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Chapter 10

Engineered Natural Longevity-Enhancing Interventions

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Abstract

Currently developing engineered biogerontological interventions promise to radically extend healthy life span. Nevertheless, the exploration of natural strategies that underlie longevity and resistance to age-related diseases in exceptionally long-living mammals, such as the bowhead whale (BW, *Balena mysticetus*) and naked mole rat (NMR, *Heterocephalus glaber*) is also rational.

This paper is an attempt to analyze the advantages and limitations of these strategies, and to elucidate their existing derived applications, as well as to estimate their future prospects in the practice of biogerontology.

Two natural longevity-modulating strategies (intermittent calorie/nutrient restriction—ICR, and intermittent oxygen restriction—IOR) utilize the universal development-and adaptational genetic programs, evolutionary "preinstalled" in all aerobic organisms. ICR and IOR synergistically diminish the basal level of mitochondria-dependent oxidative stress that is supposed to be the key factor, modulating life span and resistance to age-related diseases in aerobes.

ICR and IOR employ a common mitochondria-rejuvenating pathway, *mitoptosis*—a selective elimination of excessively ROS-producing mitochondria in the cells. Mitoptosis is a natural process that maintains the "quality" of mitochondria in the female germinal cells during early embryogenesis, and can be stimulated and maintained by IOR and ICR also in the postmitotic cells of adult organisms. Behaviorally induced continuous

mitoptosis seems to be the key mechanism responsible for exceptional longevity and resistance to age-related diseases in BW and NMR.

Additionally, ICR and IOR influence the longevity and tempo of development of age-related diseases via several mitochondria-independent pathways, such as suppressed protein glycation, enhanced DNA repair, accelerated protein turnover, stimulation of EPO, GH, HSP70 and other functional proteins. In addition, IOR distinctively intensifies stem-cell-dependent tissue repair.

The unified positive effect of IOR and ICR on the cells and organisms manifests in enhanced genome stability and increased non-specific resistance to multiple stressors.

Various forms of ICR and IOR have an impressively positive account of empiric and evidence-based use in the health-improving practices of various human cultures. Recently developed engineered techniques, such as short-termed, or alternate-day fasting (ADF, derivative of ICR) and intermittent hypoxic training/therapy (IHT, derivative of IOR) induce measurable mitochondrial and systemic rejuvenation.

The vitamin-nutriceutical supplementation synergistically enhances the efficiency of IOR and ICR.

Further development of engineered ICR and IOR protocols should facilitate their advanced clinical implementation and user-friendly, self-help applications.

Keywords: adaptation, autophagy, cancer, hypoxic preconditioning, intermittent hypoxic therapy, life span, mitochondria, mitoptosis

Introduction

This paper aims to elucidate two natural mitochondria-rejuvenative strategies and to outline their current derivative applications in clinic. The authors explore the evolutionary strategies and pathways that underlie the extraordinary longevity of some mammals. On the other hand, we make it known that the corresponding engineered life-extending natural interventions have already been successfully used in clinic. This article is neither a systemic review nor a summary of controlled double blind clinical interventions; it is more an unfolding reflection and analysis of both authors' ideas and experiences during nearly two decades of practical application of described regenerative interventions.

It is agreed that any efficient prophylactic and therapeutic strategy that aims to increase healthy lifespan, retard the aging process and suppress age-related pathology shall address superior preservation and continuous rejuvenation of mitochondria in the postmitotic cells [1].

Mitochondria carry out multiple functions other than ATP production [2]. Among these are participation in apoptosis and cellular proliferation, generation and transmission of a transmembrane potential, oxygen sensing, regulation of the cellular redox state and the level of second messengers, heme and steroid syntheses, calcium storage and release, detoxification and heat production. In the majority of the listed functions, ROS and RNS modulate vitally important non-destructive cellular activities; hence the importance of integrity of mtDNA.

On the other hand, the oxidative mutational damage to the nuclear genome (nuDNA) and, particularly, to the mitochondrial genome (mtDNA) are believed to be the culprit of aging-related genomic instability that is associated with degenerative disease and frailty. Reactive oxygen and nitrogen species (ROS and RNS) are abundantly produced in oxidative

phosphorylation (OXPHOS) in the mitochondria, inducing mutational deletions in mtDNA [3]. Modulation of lifespan by mitochondrial ROS production was shown in numerous studies on different species [4, 5].

Currently used mitochondria-supportive interventions are largely limited to slowing down ROS- and RNS-induced damage either by dietary supplementation of antioxidants [6], or by engineered overexpression of genes encoding antioxidant enzymes (e.g., SOD, catalase, glutathion-peroxidase). However, the antioxidative supplementation shows controversial results [7], while the engineered enhancement of antioxidant enzymes-encoding genes is still far from practical use.

Perhaps the most proficient bioengineered intervention that is currently under clinical testing employs the SkQ—a completely new type of synthetic mitochondria-targeting antioxidant based on mitochondrial membrane-penetrating ions [8]. This radically new approach promises to dramatically alleviate the burden of age-related diseases, as well as probably extend the healthy life span in humans in the observable future.

In the meantime, the analysis of the exceptional longevity phenomenon found in some mammals is also important if we are to evaluate practical interventions designed to postpone or alleviate aging when begun late in life. Elucidation of constituting pathways may help to synergistically increase the effectiveness of current and prospective, straightforwardly pharmaceutical, and/or genomic therapies.

The Challenge of Bowhead Whales and Naked Mole Rats

The extraordinary longevity of two mammals: bowhead whales (BW, *Balena mysticetus)* and naked mole rats (NMR, *Heterocephalus glaber*), as well as their remarkable resistance to cancer, attracted attention recently. These enormously different animals seem to have at least two key denominators in common: 1) they both occupy ecological niches in rather unproductive environments that offer season-dependent nutrition and have relatively few predators (killer whales and humans in BW and snakes in NMR); 2) both animals regularly experience significant oscillations of cellular O_2 and CO_2 tensions (diving in BW and burrowing in NMR), combined with seasonal (winter months in BW and dry season in NMR) severe calorie restriction or fasting.

Diving in BW, and living in underground, poorly-ventilated burrowing tunnels in NMR creates in both animals the intermittent hypoxic-hypercapnic state, which is interspersed by periods of normoxia and normocapnia. Earlier it was elucidated in detail [9] how these conditions may result in diminished mtDNA mutations and continuous elimination of mutated mtDNA in somatic cells, in increased autophagy, improved genome stability, increased allocation of stem cells for tissue maintenance and, ultimately, in the expression of a neotenic, life-span-enhanced and neoplasia-resistant phenotype in both animals.

Enhanced Longevity and Low Cancer Morbidity

George et al. [10] conducted aspartic acid racemization measurements of the eye lenses of 48 BW harvested between 1978 and 1996 to estimate the whale's age at the time of death. It was found that four animals were older than 100 y.o., and one was estimated to be 211 y.o. (the method has an accuracy range of about 16 %, which means this whale could have been from 177 to 245 y.o.). Amazingly, one of 100 + males was killed during mating.

The oldest living person with a birth certificate was a 122 y.o. French woman who died in 1997. Elephants have lived to 70 years in captivity, so bowheads appear to hold "the longevity record" for mammals.

Of 130 harvested BW examined between 1980 and 1989, only one exemplar had a benign tumor, found in the liver. According to Philo et al. [11], "It is unlikely that tumors are major contributors to bowhead whale morbidity or mortality."

