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REVIEW

University of Alabama at Birmingham Nathan Shock Center: comparative energetics of aging

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Abstract The UAB Nathan Shock Center focuses on comparative energetics and aging. Energetics, as defined for this purpose, encompasses the causes, mechanisms, and consequences of the acquisition, storage, and use of metabolizable energy. Comparative energetics is the study of metabolic processes at multiple scales and across multiple species as it relates to health and aging. The link between energetics and aging is increasingly understood in terms of dysregulated mitochondrial function, altered metabolic signaling, and aberrant nutrient responsiveness with increasing age. The center offers world-class expertise in comprehensive, integrated energetic assessment and analysis from the level of the organelle to the organism and across species from the size of worms to rats as well as state-of-the-art data analytics. The range of services offered by our three research cores, (1) The Organismal Energetics Core, (2) Mitometabolism Core, and (3) Data Analytics Core, is described herein.

Keywords Analytics · Aging · Energetics · Bioenergetics · Comparative biology · Metabolism · Mitochondria

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Introduction

The University of Alabama at Birmingham (UAB) Nathan Shock Center of Excellence in the Basic Biology of Aging, co-directed by Steven N. Austad and Thomas W. Buford, in collaboration with the Indiana University School of Public Health, focuses on comparative energetics and aging. For this purpose, energetics is defined as the study of the causes, mechanisms, and consequences of the acquisition, storage, and use of metabolizable energy. Comparative energetics is the study of metabolic processes at multiple

scales and across multiple species as it relates to health and aging. Nearly a century of aging research has reinforced the link between energetics and aging. In modern terms, this link is understood as increasingly dysregulated mitochondrial function, altered metabolic signaling, and aberrant nutrient responsiveness with increasing age. The twin objectives of the Center are to (1) explore in greater depth and detail than previously possible the complex relationship among cellular energetics and its relationship to organ and whole organisms' energetics, health, and aging and (2) provide quantitative, state-of-the-art technologies and novel methods in the assessment and analysis of energetics as it relates to health and aging to the geroscience community at large. An additional educational objective is addressed by the center's subscribable monthly e-newsletter (https://www.uab.edu/shockcenter/resources/aging-biology-update), *Aging Biology Update*, which catalogues new and often unique publications of interest to the basic aging researchers.

The UAB Nathan Shock Center has taken advantage of its unique strengths in comparative biology of aging and world-class expertise in cellular and tissue bioenergetics with similar expertise in the dissection of whole-animal energetics—in addition to an exceptionally innovative and adept Data Analytics Core—to offer the most comprehensive, integrated energetic assessment and analysis available anywhere, from the level of cells to organisms and across species from the size of worms to rats. We also now offer newly developed techniques that significantly enhance our ability to provide our unique constellation of services to researchers outside UAB.

Our contributions to geroscience, the recently defined field that links aging and age-related diseases [1], can best be described by specifying and describing the range of services we offer in our three research cores: (1) The Organismal Energetics Core, (2) Mitometabolism Core, and (3) Data Analytics Core.

Organismal Energetics Core (leader: Timothy R. Nagy, co-leaders Daniel L. Smith, Jr. and Christy S. Carter)

The Organismal Energetics Core provides specialized expertise in the development and utilization of methods, state-of-the-art instrumentation, and the selection of animal models to facilitate understanding the contribution of energetics to biological aging. This includes nutritional, genetic, behavioral, and pharmacological interventions that impact aging research. The core routinely measures energy acquisition, storage, and utilization in a variety of large and small, terrestrial, and aquatic animal models including *C. elegans*, *Daphnia*, *Drosophila*, zebrafish, and rodents under environmental conditions tailored to the researchers' experimental needs.

Importantly, the core has a standard operating procedure for assuring quality control and consistent results for all assays. Phenotyping is conducted using unbiased experimental design with appropriately matched controls and in a blinded fashion. The core encourages researchers to use both male and female animals whenever possible.

