto de Fisiología Celular, UNAM<br><br>**Ailed Pérez-Sánchez**<br>Modification of the ROS profile in lovastatin solid-state fermentation by genetic means or by environmental manipulation.<br>Universidad Autónoma Metropolitana, Iztapalapa<br><br>**Rocío del Carmen Pérez-Aguilar**<br>Dynamics of the intracellular $H_2O_2$ levels in the root of *Arabidopsis thaliana* in response to a specific NADH inhibitor.<br>Instituto de Biotecnología, UNAM |
| 17:00-19:00 | **Poster viewing and discussion 1** |

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| 19:00-20:00 | Business meeting of the Free Radicals and Oxidative Stress Branch of the Mexican Biochemical Society |
| 20:00-22:00 | Dinner |
| FRIDAY, MARCH 20TH, 2015 | |
| 07:00-08:45 | Workshop: Early Morning graduate course – day two<br>"El rol del proteosoma en la eliminación de proteínas oxidadas."<br>**Dr. Claudio Torres**<br>Pathology and Laboratory Medicine Department. Drexel University, Philadelphia, USA. |
| 08:45-10:00 | Breakfast |
| | **Topic: Free radicals in plants and mitochondria** |
| 10:00-11:00 | **Gerardo Rangel-Sánchez**<br>Characterization of the effects of chromium on the production of reactive oxygen species and hormone response in *Arabidopsis thaliana.*<br>IIQB, Universidad Michoacana de San Nicolás Hidalgo<br><br>**Joel Herrera-Martínez**<br>A gene regulatory network underlying ROS patterns in *Arabidopsis* roots.<br>Instituto de Ecología, UNAM<br><br>**María Alejandra Sánchez-Muñoz**<br>The formation of supercomplexes is preserved by *Moringa oleifera* hydroalcoholic extract in liver during early diabetes.<br>Facultad de Ciencias Químicas, Universidad Juárez del Estado de Durango |
| 11:00-12:00 | Invited Plenary Lecture<br>**Dr. José Antonio Enríquez Domínguez**<br>"Mitochondrial supercomplexes and ROS"<br>Centro Nacional de Investigaciones Cardiovasculares Carlos III<br>Madrid, España |
| 12:00-12:15 | Break |
| | **Symposium: Free radicals in cell biology** |
| 12:15-12:45 | **Dr. Emilio Rojas**<br>"Antioxidant response regulation by MicroRNAs contributes to cellular transformation"<br>Departamento de Medicina Genómica y Toxicología Ambiental<br>Instituto de Investigaciones Biomédicas, UNAM. México |

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| 12:45-13:15 | **Dra. Emma Saavedra**<br>"Metabolic modeling of the antioxidant pathway in *Trypanosoma cruzi*: in search of therapeutic targets".<br>Instituto Nacional de Cardiología Ignacio Chávez, México |
| 13:15-13:45 | **Dr. Armando Luna López**<br>"Hormetic response mechanisms that prevent oxidative damage during aging"<br>Instituto Nacional de Geriatría, México |
| 13:45-16:00 | Lunch |
| 16:00-18:00 | **Poster viewing and discussion 2** |
| 18:00-19:00 | Invited Plenary Lecture<br>**Dr. Luis Cárdenas Torres**<br>"Dynamic of reactive oxygen species in root hair cells and pollen tubes are essential for polar growth"<br>Instituto de Biotecnología, UNAM. México |
| 19:00-21:00 | Dinner |
| 21:00 | Cultural event – to be announced |
| SATURDAY, MARCH 21TH, 2015 | |
| 07:00-08:45 | Workshop: Early Morning graduate course – day three<br>"Aspectos metabólicos de la respuesta antioxidante"<br>**Dr. Emma Saavedra**<br>Instituto Nacional de Cardiología, Ignacio Chávez. México |
| 08:45-10:00 | Breakfast |
| | **Topic: Free radicals and cell signaling** |
| 10:00-11:00 | Invited Plenary Lecture<br>**Dr. Claudio Torres**<br>"Senescent astrocytes and neurodegenerative disease"<br>Pathology and Laboratory Medicine Department<br>Drexel University, Philadelphia, USA. |
| 11:00-12:00 | **Denise Clavijo-Cornejo**<br>HGF induce the reactive oxygen species production via NADPH oxidase in primary mouse hepatocytes<br>Universidad Autónoma Metropolitana, Iztapalapa |

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| | **Marco Antonio Zaragoza-Campillo**<br>Role of reactive oxygen species in the regulation of a MAP3K during the cell death of neurons.<br>Instituto de Fisiología Celular. UNAM<br><br>**Enrique Salas-Vidal**<br>Reactive oxygen species dynamics in developing zebrafish embryos.<br>Instituto de Biotecnología, UNAM |
| 12:00:12:15 | Break |
| | **Symposium: Oxidative stress and degenerative diseases** |
| 12:15-12:45 | **Dr. Abel Santamaria del Ángel**<br>"Antioxidant and neuroprotective properties of S-alilcysteine, an aged-garlic compound"<br>Instituto Nacional de Neurología y Neurocirugía – SSA. México |
| 12:45-13:15 | **Dr. Ivonne Olivares-Corichi**<br>"Dysfunction of insulin by oxidative stress in diabetic and obese patients"<br>Escuela Superior de Medicina, IPN. México |
| 13:15-13:45 | **Dr. Guillermo Ceballos**<br>"Study of the flavanol (-)-epicatechin effects on oxidative markers in aged mice"<br>Escuela Superior de Medicina, IPN. México |
| 13:45-14:00 | Closing remarks |
| 14:00-16:00 | Lunch |

*Organizing Committee*

*Dr. Luis Cárdenas Torres*
*Instituto de Biotecnología, UNAM*

*Dr. Mina Königsberg Fainstein*
Universidad Autónoma Metropolitana-Iztapalapa

*Dr. Luis Enrique Gómez Quiroz*
Universidad Autónoma Metropolitana-Iztapalapa

*Dr. Ana Cecilia Zazueta Mendizábal*
Instituto Nacional de Cardiología, Ignacio Chávez

**POSTER SESSION 1**
**THURSDAY 17:00 – 19:00**

**ESTRÉS OXIDANTE EN LA DIFERENCIACIÓN Y EL DESARROLLO**

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| **1.** | EFFECT OF OMEGA 3 FATTY ACIDS IN LIPID COMPOSITION AND LIPOPEROXIDATION OF PLACENTAL MITOCHONDRIA FROM DIABETIC RATS. **FIGUEROA-GARCÍA MC**, CORTES-ROJO C, SAAVEDRA-MOLINA A, PALOMAR-M M AND MEJÍA-ZEPEDA R. UNIVERSIDAD MICHOACANA |
| **2.** | CADMIUM: ANTIOXIDANT ACTIVITY OF SOD AND CAT ON THE REPRODUCTIVE SYSTEM IN PERIPUBERAL MALE RATS. **HERNÁNDEZ-RODRÍGUEZ J**, LÓPEZ-LÓPEZ AL, ARENAS-RÍOS E, LEÓN-GALVÁN M, DAMIÁN-MATZUMURA P, VIGUERAS-VILLASEÑOR RM, BONILLA-JAIME H, ARTEAGA-SILVA M. UAM – IZTAPALAPA |
| **3.** | NADPH OXIDASE ACTIVITY IS REQUIRED FOR EPIBOLY CELL MIGRATION IN ZEBRAFISH EMBRYOS. **FRANCISCO JAVIER MÉNDEZ-CRUZ**, MARIO ADÁN MENDIETA-SERRANO, LUIS CÁRDENAS, HILDA LOMELÍ AND ENRIQUE SALAS-VIDAL. INSTITUTO DE BIOTECNOLOGÍA, UNAM |

**MÉTODOS Y MODELOS PARA EL ESTUDIO DE LAS ESPECIES REACTIVAS**

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| **4.** | MEASUREMENT OF THE OXIDATIVE STRESS INDEX IN CRONICALLY EXPOSED TO VOLATILE ORGANIC COMPOUNDS (VOC) PEOPLE AND ITS RELATIONSHIP WITH METABOLIC POLYMORPHISMS. **LÓPEZ VARGAS MR** & MONTERO MONTOYA R. INSTITUTO DE INVESTIGACIONES BIOMEDICAS, UNAM |
| **5.** | PURIFICATION AND CHARACTERIZATION OF TAENIA CRASSICEPS CYSTICERCI (CESTODA) THIOREDOXIN: INSIGHT INTO THIOREDOXINGLUTATHIONE-REDUCTASE (TGR) SUBSTRATE RECOGNITION. **MARTÍNEZ-GONZÁLEZ JJ**, GUEVARA-FLORES A, RENDÓN JL & DEL ARENAL IP. FACULTAD DE MEDICINA, UNAM |

**ESTRÉS OXIDANTE EN MICROORGANISMOS**

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| **6.** | PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF DEAMINATED- DNA REPAIR PATHWAYS IN *BACILLUS SUBTILIS*. **AYALA-GARCÍA VM** AND PEDRAZA-REYES M. UNIVERSITY OF GUANAJUATO |
| **7.** | IDENTIFICATION AND SILENCING GENES *SRRA* AND *MSNA* IN A LOVASTATIN HIGH-PRODUCING STRAIN OF *ASPERGILLUS TERREUS.* **BIBIÁN LEÓN ME**, BARRIOS GONZÁLEZ J. UNIVERSIDAD AUTÓNOMA METROPOLITANA UNIDAD IZTAPALAPA |
| **8.** | CELULAR RESPONSE OF *YARROWIA LIPOLYTICA* TO OXIDATIVE STRESS CONDITIONS. **DESENTIS DESENTIS MF**, HUERTA OROS J, JIMÉNEZ SALAS Z, CAMPOS GÓNGORA E. UNIVERSIDAD AUTÓNOMA DE NUEVO LEÓN |

**ESTRÉS OXIDANTE EN LA MUERTE CELULAR Y LA ENFERMEDAD**

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| **9.** | EFFECT OF AQUEOUS EXTRACT FROM *SPIRULINA MAXIMA* ON INDUCED OXIDATIVE STRESS IN MICE SPERM. **AGUILAR-GONZÁLEZ LJ**, MOJICA-VILLEGAS MA, CHAMORRO-CEVALLOS GA, SÁNCHEZ-GUTIÉRREZ M, IZQUIERDO-VEGA JA. NATIONAL SCHOOL OF BIOLOGICAL SCIENCES, NATIONAL POLYTECHNIC INSTITUTE |

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| **10.** | EFFECT OF CURCUMIN ON MITOCHONDRIAL FUNCTION IN THE EXPERIMENTAL RENAL INSUFFICIENCY. **APARICIO-TREJO OE**, TAPIA E, MEDINA-CAMPOS ON, PEDRAZA-CHAVERRI J. DEPARTMENT OF BIOLOGY, FACULTY OF CHEMISTRY, UNAM |
| **11.** | HYPOXIA INTERFERES WITH RESPONSE TO 2-METHOXYESTRADIOL TREATMENT IN HUMAN NON-SMALL LUNG CANCER CELLS. **AQUINO-GÁLVEZ A**, GONZÁLEZ-ÁVILA G, GUTIÉRREZ-GONZÁLEZ LH, DELGADO TELLO J, CASTILLEJOS-LÓPEZ M, MENDOZA-MILLA C, CHECA M, TRINIDAD LÓPEZ A, TEJAS C, ZÚÑIGA J, CISNEROS J, TORRES-ESPÍNDOLA LM, HERNÁNDEZ-JIMÉNEZ C, GARCÍA DEL VALLE A. NATIONAL INSTITUTE OF RESPIRATORY DISEASES " ISMAEL COSÍO VILLEGAS |
| **12.** | AGED GARLIC EXTRACT INCREASES NEUROPEPTIDE Y, SUPEROXIDE DISMUTASE, CATALASE AND GLUTATHIONE PEROXIDASE MRNA LEVELS IN HYPOTHALAMUS OF DIABETIC RATS. **BARRAGÁN-BONILLA MI**, AGUILERA P, ESPINOZA-ROJO M. UNIDAD ACADÉMICA DE CIENCIAS QUÍMICO BIOLÓGICAS. UNIVERSIDAD AUTÓNOMA DE GUERRERO |
| **13.** | EFECTO PROTECTOR DE LA S-ALILCISTEÍNA (SAC) EN UN MODELO DE ESTRÉS POR INMOVILIDAD EN RATAS. **BECERRIL-CHÁVEZ H**, COLÍN-GONZÁLEZ AL, SANTAMARÍA A. LABORATORIO DE AMINOÁCIDOS EXCITADORES, INSTITUTO NACIONAL DE NEUROLOGÍA Y NEUROCIRUGÍA |
| **14.** | PORCIN PEPTIDES ATTENUATE 3-NITROPROPIONIC ACID- INDUCED BRAIN DAMAGE IN YOUNG RATS. **CALDERÓN GUZMÁN D**, OSNAYA BRIZUELA N, ORTÍZ HERRERA M, HERNÁNDEZ GARCÍA E, BARRAGÁN MEJÍA G, JUÁREZ OLGUÍN H, VALENZUELA PERAZA A, LABRA RUÍZ NA. LABORATORY OF NEUROCHEMISTRY. NATIONAL INSTITUTE OF PEDIATRICS |
| **15.** | THE EFFECT OF PROTEIN SUPPLEMENTATION, THE EXERCISE OF RESISTANCE AND ITS RELATIONSHIP WITH THE OXIDATIVE STRESS IN OLDER ADULTS WITH SARCOPENIA. **CALDERÓN GUERRERO GZ**, POLANCO FIERRO JA, GUTIÉRREZ LÓPEZ L, GARCÍA SÁNCHEZ JR, OLIVARES CORICHI IM. SEPI-ESM-IPN |
| **16.** | EFFECT OF THE OBESITY IN PRESCHOOL AGE ABOUT REACTIVE SPECIES OXYGEN AND ANTIOXIDANT SYSTEMS, IN A PEDIATRIC HOSPITAL OF THE SECRETARIA DE SALUD DEL DISTRITO FEDERAL, MEXICO. **CARMONA-MONTESINOS E**, VELÁZQUEZ PÉREZ R, GOROSTIETA SALAS E, PICHARDO-AGUIRRE E, PONCE-HINOJOSA G, HERNÁNDEZ-ZIMBRÓN L, RIVAS-ARANCIBIA S. FACULTAD DE MEDICINA, UNAM |
| **17.** | ANTIPROLIFERATIVE AND NEUROTOXIC EFFECT FROM EXTRACTS OF SOURSOP (*ANNONA MURICATA*) ON ASTROCITOMA CELLS (U87-MG). **CHUZEVILLE-MUNGUÍA C**, VÁZQUEZ-LUNA A, ÁLVAREZ-SÁNCHEZ E, MORALES-MONTOR J, VILLALPANDO-AGUILAR JL, MORENO-LEÓN G, FERNÁNDEZ-SÁNCHEZ J, AND DÍAZ-SOBAC R. INSTITUTO DE CIENCIAS BÁSICAS. UNIVERSIDAD VERACRUZANA. |
| **18.** | EFFECT OF AGED GARLIC EXTRACT ON THE EXPRESSION OF GLUT1 AND GLUT3 MRNA IN BRAIN OF DIABETIC RATS. **DE LA CRUZ-CONCEPCIÓN B**, BARRERA-NAVARRETE P, BARRAGÁN-BONILLA MI, ESPINOZA-ROJO M. UNIDAD ACADÉMICA DE CIENCIAS QUÍMICO BIOLÓGICAS. UNIVERSIDAD AUTÓNOMA DE GUERRERO |
| **19.** | ROLE OF REACTIVE OXYGEN SPECIES AND NOX IN THE CELL DEATH AND MORPHOLOGY OF CULTURED CEREBELLAR ASTROCYTES. **DOMÍNGUEZ G**, OLGUÍN M AND MORÁN J. INSTITUTO DE FISIOLOGÍA CELULAR, UNAM |
| **20.** | HIGH CHOLESTEROL DIET INDUCES MITOCHONDRIAL INJURY, OXIDATIVE STRESS, AND LIVER DAMAGE. **DOMÍNGUEZ-PÉREZ M**, NUÑO LÁMBARRI N, ROSAS-LEMUS M, RODRÍGUEZ-OCHOA JI, MIRANDA RU, URIBE-CARVAJAL S, SOUZA-ARROYO V, BUCIO-ORTÍZ L, GÓMEZ-QUIROZ LE, GUTIÉRREZ-RUIZ MC. CIENCIAS DE LA SALUD, UAM IZTAPALAPA |

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| **21.** | SUPPLEMENTATION WITH L-ARGININE PROVIDES PROTECTION AGAINST DNA DAMAGE CAUSED BY OXIDATIVE STRESS IN CULTURES OF HUMAN VASCULAR ENDOTHELIAL CELLS. **AURORA ESPEJEL-NUÑEZ**, FLORES-PLIEGO ARTURO, MANJARREZ-JANTES K, SOLIS-PAREDES JM, PARRA-HERNANDEZ S, GUADALUPE ESTRADA-GUTIERREZ. DEPARTMENT OF IMMUNOBIOCHEMISTRY, INSTITUTO NACIONAL DE PERINATOLOGIA |
| **22.** | SUBCELLULAR LOCALIZATION OF NRF2 IN THE DIABETIC RAT RETINA. **ALBERT-GARAY JS**, SÁNCHEZ-CHÁVEZ G, SALCEDA-SACANELLES R. INSTITUTO DE FISIOLOGÍA CELULAR, UNAM |
| **23.** | CURCUMIN PRETREATMENT AVOIDS CR(VI)-INDUCED HEPATOTOXICITY. **GARCÍA-NIÑO WR**, ZATARAIN-BARRÓN ZL, VEGA-GARCÍA CC, HERNÁNDEZ-PANDO R, TAPIA E, ZAZUETA C, PEDRAZA-CHAVERRI J. FACULTY OF CHEMISTRY, UNAM |
| **24.** | COCOA INTAKE INCREASES PHYSICAL FITNESS IN ATHLETES AND REDUCES MUSCLE DAMAGE AND OXIDATIVE. **GONZÁLEZ GARRIDO JA**, GARCÍA SÁNCHEZ JR, GUTIÉRREZ SALGADO DY, GARRIDO LLANOS S, PÉREZ RUIZ AG, OLIVARES CORICHI IM. ESCUELA SUPERIOR DE MEDICINA, IPN |
| **25.** | EFFECT OF A SPORTS MEDICAL PROGRAM OF FORCE ON OXIDATIVE STRESS IN HEALTHY ELDERLY AND SARCOPENIA. **GUDIÑO CASTRO L**, MEJÍA MUÑOZ E, MARTÍNEZ ARELLANES LY, GUTIÉRREZ LÓPEZ L, GARCÍA SÁNCHEZ JR, OLIVARES CORICHI IM. SEPI-ESM-IPN |

**ESTRÉS OXIDANTE EN PLANTAS**

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| **26.** | HYDROTROPIC RESPONSE DEFICIENCY IN THE *AHR1* MUTANT AFFECTS REACTIVE OXYGEN SPECIES REGULATION IN ROOTS OF *ARABIDOPSIS THALIANA*. **CASTILLO-OLAMENDI LG**, CASSAB G AND PORTA H. INSTITUTO DE BIOTECNOLOGÍA, UNAM |
| **27.** | REGULATION OF AUTOPHAGY BY ROS DURING HYDROTROPIC RESPONSE OF *ARABIDOPSIS THALIANA.* **JIMÉNEZ NOPALA GE,** CEVALLOS PORTA D, CASSAB LOPEZ G, PORTA DUCOING H. INSTITUTO DE BIOTECNOLOGÍA, UNAM |

**ESPECIES REACTIVAS Y SEÑALIZACIÓN**

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| **28.** | SENESCENCE-ASSOCIATED SECRETORY PHENOTYPE (SASP) FROM STRESS-INDUCED PREMATURE SENESCENCE (SIPS) PRIMARY LUNG MICE FIBROBLASTS INDUCES POTENTIAL CHANGES IN CELLULAR SENESCENCE, PROLIFERATION, AND CELL MIGRATION IN CELL LINE L929. **AQUINO CRUZ ANGÉLICA ALEJANDRA**, MACIEL BARÓN LUIS ÁNGEL, MINA KÖNIGSBERG, NORMA EDITH LÓPEZ-DÍAZ GUERRERO, ARMANDO LUNA LÓPEZ. CIENCIAS BIOLÓGICAS Y DE LA SALUD. UAM IZTAPALAPA |
| **29.** | CHRONIC OBESITY CONDITION INCREASE THE INCIDENCE OF MTDNA OXIDATION. **CANTÚ VALDEZ JA**, RIVERA-ÁLVAREZ I, AND GARCÍA N. INSTITUTO DE CARDIOLOGÍA Y MEDICINA VASCULAR TECNOLÓGICO |
| **30.** | HGF/C-MET DECREASES NADPH OXIDASE ACTIVITY BY A MECHANISM MEDIATED BY THE PROTEASOME 26S. **SIMONI-NIEVES ARTURO**, CLAVIJO-CORNEJO DENISE, SALAS-SILVA SORAYA, PALESTINO-DOMÍNGUEZ M, BUCIO L, SOUZA V, MIRANDA RU, GUTIÉRREZ-RUIZ MC, GÓMEZ-QUIROZ LE. CIENCIAS BIOLÓGICAS Y DE LA SALUD. UAM IZTAPALAPA |

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| **31.** | REDOX AND ERK SIGNALING EFFECT ON NRF2 PATHWAY ACTIVATION IN POSTCONDITIONED HEARTS. **GUEVARA-CHÁVEZ JG**, BUELNA-CHONTAL M AND ZAZUETA C. NATIONAL INSTITUTE OF CARDIOLOGY "IGNACIO CHÁVEZ" |
| **32.** | GLUTATHIONE (GSH) AND L-CYSTEINE, CONCENTRATIONS EFFECT ON EQUINE SPERM STORED AT 4°C. **LÓPEZ-TRINIDAD BP**, RETANA F, RODRÍGUEZ-TOBÓN A, LEÓN-GALVÁN MA, MARTÍNEZ AJ Y ARENAS-RÍOS E. CIENCIAS BIOLÓGICAS Y DE LA SALUD. UAM IZTAPALAPA |
| **33.** | REACTIVE OXYGEN SPECIES FROM A RAC1 INDEPENDENT NADPH OXIDASE REGULATE THE MOTILITY AND CAPACITATION OF *CAVIA PORCELLUS* SPERMATOZOA. **ORTIZ-GARCÍA CI**, ROA-ESPITIA AL, HERNÁNDEZ-GONZÁLEZ ENRIQUE O. CINVESTAV – IPN ZACATENCO |

**TÓPICOS EMERGENTES EN EL CAMPO DE LAS ESPECIES REACTIVAS**

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| **34.** | OXIDATIVE STRESS DUE CADMIUM SUBLETHAL EXPOSURE IN THE AXOLOTL (*AMBYSTOMA MEXICANUM*). **GARCÍA ÁVILA AG**, ROSAS PERÉZ I, MIRANDA MARTÍN DEL CAMPO J, MORTON BERMEA O, ZÚÑIGA LAGUNES S AND VANEGAS PÉREZ C. POSGRADUATE PROGRAM IN MARINE SCIENCES AND LIMNOLOGY. NATIONAL AUTONOMOUS UNIVERSITY OF MEXICO |
| **35.** | BIOMARKERS OF EXPOSURE AND OXIDATVE STRESS IN THE CRAYFISH *CAMBARELLUS MONCTEZUMAE IN SITU* EXPOSED TO METALS**. HERNÁNDEZ CE**, ZÚÑIGA SR, ROSAS I, CRAM S, PONCE DE LEÓN C, HERNÁNDEZ M, FERNÁNDEZ P, MORTON O AND VANEGAS RC. FACULTY SCIENCES. NATIONAL AUTONOMOUS UNIVERSITY OF MEXICO |

**POSTER SESSION 2**
**FRIDAY 16:00 – 18:00**

**ESTRÉS OXIDANTE EN LA DIFERENCIACIÓN Y EL DESARROLLO**

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| **36.** | IMMUNO AND CONFOCAL MICROSCOPY LOCALIZATION ANALYSIS OF GLUTATHIONE PEROXIDASE 4 INDICATES DIVERSE FUNCTIONS IN EARLY ZEBRAFISH DEVELOPMENT. **MENDIETA-SERRANO MA**, SCHNABEL-PERAZA D, LOMELÍ H, AND SALAS-VIDAL E. INSTITUTO DE BIOTECNOLOGÍA, UNAM |
| **37.** | AN *IN VITRO* MODEL TO STUDY NEURONAL SENESCENCE**. MORENO-BLAS D**, MUCIÑO-HERNÁNDEZ G, GERÓNIMO-OLVERA C, MASSIEU-TRIGO L, KÖNIGSBERG FM & CASTRO-OBREGÓN S. INSTITUTO DE FISIOLOGÍA CELULAR, UNAM |
| **38.** | INSULIN ACTIVATES GROWTH AND $H_2O_2$ LEVELS IN ROOT HAIRS OF ARABIDOPSIS. **PASCUAL MORALES EJ**, MELLADO ROJAS ME, CÁRDENAS TORRES L, REYES DE LA CRUZ H, GARCÍA PINEDA E, BELTRÁN PEÑA E. IIQB, UNIVERSIDAD MICHOACANA DE SAN NICOLÁS DE HIDALGO |

**MÉTODOS Y MODELOS PARA EL ESTUDIO DE LAS ESPECIES REACTIVAS**

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# CONTROL OF BREAST CANCER CELL BIOENERGETICS BY NITRIC OXIDE

**Dr. Neil. Hogg.** Key-Note Speaker
Medical College of Wisconsin. Milwaukee, WI.
President Society of Free Radicals in Biology and Medicine SFRBM

Inducible Nitric Oxide Synthase (iNOS) has been associated with increased mortality in estrogen aggressive breast cancer sub-types. In contrast, others are examining if iNOS induction can be targeted to breast cancer as a therapy.

Clearly there is a need to better understand the role of NO in cancer biology. How nitric oxide, generated from this enzyme alters cancer cell aggressiveness is currently poorly established. One of the hallmarks of cancer is an alteration in cellular bioenergetics, the so-called 'Warburg effect', where cancer cells appear to derive ATP from glycolysis even in the presence of adequate oxygen to drive oxidative phosphorylation. It has been observed that NO can alter cellular metabolism in a similar way, resulting in an inhibition of oxidative phosphorylation and a stimulation of glycolysis. It has our working hypothesis that NO contributes to the bioenergetic phenotype of cancer cells by creating a more permissive environment for proliferative and aggressive oncogenic mutations. Using bioenergetic measurements we have shown that overexpression of iNOS in MCF-7 cells alters their bioenergetic phenotype to a more glycolytic one, and results in increased invasiveness in vitro and in vivo. However, using a lentiviral approach, we have transfected iNOS in a dose dependent manner and show that the effect of NO on cancer cell metabolism is biphasic. At low NO production rate, a stimulation of mitochondrial function is observed that is likely due to increased mitochondrial biogenesis.