In general, the 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, show inexplicably low cancer morbidity compared to humans or terrestrial mammals. Thus: "A single cancer was found in over 1,800 other cetaceans examined, and tumors were not found in approximately 50 beluga examined in the Canadian Arctic" [12].

A single benign tumor was observed in 55 slaughtered pilot whales in Newfoundland [13], and only two benign tumors (0.1%) were reported in 2000 baleen whales hunted in South African waters [14]. Only three cases of cancers (0.7%) were found during the postmortem examination of 422 odontocetes from British waters [15]. Among few cancerous tumors ever discovered in baleen whales there were no metastatic ones and those found were small and encapsulated [16].

Naked mole rats can live more than 28 years in captivity, which is about nine times longer than similarly sized mice. There was neither a single cancer reported in the large 25 year-old captive colony [17], nor there are known NMR cancer cases described by other researchers.

What biological mechanisms provide such an extraordinary combination of increased cancer-resistance and enhanced longevity?

Peculiarities of BW Physiology

Bowhead whales live in water temperature near 0°C and feed while diving up to 33 minutes long. Diving hypoxia (that is essentially intermittent and accompanied by hypercapnia) is common to all diving mammals and birds.

According to George et al. [18], because of very thick blubber, BW may experience a "heat load" even at rest. However, the mean deep body temperature measured in 30 killed BW was 33.6 +/- 0.82°C. This is significantly lower than reported for other large cetaceans (baleen whales maintain a core body temperature between 36.6 and 37.2°C) and for other non-hibernating mammals.

We did not find neither published studies evaluating oxidative damage and mitochondrial functions in BW; however, a comparison of their known physiological and biochemical

parameters with those of other baleen whales reveals no significant differences, except notably lower core body temperature in BW.

Peculiarities of NMR Physiology

In their natural environment in Kenyan and Ethiopian plains, NMR live in hypoxic, hypercapnic, subterranean burrows [19]. Their lungs are minimally developed [20], and, alike to fetal hemoglobins, their hemoglobin has high oxygen affinity [21]. NMRs basal metabolic rate is near 0.70 ml O2/g-h, which is extremely low for their body mass [22]. Under food restriction, NMR metabolic rate further decreased by 25% [23,24].

There is evidence that NMR´s maintain and remodel bone structure with much higher efficacy compared with similar- sized mice [25].

It was also found that NMR retain a youthful vascular function and protective cellular oxidant/antioxidant balance much longer than shorter-living rats; which indicates that NMR are better protected against aging-induced oxidative stress [26].

Physiological Pathways Common in the BW and NMR

What unifying factors, common in the both extremely dissimilar mammals permit so much greater longevity and cancer-resistance compared with animals about the same size (at least in the case of NMR) and similar living conditions? As oddly as it may seem, in general - it is the oscillations of tension of O_2 and CO_2 in their cells and their patterns of oxygen metabolism.

In their natural habitats both NMR and BW continuously undergo the *intermittent oxygen restriction* (IOR), characterized by hypercapnic hypoxia that all mammals normally exposed to during embryonic and prenatal period. IOR is dubbed to emphasize its deep relationship with the established term: *intermittent calorie restriction* (ICR). The behavioral IOR in BW and in NMR induces and maintains a universal phenotypic adaptation to hypoxia, 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 [27, 28].

It is obvious that IOR and ICR governed phylogenesis of both animals in their particular environmental niches.

Life Span, Cancer and Mitochondria

All metazoan face the problem of controlling cancer, which is by-product of one of the major evolutionary events, the advent of multicellularity [29]. The chance of malignant transformation is proportional to the number of cells multiplied on the lifespan of the organism [30]. So humans have much higher cancer-control capacity then mice (about 2/3 of 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 progressively more 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 BW that can weight 2000 times more and live twice longer than humans, obviously have much better cancer control. The same relates to the NMR, which have body mass equal to mice but live without cancer a magnitude longer.

It is recognized that malignous 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 [31]. Characteristically, most of these events are mediated by mitochondria-produced ROS and RNS.

On the other hand, it is assumed 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, affluent in fuel and oxygen, stabile metabolic conditions the damaged, partially deleted mtDNA copies acquire replicative advantage and increase their number more rapidly than intact and less damaged ones, thus progressively escalating accumulative ROS burden [32].

Genomocentristic Viewpoint

In contrast to often-prevailing cellulocentristic image of an organism, the following argumentation employs the evolutionary-based genomocentristic viewpoint. We 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 to enable transferring derivative genome copies into the next generations [33]. According to Dawkins' "Selfish Gene" theory, adaptations are the phenotypic tools through which genes secure their propagation.

The interactions among nuclear and mitochondrial genomes in mammalian cells, the mutual cooperation of both genomes is similar to that one, 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 mtDNA mutations would within a very small number of generations become incompatible with survival, there should exist some common mechanisms for preservation of innate, wild-type mtDNA and selection against harmful, ROS-enhancing mtDNA deletions.

Evolutionary Maintenance of mtDNA

According to Allen [34], the *mtDNA evolutionary maintenance mechanism* relies on the repressed oxidative function of female germline mitochondria (*promitochondria*). The obligatory matrilineal mitochondria inheritance is found in the vast majority of species. This mechanism conserves germline promitochondria, preventing them from entering postmitotic

oxidative phosphorylation, which is followed by oxidative mutational damage. The irreversible differentiation of promitochondria into fully functional mitochondria of somatic cell results in increased ATP production that is necessary for a multitude of developmental events.

Degradation of Somatic Mitochondria

MtDNA of functional mitochondria is more vulnerable to oxidative damage than nuclear DNA because it is not protected by histones and mitochondria are the primary sites of ROS generation. [35] This leads to mutations of mtDNA, involving the genes coding for respiratory chain proteins and also may disturb the continuous fission and fusion of mitochondria, resulting in their enlargement. Larger mitochondria are less autophagocytosed and undergo further oxidative damage, as well as produce more ROS [36]. As the oxidative mtDNA damage gradually progresses, and heteroplasmy—the proportion of deleted mtDNA to wild-type mtDNA, increases [37]. Critically damaged mitochondria undergo *mitoptosis* (self-destruction of mutated and "worn out" mitochondria) [38,44] and mitophagy; whereas the *less damaged mtDNAs multiply continuously and more rapidly than wild-type mtDNAs, thus achieving selective replicative advantage* [39].

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 [40].

Another mechanism underlying cellular senescence is telomere attrition [41]. It is also found that a high level of oxidative stress shortens telomeres and triggers the senescence [42].

Functional activity stimulates mitochondrial biogenesis in the postmitotic cells. The primary messenger NO, thyroid and steroid hormones and mitochondria-specific nutrients and cofactors (L-carnitine, alpha-lipoic acid, taurine, coenzyme Q10, etc.) stimulate and support mitochondrial proliferation non-specifically, irrespectively of the degree of mutational burden presented in a particular clone of mtDNA.

Natural Selection of Better Quality Mitochondria

The *mtDNA selection and purification mechanism* is presented by the follicular atresia [43]**.** This mechanism selects for germline mtDNA quality in vertebrates. Follicular atresia (apoptic and/or necrotic elimination of about 90% of germinal cells in the ovaries of early female embryos) is an efficient "quality control" tool [44]. It eliminates the majority of ROS – producing mitochondria in the female germinal cell lines, thus preventing them from entering future generations, which could reduce offspring evolutionary fitness.