The core is led by Tim Nagy, who has been instrumental in advancing the field of small animal phenotyping. He helped create and validate the use of dual-energy X-ray absorptiometry (DXA) in mouse-sized rodents and has validated DXA for use with fish, snakes, rats, and humans. Dr. Nagy has also validated the use of micro-computed tomography (μCT) for determining in vivo liver fat in small animals and has utilized quantitative magnetic resonance (QMR) to determine fat content of Drosophila, zebrafish, lizards, toads, mice, and rats as well as validated the use of bioelectrical impedance spectroscopy in mice and rats. He also pioneered the use of magnetic resonance imaging (MRI) for the quantification of brown adipose tissue in mice and total fat content in organs using a pig model. Dr. Smith, extensively involved in these validations, and Dr. Carter, with her behavioral expertise, have extensive experience in implementing all core services. These services include consultation and advice on experimental design using all of its instruments.

Core services include the following.

Chemical carcass analysis (CCA)

CCA is still the "gold standard" for the determination of whole-body and individual organ composition (fat mass, fat-free mass, water, and ash content). The core is well versed in this method and still uses CCA as the standard for validating new instruments and techniques. CCA can be employed to assess body composition in animals as small as *C. elegans*. In addition, this method

is useful when animals have been euthanized and frozen prior to body composition analysis. This service may be particularly helpful for users external to UAB.

Quantitative magnetic resonance (QMR)

QMR is the gold standard for quantification of body composition in vivo. Animals do not require anesthesia, which is optimal for energy balance studies, as anesthesia carries risks of complication with aged animals and can influence food intake, body weight, and body composition. The core has the ability to determine fat, lean, and water content of *unsedated* animals ranging in size from 7 mg up to 900 gm. This core is the first lab to measure and validate in vivo fruit fly and zebrafish body composition by QMR [2, 3]. It is also the first to report using QMR that even small degrees (5%) of caloric restriction paradoxically increases relative body fat in female C57BL/6 mice [4].

Dual-energy X-ray absorptiometry (DXA)

DXA allows for the determination of total and regional body fat, soft-lean tissue, and bone (bone mineral content and density) in live animals. In addition, to use with laboratory rodents, the core has validated and published data on body composition of unique animal models such as snakes, lizards, and catfish [5–7].

Assessment of brown adipose tissue *in vivo*

Thermogenic, primarily fat-utilizing brown adipose tissue (BAT) can be assessed in living sedated animals using MRI [8]. This technique is particularly useful in longitudinal studies of the energetics in relation to health and longevity, including before and after interventions. In particular, it is a useful complement to studies of mitochondrial function in BAT with significant correlation between BAT fat-fraction signal and the expression of the mitochondrial uncoupling protein UCP-1.

Bomb calorimetry

Caloric content of food, feces, and animal tissues is determined using a micro-bomb calorimeter. By comparing energy content of food and feces, it is the gold standard for determining the amount of energy extracted during digestion and absorption. Bomb calorimetry is becoming increasingly important for measuring a comprehensive energy balance/energetics budget in studies focusing on the nutritional, genetic, and/or pharmacological interventions which impact aging, as well as microbiome-related outcomes.

Comprehensive assessment of energy balance

The core's indirect calorimetry systems (TSE systems) allow simultaneous collection of data on oxygen consumption, carbon dioxide production, food consumption, and locomotor activity in up to eight mice or rats at a time. The entire unit is housed in an environmental chamber allowing precise control of ambient temperature and photoperiod. Although the core can only conduct the indirect calorimetry studies on eight rodents at a time, methods and protocols have been developed to optimize throughput and reducing variability due to acute housing changes that occur with other measurement approaches. To this end, the core has 16 additional TSE metabolic cages that are available for staging a two-day acclimation period prior to assessment. Body composition is always measured in conjunction with the indirect calorimetry measures given the significant differences in metabolic rate between fat and lean mass. The method of choice for measuring body composition for this is QMR; however, if the investigator is also interested in bone phenotyping, then DXA can be used.