However at higher levels of NO the switch to glycolysis and away from oxidative phosphorylation is observed. This suggests that changes in NO production rate, due to local inflammation for example, could alter tumor progression. In conclusion, NO can alter cancer cell bioenergetics to a more glycolytic phenotype, and this is associated with increased invasiveness. The personalized therapeutic targeting of iNOS may be part of a combinatorial therapeutic strategy.

Regresar al programa

## *Protein Disulfide Isomerase: novel roles in redox signaling and homeostasis*

Francisco RM Laurindo - Heart Institute (Incor), University of São Paulo School of Medicine

Working models of redox-dependent signaling involve subcellular compartmentation and enzymatically controlled reactive species generation. In this context, thiol proteins may potentially act as redox signaling adaptor proteins, adjusting reactive intermediate generation to specific signals, and redox signals to cell homeostasis. Our work has focused on protein disulfide isomerase(PDI), a thioredoxin superfamily oxidoreductase from endoplasmic reticulum (ER). PDI is ubiquitous, abundantly expressed and is the founding member of a family with >20 members with increasingly described synergic and complementary actions. Such PDIs may have oxidoreductase, isomerase and chaperone effects, the latter not directly dependent on redox thiols. PDI main function is to act as a converging hub of several pathways promoting disulfide bond introduction into ER-processed proteins, while delaying ER processing of some proteins (e.g., fibrillin-1) in a holdase-like fashion. However, PDI may also display redox effects not strictly related to its ER function. Emerging information from our laboratory suggests a convergence between PDI and Nox family NADPH oxidases, the main enzymatic complex dedicated to signaling reactive species generation. PDI silencing prevents Nox1 responses to angiotensin-II and platelet-derived growth factor (PDGF) vascular cells and Nox2-related parasite phagocytosis in macrophages. PDI overexpression spontaneously enhances Nox activation and expression. In neutrophils, PDI redox-dependently associates with p47phox and supports the respiratory burst. Recent work indicates that PDI-mediated support of Nox NADPH oxidases may reflect a convergence between PDI and RhoGTPases. Silencing PDI promotes a robust loss of Rac1 and RhoA activation responses to PDGF, an effect associated with cytoskeletal dysruption and nearly complete loss of directional migration. The activation of Nox1 by PDGF involves sequential steps of Rac1 convergence with RhoGDI at membranes and a later association with PDI in an undisclosed intracellular location. In vitro, PDI associates with Rac1 with high affinity, as shown by biophysical techniques. Recently, there has been much attention to the cell surface / secreted pool of PDI due to its involvement in thrombosis, cell adhesion, immune functions and virus internalization. At the cell surface, PDI exerts trans- nitrosation, thiol reductase and apparent isomerase activities towards targets including adhesion and matrix proteins and proteases. The route of PDI externalization remains elusive and shows an apparently highly regulated feedback. We showed that cell surface /secreted PDI is highly expressed after vascular injury and mediates an anti-constrictive remodeling effect in vessels via matrix and cytoskeletal organization. Integrin beta-1 is a target of PDI in such effect, in a way that cell-surface PDI may behave as a mechanoadaptation transducer. Together, such multiple redox effects of PDI (and likely of its other family members) may render these proteins as putative redox cell signaling adaptors, with conspicuous roles in physiology and pathophysiology. (Research supported by FAPESP – Individual projects and CEPID *Redoxoma* and CNPq – INCT *Redoxoma*)

Regresar al programa

# CONSEQUENCES OF FAITHFUL OR ERROR-PRONE PROCESSING OF ROS-PROMOTED DNA DAMAGE IN *BACILLUS SUBTILIS*.

Pedraza Reyes M*.

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The metabolic conditions that prevail during bacterial growth favor the correct operation of repair systems to properly eliminate DNA lesions inflicted by intracellular and exogenous agents, including reactive oxygen species (ROS) and solar light.

The low rate of spontaneous mutations ($10^{-9}$) occurring in replicating cells strongly support the concept that repair systems faithfully reestablish the original DNA sequence after eliminating genetic insults. In contrast, when growth and/or replication ceases bacteria frequently process DNA lesions in an error-prone manner. These processes provide cells with tools to escape from stressful conditions and depending on the developmental context in which such processes occur different physiological scenarios can be anticipated.

In nutritionally stressed bacteria different components of the base excision repair machinery (BER) may process damaged DNA bases in an error-prone manner promoting genetic variability. Interestingly, suppression of the mismatch repair machinery and activation of specific DNA glycosylases promote stationary-phase mutations.

Current evidence also support the concept that in resting cells, coupling of repair processes to actively transcribed genes (TCR) may promote multiple genetic transactions that are advantageous for stressed cells.

These non-canonical ways of DNA repair contributing to mutagenesis, survival and evolution in the Gram-positive bacterium *Bacillus subtilis* will be discussed in this talk.

Work supported by CONACyT (Grant. 221231) and University of Guanajuato (Grant: DAIP-2014).

Regresar al programa

# OXIDATIVE STRESS RESPONSE, ADHERENCE AND RESISTANCE TO AZOLES IN THE OPPORTUNISTIC FUNGAL PATHOGEN *Candida glabrata*.

De Las Peñas, A., Juárez, J., Briones, M., Orta, E., Gutiérrez, G., Cañas, I., Alvarado, G., and Castaño, I. Instituto Potosino de Investigación Científica y Tecnológica División de Biología Molecular Camino a la Presa San Jose 2055 San Luis Potosí, SLP 78216 México cano@ipicyt.edu.mx

*Candida glabrata*, an opportunistic fungal pathogen, is capable of invasive infections in inmunocompromised individuals. *C. glabrata* accounts for 18-26% of all *Candida* systemic infections. *C. glabrata* is capable to respond to environmental stimuli, thus adapting to the ever changing environment within the host. Important virulence traits of *C. glabrata* have been described: a) *C. glabrata* is capable of adhering to epithelial cells mediated GPI-anchored cell wall proteins encoded in the *EPA* gene family, b) it is extremely resistant to oxidative stress and this resistance is mediated by the catalase (*CTA1*), glutathione (*GSH1* and *GSH2*), the superoxide dismutases (*SOD1* and *SOD2*) and regulated by the concerted action of the transcription factors Yap1, Skn7, Msn2 and Msn4, and c) it is naturally more resistant to azoles and this resistance is mediated by the transcription factor Pdr1 and the ABC transporters Cdr1 and Cdr2. Adherence to host cells, repsonse to oxidate stress, and resistance to xenobiotics, are important traits for a succefull infection. We have shown based on genetic and biochemical analysis that *C. glabrata* poses regulatory networks that control the expression of different genes required to respond to these stresses. We have shown that the sirtuin Hst1 (a $NAD^+$ - dependent histone deacetylase) negatively controls the expression of *PDR1* and *CDR1* (resistance to fluconazole), the expression of the transcriptional activator Msn4 and the *CTA1* gene (resistance to oxidative stress) and the expression of *EPA6* (adherence). In addition, oxidative stress induces the expression of *EPA2* whereas Pdr1 control the expression of *GSH1* (oxidative stress response). In summary, our findings propose an interesting relation between adherence, resistance to oxidative stress and resistance to xenobiotics.

Regresar al programa

# IN SEARCH FOR THE ENVIRONMENTAL STIMULI THAT INDUCE HIGHER LOVASTATIN PRODUCTION IN SOLID-STATE FERMENTATION: REACTIVE OXYGEN SPECIES (ROS) REGULATE PATHWAY GENES

J. Barrios-González
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Industrial production of antibiotics and other secondary metabolites (SM) is conventionally performed by liquid submerged fermentation (SmF); however solid-state fermentation (SSF) is rapidly becoming an alternative industrial production system.

SSF is a microbial culture system that has been used in several oriental countries since antiquity, but modernized during the last 25 years. Modern SSF systems have a record of successful applications for the production of microbial products. In the case of secondary metabolites, production is very often associated with higher yields in shorter time periods. Moreover, some antibiotics are only produced in SSF, even though the corresponding producer fungi can be readily cultivated in SmF. The reason for this different physiology in SSF is not fully understood, but it is often called “physiology of solid medium”.

Lovastatin (LOV) is secondary metabolite, produced by *Aspergillus terreus,* with great medical and economic importance, since it lowers cholesterol levels in blood.It is also the precursor for its successful semi-synthetic derivativesimvastatin. Our group developed a novel lovastatin production process by SSF on an artificial inert support: polyurethane foam (PUF). In this system, physiology of solid medium is clearly manifested, as a 30-fold higher lovastatin production than in SmF was obtained. Later we showed that one key rationale for higher lovastatin yield in PUF SSF, relative to the yield obtained by SmF, is a higher expression of the LOV genes. This and other evidence indicate that environmental stimuli in solid cultures induce physiology of solid medium.
Later work identified direct contact with air as an important environmental cue inducing this physiology, and it was considered that ROS formation could be involved in this phenomenon.

Recent results, showed a link between reactive oxygen species (ROS) and LOV biosynthesis in submerged (SmF) and solid-state fermentation (SSF). It was shown that *sod1* gene (oxidative stress-defense enzyme) was intensely expressed during rapid growth phase (or trophophase), but it was down regulated in production phase (idiophase). In that moment, ROS levels increased to high levels that remained during production phase. In a subsequent work we showed that ROS regulated LOV biosynthesis at a transcriptional level. Current work in our lab aims to understand the mechanism by which ROS regulate LOV genes.

Although ROS accumulation in idiophase happens in both culture systems, there are differences that could explain higher production in SSF. Hence, another line in our research explores the relation between ROS profiles and LOV biosynthesis.

From a practical point of view, these findings can be applied to design novel production culture systems, as well as genetic improvement methods to obtain overproducing strains for SSF and/or SmF.

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# MITOCHONDRIAL SUPERCOMPLEXES AND ROS

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Electrons feed into the mitochondrial electron transport chain (mETC) from NAD- or FAD-dependent enzymes. A shift from glucose to fatty acids increases electron flux through FAD, which can saturate the oxidation capacity of the dedicated coenzyme Q (CoQ) pool and result in the generation of harmful reactive oxygen species. To prevent this, the mETC superstructure reconfigures through to increase electron flux via FAD at the expense of NAD. This adaptation is driven by the ratio of reduced to oxidized CoQ, regulated reverse electron transport from reduced CoQ to complex I, and the resulting local generation of superoxide. CoQ redox status thus acts as a metabolic sensor that fine-tunes mETC configuration to match the prevailing substrate profile.

Regresar al programa

# Antioxidant response regulation by MicroRNAs Contributes To Cellular Transformation

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The recent inclusion of experimental data suggesting that altered microRNA (miRNA) expression under As, Cd, and Pb exposure may alter several critical cellular processes have been enriched our knowledge in heavy metal-associated disease. Our previous works have proposed miRNA expression profiles as suitable starting point for this kind of disease research, including cancer. Among the possible impaired cellular functions that miRNA-regulation may provoke is antioxidant barrier, which has been described as a key event leading to the initiation of metal-induced carcinogenesis. Here, we demonstrate that the mixture of As-Cd-Pb at epidemiologically relevant concentrations induces miRNAs that directly regulates, in a negative manner, different transcription factors involve in the antioxidant response. We also evidence that such inhibition triggers morphological transformation in a murine two-stage Balb/c 3T3 cell assay, suggesting that antioxidant response inhibition could play a role as initiator of the carcinogenesis process.

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# METABOLIC MODELING OF THE ANTIOXIDANT PATHWAY IN *Trypanosoma cruzi*: IN SEARCH OF THERAPEUTIC TARGETS

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BACKGROUND: *Trypanosoma cruzi* is the protist parasite that causes human American trypanosomiasis, a disease endemic of LatinAmerican countries. In trypanosomatids, trypanothione [$T(SH)_2$], a conjugate of two glutathione and one polyamine moieties, is the main antioxidant metabolite; hence the $T(SH)_2$-based antioxidant enzymatic machinery replaces that of the glutathione system in mammals. The aim of this work is to identify the enzymes that mainly control the $T(SH)_2$ metabolism in *Trypanosoma cruzi* by applying the quantitative analyses of metabolic modeling and the fundamentals of Metabolic Control Analysis.

METHODS: A kinetic model of $T(SH)_2$ synthesis was constructed based on the kinetic parameters of the recombinant pathway enzymes determined under near-physiological conditions as well as the enzyme activities in the cells. The model validity was established as its ability to simulate the metabolite concentrations and fluxes of the whole pathway in the cells. Further, the $T(SH)_2$-dependent peroxide detoxification system was reconstituted *in vitro* with the recombinant enzymes to determine their degree of control on the pathway flux. The *in silico* and *in vitro* predictions obtained with both approaches regarding the pathway´s flux control distribution were evaluated in supplementation experiments with thiol-molecules and polyamines in the parasites.

RESULTS: The model could robustly simulate the fluxes and metabolite concentrations found in the parasites. The model indicated that gamma-glutamylcysteine synthase > trypanothione synthase >>> polyamine supply were the most controlling steps of trypanothione synthesis. Supplementation of parasites with cysteine and GSH, but not with spermidine or putrescine, increased the $T(SH)_2$ pool. In the peroxide detoxification system, the tryparedoxin/tryparedoxin-peroxidase redox pair totally controlled the pathway flux, with negligible control exerted by trypanothione reductase.

CONCLUSIONS: The most controlling steps of the $T(SH)_2$ metabolism in *T. cruzi* were identified. The results indicated that inhibition of γ-glutamycysteine synthetase, trypanothione synthetase and tryparedoxin will have much stronger adverse effects on the parasite antioxidant defense than inhibition of low-controlling enzymes such as trypanothione reductase, being the latter, the most popular enzyme for drug-target studies.

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# HORMETIC RESPONSE MECHANISMS THAT PREVENT OXIDATIVE DAMAGE DURING AGING

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Aging is a process characterized by the decline on physiological, biochemistry and structural cellular function. There are different theories to explain this process, but currently, one of the most plausible and acceptable explanations is the free radical theory of aging, postulated by Harman in 1956. This theory postulates that aging and the related diseases are the consequence of free radical-induced damage to cellular macromolecules and the inability to counterbalance these changes during lifespan. Throughout evolution, living organisms have had to adapt to adverse conditions and agents in order to survive, so they have developed diverse and complex mechanisms to deal with them. Hence, there have been described a series of preserved processes, where a low or sub lethal dose of an agent or a stressful stimulus is capable of activate adaptive responses that increase the resistance of a cell or organism against a more severe stressor. That response is known as "hormesis". There are different hormetic agents such as radiation, heat, heavy metals, antibiotics, ethanol, pro-oxidant agents, exercise and food restriction. Hormetic response involves the expression of a large number of genes encoding proteins as cytoprotective chaperones that respond to heat stress, antioxidant enzymes, growth factors, metallothioneins, among the most important. In recent years, we have designed different hormetic models to counteract oxidative damage. Some of them are cell lines, primary cultures and recently have developed a model in Wistar rats. In these models, we tested different compounds that induce an hormetic response and with which we were able to increase the antioxidant response capacity. Furthermore, we have described some hormetic response mechanisms and identified some of the proteins involved in the cytoprotective response against at different molecules that generate oxidative damage. The results we have obtained are really encouraging and interesting in order to counteract oxidative damage during aging. Mainly because the mechanisms we have described might be used to design strategies that will allow us to protect the elderly and enable them to counteract the oxidative damage observed in aging and aging related diseases.

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# DYNAMIC OF REACTIVE OXYGEN SPECIES IN ROOT HAIR CELLS AND POLLEN TUBES ARE ESSENTIAL FOR POLAR GROWTH

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In plant cells ROS accumulation have been involved in several processes such as: development, hypersensitive response, hormonal perception, gravitropism and stress response. In guard cells from *Vicia faba* regulates the opening of stomata and more recently in root hair cells from *Arabidopsis* ROS levels generate and maintain an apical calcium gradient. This ROS accumulation plays a key role in root hair tip growth and suggested to play a similar role in pollen tubes and other tip growing cells. Herein we report a new molecular probe to depict the ROS dynamic during root hair cell and pollen tube apical growth. Hyper is a new generated GFP fused to the OxyR domain that result in a hydrogen peroxide specific probe. This molecular probe was expressed in root hair cells from Arabidopsis and tobacco pollen tubes (1). By using high resolution microscopy we depicted an apical $H_2O_2$ gradient at the tip dome where the polar growth occur, furthermore we were able to visualize dynamic ROS oscillations in root hair cells, which are couple to growth. In pollen tubes we also found a particular ROS distribution, with clear oscillations couple to growth fluctuations. In both tip growing cells, the apical regions are the site where the more dynamic ROS changes were observed, suggesting a pivotal role in polar growth.

1. Hernandez-Barrera, A., Quinto, C., Johnson, E. A., Wu, H. M., Cheung, A. Y., & Cardenas, L. (2013) *Methods Enzymol* **527,** 275-290.
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# SENESCENT ASTROCYTES AND NEURODEGENERATIVE DISEASE

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Our research focuses on the role of cellular senescence in the development of brain pathology. Aging is the greatest risk factor for the development of neurodegenerative disease, however the aspects of the aging process that predispose to the development of brain pathology are largely unknown. Research from our laboratory indicates that human astrocytes are highly sensitive and trigger the senescence program response to cellular insult in vitro. Our data indicate that the senescence program may be induced by oxidative stress, proteasome inhibition, exhaustive replication, beta amyloid, and HIV factors. In vivo, we have demonstrated that the population of senescent astrocytes is significantly elevated in the frontal cortex of the brain of aged individuals. Additionally, brain sections from Alzheimer's disease patients contain greater numbers of senescent astrocytes than age-matched controls. Based on these results, and the sensitivity of astrocytes to enter into senescence, we have hypothesized that there is a functional relationship between induction of the astrocyte senescent program and neuropathology, and we have proposed that one of the mechanisms by which aging contributes to age- and disease-related cognitive decline is via astrocyte senescence. The rationale of our studies is partly based on the recent demonstration that cell senescence can propagate between cells via paracrine mechanisms. Thus, we propose a model where the inflammatory environment created by senescent astrocytes in the aged brain might contribute to senescence of neighboring cells, leading to a feed-forward mechanism of senescence-inducing senescence involving neurons, astrocytes and microglia, which may contribute to cellular dysfunction and neurocognitive decline during aging. Factors such as amyloid deposition and neuronal tangle formation may exacerbate this process by accelerating the appearance of senescent cells. This suggests that interventions that delay senescence would be relevant and beneficial.

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# Antioxidant and neuroprotective properties of S-allylcysteine, an aged garlic compound

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S-allylcysteine (SAC) as its most abundant compound found among aged garlic extract (AGE) components. SAC is an odorless garlic preparation that has been largely demonstrated to exert antioxidant activity under both *in vivo* - experimental animal models associated to oxidative stress - and *in vitro* conditions – in cell cultures and biological preparations -. This organosulphur compound has shown to be effective scavenging reactive oxygen species (ROS), also preventing or mitigating oxidative damage. Consequently, the protective effects of SAC are currently associated with its capacity to prevent the deleterious actions of oxygen toxicity. More recently, beyond its well characterized antioxidant mechanisms (scavenging of free radicals and pro-oxidant species, induction of antioxidant enzymes, activation of Nrf2 factor, inhibition of pro-oxidant enzymes, and chelating effects) SAC has shown to induce neuroprotective effects in different neurotoxic paradigms, thereby emphasizing its potential as a therapeutic tool to be considered for the design of novel and coadjutant therapies in the CNS. This presentation highlights the properties of SAC to induce an integral pattern of antioxidant and neuroprotective features comprising the regulation of redox activity through the coordinated activity of different cell types in the CNS to preserve cell homeostasis and/or to counteract pathological processes. It can be concluded that the therapeutic properties of SAC comprise molecular mechanisms at different levels, which are promising for future studies.

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# DYSFUNCTION OF INSULIN BY OXIDATIVE STRESS IN DIABETIC AND OBESE PATIENTS.

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Several studies have shown a relationship between obesity and insulin resistance. Therefore obesity is considered a risk factor to development of Diabetes Mellitus type 2. Nowadays, the mechanisms that generate insulin resistance involve an inhibition of signal pathway or a decrease in the synthesis of the hormone. However it is unknown whether a modification of insulin could be involved in this mechanism. In this context, our group has demonstrated that the incubation of human recombinant insulin in blood from obese (grade 1 or 3) and diabetic patients induce chemicals modifications in the hormone and its polymerization. This polymer showed a molecular weight of 70 kDa, which was detected by polyacrylamide gel and western blot. Interestingly, insulin polymerization generated a loss of its biological activity, event that was attributed to oxidative stress present in the patients. Furthermore, we found that oxidative damage to hormone was in accordance with the presence of quinones and carbonyl groups, suggesting the participation of these groups in the formation of polymers. Currently, we generated a polyclonal antibody against insulin polymers, which it was coupled to magnetic particles, and incubated in plasma from obese patients. The data obtained showed for the first time, the presence of these polymers in obese patients, indeed an association of their presence with parameters of insulin resistance was established. Whether well, our studies have shown that oxidative stress can generate structural and functional changes to insulin in blood, and underline the importance of oxidative stress in the pathogenesis of obesity and its relationship with the development of type 2 diabetes. We also have shown that this molecular damage can be decreased by a treatment with hypocaloric diet and/or aerobic exercise.

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# STUDY OF THE FLAVANOL (-)-EPICATECHIN EFFECTS ON OXIDATIVE MARKERS IN AGED MICE

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# "tBHQ/Nrf2 CONFERS NEUROPROTECTION TO OLD RATS AGAISNT AN OXIDANT TREATEMENT WITH 3-NITROPROPIONIC ACID"

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With the population pyramid shift and the increase in elderly population in then last years, aging studies have become relevant because of the escalation in old age illnesses, in particular neurodegenerative diseases. During normal aging the brain decreases its antioxidant defenses becoming very susceptible to oxidative damage (OxD). Therefore, we decided study if old animals were capable to trigger the Nrf2 transcription factor in response to a particular inductor such as tert-butylhydroquinone (tBHQ), and activate the antioxidant pathway in order to reduce the OxD generated by the neurotoxic 3-nitropropionic acid (3NP). Old (24m) and adult (9m) rats were pre-conditioned for 7 days with tBHQ (100mg/Kg) and were then injected with 3NP (10mg/Kg) twice a day for 4 days. A mobility test was performed in order to evaluate their behavior, followed by a histological analysis of the caudate-putamen region and reactive gliosis. Nrf2 content in the nuclear (NF) and cytosolic (CF) fractions was also determined along with some antioxidant enzymes regulated by this factor (SOD, HO-1, GST). Our results showed that under these experimental conditions tBHQ-treated adult and old animals manage to recover their mobility in comparison to the 3NP group. These animals were also protected against the OxD observed in the striatum. Quantitatively 3NP group showed 70% damaged neurons, while tBHQ+3NP only 10% in adults and 20% in old rats. In regard to the reactive gliosis, old rats showed 4 times more glial fibrillary acidic protein (GFAP) staining than adult rats prior to any treatment, and when treated with 3NP they increased the reactive gliosis in 30%, these effect declined (10%) when old animals were pre-treated with tBHQ. In a similar manner, tBHQ-pretreatment augmented Nrf2 in NF, which correlated with the increase in SOD. Hence, our results suggest that old rats indeed increased Nrf2 content and nuclear translocation as a protective mechanism against the oxidative stress produced by 3NP, allowing the striatal tissue protection and decreasing reactive gliosis. These results are very important because they imply that senile animals are also capable to activate their antioxidant protection mechanism in response to an inductor, contrary to what was previously thought.

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# EFFECT ANTIPROLIFERATIVE OF (-)-EPICATECHIN AND ITS RELATIONSHIP WITH REACTIVE OXYGEN SPECIES IN BREAST CANCER CELL

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**Introduction:** Breast cancer is the neoplasia of increased morbidity and mortality in women from Mexico and the world. The search of new therapeutic strategies against this neoplasia has focused on natural products such as polyphenols. Our research group has shown that (-)- epicatechin (a polyphenol) present an antiproliferative effect in cell lines from breast cancer. It is well documented that cancer cells have an increased metabolism, which induce higher production of reactive oxygen species (ROS). However cancerous cells are able to evade the damage by ROS, process that it has been related with the overexpression of a protein called uncoupling protein 2 (UCP2). This protein has been found overexpressed in several cell lines and biopsies from breast cancer**. Aim:** To determine if antiproliferative effect of (-)-epicatechin is related with a downregulation in UCP2 expression, an increase in ROS production and apoptosis induction. **Material and methods:** MCF-7 and MDA-MB-231 breast cancer cells and endothelial cells (non-transformed cells) were used. Half maximal inhibitory concentration (IC50) of (-)-epicatechin was determined in breast cancer cells by 1-(4, 5-Dimethylthiazol-2-yl)-3,5- diphenylformazan (MTT) assays. UCP2 expression was established by semi-quantitative RT- PCR and western blot. ROS production was determined by the values of biomarkers of oxidative damage such as carbonyl groups and malondialdehyde (MDA); superoxide anion production was determined by fluorescence microscopy by MitoSOX$^{TM}$ in absence and presence of (-)-epicatechin; also the activity of glutathione peroxidase (GSH-mPx) was analyzed. Finally, DNA fragmentation assay was performed to determine the induction of apoptosis. **Results:** The data obtained showed higher UCP2 expression in MCF-7 and MDA- MB-231 than endothelial cells (non-transformed cells). Antiproliferative effect of (-)-epicatechin showed an IC50=350 µM, effect that was coordinated with a downregulation in UCP2 expression, increase in the values of biomarker of oxidative damage, high production of superoxide anion, decreasing in GSH-Px activity and DNA fragmentation. All these data were related with an induction of apoptosis generated by (-)-epicatechin. Interestingly, when (-)- epicatechin was used in combination with chemotherapeutics drugs we observed a major effect, suggesting the possibility to be used as adjuvant in cancer treatment. **Conclusions:** The data obtained in this work, suggest that the effect antiproliferative of (-)-epicatechin is mediated by an induction of apoptosis, an increase in the production of ROS, decreasing of antioxidant defenses and downregulation in UCP2 expression.

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# Undesaired toxicity of cyclophosphamide associated with increased oxidative stress in cancer-free organs.

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Cyclophosphamide is one of the most effective antineoplasic drugs used as part of treatment for a wide types of cancers, is an alkylating agent able to induce cross-linking of DNA.

Cyclophosphamide may also affect non-cancer cells, these effects reduce efectivity, dose and time of the treatment and the quality of life of patients. The toxicity of antineoplastic treatment can be induced by oxidative stress; however few studies has been performed in this sense and the molecular mechanisms are not yet fully understood.

In this work, male rats Wistar (200-250 g) were treated with 3 weekly doses of cyclophosphamide (60 mg/kg, intraperitoneal); other rats group were administered simultaneously a daily dose of the antioxidants: alpha-tocopherol (100 mg/kg, ip), ascorbic acid (100 mg/kg, ip) and N-acetylcysteine (100 mg/kg, ip). Control groups that received only saline, and a control group of antioxidants that are only administered daily mix of antioxidants were included. At the indicate time, liver, kidney and brain were removed and homogenized to assess lipid peroxidation by measuring thiobarbituric acid (TBARS) species; total antioxi- dant capacity using the myoglobin-induced oxidation of ABTS. Induction of apoptosis was evaluated by caspase 3 activity. Likewise, the activity of antioxidant enzymes superoxide dismutase (SOD), gluthatione peroxidase (GPX) and catalase (CAT) were measured by spectrophotometric methods.