The clonal expansion of mutated and partially deleted mtDNA copies that occupy more intensively ROS-producing mitochondria correlates with advance of senescence and aging. It is found that with *ad libitum* available nutrition and with uninterrupted supply of sea level O_2 (21%, 160 torr), the damaged mtDNA enjoy replicative advantage over wild-type (non-mutated) mtDNA, which ultimately accelerates senescence [32, 39]. This pathway employs the

chemo-kinetic advantages for shorter mtDNA molecules replication during mitochondria reproduction cycle.

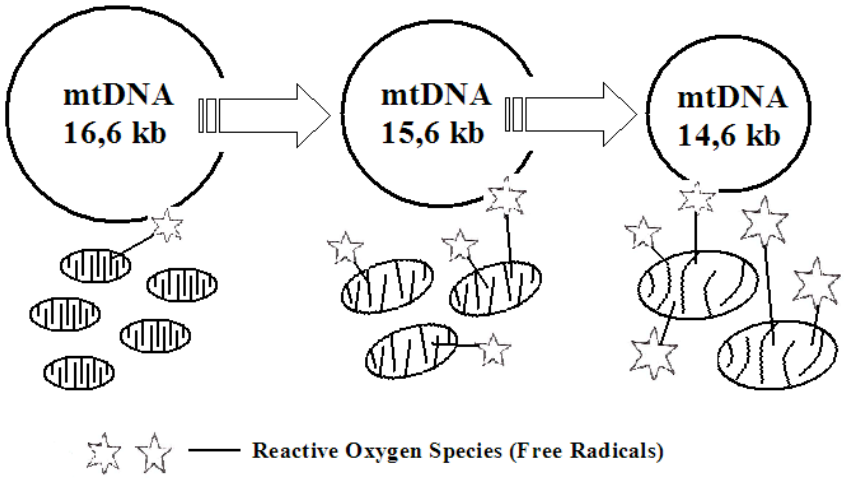

Figure 1. Some mtDNA mutations/deletions produce less efficient and more polluting mitochondria. MtDNA (ring-shaped molecule, approximate size in kb.) suffers about a magnitude higher oxidative mutational damage, compared to nuDNA. During insufficient self-repair the mutated segment of mtDNA ring would be excised/deleted and free ends fused together. The mtDNA looses information, its ring molecule becomes smaller and the mutated mitochondrion turns to be less efficient and more polluting. Wild-type mitochondria are small and dense; they move and fuse easily into the mitochondrial network. The mutated mitochondria are inefficient, large, and sluggish; they do not fuse together and produce increased amounts of ROS.

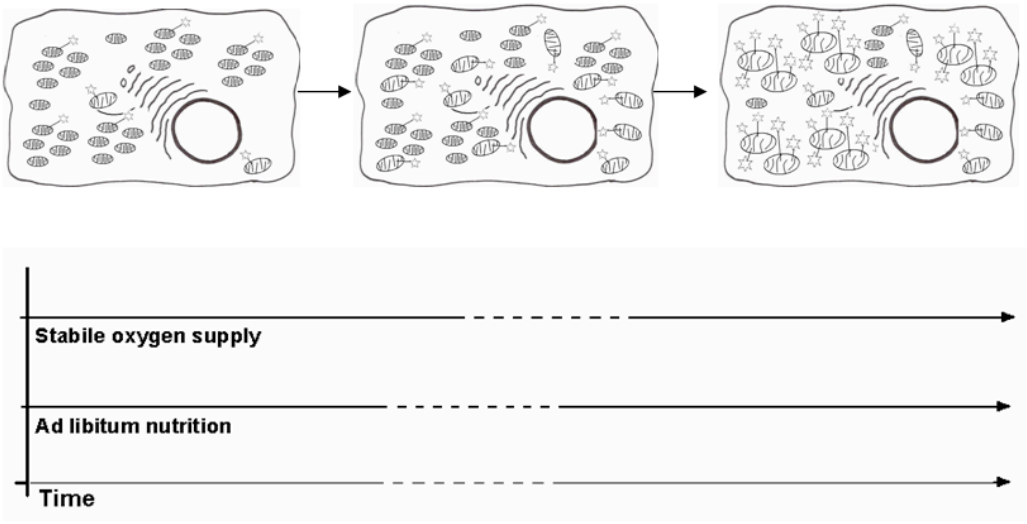

Figure 2. Affluent and uninterrupted supply of oxygen and nutrients (calories) accelerates mitochondrial decay and cellular senescence. With stabile oxygen delivery and ad libitum nutrition the mutated mitochondria out compete wild-type mitochondria for room and resources. They multiply faster, because their deleted mtDNA molecules are shorter. This vicious circle brings up the basal ROS output, which accelerates cellular senescence and the development of age-related pathology.

However, there is evidence that nuclear genome can indirectly preserve higher quality mtDNA also in somatic cells of adult animals, which may significantly increase their life span and delay the onset of age-related diseases. This would be typically achieved as a "side effect" of behavioral adaptational strategies, described by Dawkins [45] as the "extended phenotype". Such evolutionary conserved strategies as adaptation to IOR and ICR provide survival in environments characterized by rhythmic fluctuations in the availability of oxygen and nutrients, which is the apparent case in BW and NMR.

Intermittent Oxygen Restriction (IOR)

Combined hypoxia-hypercapnia is a primary physiological state in a developing mammalian embryo, and is essential to support its growth [46, 49]. The corresponding redox potential of embryonic tissues differs significantly from that of newborn and adult, and is a necessary condition for growth and development [47]. On the other hand, the gradual increase of cellular oxygen tension during the later phases of fetal development induces differentiation and maturation of tissues and organs [48, 50]. However, even the first observations of life under increased oxygen partial pressure revealed that "Oxygen might burn the candle of life too quickly, and soon exhaust the animal power within" (J. Priestley, 1775).

One possible strategy to slow down the ongoing oxidative mtDNA damage in somatic cells could employ 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 is known to protect

both germinal and somatic cells from excessive mutational damage and to support their proliferation [51, 52].

A complementary strategy, useful in qualitative selection of mtDNA would be a periodical exposure of a pool of heteroplasmic mitochondria in somatic cells to a critical functional load; such as increased energy demand combined with limited availability of fuel and/or oxygen. For instance, by exposing an adult organism to a controlled multiple ischemia-hypoxia-reoxygenation episodes, which yet remain under threshold of a massive apoptic damage. Such O_2 oscillations boost mitochondrial ROS production that consequently stimulates enhanced enzymatic antioxidative defense in healthy mitochondria [53], while destroying mutated mitochondria via *mitoptosis* [38]. Mitoptosis is not only a key mechanism of germinal follicles atresia [54], but also plays an important role in the erythrocyte maturation cycle [55] and underlies apoptic remodeling in normal tissue development and healing.

