IACUC Issues Related to Animal Models of Aging

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The articles in this issue provide an excellent introduction to and overview of the use of various animal species as models for the study of human aging. Because of the welfare issues associated with such studies, we identify potential concerns that require attention to ensure the appropriate use and humane treatment of the animals involved. We also discuss the importance of model selection and endpoints for use by investigators, institutional animal care and use committees (IACUCs), and others.

Relevance of Research on Aging

The aging process is an inevitable part of life for humans and all other living organisms. It is accompanied by loss of normal function and, often, the onset of degenerative and debilitating disease ultimately resulting in death.

The human population is aging at an unprecedented rate: in 2000, 10% of the world’s population was over 60 years of age; by 2050, that number is estimated to reach 21% (DESA-PD 2001). Yet much remains unknown about the aging process and ways to minimize the debilitating effects associated with it. For example, human life expectancy in the United States reached a record high of 77.9 years in 2007 (Minino et al. 2009), but aging-related health problems continue to seriously compromise quality of life and are a financial burden for individuals and society. Four of the top five leading causes of death in the United States are chronic diseases that disproportionately affect older persons (Minino et al. 2009), and recent US statistics indicate that 36% of total personal healthcare dollars are spent on people 65 years and older (Stanton and Rutherford 2006). Thus the development of preventive measures or interventions and treatments that slow aging and postpone or shorten the associated period of deterioration and disease would not only alleviate suffering and improve the quality of life for millions but also vastly reduce the costs associated with end-of-life health care.

The essential aim of biogerontological research is not merely to extend lifespan but to maintain good health and quality of life—mobility, mental acuity, productivity, and well-being—during the aging process. Consequently, the development and validation of animal models relevant to improving healthspan (the length of time an aging individual remains healthy, functional, and free of disability or disease) are critical to this important area of biomedical research.

Selection of Animal Models

Many animal species—some phylogenetically close to humans, others not—are long-lived and remain physiologically and behaviorally functional well into old age and so may be useful animal models for the study of human aging.

Species lower on the phylogenetic scale, such as fish (Gerhard 2007) and mice (Yuan et al. 2011), are effective models for the study of basic mechanisms of aging and contribute to a better understanding of the physiological and genetic basis for functional longevity. Species with close genetic homology to humans or analogous aging phenotypes may serve as translational models to help link research findings between lower species and humans and thus support the development and evaluation of interventions that successfully increase healthspan.

As with any biomedical research using animals, it is essential to justify the use of an animal model (i.e., the potential knowledge gained and benefits to society must outweigh the potential pain and distress to the animals) and to validate the appropriateness of the selected model. Although the typical advantages of using animal models for the study of human disease (e.g., the ability to manipulate genetic and physiological parameters, short generation time, and control of variability) apply to animals used in aging research, additional caveats may apply regarding the selection of a species.

Even among species commonly used for aging research, it is crucial to be aware of physiological and behavioral differences associated with varying genetic background, sex, environment, and other factors to ensure appropriate model selection. Gallagher and colleagues (2011) note that albino mice and rats are frequently used as models of neurocognitive aging even though the loss of retinal function associated with albinism negatively affects performance in visually based cognitive tests. And Fischer and Austad (2011) point out that the reproductive biology of small nonhuman primates differs from that of humans: unlike women, postreproductive tamarin females maintain circulating levels of estrogen and progesterone, limiting their usefulness as models of human reproductive

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aging. Thus phylogenetic relatedness does not guarantee validity as a model for human aging research.

Aging studies often require lifelong care and monitoring of the animals involved, so, in addition to the selection of an appropriate species and/or strain, consideration must be given to the long-term availability of adequate funds and the ability to provide appropriate housing and husbandry. Species such as mice and rats may involve a relatively short-term commitment of resources; others, such as nonhuman primates, dogs, and long-lived rodents (Edrey et al. 2011), require a commitment of many years or even decades.

The psychological and social needs of the species must also be addressed and may be difficult to fulfill for long-lived species such as nonhuman primates. For example, aggression between male rhesus macaques (Macaca mulatta) used in the National Institute on Aging intramural study (Kemnitz 2011) necessitated the individual housing of all male monkeys in the study from year 6 of the study onward. Considering that rhesus macaques can live for 30 years or more, some of these animals may have been individually housed for decades. What is the best way to address the lifetime psychological and social needs of such animals? Is long-term individual housing of a nonhuman primate—or any social species—a humane and appropriate use of an animal for research? Issues such as these require serious thought and discussion by all parties involved in the protocol development and review process.

Protocols should clearly define measures to maintain stability and disease-free conditions in research animal colonies for the duration of their use. Adequate health surveillance and disease prevention practices (e.g., barrier housing for rodents, careful quarantining and screening of new arrivals) are essential to ensure that infectious diseases are not introduced into a colony during the study period. Other factors can also influence study results; Tardif and colleagues (2011) describe a study in which adult mortality in a marmoset (Callithrix jacchus) population used for aging research varied greatly over time because of changes in colony social structure, movement to a new facility, and exposure to infectious agents when new animals were added to the colony. Any such variability can skew the research results and cause the needless loss of the animals involved.

