

# Successful Aging and Sustained Good Health in the Naked Mole Rat: A Long-Lived Mammalian Model for Biogerontology and Biomedical Research

Yael H. Edrey, Martha Hanes, Mario Pinto, James Mele, and Rochelle Buffenstein

## Abstract

Naked mole rats (NMRs; *Heterocephalus glaber*) are the longest-living rodents known, with a maximum lifespan of 30 years—5 times longer than expected on the basis of body size. These highly social mouse-sized rodents, naturally found in subterranean burrows in the arid and semiarid regions of the horn of Africa, are commonly used in behavioral, neurological, and ecophysiological research. Very old NMRs (>28 years), like humans, show signs of age-associated pathologies (e.g., muscle loss) as well as the accumulation of lipofuscin pigments, but no signs of tumorigenesis. Indeed, for at least 80% of their lives NMRs maintain normal activity, body composition, and reproductive and physiological functions with no obvious age-related increases in morbidity or mortality rate. Their long lifespan is attributed to sustained good health and pronounced cancer resistance. Clearly physiological and biochemical processes in this species have evolved to dramatically extend both their good health- and lifespan. We and others have tested various current theories using this species as an exceptionally long-lived animal model of successful abrogated aging. Surprisingly, NMRs have high levels of oxidative stress and relatively short telomeres, yet they are extremely resilient when subjected to cellular stressors and appear capable of sustaining both their genomic and protein integrity under hostile conditions. The challenge is to understand how these animals are able to do this. Elucidating these mechanisms will provide useful information for enhancing human life- and health-span, making the naked mole rat a true “supermodel” for aging research and resistance to chronic age-associated diseases.

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## Introduction: A Historical Perspective on Naked Mole Rats and Their Taxonomy

Naked mole rats (Rodentia: Bathyergidae, *Heterocephalus glaber*) were first described in Ethiopia by German naturalist Eduard Rüppell (1842) in his studies documenting the African mammals. He aptly named them “different-headed” (*Heterocephalus*) and smooth-skinned/hairless (*glaber*) because naked mole rats (NMRs<sup>1</sup>) lack a furry pelage and have an odd-shaped skull. Initially there was some question as to whether these furless specimens were pups of a larger haired adult or were diseased animals that had lost their fur as part of their pathology. These questions were answered definitively in 1893 after the collection of multiple specimens of these miniature walrus-like creatures by both Bottego and Parona and Cattaneo and others (Brett 1986; Jarvis and Bennett 1991). For the next 80 years NMRs seemed to be largely forgotten, the subject of only a few structural morphology and ecological studies (see Preface in Sherman et al. 1991).

Naked mole rats belong to the suborder hystricognathi in the order Rodentia. Members of this diverse suborder are characterized by multiseriate incisor tooth enamel, similar fetal membranes and sacculus urethralis, fused incus and malleus bones in the ear, and the lack of an internal carotid artery. The suborder includes caviomorphs (e.g., guinea pigs), hystricidae (e.g., porcupines), and phiomorphs (e.g., mole rats, rock rats, and cane rats). Naked mole rats belong to the family Bathyergidae, which includes six genera and more than 15 species (Deuve et al. 2008). Molecular phylogenetic analyses suggest that NMRs are a sister lineage to the other bathyergids (Deuve et al. 2008)—*Heterocephalus* is a monotypic genus with a fixed chromosome number (60) and no morphological variation.

In the following sections we introduce readers to the naked mole rat with descriptions of its social behavior and ecophysiology (ecological and physiological adaptation to

<sup>1</sup>Abbreviations used in this article: LQ, longevity quotient; MLS, maximum lifespan; NMR, naked mole rat; PI, peroxidation index; ROS, reactive oxygen species

its habitat) that may be pertinent to aging and biomedical research. We then set the stage for our focus on aging research by considering first traditional and then alternative animal models in this field. The subsequent discussions consider specific aspects of the NMR's basic aging biology and present examples of this remarkable animal's contributions to important discoveries in the field of aging research.

## Naked Mole Rat Social Behavior

Jennifer Jarvis in 1967 was the first person to keep NMRs in captivity for research purposes. She observed that although these animals were highly social and exhibited a complex suite of cooperative behaviors, breeding in captivity was difficult, with very few adult females successfully becoming breeders. Subsequently, several biologists working with Jarvis (RA Brett, R Buffenstein, CG Faulkes) began studying and reporting on the unique biology of this animal.

## Eusociality

In the mid-1970s Richard Alexander, an evolutionary biologist, was studying eusociality, defined as a colonial lifestyle with a strict division of labor and the presence of a single breeding female. His research focused on the evolution of eusociality in insects, and he proposed the hypothetical traits needed for a mammalian equivalent. At that time no eusocial mammals had been identified; the system was observed only in wasps, ants, termites, and bees (Alexander et al. 1991). Alexander described his hypothetical eusocial mammal as a strictly subterranean inhabitant, living in hard soils and having to rely on group efforts to excavate burrows and cooperatively forage for large underground tubers that could feed the entire colony (reviewed in Alexander et al. 1991). Terence Vaughan, a colleague and friend of both Jarvis and Alexander, told Alexander that he had aptly described naked mole rats, which Vaughan had seen in the office of Jarvis. Alexander, his doctoral student Paul Sherman, and Jarvis then began a collaborative study of eusociality in naked mole rats.

Jarvis observed that, like termites, male and female NMRs carry out communal tasks, live in colonies of up to 295 individuals, and restrict reproduction to one breeding female and 1 to 3 breeding males (Jarvis 1981; Lacey and Sherman 1991). Thereafter, studies of their breeding, behavior, and physiological ecology began in earnest, and members of Jarvis' lab conducted expeditions to collect animals from their natural habitat in the eastern horn of Africa. Early work on NMRs included mapping their large underground burrows and documenting the ecological variables in their natural habitat in the African horn in an attempt to understand the evolution of eusociality. Soil hardness, temperature, and rainfall were found to limit dispersal and influence reproduction and may also account for the animals' unique social system (Alexander et al. 1991; Brett 1991).

