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Theoretical Paper

Exploring Overlooked Natural Mitochondria-Rejuvenative Intervention: The Puzzle of Bowhead Whales and Naked Mole Rats

Arkadi F. Prokopov

ABSTRACT

There is an imperative need for exploring and implementing mitochondria-rejuvenative interventions that can bridge the current gap toward the step-by step realization of strategies for engineered negligible senescence (SENS) agenda. Recently discovered in mammals, natural mechanism mitoptosis—a selective "suicide" of mutated mitochondria—can facilitate continuous purification of mitochondrial pool in an organism from the most reactive oxygen species (ROS)-producing mitochondria. Mitoptosis, which is considered to be the first stage of ROS-induced apoptosis, underlies follicular atresia (a "quality control" mechanism in female germline cells that eliminates most germinal follicles in female embryos). Mitoptosis can be also activated in adult postmitotic somatic cells by evolutionary conserved phenotypic adaptations to intermittent oxygen restriction (IOR) and synergistically acting intermittent caloric restriction (ICR). IOR and ICR are common in mammals and seem to underlie extraordinary longevity and augmented cancer resistance in bowhead whales (*Balena mysticetus*) and naked mole rats (*Heterocephalus glaber*). Furthermore, in mammals IOR can facilitate continuous stromal stem cells-dependent tissue repair. A comparative analysis of IOR and ICR mechanisms in both mammals, in conjunction with the experience of decades of biomedical and clinical research on emerging preventative, therapeutic, and rehabilitative modality—the intermittent hypoxic training/therapy (IHT)—indicates that the notable clinical efficiency of IHT is based on the universal adaptational mechanisms that are common in mammals. Further exploration of natural mitochondria-preserving and -rejuvenating strategies can help refinement of IOR- and ICR-based synergistic protocols, having value in clinical human rejuvenation.

INTRODUCTION

If aging is an integral of genomic information loss over time, then oxidative damage to DNA and particularly, to the mitochondrial genome, is the culprit. Reactive oxygen- and nitrogen species (ROS and RNS) are commonly produced during oxidative phosphorylation (OXPHOS) in the mitochondria. If the amount of ROS and RNS persistently surpasses the antioxidant system capacity, then various cellular and systemic age-related pathologies develop, such as certain types of cancer, atherosclerosis, and a host of chronic inflammatory–degenerative conditions.

Physician for Integrative Medicine, Heidelberg, Germany.

It is known that mitochondria carry multiple functions other than just adenosine triphosphate (ATP) production.[1] Among these are participation in apoptosis and cellular proliferation, production and transmission of a transmembrane potential, oxygen sensing, regulation of the cellular redox state and level of second messengers, hemesynthesis and steroid synthesis, calcium storage, detoxification, and heat production. In most of the listed functions, ROS and RNS modulate a number of vitally important nondestructive cellular activities, hence, the importance of integrity of mtDNA.

However, ultimately it is the rate of accumulation of mutations in mtDNA seem to act as an engine of the molecular clock of aging, and thus modulates both timing of onset and progression of age-related dysfunctions and diseases. Blocking this mechanism is suggested as possible modulator of aging.[2] It is agreed that any efficient prophylactic and therapeutic strategy that aims at retarding aging and age-related pathology shall address continuous rejuvenation of mitochondria, above all in post-mitotic tissues.[3] Currently applied mitochondrial interventions are largely limited to slowing down ROS-RNS–induced oxidative damage either by dietary supplementation of antioxidants,[4] or by engineered overexpression of genes encoding antioxidant enzymes (e.g., superoxide dismutase [SOD], catalase, glutathione peroxidase). However, to date, interventions aimed at reducing oxidative damage in mammals, primarily through supplementation of the antioxidant system, have yielded disappointing results.

THE CHALLENGE OF BOWHEAD WHALES AND NAKED MOLE RATS

The remarkable longevity of bowhead whales (*Balena mysticetus)* and naked mole rats (*Heterocephalus glaber*), as well as their unusual resistance to cancer attracted attention only recently. In spite of enormous differences between these animals, they seem to have at least two key denominators in common: (1) both retain ecological niches in rather unproductive environments that offer season-dependent nutrition and relatively few predators (killer whales and humans in bowhead whales and snakes in naked mole rats) and (2) in their particular natural habitats both animals regularly experience significant oscillations of cellular oxygen and carbon dioxide tensions, combined with intermittent calorie restriction. Diving in bowhead whales, and living in underground, poorly ventilated burrowing tunnels in naked mole rats creates in both animals intermittent hypoxic–hypercapnic state, which is interspersed by periods of normoxia and normocapnia. My hypothesis suggests that these conditions result in diminished mtDNA mutations and continuous elimination of mutated mtDNA in somatic cells, in increased allocation of stem cells for tissue maintenance, and ultimately, in expression of a neotenic, longevity, and neoplasia-resistant phenotype in both animals.

Since 1981, Inuit whale hunters in Alaska have discovered a number of stone and ivory harpoon heads in bowhead whales that had been killed in the Beaufort Sea. It was puzzling because hunters knew that such harpoon heads had not been used for more then 100 years.

George et al.[5] analyzed the eyes of 48 bowhead whales harvested between 1978 and 1996. By conducting aspartic acid racemization measurements, they analyzed the eye lenses to estimate the whale's age at the time of death. It was found that 4 whales were more than 100 years old, and 1 was estimated to be 211 years old (the method has an accuracy range of approximately 16%, which means this whale could have been from 177 to 245 years old). Amazingly, one of the 100+ males was killed during sexual activity.