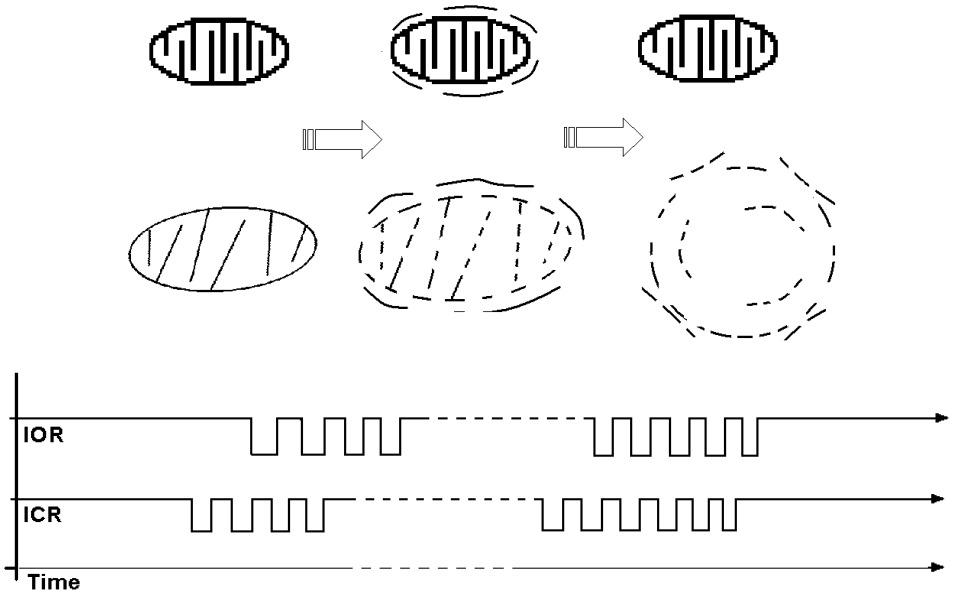

Figure 3. Mitoptosis is a physiological response to hypoxia-reoxygenation and calorie restriction. Oscillations of oxygen and nutrients (intermittent oxygen restriction—IOR and intermittent calorie restriction —ICR) stimulate impulse ROS production in mitochondria, consequently overloading mitochondrial antioxidative defense. Wild-type mitochondria (above) respond by increased production of antioxidative enzymes and survive. Mutated mitochondria (below) are much more sensitive to oxidative stress; they would be selectively eliminated in mitoptosis.

One can hypothesize that mitoptosis, being repeatedly induced by IOR in an adult organism, could purify mitochondrial populations of postmitotic somatic cells from the constantly appearing, oxidatively damaged, ROS-producing mtDNA copies. This should bring replicative advantage for the wild-type, non-mutated mtDNA's that are significantly less ROS-producing, but multiply slower than mutated mtDNA copies [37, 39, 56].

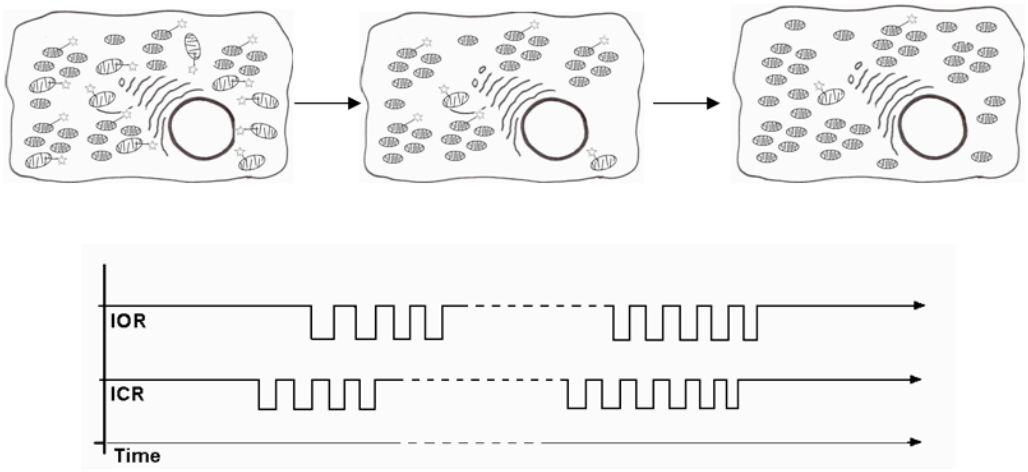

Figure 4. Oscillations of oxygen and fuel supply selectively eliminate ROS-producing mitochondria. Multiple oscillations of O_2 and nutrients purify postmitotic cells from mutated mitochondria via mitoptosis. In the absence of competition from deleted mitochondria, wild-type mitochondria quickly repopulate cells.

The IOR Stimulates Multiple Genome-Stabilizing Mechanisms

It is recognized that within the physiological range hypoxia is a universal challenge rapidly triggering multiple compensatory strategies that support genome integrity [57, 62].

Most eukaryotic cells maintain biological functions under hypoxia by switching energy source from fat acids to glucose and shutting down mitochondria. The switch is virtually instant and occurs simultaneously at the level of enzyme activity and gene expression [57]. The first-line antioxidative defense is triggered by hypoxia-induced mitochondrial ROS production and employs the glucose metabolism alteration. Underlying mechanism is based on a redirection of the metabolic flux from glycolysis to the pentose phosphate pathway, altering the redox equilibrium of the cytoplasmic NADP(H) pool [58]. The reversion to hypoaerobic metabolism is not limited to bioenergetic pathways, it stimulates expression of multiple genes and their products; numerous systems integrate to provide improved oxygen absorption, transportation and utilization.

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 [59].

However, the IOR in form of physiological intermittent hypoxia evokes particularly beneficial adaptations, not seen in continuous hypoxia. On the other hand, the obstructive sleep apnea (OSA) presents a pathological IOR pattern.

Adaptation to IOR elicits upregulation of cytoglobins (myoglobin and neuroglobin), which function as intracellular O_2 buffer and provide protection against RNS [60].

IOR stimulates insulin-independent glucose transport and accumulation of glycogen in the oxygen-sensitive cells, including cardiomyocytes and neurons; thus increasing instantly available intracellular energy reserves [61].

IOR is more efficient than chronic hypoxia in stimulating activator protein-1 and HIF-1, the master proteins, responsible for numerous adaptational pathways [63].

IOR efficiently stimulates erythropoetin (EPO) production [63]. EPO is not only the main regulator of erythropoesis, but also provides multiple adaptogenic and protective effects, particularly in the CNS [64].

HSP70, one of the majors in the chaperons family, is also stimulated by IOR [65]. It was demonstrated recently, that lifelong overexpression of HSP70 in skeletal muscle provided protection against injury and facilitated successful recovery after damage in muscles of old mice [66].

IOR is shown to stimulate growth hormone and IGF-1 release, while chronic hypoxia suppresses both [67].

IOR stimulates increased production of endogenous antioxidative enzymes [68].

IOR modulates humoral and cellular immunity [69, 70].

IOR stimulates brain-derived growth factor (BDGF) and glial cells-derived growth factor (GDNF) that provide neuronal protection and regeneration [71].

Hypoxia Facilitates Stem Cell-Based Tissue Repair

Remarkably that IOR modulates production and release of not only hematopoetic, but also stromal stem cells. Stromal, or mesenchimal stem cells (MSC) are known to convert into specialized postmitotic cells (neurons, cardiomyocytes, chondrocytes and osteocytes) in damaged tissues [72]. Normal 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-cells-dependent repair of tissues damaged with age [73].

Physiological hypoxia universally protects SC and stimulates release and homing of MSC [74]. It is found that MSC reside not only in the bone marrow, but also in perivascular tissues [75]; thus their activation by IOR seems to be a part of natural tissue-repair mechanism.

At least in some occasions, MSC can donate wild-type mtDNA by fusion with alternated cells without actually transforming into them [76]. In all variants, the IOR opens opportunity for enhanced MSC - dependent mitochondrial rejuvenation of damaged, non-replaceable cells (myocytes, cardiomyocytes, neurons, hormone-producing cells).

Protective Hypercapnia vs. Harmful Hypocapnia

In diving animals IOR is accompanied by simultaneous intermittent hypercapnia. Compared to humans, diving mammals have increased basal CO_2 values, but similar upper hypercapnia tolerance limit (37–60 torr vs. 45–60 torr, respectively) [77].