High lipid peroxidation and apoptosis were found in liver, brain and kidney of rats treated with cyclophosphamide. Whereas, treatment with antioxidants was able to prevent lipid peroxidation and apoptosis induced by cyclophosphamide treatment.

Total antioxidant capacity induced by cyclophosphamide treatment was high in liver, low in brain and unaffected in kidney. Antioxidant treatment was able to avoid changes in total antioxidant capacity in the organs.

Cyclophosphamide treatment altered antioxidant enzymes activity. SOD activity was lower in liver and higher in kidney and brain of rats treated with cyclophosphamide. The activity of CAT was higher in liver and kidney of treated rats with the antineoplastic; while in brain we were unable to detect CAT with the method used. GPX activity was lower in liver and higher in kidney and brain of treated rats.

The antioxidant treatment was able to prevent changes in SOD activity of kidney and brain; Changes in CAT and GPX activities of liver and kidney also were prevent with the antioxidant treatment, in rats treated with cyclophosphamide. The activities of SOD and GPX in brain of rats treated with cyclophosphamide remain high even with treatment with antioxidants.

The results indicate that the treatment with three weekly doses of cyclophosphamide was able to induce oxidative damage and apoptosis, and modify the total antioxidant capacity in liver, kidney and brain. Daily administration of antioxidants during cyclophosphamide treatment protects against oxidative damage and induction of apoptosis caused by the treatment. Oxidative stress was partially corrected by treatment with antioxidants.

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# THE ROLE OF Slm35 DURING AGING AND OXIDATIVE STRESS IN THE YEAST *Saccharomyces cerevisiae*

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Cellular aging is determined by a large number of genes and proteins involved in conserved pathways.

Due to its easy manipulation, the yeast *Saccharomyces cerevisiae* has been used as a model for studying mechanisms of cell aging and longevity.

In *S. cerevisiae* two types of cell life span can be studied, the chronological life span (CLS) which is defined as the time that a cell remains viable in stationary phase; and replicative life span (RLS), which refers to the number of daughter cells produced before death (Garay, E., et al., 2013).

Previous studies have shown that genes such as *RAS2, TOR1* and *SCH9* are activated in response to nutrient availability, regulating growth and cell division. Deficiency in any of these genes promotes the extension of the CLS and RLS, as well as protection against oxidative and thermal stress (Wei, M., et al., 2008).

Free radicals that mediate oxidative damage to DNA, lipids and/or proteins are an important -but not the main- cause of cell aging. In addition growth conditions in low concentrations of glucose (calorie restriction) increases cellular respiration, but faster and more efficient electron transport promotes decreased production of mitochondrial reactive oxygen species (ROS). Process that has been associated with increased CLS (Barros, M., et al., 2004).

In *S. cerevisiae*, the protein encoded by the gene *SLM35* has been identified in purified mitochondria and has been involved in biogenesis, organization and mitochondrial inheritance (Hess, Myers et al. 2009).

In this work we analyze the relevance of Slm35 during cell longevity and cell response to oxidative stress.

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# MODIFICATION OF THE ROS PROFILE IN LOVASTATIN SOLID-STATE FERMENTATION BY GENETIC MEANS OR BY ENVIRONMENTAL MANIPULATION.

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Lovastatin (LOV) is a secondary metabolite, produced by *Aspergillus terreus* that has great commercial importance since it lowers cholesterol levels in blood. As often happens with other secondary metabolites, higher LOV production is obtained in solid-state fermentation (SSF).

Earlier studies from our group showed a link between reactive oxygen species (ROS) and LOV biosynthesis in submerged (SmF) as well as in SSF. Results showed that *sod1* gene (oxidative stress-defense enzyme) was intensely expressed during rapid growth phase (or trophophase) of LOV fermentations, but it was down regulated in the production phase (or idiophase). Apparently because of this, in that moment ROS levels increased, generating an oxidative state during most of the idiophase (Miranda et al 2013). In a subsequent work we showed that ROS positively regulate LOV biosynthetic genes in both culture systems.

It is important to note that the novel type of SSF, in which this studied were made, uses polyurethane foam as inert support and does not have free aereation.

The objective of this work was to establish the effecto of 2 factors on the ROS accumulation profile and the effect, of this change in profile, on lovastatin biosynthesis. The factors considered as candidates to modify the ROS profile were: 1) silencing gen At*yap1*; and 2) the degree of aeration of the SSF.

Gene *yap1* encodes an important oxidative-stress-response transcription factor in fungi. The silencing vector was constructed by ligation of a fragment of *A. terreus yap1* (At*yap1*) gene to pGdpPki-RNAi vector. This vector has a cloning site in the middle of a system of 2 promoters in opposite directions, so that it generates a double stranded RNA that activates the RNA silencing system. The construction was then transformed in *A. terreus* TUB F-514. Lovastatin was quantified by HPLC, and ROS concentration by dicloroflurоesein and gene expression by Northen Blot.

Lovastatin SSF was performed in fermenters with different aeration rates, and ROS accumulation profile was measured in the different samples.

**Conclusions.**

AT*yap1* silencing in *A. terreus* caused, as expected, decreased expression of At*yap1*; but also of gene *sod1*, suggesting that AtYap1 regulates *sod1*. The silencing of At*yap1* also provoked sensitivity to oxidative stress.

In the transformants, the ROS build up began before schedule in both culture systems, reaching higher levels than in the parental strain. This brought about earlier and stronger expression of LOV gene: *lovE* and of sporulation gene: *brlA*. This manifested in early start LOV biosynthesis that reached higher production than the parental strain (70% increase in SSF and 60% in SmF). Also, conididation started earlier and reached a surprising 6-fold increase in sporulation index.

Results with SSFs with different areation rate showed that ROS profiles form and timing, does not change with the areation rate. However, ROS accumlation level was lower in cultures with higher areation rate, and lower LOV yields were obtained. These results show that LOV production can be changed by manipulating the ROS profile by genetic means or by manipulating the areation.

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# DYNAMICS OF THE INTRACELLULAR $H_2O_2$ LEVELS IN THE ROOT OF *Arabidopsis thaliana* IN RESPONSE TO A SPECIFIC NADPH INHIBITOR

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Arabidopsis thaliana is a small plant widely used as an experimental model for the study of a variety of fundamental biological processes; the root development is one of them. The plant root and the root hairs are key players responsible for the nutrient uptake from the soil. The apical growth of root hairs involves the regulation of the ions flow, calcium homeostasis, exocytosis, and cytoskeleton. Reactive Oxygen Species (ROS) also play an important role in the growth of the root and the root hairs. The production of the ROS occurs as a result of aerobic metabolism during respiration and photosynthesis, and its distribution is higher in organelles such as mitochondria, chloroplasts, and peroxisomes. However, ROS can be produced by the enzymatic activity of NADPH oxidase (RBOHs in plants for respiratory burst oxidase homologue), these enzymes transfer electrons from NADPH to an acceptor, the oxygen, to form the superoxide radical from which other ROS originate as $H_2O_2$. The inhibition of these enzymes have been addressed using many general inhibitors, the DPI is probably the most popular. In this work we measured the dynamics of $H_2O_2$ levels in living plant roots and root hairs from Arabidopsis expressing Hyper, a specific $H_2O_2$ sensor. The $H_2O_2$ distribution and responses under the effect of specific and newly developed inhibitor of the NADPH oxidase (VASP2870) were determined. The responses were very different depending on the inhibitors, suggesting a more specific role for the VASP2870.

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# CHARACTERIZATION OF THE EFFECTS OF CHROMIUM ON THE PRODUCTION OF REACTIVE OXYGEN SPECIES AND HORMONE RESPONSE IN *Arabidopsis thaliana*

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Chromium [Cr(IV) or chromate] is an element that has been increasing its concentration in the soil due to human activities, which represents a danger for the toxicity of this element to several organisms, including plants. On the other hand, plants responses to Cr(VI) are similar to those induced by other metals, so have been proposed common mediators, mainly reactive oxygen species (ROS) and hormonal signaling pathways such as ethylene and auxin. In a previous analysis, the *Arabidopsis thaliana* mutants *ein2* (*ethylene insensitive 2*) and *slr1* (*solitary root1*) affected in the ethylene and auxin response respectively, showed resistance to sublethal concentrations of Cr(VI) compared to wild-type (Col-0) seedlings. In this study, we evaluated the primary root growth of the *ein2* mutant at concentrations of 20-100 µM Cr(VI), finding that *ein2* has a partial resistance to 100 µM Cr(VI) concentration, while in control (Col-0) seedlings concentrations higher than 60 µM Cr(VI) repressed primary root growth and increased formation of root hairs, lateral roots and adventitious roots. The inhibition of primary root growth by high Cr(VI) concentrations, was related to a decrease of cell division as evidenced by arrested expression of the cell cycle marker *CycB1:uidA* in the root meristem. In contrast, *ein2/CycB1:uidA* still showed expression of this marker. Using a histochemical analysis with 3,3'-diaminobenzidine (DAB), it was found that the *ein2* mutant showed lower accumulation of $H_2O_2$ than wild-type (Col-0) seedlings. Moreover, using the *Arabidopsis* transgenic line HyPer (which is using for the *in vivo* detection of $H_2O_2$), was observed a higher production of $H_2O_2$ in response to Cr(VI) in a dose-manner. Regarding to auxin response, an initial evaluation was performed in *slr1* mutant, founding that its primary root growth was not affected by Cr(VI). Moreover, using DHE (dihydroethidium), *slr1* showed a lower accumulation of $O_2$ as compared to control seedlings (Col-0). Our findings suggest that Cr(VI) is an important factor modulating the interplay between ROS production and root system architecture by an ethylene and auxin-dependent signaling.

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# A GENE REGULATORY NETWORK UNDERLYING ROS PATTERNS IN *ARABIDOPSIS* ROOT

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**Introduction**. Reactive Oxygen Species (ROS), that are molecules with at least one oxygen atom, have been proven to play a major role elicitors of cell decisions (proliferation vs differentiation) during development, and the *Arabidopsis* root is a useful system to address their role *in* vivo[2]. In *Arabidopsis thaliana*, more than 120 genes that belong to different enzyme families such as NADPH oxidases, Superoxide dismutases or catalases participate in ROS homeostasis and are important for ROS production and scavenging [2]. The interactions among these and other molecular components are important for proper root development and biotic and abiotic stress response, but until now just a few Transcription Factors (TF) that controls the expression of ROS pathway genes, have been characterized[3]. Gene Regulatory Networks (GRN) can be inferred from well-curated data and also from genomic data bases[4]. GRNs are useful tools in understanding the complex logic underlying the regulation of biological processes, such as ROS homeostasis. Such integrative and systemic approaches may be useful for designing crucial experiments in a more efficient and systems-approach framework. In this work, we used a top-down systemic approach to integrate a GRN that controls genes responsible for ROS homeostasis in the *Arabidopsis* root. **Methods.** We used a similar approach to that reported by Chávez-Montes *et al* (2014)[5], First we retrieved all microarray data for *Arabidopsis* root available at EBI ArrayExpress database until January 2015. We only used data from ATH1-121501 array and discarded all the samples that came from mutants or over- expression lines and ecotypes other than Columbia. We ran the arrayQualityMetrics Bioconductor package on our database in order to delete CEL files with poor quality. We used the celutil script to convert binary CEL files to ASCII format and a CDF file was created, containing 2088 TFs and all genes of ROS pathway with USE_ME.pl script from Xspecies and then packaged with mackecdfenv and AnnotationDbi R packages. Custom CEL file was normalized with gcRMA and used as input for ARACNe algorithm[6]. **Results.** We inferred a GRN for ROS pathway in Arabidopsis root. Analyzed its systemic properties and predict new functions for some TFs included in our inferences. This first hypothesis will be used for further top-down inferences and also bottom-up procedures to integrate a well-grounded GRN underlying ROS homeostasis in the Arabidopsis root.

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# THE FORMATION OF SUPERCOMPLEXES IS PRESERVED BY *Moringa oleifera* HYDROALCOHOLIC EXTRACT IN LIVER DURING EARLY DIABETES.

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**Background:** The increasing prevalence of Diabetes mellitus worldwide highlights the few effective treatment strategies to combat this disease. The role of mitochondria in human health and disease is recognized by the emergence of “Mitochondrial Medicine”. Mito Q and Coenzyme $Q_{10}$, which are ubiquinone-based antioxidants that focus on targeting electron transport chain (ETC) to decrease ROS production. The four protein complexes of the ETC are located in the cristae membrane and complexes I, III and IV are organized in respiratory supercomplexes called respirasomes. Respirasomes enhance the efficiency of electron transfer and the generation of the proton motive force that is used by ATP synthase, to produce ATP. The conditions that drive the formation of supercomplexes are unknown, but it is known that complex I assembly and activation is critical for this process. *Moringa oleifera* is a medicinal plant recognized by its antihyperglycemic and antioxidant properties. Among its polyphenols, it has been shown that quercetin can behave as a “coenzyme Q- mimetic” molecule, preventing a possible electron leak, lipoperoxidation and protein oxidation. **Aim:** In this work, we studied the impact of the hydroalcoholic leaves extract of *M. oleifera* on liver mitochondrial respiratory complexes from STZ- induced diabetic rats. **Methods:** After treatment, animals were sacrificed and liver mitochondria were isolated to evaluate oxygen consumption, specific respiratory activities, supercomplex formation, ROS production, protein carbonylation and lipoperoxidation. **Results:** As far as we know, no reports are available concerning alterations in supercomplexes formation during diabetes. Analysis by BN-PAGE showed that diabetic animals had a lower content of I-III-IV supercomplexes and the individual complex I is more abundant compared with control or *M. oleifera* groups. Furthermore, the activity of complex I and the hydrolytic activity of complex V were increased during diabetes and no change was observed for complex II activity. Supporting the previous data, oxygen consumption increased in complexes I and II, with NADH and succinate, respectively. *M. oleifera* administration diminished such modifications and protected mitochondria against lipoperoxidation and protein carbonylation, however ROS production generated by complex III, does not change in diabetic-treated group. **Conclusion:** These results suggest that *M. oleifera* polyphenols target mitochondria to prevent supercomplexes dissociation during hyperglycemia reestablishing their activity and protecting from oxidation of lipids and protein by ROS.

Regresar al programa

# HGF INDUCE THE REACTIVE OXYGEN SPECIES PRODUCTION VIA NADPH OXIDASE IN PRIMARY MOUSE HEPATOCYTES

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**Introduction.** Our group reported that the hepatocyte growth factor (HGF) and its receptor c-Met are involved in the antioxidant response and protect against oxidative stress-induced cellular damage. However recently we report that HGF/c-Met induce the NADPH oxidase activation and therefore the ROS production, the assessment of the NADPH activity showed an increase at early times after the treatment that contribute to ROS formation and leads to cell survival in primary mouse hepatocytes.

**Objective.** In the present work we were focused in determine the NADPH oxidase homologous expression in hepatocytes at short times and to elucidate the different ROS production induced by HGF indicating a possible differential regulation of the NADPH oxidase isoform.

**Methods**. Primary mouse hepatocytes were isolated by the two-step collagenase perfusion and pretreated or not with 50 ng/ml HGF (0-24h) and DPI (10mM) as NADPH oxidase inhibitor. We analyze by qRT-PCR the RNA expression (Figure 1) and by Western Blot (WB) the protein content of the NADPH oxidase subunits (Figure 2A), the protein expression was confirmed by a confocal assay (Figure 2B), the contribution of ROS in HGF/c-Met-induced Nox activation in the hepatocyte was detected with DHE for $O_2^{\bullet-}$ (Figure 3) or DCFH-DA for $H_2O_2$ (Figure 4A), to verify the $O_2^{\bullet-}$ the 2HE fluorescence was determined by confocal microscopy in vivo (Figure 4B). Finally the c-Met receptor activation and p22phox localization induced by HGF was analyzed by a confocal assay (Figure 5A and 5B, respectively).

**Results.** The data show that primary mouse hepatocytes express 5 catalytic homologous of NADPH oxidase (Nox1, Nox2, Nox4, Duox1 and Duox2) and the regulatory subunits like p60phox, p40phox, p47phox, p67phox y p22phox (Figure 1 and 2B). Previous data show that HGF induced a biphasic mechanism of NADPH oxidase regulation, a WB was performed in order to identify if the increase in enzyme activity at short times is not related with protein content (Figure 2A).It has been describe that Nox homologous have a different ROS contribution, in the hepatocytes we found a pick at 15min in $O_2^{\bullet-}$ production and 30 min in $H_2O_2$ this data was verified by confocal microscopy. Finally we describe that HGF induce the c-Met activation

**Conclusions**. Our results suggest a differential regulation of the NADPH oxidase isoforms by HGF and c-Met receptor not only liver, but practically any organ or system in hepatocytes.

Conacyt #131707**.**

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# ROLE OF REACTIVE OXYGEN SPECIES IN THE REGULATION OF A MAP3K DURING THE CELL DEATH OF NEURONS.

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Reactive oxygen species (ROS) modulate apoptosis of cerebellar granule neurons (CGN), but the mechanisms implicated have not been clarified. According to a previous study, the mitogen- activated protein kinases (MAPK) pathway is activated by oxidative stress in CGN and may participate in the apoptotic death of CGN. However, the mechanism by which the MAPK pathway is activated by ROS in CGN is still unknown. In non-neuronal cells, it has been suggested that the MAPKs JNK and p38 could be activated by the interaction of the ROS with ASK1, a kinase kinase of the MAPK (MAP3K). In particular, it is known that the ROS act by modulating different regulators of ASK1: Trx1, Trx2, Akt, Grx1 and TXNIP. It has been demonstrated that the reduced form of thioredoxin (Trx1) binds to ASK1 in basal conditions, keeping ASK1 in an inactive state. Under oxidizing conditions, Trx1 dissociates from ASK1 allowing the activation of ASK1 and then the activation of JNK and p38. On the other hand, under the presence of ROS Trx1 is negatively modulated by TXNIP. Furthermore, it has recently been reported that Akt (a kinase of survival) is redox sensitive and that it phosphorylates serine 83 (Ser83) of ASK1 causing its inactivation. Based on these studies, one possible scenario in the CGN is that ROS generated early by apoptotic conditions induce the dissociation of Trx1 from ASK1 and regulates the activation of Akt, which would modulate the activation of ASK1. In addition to that, the ROS would regulate the activity of Trx1 (negative regulator of ASK1) through its binding to TXNIP. Taken together, all these events would lead the apoptotic death of CGN. In this study, we evaluated this possibility by using a model of apoptotic death of cultured CGN induced by high potassium deprivation (K5). Under these conditions, we found an early increase in the generation of ROS induced by K5 treatment. In addition, we found that the death of CGN stimulated by K5 was also dependent on time. On the other hand, using Western blot assays we found a decrease in the phosphorylation of Ser83 of ASK1 (activation of ASK1) from very short times and, in agreement to this result, we found a decrease in the activation of Akt induced by K5. In addition, in line with these observation we found an increase in the phosphorylation of threonine 845 (T845, phosphothreonine that monitors the state of activation of ASK1), at short times. Based on assays of co-immunoprecipitation we observed that K5 significantly reduced the interaction between Trx1 and ASK1 after 30 minutes of treatment, suggesting that ROS generated by this stimulus could be modulating, at least partially, the complex Trx1-ASK1. Finally, we found that K5 induces the expression of TXNIP from 2 hours of treatment and that this expression decreases by using antioxidants and NADPH oxidase inhibitors. These data suggest that ROS generated by K5 could regulate the activation mechanism of ASK1 and thus activate the signaling pathways involved in the control of the apoptotic machinery of CGN.

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# REACTIVE OXYGEN SPECIES DYNAMICS IN DEVELOPING ZEBRAFISH EMBRYOS.

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Reactive oxygen species (ROS) are natural oxygen derivatives generated during aerobic metabolism and specific enzymatic activities. ROS play pivotal roles in the regulation of major cellular behaviors such as: proliferation, cell death, migration, differentiation and aging. Previously we found in mouse embryos, that ROS participate in the control of cell death during remodeling of different tissues, suggesting an extensive role in development. To gain further insight into ROS functions in animal development, zebrafish embryos were labeled with a widely used ROS-sensitive fluorescent dye (CM-H2DCFDA) and visualized by wide field fluorescence and confocal microscopy. We found that the fluorescent signal which indicates the ROS distribution present highly dynamic patterns that correlate with key developmental process. During the early cleavages, the ROS signal localized at the cleavage furrows. In later 32-cell to 64-cell stage embryos, ROS distribution was still present at cleavage furrows with fluctuations that seems to be coordinated with cell divisions that later on result in a “wave” pattern. In embryos undergoing epiboly, the ROS distribution was still very dynamic among deep cells; however, a distinctive intense signal was found at the blastoderm migration front throughout the whole process. The observed ROS distribution is derived from the NADPH oxidases activity since treatment with the inhibitor VAS2870 lead to decreased ROS and interfered with epiboly progression. These results demonstrate the importance of ROS in the control of epiboly cell migration in zebrafish. Furthermore, we suggest that NADPH oxidases activity is the main source of ROS that participate in fundamental developmental process in vertebrates.

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Regresar al programa

# Effect of Omega 3 Fatty Acids in Lipid composition and Lipoperoxidation of Placental mitochondria from diabetic rats.

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**Introduction:** Diabetes is a multifactorial chronic degenerative disease. The damage to biological molecules by Oxidative stress, through the production of reactive oxygen species (ROS) has been proposed as a major cause of alterations observed during diabetes. Gestation is a physiologic state that requires metabolic adaptations, characterized by a higher demand of oxygen and energy; to accomplish this demand, the mother cellular metabolism increases, generating higher amounts of free radicals; the overproduction of free radicals plays and important role as stress keepers, which derive in lipid peroxidation in cellular membranes altering transport and cellular signal transduction. Several clinical, experimental, and epidemiologic studies have confirmed that the intake of omega-3 polyunsaturated fatty acids from fish oil exerts a favorable effect on diabetes development and progression. Increased interest in using omega-3 fatty acids led us to examine their metabolic effects in placental mitocondria from Wistar rats with Type 2 diabetes (T2D). It was evaluated the effect of omega 3 fatty acids during maternal hyperglycemia on fatty acid composition, free radical generation in placental mitochondria from rats with 19 days of gestation induced or not to diabetes.

**Methods:** 48 hours female newborn rats were induced to T2D by a unique intraperitoneal injection of streptozotocin (STZ) of 135 mg/kg of body weight in 50 µl of citrate buffer. The control groups were injected only with 50 µl of citrate buffer. After weaning, from STZ groups one was supplemented with flax seed oil (125 mg/kg body weight daily) (STZ-□3) and the other one not (STZ). The same criterion was applied for control groups (C-□3 and C). Periodical measurements of blood glucose concentration, cholesterol, triglycerides, and glucose tolerance curve (GCT), were taken as the indicative parameters of the metabolic alterations. Fatty acids were analyzed by gas chromatography.

**Results:** Blood glucose was recorded every week, and at the fourth month glycaemia was 106 mg/dl for C group, 117 mg/dl for C-□3, 145 mg/dl for STZ, and 106 mg/dl for STZ-□3. Induced hyperglycemia was not so high, and at this time, it was apparently controlled by omega-3, but not for so long. The fatty acid composition of mitochondrial placenta from diabetic rats showed higher percentage of saturated fatty acids. The MDA increased in C-□3 group 5.1 fold compared to C group and 2 fold compared to STZ and STZ-□3 ($P > 0.02$). In such conditions, it is clear that female lipid metabolism was not altered significantly, except in group C-□3 and the MDA production is very high. In this work, it will be shown that omega-3 fatty acids had only limited beneficial effects on controlling diabetes in female Wistar rats. Thus, since benefit to risks of modifying maternal fat intake in pregnancy are not yet completely understand, additional studies are needed before recommending ω-3 fatty acids intake during pregnancy.

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Regresar al programa

# CADMIUM: ANTIOXIDANT ACTIVITY OF SOD AND CAT ON THE REPRODUCTIVE SYSTEM IN PERIPUBERAL MALE RATS

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The heavy metal cadmium (Cd) is a pollutant associated with several modern industrial processes. Cd is absorbed in significant quantities from cigarette smoke but food is the main source for the non-smoking population, it is estimated that the intake of Cd in the diet is in an extensive range from 10–40 µg/day in no polluted areas to several hundred micrograms in polluted regions and is known to have numerous undesirable effects on health in both experimental animal s and humans, targeting kidneys and liver, in the others and there is no mechanism for the excretion of Cd in humans, thus Cd accumulates in tissues. Cd itself is unable to generate free radicals directly, however, indirect formation of reactive oxygen species (ROS) involving the superoxide radical, hydroxyl radical and nitric oxide. The toxic mechanisms of Cd are not well understood, but it is known to act intracellularly, mainly via free radical-induced damage, particularly in reproductive organs like testes, because Cd induces testicular damage and reproductive toxicity in rat. The intoxication with Cd in rats alters the activity of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT), both important in the reproductive system to form an antioxidant defense system that acts in the presence of high concentrations of superoxide radicals to reduce hydrogen peroxide, and together with other antioxidant enzymes maintain the balance between the generation and degradation of ROS within the tissues of the reproductive tract, a constant imbalance alters the structure and consequently the reproductive function in individuals exposed to Cd. However, a wide spectrum of deleterious effects on the reproductive tissues of the animals has not been investigated in peripuberal periods. The aim of this study was to determine the effect of Cd in the antioxidant enzyme activity in reproductive tissues. 1 mg/kg of $CdCl_2$ was administered intraperitoneal, from the first day of life (DL) until the days of euthanasia (35, 49, 56 and 70 DL), the activity of SOD and CAT was evaluated in testes (t), seminal vesicle (sv), epididymis (epi), prostate (pr) and penis (pn) on the peripuberal periods by the methods of Winterbourn (1975) and Beers & Sizer (1952), respectively. In our results, we observed that the activity of SOD and CAT are specific in each organ in the animals with Cd. So that SOD increased in t, sv and pn, and decreased in epi and pr. In the activity of CAT, we observed an increased in t, sv and pr, and decreased in epi and pn. However, is important to point that in 56 and 70 DL of the animals with Cd, the activities of these enzymes are decreased. In this study, the changes in the activity of the antioxidant enzymes can be considered an important indicator of oxidative stress in a given tissue.