The oldest known ages for mammals are 110 years for a blue whale and 114 years for a fin whale, based on Japanese studies[6] of waxy laminates on the inner ear plug of the whales, a method that does not work for bowheads. The oldest living person with a birth certificate was a 122-year-old French woman who died in 1997. Elephants have lived to 70 years in captivity, so bowheads appear to hold the longevity record for mammals.

LOW CANCER MORBIDITY

Of 130 dead bowhead whales examined between 1980 and 1989, only 1 exemplar had a

benign tumor, located in the liver. According to Philo et al.,[7] "It is unlikely that tumors are major contributors to bowhead whale morbidity or mortality."

In general, necropsy studies of numerous baleen whales and odontocetes, harvested during decades of industrial whale hunting in the North and Antarctic regions, or stranded on the shores, point to inexplicably low cancer morbidity compared to the terrestrial mammals. Thus, "A single cancer was found in over 1800 other cetaceans examined, and tumors were not found in approximately 50 beluga examined in the Canadian Arctic."[8]

A single benign tumor was observed in 55 slaughtered pilot whales in Newfoundland,[9] and only two benign tumors (0.1%) were reported in 2000 baleen whales hunted in South African waters.[10] Only three cases of cancers (0.7%) were found during the postmortem examination of 422 odontocetes from British waters.[11] Among few cancerous tumors ever discovered in baleen whales there were no metastatic ones and those found were small and encapsulated.[12]

Naked mole rats can live more then 28 years in captivity, which is approximately 9 times longer than similarly sized mice. There was neither a single cancer reported in the large 25-year-old captive colony,[13] nor are there known cases of cancer in naked mole rats described by other researchers.

PECULIARITIES OF BOWHEAD WHALE PHYSIOLOGY

Bowhead whales live in water temperature near 0°C and feed while diving for periods up to 33 minutes long. Diving hypoxia (which is essentially intermittent and accompanied by hypercapnia) is common to all diving mammals and birds. Biochemical and hormonal studies of bowhead whales are fragmentary. Compared to terrestrial mammals, bowhead whales have similar values of serum glucose (86.8 ± 25.9 mg/dL), but significantly higher cholesterol (409.7 ± 86.3 mg/dL) and triglycerides (287.0 ± 139.4 mg/dL), which is similar in all baleen whales.[14]

Levels of thyroid hormones (TH) in bowhead whales do not differ radically from marine or terrestrial animals.: total thyroxine (TT_4) nmol/L: 83.30 ± 20.5; total triiodthyronine (TT_3) nmol/L: 1.14 ± 0.42; free thyroxine (FT_4) pmol/L: 25.80 ± 7.63; free triiodthyronine (FT_3) pmol/L: 4.22 ± 1.63. Age, gender, or seasons do not significantly affect their serum TH levels, except for lower FT_3 found in pregnant females.[15]

According to George et al.,[16] because of very thick blubber, bowhead whales may experience a "heat load" even at rest. However, the mean deep body temperature measured in 30 killed bowhead whales was 33.6°C ± 0.82°C.[16] This is drastically lower than reported for other large cetaceans (baleen whales maintain a core body temperature between 36.6°C and 37.2°C) and for other nonhibernating mammals.

Compared to other diving mammals, including migrating whales, the relatively slow moving bowhead whales may remain within a less physiologically challenging, mixed anaerobic–aerobic metabolic range. Only during short intervals of intensive swimming and deep diving can this metabolic state be interspersed with episodes of deeper hypoxia that are followed by acute reoxygenations.

I did not find either published studies in hypoxic–hypercapnic responses in bowhead whales or studies on tissue oxidative damage and mitochondrial functions; however, a comparison of their known physiologic and biochemical parameters with those of other baleen whales does not reveal significant differences, except notably lower core body temperature in bowhead whales.

PECULIARITIES OF NAKED MOLE RAT PHYSIOLOGY

In nature, naked mole rats live in hypoxic, hypercapnic, subterranean burrows.[17] Their lungs are minimally developed[18] and similar to fetal hemoglobins, their hemoglobin level has high oxygen affinity.[19] The basal metabolic rate of naked mole rats is near 0.70 mL O_2/g-h, which is extremely low for their body mass.[20] Compared to shorter-living rodents, naked mole rats show lowered FT_4 fraction (0.39 ± 0.09 ng/dL) and downregulated thermal homeostasis that is close to poikilothermy. FT_4

levels in naked mole rats are considerably lower than those reported for a wide variety of vertebrate species and are one order of magnitude lower than for other mammals.[21] Under food restriction, the metabolic rate of naked mole rats is further decreased by 25%.[22]

There is evidence that naked mole rats maintain and remodel bone structure with much higher efficacy then similar-sized mice.[23] It was also found that naked mole rats retain a youthful vascular function and cellular oxidant/antioxidant phenotype much longer than shorter-living rats, which indicates that they are better protected against aging-induced oxidative stress.[24]

SOME PHYSIOLOGIC FEATURES COMMON IN THE BOWHEAD WHALE AND NAKED MOLE RAT

What factors are common to both of those extremely dissimilar mammals that afford so much greater longevity and cancer-resistance compared to animals approximately the same size (at least in the case of naked mole rats) and similar living conditions? As odd as it may seem, in general it is the oscillations of tension of oxygen and carbon dioxide in their cells and the way they metabolize oxygen. In their natural habitats both naked mole rats and bowhead whales persistently undergo intermittent oxygen restriction (IOR), which is related to the type of hypercapnic hypoxia that all mammals are exposed to during the embryonic and prenatal period. IOR is so named to emphasize its logical relationship with the established expression: intermittent or short-termed calorie restriction (ICR). The IOR in bowhead whales and in naked mole rats may induce and maintain a universal phenotypic adaptation, or a life-long phenomenon of hypoxic preconditioning that is well known to reduce and/or prevent apoptic and necrotic damage caused by acute hypoxia-reoxygenation.[25,26] On the other hand, it is obvious that the IOR and ICR govern phylogenesis of both animals in their particular environmental niches.