Extended longevity is a common feature associated with group living—cave-roosting bats, humans, mole rats, and

eusocial insects (honey bees, wasps, and ants) all exhibit longer lifespans than expected on the basis of body mass. Indeed, even though solitary species of mole rats inhabit similar thermally buffered subterranean habitats that facilitate low extrinsic mortality, their reported lifespan is approximately half that of the NMR, suggesting that colonial lifestyle may contribute to the NMR's extraordinary longevity. Although humans are not eusocial, there are certain parallels between long-lived eusocial species and human society: extended care of young, intergenerational transfer of information, and a division of labor, features that may enhance inclusive fitness by kinship and contribute to prolonged lifespan.

DNA fingerprinting shows that there is very low genetic variation in a colony, suggesting a high level of inbreeding similar to those of monozygotic twins or sibling-mated lab mice after 60 generations (Honeycutt et al. 1991; Reeve et al. 1990). Yet morphological differences (e.g., body mass) are evident even among littermates and determine their individual roles in the colony (Jarvis et al. 1991). The morphological differences are likely due to epigenetic mechanisms that have not been studied and may contribute to differential longevity.

## Breeders versus Workers

In the wild, nonbreeding worker animals that forage and extend or defend the burrow system live approximately 4 years—considerably longer than other free-living rodents. For instance, the striped field mouse (*Rhabdomys pumilio*) of southern Africa has a maximum lifespan (MLS<sup>1</sup>) of about 4 years in captivity. In population demographic studies in the wild involving the tagging of animals with a unique identification and their subsequent recapture, the estimated lifespan of striped field mice is 2 months (David and Jarvis 1985). Using similar techniques, mean lifespan for bank voles (*Clethrionomys glareolus*) in the wild is less than 3 months, whereas in captivity it is about 5 years (Bobeck 1969). Lower longevity is common in the wild, where many factors contribute to higher extrinsic mortality. Not surprisingly, given that NMR breeders in a colony are not subject to the same predation risks as nonbreeders, in the wild they live to ~17 years—4 times longer than their nonbreeding counterparts. This difference between breeders and workers diminishes in captivity, where both live well into their third decade.

Any female in the colony that is older than 6 months of age is capable of becoming a breeder. Should the dominant female in a colony die, the remaining females may fight to death to establish dominance and become reproductively active (Jarvis 1981; O'Riain et al. 2000). When a female becomes a breeder she exhibits an estrogen-dependent "pubescent" growth surge and a substantial increase in her body length (Buffenstein 1996; Dengler-Crish and Catania 2009). The subordinate females in the colony, regardless of age, remain anovulatory with very low levels of sex steroids (Faulkes et al. 1990). Similar suppression of the hypothalamic-pituitary-gonadal axis is

evident in subordinate males, which have low luteinizing hormone and testosterone levels, extremely small cryptorchid testes, abnormal sperm production, and impaired fertility (Clarke and Faulkes 1998).

Given the low sex steroid profiles in subordinate NMRs, it is not surprising that these nonbreeding individuals are sexually monomorphic. They have similar behavioral patterns, body size, anogenital distance, perineal musculature and bone traits; even the brain and spinal cord cannot be distinguished on the basis of gender (Edrey et al. 2010; Goldman et al. 2006; Holmes et al. 2007, 2008; Peroulakis et al. 2002; Pinto et al. 2010; Seney et al. 2006). Subordinate features differ markedly from those of breeders, regardless of gender.

## Ecophysiology

Researchers interested in ecophysiology originally focused on the NMR responses that enable the animals to survive and thrive in their dark, dank environment in the arid and semi-arid regions of northeast Africa. Fossil evidence revealed that NMRs have occupied a subterranean niche since the early Miocene era some 24 million years ago (Lavocat 1978). Not surprisingly, NMRs have evolved a suite of adaptive responses to this hostile habitat (Buffenstein et al. 1994). These include tolerance of vitamin D deficiency and efficient mineral metabolism in the absence of sunlight (Buffenstein et al. 1988, 1994), independence of free water sources (Urison and Buffenstein 1994), and microbe-dependent digestion of poor-quality foods with concomitant reliance on volatile fatty acids as a source of energy and microbes as a source of protein (Buffenstein and Yahav 1991; Yahav and Buffenstein 1992).

Life in sealed, deep (2 m), humid burrows in equatorial northeast Africa provides a thermally stable milieu throughout the year. Mechanisms for metabolic heat loss are limited to thermal conductance because the high humidity precludes evaporative cooling and the lack of air movement reduces the efficacy of convective heat exchange (Buffenstein and Yahav 1991); therefore, like most subterranean endothermic inhabitants, NMRs have low basal metabolic rates and high rates of thermal conductance (McNab 1979). Individuals isolated from colony members show pronounced thermolability (Buffenstein and Yahav 1991; Withers and Jarvis 1980).

Gas exchange in the burrows is also limited by the lack of air flow and slow rates of diffusion through soil, making the atmospheric conditions of the burrows largely hypoxic (with low levels of oxygen) and hypercapnic (with high levels of carbon dioxide). An unusual lung morphology enables NMRs' extreme tolerance of these conditions (Maina et al. 2001; Park et al. 2008).

Many of these evolved traits for survival in subterranean conditions may also be important in prolonged longevity. Neurophysiologists have recently analyzed the NMR nervous and sensory systems and identified many unique

features that enable the animals to thrive in their underground milieu (Catania and Remple 2002; Credner et al. 1997; Crish et al. 2006; Henry et al. 2006; Hetling et al. 2005; Riccio and Goldman 2000). Among these features are degeneration of the visual system and greater reliance on the somatosensory system (Catania and Remple 2002; Hetling et al. 2005; Nemeč et al. 2008) and an apparent inability to sense chemical (capsaicin and acid) pain (Park et al. 2008), although the animals respond normally to acute pinch and heat (Kanui and Hole 1990; Kanui et al. 1993; Park et al. 2008; Towett et al. 2006).