Oxygen consumption rates in aquatic organisms such as zebrafish and *Daphnia* are measured using the Loligo® respirometry system (Loligo Systems ApS, Denmark) with static chambers appropriately sized for the individual organism being measured. The chambers can also be used to measure oxygen consumption in terrestrial organisms by flushing with oxygenated air using either the timed exchange or chamber oxygen level. Blanks (chambers without animals) are run in parallel as well as before and after animals are introduced into the static chambers to provide internal controls. A 24-well microplate system has now been validated for measures of oxygen consumption down to the individual animal level for organisms including worms and zebrafish embryos in liquid media and *Drosophila* in air using the Loligo® system platform.

Energetic measurements are available using many other instruments and methods including mouse and rat running wheel cages, treadmill and rolling wheels for forced exercise, capability to measure VO_2 max in rodents, glucose and insulin tolerance testing, home cage activity, and core temperature using implantable transmitters.

Healthspan assessments

The core is also capable of assessing a variety of short-term physical activity measurements as well as measures of cognition (memory and anxiety) using EthoVision® XT, a system for automated tracking and analysis of animal activity and behavior. The system is suited for everything from straightforward open field tests to high-throughput research, to sophisticated protocols including external equipment control. EthoVision XT can be used to track animals including rodents, flies, or fish, in a wide range of test set ups. A basic EthoVision XT set up allows a researcher to automate standard behavioral tests such as the water maze, plus maze, and open field test. In addition, EthoVision XT is easily used to automate high-throughput experiments, such as monitoring zebrafish larvae activity in 96-well plates. The added benefit of this system is that it may be used to help local and external investigators add healthspan measures to their portfolio of assessments. This is because the core is able to guide investigators through how to design and set up behavioral "arenas" to assess their healthspan measure of interest, in their local environment and video record the behaving animals. The core can then receive those videos and run them through the EthoVision XT system. Thus, investigators without access to this state-of-the-art equipment, or without knowledge of designing and performing behavioral assays, may benefit from the expertise of this core regardless of the location of their home institution. In addition, local assistance with rotarod, grip strength, and exercise training as well as various intervention methods (food, water, injection, or gavage delivery) is available.

Mitometabolism Core (leader: Jianhua Zhang, co-leaders Victor M. Darley-Usmar and Scott W. Ballinger)

The Mitometabolism Core provides state-of-the-art services to assess age-related changes in metabolism, mitochondrial function, mitophagy, and redox biology. Specifically, the core focuses on the interface between mitophagy and mitochondrial function and integrated interpretation of bioenergetic and metabolomics data in which we have unique expertise. The core builds on the emerging concept, developed at UAB, of integrating bioenergetic function with mitochondrial health (e.g., the Bioenergetic Health Index, BHI) [9–12]. This approach also allows for the integration of metabolism and mitochondrial function with mitophagy metrics [13–15]. The core also offers techniques needed to determine the impact of mitochondria-nuclear interactions (which also were pioneered at UAB) [16]. In addition, the core offers three recently developed approaches: (1) measurement of mitochondrial function in frozen samples; (2) an integrative analysis method so that aspects of mitochondrial function can be integrated with metabolomics, as well as aspects of mitophagy that are important for mitochondrial quality control (10–12); and (3) the quantitative measurement of mitochondrial damage-associated molecular patterns (mtDAMPs) from plasma/serum samples. The newly developed techniques will greatly extend the application of bioenergetic measurements to stored frozen samples and alleviate the current limitations that require fresh samples for mitochondrial measures.

Specifically, core services include the following:

Bioenergetic analyses in diverse fresh tissues and diverse organisms

Many of the probes to test autophagy or mitochondrial function are based on biochemical properties such as metabolite profiles or pH change/membrane potential that can easily be employed across species. The respirometry platform we use for mitochondrial analysis is the extracellular flux analyzer (a.k.a. the Seahorse) for intact and plasma membrane permeabilized cells, tissues, and organisms [17–19]. We have applied the mitochondrial stress test using the sequential addition of inhibitors of oxidative phosphorylation as described in our methods articles and reviews for a broad range of cell types including human platelets, cardiomyocytes, and monocytes [9, 10, 18, 20, 21]. We have had similar success in applying these types of methods (some of which we have developed and some using literature protocols) to pancreatic islets, arterial sections, and adipose tissue.