Finally, although most aging studies extend for the life of the animal, some do not, and in these cases it is important to determine what will happen to the animals after the study. Some animals may be suitable for use in another study, euthanasia may be appropriate for some species, “retirement homes” are available for the care of nonhuman primates, and adoption may be a possibility for other species.

Importance of Monitoring and Humane Endpoints

Animal welfare issues in aging research are often similar to those associated with other types of biomedical research, but concerns unique to research with aging animals warrant careful examination by the IACUC.

The potential for individual animals in aging studies to experience chronic pain and distress caused by age-related debilitation and/or disease is high and must be balanced with the acquisition of beneficial data and scientific information. All such studies must include experimental endpoints that specify physiological or behavioral signs defining the point at which an animal will be removed from the study. Establishing appropriate endpoints before beginning the study prevents unnecessary pain and distress for the animal subjects and ensures accurate and timely data collection. To be effective, experimental endpoints must be based on relevant and objective criteria specific to the study.

In some studies of aging, however, it may be desirable or necessary to keep individual animals until the end of their life. Spontaneous death then becomes the de facto endpoint. But the use of this “endpoint” raises significant questions that must be addressed. Will ill or injured animals receive treatment or special housing or care? Is there any point at which an animal can be removed from the study before spontaneous death? Investigators who require longevity data are often reluctant to jeopardize their data by removing animals from aging studies before death, but the risk of animal suffering under these standards is high, so either death as an endpoint must be scientifically justified or alternative endpoints identified.

In certain circumstances, endpoints other than spontaneous death may be effective and should be considered as a means to reduce the potential for end-of-life pain and distress. For example, it is possible to predict death in aged mice to within 1 week or less by using biomarkers such as precipitous weight loss, decrease in body temperature, and respiratory difficulty (Ray et al. 2010). In Ray’s study, 1 week represented approximately 1% of the median lifespan; other studies (Black et al. 2003; Redgate et al. 1991) have also reported senescent terminal weight loss periods in rodents that can be used to predict the likelihood of death and allow early removal of animals without significantly affecting longevity data. A similar geriatric syndrome of terminal weight loss, called failure to thrive, affects elderly humans and may occur in other mammalian species (Black et al. 2003).

If euthanasia is not an option, the investigator must determine the acceptability of medical or other interventions to reduce pain or distress caused by illness and/or debilitation. In some cases, simple changes in housing, bedding, or food type or the provision of analgesics or anti-inflammatory medication may greatly alleviate suffering while allowing an animal to remain in a study without compromising data collection.

In an attempt to avoid the use of death as an endpoint, protocols sometimes call for euthanasia of animals when they become moribund. It is important to clarify that the term “moribund” typically refers to a severely impaired mental and physical state that occurs just before death (Toth 2000). Moribund animals may not be consciously aware of pain and distress but it is highly likely that significant pain and distress occur during progression to a moribund state. Euthanasia before an animal is moribund is a more humane alternative.
The definition of clear and objective endpoints before the start of a study will go far in preventing misunderstandings and delays in treatment for ill or injured animals. However, the traditional endpoints used in acute studies are not always appropriate for aging studies. Elderly but normally functioning animals may show poor body condition, physical disabilities, or neoplastic lesions, yet may remain in an aging study if they are not judged to be suffering or if appropriate interventions are provided to reduce suffering. For example, an aged rat that continues to eat, drink, and ambulate normally in the presence of a clinically obvious neoplastic mass might be kept on study if there is no evidence of pain or distress.

Personnel responsible for monitoring research animals must receive training to recognize abnormal behavior or appearance and know what measures to take when abnormalities are observed. Painful, frail, or anxious animals require more frequent observation and may benefit from alternative husbandry or housing. In some cases, individual housing may provide a debilitated, elderly animal more comfort than maintenance in a group; in others, a change in access to food and water or the provision of soft food may be sufficient to allow an animal with limited mobility or missing teeth to eat and drink and maintain body condition. Palliative treatments such as analgesics or anti-inflammatories may also be appropriate.

Use of Calorie Restriction

Research has demonstrated that chronic restriction of caloric intake without malnutrition (calorie restriction, CR) extends lifespan in rodents, nonhuman primates, fruit flies, nematode worms, and yeast and that it may also be effective in rabbits, dogs, and other mammalian species (Barzilai and Bartke 2009; Duffy et al. 1997; Kemnitz 2011), although differences in genetic background may lead to varying results in these species (Yuan et al. 2011). Such studies have become a major thrust of biogerontological research and there are several potentially useful animal models of CR. However, investigators have not yet identified the underlying mechanisms that lead to enhanced longevity (Barzilai and Bartke 2009; Ingram et al. 2005; Kemnitz 2011) and the resulting variability in CR study protocols raises welfare concerns for the animals involved.