In 2002, surviving members of a wild-caught NMR colony collected in Kenya in 1974 and now in the Buffenstein lab were more than 28 years old. With this finding, these mouse-sized rodents were documented as the longest-living rodents (Buffenstein 2005; Buffenstein and Jarvis 2002), exhibiting a maximum lifespan now known to be at least 30 years (Buffenstein 2008; Buffenstein et al. 1988)—almost an order of magnitude longer than mice!

In addition to their impressive maximum lifespan, attenuated aging phenotypes, and resistance to cancer, these mouse-sized rodents continue to astound researchers in many other areas (Buffenstein and Yahav 1991; Catania and Remple 2002; Park et al. 2008; Reeve et al. 1990). Here we address several traits of aging naked mole rats, examine why they may be important in aging research, and document their unique contributions to the field of biological aging.

## Traditional Aging Models: Strengths and Shortcomings

The most frequently used animal models in biogerontology are *Caenorhabditis elegans*, *Drosophila melanogaster*, *Mus musculus* (particularly the inbred C57Bl/6 strain), and *Rattus norvegicus*. Because these traditional animal models exhibit common traits of rapid aging, such as genomic instability and damage to macromolecules with increasing age, they have yielded important insights into the genetics and molecular biology of aging across the animal kingdom. Furthermore, since their genomes have been well characterized, experimental manipulations involving the overexpression and/or deletion of a particular gene are possible, facilitating studies that can address questions about lifespan extension and the biological mechanisms involved in the aging process (Buffenstein et al. 2008a).

Significant advances in the field of aging have resulted from the study of these model species, including the alteration of lifespan through genetic means. For example, by manipulating the Methuselah gene identified in *Drosophila*, researchers have extended the organism's lifespan by 35% (Lin et al. 1998) with concomitant resistance to stress (heat, starvation, and oxidative stress; reviewed in Paaby and Schmidt 2008). Single gene mutations that influence MLS have been discovered in a wide range of classic animal models (reviewed in Aigaki et al. 2002). Collectively these led to the hypothesis that an evolutionarily conserved genetic program

may link nutrient utilization and the partitioning of energy with lifespan (reviewed in Guarente and Kenyon 2000; Longo et al. 2005).

Thus traditional animal models have many advantages for biomedical research, most notably the vast amount of information available for these species and the fact that their genome has been sequenced to full 7x coverage (e.g., Mouse Genome Sequencing Consortium 2002). Furthermore, because of the relatively short lifespan of these species, studies assessing genetic and experimental manipulations can be completed in a relatively short time frame without incurring too large a cost in animal maintenance.

Yet the features that make traditional models ideal for grant-based research may be problematic for studies relevant to human aging: these species are short-lived (with longevity quotients half that expected on the basis of their body size), indicating that they must not have evolved good defenses against aging. Moreover, some experimental manipulations that result in an enhanced lifespan are frequently accompanied by undesirable attributes such as decreases in fertility or pathogen resistance (Gardner et al. 2005). Even experimental manipulations that lead to lifespan extension in short-lived species and enhance their defenses may not be pertinent to attenuating rates of aging in humans and other long-lived organisms (Finch 1990; Miller and Nadon 2000). Thus the traditionally used animals may not yield relevant insights into the aging process of long-lived species.

## Alternatives to Traditional Animal Models

Rates of aging in the animal kingdom diverge more than 40,000-fold (Finch 1990). Even among mammals, aging rates (indicated by maximum species lifespan potential) vary by 2 orders of magnitude, exceeding even the most robust experimental lifespan enhancements (examples in Ayyadevara et al. 2008; Guarente and Kenyon 2000; Masoro 2005). Species with naturally divergent rates of aging have evolved mechanisms to facilitate prolonged good health, sustained activity, and robust immune systems in order to naturally lead long lives and should therefore be added to the animal models used in aging research. Studies of such species (e.g., Austad 2011; Tardif et al. 2011; and Waters 2011, in this issue) may yield useful information about the mechanisms of aging in long-lived species and about possibilities for retarding the aging process to promote healthier, attenuated human aging.

It is usually fairly easy to recognize species that have evolved a long lifespan, with a simple calculation based on the direct positive relationship between maximum lifespan and body size at adulthood: generally, for every doubling of body mass, MLS increases by approximately 16% (Buffenstein et al. 2008a; Hulbert et al. 2007). Using this allometric relationship, and comparing lifespan values predicted on the basis of body size with reported MLS (known as the longevity quotient, LQ; Austad and Fischer 1991; Prothero and Jürgens 1987), species that are outliers to this relationship are readily identifiable.