It will be further elucidated how IOR not only protects mtDNA from excessive auto-oxidative damage, but also may favor selection and multiplication of less ROS-producing, "fittest" mitochondria that host nonmutated mtDNA copies. Second, how the IOR can enhance stromal stem cell-dependent tissue repair will be examined. Third, how the interrelated, synergistically functioning ICR mechanisms can augment these beneficial effects will be explored. Later how the same underlying mechanisms may be responsible for the notable clinical efficiency of intermittent hypoxic training/therapy (IHT), a novel prophylactic, therapeutic, and rehabilitative modality will be examined.[27]

LIFESPAN, CANCER, AND MITOCHONDRIA

All metazoan face the problem of controlling cancer, which is a byproduct of one of the major evolutionary advances, the advent of multicellularity.[28] The chance of malignant transformation is proportional to the number of cells multiplied on the lifespan of the organism.[29] Thus humans have much higher cancer-control capacity than mice (approximately 2/3 wild mice kept in a laboratory setting naturally die from cancer). Prevention and suppression of malignancy in constantly proliferating tissues (epithelial, liver, bone marrow) becomes increasingly difficult as body size increases, requiring the accelerated recruitment of additional controls that supposed to operate efficiently during initiation, promotion, and progression at all three levels of cancerous genome evolution in the host. Therefore, bowhead whales that can weight 2000 times more and live two times longer than humans obviously have much better cancer control. The same relates to the naked mole rats, which have body mass equal to mice but live without cancer a magnitude longer.

It is recognized that malignant cells and tumors in an organism are products of multistage evolution of instable copies of the "selfish" mutated genome that escape immune surveillance and apoptosis.[30] Most of these events are mediated by mitochondria-produced ROS and NOS.

On the other hand, one can assume that each somatic cell initially contains a pool of mtDNA

copies having various degrees of oxidative/mutational deletions (heteroplasmy). It was found that under normal, stabile metabolic conditions rich in fuel and oxygen, the fastest (oxidatively damaged) mtDNA copies can acquire multiplicative advantage and increase their number more rapidly then less damaged ones, thus increasing accumulative ROS burden. [31]

GENOMOCENTRISTIC VIEWPOINT

In contrast to often prevailing cellulocentristic and mitochondriocentristic image of an organism, the following argument uses the evolutionary-based genomocentristic viewpoint. I believe that bodies, cells, and cellular organelles can be logically viewed as molecular machines that are designed, assembled, and used by their genomes with the single purpose: enable transferring genome copies into the next generations.[32] According to "The selfish gene" theory, adaptations are the phenotypic tools through which genes secure their propagation. The interactions between nuclear and mitochondrial genomes in mammalian cells, the mutual cooperation of both genomes can be compared to that which exists between a shepherd and his cattle. Both benefit from each other, but it is the shepherd who governs his herd and controls the cattle's quantity and quality.

Since uncorrected accumulation of mutations would within a very small number of generations become incompatible with survival, there should exist common mechanisms for selection against harmful, ROS-enhancing mtDNA mutations. The amount of mitochondria depends on the energy demand, imposed on cells, tissues, and organs. The primary messengers, nitric oxide (NO), thyroid and steroid hormones, and mitochondria-specific nutrients (L-carnitine, α-lipoic acid, coenzyme Q10, etc.) can stimulate and support mitochondrial proliferation nonspecifically, irrespective of the mutational burden of a particular clone of mtDNA. On the other hand, except for follicular atresia, there is no known mechanism that clearly governs mtDNA quality in vertebrates. It is supposed that atresia is an efficient but still not ultimately perfect quality control tool, which eliminates most ROS-producing mitochondria in female germline cells during early embryogenesis.[33]

The clonal expansion of mutated and partially deleted mtDNA copies, which inhabit more intensively ROS-producing mitochondria correlates with advance of senescence and aging. It is found that at the conditions of *ad libitum* available nutrition and oxygen, some damaged mtDNA enjoy replicative advantage over wild-type (nonmutated) mtDNA that ultimately accelerates senescence.[31,34] Furthermore, it was suggested that microheteroplasmy (accumulation of acquired mutations in mitochondria of somatic and germinal cells that begins already in early embryonic period) is the primary cause of the exhaustion of the tissue renewal capacity in advanced age.[35]

Nevertheless, one can hypothesize that the nuclear genomes can indirectly "select" the mtDNA of better quality via behavioral adaptational strategies, described by Dawkins[36] as "extended phenotype."