Regresar al programa

# NADPH OXIDASE ACTIVITY IS REQUIRED FOR EPIBOLY CELL MIGRATION IN ZEBRAFISH EMBRYOS.

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NADPH oxidase (Nox) enzymes catalyze the formation of reactive oxygen species (ROS), superoxide and/or hydrogen peroxide. ROS are important signaling molecules involved in the regulation of major cellular behaviors such as cell migration. In zebrafish five Nox genes have been reported Nox1, Nox2, Nox4, Nox5 and Duox, from which Duox was found to participate in hydrogen peroxide formation during wound response using 3 days post fertilization fishes. However, detailed studies on the roles played by Nox genes in early zebrafish development are still lacking. During zebrafish gastrulation a major cell migration developmental process occurs, which is known as epiboly. During epiboly cells migrate from the animal pole into the vegetal direction covering the yolk cell. Recently we detected a distinctive ring of deep blue formazan deposition at the epiboly leading edge in zebrafish embryos stained with nitroblue tetrazolium salt (NBT). This pattern indicates the presence of superoxide in the leading epiboly region. Due to the particular capacity of Nox enzymes to form superoxide we propose that Nox are responsible for ROS formation at the epiboly leading edge and these molecules could participate in the control of cell migration. To test this hypothesis sphere stage embryos at epiboly onset, were exposed to VAS2870, a general Nox inhibitor. Our results indicate that this compound interfere with epiboly progression and suggest a role for the NADPH oxidase during this process. To identify the particular Nox enzyme(s) that is (are) involved in superoxide formation and epiboly control, we are analyzing the expression patterns of the different reported Nox genes and other important regulatory proteins. Currently we are carrying loss of function analysis by gene knock down of Duox and p22phox. In particular Duox knock down embryos show a slight delay in epiboly progression. These results indicate that other Nox genes are required for the epiboly to proceed. These functional analyses will be presented and discussed.

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Regresar al programa

# Immuno and confocal microscopy localization analysis of glutathione peroxidase 4 indicates diverse functions in early zebrafish development.

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Aerobic organisms use a variety of molecular and enzymatic antioxidants to metabolize and modulate reactive oxygen species (ROS) accumulation. In mouse the targeted disruption of glutathione peroxidase 4 gene (*gpx4*) presents mid gestation lethality between days 7.5 and 8.5, highlighting the relevance of this particular antioxidant enzyme in development. Previously we reported in mouse embryonic limbs that GPx4 expression and activity protects the forming digits from undergoing extensive cell death by limiting ROS accumulation to the interdigital tissues during limb remodeling. This previous evidence suggests that antioxidant enzymes localization pattern and activity participate in setting the regions of ROS accumulation in developing tissues. However detailed analysis of spatial and temporal patterns of GPx4 protein localization dynamics during whole organism development is still lacking, for this purpose the zebrafish embryos are suitable organisms due to its small size, transparency and external development. Therefore in the present study we characterized by immunofluoresence microscopy the GPx4 protein localization during the first 24 hours of zebrafish development. We found that GPx4 present an interesting localization at particular developmental stages that are complementary to the patterns of ROS distribution. In early cleaving embryos GPx4 is found in all blastomeres and excluded from the cleaving furrows. By the 128- to 512-cell stage, GPx4 is still found in all blastomeres but start to show a distinct nuclear localization in clusters of marginal blastomeres that eventually extend to the whole embryo. During epiboly, GPx4 is observed in all blastoderm cells and absent in the yolk cell. By 24 hour of development, GPx4 is found at the center of myotomes with decreased signal at the myosepta. In conclusion, the present study provides evidence that GPx4 protein present dynamic localization that could participate in determining the patterns of ROS accumulation. We are currently characterizing the mechanisms that are regulating the *gpx4* expression patterns and the developmental effects of GPx4 loss of function.

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Regresar al programa

# AN *IN VITRO* MODEL TO STUDY NEURONAL SENESCENCE

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Cellular senescence is a biological state in which cells permanently lose the ability to divide and whose most distinctive feature is the secretion of various proteins such as proinflammatory cytokines, chemokines, growth factors and proteases. Which altogether are called senescence-associated secretory phenotype (SASP). SASP mediates paracrine interactions between senescent cells and their surrounding microenvironment and has been implicated in tumor progression, inflammatory responses, embryonic development, aging and other cellular processes.
Cellular senescence seems to contribute to organismal aging, since senescent cells accumulate during life span, moreover, senescent cells selective elimination delays tissues dysfunction and extends healthspan in mouse.
Cellular senescence has thought to be an unexpected outcome in post-mitotic cells. Therefore, there are very few studies about neuronal senescence either *in vivo* or *in vitro,* hence the mechanisms and senescence-inducing stimuli in neurons remain unknown.
Our goal is to develop a model of neuronal senescence *in vitro* that might allow us to investigate the molecular basis of neuronal senescence and to evaluate their similarity to senescent phenotype described for mitotic cells.
Therefore, cortical cells derived from Wistar rat embryos isolated and maintained up to 40 days *in vitro* and several cellular senescence markers were determined. Our data showed that cortical primary cultures can be used as a model to investigate neuronal senescence at the cellular and molecular levels.

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# INSULIN ACTIVATES GROWTH AND $H_2O_2$ LEVELS IN ROOT HAIRS OF ARABIDOPSIS

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## ABSTRACT

The TOR kinase activity in Arabidopsis has been implicated in the root hairs growth; its inactivation with alteration in some cell-wall component and decreasing ROS levels in these organs (Leiber et al., 2010; Ren et al., 2012). In mammals, insulin and insulin-like growth factors (IGFs) stimulated cell growth through PI3K/TOR/S6K pathway (Zoncu et al., 2011), as well as ROS production. In plants has also been reported that insulin activates the aforementioned pathway (Sánchez de Jiménez et al., 1999), on the other hand, high levels of $H_2O_2$ at the root hairs apex promotes their growth (Cardenas, 2009). In our laboratory, we have observed that insulin stimulates growth and increases intracellular levels of $H_2O_2$ in root hairs of Arabidopsis. Interestingly, this effect does not occur in seedlings treated with inhibitors of TOR (rapamycin and Torin1), or mutants of this kinase (*tor-es1*). Since one of the effects of insulin in mammals is increase ROS levels through activation of NADPH OXIDASE, in the present study, was assessed inhibition of activity of NADPH OXIDASE (DPI), peroxidases (SHAM) and mitochondrial activity (antimycin and rotenone) on ROS production stimulated by insulin in Arabidopsis root hair. In the treatment with DPI, we saw a sharp drop in $H_2O_2$ levels, cessation of growth and an explosion of radical hairs apex, such effects were delayed in the presence of insulin. Regarding to inhibition of peroxidases, was observed a gradual decrease in growth and ROS levels. Experiments of inhibition on the system I and III of the respiratory chain are in process.

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# MEASUREMENT OF THE OXIDATIVE STRESS INDEX IN CRONICALLY EXPOSED TO VOLATILE ORGANIC COMPOUNDS (VOC) PEOPLE AND ITS RELATIONSHIP WITH METABOLIC POLYMORPHISMS

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Populations living nearby pollution sources are under a chronic exposure to diverse substances, some of them highly toxic. These substances contaminate lakes and rivers as a result of industrial activities and directly affect living creatures and human beings (Miller *et al*., 2004; Verkasalo *et al*., 2004; Motta *et al*., 2008). It has been informed that the Atoyac River located in the border limit of the Tlaxcala and Puebla states, is receiving domestic and industrial discharges without a proper treatment, causing a dramatic deterioration of the environment; various diseases have been reported affecting mainly the young, including leukemia and renal problems (J. Belmont, 2014).

The objective of this project is to study vulnerable groups (children age 8-12) from the settlements near the Atoyac River who have been chronically exposed to volatile organic compounds (VOC) like gasoline, toluene, benzene, chloroform, heavy metals, etc. from the industrial waste discharges carried by the river (Montero *et al.,* 2006; Palma y Morales, 2010). The oxidative stress index (OSI) will be determined in plasma samples to characterize one of the main defenses of the organism against an environmental aggression, just as the one that is occurring right now in this part of the country. The oxidative stress index (OSI) will be calculated using the total antioxidant capacity (TAC) and the total oxidant status (TOS) as determined by the Erel method (2004, 2005). These data will give information about the antioxidant response induction status. Furthermore, the genetic polymorphisms of enzymes involved in the metabolism of VOCs will be determined, like cytochrome P450-2E1*5, glutathione transferases (GSTT1 y GSTM1) (Montero *et al.,* 2007), and the NADPH dehydrogenase quinone (NQO1*2), to analyze whether any of these polymorphisms contributes to the oxidative status, increasing the susceptibility to VOC exposure.

During the standardization of the method to obtain OSI trough TOS and TAC, blood samples from students living in Mexico City were used, with the following characteristics: healthy active smokers (≥5 cigarettes per day) and healthy non-smokers, between 23 and 32 years old. TOS and TAC were spectrophometrically determined and the oxidative stress index (OSI) was calculated based on the relation between TOS and TAC, TOS as micromol $H_2O_2$ equiv/L and TAC as mmolTrolox equiv/L. OSI has no units and it's an indicator of the oxidative stress level in the sample (Altay *et al*., 2011). A significant difference ($p<0.05$) was found between the OSI from the active smokers samples (5.30 ± 0.746 N=22) and the non-smoker samples (2.94 ± 0.216 N=20), which was expected due the exposure to toxics of active smokers.

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# PURIFICATION AND CHARACTERIZATION OF TAENIA CRASSICEPS CYSTICERCI (CESTODA) THIOREDOXIN: INSIGHT INTO THIOREDOXIN- GLUTATHIONE-REDUCTASE (TGR) SUBSTRATE RECOGNITION.

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Thioredoxin (Trx) is an oxidoreductase central to redox homeostasis in cells and is involved in the regulation of protein activity through thiol/disulfide exchanges. Based on these facts, our goal was to purify and characterize cytosolic thioredoxin from Taenia crassiceps cysticerci, as well as to study its behavior as a substrate of thioredoxin-glutathione reductase (TGR). The enzyme was purified >133-fold with a total yield of 9.7%. A molecular mass of 11.7kDa and a pI of 4.84 were measured. Native electrophoresis was used to identify the oxidized and reduced forms of the monomer as well as the presence of a homodimer. In addition to the catalytic cysteines, cysticerci thioredoxin contains Cys28 and Cys65 residues conserved in previously sequenced cestode thioredoxins. The following kinetic parameters were obtained for the substrate of TGR: a Km of 3.1µM, a kcat of 10s-1 and a catalytic efficiency of 3.2×106M-1s-1. The negative patch around the α3- helix of Trx is involved in the interaction with TGR and suggests variable specificity and catalytic efficiency of the reductase toward thioredoxins of different origins.

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# MOLECULAR EVOLUTION AND CHEMICAL PROPERTIES OF THIOREDOXINS

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Thioredoxins (Trx) are small oxidoreductase enzymes that play a role in cellular redox systems by facilitating reduction of other proteins via their dithiol-disulphide active site. Thioredoxins act as antioxidants that reduce oxidative stress and other environmental stresses, protect proteins from oxidative aggregation and inactivation to promote protein folding, regulate apoptosis via denitrosylation, and modulate inflammation [1]. Thioredoxins have a similar three-dimensional fold comprising a central core of five β-strands surrounded by four α-helices. All feature a conserved active-site loop containing two redox-active cysteine residues in the sequence Cys-Gly-Pro-Cys (CGPC). Cys residues are the key to explain the biological activity of Trx because they are located in the active site of the enzyme. Recently, the X-ray crystal structures of seven laboratory resurrections of Precambrian thioredoxins dating up to approximately four billion years ago, has been reported [2]. In this work we present a theoretical study of the dynamical properties of the seven Trx, in addition with the *Escherichia coli* and the human thioredoxins. Using classical molecular dynamics simulations, structural properties on the region Cys-Gly-Pro-Cys (CGPC) of the nine thioredoxins were studied by assessing the root-mean-square deviation (RMSD), for each residue of CGPC region. The results show important changes in the conformation of the CGPC active site and of other regions between human, *E. coli* and ancestral Trx enzymes, which could be related to differences on their biological activity. Additionally, we calculated the electronic structure of the human Trx in order to explore the chemical reactivity properties of the active site of this enzyme. The objective of this project is to validate a computational method for protein families systematic study, integrating physicochemical parameters and structural properties of these biomolecules in their natural history under different pressures of environmental selection.

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# KINETIC BEHAVIOR OF WATERCRESS (*Nasturtium officinale*) AQUEOUS, ACETONIC AND ALCOHOLIC LEAVES EXTRACTS

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Reactive oxygen species (ROS) produced in aerobic organisms by internal and external agents, causes damage such as lipid peroxidation and DNA damage, these negative effects can be offset by the effect of antioxidant mechanisms due to its ability to capture free radicals. The **aim** was to analyze the kinetic behavior of antioxidants present in the aqueous, acetonic and alcoholic extracts of watercress (*Nasturtium officinale*). **Method**: The kinetic behavior of polyphenols was studied using 1,1-diphenyl-2-picrylhydrazyl (DPPH). After addition of different standard concentrations to DPPH (0.025 g litre$^{-1}$), the percentage of remaining DPPH was determined at different times from the absorbances at 517 nm. The percentage remaining DPPH against reaction time followed a multiplicative model equation: $\ln [DPPH_{REM}]=b \ln t+\ln a$. The slopes of these equations may be useful parameters to define the antioxidant capacity. The steeper the slope, the lower the amount of antioxidant necessary to decrease by 50% the initial DPPH concentration ($EC_{50}$). This parameter, $EC_{50}$, is widely used to measure antioxidant power, but it does not takes into account the reaction time. Time needed to reach the steady state to the concentration corresponding at $EC_{50}$ ($T_{EC50}$) was calculated, and antiradical efficiency (AE) as a parameter to characterize the antioxidant compounds where $AE=1/EC_{50}T_{EC50}$. AE is more discriminatory than $EC_{50}$. AE values are more useful because they also take into account the reaction time. **Results:** Extracts of Watercress presented a higher AE compared to the positive control ascorbic acid. The acetonic extract and the aqueous extract presented no differences in the AE. The alcoholic extract presented higher AE due to the polyphenol content. **Conclusion:** Watercress aqueous, alcoholic and acetonic extracts contains antioxidant activity. The alcoholic extract presented the highest antiradical efficiency.

**Keywords**: Antioxidant activity, kinetics, *Nasturtium officinale*, polyphenols.

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# PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF DEAMINATED-DNA REPAIR PATHWAYS IN *Bacillus subtilis.*

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DNA reacts continually with $H_2O$ (hydrolysis) and reactive oxygen species, resulting in multiple spontaneous DNA modifications. Three bases normally present in DNA contain exocyclic amino groups (cytosine, adenine and guanine). Deamination of these three bases generates the base analogues uracil, hypoxanthine and xanthine, respectively[1]. As deaminated bases in DNA have pairing specificities different from the original bases, if left unrepaired, these lesions are not only mutagenic but also potentially lethal. To avoid the noxious effects of amino group's loss in DNA, cells possess excision repair pathways to prevent mutagenesis and proteins belonging to these pathways are highly conserved in all three domains of life[2]. Recently, in our laboratory, it was reported that in the sporulating bacterium *Bacillus subtilis*, uracil in DNA can be processed by: *i*) Base excision repair system (BER) by the action of uracil-DNA glycosylase; *ii*) Mismatch repair pathway (MMR) through MutSL complex, and, *iii*) Alternative excision repair (AER) whose pathway apparently is initiated by the Endonuclease V (YwqL)[3]. In *Escherichia coli,* Endonuclease V (Nfi) is able to process, uracil and other deaminated bases including hypoxanthine and xanthine as well as other detrimental DNA lesions[4], presumably through an alternative repair pathway that requires the 3'□5' exonuclease activity of DNA polymerase I followed by gap-DNA synthesis[5]. Interestingly the DNA polymerase I of *B. subtilis* lacks a proofreading 3'□5' exonuclease activity; therefore, in addition to analyzing the spectrum of lesions that are recognized and processed by YwqL, we are investigating the mechanism by which the AER pathway, initiated by this enzyme, proceeds in *B. subtilis*. Since our results have shown that *ywqL* as well as *yxlJ* (the latter encoding a protein homologous to mammalian 3-methyladenine DNA glycosylase involved in processing hypoxanthine[6]) are expressed during sporulation, we are also analyzing the role played by these proteins in protecting spores from the genotoxic effects of the deaminating agents nitrous acid and sodium bisulfite. Current results showed that *ywqL* disruption decreased spore survival and that such effect was greater than that observed in spores lacking Ung and/or Yxlj as compared to wild type spores.

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# IDENTIFICATION AND SILENCING GENES *srrA* AND *msnA* IN A LOVASTATIN HIGH-PRODUCING STRAIN OF *Aspergillus terreus*

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Earlier studies from our group showed a link between reactive oxygen species (ROS) and lovastatin (LOV) biosynthesis in submerged (SmF) as well as in SSF. Results showed a ROS build up during production phase (idiophase) in both culture systems (1). In a subsequent work we showed that ROS regulate LOV biosynthesis at a transcriptional level, although the mechanism is presently unknown. It is considered that stress response transcription factor(s) could be this link between ROS and genes. Moreover, that work showed that *srrA* was up regulated in idiophase, and that the promoter regions of LOV biosynthetic- genes *lovE* and *lovF* contained putative binding sites for SrrA (Skn7) and MsnA (Msn2/Msn4) (2). SrrA and MsnA are transcription factors that are part of the cellular response to oxidative stress and other types of stresses (3).

The objective of this work is to study the possible involvement of SrrA and MsnA in the regulation of LOV biosynthesis.

Constructions for silencing gene *srrA* or gene *msnA* were based in our RNA interference vector pGdpki-RNAi that contains a double-stranded RNA expression cassette and a phleomycin resistant marker. A small fragment (exon) of the corresponding gene (obtained by PCR) was cloned between the two opposing promoters of the vector. The identification of the homologous genes in *A. terreus* was as follows: *srrA* was found in *Aspergillus terreus* NIH2624 genome, as hypothetical protein, with a note="similar to stress response regulator SrrA" (ATEG_03268.1) with accession number: XM_001212446.1. It was 89 % similar (537/645), e-value 0, to *A. oryzae* RIB40. On the other hand, MsnA protein sequence from *Aspergillus parasiticus* was used to search for its homologous in *A. terreus*. MsnA was found as a "Conserved hypothetical protein" in (ATEG_05308) AN: XP_001214486. And turned out to be highly similar (70%) (436/621) to the sequence of *A. parasiticus*.

*A. terreus* TUB- F514, a lovastatin high-producing strain was transformed (protoplasts) with srrA silencing construction: pGdpKiRNAi-srrA.

Some transformants, resistant to phleomycin have already been obtained. Preliminary results indicate that most of them show moderate sensitivity to $H_2O_2$. Interestingly, all of them displayed lower LOV production, although in different degrees.

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Regresar al programa

# CELULAR RESPONSE OF *Yarrowia lipolytica* TO OXIDATIVE STRESS CONDITIONSDESENTIS DESENTIS,

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The majority of living organisms depend on oxygen for survival; however, reactive oxygen species (ROS) may affect the cell metabolism. Oxidative stress can be defined as an imbalance between pro oxidants and antioxidants which potentially leads to a situation where important biomolecules undergo oxidative damage, thus compromising the cell viability[1]. Organisms also had to evolve a multitude of mechanisms to protect their cells from toxic effects of oxygen; these mechanisms involve non-enzymatic and enzymatic antioxidant defenses. Enzymatic antioxidant defense includes catalases, peroxidases, superoxide dismutases, among others[2]. In this study we use *Yarrowia lipolytica,* a yeast with biotechnological importance as an experimental model to analyze the effect of two oxidant agents ($H_2O_2$ and menadione) in the genetic expression of 3 catalases, 2 superoxide dismutases and 1 glutathione peroxidase genes, which are involved in the cellular response to oxidative stress in this yeast[3]. And also show if the use of gallic acid as an antioxidant agent has an impact in the expresion of the genes already mentioned. To determinate the proper peroxide and menadione concentration that was able to produce oxidative stress *Y. lipolytica*, serial dilutions of cells growing in logarithmic phase were spotted in YPD – plates containing different concentration of the oxidant agents. It was established that the proper concentration to use were 4.5 mM of $H_2O_2$ and 0.1 mM for menadione[4]. On the other hand, an in silico analysis from *Y. lipolytica* genome allowed to identify the sequence of mentioned genes and specific primers were designed. Total RNA from cells grown in different treatments was obtained and genes expression patron was determined by semi quantitative RT
– PCR.

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387.

Regresar al programa

# OXIDATIVE STRESS RESPONSE GENERATED BY HEAVY METALS IN *Candida glabrata*

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*Candida glabrata* has emerged as an important opportunistic pathogen in both mucosal and bloodstream infections. The description of its oxidative stress response (OSR) has been studied before and is known the contribution of enzymes such as catalase and superoxide dismutases and antioxidant molecules like glutathione. Moreover, the transcriptional factors Yap1, Skn7, Msn2 and Msn4 are key regulators in the OSR. Glutathione is an essential tripeptide-like thiol- containing molecule required to keep the redox homeostasis and also participates in the detoxification of metal ions. GSH is synthesized from glutamate, cysteine, and glycine by the sequential action of Gsh1 (γ-glutamyl-cysteine synthetase) and Gsh2 (glutathione synthetase) enzymes. GSH detoxifies metal ions and xenobiotics because of the high affinity of metals to thiols. GSH acts like a precursor for phytochelatins (PCs). PCs, (γ-Glu-Cys)(n) Gly polymers, which serve as high-affinity, thiol-rich cellular chelators and contribute to the detoxification of heavy metal ions, are derived from GSH and related thiols. We are interested in the study of the regulation of *GSH1* and *GSH2* genes in *C. glabrata* in response to heavy metals. For this purpose, we analyzed the growth of parental and *gsh2*Δ and *gsh1*Δ *pro2-4* (suppressor mutant) in presence of different heavy metals (cadmium, arsenic and mercury). We carried out transcriptional fusions of promoters with GFP protein and these plasmids were introduced in transcripcional factors mutants. The strains were analyzed by flow cytometry. We found that mutants in GSH synthesis are more sensitive to stress by heavy metals that parental strain. *GSH1* and *GSH2* are induced by cadmium and even more by arsenic.

Regresar al programa

# DNA DAMAGE AND EVALUATION OF GENE EXPRESSION OF THE BASE EXCISION REPAIR (BER) MACHINERY WHEN SUBMITTING TO OXIDATIVE STRESS A *ENTAMOEBA HISTOLYTICA*

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The protozoan parasite *E. histolytica* is exposed to oxidative stress during colonization, tissue invasion and metronidazol drug treatment. Although the mechanisms of DNA repair in this parasite are largely unknown, the genome of *E. histolytica* contains enzymes involved in several DNA repair pathways like the Base Excision Repair (BER). The BER pathway contains several proteins such as DNA glycosylase, a nuclease, a polymerase, PCNA and a DNA ligase. To date in this parasite the only characterized are the EhDNAlig I and EhPCNA proteins. In this work we induce oxidative stress using $H_2O_2$ in *E. histolytica* trophozoites. Viability and growth curves with recovery times were performed after exposure to different $H_2O_2$ concentrations. We determine that in 4 mM of $H_2O_2$ trophozoites maintained 70% of its viability and were able to grow. In this conditions we determine the formation of the 8-oxodeoxyguanosine adduct indicating oxidative DNA damage. In order to investigate the mRNA expression of BER genes we isolated RNA from *E. histolytica* and performed a semi- quantitative RT-PCR analysis. All the genes that could be involved in BER pathway are expressed under basal culture conditions. Although we only found upregulation of the EhOGG1 gene, the bifunctional glycosylase, which can attack the abasic site after the removal of the 8-oxodG base and the major enzyme for DNA repair via BER pathway To explore localization changes of EhDNALigI and EhPCNA after DNA damage we carried out inmunofluorescence assays to co-localized EhDNAligI with EhPCNA during recovery after $H_2O_2$ exposure**.** In basal condition, confocal images revealed the presence of EhDNAligI at the nucleus co-localizing with DAPI stain. EhDNAligI is mainly at the nuclear periphery where it co-localized with EhPCNA. After 1 and 3 h of recovery from $H_2O_2$ insult, the EhDNAligI showed a homogenously distributed at the nucleus and in foci-like structures, co-localizing with EhPCNA. Our findings suggest that BER pathway could be functional in *E. histolytica* and could have a role in the resistance of this parasite to oxidative stress.

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# A DNA DAMAGE DEPENDENT MECHANISM REGULATES THE RETURN OF *Bacillus subtilis* SPORES TO VEGETATIVE GROWTH.

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In response to DNA damage, cells activate checkpoint signaling mechanisms to control cell cycle progression and elicit DNA repair to maintain genomic integrity. In *Bacillus subtilis,* it has been described a protein termed DisA, which scans the chromosome for damage delaying the initiation of sporulation in response to chromosomal damage[1]. A recent work from our laboratory reported that a DisA-dependent checkpoint responds to oxidative DNA damage during spore germination/outgrowth to ensure the successful return of *B. subtilis* spores to vegetative growth[2]. In this stage the uptake of water into the spore core and the resumption of the aerobic metabolism promote the synthesis of reactive oxygen species (ROS) generating oxidative DNA lesions that can be processed by the AP endonucleases Nfo/ExoA in combination with the nucleotide excision repair system (NER) and the protein RecA[3]. *B. subtilis* spores lacking Nfo and ExoA were slow in returning to vegetative growth and disruption of *disA* suppressed this phenotype.

During germination/outgrowth, DisA-GFP fluorescent foci were found associated with the chromosome of outgrown spores; notably, the delayed outgrowth of *nfo exoA* spores was accompanied by a delay in chromosome segregation as well as in DNA replication. Moreover, repair of oxidative lesions in DNA and the slow germination outhgrowth phenotype were suppressed by *disA* disruption in spores deficient for Nfo and ExoA and there was increased mutagenesis in hydrogen peroxide-treated outhgrown *nfo exoA disA* spores compared outgrown *nfo exoA* spores challenged with the same oxidizing agent. Finally, the interaction of DisA with another proteins involved in DNA repair and/or recombination is currently under investigation in our laboratory.

1) Bejerano-Sagie et al. 2006. Cell, 125, 679–690.
2) Campos et al. 2014. J. Bacteriol. 196, 568-578.
3) Ibarra et al. 2008. J. Bacteriol. 190, 2031-2038.

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# EFFECT OF AQUEOUS EXTRACT FROM *SPIRULINA MAXIMA* ON INDUCED OXIDATIVE STRESS IN MICE SPERM

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ABSTRACT

*Spirulina maxima* is a blue-green alga widely used for therapeutic and health supplements foods, because of its various pharmacological properties, among them their antioxidant activity. Oxidative stress is an imbalance between the production of reactive oxygen species (ROS) and antioxidants. This excessive production of ROS or impaired antioxidant defense mechanisms in spermatozoa has become a major threat to spermatozoa functionality in humans. Sperm are extremely susceptible to damage by ROS because they exhibit in their membranes polyunsaturated fatty acids, in addition to, sperm can generate ROS derived from
their normal metabolic activity. Besides, the presence of low concentrations of $Fe^{2+}$ are sufficient to catalyze the formation of hydroxyl radical (•OH) and initiate lipid peroxidation. This study aimed to investigate the antioxidant activity of *Spirulina* extract on oxidative damage induced by ferrous iron/ascorbate (100 µM/150 µM) in sperm of CD1+ mice. We evaluated sperm motility and lipoperoxidation in spermatozoa treated with or without *Spirulina* extract. Treatment groups included:
1) Control, 2) ferrous iron/ascorbate (100 µM/150 µM), 3) *Spirulina maxima* extract (250 µg/ml), and 4) *Spirulina maxima* extract (250 µg/ml) + ferrous iron/ascorbate (100 µM/150 µM). Spermatozoa pre-treated with *Spirulina* extract 15 minutes before ferrous iron/ascorbate treatment exhibited a significant increase in motility (4.0-fold) and a significant decrease in lipid peroxidation concentration (4.3-fold) relative to spermatozoa treated with ferrous iron/ascorbate. These results suggest that aqueous extract of *Spirulina* may protect sperm quality (motility) from oxidative damage.