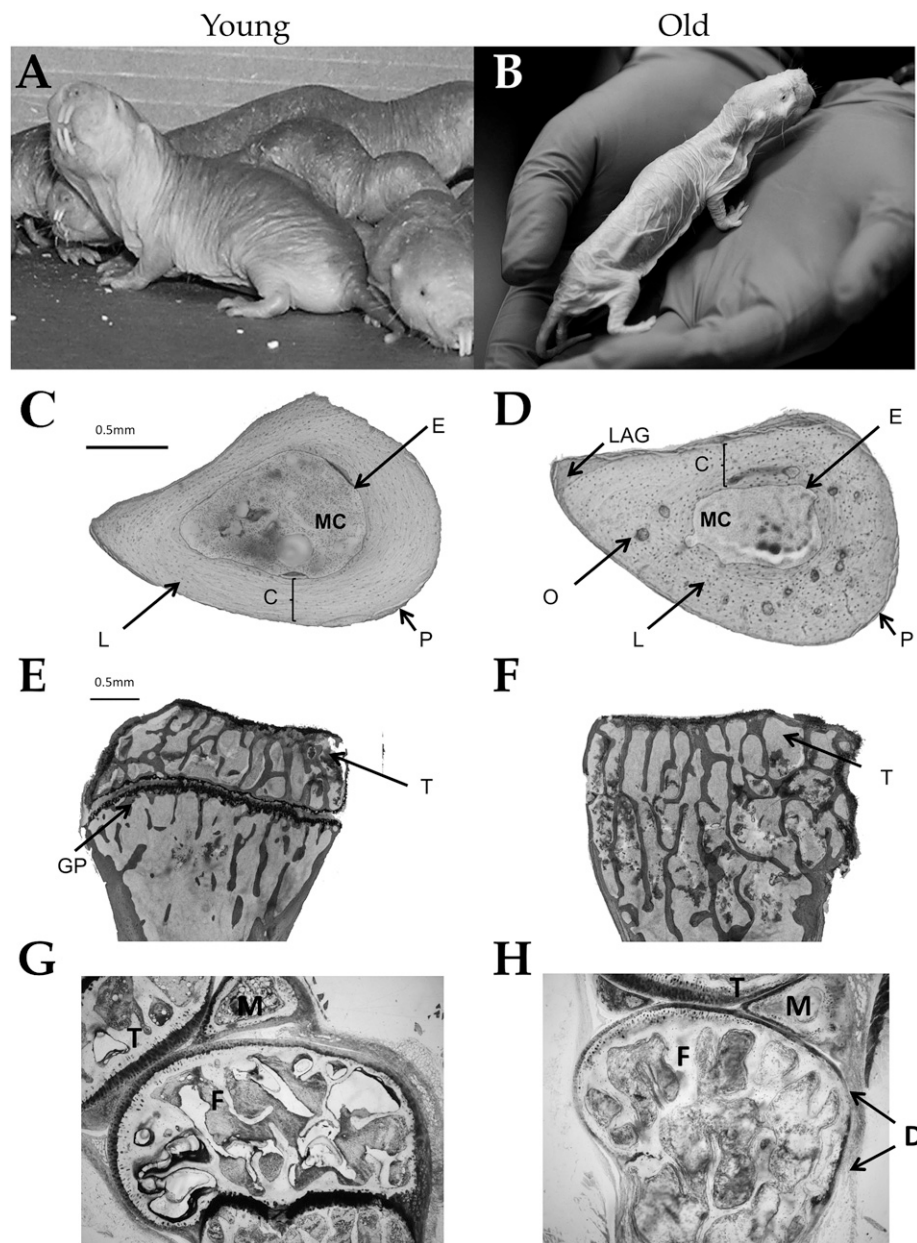
Data for a wide range of mammalian species reveal that enhanced longevity has evolved in a number of mammals—for example, humans, bats, and NMRs all live at least 5 times longer than expected on the basis of their body size. Ironically, short-lived outliers include mice and rats, which both live about 50% as long as would be expected by body mass, possibly because of their selective inbreeding in captivity for more than a century. Inbreeding weakens the forces of natural selection (indeed, one need look no further than the health challenges characteristic of purebred dogs, in contrast to the generally healthier lives of mutts), reduces adaptive genomic information essential for survival, and may lead to numerous failures in physiological function and early death (as indicated by low LQs). Thus the use of inbred rodent strains, which lack natural defenses against aging, may obscure the essential features of aging as a period of progressively impaired adaptation (Rose 2009), constraining the research to areas directly relevant to a particular mouse strain that may not be pertinent to human aging. In contrast, NMRs, although naturally inbred, do not suffer from common rodent pathology and exhibit a naturally long life.

## Basic Aging Biology of the Naked Mole Rat

Captive NMRs have recently surpassed their own record of longevity (28 years; Buffenstein and Jarvis 2002) and now carry an MLS value of at least 30 years (Buffenstein 2008). Adult mortality up until the age of 24 years does not appear to increase with age, and deaths occur with equal frequency among all age groups (Buffenstein 2008). The highest mortality rates are evident in the first 2 months of life; thereafter adult animals are seldom sick. Moreover, their physiology remains youthful as they maintain body composition, basal metabolic rate, and gastrointestinal absorption for at least 70% of their impressive lifespan (Buffenstein 2008; O'Connor et al. 2002). Given their exceptional longevity, and only a modestly reduced basal metabolic rate (75% of that expected on the basis of body size) relative to aboveground-dwelling similar-sized animals, it is not surprising that naked mole rats have the highest mass-specific lifetime energy expenditure of any known mammal (O'Connor et al. 2002).

## Activity Levels and Muscle and Bone Health

Only a slight decrease in activity levels is evident until at least 24 years of age, at which point NMRs are considered old. In our colony (Figure 1A), for example, the oldest individual (Figure 1B) is a male caught from the wild in June 1980; like other very old animals, he has recently become markedly sarcopenic but nevertheless commands alpha status as the dominant breeding male. Although less active than he was in younger years, he is capable of moving rapidly through the burrow system when food is supplied (as alpha he's entitled to early access) and is thought to be still siring pups.



**Figure 1** Naked mole rats (NMRs) in captivity. (A) A colony of young NMRs. Note large procumbent incisors, darker dorsal skin than ventral, and lack of external ear pinnae. (B) A 30-year-old NMR breeding male. This animal is sarcopenic and has also lost most of his subcutaneous fat layer, revealing a fine parchment-like transparent skin and his underlying organs. A transponder (for identification) is apparent through the translucent skin. (C,D) Femoral bones from young and old NMRs stained with toluidine blue. Cortical bone through the midshaft of the femur from a 2-year-old (C) and a 24-year-old (D) (original magnification 5x). There are organized osteocyte lacunae (L) arranged throughout the bone. Extensive signs of periosteal (P) and endosteal (E) remodeling as well as intracortical (C) remodeling with age are evident. Cortical bone area and density are unchanged with age. No significant change in medullary cavity area (MC) with age. There is considerable variability in osteon (O) number and size; the number is closer to that seen in humans than in mice. Lines of arrested growth (LAG) indicate fast growth patterns. The growth plate (GP) in the epiphysis is evident in a 2-year-old (E) but is completely resorbed at 24 years (F). This is clear evidence of a well-structured remodeled trabecular bone (T) that extends from the metaphysis to the epiphysis. NMRs show no indication of trabecular thinning or loss and thereby maintain trabecular connectivity. Trabecular bone morphology is sustained in a 24-year-old NMR. (G,H) Articular cartilage in young and old NMR in the left knee joints. Articular cartilage integrity generally persists from 2 to 24 years of age. This image shows the ossified meniscus (M) adjacent to the medial condyle of the tibia (T) and femur (F); unique to NMRs, the ossified meniscus may contribute to enhanced stability, reducing the amount of stress on the knee joint and assisting in the maintenance of cartilage integrity. In one instance we observed signs of damage (D) to the articular cartilage, a possible sign of osteoarthritis as indicated by the thinning and fibrillation of articular cartilage and subchondral bone changes in a 26-year-old NMR (H). Photos courtesy of Rochelle Buffenstein (A); Eric Gay, Associated Press (B); Yael Kramer, Department of Biology at the City College of New York (C-F); Mario Pinto (G-H). The original figure is in color and is available in the online posting of this article at [www.ilarjournal.com](http://www.ilarjournal.com).