INTERMITTENT OXYGEN RESTRICTION

Combined hypoxia-hypercapnia is a primary physiologic state in a developing mammalian embryo, and is essential to support its growth.[37,38] The redox potential of embryonic tissues differs significantly from that of newborn and adult, and is a fundamental characteristic of growth and development.[38] On the other hand, higher cellular oxygen tension during the later phases of fetal development correlates with differentiation and maturation of tissues and organs.[39,40]

The possible strategy to slow down the ongoing oxidative mtDNA damage in mammalian somatic cells can be maintaining and/or constantly returning, back to more economical, embryonic-type pattern of oxidative metabolism with its hypoxia-resistance and more youthful, chronologically earlier gene expression profile. This type of metabolism protects both germinal and somatic cells from mutational damage and stimulates their proliferation.[41,42] A complementary strategy, effective in qualitative selection of mtDNA can be a periodic exposure of a pool of heteroplasmic mi-

tochondria to a critical functional load such as increased energy demand combined with limited availability of fuel and/or oxygen. For example, by exposing tissues to controlled multiple ischemia-hypoxia-reoxygenation episodes, which yet remain under apoptic threshold, such oscillations enhance mitochondrial ROS production that consequently stimulates enzymatic antioxidative defense (hypoxic preconditioning) in healthy mitochondria[43] while destroying mutated mitochondria via mitoptosis.[44] Mitoptosis is a key mechanism underlying follicular atresia[45] and also plays an important role in erythrocyte maturation cycle.[46]

One can hypothesize that mitoptosis, being induced by IOR in the postnatal, postmitotic somatic cells, could continuously purify their mitochondrial populations from the constantly accumulating, most damaged, ROS-producing mtDNA copies, thus providing replicative advantage for the wild-type, nonmutated mtDNAs that are significantly less ROS-producing, but replicate slower then mutated mtDNA copies.[31,47]

Within the physiologic range, hypoxia is a major organismal stressor triggering rapid compensatory strategies. Cortical and hippocampal neurons from oxygen-sensitive mammalian species are hypoxia-tolerant during the embryonic and neonatal periods. Oxygen tension in fetal brain is less than half the normal value for adults.[48] Most eukaryotic cells can maintain biologic functions under hypoxia by switching energy source and shutting down mitochondria. The switch is almost immediate and occurs simultaneously at the level of enzyme activity and gene expression.[49,50] The reversion to hypoaerobic metabolism is not limited to bioenergetic pathways but extends to multiple unrelated genes and their products; numerous systems integrate to provide improved oxygen absorption, transport, and utilization.

Adaptation to IOR elicits upregulation of cytoglobins (myoglobin and neuroglobin), which function as intracellular oxygen buffer and provide protection from RNS.[51] IOR stimulates accumulation of glycogen in the oxygen-sensitive cells including cardiomyocytes and neurons, thus increasing intracellular energy reserves.[52] IOR is more efficient than chronic hypoxia in stimulating activator protein-1 and hypoxia-inducible factor-1, the master proteins, responsible for numerous adaptational pathways.[53] IOR significantly increases erythropoetin (EPO) production.[54] EPO is not only the main regulator of erythropoesis, but also provides multiple adaptogenic and protective effects, particularly in the central nervous system (CNS).[55] Heat-shock protein (HSP)-70, one of the major chaperone proteins, is also stimulated by IOR.[56] It was demonstrated recently that lifelong overexpression of HSP-70 in skeletal muscle provided protection against damage and facilitated successful recovery after damage in muscles of old mice.[57] IOR is shown to stimulate growth hormone and insulin-like growth factor (IGF)-1 release, while chronic hypoxia suppresses both.[58] IOR induces increased production of antioxidative enzymes.[59] The unified positive effect of IOR on the cell is called cross-adaptation (induction of nonspecific resistance to multiple stressors)[60] and is a conserved trait controlling fundamental regulatory pathways that were established at the beginning of evolution of aerobes.

PROTECTIVE HYPERCAPNIA VERSUS HARMFUL HYPOCAPNIA

In diving animals IOR is accompanied by intermittent hypercapnia. Hypercapnia *in vivo* protects against the damaging effects of ischemia or hypoxia, which is known in clinic for decades.[61] Compared to humans, diving mammals have increased basal carbon dioxide values, but similar upper hypercapnia tolerance limit (37–60 mm Hg versus 45–60 mM Hg, respectively).[62] Several mechanisms have been offered to explain the protective role of carbon dioxide *in vivo*. One of the most significant appears to be the stabilization of the iron-transferrin complex, which prevents the involvement of iron ions in the initiation of free radical reactions.[63] Also a unified underlying mechanism is suggested: even moderately elevated pCO_2 directly suppresses mitochondrial ROS production in animals.[64] It was shown in human blood phagocytes and alveolar macrophages, in the cells of the liver, brain, myocardium, lungs, kidneys, stomach, and

skeletal muscle, tissue phagocytes, and liver mitochondria of mice. Generation of ROS was measured in the cell cultures and biopsies using different methods after exposure of cells and whole body to hypercapnia. The results obtained suggest that carbon dioxide at a tension close to that observed in the blood (37.0 mm Hg) and higher (60 or 146 mm Hg) is a potent inhibitor of mitochondrial ROS generation. The mechanism of carbon dioxide effect appears to depend, partially, on the inhibition of the NADPH-oxidase activity.[64] Carbon dioxide also efficiently scavenges peroxynitrite, which diminishes or prevents relevant nitration and oxidative damage.[65]

In contrast to hypercapnic IOR in diving mammals, the continuous altitude hypoxia is coupled with hypocapnia caused by altitude hyperventilation. Furthermore, compared to consistently intermittent diving hypoxia, the altitude hypoxia is a constant factor, which poses on the body "higher price of adaptation" due to combined hypocapnia, hypohydration, UV rays, low temperatures, and insufficient rest, additionally aggravated by nutritional deficiencies, typical in mountains. It was shown that some mountain-climbers who completed Everest trail without supplementary oxygen suffer long-term CNS damage. The extent of this damage was proportional to degree of altitude hypocapnia in probands.[66,67] It has been shown that continuous hypoxia causes accelerated accumulation of mitochondrial damage, seen as lisosomal mitochondrial "junk" in muscle and nerve cells.[68] All this points out that deviation from evolutionary shaped intermittent hypoxia/hypercapnia adaptation pattern results in functional overload and accelerated structural damage to mitochondria.