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# EFFECT OF CURCUMIN ON MITOCHONDRIAL FUNCTION IN THE EXPERIMENTAL RENAL INSUFFICIENCY

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**Background:** The pathogenesis of chronic kidney insufficiency (CKI) involves a complex
interaction of oxidative stress, inflammation and fibrotic processes that lead to subsequent progression toward end-stage renal disease The remnant kidney induced by
5/6 nephrectomy (5/6NX) is a widely used model to study the progression of CKI, which is characterized by the increase of oxidant stress and decrease in the activity of antioxidant enzymes. It has been reported that administration of curcumin (a bifunctional phenolic antioxidant) is able to reverse and/or reduce the alterations in the NX5/6 model; also it has been showed that curcumin has activities against mitochondrial dysfunction in several renal pathologies, in which the damage in mitochondria has been implicated in the pathogenesis. However studies at longer times (30 and 60 days after nephrectomy), have not observed variations in the parameters of mitochondrial respiration in the remaining kidney.
**Objective:** The objective was determined whether there were alterations in mitochondrial function at short times (24 hours) in the model of experimental IRC NX5 / 6 and if the curcumin was able to attenuate these alterations and the renal damage progression. **Method:** The *in vivo* model consisted of 3 groups of male Wistar rats with n = 7: Group control, nephrectomized and nephrectomized with a pretreatment of curcumin (60 mg/kg/day) per 7 days. The amount of creatinine and BUN (blood urea nitrogen) in plasma was quantified at 24 hours. The decrease in the total antioxidant power was evaluated by the total antioxidant capacity (TAC) corresponding to low molecular weight antioxidants in kidney and plasma using cyclic voltammetry (CV). The TAC in plasma also was evaluated by oxygen radical absorbance capacity (ORAC). Kidney isolated mitochondrias (24 hours after surgery) was used for the determination of mitochondrial parameters state 3, state 4, respiratory control index (RCI) and ADP/O using malate/glutamate as substrate. The activity of the mitochondrial complex I,II, III and IV also were estimated.
**Results:** A higher concentration of plasma creatinine and BUN, increasing in plasma TAC as well as decreasing in TAC of the kidney were found in nephrectomized rats. On the other hand, were found a decrease of the state 3, ICR and ADP/O and increase in the state 4 during respiration induced by malate/glutamate and a decrease in the complex I activity in Nx5/6 rats. All of these alterations were significantly attenuated by the administration of curcumin to 60 mg/kg/day.
**Conclusion:** Curcumin (24 hours after surgery) prevents the increase in blood levels of creatinine and BUN and observed changes in the kidney and plasma TAC in nephrectomized rats. Curcumin was also able to prevent uncoupling in mitochondria by the preservation of the activity of complex I which was evidenced by the decrease in state 3 and state 4 increases in nephrectomized rats.

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# HYPOXIA INTERFERES WITH RESPONSE TO 2-METHOXYESTRADIOL TREATMENT IN HUMAN NON-SMALL LUNG CANCER CELLS.

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**Abstract**

***Background:*** 2-Methoxyestradiol (2ME) is an anti-angiogenic, antiproliferative, and pro-apoptotic drug that, since it inhibits the proliferation of many human cancer cell lines in vitro, is considered to have potential clinical benefit in the treatment of cancer. Hypoxic tumor cells are known to be more resistant to current treatment modalities and more resistant to radiation than normoxic cells. ***Objective:*** The aim of this study was to compare the response in the cell growth of normoxic and hypoxic cells exposed to different concentrations of 2- ME. ***Methods:*** The percent of cell growth was determined by N-hexa- methylpararosaniline (crystal violet) staining assays, the apoptotic cells were analyzed by flow cytometry. Group comparisons for cell growth were performed by using the Mann–Whitney U test, The protein detection of HIF-1α, HIF-2α in total cell extracts it was through Western Blot. The expression assay for HIF-1α, HIF-2α was determined by real-time-PCR using the TaqMan Gene Expression Assay. ***Results:*** Treatment with 2-ME at 10 M is effective in increasing the levels of apoptosis in A549 cells under normoxic conditions. However, no effect of 2-ME on the cell growth inhibition under hypoxic conditions was observed. ***Conclusion*****:** Hypoxia is a factor in the resistance to 2-ME treatment in A549 cells.

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# AGED GARLIC EXTRACT INCREASES NEUROPEPTIDE Y, SUPEROXIDE DISMUTASE, CATALASE AND GLUTATHIONE PEROXIDASE mRNA LEVELS IN HYPOTHALAMUS OF DIABETIC RATS.

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Neuropeptide Y (NPY) is an important hypothalamic orexigenic neuropeptide. It´s related with regulation of feeding and promotion hyperphagia. Moreover, hypothalamic reactive species oxygen (ROS) production are key to the central regulation of satiety. However, during diabetes mellitus (DM), has been reported that exist hyperphagia and oxidative stress. The oxidative stress is characterized by excessive production of ROS and reduction of antioxidant defense mechanisms. It has been shown that substances with antioxidant capacity, revert several alterations found in the DM, but still not known molecular mechanism by which they exert this effect. Therefore, the aim of this study was to determine whether an antioxidant as aged garlic extract (AGE) has effect on mRNA levels of NPY, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), in hypothalamus of normal and diabetic rats in association with blood glucose concentration and ROS level. For this purpose, male Wistar rats (280-350g) were used. The animals were given food and water *ad libitum* and were maintained under static environmental conditions (12:12-h light/dark cycle). The rats were divided randomly into 6 groups: control (Control), control + 100 mg of AGE (C-100), control + 200 mg AGE (C-200), diabetics (D), diabetics + 100 mg of AGE (D-100), diabetics + 200 mg of AGE (D-
200). Diabetes was induced by a single injection of streptozotocin (60mg/kg bw) intraperitoneally (*i.p.*). AGE was administered at dose of 100 and 200 mg/kg bw/day *i.p.* for 4 weeks. Twenty-four hours after last treatment, blood was collected for estimation of glucose, and the animals were killed by cervical decapitation and hypothalamic region was separated from brain tissue to determinate NPY, SOD, CAT and GPx mRNA levels by performing real-time PCR, and to evaluate ROS content by flow cytometry. We found that in our study model, DM induces an increase in blood glucose levels ($p<0.05$) without significant changes in the level of ROS or in the mRNA levels expression of NPY and antioxidant enzymes, when compared to control animals ($p>0.05$). Administration of 200 mg of AGE attenuated the elevation in blood glucose levels in diabetic rats, and induced an increase in the level expression of NPY, MnSOD, GPx and CAT in the rats of group D and C-200 ($p<0.05$). AGE treatment not induces changes in the level of ROS in any group ($p>0.05$). These findings suggest that AGE has an important effect on the regulation of gene expression, and the daily administration of 200 mg/kg bw but, it does not affect the level of ROS.

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# EFECTO PROTECTOR DE LA S-ALILCISTEÍNA (SAC) EN UN MODELO DE ESTRÉS POR INMOVILIDAD EN RATAS

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Nowadays, the responses of the Central Nervous System to chronic stress remain unknown, although a considerable number of studies have demonstrated that responses to stress comprise a wide variety of neurochemical and morphological modifications in the brain. Noteworthy, a common component of chronic stress is oxidative stress, which is defined as the imbalance between reactive oxygen species (ROS) formation and the response of endogenous antioxidant systems. In this regard, several studies have shown that S-allylcysteine (SAC) a garlic-derived compound, possesses promising antioxidant properties, including its well-known ability to scavenge ROS, to augment the levels of enzymatic and non-enzymatic endogenous antioxidants, to increase the activation of the master redox regulator Nrf2, and to inhibit the activity of pro-oxidant enzymes; thereby SAC is a good natural candidate to test in protocols involving oxidative stress. In this work, we tested the behavioral, morphological and biochemical effects of a daily administration of SAC in the brain of rats submitted to chronic restrain stress. Rats were immobilized in a restrain device for 7 h per day during 21 days. SAC was given daily for 21 days at a dose of 50 mg/kg (i.p.), starting at day 1 of restrain, just 10 min before immobilization. Different endpoints were estimated at the end of the restrain protocol, including: 1) brain morphology,
2) ratio of cell death, 3) behavioral tests (forced swimming test, locomotor alterations and elevated "t" maze), 4) body weight, 5) antioxidant enzyme activities (glutathione reductase (GR)), and 6) oxidative damage to lipids. SAC protected the brain from cell death induced by stress at the morphological level in CA3 (hippocampus), striatum and cortex. In addition, the number of visits that stressed rats made to closed arms in the "t" maze as an index of anxiety was significantly reduced by SAC. In regard to enzyme activities and oxidative damage to lipids, both were increased in stressed rats mainly in cortex, and were reduced by SAC up to basal levels. Our preliminary results suggest that the protective effects of SAC on chronic restrain stress are due to the antioxidant properties of this natural agent.

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# PORCIN PEPTIDES ATTENUATE 3-NITROPROPIONIC ACID- INDUCED BRAIN DAMAGE IN YOUNG RATS

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Abstract
3-Nitropropionic Acid (3-NPA), a complex II inhibitor of the electron transport chain, causes Huntington disease-like symptoms after administration into animals. However, primary mechanisms of cell death and free radicals are not clearly understood. This study tested the hypothesis that porcin peptides (cerebrolysin) leads to the protection of brain against free radicals as consequence of 3-NPA administration, measuring the levels of dopamine and GSH biomarkers.
Methods. Male young Fisher rats (weight 80g) received the next treatments as follow: NaCl 0.9% (group A). Group B, cerebrolysin (200mg/rat); group C, cerebrolysin (200µg/rat) + 3-NPA 20mg/kg; all administered intraperitoneally daily for 3 days and 3-NPA in single doses, in the noticed doses. At the moment of sacrifice the blood was obtained to assess haemoglobin levels. Brain were obtained to dissected and measure dopamine and GSH concentrations, through fluorescence methods.
Results. Dopamine levels diminished significantly ($p = 0.03$) in cortex, hemispheres and cerebellum/medulla oblongata regions of animals that received cerebrolysin plus 3-NPA. GSH concentration increased significantly ($p = 0.02$) in cortex regions of animals treated with cerebrolisyn plus 3-NPA.
Conclusion. Porcin peptides attenuate the damage against free radicals as consequence of 3-NPA administration, and alter the dopaminergic metabolism in brain of young rats.

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# THE EFFECT OF PROTEIN SUPPLEMENTATION, THE EXERCISE OF RESISTANCE AND ITS RELATIONSHIP WITH THE OXIDATIVE STRESS IN OLDER ADULTS WITH SARCOPENIA

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**Introduction:** Aging is a deleterious process associated with a number of changes in physiological function and increased mortality. Based on the theory of aging free radical production is proposed that a large damage to biomolecules by reactive oxygen species (ROS), which leads to diseases such as sarcopenia, a syndrome characterized by a mass loss muscle, decreased strength and lower physical performance. The aim of this study was to evaluate the effects of a sports doctor program force on ROS production and muscle mass in healthy elderly and sarcopenia.
**Methods**: Two groups of elderly females 60 to 75 years were included. Formed by theoretically healthy older adults (ATS, n = 30) and the second group for older adults with sarcopenia (AS, n = 10). Both groups underwent a sports physician strength program for 12 weeks and anthropometric assessment, body composition, strength, physical performance, reaction times, flexibility and clinical chemistry profile (glucose, cholesterol and triglycerides) and markers of oxidative damage (MDA, carbonyls and SH groups) were determined.
All these measurements were performed before and after application of sports medical program.

**Results:** The implementation of the sports physician strength program generated a significant decrease in the percentage of body fat in both groups. Clinical chemistry profile in a significant decrease in glucose, cholesterol and triglyceride values was achieved leading to normal levels. Markers of oxidative damage to lipids (MDA) decreased significantly in both groups, however only a decrease in markers of damage to proteins was observed in the ATS group actually increased this marking was observed in the AS group. An increase in antioxidant defense systems (SH groups) was observed in both groups. Morphofunctional regarding capabilities in a diminution of fragility, increased reaction rate, motion, strength and flexibility was observed in both groups.

**Conclusions:** Resistance exercise without protein supplementation improves strength and physical performance in individuals with sarcopenia, but does not increase the percentage of muscle mass and elevated markers of damage to proteins (carbonyl groups) .The resistance exercise and protein complementation patients with sarcopenia leads to a state of reduced oxide (homeostasis), which leads to improved strength and increase the percentage of muscle mass.

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# EFFECT OF THE OBESITY IN PRESCHOOL AGE ABOUT REACTIVE SPECIES OXYGEN AND ANTIOXIDANT SYSTEMS, IN A PEDIATRIC HOSPITAL OF THE SECRETARIA DE SALUD DEL DISTRITO FEDERAL, MEXICO.

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**Introduction.** Obesity is the main risk factor for metabolic syndrome, and preschool age is one of the main stages for the genesis, development and programming of adipocytes. Based on the above, it can be said that children that are obese at 3-5 years of age, will be obese adults, as it will be very difficult for them to reduce weight, even if they make lifestyle changes.
**Methods.** Two groups of children aged 3 to 5 years were compared, one with normal weight (<85 percentile) and the other with obesity (≥ 95 percentile); subsequently, an anthropometric assessment was carried out, as well as a Z-score evaluation, quantification of lipid peroxidation, superoxide dismutase, catalase and glutation system.
**Results.** The group of preschoolers obese showed significantly higher values for weight, body mass index, lipids and proteins oxidized, total glutathione, and decreased superoxide dismutase and catalase compared with children with normal weight.
**Conclusions.** The metabolic changes caused by early obesity are characterized by increased lipid peroxidation and protein oxidized such as observed and described in the adult obese, however based on the results it can be inferred that obese children present changes of balance oxide-reduction, that participate in the pathologies associated in the obesity.

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# ANTIPROLIFERATIVE AND NEUROTOXIC EFFECT FROM EXTRACTS OF SOURSOP (*Annona muricata*) ON ASTROCITOMA CELLS (U87-MG)

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**Introduction:** The acetogenins are included in the *Annonaceae* family are secundary metabolites of the soursop, currently they have demonstrated a great contribution against oxidative stress and free radicals, combinet with a selective effect and antiprolipherative. Recently it was reported for the *European community* an atypical Parkinsonism case in the French Islands, because excessive consumption of tea or medicinal extracts, this effect could be involved in the phosphorilation of the Tau protein, it is described as a marker in neurodegenerative taupathies such Alzheimer and Parkinson diseases. The **aim** of this work was determined the antioxidant, antiproliferative and neurotoxic capacity of the aqueous extracts from leaf, seed and pulp of soursop was tested on astrocytome monolayers cells (U87-MG). **Methods**: In this work it was measured the antioxidant capacity by captation percentage and Trolox equivalent (TE) of soursop extracts with the radical 1,1-diphenil-2-picryl-hidrazilo (DPPH) through colorimetric method. The effect antiproliferative was determined *in vitro*, employing the reduction method of 3-(4-5-dimethylthiazol-2- yl)-2-5-diphenyltetrazolium bromide (MTT) on U87-MG cells used letal dose 50 ($LD_{50}$). **Results:** The antioxidant capacity values were 88, 55 y 53% for leaf, seed and pulp respectively, and a Trolox equivalent were 0.155±0.06, 2.5±0.01 and 0.012±0.001mM for the same extracts. The antiproliferative effect showed a $DL_{50}$of 0.38, 0.28 and 9.49µg/mL for soursop extracts used. **Conclusion:** The soursop extracts have a potencial effect antioxidant and antiproliferative in glioblastoma.

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# EFFECT OF AGED GARLIC EXTRACT ON THE EXPRESSION OF GLUT1 AND GLUT3 mRNA IN BRAIN OF DIABETIC RATS.

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Hyperglycemia in diabetes mellitus generates oxidative stress due to imbalance between the levels of antioxidants and reactive oxygen species, these species oxidized transcription factors than regulate gene expression of cerebral glucose transporters (GLUT1 and GLUT3), and induce dysregulation, neuronal damage and the possible onset of diabetic neuropathy. The purpose of this study was to evaluate the effect of aged garlic extract (EAE) on the expression levels of mRNA of GLUT1 and GLUT3 in brain of diabetic rats. We used 20 male Wistar rats, grouped in: control, diabetic, diabetic treated and treatment. The diabetes was induced with 60 mg / kg streptozotocin; the treatment of EAE was administered daily at a dose of 360 mg / kg for 42 days, the blood glucose levels was monitored, the rats was sacrificed and the brain was dissected, and we realized total RNA extraction, RT-PCR and Real-time PCR. The glucose levels in the diabetic group treated EAE decreased significant ($p < 0.05$) compared with control. The mRNA expression level of GLUT1 did not present changes in diabetic treatment group, but decreased significantly in the control group treated ($p < 0.05$). In the other hand, we did not find significant changes in the level of expression of GLUT3 in any of groups. It has been reported that the level of neuronal glucose, are tightly regulated in the BBB, and that during periods of hyperglycemia this regulation can occur, which explains the absence of changes in the messenger, since these carriers are regulated by glucose concentration. In conclusion, the EAE administered for 42 days in a dose of 360 mg / kg in diabetic rats results in an anti-hyperglycemic effect which has no significant effect on mRNA expression of GLUT1 and GLUT3.

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# ROLE OF REACTIVE OXYGEN SPECIES AND NOX IN THE CELL DEATH AND MORPHOLOGY OF CULTURED CEREBELLAR ASTROCYTES

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Reactive oxygen species are responsible of numerous cellular processes, including programmed cell death. Cell death implies morphological changes that may contribute to the progression of this process. In the nervous system, ROS are involved in physiological and pathological cell death processes. NADPH-oxidases (NOX) are a major source of ROS in many cell types including neurons and astrocytes. It has been reported that in cerebellar granule neurons, NOX2 is activated in response to staurosporine, leading to apoptotic cell death dependent on ROS. Although it is well known that astroglial cells have a relatively high antioxidant capacity, there is little information about the effect of ROS and NOX in astroglial cells during cell death. In this study, we explored the effect of staurosporine (St), ROS and NOX on the cell death and the morphology associated with cell death of cerebellar astrocytes. We found that St induced an early ROS production and NOX activation, which seem to participate in the death of astrocytes since NOX inhibition led to the protection of these cells under this conditions. We also found that astrocytes express NOX1, NOX2 and NOX4, suggesting that the cell death process could involve at least one of these homologues. Using astrocytes devoid of NOX2 and NOX3 we discarded that these homologues participated in the cell death process induced by St. Finally, we showed that during the cell death process, astrocytes change drastically their morphology, which seems to be due to the reorganization of tubulin and actin, and not by changes in the expression of these proteins. Although exogenous administration of ROS induced morphological changes similar to those observed with St in astrocytes, we found that the morphological changes produced by St are independent of ROS. We conclude that ROS generated by a NOX is required for cell death in astrocytes, but not for the morphological alterations induced by St.

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# HIGH CHOLESTEROL DIET INDUCES MITOCHONDRIAL INJURY, OXIDATIVE STRESS, AND LIVER DAMAGE

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**Background:** NAFLD is defined as infiltration of fat in hepatocytes, by more than 5% of them in the absence of alcohol consumption. Mitochondria are organelles with low colesterol content, that play an important role in reactive oxygen species (ROS) production. Alterations in mitochondrial function leads to energy depletion, and oxidative stress, playing a prominent role in NAFLD liver damage progression. **Objective:** To study mitochondrial role in liver damage by a high cholesterol diet. **Methods:** C57BL/6J male mice were fed with high cholesterol diet (HC, 2% cholesterol and 0.5% sodium cholate) or normal diet (Chow) for thirty days. Liver function test was determined in serum. Lipids content were performed by spectrophotometric assay. Global transcriptomic analysis was performed in an Illumina platform. DHE and DCFH were used to measure ROS production. Protein oxidation was determined using OxyBlot kit. Mitochondrial membranes potential (ΔΨ) were detected spectrophotometrically using safranine-O and by confocal microscopy using a MitoRed probe. Mitochondria morphology was analyzed by transmission electron microscopy. Mitochondrial proteins content were assayed by Western blot. **Results:** Cholesterol overload induced steatosis in liver tissue, with an increase in AST, ALT and ALP. Global transcriptomic analysis revealed 379 down regulated genes and 414 upregulated, particularly oxidative stress response among others. An increase in peroxides production in situ was observed judged by an increase of fluorescence. HC induced a differential expression of GPx isoforms, an increment of GPx-4 and a decrease in GPx-1. A decrease in mitochondria size, but an increase in mitochondria number was observed. ΔΨ was reduced in HC mitochondria, corroborated by Mitored fluorescence decrease by confocal microscopy, which confirmed mitochondria uncoupling. BAX, a pro-apoptotic protein, was increased, although Bcl-XL and Mcl-1, two anti-apoptotic proteins were also increased in HC livers. **Conclusion:** HC diet induced liver damage and dysfunction, associated with a decreased in mitochondrial membrane potential, although an increase number of mitochondria were observed. Mitochondrial Gpx-4 increased as a result of a peroxide production increment, while this response did not decreased oxidative damage. Finally, although BAX was increased an anti- apoptotic response was present in HC liver, which could be interpreted as a compensatory response.

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# Supplementation with L-Arginine provides protection against DNA damage caused by oxidative stress in cultures of human vascular endothelial cells

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**Introduction:** Preeclampsia is*!* a multisystem disorder of unknown etiology, associated with raised blood pressure, proteinuria and endothelial dysfunction during pregnancy. It has been described the participation of neutrophils in the induction of oxidative stress during preeclampsia, through secretion of pro- inflammatory cytokines and reactive oxygen species leading to endothelial dysfunction. On the other hand, it is well known that oxidative stress generates mutagenic base modifications in DNA, and 8-oxo-dG is one of the major products of DNA oxidation. Recently, it has been proposed that supplementation with L- Arginine reduces the incidence of preeclampsia in high risk women, probably due to its antioxidant activity. However, the molecular mechanisms involved in this protective effect need to be elucidated.

**Objective:** To address if supplementation with L-Arginine provides protection against DNA oxidation in cultures of human vascular endothelial cells.

**Methods:** Human vascular endothelial cells (HUVECs) were isolated from umbilical cord veins obtained from healthy women underwent cesarean sections at term, with no evidence of hypertension through the pregnancy. HUVECs were cultured in EndoGro media with LS supplement kit and 1% antibiotic, with or without 200 uM L- Arginine. Confluent HUVECs were stimulated with neutrophils activated with 50umol/L arachidonic acid (1:16 ratio of neutrophil/cells), TNF alpha (1 ng/mL), or 10% preeclamptic serum during 24 hrs at the same conditions; a fourth group was used as a control whithout stimulation. After incubation, cells were rinsed in PBS and fixed. Presence of 8-oxo-dG as product of DNA oxidation was determined by immunofluorescence.

**Results:** The proportion of nuclei that stained positive for 8-oxo-dG was significantly higher in the three experimental conditions compared to the control group. This proportion was decreased in cultures supplemented with L-Arginine.

**Conclusion:** Supplementation with L-Arginine provides protection against DNA damage caused by oxidative stress, as antioxidant defense in primary cultures of endothelial cells. This finding provides a novel insight about the molecular mechanisms involved in the protective role of L-Arginine during preeclampsia.

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# SUBCELLULAR LOCALIZATION OF NRF2 IN THE DIABETIC RAT RETINA

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Diabetes mellitus leads to several complications, including retinopathy; however, its pathogenesis is unknown. There is evidence in a variety of systems that hyperglycemia produces oxidative stress. Indeed, it has been reported oxidative stress in retina at long standing diabetes; then, it is not clear if oxidative stress is cause or effect of retina alterations in diabetes. The Nuclear Factor Erythroid 2- related (Nrf2) is known to regulate the transcription of antioxidant enzymes. In normal conditions, Nrf2 is in the cytoplasm, bound to Keap1 protein. Under oxidative stress conditions, Nrf2 is released from Keap1 and translocated to the nucleus, where it binds to the antioxidant response element (ARE), promoting the transcription of antioxidant proteins. Therefore, we studied the subcellular localization of Nrf2 in the rat retina at early induced diabetes. Retinas from normal and 7-45 days streptozotocin- diabetic rats were used. Nrf2 was determined by Western blot in nuclear and cytoplasmic fractions, and by immunohistochemistry in retinal sections, following conventional procedures. Positive immunoreactivity for Nrf2 was found in nuclear layers of the retina, and not differences were found at any condition. Western blot studies revealed a continuous increase in Nrf2 levels in the cytoplasmic fraction, which was statistically significant (25%) at 45 days diabetes. On the other hand, Nrf2 expression levels in the nucleus fraction were only changed at 20 days diabetes, where its levels were significantly decreased by
24%. Our results indicated that the normal redox state of the retina is maintained at early diabetes stages, suggesting that oxidative stress did not trigger the pathogenesis of diabetic retinopathy.

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# CURCUMIN PRETREATMENT AVOIDS Cr(VI)-INDUCED HEPATOTOXICITY

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Curcumin is a polyphenol derived from turmeric with recognized antioxidant properties. Hexavalent chromium compounds [Cr(VI)], such as potassium dichromate ($K_2Cr_2O_7$), have been widely recognized as an environmental toxic and carcinogen compound that induces oxidative stress. In liver, it has been described that the $K_2Cr_2O_7$ increases levels of reactive oxygen species (ROS) and induces damage to lipids and proteins generating tissue damage, ascites and mitochondrial dysfunction. The objective of this study was to evaluate the hepatoprotective effect of curcumin on the hepatic damage generated by $K_2Cr_2O_7$ in rats. Thus, animals were pretreated daily by 9-10 days with curcumin (400 mg/kg b.w.) by gavage before of a single intraperitoneal injection of $K_2Cr_2O_7$ (15 mg/kg b.w.). Groups of animals were sacrificed 24 and 48 h later, the ascites fluid was obtained and the liver was removed and weighed. $K_2Cr_2O_7$-induced damage to the liver was evident by histological alterations, ascites and increase in the liver weight as well as in the activity of alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase and alkaline phosphatase in plasma. At respect, curcumin successfully protected against $K_2Cr_2O_7$-induced hepatotoxicity. Moreover, curcumin attenuates $K_2Cr_2O_7$-induced oxidative damage in liver and isolated mitochondria, which was evident by the reduction in the content of malondialdehyde and carbonylated proteins and the reestablishment of the glutathione content and the activity of several antioxidant enzymes. In addition, curcumin restored mitochondrial function, maintaining mitochondrial respiration and the activity of mitochondrial enzymes aconitase, NADH:ubiquinone oxidoreductase and $F_1F_0$ ATPase, and maintained the ATP levels. The activity of complex II was not completely reestablished by curcumin, whereas complexes III and IV activities were unchanged. Besides, curcumin inhibited the opening of the mitochondrial permeability transition pore, evaluated as the depolarization of mitochondrial membrane potential and matrix swelling induced by $Ca^{2+}$. Therefore, curcumin avoided the rupture of the outer mitochondrial membrane and cytochrome c release. In conclusion curcumin's protective effect against $K_2Cr_2O_7$-induced liver toxicity is associated with prevention of oxidative stress and with preservation of mitochondrial function.