NMRs take on their communal tasks beginning shortly after weaning and continue until death, without any apparent interruption (RB, personal observation). Among these tasks is the digging of burrows. NMRs use their teeth and large jaw muscles as powerful chisels, and their hind limbs to brace themselves while digging as well as to provide the power for locomotion and backward thrusts while shifting chamber contents. Studies of bone quality in these rodents reveal little degeneration and, unlike mice, very efficient bone remodeling (Figure 1C-F) and maintenance of cortical bone quality (Pinto et al. 2010). This remodeling allows older animals to carry out the same activities they did in their youth and may also contribute to sustained bone quality in breeding females that continue to reproduce throughout their long lives despite the high mineral demands of both pregnancy and lactation (26 years; Buffenstein 2008).

Older individuals show a substantial decline in articular cartilage thickness of the femoral and tibial condyles and in the patella (Figure 1G,H). Surprisingly, this decrease in cartilage thickness is not accompanied by significant age-related changes in calcified cartilage thickness, subchondral bone thickness, or chondrocyte number. The appearance of osteophytes (small bone protrusions), severe loss of cartilage, and cell cloning in the anterior region of the medial condyle of a 26-year-old individual suggests that old animals may naturally be afflicted with osteoarthritis (Figure 1H; unpublished data, Pinto, Jepsen, and Buffenstein). No indications of osteoarthritis were evident in the younger (<24 years) animals examined.

## Age-Related Effects on Internal Organs

The recent deaths of two females 29 and 30 years old revealed many age-associated, nonterminal pathologies commonly seen in elderly humans (Figure 2). These data, together with pathological reports from other old individuals, show that, like mice and humans, NMRs accrue the age-associated yellowish-brown pigment lipofuscin, which accumulates in various organs as a result of incomplete lysosomal digestion of cell products. Similarly, old NMRs show signs of infarcts in tissues and in tissue regeneration, especially in the liver and kidney. Although we have not as yet observed clinical manifestations of heart disease, histological signs are evident in aged NMRs. The oldest animals have increased numbers of cardiac cells with large nuclei (Figure 2A), which are also seen in the hearts of aged human and other rodents and are thought to represent either cells that have duplicated nuclear material or individual cell hypertrophy in response to increased cardiac load. Areas of accumulation are often in the perinuclear or polar position of the cardiac myocyte. Fibrosis in aged animal hearts is related to the replacement of cardiac myocytes, often considered postmitotic and terminally differentiated, by fibroblasts and increased extracellular matrix consisting of collagenous connective tissue.

Older animals may also have pronounced deposits of hepatocellular pigments, which often present as a combina-

tion of iron, lipofuscin, and sometimes bile and show signs of hepatocellular degeneration, with large misshapened, multinucleated cells (Figure 2B,C). The liver is typically capable of regeneration. As the primary organ for food metabolism, it is exposed daily to natural toxins (e.g., aflatoxins and mycotoxins in the captive NMR diet of fresh yams and corn). Over time, as with aging, the lysosomal residues accumulate, as well as the byproducts of hemoglobin digestion. Hepatocellular degeneration occurs when hepatocytes swell in response to nonlethal insults, impeding bile flow and resulting in a corrosive accumulation. Animals that have decreased food intake or metabolic disease (fatty liver, hepatic lipidosis) may be subject to large numbers of fat-laden vacuoles.

Surprisingly, very little pathology has been observed in the kidneys of aged NMRs. Whereas lab rats develop severe chronic interstitial and tubular disease that leads to renal failure and the fatal buildup of toxins, NMR kidneys occasionally show only mild mineral deposition in the collecting ducts and relatively small focal areas of infarction with fibrosis (periglomerular and tubular) and tubular regeneration (Figure 2D,E).