HYPOXIA AND STEM CELLS

Stromal, or mesenchimal stem cells (MSC), are able to convert into specialized postmitotic cells (neurons, cardiomyocytes, chondrocytes, and osteocytes) in damaged tissues.[69] Autoreparative processes in the body seem to be highly dependent on MSC. Thus, progeria particularly affects stem cells, reducing their resistance to oxidative stress and preventing stem cell-dependent repair of tissues damaged with age.[70]

Physiologic hypoxia is known to protect stem cells and stimulate release and homing of MSC.[71] It is found that MSC reside not only in the bone marrow, but also in perivascular tissues[72]; thus their activation by IOR seems to be a part of natural tissue-repair mechanism. In some occasions, MSC can donate wild-type mtDNA by fusion with alternated cells without actually transforming into them.[73] In all variants, the IOR opens window of opportunity for enhanced MSC-dependent mitochondrial rejuvenation.

INTERMITTENT CALORIC RESTRICTION

Bowhead whales are the only baleen whales that spend their entire lives near the polar ice edge and do not migrate to temperate or tropical waters to calve. Bowhead whales are well adapted for living in arctic waters: they have very thick blubber, up to 0.5 meter, which provides insulation and energy storage. Nutritional and energy balance in bowhead whales is characterized by deep excursions into stored nutrients during winter months and summer periods of great abundance. This pattern of ICR makes bowhead whales fully dependent on the effluent nutrients accumulation during summer, while survival throughout winter months under extreme nutritive-caloric restriction relies on autophagy (especially in pregnant and nursing females), which is a recognized tissue-rejuvenating and cancer-suppressing strategy.[74-76]

Similar to other baleen whales, bowhead whales thrive on fat- and protein-rich zooplankton food. Fat-based OXPHOS has distinctive advantages compared to glucose-dependent OXPHOS (the latter prevails in mitochondrial energy pathways in terrestrial herbivores). Marine mammals do not drink seawater; instead they produce it metabolically (oxidation of 1 g of fat gives 1.07 g of water). During summer periods of plentiful nutrition, as well as during fasting months, the blood glucose in bowhead whales corresponds to the levels found in terrestrial animals.[14]

As a result of the absence of carbohydrates

in their food, in bowhead whales glucose is synthesized from amino acids and lactate in gluconeogenesis, thus providing mitochondria with optimal amount of this essential energy substrate and important metabolic precursor. However, under starvation-induced hypoglycemia, mitochondria basically metabolize fat-derived ketones for energy production. This is a highly conserved physiologic adaptation to prolonged food restriction that evolved to enhance survival and maintain adequate functions while sparing proteins.[77-79]

Ketone bodies, consisting of acetoacetate and β-hydroxybutirate are derived from fat in the liver and their concentration in blood is inversely related to that of glucose. Ketone bodies are more energetically efficient than either pyruvate or fatty acids because they are more reduced (greater hydrogen/carbon ratio) than pyruvate and do not uncouple the mitochondrial proton gradient as occurs with fatty acid metabolism.[80] In contrast to glucose, ketone bodies bypass cytoplasmic glycolysis and directly enter the mitochondria where they are oxidized to acetyl-CoA. The amount of acetyl-CoA formed from ketone body metabolism is also greater than that formed from glucose metabolism.[81] This increases TCA cycle metabolites and enhances the mitochondrial proton gradient, resulting in increased ATP production. Remarkably, the ketone body-induced boost in the ATP production is also accomplished using less oxygen.[80] In addition to increasing ATP production while sinking oxygen consumption, ketone metabolism can also lessen production of damaging free radicals, which diminishes tissue inflammation provoked by ROS.[79–82]

It is noteworthy that compared to oxidation of fat acids and ketones, glucose oxidation in mitochondria results in significantly higher ROS production.[79–83] Conversely, it is known that physiologic hypoglycemia selectively induces mitochondria-triggered apoptosis of malignant cells, while mitochondria of normal cells easily tolerate even deeper hypoglycemia.[84] Thus, fat-derived ketone bodies are not only a more efficient metabolic fuel than glucose, but also provide anti-inflammatory and antineoplastic effects.

Bowheads grow to approximately 8 meters during their first year then grow very slowly after weaning. Plentiful protein and fat-rich nutrition during weaning and the growth period, followed by a life-long, rhythmically predictable, season-dependent ICR results in downregulation of longevity, modulating genes daf-2 and daf-16,[85,86] a highly conserved genomic response found in yeasts, *C. elegans*, mice, and men. Kenyon et al.[85] also found that in adulthood only daf-2–deficient *C. elegans* are both longer-lived and resistant to oxidative stress. Noteworthy that daf-2 product deficiency can be phenotypically induced by ICR.

Spindler[87] has found that acute caloric restriction partially or completely reverses age-related alterations of liver, brain and heart proteins. Caloric restriction also rapidly and reversibly mitigates biomarkers of aging in adult rhesus macaques and humans. He concludes that, " . . . highly conserved mechanisms for the rapid and reversible enhancement of life- and health-span exist for mitotic and postmitotic tissues."