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# COCOA INTAKE INCREASES PHYSICAL FITNESS IN ATHLETES AND REDUCES MUSCLE DAMAGE AND OXIDATIVE

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## ABSTRACT

**Introduction**: Previous studies have demonstrated the protective effects of cocoa consumption, which it has been attributed to its anti-inflammatory and antioxidant properties. Exercise of sufficient intensity and duration result in increased production of reactive oxygen species in various tissues. The enhanced production of these species leads a cellular loss of redox homeostasis, generating conditions of oxidative and tissue damage.

**Aim**: To determine the effect of cocoa consumption on muscle enzyme as indirect markers of muscle damage, oxidative stress biomarkers and physical fitness in professional soccer players.

**Material and methods:** Fifteen players were evaluated before and after consumption of cocoa. Biochemical parameters were assessed to determine the health status of the subjects, biomarkers of muscle damage and oxidative were measured in plasma to establish muscle and redox status. In addition, physical performance was evaluated using the Cooper test.

**Results:** Before cocoa consumption, biochemical parameters showed the metabolic status healthy of study group however, muscle damage was detected. Interestingly, cocoa intake decreased the values of biomarker of muscle damage in 39.4% (CK) and 23.03% (LDH). Also, redox status was modified by the decreasing of carbonyl groups (26.31%), thiol groups (27.52%), MDA (32.42%) and increasing of total antioxidant capacity (TAC) (15.98%) and GSH-Px activity (26.37%). These data were in concordance with the increase of 4% in Cooper test, suggesting an improves in physical performance.

**Conclusion**: Our findings suggest that cocoa consumption by short period of time; it could be useful in the maintaining of a good physical fitness, due to the favourable effects on muscle and redox status in athletes with exhaustive exercise.

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# EFFECT OF A SPORTS MEDICAL PROGRAM OF FORCE ON OXIDATIVE STRESS IN HEALTHY ELDERLY AND SARCOPENIA.

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**Introduction:** Aging is a natural process of every living involves a number of degenerative changes such as reduced physiological function and increased likelihood of illness and death. There are many theories of aging, but the most accepted is the free radical oxidative stress damage to biomolecules, related to diseases such as obesity, hypertension, diabetes mellitus type 2 and sarcopenia. The strategy of resistance exercise and protein complementation arises to improve the quality of life of the elderly with sarcopenia. **Objective:** To evaluate whether protein supplementation and resistance exercise increases muscle mass, strength and physical performance in older adults with sarcopenia and determine its effect on oxidative stress. **Material and Methods:** A group of theoretically healthy older adults (CT3 n = 10) and a group of older adults with sarcopenia was formed randomly formed two groups, a control group PSP (n = 10) which was administered placebo and problem group (PCP n = 10) which was given a supplement of whey proteins. Both groups of older adults with sarcopenia were applied to a program of physical endurance activity for 12 weeks. Markers of oxidative stress and molecular damage were evaluated, and carried out the analysis of body composition, strength and physical performance. Before and after the program the same variables were determined.
**Results:** At the end of surgery, a decrease was observed in the percentage of body fat and an increase in the muscle percentage PCP group. Muscle strength and physical performance increased in both groups. Decreased serum glucose, cholesterol and triglycerides, markers of damage to lipids decreased in both groups. Markers of protein damage in the PSP group increased 65.8%. The antioxidant defense systems in the PCP group increased 75.9%. **Conclusions:** Resistance exercise without protein supplementation is a strategy to reduce levels of glucose, total cholesterol and triglycerides and improve strength and physical performance in individuals with sarcopenia, but does not increase the percentage of muscle mass and elevated markers protein damage. The association of resistance exercise and protein supplementation in older adults with sarcopenia leads to a redox state (homeostasis), it does increase the percentage of muscle mass and its contribution to improving strength gains independence in older adults with sarcopenia.

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# A HIGH CHOLESTEROL DIET ENCHANCES OXIDATIVE STRESS AND THE CARCINOGENIC EFFECT OF N-NITROSODIETHYLAMINE IN THE LIVER

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**Background**: Liver cancer represents one of the main adult malignances and leading cause of deaths worldwide. Nowadays, several liver cancer risk factors are known, such as, hepatitis virus B (HVB) or C (HVC) infection, insulin resistance and aflatoxin. Nevertheless, there is an emerging battery of information that points out that excessive cholesterol ingestion could be considered as another important pre-condition for hepatic cancer development; however, the particular carcinogenic effect of this lipid remains elusive. **The aim** of this work was to identify whether or not a high cholesterol diet is capable to disrupt the normal liver physiology setting up the microenvironment for cancer development. **Methodology:** A single dose of N-nitrososdiethylamine (10□g/g, ip) was administrated or not on 14-day old mice followed or not by hypercholesterolemic diet (HC) feeding (cholesterol 2%, sodium cholate 0.5%, at 16-days old, *at libitum*) for different times (0,2,5,7, 14 days and 8 months) mice were sacrificed and weighted; blood serum was recuperated and storage (-80°C) for further analysis; livers were weighted and divided for different biochemical assays (western blot, immunofluorescence, reactive species detection (ROS) *in situ* detection, HPLC). **Results:** Our data indicate that our model results in serum and liver tissue cholesterol accumulation after 7 days of the HC diet regimen. Moreover, increased lipid, protein and DNA oxidative damage were significant after 7 days of the DEN injection that were augmented when mice also under the HC diet regimen correlating with ROS content determined by fluorescent probes. Accordingly, the antioxidant enzymes battery were significantly increased when expose to DEN and HC. Importantly, the DNA damage repair-related proteins were significantly diminished in both groups HC and DEN and HC indicating that the HC diet alone is capable to impair DNA repairing processes that allow mutations accumulation and eventually to cancer. Finally, livers after 8 months of treatment were analyzed showing bigger, more vascularized tumors were found on DEN and HC groups. Our results strongly suggest that a liver cholesterol overload is a risk factor for liver cancer development.
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# METABOLIC, INFLAMMATORY AND OXIDATIVE STRESS PARAMETERS THROUGHOUT THE LIFE OF OBESE MICE GMS

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**Introduction:** Obesity is a major factor in the development of metabolic abnormalities such impaired glucose tolerance, insulin resistance, oxidative stress and inflammation. A similar scenario occurs during the aging process. Although some studies have been conducted on the pathophysiological effects of obesity, its impact associated with gender and throughout life, especially during aging, has not received attention.

**Objective:** To know the contribution of the obesity over the metabolic state through the life.

**Method:** In a model of induced obesity by neonatal neurointoxication using CD-1 female and male mice, the biochemical parameters, cytokine concentration, glucose tolerance and insulin sensitivity were quantified. In addition, oxidative damage was evaluated from 4 to 20 months of age.

**Results:** The Lee index (IL) and the levels of cholesterol and triglycerides gradually increased in all groups over time. GMS obese mice showed the highest levels of cholesterol; additionally, the highest levels of the triglyceride content were found in GMS female mice. Liver function increased according the age in all groups, showing a possible liver damage, especially in female mice. In terms of inflammatory profile, the group of males, particularly GMS obese mice presented elevated levels of TNF-α, IL-10, and IL-6 at 12 months of age, compared with the group of female mice. Adiponectin levels decreased gradually over the life; however male mice showed lower levels of adiponectin compared with female mice. Alterations in glucose tolerance and insulin sensitivity were found in female and male mice from young stages to aging. However, obese mice showed the highest levels, which decreased at 20 months of age. Oxidants and antioxidants parameters in plasma, liver, lung, heart and kidney allowed to know the influence of obesity damage during aging.

**Conclusion:** The metabolic parameters, low-grade inflammation, and oxidative stress gender, reflect the effects of obesity associated with aging. The obesity model GMS can be useful for studying the metabolic changes in obese organisms throughout life, also for the study of therapeutic agents that could prevent or reduce the mentioned alterations.

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# ANTIOXIDANT DEFENCES AND IMMUNOCOMPETENCE IN THE FISHING BAT *MYOTIS VIVESI* UNDER HARSH ENVIRONMENT CONDITIONS

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The fishing bat Myotis *(Myotis vives*) is an endemic species that lives in the desert islands in the Gulf of California, Mexico. This bat is accustomed to the extreme weather in the island, where summers are exceptionally hot (>45$^{o}$C) and winters are extremely cold (~2$^{o}$C) and windy.
It's known that during winter a los of bats enter torpor and some hibernate for several days in order to confront these environmental conditions. However, many aspects related to the hibernation process are highly stressing or even lethal for non-hibernating animals, so this adaptive hypothermia must come with a set of protective adaptations that allow a rapid recuperation from the low temperatures, metabolic depression and oxygen deficit. The aim of this work is to determinate if antioxidant activity in blood, and immunocompetence varies between autumn and winter seasons. We expect to find an enhanced antioxidant activity, and diminished immunocompetence in bats during winter. Therefore the activity of some antioxidants proteins such as superoxide dismutase and catalase were determinate, along with blood immunocompetence.

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# EFFECT OF ASCORBIC ACID AND ALPHA-TOCOPHEROL ON THE INDUCTION OF MICRONUCLEOUS AND APOPTOTIC CELLS IN MICE TREATED WITH VANADIUM PENTOXIDE

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Vanadium is a heavy metal considered by IARC (International Agency for Research on Cancer) as a possible carcinogen for humans, and although it has shown cytotoxic effects and widespread toxicity, there is few evidence of its genotoxic effects *in vivo*. However, some vitamins like ascorbic acid and alpha-tocopherol have antioxidant properties, so they have called attention in studies related to the modulation and the treatment of chronic-degenerating diseases related to DNA damage and oxidative stress such as cancer. In this study, we evaluated the effect of ascorbic acid and alpha-tocopherol on the induction of micronucleous and apoptotic cells in mice treated with vanadium pentoxide [$V_2O_5$]. Groups of five CD-1 mice were treated with: a) 100 mg/kg of ascorbic acid or 20 mg/kg of alpha-tocopherol; b) 40 mg/kg of $V_2O_5$ and c) with ascorbic acid or alpha-tocopherol prior to $V_2O_5$. The micronucleous were evaluated in agreement to the technique proposed by Hayashi *et al* (1990)[1], whereas the apoptosis and cellular viability was realized in agreement to the technique modified by García-Rodríguez *et al* (2013)[2]. Blood samples were obtained from the tail vein 0, 24, 48 and 72 h after each treatment. The results showed a significant increase in the frequency of micronucleous and the apoptotic cells in the group treated only with $V_2O_5$. While in the groups treated only with ascorbic acid or alpha-tocopherol decreased basal frequencies of micronucleous and increased the cell viability. In the mice treated with $V_2O_5$ which were administered previously with ascorbic acid or alpha-tocopherol it was observed that this was able of modulate the effect of high levels of genotoxic like cytotoxic because it decreased the frequency of micronucleous and apoptotic cells. Particularly ascorbic acid had a more consistent effect on the modulation of the genotoxic damage induced by vanadium pentoxide, probably to its antioxidant potential. With base in these results, it is suggested that, although exposure of human populations to metallic compounds such as vanadium may represent a genetic and cytotoxic risk, the substances with antioxidant potential such as ascorbic acid and alpha-tocopherol can prevent or modulate these effects.

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Regresar al programa

# ADDITION OF ANTIOXIDANTS IN THE EXTENDER FOR THE CONSERVATION OF SPERM QUALITY OF BOAR SEMEN FRESH

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Thawing and freezing sperm can increase the generation of reactive oxygen species (ROS), resulting in DNA damage, cytoskeletal alterations, inhibition of sperm-oocyte fusion axonema affecting sperm associated with loss of the motility. The aim of this study was to evaluate the effect of the addition of antioxidants vitamin C, E and their combination on sperm quality: motility, viability and acrosome integrity (NAR) of boar semen fresh. They used ejaculates of boars York race, motility, viability and acrosome integrity was evaluated and the MR-A diluent was used for conservation per six day. The diluted semen will be added the antioxidants vitamin C, E and their combination (vitamin C + E) in amount of 0, 1, 2 and 4 mg / ml, respectively. The results of motility, viability and NAR, were achieved with vitamin E at a concentration of 4 mg / ml of 39.33, 63 and 75.33% respectively as average of three replicates. In conclusion, the addition of vitamin E in the process of preservation of boar semen fresh, it is favorable on sperm quality in pigs.

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# EFFECT OF RACE AND THE ADDITION OF ANTIOXIDANTS ON SPERM QUALITY OF BOAR SEMEN FROZEN-THAWED

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The use of frozen semen in Swine Production Units, may be an alternative, especially to overcome the transport over long distances where the shelf life of sperm characteristics is of vital importance. The freezing of semen has five fundamental effects on sperm function and structure: changes in cell membranes, internal environmental changes, effects on the cytoskeleton, impact on mobility and effects on the heart. The sperm oxidative stress is the damage they may suffer in the integrity of structural components and physiological effect of which is directly related to decreased survival and fertilizing capacity after being ejaculated. Oxidative stress is caused by the formation of large amounts of reactive oxygen (ROS) or molecules containing free radicals, which are present during the handling and manipulation of the ejaculate. The aim of this study was to evaluate the effect of race and the addition of antioxidants to the extender on sperm quality of boar semen frozen-thawed. 6 ejaculates were used boars Pietrain Landrace and 1.5 years of age. Antioxidants used were vitamins C, E and their combination at concentrations of 2, 4 and 6 mg / ml, the freezing method was proposed by (2) in
0.25 ml plastic straws and assessed motility, viability and acrosomal integrity ( NAR) after thawing. The results obtained after thawing of semen were 72% motility, viability, NAR 95% and 57% in semen from Landrace, with Pietrain semen of males, the results were 68% motility, viability NAR 83% and 57% with concentrations of 2, 4 and 6 mg / ml vitamin C, E and C + E, respectively. The breed of boars is crucial to the success of the freezing of semen, the use of antioxidants in the conservation of boar semen by freezing, is a promising area, since it preserves indefinitely sperm characteristics related to their fertilizing ability.

Regresar al programa

# "Induction of premature senescence by different stimuli in a rat astrocyte primary culture"

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Senescence is recognized as a cellular state in which cells lose their ability to replicate. However, it is known that cells can achieve this state as a response to different stimuli, such as telomere shortening (Replicative Senescence, RS) or oxidative stress (Stress Induced Premature Senescence, SIPS). Actually, some other stimuli have been recognized to induce the senescence response, such as oncogenic stress, proteasome impairment, autophagy impairment, hsp70 depletion, strong mitogenic signals and □/UV exposure.

The aim of this work is compare different senescent induction pathways in primary astrocytes. Hence, we induced premature senescence in primary rat astrocytes using different stimuli in order to characterize different types of senescence. Senescence was induced by oxidative stress ($H_2O_2$), proteasome inhibition (epoxomicin) and autophagy inhibition (cloroquine) and senescence classic parameters were evaluated, cellular proliferation, DNA synthesis and □- Galactosidase activity assay (SA-□-Gal), as well as some cell cycle inhibitors such as p16, p21 and p27 expression.

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# ANTIOXIDATIVE EFFECT OF DOCOSAHEXAENOIC ACID (DHA) ON INDOMETHACIN INDUCED GASTRIC DAMAGE IN MOUSE

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Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used to treat rheumatoid arthritis, acute pain or fever. However, NSAIDs usually produce side effects during prescription, including gastric erosion, ulceration and bleeding of the tissue. Previous studies have shown gastroprotective effects of docosahexaenoic acid (DHA, an omega-
3 long-chain polyunsaturated fatty acid) on indomethacin-induced gastric damage. The aim of this study was to evaluate the antioxidative effect of DHA in indomethacin-induced gastric damage in mouse. DHA (100 mg/kg) was administered orally 2 h before oral administration of indomethacin (30 mg/kg). Gastric ulcer formation was estimated macroscopically 5 h after indomethacin administration. Carbonyl groups were determined in mice serum, while malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) were determined in gastric tissue. Macroscopically and microscopically DHA administration demonstrated a reduction of the ulcer severity during indomethacin-induced gastric damage. Pretreatment with DHA induced an augmented of GSH and SOD levels compared with the group only treated with indomethacin however CAT showed no significant difference. On the other hand, we observed that indomethacin-induced gastric damage was accompanied by the development of oxidative stress, evidenced by the accumulation of carbonyl groups and MDA. These data suggest that DHA pretreatment induces antioxidant effects.

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# COMPARATIVE PROFILE OF OXIDATED FATTY ACIDS IN ADIPOCYTES, SUBCLINICAL ATHEROGENESIS AND METABOLIC RISK BETWEEN NON-OBESE AND OBESE PATIENTS.

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Endothelial dysfunction and metabolic disorders leading to progressive atherogenesis are considered cardiovascular risk factors in obese patients; whereas participation of nitric oxide (NO) and lipid oxidation in adipose tissue is yet not clear. The aim of this research was to estimate whether plasma NO and oxidized fatty acids profile, as well as *in vitro* NO from isolated adipocytes are related to endothelial dysfunction, subclinical atherogenesis and metabolic risk in non-obese and obese patients.

**Methods.** This research was performed as a cross-sectional study. Obese group was constituted by morbid obese patients programmed for bariatric surgery. Non-obese patients group was constituted of healthy individuals who underwent surgery due to abdominal hernia. Obese group was further divided as: Metabolically Unhealthy Obese (MUO, defined according to metabolic syndrome, using NCEP-ATPIII criteria) and Metabolically Healthy Obese (MHO). Adipocyte isolation was performed according to a standard protocol and further stained with oil red as a marker for adipocytes. NO was measured through nitrate-nitrite reaction, reflecting nitric oxide production, and determined either in plasma or in 2-weeks cultured adipocytes isolated from visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT). Endothelial dysfunction and subclinical atherogenesis were measured through ultrasonographic determination of brachial artery Flow Mediated Dilatation (FMD) and Carotid Intima Media Thickness (CIMT), respectively. The oxidized fatty acids profiles were determined by commercially available Multiplexing kit.

**Results.** Ten patients were eligible for this study, aged 40 ± 9 years-old, gender male 4, female 6; whereas 6 obese patients were classified as MUO. Plasma levels of oxidized fatty acids were different between non-obese and obese patients. Concentration of NO directly correlated with NO from cultured adipocytes isolated from VAT, and inversely correlated with NO from SAT isolated adipocytes. In order to include both NO determinations *in vitro*, the ratio of NO from VAT adipocytes / NO from SAT adipocytes was used for further analysis. Plasma NO levels showed correlation with Body Mass Index (BMI) (r=-0.6, p=0.4) and higher values were observed in UHO patients; while NO produced by cultured VAT/SAT adipocytes showed particular correlation with FMD and CIMT.

**Conclusion.** Results from our study suggest early evidence that plasma oxidized fatty acids as well as NO levels and NO produced by VAT/SAT cultured adipocytes are related with metabolic risk, endothelial dysfunction and/or subclinical atherogenesis in obese patients.

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# TROLOX AND DHEA EFFECT ON SENESCENCE ASSOCIATED SECRETORY PHENOTYPE MODULATION

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Cellular senescence is a phenomenon characterized by the irreversible growth arrest in G1 phase of the cell cycle, usually mediated by p16 and p21. Furthermore senescent cells secrete several molecules that modify their microenvironment and that altogether are called senescence associated secretory phenotype (SASP). SASP is characterized to content pro-inflammatory molecules such as cytokines, growth factors and chemokines that can be involved in several age-related pathologies.

Senescence can be achieved by diverse pathways. The classical senescence is attained telomeres shortening and is called Replicative Senescence (RS). Moreover senescence can be prematurely induce as a response to different stressors: oxidative stress (Stress-induced premature senescence SIPS) and proteasome impairment (Proteasome inhibition-induced premature senescence, PIIPS) and others; depending on the senescence induction pathway, SASP molecules are expressed in a different way. The aim of this work was to determine if some molecules such as the antioxidant trolox and the hormone dehydroepiandrosterone (DHEA), are able to modulate SASP secretion in the diverse kinds of senescence. RS, SIPS and PIIPS were characterized and cytokines IL-2, IL-4, IL-6, IL8, IL10 GM-CSF, IFN-γ and TNF-α were determined in SASP obtained from the different kinds of senescent cells.

Interestingly, senescence cells induced with the dives stimulus produced SASP with dissimilar cytokine components.

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# ASSESSMENT IN-VITRO OF ANTIOXIDANT CAPACITY FROM NANCHE EXTRACT (*BYRSONIMA CRASSIFOLIA*)

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Nowadays one of the causes that plays an important role in the development of chronic degenerative diseases like cancer is the cellular oxidative stress, which is caused by an increase of free radicals. It has been recently suggested that consumption of antioxidant- rich fruit may help reduce the negative effects of cellular stress. The nanche (*Byrsonima crassifolia*) contains bioactive compounds such as vitamin C, carotenoids, and polyphenols considered as major natural antioxidants. The aim of this work is to demonstrate the antiproliferative effect of bioactive compounds in nanche on breast cancer.

**Methodology:** A colorimetric method was used by employing the radical 1,1- picryl-hidrazilo difenil2 (DPPH) to measure the antioxidant capacity. The antioxidant capacity of the nanche extract was determined by reducing the complex $Fe^{3+}$ to $Fe^{2+}$ (FRAP), using Trolox equivalents (TE) as reference standard. The antiproliferative effect was carried out from in-vitro assays by using as method the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and Alamar-Blue Reagent (7-hydroxy-3H-phenoxazin-3-one-10-oxide) on breast cancer cell lines MDA-MB 231 (metastatic) and MCF-7 (non-metastatic).

**Results:** The proteins involved in the antiproliferative effect were determined by searching carbonylated protein in breast cancer cell lines treated and untreated with nanche extract. The nanche extracts developed a high antioxidant activity, with values of 223.96±22.9 and 184.16±22.2 µMeqTrolox/g DPPH and FRAP respectively. For in vitro assays, two trials were determined, the LD50 (MTT) with a value of 110±01 and 122±04 mg /mL for MDA-MB 231 and MCF-7 respectively, and the reduction (Alamar-Blue) which for MDA MB-231 was 44% and 54% for MCF-7 in a period of 48 hours for both lines.

**Conclusion:** The nanche extract has antioxidant activity, due to the content of bioactive compounds present in the fruit (polyphenols, carotenoids and vitamin C), which have a cytotoxic effect on breast cancer cell lines.

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# STUDY OF MODULATION OF DNA REPAIR BY AUTOPHAGY AND THE NUCLEAR RECEPTOR FAMILY NR4A.

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Along embryo development and throughout life, the genome is constantly modified by endogenous and exogenous reactive molecules. It is essential to maintain genome integrity to sustain cell viability, to ensure proper development and to promote a healthy life span. Hence, cells have evolved several mechanisms to detect and repair damaged DNA. Mutations in some DNA repair genes cause neural development defects, show features resembling accelerated aging or correlate with cancer and neurodegenerative diseases.

Autophagy is a catabolic process that eliminates, among other substrates, dysfunctional mithocondria producers of reactive oxigen species; hence autophagy protects nuclear and mitochondrial genomes. Notably, autophagy also keeps genome integrity by controling cytokinesis and retrotransposition rate. Once DNA is damaged, autophagy promotes its repair. Accordingly, autophagy defects result in genome instability.

In previous work we found that nuclear receptor NR4A1 is essential for autophagic cell death induced by three different stimuli, leading us to propose that NR4A1 modulates autophagy. Interestingly, members of the NR4A family also regulate several DNA repair pathways. Our goal in this work is to determine whether autophagy and NR4A promote DNA repair in a coordinate way.

We will present results comparing the response of nomal cells (mouse embryonic fibroblasts) with cancerous cells known to express NR4A (mouse melanoma cell line B16-F10 and human lung carcinoma cell line A549), exposed either to Irinotecan, which induces single strand DNA breaks or to Etoposide, which induces double strand DNA breaks. The contribution of NR4A1 and autophagy to stimulate repair DNA will be discussed.

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# CHOLESTEROL OVERLOAD ENHANCES LIVER DAMAGE DUE TO A DECREASE IN ANTIOXIDANT PROTEINS IN MICE SUBJECTED TO BILE DUCT LIGATION

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It is well known that lipid overload, particularly cholesterol, sensitizes to hepatocellular damage. NAFLD is defined as infiltration of fat in hepatocytes in the absence of alcohol consumption. The accumulation of fat in the liver induces cytotoxicity and sensitizes the organ to a second aggression. Mitochondria are organelles with a very low content of cholesterol and are considered the main source of reactive oxygen species (ROS). Alterations in mitochondrial function leads to energy depletion, and oxidative stress, playing a prominent role in liver damage and the progression of NAFLD. Moreover cholestasis is the condition in which the bile flow from the liver is slowed or blocked, causing bile salts, bilirubin, and lipids to accumulate in the blood stream and the liver. We were focused to figure out the effect of cholesterol in liver damage after bile duct ligation (BDL) and the involvement of the antioxidant system in the healing process. *Methods.* C57Bl/6 male mice were fed with the HC diet (Cholesterol 2% and Sodium cholate
0.5%), control animals were fed with regular diet (Chow) for two days. BDL surgery was conducted following standard methods. Liver function tests, and bile acids in tissue and serum were evaluated. H&E, TUNEL, ORO and IH stains were accomplished, also antioxidant proteins were analyzed by WB. *Results.* Data show that HC animals were more susceptible to both insults; all animals in the HC-BDL group (n=6) died during the first 72 h after surgery, while Chow-BDL mice presented a 100% of survival (n=7). Liver macroscopic inspection of HC mice showed the characteristic pale color in steatosis and changes in gallbladder. Although AST, ALT and ALP were increased as a consequence of BDL, animals fed with the hypercholesterolemic diet increased significantly these values (ranging from 20- to 200-fold), these data were in agreement with an elevation on bilirubin and bile acids. H&E staining show how BDL aggravates damage in chow mice liver by necrosis, improving the repair process at the third day, not so HC animals. TUNEL staining indicates an increment of apoptosis in HC mice, also Ki67 immunohistochemestry show a rise in proliferation in chow animals but not in HC mice, suggesting an exacerbation of cholestatic damage. On the other hand examination of antioxidant enzymes such as catalase, SOD 1, SOD 2, GST, γGlutamil, and gpx1-2, were increased in chow mice over control, meanwhile HC animals presented a decrease regarding the HC control. In conclusion our data suggest that cholesterol overload aggravates cholestasis and leads to dead as a result of increase oxidative stress generation, due to a decrease in expression of mitochondria antioxidant enzymes.

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# DIFFERENTIAL MITOCHONDRIAL DYNAMICS IN REPLICATIVE SENESCENCE (RS) AND STRESS INDUCED PREMATURE SENESCENCE (SIPS) IN HUMAN LUNG FIBROBLASTS WI38

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Aging is a universal an irreversible physiological process that is related to continued deterioration as well as to the progressive loss of the organism adaptive capacity. Senescence is a cellular phenomenon related to aging, which occurs when the cells reach their maximum replicative capacity and therefore irreversibly stop the cell cycle in G1 phase. Cells can achieve senescence by different pathways or stimulus, such as telomere shortening during cellular proliferation, in which case this event is called replicative senescence (RS), or due to bimolecular oxidative damage, and it is then called stress-induced premature senescence (SIPS).