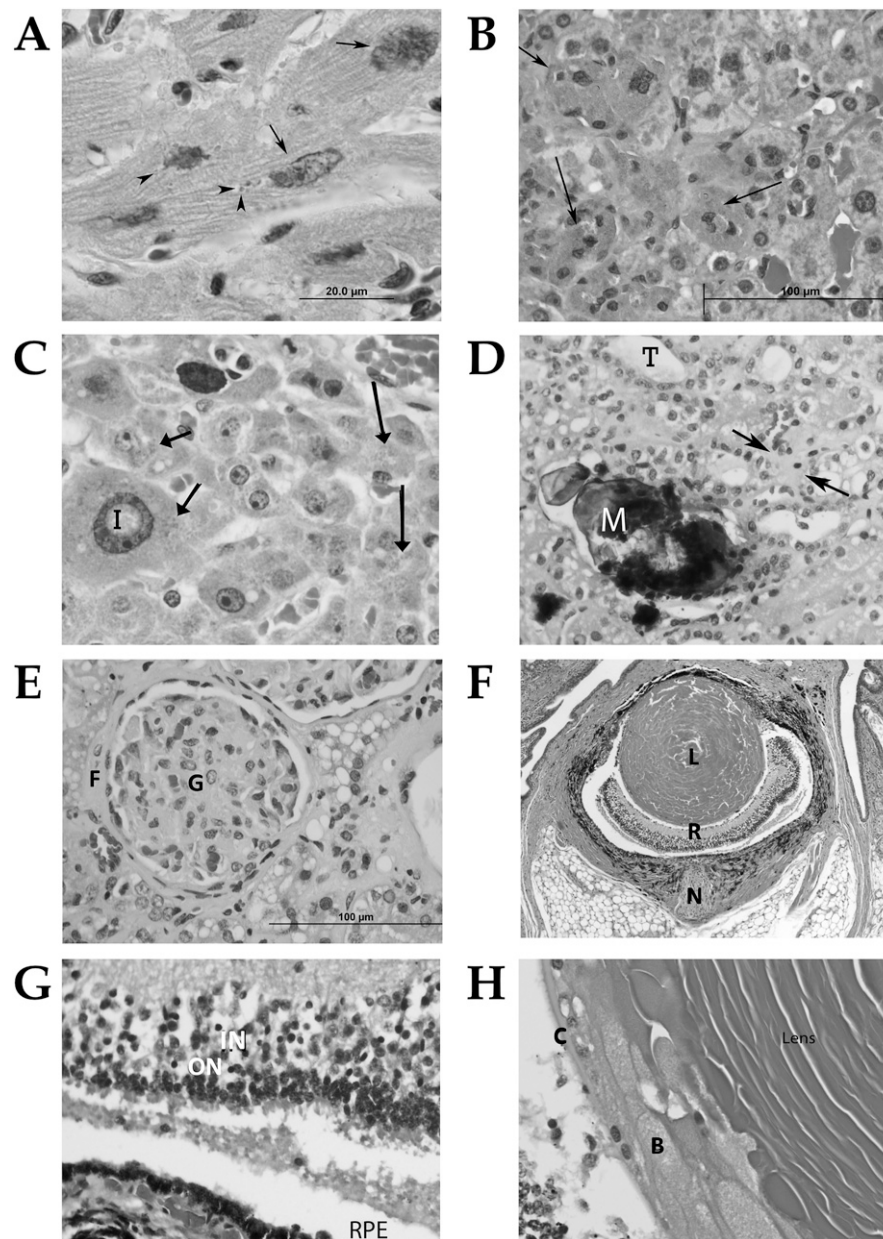
## Age-Related Effects on Neurosensory Systems

NMRs show no apparent age-related decline in cognitive function, although as yet we do not know whether, like humans, they suffer from extracellular plaques and/or other associated brain pathology. Neurotrophic growth factors, sustained at similar levels throughout adult life (YHE, unpublished data), may contribute to the maintenance of neuronal integrity and possibly also enable neurogenesis even in old age.

Although NMRs have a highly degenerate visual system (Crish et al. 2006; Hetling et al. 2005; Mills and Catania 2004), in keeping with life in a light-free environment, the eyes of young, middle-aged, and old animals reveal a seemingly age-associated progressive degeneration of the retina. This is characterized by the loss of nuclei in both the inner and outer nuclear layers, loss of the outer plexiform layer, and “mixing” of remaining nuclei. There are also age-associated cataractous changes in the eye lens (Figure 2F-H).

## Other Age-Related Pathologies: Cancer

There are as yet no reports of spontaneous neoplasia in NMRs. This lack of cancer may be an important component of the exceptional longevity of NMRs and is most unusual among both captive wild-caught rodents (such as *M. musculus* and *Peromyscus* sp.) and domesticated laboratory mice and rats—approximately 70% of domesticated laboratory rodent deaths are attributable to various types of cancers (Ikeno et al. 2005). Understanding the mechanisms that facilitate cancer resistance in captive NMRs may reveal important insights into cancer prevention.



**Figure 2** Images from key organs of very old (29-30 years) naked mole rats (NMRs), stained with hematoxylin and eosin, reveal that these animals present with many age-associated pathologies commonly seen in humans. **(A) Aged NMR heart.** Although clinical cardiac disease has not been noted, older NMRs may demonstrate histological signs of cardiac aging. Myocardocytes are branched striated muscle cells, with one or two nuclei. Several enlarged myocardial nuclei (arrows) are 2 to 4 times larger than those of typical myocardocyte nuclei in young animals. Lipofuscin pigment (arrow tips) is visible in the cytoplasm in a perinuclear fashion (original magnification 1000x). **(B) Aged NMR liver.** Yellow-gold intracellular pigment accumulation (arrows) is likely to be lipofuscin in hepatocytes. Degeneration of hepatocytes is characterized by variably sized cells. Hepatocytes contain both lipofuscin and iron pigment (arrows). **(C)** One large nucleus contains an invagination of the cytoplasm (cytoplasmic inclusion; I) (original magnification 1000x). Arrows represent accumulation of lipofuscin pigments, which are common in aged animals. **(D) Aged NMR kidney.** In the medulla of the kidney, a large focus of mineral (M) is present. Several areas of the interstitium demonstrate fibrosis (between arrows). Medullary tubules (T) are present (original magnification 200x). **(E)** A glomerulus (G) shows increased mesangium in the tuft and increased fibrosis (F) around the capsule. Progressive glomerular change with loss of function is part of the renal aging process (original magnification 400x). **(F) Aged NMR eye.** Low magnification (40x) of the eye demonstrates the size of the lens (L) relative to the size of the entire orbit. The retina (R) is detached, with some fusion of the retinal nuclear layers. The optic nerve (N) is present but much smaller than that of similar-sized rodents. **(G)** The retina in this high magnification (200x) shows loss of the normal numbers of granular cells from both its inner (IN) and outer nuclear (ON) layers and is detached from the retinal pigmented epithelium (RPE). These are hallmarks of retinal degeneration. **(H)** The high magnification (400x) of the lens reveals balloon cells (B) adjacent to the lens capsule (C). This shows the development of cataracts in the aged NMR. The original figure is in color and is available in the online posting of this article at [www.ilarjournal.com](http://www.ilarjournal.com).

## Contributions of Naked Mole Rats to Important Discoveries in the Field of Aging

Research on naked mole rats has contributed data to some key areas of aging research.