Bioenergetic analyses using frozen cells and tissues

One of the challenges of making the XF technology available to researchers in the aging field external to sites with the instrumentation and expertise is that the methods have previously been limited to fresh cells or tissue. This restricts mitochondrial assessments to spectrophotometric assays of single enzymes, which does not provide data in which complex activities can be compared using the same units, is not an integrated measure of inter-complex electron transfer, and is low throughput. In collaboration with Dr. Orian Shirihai at UCLA, we developed new methods to allow the measurement of mitochondrial function in frozen samples using the XF platform [22]. The assay is approximately 100 times more sensitive than oxygen electrode–based assays and 10–20 times more sensitive than standard spectrophotometric assays with increased throughput (approximately 20–50×) and improved precision. An additional advantage is that the method is sufficiently sensitive to measure mitochondrial electron transport activity in cell lysates, so avoiding artifacts during mitochondrial isolation. This new method will greatly extend the application of bioenergetic measurements to stored samples and decrease the current limitations that require fresh samples for mitochondrial measures and so allow services to be offered to aging researchers outside of UAB. All complex activities can be measured with a high degree of reproducibility in as little as 10 mg of frozen wet weight tissue.

In addition to the methods above, we employ the mass spectrometry Core at UAB to provide targeted metabolomics data as needed by investigators. For complex data sets, we have used advanced informatics techniques such as the xMWAS analytical package that can integrate metabolomics/bioenergetics data with aging-relevant biological endpoints [23].

Integration of variables key to mitochondrial quality control

Mitophagy and mitochondrial quality control assessments

Defective mitochondria are removed from the cell through the controlled process of mitophagy, which becomes defective during aging [24, 25]. The challenge for investigators has been to link mitophagy and metabolism and bioenergetic health. Because of its complexity, optimal experimental design and interpretation need considerable thought and expertise. Our core assists investigators in the aging field from the initial conceptualization of ideas to experimental design and data interpretation in assessing mitophagy and mitochondrial dynamics. Multiple complementary methods are an approach we recommend, as we have detailed in our methods articles and reviews [26–30] If an investigator finds differences in mitochondrial dynamics or number, we can then guide them in defining mechanisms including changes in mitochondrial biogenesis or mitophagy. We detailed these approaches in a recent methods article [26].

Isoprostanes and mitochondrial DNA (mtDNA) damage as markers of oxidative stress

The non-specific process of lipid peroxidation generates a series of characteristic products, the levels of which are well established, unusually stable, markers of oxidative stress. The oxidation products derived from arachidonic acid are isoprostanes [31, 32]. We offer this measurement to core users through the mass spectrometry core at UAB. It is likely that the unsaturated fatty acids in other species than mice and humans will be different, and in this case, we will determine the analogs of the isoprostanes generated so that they can be used as indices of oxidative stress across species. We have considerable experience in the mechanisms of lipid peroxidation and their detection [32, 33] and are well versed in the techniques necessary to purify and identify these products and measure them in the low pg/ml range using isotope dilution analysis LC-ESI-MRM-MS. These methods also can be complemented by the more commonly used measurement of protein carbonyls and thiol oxidation (both protein and free).

The core assesses DNA damage using quantitative PCR (QPCR). Damage is detected in a gene-specific manner that does not require large amounts of DNA. The principle of this gene-specific assay is that DNA lesions will block the polymerase and therefore will lead to a decrease in amplification [29, 34]. Sensitivity of the assay increases through amplification of large targets (thereby increasing the probability of encountering a DNA lesion). Briefly, genomic DNA is extracted (Qiagen) and quantified fluorescently (PicoGreen, Molecular Probes) on a Cytofluor 4000

Series fluorimeter and used for QPCR. We have successfully used this assay to show age-related accumulation of mtDNA damage in diverse cells and tissues including vascular tissues [26, 28, 35].