In the latest calorie restriction human study by Civitarese et al.[88] mtDNA content increased by 35% $\pm$ 5% in the caloric restriction group and 21% $\pm$ 4% in the caloric restriction plus exercise group, with no change in the control group. The authors show that in overweight nonobese humans, short-term calorie restriction lowers whole-body energy expenditure and oxygen consumption in parallel with an induction of mitochondrial biogenesis, PPARGC1A and SIRT1 mtRNA, and a decrease in DNA damage with a tendency toward lower SOD activity. The authors conclude that caloric restriction directly stimulates biogenesis of more efficient mitochondria in human skeletal muscle, which also diminishes oxidative stress.

ICR is common in nature and is more efficient compared to continuous caloric restriction, because the chronic limitation of available energy and structural resources in the extreme and fluctuating, or low-productive natural environments can diminish adaptational potential. On the other hand, the predictably oscillating availability of oxygen and nutrients induces multiple "saving" strategies (from intracellular to behavioral), which seem to be especially effective with growing body size as in the case of bowhead whales, or with eusocial structure as in naked mole rats. ICR also may

efficiently minimize potential threats that are imposed on an organism by the constantly appearing mutated genome copies. During caloric restriction periods first the alternated and mutated cells undergo apoptosis and autophagy, while surviving healthy cells recover and proliferate in feasting times. It has been found that tumors that have lost self-eliminatory apoptic mechanisms still can undergo immune attacks, and if not destroyed by them, they can be encapsulated and stay dormant.[89,90] These processes can be facilitated in bowhead whales by seasonal ICR.

In nature, foraging by naked mole rats is severely restricted during dry seasons. Animals cannot search extensively for new food sources unless the ground has been sufficiently moistened by rainfall.[91] During brief periods after rain, naked mole rats dig intensely to find enough food to sustain them through the long, irregular droughts. This foraging pattern may keep wild naked mole rats in the state of ICR. On the other hand, some colonies, once they find large tubers, tend to farm these. The naked mole rats colonies feed on plant roots and tubers rich with carbohydrates, but relatively poor in proteins and fats. Naked mole rats practice coprophagy that allows "fair distribution" of scarce nutritional resources and contributes to digestive efficiency, as well as reinoculates naked mole rats with endosymbionts that supply an additional source of protein and energy from digestion of the microbes themselves.[92] Endosymbionts ferment indigestible cellulose and fibers into short-chain fatty acids and other nutrients, which supply a fraction of basal energetic rations, and also may provide precursors that underlie increased cellular/mitochondrial membrane resistance to oxidative damage. Naked mole rats also drink no water, deriving it exclusively from food.

A COMPLEMENTARY UNCOUPLING MECHANISM

The other nonexclusive, complementary mechanism that may work in both animals can rely on mild, ROS-triggered uncoupling that is shown to modulate mitochondrial redox state.[93] Because ROS signaling plays a major role in the normal physiology of the cell, birds reduce oxidative stress by cutting leakage from complex I, and not by raising intramitochondrial antioxidant levels. Therefore, not only antioxidants but the redox state of complex I, which is the major source of ROS, is important.[94] Redox state is dependent on numerous factors like substrate supply, ATP use, uncoupling, amount of active complexes, and allosteric influences. Physiologic hypoxia generally develops in muscles during exercise, enhancing mitochondrial ROS production that, in turn, stimulates increased enzymatic antioxidative defense. Artificially applied hypoxia potentiates effects of exercise via multiple mechanisms, and is used for decades by athletes in the form of altitude (hypoxic) training.[95] During exercise, the flow of electrons down the respiratory chain increases, as does oxygen consumption. The overall effect is greater oxidation of complex I, lower proton leakage, and increased stabilization, or enhanced resistance of mitochondrial permeability transition pore (mPTP) to ROS. A decrease in the reduction state of complex I can explain increased lifespan of mice with high resting metabolic rates. It was shown that they had more uncoupling proteins in their mitochondria, which enables electron flow to be uncoupled from ATP production and dissipating more energy as heat.[96] The mPTP is shown to participate in hypoxic preconditioning.[97]

HORMETIC RESPONSE

Both IOR and ICR, as ROS-modulating interventions can be also viewed, as hormetic ones.[98] Hormesis is an adaptive response to low doses of otherwise harmful agents and influences by triggering a cascade of stress-specific resistance pathways. Evidence from protozoa, nematodes, flies, rodents, and primates indicate that stress-induced tolerance modulates neoplasia and longevity. By tracing each hormetic intervention back to their molecular mechanisms, we can see that nearly all of them involve ROS hyperproduction, mostly mitochondria-mediated. From this viewpoint hormesis can be described as a phenomenon of induced augmented antioxidative defense and increased genomic stability via

various ROS-enhancing interventions that remain under irreversible mtDNA and nuDNA damage threshold.

