

UF Pepper Center

RC2: Metabolism and Translational Science Core

Specific biological analyses useful for geroscience studies and trials are critical in determining whether targeted interventions can delay the onset and progression of impaired mobility. For each REC applicant or pilot study, all scientists involved (Core leaders, staff, and study Principal Investigator (PI)) will consider all potential measures in detail and determine the most appropriate analyses.

Examples of Key Biomarkers Directly Linked to Selected Domains of Biology of Aging.

Biological Domains of Aging	Proteins (Immunoblot/ELISA/Jess System/Luminex)	RNA (qRT-PCR)	DNA (qRT-PCR and q-PCR)	Function (Oxygraph-2k, XFe96 Flux Analyzer, Spectrophotometry)
Mitochondrial Biology (including Oxidative Stress)	PGC1a, TFAM, NRF1, Opa1, Fis1, Mfn1, Drp1, Sirtuin-3; total OXPHOS cocktail (includes subunits of all five ETCs), GDF15; MPO, oxLDL, protein carbonyls, ferritin; Nitrotyrosine, 4-HNE	PGC1a, TFAM, NRF1, Sirtuin-3, TNFa, IL-6, NF-κB, ETC enzymes; GDF15	Quantification of Mt DNA copy number Mt DNA strand breaks and oxidized purines within specific regions encoding ETC (NADH dehydrogenase subunit 1/2 (ND1/2), NADH dehydrogenase subunit 4/5 (ND4/5), Cytochrome Oxidase subunit II and ATPase subunit 6/8 (COII/ATPase 6/8), Cytochrome B6, Mt DNA 4977-bp common deletion (Mt DNA ⁴⁹⁷⁷)	Mt respiratory function in WBCs, fat, and muscle fibers (high-resolution respirometry, Oxygraph-2k; high-throughput respirometry, XFe96 Flux Analyzer); ETC enzyme and citrate synthase activity; ROS production (H ₂ O ₂); aconitase activity
Senescence and Inflammation	Senescence proteins: GDF15, Fas, OPN* ² TNFR1, CCL3, IL-15, activin A NF-κB; adiponectin, MPO, soluble E-Selectin, Soluble ICAM-1, soluble VCAM-1, Total PAI-1, IFN-γ, IL-10, IL-12, IL-12, IL-13, IL-1β, IL-5, IL-6, IL-8, TNF-α, IL-15	TNFa, IL-6, NF-κB, GDF15	DAMPs; Mt DAMPs like <i>Mt DNA</i> and nuclear fragments	
Autophagy	Beclin-1, Atg proteins, LC3, LAMP1 & 2A, PINK1, Parkin, p62, BNIP3, TFEB	Same as proteins		
Circadian Biology	Bmal1/Arntl, Per1, Per2, Rev-erb alpha, beta (Nr1d1 and Nr1d2), Nfil3, and Dbp	Same as proteins		
NAD⁺ Homeostasis	NAMPT, CD38, PARPs, SARM1, sirtuins	Same as proteins		NAD/NADH and NADP/NADPH; NAMPT enzyme activity

To achieve our goals, RC2 pursues the following aims:

Aim 1: To support protein, RNA, and DNA isolation and analysis of specific biomarkers of aging.

Aim 1A: To provide services that extract protein from biospecimens (tissues and blood) as requested or required, and to perform biochemical protein analyses (standard and multiplex) that suit the specific needs of a study. RC2 uses traditional western blot or capillary-based immunoassays (Jess, ProteinSimple, San Jose, CA) for protein analysis of tissues (i.e., muscle, fat, and brain). Jess is a novel automated capillary nano-immunoassay capable of fluorescent multiplexing that is used to analyze the expression of multiple proteins simultaneously in small tissue samples. For protein analysis in plasma/serum, Luminex xMAP® multiplex technology (Luminex, Austin, TX), and ELISAs will be used to analyze applicable biomarkers.

Aim 1B: To support RNA isolation from biological samples (e.g., blood, muscle, fat) as requested or required, and related biochemical analysis. RNA will be isolated and cDNA prepared. RC2 uses quantitative real-time PCR (qRT-PCR) to analyze gene expression in these biological samples (Bio-Rad Laboratories C1000 Touch™ Thermal Cycler with CFX96 Touch™ Real-Time PCR Detection System, Bio-Rad Laboratories, Inc., Hercules, CA).

Aim 1C: To support DNA isolation from tissues or whole blood as requested or required, and related biochemical analysis. This core uses qRT-PCR and quantitative PCR (q-PCR) for the analysis of Mt DNA copy number, molecular damage to nuclear and Mt DNA in tissues, and damage-associated molecular patterns (DAMPs) in plasma. qRT-PCR is performed as described above, and q-PCR is performed via Bio-Rad Laboratories C1000 Touch™ Thermal Cycler with Dual 48/48 Fast Reaction Module.

Aim 2: To support analysis of Mt respiration, Mt enzyme activities, and NAD coenzymes.

Aim 2A: To measure Mt respiratory function in freshly isolated tissue (muscle, brain, sensory cells, etc.) with high-resolution respirometry.

Aim 2B: To measure Mt respiratory function of cells (i.e., WBCs) using high-throughput respirometry.

Aim 2C: To measure Mt electron transport complex (ETC) activities (complexes I–V) and NAD coenzymes (NAD⁺, NADH, NADP⁺, and NADPH) in frozen tissue samples using a multimode microplate reader.