Integration of mitochondrial bioenergetics, metabolomics, and programs of mitochondrial quality control

Great progress has been made in recent years that enabled unbiased large data set collection and analyses. These advances are expected to have high impact on complex processes including aging. Furthermore, with the development of sensitive methods such as the XF96 extracellular flux analyzer, we are now able to measure multiple interconnected parameters of mitochondrial function, using both the mitochondrial stress test and electron transport chain complex-specific substrates. These variables can then be subjected to integrative analysis, differential network analysis, and community detection to improve our understanding of complex molecular interactions and disease mechanisms [36]. We have in place at UAB a targeted Metabolomics Core and, as mentioned above, are able to enlist it for use by investigators interested in aging research. Data generated can be incorporated with bioenergetics and mitophagy/mitochondrial dynamics studies using the freely available xMWAS platform. Such analyses have been successfully used in detecting new connections of transcriptome-metabolome interaction networks in SH-SY5Y cells [37] and the strong association of selective metabolites with mitochondrial parameters in platelets [23]. We have introduced to the community the concept of the bioenergetic-metabolite-interactome as a framework to integrate complex data sets and reveal metabolic programs and how they adapt during aging and now offer these methods to aging researchers [38].

Mitochondrial nuclear exchange (MNX) models, haplotyping and mtDAMPs

MNX models

The core offers MNX models (originally developed at UAB [16]), whereby isogenic (nuclear) backgrounds having different mtDNA genetic backgrounds are generated. This enables assessment of the impact of different nuclear, mitochondrial, and nuclear-mitochondrial genetic combinations on disease susceptibility, pathology, molecular measures, and gene expression in vivo. Using these models, we have shown that specific nuclear-mtDNA combinations modulate susceptibility to many forms of age-related diseases such as heart failure [39], cancer [40], obesity [41], and pulmonary dysplasia [42]. These changes also are accompanied by major changes in gene expression when challenged with stressors associated with unhealthy aging (e.g., high-fat diet). Using this approach, we have been able to directly assign the impact of either the nucleus, mtDNA, or nucleus-mtDNA combination on whole animal metabolism and gene expression [41]. These approaches provide a new means for interpreting the genetics for disease susceptibility [43, 44] and assessing the role(s) of nuclear and/or mitochondrial genetic backgrounds in aging and related disease processes. Note that the MNX models are not standard conplastic models in that they are generated directly with 100% of the desired nuclear and mtDNA complements from respective donor strains through nuclear transfer [16]. This approach allows for unambiguous assessment of mtDNA contributions to the pathway of interest as there is no complexity introduced by recombinational events between different nuclear backgrounds from the F_1 hybrid and subsequent filial generations associated with standard backcrossing methods in conplastic models [45].

Mitochondrial DNA haplotype analysis

At times, it will be necessary to confirm mtDNA haplotype to determine the impact of the mtDNA on aging processes and/or disease pathology. In these instances, mtDNA haplotype analysis will be performed by direct sequencing at the UAB Heflin Center for Genomic Science Core Laboratories.

mtDAMPs

Recent studies focusing upon the factors underlying inflammatory response have identified damage-associated molecular patterns (DAMPs) as initiating factors. DAMPs are endogenous molecules released by cells in response to stress factors (e.g., ischemia, environmental factors) that initiate or enhance inflammatory response. DAMPS can be nuclear or cytosolic proteins or molecules. They can also be mtDNA. It

has been shown that release of extramitochondrial mtDNA fragments (mtDAMPs) directly induce innate immune response [46, 47]. Evidence suggests that release of these factors is associated with aging, chronic inflammation, and the pathogenesis of age-related disease in humans, mice, and rats [48, 49].