IOR IN NONDIVING AND NONBURROWING MAMMALS

While lifespan-extending effects of ICR have been extensively demonstrated in numerous studies and in diverse species, there is also growing data on similar effects of IOR. Experiments show that prolongation of lifespan can be induced by exposure to IOR in species that usually live in normoxic and normocapnic atmosphere.[99] Since the pioneer publication of Meerson et al.[100] multiple aspects of adaptation to IOR were elucidated. Recently, the IOR was shown to prevent mtDNA deletions and mitochondrial structure damage in ischemia-reperfusion *in vivo*.[101] One of the recent studies was focused on the difference in adaptation to chronic hypoxia compared to intermittent hypoxia.[102] Authors tested the hypothesis that repeated brief reoxygenation episodes during chronic hypoxia improve myocardial tolerance to hypoxia-induced dysfunction. Three groups of male rats were exposed for 2 weeks to chronic hypoxia (10% oxygen and 90% nitrogen), intermittent hypoxia (same as chronic hypoxia, but 1 hour per day exposure to room air), or remained in normoxia (room air, 21% oxygen). To evaluate myocardial tolerance to reperfusion, hearts of sacrificed animals were isolated and perfused for 30 minutes; initially with hypoxic and then with hyperoxic medium. Exposure to either chronic hypoxia or intermittent hypoxia increased hematocrit, hemoglobin concentration, and erythrocytes count. Hypoxia decreased food and water intake with respect to normoxia. As a result, normoxic rats experienced net weight gain in 2 weeks. In contrast, chronically hypoxic rats underwent weight loss, whereas intermittent hypoxia rats neither gained nor lost weight. As the energy expenditure in caged rats can be assumed to be the same in all animals, the efficiency in food assimilation should have been greater in intermittent hypoxia group. In normoxia and especially in intermittent hypoxia group, the deleterious effect of reperfusion stress was apparently less than in continuous hypoxia group. Thus, despite differing only for 1 hour daily exposure to room air, chronic and intermittent hypoxia induced different responses in animal homeostasis, markers of oxidative stress, and myocardial tolerance to reoxygenation. Authors conclude that the protection in rats exposed to intermittent hypoxia appears conferred by the hypoxic preconditioning due to the reoxygenation, rather than by hypoxia *per se*.

A number of animal studies show that beneficial effects of IOR can be achieved during short hypoxic exposures, varying from half an hour to several hours a day.[103,104]

WHY NORMOXIC AND *AD LIBITUM*-FED NAKED MOLE RATS DEMONSTRATE LONGEVITY AND CANCER-RESISTANT PHENOTYPE

The study of oxidative damage in lipids, DNA and proteins in *ad libitum*-fed captive or laboratory-born naked mole rats, which in contrast to wild naked mole rats live in normoxic and normocapnic conditions, showed much greater oxidative stress markers compared to mice.[105] The level of isoprostanes in the urine was 10 times higher, the level of malondialdehyde in liver tissue was twice as high and isoprostane levels in heart tissue in the naked mole rats was 2.5 times higher then in mice. It was also found significantly more DNA and protein damage in their kidney, liver and in the heart. Nevertheless, captive naked mole rats live an order of magnitude longer than predicted based on their body size and suffer no cancer. What mechanisms may explain these striking findings?

According to Buffenstein,[106] "In preliminary studies we have found that naked mole rats cells are more resistant to neoplastic transformation by combinations of oncogenes (SV40 large T antigen and activated Ras) that routinely convert both mouse and human cells into cancer cells. The remarkable resistance of the naked mole rats to cancer may also result from interspecies differences (from mice, in particular) in intracellular pathways that must be tar-

geted in order to convert a normal cell to a cancer cell. We hypothesize that naked mole rats have both more efficient DNA repair, and reduced mutagenesis leading to genomic stability."

Remarkably, that the blueprint of protective pathways and mechanisms, integrated by IOR and ICR evolutionary-shaped genotype, synergistically provides expression of the longevity and cancer-resistant phenotype even under increased oxidative stress markers. The question arises, what amount of naturally occurring IOR and ICR is necessary to express the innate, unique naked mole rats phenotype in an affluent, pro-oxidative laboratory conditions?

The answer may be found in the functional hypometabolic states that are known to induce relaxation response and relive stress in mammals. I presume that captive naked mole rats are still capable to maintain in an affluent environment their behavioral pattern and natural circadian rhythm. The intermittent periods of behavioral hypoxia-hypercapnia, hypometabolism and hypothermia may still be present in their daily life cycle. For instance, in naked mole rats a typical resting-sleeping pattern (when many animals huddle in the sleeping chamber) can facilitate in their bodies temporary state of physiologic hypoventilation, hypoxia-hypercapnia and hypometabolism that stimulate cellular reparative activity. The studies of the interaction between sleep and thermoregulation in naked mole rats revealed functional poikilothermia for the period of the rapid-eye movement (REM).[107,108] Such "rejuvenative hypoxic-hypothermic naps" may correspond to the naturally occurring IOR periods.

Additionally, both wild and captive naked mole rats hav naturally low levels of vitamin D_3; this condition is known to evoke metabolic deficiency, similar to caloric restriction[109] and in *ad libitum*-fed naked mole rats it can act as a natural CR – inductor.

The other protective mechanism may involve the microbial symbionts-produced volatile fatty acids, proteins and lipids, which can significantly impact the lifespan and cancer resistance in naked mole rats. Among them the butyric acid and its derivates are known to offer antineoplastic and lifespan-prolonging effects.[110,111]

Finally, remembering that mitochondrial and bacterial lipids belong to the same family, one can deduce that digested bacterial endosymbionts may supply valuable precursors and support mitochondrial biogenesis in naked mole rats.

Whether synergism of these and other mechanisms can explain the paradox of a remarkable cancer-resistance and longevity of captive naked mole rats on the background of significantly increased OS markers remains a challenging question calling for further studies.

DISCUSSION

There is little doubt that the whole complex of lifespan-increasing and antineoplastic adaptations in bowhead whales and naked mole rats evolved in their particular natural, variable hypoxic-hypercapnic and calorie-restricted environments. In general, these numerous adaptations may be interpreted as signs of neoteny. Neoteny[112] (pedomorphosis, fetalization) is described as a prolongation of an organism's juvenile phase that nevertheless allows sexual reproductive maturation. It is generally accepted that neoteny is a backward step in evolution, because the neotenic species typically originate from those that evolved to live in affluent environments, but for various reasons (such as climatic change, environmental hypothermia, ICR and IOR) adapted to survive in less productive, but more protected niches.