Physiological hypercapnia in vivo prevents damaging effects of ischemia or extreme hypoxia, which is widely used in clinic [78]. Several mechanisms may explain the protective role of CO_2 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 [79].

It is found that even moderately elevated pCO_2 directly suppresses mitochondrial ROS production [80]. It was shown in human blood phagocytes and alveolar macrophages, in the cells of the liver, brain, myocardium, lungs, kidneys, stomach, and skeletal muscle, as well as in mice tissue phagocytes and liver mitochondria. Generation of ROS was measured in the cell cultures and biopsies using different methods after exposure of cells and whole body to hypercapnia. CO_2 at a tension close to that observed in the blood (37.0 torr) and higher (60 or 146 torr) is a potent inhibitor of mitochondrial ROS generation. The mechanism of CO_2 effect appears to depend, partially, on the inhibition of the NADPH-oxidase activity [80].

In addition, increased CO_2 efficiently scavenges peroxynitrite, which diminishes or prevents relevant nitration and oxidative damage, particularly in neurons [81].

In contrast to hypercapnic IOR in diving mammals, the continuous altitude hypoxia (such as in high mountains) is coupled with persistent hypocapnia caused by altitude hyperventilation. Furthermore, compared to consistently intermittent diving hypoxia, the constant altitude hypoxia poses on the body significantly 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 many mountain-climbers that completed Everest trail without supplementary oxygen suffer long-term CNS damage. The extent of this damage was proportional to degree of altitude hypocapnia [82, 83]. It is found that continuous hypoxia caused accelerated mitochondrial damage, seen as lisosomal mitochondrial "junk" – lipofuscin [84].

Compared to beneficial effects of IOR the continuous exposure to high altitude hypoxia, combined with hypocapnia accelerates development of age-related pathology in humans, as it was demonstrated in a study focused on the relationship linking human aging and altitude [85].

The author examined cardiovascular, respiratory, neurological, immune and endocrine systems of the subjects at different altitudes. Study showed that memory (in particular, short-term memory) declined with altitude. The age of memory loss at high altitude began several years earlier than that of the subjects in lowland areas. The altitude also negatively influenced cardiac functions. The lung function of middle and old aged subjects living at altitude and then moving to lowland areas for 4–7 years were still lower than those of resident subjects in lowland areas. Their immune and endocrine functions were suppressed as well. These changes indicated that environmental stresses such as chronic hypoxia, hypocapnia, ultraviolet irradiation and cold exposure result in more rapid aging at high altitude.

The underlying molecular mechanisms have been elucidated in the study of Jefferson et al. [86]. The authors verified that oxidative stress is increased following both acute exposures to high altitude without exercise and with chronic residence at high altitude. The limit of human altitude hypoxia adaptability is believed to be located around 3500–4000 m.

Summarizing, these data indicate that a radical deviation from the evolutionary shaped intermittent hypoxia/hypercapnia pattern, found in normal embryonic development might cause

oxidative distress, disadaptation, functional overload of mitochondria and their accelerated structural degradation.

IOR in Non-Diving and Non-Burrowing Mammals

Whereas longevity-inducing and health-benefiting 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 beneficial adaptations and prolongation of life span may be induced by IOR in species that habitually live in normoxic and normocapnic atmosphere.

Honda [87] has shown that in *C. elegans*, changes in the generation and destruction of free radicals are implicated in life span modulations. The life spans under high and low oxygen concentrations were shorter and longer, respectively, than those under normoxic conditions. Short-term exposure to high and low oxygen concentration lengthens the life span. This is considered to be the result of an increase in antioxidant defense induced by short-term oxidative stress.

Since the pioneer publication of Meerson et al. [88] multiple aspects of adaptation to IOR were elucidated. Recently, the IOR was shown to directly prevent mtDNA deletions and mitochondrial structure damage in ischemia-reperfusion in vivo [89].

An important study of Milano et al. [90] focused on the difference in adaptation to continuous, compared with intermittent hypoxia. 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 two weeks to chronic hypoxia (10% O_2 and 90% N_2), intermittent hypoxia (same as chronic hypoxia, but 1 hr/day exposure to room air), or remained in normoxia (room air, 21% O_2). To evaluate myocardial tolerance to reperfusion, hearts of sacrificed animals were isolated and perfused for 30 min.; 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 two 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 hr 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 repetitive reoxygenation, rather than by hypoxia per se.

A number of animal studies show that beneficial effects of IOR can be achieved during short and/or multiple hypoxic exposures, varying from half an hour to several hours a day [91, 92].

The Difference between Sanogenic and Pathogenic IOR

There are contrasting differences in physiological outcomes of various intermittent hypoxic regiments and protocols. Persistent hypertension is a common disorder found in patients and animals exposed to a severe, brief IOR, as occurs in obstructive sleep apnea (OSA) [93]. Alternatively, the adaptation to mild, physiological, normo - or hypobaric IOR has been repeatedly demonstrated to prevent development of experimental hypertension, and reduce blood pressure of hypertensive animals and patients [94].

The main reason for this discrepancy is that the cardiovascular and cellular response to IOR greatly depends on the parameters of hypoxic intervention. Experimental protocols vary greatly in duration and intensity of hypoxia. Thus, protocols that simulate OSA, inducing systemic hypertension and impairing endothelium-dependent vasorelaxation generally employ brief, repetitive, severe hypoxia episodes for prolonged periods. For instance, the inspiration of 2–3% O_2 for 6 sec. at 30 sec. intervals, several hours per day for 4–7 weeks, or inspiration of 10% O_2 for 1 min. at 4 min. intervals, 12 h per day, for 14 days.

In contrast, the protocols that stimulate beneficial long-termed adaptation to hypoxia (hypoxic preconditioning) are based on the physiological IOR; for instance an intermittent inspiration of 9–12% O_2 for one hour a day during 3–12 weeks. Such treatments prevent development of endothelial dysfunction and abolish rise of blood pressure in spontaneously hypertensive rats [94].

The physiological justification of the most efficient intermittent hypoxia protocols stems from the study of naturally occurring IOR (hypoxic cycles) in non-pregnant and pregnant mammalian uterus by Chizov et al. [95, 96]. The authors discovered series of rhythmical plummeting of oxygen tension in the uterus (- 4 ± 2 torr from 6–8 pO_2 torr baseline, duration for 3–5 min.) with subsequent return to baseline that appeared several times a day, and continued for about an hour in each series. It is suggested that these oscillations serve as an evolutionary conserved cellular "hypoxic training" mechanism that assists embryogenesis, development and maturation of embryo's enzymatic antioxidative defense. Logically, one can presume that these O_2 oscillations, caused by rhythmical spasms of uterine arteries may serve as an instrument of mitoptosis execution in follicular atresia. Similar spontaneous pO_2 oscillations were also found in the various tissues of adult mammals [97, 98]. The IOR protocols based on this discovery are currently known under the name of "The Intermittent Hypoxic Training/Therapy" (IHT) and used in clinic, as well as serve to enhance athletic training [99]. The IHT efficiently induces hypoxic preconditioning, or long-term adaptation to hypoxia in oxygen-sensitive organs [100, 101].

Hypoxic preconditioning (hypoxia adaptation) presents a common physiological pathway that involves adaptive genexpression and synthesis of corresponding proteins; it modulates

multitude of cellular functions both in health and disease. The mitochondria-produced ROS and RNS triggering the adaptation in hypoxic preconditioning [102].