We have developed protocols for quantifying specific mtDAMPs from both human and mouse plasma/serum samples. We have developed these methods for both human and mouse samples. We have been able to quantify mtDAMPs at levels as low as 1–1,000 copies per individual sample, which enables quantitative comparisons between individuals. As recent studies have delineated the molecular pathways linking mtDAMPs to the initiation of innate immune response, this strategy provides researchers with a mechanistic approach for connecting changes in mitochondrial bioenergetics, quality control, and genetics with inflammatory response. Increased mitochondrial dysfunction and/or oxidative stress should impact levels of mtDAMPs and therefore activate inflammatory pathways.

Education and training in the measures of bioenergetic health and mitochondrial health and quality control

In conjunction with the other cores, we will continue to develop webinars and educational video content to extend the user base of the state-of-the-art techniques featured in this core. As an example, we have developed an educational video describing methods for bioenergetic analysis that was published in the journal JOVE and has been downloaded approximately 50,000 times. We continue to update these offerings, links to which can be found on the UAB Nathan Shock Center website.

Data Analytics Core (leader: David B. Allison, co-leader Andrew Brown)

Statistical analyses for biology of aging research data provide many challenges. For example, aging research involves longitudinal analyses requiring researchers to model the dependency of multiple observations for the same organism taken over time. Second, missing data commonly occur with longitudinal study designs. Third, these issues are compounded by how to model the dependence among multiple samples taken from each organism even at a single time point. Fourth, with time-to-event (e.g., survival) outcomes, issues of left, right, and interval censoring add further complexities. Statistical approaches in comparative biology face similar issues, e.g., phylogenetic dependence (non-uniformity on the residual covariance structure). Given that evolutionary biology suggests that all taxa are related, there are other unique, species-level developmental and anatomical aspects to consider. Furthermore, how to model comparisons across species over a lifespan is a major statistical challenge.

Recently, concerns have been raised about the reproducibility of basic science investigations. Although sometimes such concerns are expressed as evidence of poor research practices, irreproducibility can be the result of genuine and interesting differences in measurement methods, statistical models, and model organisms. Furthermore, scientists, who work with model organisms, frequently use basic statistics to analyze their data. However, there is a growing realization that reported statistical claims in scientific publications are often mistaken [50, 51]. Aging research is not immune to such reproducibility challenges. The Data Analytics Core assists researchers in addressing such issues by supporting rigorous design and analysis implementation and training for researchers both within and beyond the UAB Nathan Shock Center.

The core, which is physically located at the Indiana University-Bloomington School of Public Health, is led by Dr. David B. Allison. Dr. Allison is a fellow of the Gerontological Society of America and the American Statistical Association, is an elected member of the National Academy of Medicine, and served on the National Academy of Sciences' recent Reproducibility and Replicability panel [52]. Dr. Andrew W. Brown, the core co-leader, is an assistant professor in the Department of Applied Health Science, and a long-time collaborator of Dr. Allison.

Core services offered include the following.

Statistical support for research on aging, including traditional, specialized, and bespoke methods

Effective statistical collaboration requires thorough involvement of statisticians in all phases of the study, from research proposal development and study

design to manuscript preparation. This involves a major and active participation in the entire research program, aimed at acquiring an accurate picture of the investigators' data and hypotheses of interest. The core strongly encourages continued engagement with researchers throughout a project, but also helps investigators even if they engage the core only at certain phases (e.g., power analysis; final analysis after data collection). Core expertise in traditional statistical techniques include study design; power and sample size estimation; strategy of individual or cluster randomization; definition of outcome variables; model selection and validation of distribution assumption; determination of frequency of repeated measurements; interim and final analyses of projects; and collaboration in manuscript and grant proposal preparation.