In nature, both bowhead whales and naked mole rats exist life-long in conditions that reproduce at the cellular level the mammalian neonatal period that is characterized by prevalence of hypoaerobic metabolism that maintains a reduced, mtDNA-protecting cellular environment.[38] Hypoxia-tolerant mammal neonates consistently demonstrate hypoxic hypometabolism and insufficient thermal homeostasis.[113] Reduction of core body temperature is shown to contribute to the increased lifespan and to the rejuvenative effects conferred by calorie restriction. The core body temperature in bowhead whales is stabilized at remarkably low 33.6°C ± 0.82°C[16] and in the naked mole rats in the range of 33.1°C ± 0.6°C.[21] Such low set point of bodily thermo-

stat seemingly affects hormonal and mitochondrial functions, and may directly influence the lifespan. In the study of Conti et al.[114] a moderate, sustained reduction of core body temperature in the range 0.3°C to 0.5°C prolonged lifespan in transgenic mice independently of altered diet or CR.

Apparently, the IOR in the perinatal period appears as a source of adaptive mechanisms that can be traced in varying combinations, in many survival strategies. Generally, it is found that under lower oxygen tension the mitochondrial ROS production is suppressed significantly, OXPHOS is more efficient and the maintenance energy is reduced because of notably lesser proton leak.[49] Thus, synergism of IOR and ICR may serve as the key mechanism that is slowing down maturation tempo and neotenically directs phenotype development.

The Mexican salamander (axolotl; *Ambystoma mexicanum*), which demonstrates an extreme example of neoteny, may offer some help in connecting the dots and in demystifying pictures of two mammals: bowhead whales and naked mole rats. Salamanders, frogs, and newts normally develop in two stages: larvae hatched from eggs transform into adult animals, tadpoles become frogs. Axolotl is descended from what were once terrestrial salamanders (*Ambystoma tigrinum*). Their origin is a high-altitude (2400 meters above sea level) body of water surrounded by a low-productive terrestrial environment. These conditions are thought to favor neoteny. Axolotl is an obligatory neotene, completing its full life cycle without metamorphosis. The suppression of maturation in this species is caused by several mechanisms that reduce efficiency of thyroid hormones. Metamorphosis in axolotl can be induced with thyroid hormone supplementation, thyroid stimulating hormone, or by stimulation of hypothalamic neurons.[115] Axolotls become sexually mature as larvae and are exceptionally long-lived: it is not unusual for animals to survive 25 years in captivity, while a metamorphosed specimen that mature into adults and migrate onto land hardly live past 5 years. Axolotls are capable of the regeneration of entire lost appendages. They can also readily accept and restore to full functionality transplants from other individuals, including eyes and parts of the brain. In metamorphosed individuals, however, the ability to regenerate is greatly diminished.[116]

According to the hypothesis of Shostak,[117, 118] neotenic genotype uses for tissue maintenance increased allocation of stem cells, " . . . redundant elements that function as backups in the event of failure."

Neotenic mechanisms seem to provide more efficient buffering of oxygen oscillations, thus diminishing total ROS and NOS output. Ultimately, it is the stabilization of oxygen oscillations at mitochondrial level may be a critical parameter, determining the efficiency of adaptation, because all body oxygen transporting systems conspire for a common goal: not to increase but stabilize oxygen tension near mitochondria, which consequently optimizes ROS oscillations and reduces oxidative stress.

More efficient mitochondria, genome stabilization, increased allocation of stem cells, enhanced reparative processes, increased lifespan and superior cancer resistance seem to emerge as mere side effects of these particular, by IOR-ICR modulated adaptations, which may have neoteny as their common denominator in bowhead whales and naked mole rats.

SUMMARY

Intermittent hypoxic hypercapnia is a primordial physiologic state, which governs physiologic development in all mammals, while remains a life-long feature only in diving and burrowing mammals. The phenomenon of increased cancer-resistance and longevity of bowhead whales and naked mole rats may be based on the essentials of cellular mitochondrial life cycle, modified by phenotypic adaptation to behavioral IOR and ICR.

Physiologically, the adaptation to IOR and ICR in bowhead whales and naked mole rats, may maintain the metabolism and corresponding gene expression profile in (or provide continuous "resetting" to) their neonatal state, which is characterized by more efficient absorption, transportation and utilization of oxygen and enhanced DNA repair.

The total outcome of this permanent preconditioning results in expression of neotenic phe-

notype that is characterized by lowered core body temperature, diminished total accumulative oxidative damage to mitochondria, increased enzymatic antioxidative defense, permanent rejuvenation of mitochondrial pool, enhanced autophagy and improved stabilization of genome. This can manifest in a dramatic reduction of neoplasia, combined with increased lifespan in bowhead whales and naked mole rats.

Mammalian genome intrinsically possesses IOR and ICR adaptational programs, and although they are usually "deinstalled" in normoxic-normocapnic and *ad libitum*-fed phenotypes during postnatal development, they also can be "reinstalled" and maintained with the help of specially designed protocols in adult mammals.

The question remains whether artificially implied IOR and ICR protocols can modulate oxidative metabolism, rejuvenate mitochondrial populations, improve genome stability, postpone senescence and retard development of age-related pathology and ultimately, increase healthy lifespan, in the other known neotenic species: humans?

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Address reprint requests to:
Arkadi F. Prokopov
Physician for Integrative Medicine
Heugasse 2
69117 Heidelberg
Germany

E-mail: altark@web.de

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