Protocols should provide detailed information about the CR procedures and monitoring of the animal subjects throughout the study. For example, target weights or caloric levels should take into account strain, sex, and age differences in nutritional requirements for the species used (Rowland 2007). In particular, bearing in mind that the daily caloric intake for CR animals is usually 60-85% of that for ad libitum—fed controls, justification of the use of CR below these levels requires serious consideration of the potential for malnutrition, severe loss of body condition and/or body fat reserves, and psychological distress due to chronic hunger.

Investigators must also include information about the nutritional composition of the CR diet and about methods to monitor the animals’ body weight, food intake, vitamin and mineral intake, body condition, or other health measures during the study period. Because food intake spontaneously declines as nonhuman primates reach middle age (Kemnitz 2011), the caloric allocations for these animals require adjustment to prevent malnutrition. In fact, Kemnitz suggests that very old nonhuman primates should not be subject to CR at all.

Use of Privately Owned Animals

Large populations of aging companion animals (cats, dogs, and horses) are a relatively untapped resource for aging studies (Waters 2011). These species are long-lived and have varying rates of senescence linked to genetic background. Obviously, the use of privately owned animals requires informed consent by owners, who, in addition to the IACUC, must receive ongoing information about the purpose and goals of the study, especially if it involves withholding potentially beneficial treatments or the use of untested interventions. The protocol should also include guidelines for the withdrawal of an animal from the study if desired by the owner or if warranted by changes in the animal’s health status.

Emerging Welfare Issues

The increasing interest in research to improve healthspan will spur the search for animal models that effectively represent the characteristic traits of human aging, such as frailty, sarcopenia, and impaired cognitive capabilities (Kirkland and Peterson 2009; Walston et al. 2006), and such studies may entail welfare challenges. Old animals with potentially diminished ability to tolerate or recover from experimental manipulations may be at greater risk of pain and distress during research procedures intended to evaluate physical and psychological parameters of aging. Protocols will therefore need to clearly define measures for more sophisticated monitoring of animal health and well-being as well as potentially more aggressive treatment of illness or disability in experimental animals to minimize this risk.

The use of unusual or atypical laboratory animal species for aging research may require the development or refinement of appropriate clinical health assessments and experimental endpoints and may also present IACUCs and investigators with distinct challenges. For example, nondomesticated species that are not bred in captivity must be obtained from the wild. Personnel involved in the capture, holding, and transport of wild animals must have specialized training in the handling and care of these species to avoid animal welfare concerns. In addition, the capture and use of some wild species are regulated and require knowledge of specific regulations such as the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES, www.cites.org; Fischer and Austad 2011). Long-lived species such as the naked mole rat (Heterocephalus glaber), the little brown bat (Myotis lucifugus), and some wild birds have been suggested as excellent aging models (Austad 2009, 2011; Edrey et al. 2006). These species may be subject to CR procedures and monitoring of the animal subjects throughout the study. For example, target weights or caloric levels should take into account strain, sex, and age differences in nutritional requirements for the species used (Rowland 2007). In particular, bearing in mind that the daily caloric intake for CR animals is usually 60-85% of that for ad libitum—fed controls, justification of the use of CR below these levels requires serious consideration of the potential for malnutrition, severe loss of body condition and/or body fat reserves, and psychological distress due to chronic hunger.

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2011), but they require specialized care and housing to thrive in captivity.

The ever-increasing use of genetically manipulated animals in research creates unique welfare issues. Manipulation of the genome sometimes results in animals with unexpected health and/or behavioral issues that require special husbandry or veterinary care. In addition, the creation and breeding of transgenic, knockout, and other mutant animals—including genetically manipulated marmosets (Tardif et al. 2011) and other larger mammals—inevitably leads to the production of excess animals due to the necessity of breeding and identifying animals of the correct genotype for the study. Caring for these excess animals and/or finding an alternative use for them (e.g., in other research studies or for training) may be problematic for investigators and IACUCs, so it is important to consider ways to minimize overproduction and thus avoid euthanasia if no alternative use can be identified.

**Conclusion**

As the world’s human population ages, research involving age-related diseases, longevity, and healthspan will become more prominent. The selection and use of appropriate animal models is critical to enhancing knowledge of both the aging process and, eventually, ways to maintain health while aging and increase longevity. The use of animals in translational research (e.g., to develop interventions that successfully increase healthspan) is also vital, and may involve nontraditional species or privately owned animals. Although studies involving aged animals present unique challenges to animal welfare, carefully planned research protocols and thoughtful attention to endpoints can help minimize welfare concerns. The use of more sophisticated and comprehensive monitoring of individual animals, flexibility in housing and husbandry procedures, and palliative medications and treatments can further refine these studies and ensure both good animal welfare and high-quality data.

**References**