Messenger nitric oxide (NO) seems to play a central role in hypoxia adaptation. The NO-dependent protective mechanisms activated by IHT include stimulation of NO synthesis, as well as restriction of NO overproduction. The availability of NO precursors and donors (arginine and ornitine) and negative feedback of NO synthesis may optimize NO production. The adaptive enhancement of NO synthesis activates other protective factors, such as heat shock proteins, enzymatic antioxidants and prostaglandins, making the adaptation to hypoxia multilevel and sustained [103].

The unified positive effect of physiological IOR on the body is called cross-adaptation (induction of non-specific resistance to multiple stressors) and is a highly conserved characteristic that employs fundamental regulatory pathways that were established at the beginning of evolution of aerobes [88].

Intermittent Calorie Restriction (ICR)

Back to bowhead whales, who are the only baleen whales that spend their entire lives near polar ice edge; they do not migrate to temperate or tropical waters to calve. BWs are well adapted for living in cold waters—they have very thick, up to 0.5 m. blubber, which provides insulation and energy storage. Nutritional, and-energy balance in BW is characterized by deep excursions into stored nutrients during winter months, followed by summer periods of great abundance. This pattern of ICR makes BWs fully dependent on the affluent nutrients buildup in summer, while their survival throughout winter months under extreme fasting relies on autophagy (especially in pregnant and nursing females). The autophagy is a well recognized tissue-rejuvenating and cancer-suppressing strategy [104, 105, 106].

As all baleen whales, BWs thrive on fat-and protein-rich zooplankton. Fat-based OXPHOS has distinctive advantages compared to glucose-dependent OXPHOS (the latter prevails in mitochondrial energy pathways in some terrestrial herbivores). Marine mammals do not drink seawater; instead they produce it metabolically (oxidation of 1g fat gives 1.07 g. water).

Remarkably that in summer periods of affluent nutrition, as well as during fasting months the blood glucose in BW corresponds to the levels found in terrestrial animals [107].

Due to absence of carbohydrates in their food, glucose in BW is synthesized from glycerin, amino acids and lactate in gluconeogenesis, thus providing mitochondria with optimal amount of essential energy substrate and important metabolic precursor.

However, under starvation-induced hypoglycemia, mitochondria switch to metabolizing fat-derived ketones for energy production. This is a highly conserved adaptation to fasting and prolonged food restriction that evolved to enhance survival and maintain adequate functions while sparing proteins [108, 109, 110].

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 [111]. 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 [112], which increases ATP production. Remarkably, the ketone body-induced boost in the ATP production is also accomplished using less oxygen [113].

In addition to increasing ATP production while sinking oxygen consumption, ketone metabolism also lessens production of free radicals, which diminishes tissue inflammation provoked by ROS [111, 112, 113]. Noteworthy that compared to oxidation of fat acids and ketones, glucose oxidation in mitochondria results in significantly higher ROS production [114].

Conversely, physiological hypoglycemia selectively induces mitochondria-triggered apoptosis of malignant cells, while mitochondria of normal cells easily tolerate even deeper hypoglycemia [115]. Thus, fat-derived ketone bodies are not only a more efficient metabolic fuel than glucose, but also provide anti-inflammatory and anti-neoplastic effects.

Bowheads grow to about 8 m during their first year; then grow very slowly after weaning.

Affluent, protein and fat-rich nutrition during weaning and growing period, followed by a life-long, rhythmically predictable, season-dependent ICR results in downregulation of longevity-modulating genes daf-2 and daf-16 [116, 117]—a highly conserved genomic response found in yeasts, *C. elegans*, mice and men. Hsu et al. have found that in adulthood only daf-2-deficient *C. elegans* are both longer-lived and resistant to oxidative stress [116]. Noteworthy that ICR can induce daf-2 product deficiency.

In nature, foraging of NMR is severely restricted throughout dry seasons. Animals cannot search extensively for new food sources unless the ground has been sufficiently moistened by rainfall [118]. During brief periods after rain, NMR dig intensively to find enough food to sustain them through the long, irregular droughts. Such foraging pattern may maintain the ICR in wild NMR. The NMR colonies feed on plant roots and tubers rich with carbohydrates, but relatively poor in proteins and fats. NMR practice coprophagy that allows “fair distribution” of scarce nutritional recourses and contributes to digestive efficiency, as well as reinoculates NMR with endosymbionts that supply an additional source of protein and energy from digestion of the microbes themselves [119]. Endosymbionts ferment indigestible cellulose and fibers into short-chain fatty acids and other nutrients, which supply a significant fraction of basal energetic rations, and also may offer precursors that provide increased cellular/mitochondrial membrane resistance to oxidative damage. NMR also drink no water, receiving it exclusively from food.

Why Do Normoxic and Ad-Libidum-Fed NMRs Still Express Longevity and Cancer-Resistant Phenotype?

The study of oxidative damage in lipids, DNA and proteins in ad-libitum fed captive or laboratory-born NMR, which in contrast to wild NMR live in normoxic and normocapnic conditions, showed greatly increased oxidative stress markers compared to mice [120]. 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 NMR 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 NMR 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 [121] " In preliminary studies we have found that NMR cells are more resistant to neoplastic transformation. We hypothesize that NMRs have both more efficient DNA repair, and reduced mutagenesis leading to genomic stability".

Remarkably, that the blueprint of protective pathways and mechanisms, synergistically integrated by IOR and ICR evolutionary-shaped genotype, provides expression of the longevity and cancer-resistant phenotype even at increased basal level of oxidative stress. The question arises, if the naturally occurring in captive NMR spontaneous IOR and ICR episodes may be responsible for the expression of innate, unique NMR phenotype in an affluent, pro-oxidative laboratory conditions?

The laboratory studies of the interaction between sleep and thermoregulation in NMR revealed functional poikilothermia for the period of the REM bout [122,123]. It is known that the functional hypometabolic states induce relaxation response and mitigate stress in mammals. It cannot be excluded that captive NMR are still capable maintaining in an affluent environment their natural behavioral pattern and circadian rhythm. For instance, a typical in NMR resting-sleeping pattern (when many animals huddle in the sleeping chamber) can facilitate in their bodies temporary state of physiological hypoventilation, hypoxia-hypercapnia and hypometabolism that stimulate cellular regeneration. Such "rejuvenative hypoxic-hypothermic naps" may represent the naturally occurring IOR episodes. So, the intermittent periods of behavioral hypoxia-hypercapnia, hypometabolism and hypothermia may still be incorporated in their daily life cycle in normoxic atmosphere.

On the other hand, there is a recent parallel finding in the knockout Glutathione peroxidase 4-deficient mice that shown higher level of basal oxidative stress, but paradoxally, had longer median life span than wild-type mice [124]. It was found that the Gpx4-deficient mice have significantly increased sensitivity to oxidative stress-induced apoptosis. Authors conclude that lifelong reduction in Gpx4 increased life span and reduced/retarded age-related pathology most likely through alterations in apoptic sensitivity of tissues, which supports the IOR-dependent mitoptosis pathway hypothesis.

Additionally, both wild and captive NMR are naturally low in vitamin D3; this condition is known to evoke metabolic deficiency, similar to caloric restriction [125] and in ad libitum fed NMR 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 life span and cancer resistance in NMR. Among them the butyric acid and its derivates are known to offer antineoplastic and life span-prolonging properties [126, 127].

And at last, remembering that mitochondrial and bacterial lipids belong to the same family, one can deduce that digested bacterial endosymbionts may supply valuable precursors, supporting mitochondrial biogenesis in NMR.