Specialized statistical approaches beyond point-and-click software packages are often needed to address specific research questions. Because our combined expertise spans decades of aging research, we are able to provide support that is content-specific. A non-exhaustive list includes the following:

1. *Cluster randomization and power analysis*. A critical but often ignored problem in animal studies is that animals may be group-maintained in cages. Outcomes from animals in the same cage may share more similarities than those from animals in different cages. Ignoring these "clustering" effects can lead to underpowered designs and flawed hypothesis testing. Generalized estimation equations or random effect models should be used.
2. *Computer-intensive, resampling-based inferences*. In the case of small sample size or highly skewed data for which current asymptotic theory–based statistical approaches are not appropriate, some resampling-based methods (bootstrap, permutation, etc.) can be provided to the researchers, utilizing the core's high-performance computing facilities as needed.
3. *Genetic data analyses*. New techniques and reduced costs make genetic analyses a powerful tool to investigate genetic and gene-environment interaction effects on the energetics of aging. Drs. Allison and colleagues have conducted genetic studies including microarray, GWAS, next-generation sequencing, and epigenetics analyses and are developing new methods and software packages to analyze high-dimensional genetic data [53–56].
4. *Longitudinal analysis*. The statistical analysis of longitudinal data presents special challenges because repeated measurements on research subjects over time violate independence assumptions. The emphasis on aging rate and accurate characterization of individual change over time require a substantial shift in approaches: from concern with heterogeneity among subjects at a given time to heterogeneity among subject's changes and measurement reliability over time. A longitudinal study is necessary to estimate subject-specific rates of change, a prerequisite to exploring patterns of change among physiological processes in the aging organism and relationships to the mortality and morbidity experiences of individuals [57]. This can be challenging in ecological settings, such as repeated fecal sample collection in wild elephant populations.
5. *Mediation analyses*. Core investigators have expertise in mediation analysis, which can help elucidate the mechanism for the effect of potential causal factors (e.g., caloric restriction) on aging-related outcomes [58].
6. *Missing data techniques*. Animals may die for predictable or unpredictable reasons, and measuring equipment may temporarily fail. Such situations lead to incomplete information, can reduce power, and may bias inference. Multiple imputation strategies will be provided to handle missing data. Dr. Allison also has conducted investigations to validate methods for handling missing data and maximizing statistical power in mediation analysis [59]. Multiple imputation is particularly challenging to implement in longitudinal analysis.
7. *Study design*. A thoroughly designed study ensures that a desired inference can be drawn from the resultant data. Sometimes, the design elements are relatively routine (power analysis; randomization; allocation concealment; blinding). Other cases are more intricate. For instance, the core worked with Dr. Chusyd on an ongoing project to create a study design and a priori data analysis plan for the early life trauma and biological aging study with orphan elephants mentioned above, with specific challenges of age- and

sex-matching orphan with non-orphan elephants and the difficulty of longitudinal analyses with expected unpredictable missing data.

8. *Survival and lifespan analysis*. Analysis of longevity and survival times is a special focus in research on aging. While many analyses are "off-the-shelf" methods such as Cox proportional hazards, log-rank tests, and Kaplan–Meier curves, often more specialized methods are required. Drs. Allison and colleagues have published on methods to expand analytical options for lifespan analysis including tests to compare maximum lifespan with the generalized lambda distribution [60], which facilitate investigating differences in various characteristics of lifespan distributions rather than only means.
9. *Bespoke statistical support*. Researchers may have immediate questions that no available statistical approach can directly address, such as when complex statistical method's distributional assumptions are violated. In such cases, the Analytics Core will adapt or create statistical solutions to appropriately address the research question (e.g., tailoring a resampling-based statistic to the task). This is highlighted in the work on the Organismal Energetics Core's Loligo system described above. We also help researchers determine the most powerful and cost-/time-efficient designs using methodological and simulation studies, and we have previously published several papers on this stimulated by our work in helping aging-research scientists design and analyze studies.