### Oxidative Stress Theory

Over the years biogerontologists have put forth various theories in an effort to explain the process of aging. The current most popular theory is the oxidative stress theory (Harman 1956), which states that as an organism carries out its basic functions it experiences oxidative damage from reactive oxygen species (ROS<sup>1</sup>) as well as oxidative stress from both endogenous (i.e., mitochondrial) and exogenous sources. Without ROS neutralization by antioxidants, damage to macromolecules may occur. Modulation of one of these variables (antioxidants, oxidative stress, and damage) should therefore affect the other factors and lifespan in a directly correlated way. Species with enhanced antioxidant defense ought to have a greater lifespan and lower levels of oxidative damage, assuming similar levels of ROS production.

Recent information from studies on NMRs and other nontraditional model organisms (e.g., birds and bats) as well as transgenic mouse models suggests that this theory is oversimplified and/or inadequate and that oxidative stress may play only a limited role in aging (Buffenstein et al. 2008b; Muller et al. 2007; Pérez et al. 2009a; Salmon et al. 2010). Data from NMRs, for example, do not support the theory on multiple levels.

NMRs produce amounts of ROS comparable to those of shorter-lived species such as mice (Labinskyy et al. 2006; Lambert et al. 2007) and yet show no significant changes in mitochondrial mass or efficiency throughout their lives (Csiszar et al. 2007). Similarly, studies assessing the effects on lifespan by increasing antioxidant defense mechanisms in genetically manipulated mice have yielded equivocal results—more commonly, no increase in lifespan is observed (Jang et al. 2009; Pérez et al. 2009a). Antioxidant levels in NMRs are not significantly elevated compared to those of short-lived species (Andziak et al. 2005), further supporting the premise that a superior antioxidant defense is not an essential component of longevity. Activity levels of antioxidants—manganese superoxide dismutase (Mn-SOD), catalase, and copper zinc SOD (Cu/Zn SOD)—do not change with age in NMRs, whereas mice show a significant age-related decline that is thought to contribute to higher levels of oxidative damage as they age (Andziak et al. 2005).

But not all our data contradict the revered Harman theory. NMR cells have an overall lower peroxidation index than do those of shorter-lived rodents, which may account for their cell membrane's resistance to oxidative stress (Hulbert et al. 2006). Furthermore, NMR membrane fatty acid composition does not change with age, suggesting that the animals are able to efficiently repair or remove damaged

phospholipids. High levels of oxidative damage to all macromolecules suggest that NMRs are capable of tolerating such damage throughout their long lives with no ill effect (Andziak and Buffenstein 2006; Andziak et al. 2006; Pérez et al. 2009b).

### Theory of Telomere Shortening

The termini of eukaryotic chromosomes carry telomeres, short, highly conserved repetitive sequences of DNA thought to protect the chromosome ends. With each DNA replication and cell division, these telomeres shorten because of the inability of DNA polymerases to completely replicate the chromosome ends (Olovnikov 1973). Telomere length is therefore considered a biomarker of aging and the theory of telomere shortening posits that the length of telomeres correlates with a species' maximum lifespan. Alternately, the reverse transcriptase enzyme telomerase, which is responsible for replenishing telomere function (Chan and Blackburn 2004), might play a part in species longevity. NMR studies, however, reveal no correlation between telomere length (Buffenstein 2005; Seluanov et al. 2007) and/or telomerase activity (Seluanov et al. 2007) and MLS. In fact, NMRs' relatively short telomeres are similar in size to those of humans, and their telomerase activity, although weakly measurable in skin fibroblasts (Seluanov et al. 2007), generally is limited to the division of tissue cells (e.g., testes, spleen, and skin) and only at very low levels of activity (Yang, Mele, Hornsby, and Buffenstein, unpublished observations). These data join a myriad of others that do not support this theory of aging.

### Glucose Regulation

One suggested lifespan modulator is the insulin/insulinlike growth factor (IGF) signaling (IIS) pathway, which is conserved in yeast, worms, flies, and mice. The genetic lowering of this signaling leads to a significant increase in lifespan (Guarente and Kenyon 2000), and a naturally low IIS may play a role in NMR longevity.

NMRs have low fasting blood glucose levels but show an impaired glucose tolerance, with prolonged elevated blood glucose concentrations, when given a supraphysiological bolus of glucose (Kramer and Buffenstein 2004). This response is similar to that seen in long-lived Snell and Ames dwarf mice (Kramer and Buffenstein 2004). Indeed, both NMR and experimental mouse models of lifespan extension share similar reduced thyroid and increased insulin sensitivity, suggesting a complex relationship between lifespan and glucose metabolism (Buffenstein and Yahav 1991; Buffenstein et al. 2001; Buffenstein and Pinto 2009). A possible mechanism that could explain this relationship may be mitochondrial biogenesis and/or cell proliferation, both of which are known to be affected by these hormonal regulators of metabolism (insulin and thyroid; Berdanier 2006).

The common result of enhanced lifespan in natural and experimentally manipulated long-lived animals may reflect a

general molecular mechanism that could contribute to anti-aging therapies. This important endocrine pathway is under investigation.

## Cell and Tissue Stability

The efficacy of proteomic maintenance has also been suggested as a mechanistic modulator of aging (Pérez et al. 2009b). Proteins are vital for every physiological process, so maintaining their structure and function is critical to an organism's existence. Altered protein expression and mutations in the protein itself (e.g., its structure) may lead to impaired cell function and cell death (reviewed in Naidoo 2009). Here, too, age-related changes are evident in mice but absent in NMRs over more than 2 decades. NMRs do better than mice at maintaining protein quality and stability: they show less irreversible oxidation damage, less urea-induced protein unfolding, and protein ubiquitination, all of which may be due in part to higher proteasome activity (Pérez et al. 2009b).

Lipids may also be critical in affecting rates of aging through cell stability (Hulbert et al. 2007). Among other functions, they compose the cell membrane bilayer; damage to membrane phospholipids compromises the cell membrane and thus impairs the ability to regulate cell contents. Lipids that are more susceptible to oxidative stress have multiple double bonds and a high peroxidation index (PI<sup>1</sup>). Oxidative damage to these polyunsaturated lipids produces a domino effect, as the products of lipid peroxidation are themselves potent ROS, resulting in further oxidative damage.