Aim 3: To facilitate and provide consultation for investigators on analyses and sample storage, and collaborate synergistically with the other Older Americans Independence Center (OAIC) cores. We will interact closely with all OAIC cores in the design of studies and selection of biomarkers, as well as support Research Education Core (REC) Scholars and pilot study investigators. In addition to the above listed biochemical analyses, we will provide consultation for investigators to employ other cores for additional analysis relevant to aging (i.e., genomics, transcriptomics, epigenetics, and proteomics (see Resource section)). Furthermore, RC2 will provide short-term sample storage and coordinate long-term storage of biological samples for future ancillary studies with the University of Florida (UF)'s Clinical and Translational Science Institute (CTSI) Biorepository.

The Metabolism and Translational Science Core is capable of performing all of the analyses listed below as well as many others that fall within each category (e.g., most ELISAs and multiplex immunoassay panels).

In addition, the Core provides

- Guidance on the selection of analyses best suited for an investigator's project
- Consultation on the development of standard operating procedures for the collection, processing and storage of biological samples as well as on IACUC/IRB protocols
- Budget estimates for both currently funded projects as well as grant proposals
- Training of young investigators

Main Measures

Analysis technique	Assay / Analytes	Specialized Equipment
ELISA	Sample type: plasma, serum, tissue	<ul style="list-style-type: none"> • Bio-Tek Synergy HTX multi-detection microplate reader (fluorescence, absorbance and luminescence measurements) with Gen5 Data Analysis Software • BioTek Instruments ELx405™ HT Microplate Washer - for 96-well plates
Oxidation	MPO, oxLDL, Protein carbonyls, ferritin	
Inflammation	TNF α , IL-6, IL-8, V-CAM, Selectins, CRP	
Apoptosis	Cell death ELISA	
Multiplex Immunoassay	Sample type: plasma, serum, tissue	<ul style="list-style-type: none"> • Millipore MILLIPLEX® Analyzer 3.1 xPONENT System with MILLIPLEX® Analyst 5.1 software (Luminex 200) • BioTek Instruments ELx405™ HT Microplate Washer - for 96-well plates
Cardiovascular Disease, Metabolic Disorders, Inflammation	Adiponectin, MPO, Soluble E-Selectin, Soluble ICAM-1, Soluble VCAM-1, Total PAI-1, IFN-gamma, IL-10, IL-12, IL-12, IL-13, IL-1beta, IL-5, IL-6, IL-8, TNF-alpha	
qRT-PCR	Sample type: cells, tissue, whole worms	<ul style="list-style-type: none"> • Bio-Rad Laboratories CFX96 Touch™ Real-Time PCR Detection System • Bio-Rad Laboratories C1000 Touch™ Thermal Cycler with Dual 48/48 Fast Reaction Module
Inflammation	TNF α , IL-6, NF- κ B	
Autophagy/Proteolysis	LAMP-2, Atg5, 7, 12, Beclin-1, MurF 1, Atrogin 1, Foxo-3	
Aging-related transcription factors	FOXO/DAF-16, FOXA/PHA-4, NRF2/SKN-1, nuclear hormone receptors, etc	
Mitochondria	mtDNA copy number, mtDNA mutation/deletion/damage	
Immunoblot	Sample type: tissue, cells, mitochondria	
Oxidative stress	Nitrotyrosine, 4-HNE	

Mitochondria	Fusions & Fission (Opa1, Fis1, Mfn1, Drp1), Biogenesis transcription/Translation (PGC1 α , TFAM, Nrf1)	<ul style="list-style-type: none"> • ProteinSimple Jess System for capillary immunoassays (chemi, IR, NIR detection for multiplexing) • Bio-Rad Criterion™ gel electrophoresis • Bio-Rad Trans-Blot® SD semi-dry transfer cell • Bio-Rad Trans-Blot® Turbo™ Transfer System • Bio-Rad Gel Doc XRS+ high-resolution gel/blot imaging system
Inflammation	TNF α Receptor I, NF- κ B	
Apoptosis	Caspases 3 & 9, PARP, Endo G, & AIF	
Autophagy/Proteolysis	LC3, Beclin-1, Atg proteins, p62, PINK-1, Parkin, MuRF-1, Atrogin-1, Foxo-3	
Activity assay/Spectrophotometry	Sample type: cells, tissue	<ul style="list-style-type: none"> • Bio-Tek Synergy HTX multidetection microplate reader (fluorescence, absorbance and luminescence measurements)
Oxidative stress / Mitochondria	ROS production (H ₂ O ₂), Enzyme activity: Aconitase, Citrate synthase, Cytochrome c oxidase	
Apoptosis	Caspase 3 & 9	
Respirometry	Sample type: isolated mitochondria, cells, tissue, whole worms	<ul style="list-style-type: none"> • OROBOROS Oxygraph-2k for high-resolution mitochondrial respiratory function assessment • Agilent/Seahorse XFe96 Flux Analyzer for high-throughput in a 96-well plate format <ul style="list-style-type: none"> ○ BioTek Cytation 1 cell imaging multi-mode reader (cell count)
Mitochondrial respiratory function	High resolution (Oroboros O2k) or high-throughput (Agilent/Seahorse Flux Analyzer XFe96) mitochondrial respiratory function measurements: oxygen consumption rate; extracellular acidification rate (XFe96 only)	