Provision of reproducibility, verification, and transparency support for aging researchers

Our efforts in this area serve to bolster scientific rigor, thereby preventing the need for post-publication error correction. In cases where correction is still needed, we help streamline the process and normalize the self-correcting nature of science, rather than unduly penalize it. We and others have benefited from these processes, too: for every aging paper over the last two years for which Data Analytics Core members were co-authors, we have caught an error in the paper before it went out of various magnitudes. Our experiences demonstrate that even professional statisticians can use a double check on analyses and reporting. Hence, we formally test the reproducibility of data, code, and results. We have facilitated reproducibility throughout the stages of study completion, including (1) bottom-up consulting; (2) at the analysis stage; and (3) post-submission or post-publication. For bottom-up consulting, the Data Analytics Core works with authors to create a data management plan up-front and establish practices to make code and data reproducible from the start. At the analysis or pre-publication stage, we rerun code directly and compare to the manuscript to ensure that the results in the paper are easily reproducible and are, in fact, reproduced. Frequently, we are not able to exactly reproduce results in the manuscript, and at least some numerical results need to be revised before publication. Revisions range from correcting rounding errors to substantially different conclusions. Our processes prevent errors from entering the literature. Finally, if requested, we check data and code for public sharing after a paper has been submitted, accepted, or published. If errors are identified at this stage, we have helped investigators navigate post-publication error correction, which can be time-consuming and difficult to navigate [51]. Frequently, the qualitative conclusions from the studies have remained relatively unchanged upon post-publication checking, but other examples exist in the literature of errors that needed substantial correction or retraction.

Verification: confirming and resolving discrepancies in existing results

We distinguish verification from reproducibility checks. Reproducibility is a simple process of independently confirming whether exact results can be reproduced. Verification involves deeper, more critical statistical evaluation of the design, data, and analytical methods. Verification has three main steps. First is reproducibility, where we ensure that we can produce the same results in the manuscript from final data provided. Second is to check that reported analytical methods are the methods actually and correctly implemented during analysis. Third is a review from a biostatistician to determine whether employed methods were appropriate for the project. If the Data Analytics Core is involved early in the research process, verification can instead start with two separate statistical teams

discussing analytical strategy, analyzing results in parallel to check robustness between teams, and teams checking each other's work. We help investigators review analysis protocols and pre-registration to support them in following through on methods that were decided a priori (see below).

Transparency: ensuring trust in science and making results go further

The core will help investigators with three separate tasks that are becoming increasingly common to support reproducible science:

(1) Data and code sharing. Investigators face numerous choices in how and where to share data and code. Various repositories exist that have unique considerations for the type of project. The core's experiences using many of these position it to facilitate their adoption by investigators. It will assist with additional considerations for how data and code should be structured and annotated, including data dictionaries and FAIR (findable, accessible, interoperable, and reusable) data principles.
(2) Pre-registration. Pre-registration supports transparent and reproducible science by allowing investigators to specify a priori study designs, outcomes, and analysis plans for more robust hypothesis articulation and testing. The core will assist with pre-registration protocols, which can vary depending on type of study design.
(3) Reporting guidelines. The core provides guidance on implementing reporting guidelines, such as by checking adherence with CONSORT for randomized trials [61], ARRIVE for animal studies [62], and PRISMA for systematic reviews [63].
(4) Text recycling or plagiarism detection. Immediately prior to submission, we will help investigators run manuscripts through text recycling or plagiarism software iThenticate (licensed to IU's SPH for this purpose) to ensure that work has been appropriately cited. The use of this service has prevented honest errors in inadequately citing one's own or others' work.

Using UAB Nathan Shock Center Services

To inquire about using UAB Shock Center services, investigators should contact Core leaders directly. Contact information for Core leaders can be found at https://www.uab.edu/shockcenter/research. Initial direct contact is critical for consultation on feasibility, experimental design, and cost. Some projects may be appropriate for the Shock Center pilot grant program. Requests for pilot grant (up to $25,000) applications are posted twice per year, and requests for small pilot awards (< $10,000) can be submitted at any time. For information on those possibilities, contact Christy Carter (cartercs@uabmc.edu), leader of the Research Development Core.

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