Saturated and monounsaturated fatty acids, on the other hand, are resistant to peroxidative damage. Fatty acid composition in tissues and mitochondria varies systematically among species and correlates both with body mass and lifespan (Hulbert et al. 2007). NMRs are one of a growing list of long-lived mammals that are outliers in the allometric relationship between PI and body mass; others are the echidna and humans (Hulbert 2008). All three of these mammals have maximum lifespans 4 to 6 times greater than predicted by body mass—their PIs concur with an inverse logarithmic relationship between PI and maximum lifespan (Hulbert 2008). Similarly, long-lived birds such as the albatross and petrel have a lower PI than shorter-lived birds (Buttemer et al. 2008), with PI values of the same logarithmic relationship as those of mammals.

Collectively these examples suggest that the doubling of MLS is accompanied by a 19% reduction in the PI of skeletal muscle fatty acids (Hulbert et al. 2007). This relationship between lipid peroxidation susceptibility and longevity is also evident within species: queen bees have lower PIs than worker bees (Haddad et al. 2007), wild strains of mice show PIs commensurate with their longevity (Hulbert et al. 2006), and human centenarians/nonagenarians and their offspring have lower PIs than the general human population (Atzmon et al. 2006; Puca et al. 2008; Rabini et al. 2002).

NMR fibroblasts, like those of other long-lived species, are remarkably resistant to a broad array of harsh toxins,

chemotherapeutic drugs, heat, and low-glucose medium (Salmon et al. 2008; JM, unpublished observations). Surprisingly, NMR cells reportedly are more sensitive than mouse cells to certain forms of stress such as H<sub>2</sub>O<sub>2</sub>, ultraviolet (UV) light, and rotenone (Salmon et al. 2008). These exceptions to their tremendous resilience may provide useful insights into the coping mechanisms of animals when faced with stressful toxins or situations. Lack of resistance to H<sub>2</sub>O<sub>2</sub> and UV light may reflect traits associated with environmental conditions belowground. Evolution in such a niche may have determined optimal levels of key cellular antioxidants, as displayed in the NMR by the dramatic 70-fold decline in cytosolic glutathione peroxidase and species-specific susceptibility to peroxidation of membrane lipids (Andziak et al. 2006; Hulbert et al. 2006). More importantly, the enhanced susceptibility to certain toxins may not be detrimental but reflect differential regulation of pathways governing the sensitivity and responses to DNA damage, such as ATR and p53 (common markers of the cellular stress response), and enhanced cell senescence and apoptotic pathways that could remove or prevent damaged cells from contributing to the physiological decline of the organism.

Finally, NMR brain slices are tolerant of nutrient deprivation (Nathaniel et al. 2009) and, perhaps most impressively, data suggest that NMR brains are extremely tolerant of a wide range of oxygen availability, surviving hypoxia and anoxia (Larson and Park 2009; Nathaniel et al. 2009). Indeed, although these animals have evolved to live under relatively hypoxic atmospheres, they appear to thrive when housed in aboveground gaseous atmospheres that are comparatively hyperoxic to those in which the species evolved (Edrey et al. 2010). Such housing may, however, contribute to the high level of oxidative damage we see in captive colonies. Hyperoxia can be as detrimental as hypoxia and both can cause neuronal death in most species.

## Cancer Resistance

Resistance to chemical stressors, DNA-damaging agents (e.g., chemotherapeutic drugs), and toxins may contribute substantially to the pronounced cancer resistance of naked mole rats. Experimental attempts to transform cells, using the oncogenic cocktail of Ras<sup>G12V</sup> and SV40 large T antigen (TAg), into tumorigenic agents have been unsuccessful in NMRs (Liang et al. 2010), despite the effectiveness of this method in other rodents, bovines, and humans, which develop aggressive cancers capable of metastasizing and killing their hosts (Livingston and Bradley 1987; Sun et al. 2004, 2005; Weinberg 1989). In NMRs, the Ras<sup>G12V</sup> and SV40 TAg-transduced cells rapidly enter crisis, as indicated by numerous nuclear abnormalities, including anaphase bridges, multinucleation, aneuploidy, and signs of incomplete cell division. Crisis is commonly caused by DNA damage or telomere dysfunction and concomitant failure of cytokinesis. The rapidity with which NMR cells enter crisis or permanent cell cycle arrest may reflect their highly

efficacious cell cycle surveillance by tumor suppressors when confronted with mutagenic agents (JM, unpublished observations).

NMR fibroblasts expressing oncogenes can, however, form tumors if they also ectopically express hTERT (human telomerase reverse transcriptase; Liang et al. 2010). Altered cancer resistance facilitated by hTERT may indicate that, unlike laboratory rats and mice and despite published reports to the contrary (Seluanov et al. 2007), NMRs lack telomerase in somatic tissues and skin fibroblasts or that hTERT has properties different from those of the rodent telomerase. It is not known whether the addition of hTERT simply facilitates telomere maintenance, but this seems unlikely as crisis was rapidly induced before cells had the chance to divide numerous times and thus critically shortened the telomeres. Rather, extratelomeric effects of hTERT, independent of telomere maintenance, may be responsible for the induced tumorigenic growth. When NMR cells normally encounter potentially mutagenic agents or cellular modifications they rapidly stop dividing and enter crisis. This response most likely plays a key role in NMR cancer resistance.

## Conclusions

We have described the unique features and aging traits of the naked mole rat and explained why this long-lived animal is an ideal animal model for aging research. This species may also prove to be an excellent model in studies exploring resistance to common chronic age-associated diseases. NMR studies have yielded data that do not support some current widely accepted theories of aging, but future studies addressing other mechanistic pathways that may modulate aging may provide more pertinent insights into the aging process. Mechanistic dissection of these pathways and their regulatory role in aging may lead to intervention studies that in turn support efforts to prolong human health and lifespan.

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