It is now know that mitochondria are dynamic organelles that are able to modify their morphology due to subcellular processes such as fusion, fission and biogenesis, which altogether are called mitochondrial dynamics (MD). During senescence this process is altered, however the exact modifications in MD are still unclear, and nothing is known about the differences among diverse senesce-induced MD. Hence the aim of this work was to understand if there are variations in MD due to the senescence induction pathway. Human lung fibroblasts WI38 were used to study MD in RS and SIPS. MD central proteins were determined by western blot: Opa1 (fusion), Drp1 (fission) and PGC1α (biogenesis), as well as the mRNA expression of some mitochondrial fusion protein such as Mfn 1 and 2 (fusion); Drp1, Fis1 and Mff (fission). Mitochondrial network was also evaluated by confocal microscopy. Our results showed that Opa1 decreased during SIPS while Drp1 increased in SR. Interestingly, SR cells maintained the normal tubular morphology, whereas SIPS cells had a different morphology suggestive of a hiperfused network in the perinuclear region with fragmentation and probable mitochondrial loss in the peripheral zones.

More experiments are necessary in order to completely characterized in senescence, however this work shows for the first time that MD in senescent cells might be different depending of the senescence induction path.

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# EFFECT OF TREATMENT WITH SIMBIOTIC ON OXIDATIVE STRESS AND METABOLIC PARAMETERS IN OBESE CHILDREN IN SCHOOLAR AGE

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**Introduction:** An imbalance of energy uptake and expenditure is only one aspect of a complex system leading obesity. Recent research has also suggested that the gut microbiota plays an important part in energy extraction, storage and expenditure. Indeed, some studies demonstrate the effects of the gut microbiota on host metabolism. Therefore, a balanced microbiota could be an important factor in the obesity treatment to regulate clinic parameters, oxidative stress, inflammation and metabolic parameters.
**Aim:** To determine the effect of a symbiotic treatment (probiotic and prebiotic) in obese children on
biomarkers of oxidative damage, inflammation, insulin resistance and metabolic parameters
**Methods:** Twenty obese children (7-9 years old, with BMI in percentile >95) were included in the study.
Children theoretically healthy with the same age and in the percentile >25 and < 85 were included as control group. Written informed consent was obtained from all participants. The obese patients were divided in two treatment groups (n=10), the first one was treated with lifestyle modification (LSM). The second group was treated with LSM and the prescription of symbiotic (LSMS). Before and after of the treatments, measuring anthropometric, physical fitness (Harvard test), biochemical parameters (Lipids, hematic biometric, transaminase etc.), biomarkers of oxidative stress (Carbonyl groups, quinones groups, thiobarbituric reactive substance (TBARS), malondialdehyde (MDA), thiol groups (SH) and glutathione peroxidase (GSH-Px)) were assessed; also inflammation markers (Arginase activity, TNFα, and Protein C reactive), glucose tolerance and insulin concentration were evaluated. Student's t-test and ANOVA were used to determine differences statistically significant. P-values < 0.05 were considered statistically significant.
**Results:** Before of treatments HDL, GGT, physical fitness, HOMA-IR, Matsuda and Protein C- reactive
were statically significant between healthy and obese children. Interestingly, post-treatments both groups showed a decreasing in weigh, BMI, waist, hip and in the Harvard test. Furthermore, in LSMS group significant changes in TGO and TGP were observed. Finally, not differences in biomarkers of oxidative stress, inflammation and insulin resistance were observed after treatment between the study groups.
**Conclusion:** The data obtained in the study showed that oxide reduction status between healthy and
obese children is similar, and that the treatment with LSM or LSMS not modifies the oxide-reduction and inflammation status of obese children; however anthropometric parameters can be modified better by LSMS than LSM treatment.

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# EFFECT OF ADMINISTRATION OF FLAVONOIDS (QUERCETIN-3-RUTINOSIDE AND MYRICETIN) ON GENOTOXIC AND CYTOTOXIC DAMAGE IN MICE CD-1 TREATED WITH HEXAVALENT CHROMIUM: MICRONUCLEUS, APOPTOSIS AND CELL VIABILITY

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Chromium is a heavy metal and it is known that its oxidation state VI (Cr VI) is its most toxic form since it induces genotoxicity and citotoxicity. The way in which the Cr VI induces DNA lesions is by generating reactive oxygen species (ROS) during intracellular reduction to Cr III. In contrast, in recent decades antioxidants have been used in diets due to its properties to prevent chronic degenerative diseases and even decelerates aging. In this study, the effect of quercetin-3-rutinoside and myricetin on the genotoxic and citotoxic damage induced by the Cr VI was evaluated. Groups of five mice CD-1 were treated with flavonoids (625 mg/kg of quercetin-3-rutinoside and 1 mg/kg of myricetin), hexavalent chromium (20 mg/kg of $CrO_3$) and the flavonoid prior to $CrO_3$. DNA damage was evaluated by analysis of micronucleus (MN) using the acridine orange technique[1], and apoptotic and cell viability using the technique described by McGahon *et al*., (1995)[2], with modifications[3]. Blood samples were obtained from the tail vein 0, 24, 48 and 72 h after each treatment. The results showed that the treatments with flavonoids (quercetin-3-rutinoside and myricetin) alone did not modify MN frequency. $CrO_3$ treatment significantly increased MN frequency after the injection. When quercetin-3- rutinoside or myricetin were administrated prior to the injection of $CrO_3$, the MN frequency decreased compared the group treated only with $CrO_3$ and his reduced more than 70% and 30% respectively at 48 h. Concomitantly there was a greater presence of MN in the group treated with $CrO_3$ alone. Regarding apoptosis by combining the treatments of antioxidants with $CrO_3$, there was a decrease in apoptotic cells; the cell viability had a slight increase in the viable cells of the experimental groups in comparison to the group administrated with $CrO_3$. We conclude that both quercetin-3-rutinoside and myricetin may modulate genotoxic and citotoxic damage induced by $CrO_3$.

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# "THE ROLE OF REACTIVE OXYGEN SPECIES IN THE ASSEMBLY AND STABILIZATION OF MITOCHONDRIAL SUPERCOMPLEXES IN HEART"

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Reactive oxygen species (ROS) are highly reactive with lipids, proteins and nucleic acids. Depending on its concentration, localization and the molecular context in which they are produced may have beneficial or damaging effects in the cell. Excessive ROS generation contributes to cell death by necrosis or apoptosis and to the development of different pathologies. Mitochondria are main source of ROS due to electron leakage at the respiratory complexes of the electron transport chain (ETC) as the superoxide radical ($O_2^{\bullet-}$) is produced by the one-electron reduction of $O_2$. Indeed, mitochondria are both source and target of ROS action. For this reason, mitochondrion plays a central role in the etiology and pathogenesis of cardiovascular diseases.

The structural and functional organization of the ETC has been subject of debate since almost 60 years ago. On one hand, it has been sustained that electron transfer is based on random collisions and that the components of the ETC diffuses freely in the membrane; other experimental evidences indicate that the complexes of the ETC maintain stable interactions and that are associated in supercomplexes which function as an unit [1,2]. Diminution in the content of such structures has been related with experimental heart failure, suggesting that the assembly of supercomplexes might be modified by oxidative stress and lead to mitochondrial dysfunction in pathological conditions[3].

Oxidative stress is a condition associated with ischemic-reperfusion damage, therefore in this project we evaluated if mitochondrial dysfunction in reperfused hearts is related with changes in the organization of supercomplexes and the effect of ROS in the assembly of such structures. To accomplish this objectives, we administrated the antioxidant N-acetilcysteine (NAC) and applied the cardioprotective strategy of postconditioning to ischemic-reperfused hearts. Hearts from the four groups: 1) Control, 2) IR (ischemic-reperfused), 3) IR + NAC and 4) IR + Post were used to obtain mitochondria. Supercomplexes assembly and the activities of individual complexes were measured in blue native gels.

Cardiac work decreased in the IR group early during reperfusion and changes in the pattern of respiratory supercomplexes were found in correlation with diminished activities of complexes I and IV as compared with control mitochondria. We also observed increased ROS and augmented levels of the lipid peroxidation markers (malondialdehyde ( MDA) and 4-hydroxynonenal (4-HNE). Reperfused hearts treated with both NAC and postconditioning maintained cardiac work, the association of the supercomplexes was similar to those observed in control mitochondria, the activities of the individual respiratory complexes were restored and ROS, MDA and 4HNE levels decreased.

Our results showed that cardioprotection is associated with diminution in oxidative stress and suggest that ROS might be related with changes in the association of supercomplexes and with loss of the activity of individual complexes.

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# INFLUENCE OF PHYSICAL ACTIVITY ON THE MITOCHONDRIAL DYNAMICS IN SKELETAL MUSCLE FROM OBESE RATS

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**Background.** Obesity is a chronic and multifactorial disease, one of its main treatments is the incorporation of daily physical activity. The stimulation of skeletal muscle through contraction and relaxation, leads to progressive changes that are reflected in the activation or suppression of specific signalling pathways[1]. Nevertheless, there is still little evidence about the physical activity effects under obesity conditions. On the other hand, if the cell energy demand gets altered, the mitochondria suffer conformational changes in order to maintain its function to attend such demands[2]. Mitochondrial dynamics is the mechanism activated during this process, and it makes reference to the constant change of the mitochondrial architecture through fusion and fission events which objective is to maintain a healthy mitochondrial population with the exchange of genetic material, and is regulated by the signalling of reactive oxygen species (ROS) influenced by the amount of oxidative stress associated to the workload and metabolic demand of the cell[3]. The objective of the present study, is centred in analysing the mitochondrial adaptive processes under chronic (obesity) and acute (physical activity) stress conditions in a murine obesity model through the identification of the related proteins.

**Materials and Methods**. Lean (n=4) and obese (n=4) gastrocnemius muscle from 12 weeks old male Zucker rats where dissected and homogenized. Protein expression related to mitochondrial fusion Mitofusin 2, (Mfn2) and mitochondrial fission dynamin-related protein 1, (Drp1) were analysed by Western blot. Oxidative stress was analysed by measuring aconitase, superoxide dismutase and catalase activity by colorimetric and oximetry assays, respectively.

**Results**. There was a difference between the weight of gastrocnemius muscle from lean and obese rats, 4.6 $\pm$ 0.37 g and 3.9 $\pm$ 0.33 g ($p<0.05$) respectively. Catalase activity was elevated in 26.9% ($p>0.05$), superoxide dismutase and aconitase activity were diminished in 27.35% (>0.05) and 28.4% ($p>0.05$), respectively in the obese rat group when matched with the lean rat group. Western blot analysis revealed that Drp1 expression was elevated in obese rats 18.36 times ($p<0.05$) when compared to lean; Mfn2 level of expression also shows a difference between groups, with an elevation of this protein in the obese rat group in 22.69 times ($p<0.05$).

**Discussion**. The elevated Drp1 expression in obese condition, due to the excess of nutrients which elevates the metabolic workload of the cell, forcing the mitochondria network to fission to improve its efficiency. Mfn2 also shows an increment in its expression as an attempt to compensate the great increase in the fission process. The decrease in the superoxide dismutase activity of obese rat group does not compensate the generation of radical superoxide because of the increase in the ROS generation as a proper obesity side effect, hence the result in the aconitase activity, nevertheless the increase in catalase activity may compensate the oxidative damage generated by hydrogen peroxide. It is important to mention that the physical activity influence in this matter, still needs to be clarified with the immunodetection of the proteins involved with mitochondrial dynamics, but the analysis of enzyme activity agrees with the fact that there is a positive impact in the management of oxidative stress under obesity conditions.

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Regresar al programa

# OXIDATIVE STRESS EVALUATION IN CANCER-INDUCED MICE TREATED WITH RUTA GRAVEOLENS L. LECTINS.

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Oxidative stress is a natural process in aerobic organisms, due to the production of free radicals during the mitochondria activity, as a consequence of aerobic respiration, thus, there are antioxidant systems in order to keep the homeostasis in the system, but, when other sources of free radicals add them to the ones existent in the organism, the antioxidant system becomes unable to scavenge those, then, they produce damage on the cells.

Cancer is an illness that, besides being multifactorial, can be suffered mostly frequently by people whose habits are unhealthy, most of them, related to free radicals production, yet, after suffering Cancer, the production of free radicals improves by the malignant cells, destabilizing even more the antioxidant system.

There are some molecules able to improve the antioxidant activity, thus, stabilizing the homeostatic system, also there are others that can skip the malignant cells´s evasiveness inducing cellular death. One of those molecules are plant lectins wich are known for inducing death selectively to malignant cells over normal cells.

In this work we test the difference in the production of Oxigen Reactive species and Nitrogen Reactive species through direct and indirect methods in order to compare the differences between normal and ill system against systems damaged but treated with *Ruta graveolens* L. lectins.

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# NICORANDIL TREATMENT ALLOWS RECOVERY IN POST-FATIGUE TENSION FROM DIABETIC SKELETAL MUSCLE.

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Diabetes is a chronic degenerative disease characterized by the development of hyperglycemia due to a deficiency in the production or action of insulin which affects the metabolism of carbohydrates, proteins and lipids. There are numerous complications associated with diabetes, including muscle fatigue. Several aspects are unknown regarding muscle fatigue in the pathology. It has been postulated that the ATP-sensitive potassium channels ($K_{ATP}$) play a fundamental role in muscle function, therefore the objective of this work was to evaluate the effect of nicorandil (mito$K_{ATP}$ opener) treatment of diabetic rats and asses postfatigue tension of skeletal muscle.

Four groups of Wistar strain rats were used: Control (C), Diabetic (D) Diabetic + Nicorandil (D+N) and Diabetic + Nicorandil + 5-hydroxydecanoate (5mg/kg) (mito$K_{ATP}$ blocker) (D+N+5HD). Diabetes was induced with streptozotocin (60 mg/kg) intraperitoneally, Nicorandil (5mg/kg) treatment last 2 weeks. Soleus muscle was dissected out and isometric tension was recorded in presence of a $K_{ATP}$ channel opener (pinacidil) and blocker (glibenclamide).

Control group in the presence of glibenclamide (150 µM) had recovery of post- fatigue tension (58.47 ± 10.48%, p=0.037). Group D had no recovery (15.68 ± 6.54%). However, D+N group showed 62.73 ± 8.17% of increase in postfatigue tension (p=0.025). Pinacidil (200 µM) had no effect in C and D groups. D+N group showed a recovery on postfatigue tension (37.06 ± 4.28%, p=0.001) in the presence of pinacidil. Regarding to D+N+5HD, we were unable to perform tension records because apparently 5HD has citotoxic effect and there was 100% mortality in several batches of rats.

In summary, according to our results, we can conclude that diabetes affects muscle function and mito$K_{ATP}$ channels through nicorandil treatment could have a protective effect during the disease.

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# HYDROTROPIC RESPONSE DEFICIENCY IN THE *ahr1* MUTANT AFFECTS REACTIVE OXYGEN SPECIES REGULATION IN ROOTS OF *Arabidopsis thaliana*.

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Stationary growth is a distinct feature of plants and distinguishes them from other organisms. Because of this feature, plants have developed and evolved a variety of movement responses that allow them to change their growth direction. These responses are called tropisms and involve bending, and inclusive growth of the organs of the plant toward or away from the stimulus.

Plant roots display tropisms in response to various environmental cues such as gravity, touch, and moisture gradients.

Hydrotropism is the tropism that involves the perception of water and consequently the change of the growth direction of the root to the water source. This process is considered an adaptive strategy to resist the drought. Hydrotropism is crucial for the establishment of the root structure and the survival of the plant under water limiting conditions. However, the fundamental mechanisms that regulate hydrotropism is still unknown.

Cassab and col. developed a screening system using a water potential gradient for the isolation of mutants and to study of the hydrotropic response of *Arabidopsis thaliana*, which roots respond negatively to hydrotropic stimulus. One of this isolated mutant: *ahr1* (altered hydrotropic response) develops longer roots in water deficient medium compared with wild type roots that stop growth in these conditions.

Oxidative stress is a consequence of water stress. However studies in ROS regulation during hydrotropic that basically is a water stress response, are inexistent. Our hypothesis is that ROS balance change in response to water stress in the *ahr1* mutant and that this change may promote root growth. In this work, we analyzed ROS production in roots growing in water stress medium and the effect of scavengers of ROS in root growth in the same conditions. Our data showed that *ahr1* produce less $H_2O_2$ than wild type plants in response to water stress. Besides we analyzed the expression of genes that are related to the production of ROS: NADPH-oxidizes and a $Mn_2$- superoxide dismutase. We analyzed the expression of genes that are related to ROS control like peroxidases (AtPer33 and AtPer34) and catalase-2. We also observe the expression of genes that participate in root, like SUMO E3 Ligase HIGH PLOIDY2 (HPY2), a glycosylphosphatidylinositol (GPI)-anchored protein (COBRA), and a tonoplast intrinsic protein (GAMMA). Results will discussed during the presentation of this work.

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# REGULATION OF AUTOPHAGY BY ROS DURING HYDROTROPIC RESPONSE OF *Arabidopsis thaliana*

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Plants have developed and evolved mechanisms that are called tropisms, which involves the bending of the plant organs and even the regulation of the growth toward or away from the perceived stimulus. Tropisms determine the root growth direction and orientation in nature, also help in the development of root system and are responsible of anchoring the root to the ground; they are also considered as fundamental to avoid drought. Hydrotropism is the tropism that involves the perception of water and consequently the change of the root's growth direction to the water source. This process is considered an adaptive strategy to resist the drought. The plants' roots have used hydrotropism to avoid areas with reduced water and grow towards wetter areas. This process is crucial for the establishment of the root structure and the survival of the plant under water limiting conditions.

Several factors have been described that are involved or regulate the hydrotropic response in *Arabidopsis thaliana*. One of them induce autophagy under hydrotropic response. Autophagy is a catabolic process that degrades and recycles cytoplasmic content, and induces a response to various kinds of stress and protects the cell until it is controlled. It is known that autophagy degrades amyloplasts of the root tip during the hydrotropic response. In this work, we measure the participation Atg8 protein (an autophagy marker) evaluating their role by ROS under water stress on root. We propose that the perception of water stress on *Arabidopsis* root induces ROS accumulation, which promotes the activation of the autophagy as a protective mechanism that allows the cells to survive until the situation becomes more favorable.

Reactive oxygen species (ROS) are toxic products of aerobic metabolism, which are harmful to aerobic life. ROS accumulation leads to oxidative stress, which may damage all cell components such as proteins, lipids, and DNA. $H_2O_2$ and $O_2^-$ have been suggested as regulators of autophagy. ROS generally induce autophagy to reduce oxidative damage in plants.

Drought stress are the most common environmental stresses that affect plant growth and development. These stress can create oxidation damage to plant cells, leading to the accumulation of ROS and oxidized proteins. The ability of autophagy to scavenge oxidized proteins and regulate the ROS levels suggests that autophagy may also be involved in water stress. Some ATG genes, such as *AtATG8* in *Arabidopsis* function in response to salt stress and osmotic stress. *ATG18* downregulation leads to the accumulation of oxidized proteins, which subsequently increases sensitivity to oxidative stress ROS may function in the induction of autophagy during hydrotropic response of Arabidopsis thaliana. Autophagy plays an important role in protecting plant cells from oxidative stresses by degrading oxidized proteins.

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# Senescence-associated secretory phenotype (SASP) from stress-induced premature senescence (SIPS) primary lung mice fibroblasts induces potential changes in cellular senescence, proliferation, and cell migration in cell line L929.

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Cellular senescence is a normal biological state characterized by irreversible growth arrest accompanied by a complex phenotype. It can be triggered prematurely by multiple mechanisms including DNA damage and oxidative stress (stress-induced premature senescence, SIPS). Senescent cells secret numerous cytokines, growth factors and proteases with beneficial or detrimental effects depending on the physiological context. This secretory profile is called senescence-associated secretory phenotype (SASP) and can promote malignancy in nearby cells.

The aim of this study was to evaluate the effect of the SASP obtained from primary lung mice fibroblasts prematurely induced to senescence with $H_2O_2$ (SIPS) on cellular migration, proliferation and senescence on L929 cell line.

Primary lung fibroblasts were obtained from CD-1 mouse and were exposed for 2h to 75 uM $H_2O_2$. The SASP from this culture was recovered at different days (9, 15 and 21) and added to L929 cells that had been previously seeded on coverslips. L929 were exposed to SASP during 72 h, and after that, cell proliferation was determined by trypan blue assay, cellular migration by wound healing technique, and cellular senescence by SA-beta-galactosidase assay.

Our results showed that SASP from prematurely induced senescent cells induces processes such as migration, proliferation and senescence as observed in cancer cells .
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# CHRONIC OBESITY CONDITION INCREASE THE INCIDENCE OF mtDNA OXIDATION

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***Introduction:*** It is well known that the obesity condition leads to increase in inflammation (tumor necrosis factor-alpha,TNF-α;, interleukin 1□, IL-1β; and IL-6) by the excessive adipose tissue. All of this cytokines promote the generation of reactive oxygen species (ROS) creating an oxidative ambient (Zhang, 2011).This high levels of ROS affects directly DNA, especially the mitochondrial DNA (mtDNA) because its lack of histones, and even more injured because it is near to the electron transport chain, oxidizing nucleotides that can result in severe transcriptional errors. The most common oxidized nucleotide by ROS is the guanine that oxidized to 7, 8-dyhidro-8-oxoguanine (8-oxo-dG) (de Souza- Pinto, 2001). DNA base excision repair (BER) pathway is responsible to treating this injury, activated by a glycosylase specific for this lesion, the 8-oxoguanine DNA glycosylase (OGG1) followed by a cascade of recognition and enzymatic reactions to restore de genome integrity (Liu, 2010). Recently, some studies have shown the relevance that OGG1 have in some pathologies, such as obesity, diabetes mellitus type 2 (DM2), infertility and neurodegenerative disease. Due all implications that OGG1 have in the restore process of damaged mtDNA, we suggest that if the obesity condition increase ROS, in mitochondrial environment, this will cause an increase in the mtDNA oxidation, promoting changes in OGG1 expression.

***Materials & Methods:*** We used a group of 12 Zucker rats (6 lean, 6 fa/fa) with 12 weeks old as obesity model. Mitochondria were isolated from cardiac tissue by differential centrifugation. Mitochondrial activity was measured by oximetry using two pairs of substrates, which were glutamate plus malate (GM) for complex I activity, and succinate plus rotenone (SR) for complex II activity. We determined OGG1 expression by western blot assay. We collected antioxidant activity by measuring catalase by oximetry and superoxide dismutase (SOD) by colorimetric assay. Aconitase activity was measured by this last method as well.

***Results:*** The oximetry assay for mitochondrial respiration activity showed significant difference only in the state 3 in the presence of GM, (Ctrl 31.62 ± 5.74 nmol$O_2$/min; Fa/Fa 23.17 ± 5.41 nmol$O_2$/min, $p<0.006$) and SR, (Ctrl 33.13 ± 8.77 nmol$O_2$/min; Fa/Fa 20.9 ± 4.79 nmol$O_2$/min, $p<0.02$) comparing both groups. The oxidative stress our results shown no significant difference between control group (Ctrl) and obese group (Fa/Fa). In contrast, we found a significant reduction of aconitase activity represented by a 55.7% of less activity comparing the control group to the obese group (Ctrl, 48.49 ±
13.66 nmol/min/mg; Fa/Fa, 21.46 ± 13.62 nmol/min/mg, $p<0.01$). Finally, we found 76.6% more expression of OGG1 in mitochondria from obese group over the control one (Fa/Fa, .28 ± .13 OD; Ctrl,
.06 ± .03 OD, $p<0.03$).

***Conclusion:*** Our results show that the obesity condition brings more oxidative stress to the mitochondrial environment and it increase the damage to its integrity, having an impact directly over the mitochondrial activity. The increased expression of OGG1 in the obese group allow us to think that in fact, the OS generated by this condition in damaging the mtDNA and promoting changes in OGG1 expression supporting our hypothesis. We still need the results of the mtDNA damage measurement to round our results.

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# HGF/c-Met decreases NADPH oxidase activity by a mechanism mediated by the proteasome 26S.

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The hepatocyte growth factor (HGF) is known as a very potent hepatocyte mitogen that triggers a wide range of cellular responses through its interaction with the c- Met receptor. Once the binding is completed, c-Met recruits different signaling proteins that are pivotal for proliferation, survival, morphogenesis, motility and antioxidant response modulation. The importance of HGF/c-Met pathway was evident when the carcinogenic effect observed on a liver c-Met specific mutant mouse due NADPH activation. The NADPH oxidase (Nox) multi-enzymatic system is one of the main sources of reactive oxygen species (ROS). The Nox-produced ROS display different roles, such as, molecules involved in hormone production chemical modification, pathogen defense, and cellular signaling mainly. **Objective:**
To evaluate the HGF/c-Met role on the NAPH regulatory subunit ($p22^{phox}$) turns over through the proteasome 26S. **Methods:** C57BL/6 male mice liver was subjected to two step collagenase (type I) perfusion for hepatocyte isolation. Hepatocytes were cultured at standard conditions and treated or not with a
proteasome inhibitor (Epoxomicin, 200nM/ml), for 30min and/or HGF addition (50ng/ml) at different times; once treatment completed protein was isolated for western bolt and immunofluorescence analysis. **Results:** Our data shows that there is significant $p22^{phox}$ degradation 12h after HGF addition, result that was further validated with the proteasome inhibition, confirming that the proteasome
26S is the $p22^{phox}$ degradation pathway by using confocal microscopy. Our data suggest that the HGF/c-Met signaling is controlling the NAPH regulatory subunit ($p22^{phox}$) protein content through the proteasome 26S. CONACYT, NO. 131707

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# REDOX AND ERK SIGNALING EFFECT ON Nrf2 PATHWAY ACTIVATION IN POSTCONDITIONED HEARTS

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Abstract: Ischemic heart diseases are the main cause of death worldwide. In these pathologies myocardium is damaged due to lack of blood flow. Reperfusion therapy is the clinical treatment aimed to rescue myocardial function by restoring blood flow through the occluded vessel. Paradoxically, restoration of blood flow can cause additional damage, which might even lead to death. Therefore, multiple cardioprotective strategies have been developed to contend against reperfusion damage. The mechanical maneuver of postconditioning (PostC) has demonstrated its protective potential in both experimental and clinical settings. It consists on the application of short cycles of reperfusion/re-occlusion just after the ischemic event and before long reperfusion, resulting in the activation of endogenous protective mechanisms in the myocardium.

Purpose**:** Contribute to elucidate the bases of endogenous protective mechanisms activation during PostC in the ischemic myocardium. Specially, the role of reactive oxygen species (ROS) as signaling molecules in the activation of the transcription factor Nrf2 (Nuclear factor E2-Related Factor 2) through the upstream cardioprotective kinase ERK (Extracellular signal- regulated kinase).