The IOR in Clinic

The IOR, optimised as designed protocols that vary in periodicity, duration and intensity of hypoxic challenge, is used for a long time to accomplish particular aims, such as preacclimatize to high altitude and improve athletic performance [128].

It is demonstrated that the intermittent hypoxic therapy/training (IHT) as the most "engineered" and particularly mitochondria-targeting among various IOR protocols, presents a feasible, compliant intervention, which is effective in prevention, treatment and rehabilitation of numerous chronic degenerative diseases [129, 130, 131].

The IHT technology has been gradually developing during the last decades [99]. It belongs to the tools of evolutionary medicine [132], which harnesses the process of adaptation to challenging environmental conditions or physical stimuli and evokes innate genetic potential to withstand these challenges. A completed and sustained adaptation to IHT may bring multiple health-benefits and alleviation or cure in numerous chronic degenerative diseases [129,130].

Technically, an IHT session consists of 6–10 repeated, 2–6 min. duration intervals of hypoxic (9–12% O_2) air inhalation, interspersed with 3–6 min. duration inhalations of normoxic or hyperoxic air. Optimally, such daily sessions shall be consequently repeated 3 to 6 days a week. Throughout each session a patient experiences controlled multiple hypoxia-reoxygenation episodes that in the course of 2–6 weeks of treatment gradually induce a systemic, long-termed hypoxia adaptation.

The Summary of IHT Experience

During the last three decades the IHT gradually progressed as a non-medication treatment, and revealed its notable preventative, curative and rehabilitative potential. While theoretical basis of IHT has been consolidating via several decades of academic research, the practical knowledge of curative power of moderately hypoxic "mountain air", as well as familiarity with breathing techniques that induce a temperate hypoxia-hypercapnia, accounts for millennia and runs through various cultures and civilizations. Current technology advancement catalyzed the evolution and development of hypoxic treatment from esoteric concepts and costly mountain sanatoriums to molecular-biological insights and high-tech, user-friendly equipment that supports individualized treatment protocols.

The IHT centers in Russia and CIS, Europe and USA, China, Japan, Australia and New Zealand accumulated nearly three decades of physiological, sport medicine-and clinical research in this modality. Up to date, only in Russia clinical scientific research in IHT resulted in hundreds of dissertations, as well as numerous publications and presentations at international conferences [133]. In the light of accumulated evidence, it is hard questioning the effectiveness and safety of IHT. The statistics of treatment of 46,723 patients (incl. 4716 children) revealed

good and satisfactory results in 75–95% of cases treated during a standard 2- to 3-week cure [134].

Contraindications to IHT include acute infections, intoxications, exacerbations of chronic inflammatory diseases, fever, acute somatic conditions and trauma (shock syndrome, myocardial infarction, stroke, asthma attack, etc.), and decompensated chronic conditions.

A Multiple-Modality Rejuvenative Intervention

The IHT modulates several underlying mechanisms of aging, such as: expression of p53 and p66 proteins that modulate apoptosis and inflammation, as well as DNA maintenance and tissue repair [64-69, 135]. These pathways underlie the pathogenesis of aging and also function as the key players in a host of common degenerative or "civilization" diseases. Those include atherosclerosis and its main manifestations (cardiovascular disease, myocardial infarction, stroke), as well as diabetes type 2, arterial hypertension, inflammatory diseases of joints and respiratory ways, allergy, gastrointestinal problems and autoimmune conditions, cancer and neurodegenerative diseases.

The potential of IHT in rejuvenative therapies is recognised [136]. However, up till now we have found only one paper aimed to evaluate effects of IHT on various biomarkers of aging. In this study, using the biochemical parameters (levels of ROS, POL and antioxidative enzymes) and psychometric tests, researchers demonstrated that in average, a single completed course of IHT results in reversal of selected markers of aging on 3 to 5 years [137].

The ICR in the Clinic

While a life long caloric restriction remains a golden standard in life–extending interventions, it is recently demonstrated that various forms of the intermittent caloric restriction (ICR) can have similar or even higher efficiency in inducing favorable gene-expression changes and corresponding health benefits.

A study of Anson et al. [138] compared intermittent fasting and continuous calorie restriction in mice. A control group was fed ad libitum; another group was fed 60% of the calories that the control group consumed. A third group was fasted for 24 hours, and then permitted to free-feed. The intermittently fasting mice didn't cut total calories at the beginning and the end of the observation period, and only slightly cut calories in between. A fourth group was fed the average daily intake of the fasting mice every day. Both the fasting mice and those on a restricted diet had significantly lower blood sugar and insulin levels than the free-fed controls. Kainic acid, a toxin that damages neurons, was injected into the dorsal hippocampus of all mice. Hippocampal damage is associated with Alzheimer's. Interestingly, less damage was found in the brains of the intermittently fasting mice than in those that were on a restricted diet, and most damage in mice with an unrestricted diet. But the control group, which ate the average daily intake of the fasting mice, also showed less damage than the mice with restricted diet.

ICR decreases incidence and increases latency of mammary tumors in mice to a greater extent than does chronic caloric restriction [139].

Human studies confirm the efficiency of ICR. A six-month caloric restriction protocol resulted in improvement of biomarkers of longevity and oxidative stress in overweight subjects [140].

Similar results were obtained by Skrha et al. in obese diabetic patients [141]. Johnson et al. investigated the efficiency of alternate day calorie restriction (ADCR) protocol in asthma patients [142]. The study aimed to determine if overweight asthma patients would adhere to this dietary regimen, and to establish the effects of the protocol on their symptoms, pulmonary function, markers of oxidative stress, and inflammation. Ten subjects with BMI>30 were maintained for 8 weeks on a dietary regimen in which they ate ad libitum every other day, while consuming less than 20% of their normal calorie intake on the intervening days. Nine of the subjects adhered to the diet and lost an average of 8% of their initial weight during the study. Their asthma-related symptoms, asthma control, and quality of life improved significantly, within two weeks of diet initiation; these changes persisted for the duration of the study. Levels of serum beta-hydroxybutyrate were increased and levels of leptin were decreased on CR days, indicating a shift in energy metabolism toward utilization of fatty acids and confirming compliance with the diet. The improved clinical findings were associated with decreased levels of serum cholesterol and triglycerides, notable reductions in markers of oxidative stress (8-isoprostane, nitrotyrosine, protein carbonyls, and 4-hydroxynonenal adducts), and increased levels of the endogenous antioxidant uric acid. Indicators of inflammation, including serum tumor necrosis factor-alpha were also significantly decreased by ADCR. Compliance with the ADCR diet was high, symptoms and pulmonary function improved, and oxidative stress and inflammation declined.

In the human calorie restriction study by Civitarese et al. [143] mitochondrial DNA content increased by 35% ± 5% in the CR group and 21% ± 4% in the CR+ exercise group, with no change in the control group. The authors demonstrated 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 mRNA, and a decrease in DNA damage with a tendency toward lower SOD activity. Authors conclude that caloric restriction directly stimulates biogenesis of more efficient mitochondria in human skeletal muscle, which diminishes basal oxidative stress.

Spindler [144] have found that acute CR partially or completely reverses age-related alterations of liver, brain and heart proteins. CR 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."

Synergism of IHT and ICR

The IOR and ICR synergistically modulate OXPHOS in the mitochondria and stimulate mitochondrial turnover and biogenesis [89, 143]. Similarly to ICR, the short-termed IOR (as

intermittent exposure to mountain altitude) induced clinical improvements in patients with metabolic syndrome and related conditions [145].