Methods and Results: Myocardial infarction was induced in an *in vivo* model using Wistar male rats. The left anterior descending coronary artery was occluded for 5 minutes followed by 10 and 60 min of reperfusion (Group IR). Other group of animals was subjected to PostC (IR+PostC) and then to 10 or 60 min of reperfusion. The third group received the ROS-scavenger N-acetylcysteine (NAC) at 2.3 mg/Kg injected into the left ventricle, previous to the application of PostC and then subjected to 10 min of reperfusion (Group IR+PostC+NAC).

We found that myocardial protection in the IR+PostC was related with the activation of several kinases, including ERK and with low ROS levels.

To determine if cardioprotection and kinase signaling activation were dependent on redox signaling, we applied NAC to delete ROS during PostC, and we evaluated the cardiac performance (Group IR+PostC+NAC). We observed that heart function decreased in this group in correlation with diminution in the ROS levels as compared with those measured in the IR+PostC group. We also observed that PKC and ERK activities decreased.

Conclusion: These results suggest that ERK activation is related with redox signaling. We are currently involved in evaluate changes in Nrf2 activation that might result from ROS-signaling inhibition in PostC hearts.

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# GLUTATHIONE (GSH) AND L-CYSTEINE, CONCENTRATIONS EFFECT ON EQUINE SPERM STORED AT 4°C

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Introduction: The sperm cooling (4° C), consists in temperature reduce for decrease the metabolic processes and the bacterial growth, this for preserving them until the time of use. Compared to cryopreservation, this methodology has helped to reduce the damage caused by the abrupt decrease in temperature. However, it has not yet found, a way to prevent damage caused by oxidative stress that occurs in this process. For this reason, the study interest is to evaluate the protective effect that could end up having glutathione (GSH) and cysteine (Cys) during the cooling process. Methodology: Samples from 3 horses-quarter mile race stallions, were obtained which remained in the Equifauna laboratory located in Tlaxco, Tlaxcala, México. The ejaculates were obtained by an artificial vagina Missouri modified type, Polish style (Krakow). Once the sample collected the seminal analysis was performed. The sperm motility percentage and their concentrations were determined, further to determining the viability using the parameters reported in the manual of the World Health Organization, customized horse semen. Subsequently they formed two groups, control and experimental this last one were the treatments (Extender, 0.5mM GSH, 2mM GSH, 5mM GSH, 0.5mM GSH + 5mM Cys + Extender, 2mM GSH + 5mM Cys + Extender, 5mM GSH + 5mM Cys + Extender, 0.5mM GSH
+ 10mM Cys + Extender, 2mM GSH + 10mM Cys + Extender, 5mM GSH + 10mM Cys + Extender, 0.5mM GSH + 5mM Cys, 2mM GSH + 5mM Cys, 5mM GSH + 5mM Cys, 0.5mM GSH + 10mM Cys, 2mM GSH + 10mM Cys, 5mM GSH + 10mM Cys). For obtain the percentage of live sperm before and after cooling was used Nigrosin-Eosin staining. Then the lipid peroxidation was evaluated through the determination of MDA by Alvarez and Storey. The same analysis was repeated after storing 72 hours the samples at 4°C. Results: The seminal examination showed that the semen samples had a volume average of 21.1ml, 70.1% motility and 88.15% of viability before refrigerating with an average concentration of 390.56x106 sperm / ml. Nigrosin-Eosin test showed that none of the treatments except the 2 mM GSH and Cys 10 mM kennie with a percentage of 64.33% (p> 0.05) mantains sperm viability even after being refrigerated for 72 hrs. None of the treatments was able to reduce the percentage of MDA a product of membrane lipid peroxidation. However, the concentration of 2 mM GSH + 10 mM Cys and 5 mM GSH + 10 mM Cys showed a significant difference (p> 0.05) in the concentration of MDA after sperm refrigerate for 72 hrs. Conclusion: The 2 mM GSH + 10mM Cys + kennie concentration maintain sperm alive for 72 hrs cooling.

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# REACTIVE OXYGEN SPECIES FROM A RAC1 INDEPENDENT NADPH OXIDASE REGULATE THE MOTILITY AND CAPACITATION OF *Cavia porcellus* SPERMATOZOA

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The spermatozoon is a highly specialized, polarized and condensed cell; unable to grow or divide, its only function is the ovule fecundation. The physiological production of reactive oxygen species (ROS) in spermatozoa, described for the first time in 1943, is necessary for the proper differentiation and maturation processes and for the adequate functioning of the cell. Once the spermatozoa are deposited in the female tract, two additional maturation processes take place: the capacitation and the acrosome reaction. In mammalian spermatozoa orthologous of Nox2/gp91$^{phox}$, a catalytic subunit of NADPH oxidases (NOX), have been identified. The production of ROS by NOX is highly regulated. Only when the cytosolic subunits p40$^{phox}$, p47$^{phox}$, p67$^{phox}$ and the Rac1 GTPase interact with the transmembrane subunits p22$^{phox}$ y gp91$^{phox}$. NOX2 is able to produce superoxide. Different NOX have been described for mammalian spermatozoa: a variant of NOX2 in *Mus musculus* and NOX3 in *Rattus norvegicus*, both regulated by Rac1 and NOX5 in human and equine, which activates in presence of $Ca^{2+}$. However, it is not well known about the participation of these NOX and ROS in capacitation and sperm motility. Therefore, the main objective of this work is to dissect the pathway by which ROS are produced during capacitation and their physiological roles in spermatozoa. We quantified the ROS production during spermatozoa capacitation by using a colorimetric method, additionally the effect on mobility was evaluated by a computer- assisted sperm analysis (CASA) in the presence of antioxidant agents or the non- selective flavoproteins inhibitor, DPI. Firstly, the ROS concentration increased during capacitation, this increase was dejected by DPI. Sperm motility was decreased when spermatozoa were capacitated in presence of DPI, several parameters of motility were affected. Using specific antibodies against NOX4, by Western blot a protein band with a Mr of 75 Kda was detected in spermatic extract of *C. porcellus,* but not in spermatic extract of *M. musculus.* By immunofluorescence, the protein was located in the middle piece of the sperm. Finally, to know the effect of ROS in capacitation, sperm were capacitated in presence or absence of DPI. When protein phosphorylation in Tyr was analyzed a decrease of protein phosphorylation was observed for spermatozoa capacitated in presence of DPI. In conclusion, we suggest that the ROS, probably produced by NOX4, are important for capacitation and sperm motility.

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# THE PROTECTIVE EFFEECT OF THE HEPATOCYTE GROW FACTOR IN THE PANCREATIC CELL LINE RINM5F TREATED WITH ALCOHOL AND ACETALDEHYDE.

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Alcohol is the most widely used psychoactive drug in the world. Prolonged use may result in progressive and irreversible damage to the pancreatic gland, leading to organ inflammation, fibrosis and acinar atrophy that can result in exocrine and endocrine insufficiency. The effect of ethanol on cells is due to a nonspecific interaction with the membrane, which translates as an increase in the fluidity of the same. Ethanol is biotransform and is obtained as a metabolite acetaldehyde which is toxic, mutagenic and carcinogenic. In addition to our research group has been extensively described the protective effect of HGF in the liver by increasing the antioxidant defence system and regulating pro-oxidant systems such as NADPH oxidase, which is capable of producing ROS signalling target. Sustained NADPH oxidase activation correlates with pathologies such as acute pancreatitis and is well known that NADPH oxidase is used by cytotoxic growth factors such as TGFβ.

**Methods:** RINm5F cell line was treated with 100 mM ethanol, or 200 mM acetaldehyde, with or without HGF 50 ng/ml by 12 h and with or without the ERK 1/2 inhibitor before the toxic stimulus, cell viability was determinate by a commercial kit (CCK-8, Dojindo), specific NADPH oxidase activity was determined by monitoring the production rate of superoxide anion, protein analysis was measured by Western blotting, apoptosis was assessed by acridine orange and ethidium bromide staining by immunofluorescence.

**Results:** pre-treatment with HGF for 12 h maintains cell viability to aggression with ethanol and its metabolite acetaldehyde, this protective effect is abrogated by the ERK1/2 inhibitor and the same effect is observed by apoptosis assay. HGF modulates antioxidant systems, increasing and decreasing GSTM, SOD1. The HGF deregulates the NADPH oxidase, the main pro-oxidant system. A possible mechanism is proposed by activating pathways ERK1/2 and AKT. Furthermore proapoptotic Bax systems, activation of p38 and even decreases transcriptionally are deregulated.

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# IODINE AND OXIDATIVE STRESS IN A RAT MODEL OF PROSTATIC HYPERPLASIA

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Benign prostatic hyperplasia is a proliferative-inflammatory disease. In animal models, oral treatment with iodine does not modify the prostate weight, but it prevents acini hyperplasia. The mechanisms associated with this protection are unknown, but in breast tumors iodine promotes the expression of antioxidant enzymes (catalase), and in a cell-free model, iodine neutralizes free radicals (FRAP assay). The aim of this study was to evaluate the effects of iodine on the oxidative stress and inflammation of the hyperplastic prostate. Hyperplasia was induced in male Wistar rats (250 g) with testosterone (3 mg/kg body weight, slow release for 4 weeks). There were 3 hyperplasia groups: water, iodine, and celecoxib (Cxb), and one control group. Cxb was used as an anti-inflammatory control (cyclooxygenase-pathway inhibitor). One week before the induction of hyperplasia, iodine (0.05%) or Cxb (5mg/kg/day) treatments were administered in the drinking water (5 weeks). Prostatic DNA content was monitored as indicator of cell proliferation. Lipoperoxidation (MDA levels), nitrites (Griess assay), iNOS activity (nitric oxide-generating enzyme, colorimetric assay), COX-2 levels (prostaglandin-generating enzyme, ELISA), and prostaglandin PGE2 (ELISA) were evaluated as markers of oxidative stress. TNF-alpha (ELISA) was measured as an inflammatory indicator. With the exception of COX-2 and TNF-alpha, all oxidative stress mediators significantly increased in hyperplastic prostate. Iodine supplementation did not modify the lipoperoxidation index or COX-2 levels, but it did prevent the proliferative/prooxidant response induced by the hyperplasia. In addition, iodine reduced prostatic TNF-alpha to below control levels. In general terms, the antioxidant and anti-inflammatory effects of iodine were comparable to those of Cxb, suggesting it could be used therapeutically without the adverse effects associated with non-steroidal anti-inflammatory drugs. Ongoing studies analyze the direct or indirect mechanisms of iodine involved in these responses.

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# EFFECT OF REDUCED GLUTATHIONE (GSH) EXOGENOUS, IN CAPACITATION PROCESS IN THE BOAR SPERM (*SUS SCROFA DOMESTICUS*).

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Sperm are produced in the testis, through spermatogenesis process. However, until this point, are not capable to fertilizing the egg, so they must pass through the epididymis to complete its maturation process, where will acquire the potential to capacitation and acrosomal reaction in female genital tract, concluding with fertilization. The sperm cells like other cells, are dependent on oxygen, essential for all aerobic organisms, since it is the main source of energy acquired from oxidative metabolism. However, the oxygen consum generate several actives forms of metabolits and peroxidative. However, oxygen consumption generates several types of actives oxygen metabolites, and peroxidative molecules, all known as reactive oxygen species (ROS), which, initially had only been considered by its harmful effects on sperm, causing damage to the plasma membrane, the DNA, and therefore mobility and affecting cell viability. But now we have information that
indicate that the generation of ROS, such as superoxide anion ($O_2^-$) and its dismutation product, hydrogen peroxide ($H_2O_2$), are involved in different signaling pathways, to acquire fertilizing capacity sperm, favoring processes such as capacitation and acrosomal reaction of sperm. The antioxidant enzyme system:
superoxide dismutase, catalase and glutathione peroxidase (GPX); is responsible to protecting sperm, against ROS. Particularly the GPX / glutathione reductase system (GR), which, metabolizes $H_2O_2$ produced endogenously, using the reduced glutathione (GSH) as substrate and GPX action which oxidized to GSH, returning to their reduced state by GR action. However, it has been suggested that high
concentrations of GSH may affect the sperm capacitation. Therefore, the aim of this study was measure the optimal concentration of GSH which was determined in boar sperm. For this purpose, was induced the capacitation in vitro in boar sperm
obtained from Genetics NS, for which were used, different concentrations of GSH [0.5, 1, 5 mM]. Subsequently, they were capacitated in Talp-Hepes medium supplemented with albumin (F5) for 4 hours at 39 ° C, 5% CO2, at the end were stained with chlortetracycline to observe different patterns of capacitation. 100 cells were counted in fluorescence microscope, apparently, resulting higher number of capacitated sperm with 1 mM GSH.

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# Bcl-2 LOCATION DURING HORMETIC RESPONSE

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Bcl-2 has been shown to suppress apoptosis and contribute to tumor development. Beyond its role in cancer, Bcl-2 is essential in developmental program cell death, tissue remodeling and defense against pathogens. Besides the above-mentioned functions, Bcl-2 seems to play a protective role during the hormetic response. Hormesis is the process whereby exposure to a low dose of a chemical agent induces an adaptive effect on the cell or organism. This response recalls the expression of cytoprotective and antioxidant proteins, allowing pro-oxidants to emerge as important hormetic agents. The anti-apoptotic protein Bcl-2 is known to protect cells against death induced by oxidants and it has been suggested that Bcl-2 might also modulate steady-state reactive oxygen species (ROS) levels.
A model to study the oxidative conditioning hormesis response (OCH) by treating cell lines with low $H_2O_2$ doses has been previously established. In particular, when L929 cells were treated with 50 µM $H_2O_2$ for 9 h, neither oxidative damage nor oxidative imbalance was induced and cells treated this way maintained a high survival rate (70-80%) compared with non-conditioned cells (10-15%). Also, an increase in Bcl-2 expression could be detected.
Thus in order to deepen on how does Bcl-2 exerts its hormetic function, WT- MEFs were treated with 50 µM $H_2O_2$ at different intervals for 9 h. Cells were fixed in 4% para-formaldehyde and immunostained against Bcl-2 and HSP70, as a mitochondrial marker. Nucleus was stained with DAPI. Bcl-2 sub-cellular localization was analyzed by fluorescence microscopy. In non-treated cells, Bcl-2 was present at the cytoplasm and surprisingly at the nucleus as well. Nevertheless, after treatment with $H_2O_2$, Bcl-2 cytosolic distribution gradually changed to a mitochondrial co-localization. Bcl-2 translocation to the mitochondria started to be detectable after 2 h treatment; and at 9h Bcl-2 could clearly be detected on the mitochondria. Bcl-2 nuclear fraction remained present throughout the experiment.
Our results indicate that Bcl-2 mitochondrial translocation might be part of the hormetic response, because by increasing its presence where ROS are being produced, Bcl-2 may be able to exert its protective function. At the same time, Bcl-2 presence at the nucleus suggests that this protein might be related with encouraging nuclear-translocation of transcription factors related to antioxidant response and cell survival such as Nrf2.

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# CADMIUM AGGRAVATES OXIDATIVE DAMAGE IN MOUSE PRIMARY HEPATOCYTE CULTURE WITH HIGH LIPID CONTENT.

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One of the major pathogenic mechanisms for progression of nonalcoholic fatty liver disease (NAFLD) is oxidative stress, and the involvement of reactive oxygen species (ROS) has been suggested, leading to cellular impairment and death by either necrosis or apoptosis. Cadmium (Cd) is a pro-oxidant heavy metal, it is has been reported that intensify the inflammation process and oxidative stress that could contribute to NAFLD progression. **Aim:** To determine the effect of Cd in hepatocytes obtained from mice fed with hypercholesterolemic diet. **Methods:** C57bl6 male mice were fed for 48 h with a hypercholesterolemic diet (HC; 2% cholesterol and 0.5% sodium cholate). Hepatocytes were obtained by using the two-step perfusion method. Hepatocyte primary culture was treated with different Cd concentrations at different periods. Lipid content was determined by using the red oil dye. Cell viability was determined by using the Cell Counting Kit 8 (CCK8®) commercial kit. ROS detection was measured by spectrophotometry, and protein carbonyl groups were determined by the Oxyblot® kit. Antioxidants and lipogenic enzymes content was assessed by western blot. **Results:** Our data showed that Cd significantly diminished cell viability in a concentration and time dependent manner. Cd exposure increased ROS production and protein oxidation, and decreased antioxidant enzymes content, such as, superoxide dismutase (SOD), glutathione peroxidase (GPX), gamma glutamylcysteine synthetase (Ɣ-GCS), while heat shock protein 70 (HSP70) was increased. Lipid content accumulation was decresased when an antioxidant was co-administrated with Cd. In addition, the lipogenic enzyme (acetyl-coA carboxilase) ACC and the transcription factor sterol regulatory element binding protein-1c (SREBP-1c) were increased. **Conclusion**: Our data suggest that Cd could favor lipid accumulation in steatotic hepatocytes through an increase in ROS generation and a decrese in antioxidant response, which could be involved in NAFLD progression. Conacyt 166042. INFR-2013-01-205941.

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# OXIDATIVE STRESS DUE CADMIUM SUBLETHAL EXPOSURE IN THE AXOLOTL (*Ambystoma mexicanum*)

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**Introduction**. The axolotl *Ambystoma mexicanum* is a neotenic amphibian, endemic of the valley of Mexico basin. Nowadays, its distribution is restricted to Xochimilco wetland where populations register a severe reduction due to serious environmental deterioration and pollution. *In situ* studies demonstrate that axolotl populations are subject to a severe oxidative stress related in part to the chronic sublethal exposure to metals. Among them, cadmium is one of the metals of major concern due its high toxicity including the induction of oxidative stress. Moreover, cadmium concentrations in water, sediment and biota in Xochimilco wetland exceed the permissible limits for the protection of aquatic life. Thus, the aim of this study was to determine in *A. mexicanum* juveniles the oxidative effect of cadmium at environmentally relevant concentrations through the evaluation of biomarkers exposure and oxidative stress as well probable alterations in the regulation of essential metals (Zn, Cu, Fe and Cu).

**Materials and methods.** Renewal static bioassays were conduct during 15 days; axolotl juveniles were expose to 20 and 200 µg Cd/L, relevant environmental levels; a control group without metal exposure was consider. At the end of the exposure period, specimens were dissect, liver was fractionate into subsamples and store at -80°C for later analysis. The oxidizing effect of cadmium sublethal exposure was establish through the hepatic evaluation of biomarkers of exposure and oxidative stress as Metallothionein (MTs), total (GSH), oxidized (GSSG) and reduced (GSH) Glutathione, GSH/GSSG ratio, Catalases (CAT), Superoxide dismutase (SOD) and Lipoperoxidation (TBARs). Cadmium concentration in the experimental medium and the hepatic levels of Cd, Fe, Zn, Cu and Mn in *A. mexicanum* were determine by ICP-MS.

**Results and Discussion.** The obtained results suggest that cadmium even at sublethal concentrations exerts a severe oxidative stress in the axolotls. Significant liver cadmium bioaccumulation was register and was relate with a significant induction of MTs suggesting its participation in processes of detoxification and protection to cadmium injury. Essential metals remain unchanged denoting the maintenance of its homeostatic regulation. All the biomarkers of oxidative stress analyzed denote a significant alteration of the cellular redox balance in the organisms related with cadmium bioaccumulation. The alterations observed at the biochemical level and the causal relationships obtained with the hepatic accumulation of cadmium demonstrate the cellular oxidant effect of cadmium, even at very low concentrations but environmentally relevant levels. The obtained information contributes to the environmental risk analyses of metals exposure in Xochimilco wetland and for the future management of the axolotl wild population.

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# BIOMARKERS OF EXPOSURE AND OXIDATVE STRESS IN THE CRAYFISH *Cambarellus monctezumae in situ* EXPOSED TO METALS

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**Introduction:** Xochimilco wetland is a peri-urban lake located south of Mexico City. Nowadays, this aquatic system is subject to a severe environmental degradation due several physical, chemical and biological pressures due the unregulated urban growth and the technification of traditional agricultural activities. Particularly, the concentration of metals in water, sediment and organisms in the system exceed the limits established for the protection of aquatic biota. Several studies demonstrate that metal ions stimulate the production of reactive oxygen species (ROS) and exerts oxidative stress. Thus, the aim of this study was to evaluate the oxidizing effect of *in situ* exposure to metals and the probable relationship with the hepatic concentration of metals in the crayfish *Cambarellus monctezumae*, a species of ecological and economical relevance.

**Materials and methods:** Four sampling sites were select considering different levels of metals contribution due urban and agricultural activities (Bordo, Cuemanco, Apampilco and Apatlaco). In adult specimens from each site cephalothorax, abdomen (muscle) and exoskeleton were dissect. In a subsample of tissues the concentration of metals was determined by ICP-MS, 7 essential (Cr, Mn, Fe, Co, Cu, Zn, Se) and 6 with unknown biological function (As, Ni, V, Sr, Cd, Pb). Biomarkers of exposure and oxidative stress were analyze in the whole cephalotorax where the hepatopancreas is located, the main organ of metabolism, accumulation and metal detoxification. Metallothioneins (MTs) was evaluate as indicator of metals exposure and oxidative stress, and total (GT), reduced (GSH), oxidized glutathione (GSSG) and GSH/GSSG were evaluate as indicators of cellular redox status.

**Results and Discussion**: Tissue metals concentration demonstrate that the cephalothorax accounts for the highest accumulation reflecting that the hepatopancreas is the main metals target organ. The obtained results of GT, GSH, GSSG and GSH/GSSH suggest that all the organisms are subject to a severe oxidative stress; nevertheless, the highest level of alteration was register in the organisms from Apatlaco site of urban and agricultural impact, where the highest tissue concentrations of metals were recorder. The apparent contradiction of the obtained results can be reflecting particularly compensatory mechanisms to the toxic action of metals.

**Conclusion:** The biomarkers of exposure and oxidative stress analyzed in this study are relevant tools to understand the levels of risk of the populations of *C. monctezumae in situ* exposed to contaminants as metals in Xochimilco wetland.

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# IMPLICATIONS ON BIOLOGICAL RHYTHMICITY IN THE AXOLOTL *Ambystoma mexicanum* JUVENILES OF SUBLETHAL CADMIUM EXPOSURE: OXYGEN CONSUMPTION AND CELLULAR REDOX BALANCE

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**Introduction.** The axolotl *Ambystoma mexicanum* is an endemic amphibian of the basin of Mexico valley, nowadays under extinction risk and distributed only in Xochimilco, a periurban wetland in Mexico City. Heavy metals contamination has being recognize as one of the main factors responsible of the reduction of their population. Among them, cadmium is one of the metals of major concern due to the high environmental concentrations above the limits of protection for aquatic life and due its known toxicity including oxidative stress and neurotoxicity. However, few studies in amphibians analyse the alteration of pivotal biological responses as biological rhythms of known neurotoxicants as cadmium. Thus, the objective of this study was to evaluate the effect of cadmium on the daily rhythmicity of the oxygen consumption and the cellular redox balance of *A. mexicanum* juveniles.

**Materials and methods.** Organisms were expose in renewal static bioassays during 15 days to 20 and 200 µg Cd/L, relevant environmental concentrations; a control group without metal exposure was consider. At the end of the assays, oxygen consumption, hepatic total glutathione (TG), reduced and oxidized glutathione (GSH, GSSG), GSH/GSSG ratio and hepatic cadmium concentration were measured each 3 h during 24 h. A COSINOR analysis was conduct to analyse the daily rhithmicity of the biological responses evaluated.

**Results and Discussion.** Obtained results in control group demonstrate the expected unimodal pattern of aerobic metabolism with a period of 23.42 h and an acrophase at 10:00 h. However, in cadmium exposed organisms the daily rhythm of routine metabolism shifted to a bimodal pattern with periods of 12.12 and 11.54 h and acrophases at 3:00 and 15:00 h and 1:00 and 11:00 h in axolotls exposed to 20 and 200 µg Cd/L respectively. Moreover, in relation with control group the metabolic amplitude and the scope for metabolic activity was significantly altered due cadmium exposure. In control group, a similar daily rhythmicity in TG, GSH, GSSG and GSH/GSSG was observed demonstrating their relationship with the aerobic metabolism pattern. However, in exposed organisms significant alterations in the daily pattern and in the evaluated antioxidant responses were registered. The results of this study suggest that cadmium is exerting a neurotoxic effect altering the normal biological rhythmicity of aerobic metabolism and antioxidant systems, affecting also the energetic status of the organisms due probable regulation, compensation and detoxification processes. The results of the patterns of the cellular oxidative stress suggests also alterations in the regulation of glutathione synthesis, results that are also supported with unpublished data of liver metallothionein concentrations and lipoperoxidation in the axolotls sublethally exposed to cadmium.

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# BIOMARKERS OF OXIDATIVE STRESS IN *Oreochromis niloticus* (CICLIDAE) *in situ* EXPOSED TO METALS

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**Introduction.** The peri-urban lake of Xochimilco, located south to Mexico City, is receptor of multiple contaminants due to urban unregulated growth and agricultural activities. Metal concentrations in this wetland represent a severe risk for aquatic populations. Metals toxicity, even at very low concentrations is relate with the production of reactive oxygen species and the induction of oxidative stress. Among the biomarkers of cellular redox state, metallothionein and glutathione concentrations are consider adequate indicators of the exposure and the oxidative stress of metals. Thus, the objective of this is study was to evaluate, in the tolerant species *Oreochromis niloticus* biomarkers of exposure and oxidative effect to metals under different scenarios of *in situ* impact to urban and agricultural activities in the wetland of Xochimilco and to analyse the probable causal relationship with the hepatic concentration of metals.
**Materials and methods.** Six sampling sites were select considering different levels of metals contribution due to urban and agricultural activities (Bordo, Apampilco, Apatlaco, La Draga, San Diego and Asuncion) where adult specimens of the tilapia *O. niloticus* were collected. In the liver, concentration of Metallothionein (MTs) was evaluate as a marker of exposure and indicator of oxidative stress to metals; the total (GT), oxidized (GSSG) and reduced (GSH) glutathione concentration and the ratio GSH/GSSG were also evaluated as markers of the cellular oxidative status. Liver concentration of metals were evaluate by ICP-MS.
**Results and Discussion.** Liver concentration of 14 metals was determined; 7 essential (Cr, Mn, Fe, Co, Zn, Se) and 7 of unknown biological function. The obtained results demonstrate that the tissue concentration of the later ones represent a sever risk for the *O. niloticus* populations. The highest levels were register in the tilapias from Apatlaco (V, Cr, Ni, Sr, Ag, Cd, Pb) urban and agricultural impact) in which the higher concentrations of hepatic MTs were obtain suggesting the participation of this metalloprotein in the protection and detoxification of metals. The obtained hepatic levels of GT, GSH, GSSG and GSH/GSSG suggest that all the organisms are subject to a severe oxidative stress. Nevertheless, the greater degree of alteration was register in the organisms from La Draga and San Diego, sites of major urban impact.
**Conclusion.** The obtained results demonstrate that the tilapia *Oreochromis niloticus* reflects local conditions of impact by metals. The biomarkers of exposure and oxidative stress to metals analysed in the present study denote its robustness for studies of environmental monitoring even in species of recognized tolerance and in scenarios of high environmental complexity as the urban-agricultural wetland of Xochimilco.

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