Current research together with earlier observations, clearly indicate a spectacular similarity in outcomes of adaptation to ICR and IOR and justify concomitant use of both. In our practice we have found that a combination of modified IHT and Extended Morning Fasting (EMF) shows synergism in the accelerated recovery of diabetes type 2 patients and in other degenerative diseases. Previous research demonstrated the possibility of increasing the number of insulin receptors in cellular membranes and their enhanced sensitivity to insulin after ICR [146].

It was also shown that the sensitivity to insulin and the number of pancreatic beta cells increases, following IHT application [147]. Physiological hypoxia upregulates the activity of the glucose transporter GLUT1 by opening the K-ATP channels for glucose transport activity [148]. The pancreatic ATP- sensitive potassium (K-ATP) channel is a critical regulator of beta-cell insulin secretion and glucose metabolism [149, 150, 151]. This information provided ground for introducing IHT in combination with ICR and other integrative modalities in the treatment of patients with diabetes type 2.

Our earlier clinical research [152] has demonstrated that ICR in 5 - 7 days clinical setting induces a significant improvement in diabetes type 2 patients. We also found that outpatients could be more conveniently treated with a partial (early daytime) fasting regime, which we dubbed Extended Morning Fasting, EMF. Eight diabetes type 2 patients (duration of illness from 3 to 12 years) were subjected to ICR (fasting during the day lasting 16/18 hours out of 24 (the first food consumption took place at approximately 14.00–16.00 and the second at 19.00–20.00). All patients (five of theme were obese) arrived at the clinic in a state of decompensated diabetes. Before treatment with ICR-IHT protocol, four of patients received regular insulin injections, and three with oral hypoglicemic medication. One patient partially controlled diabetes by diet. The basic condition for starting the IHT element of our therapy was normoglycaemia, which was achieved by a low-calorie diet and oral medication, or insulin injections. During the first 2–3 days of IHT treatment the oral medication or/and insulin dosage were decreased gradually.

As a result of the two to three weeks' daily treatment in four patients on insulin injections, the diabetes was compensated by diet and lower doses of insulin (reduction, respectively from 180 to 30 units; from 100 to 20 units; from 70 to 40 units and from 64 to 40 units). The three patients formerly on oral hypoglycaemics were compensated with diet alone after the IHT treatment. One patient with mild diabetes achieved stable compensation on the diet as well. Patients were observed for from five months to five years. Regular morning fasting and hypoglycaemic diet maintained the achieved improvements indefinitely.

We hypothesise that IHT with concomitant glucose deprivation via EMF protocol helps overcome the cellular glucose/insulin resistance, probably as a result of synergistic upregulating effect on the membrane and nuclear binding sites such as the peroxisome proliferator-activated receptors. These candidate receptors mediate insulin-sensitivity. We believe that the effect of ionic K-ATP channel activation upregulates metabolic activity generally and also the overexpression of the glucose transporter GLUT1 participate in the total therapeutic effect.

To achieve the optimum results from this intervention, it is necessary to individualise the correct intensity, dose, frequency and timing between the applications of hypoxic stimuli and also monitor the patient response. Fluctuations of blood oxygen saturation (SpO2) during an IHT session serve as a valuable indicator of sensitivity to treatment.

Authors's practical experience with the IHT and our clinical outcomes in general corresponds to the published results of other researchers. A summary table below shows the results of synergistic application of IOR and ICR. As both authors are privately practicing holistic physicians, we use the reasonable evaluation criteria of evidence-based medicine and do not apply controlled randomized double-blind experimental clinical study design.

Table 1. The results of treatment (individualized ICR-IOR protocols) of 102 patients in 1998–2006

Diseases and conditions ***Nr. of patients***		**Outcome**		
		Improvement or cure	**No effect**	**Worsening**
Cardiovascular disease	8	6	1	1
Arterial hypertension	12	11	1	0
Type 2 diabetes	16	15	1	0
Burnout syndrome	4	4	0	0
Depression	2	2	0	0
Bronchial asthma	7	7	0	0
Emphysema	2	1	1	0
Multiple sclerosis	3	2	1	0
Neuroborreliosis	3	2	1	0
Epilepsy	1	1	0	0
Premenstrual syndrome	12	10	2	0
Postmenopausal syndrome	9	9	0	0
Infertility	4	2	2	0
Hypothyroids	11	10	1	0
Irritable bowel syndrome	5	5	0	0
Wound healing	3	3	0	0
Total	102	90	11	1
	100%	88,2%	10,8%	0,98%

Discussion

Aging bears the general characteristics of gradual depletion of functional and structural reserves at the cellular level. Aging is also associated with the lowered ability of an organism to respond to stress and maintain homeostatic regulation when given a challenge, thereby decreasing the organism's capacity to resist detrimental changes occurring in adult life. Consequently, increased resistance to degenerative disease, as well as extended life span depend on the capacity and plasticity of intracellular structural – and energy reserves.

At the molecular level, any physiological activity induces some degree of functional damage and depletion of reserves, which should be repaired and, under certain conditions may be consequently (over) compensated, thus increasing amount of available cellular reserves. The same pathways and same cellular energy-and structural reserves participate in normal aging; they may decline if they are not used and do not go through continuous functional repair. On the other hand, they degrade if overused and chronically under repaired. In both cases, mitochondria-modulated oxidative stress seems to be the culprit.

Currently, the ultimate test of mitochondrial-oxidative stress theory of aging is underway in the Skulachev project [153]. Mitochondria-specific antioxidant SkQ, which selectively accumulates in mitochondria, applied in nanogram concentrations inhibited development of such age-related conditions as osteoporosis, involution of thymus, cataract, retinopathy, tumors, etc. SkQ1 has a strong therapeutic action on some already developed retinopathies, in particular, congenital retinal dysplasia. With drops containing SkQ1, vision is recovered in 50 of 66 animals that became blind because of retinopathy. SkQ1-containing drops instilled in the early stage of the disease prevent the loss of sight in rabbits with experimental uveitis and restore vision to animals that had already become blind. Alleviation is also achieved in experimental glaucoma in rabbits. Further, the pretreatment of rats with SkQ1 significantly decreases the H2O2-induced arrhythmia of the isolated heart. SkQ1 strongly reduces the damaged area in myocardial infarction or stroke and prevents the death of animals from kidney infarction. In p53-deficient mice, SkQ1 decreases the ROS level in the spleen cells and inhibits appearance of lymphomas, which are the main cause of death of such animals. These data support the main postulates of the mitochondrial-oxidative stress theory of aging.

According to the theory of stress-induced premature senescence [154], sublethal doses of various stressor agents (such as environmental and behavioral stress, H2O2, hypoxia and hyperoxia, ionizing irradiation, UV light, etc.) lead to the exhaustion of the replicative potential of the proliferative normal cell types and the accumulation of senescent cells, which might be responsible for the creation of a micro-inflammatory state, thereby participating in tissue ageing.

On the other hand, the same agents and interventions being applied in proper doses and timing may induce increased non-specific resistance to multiple stressors and increase life span in various species via hormetic effect [155]. This is fully applicable to both IOR and ICR and their synergistic combination.

ICR is common in the nature and is more beneficial for survival compared with continuous calorie restriction, because a chronic restriction of availability of energy –and structural resources in the extreme and fluctuating, or low-productive natural environments diminishes adaptational potential. Calorie-restricted and chronically hypoxic animals and humans are more sensitive to cold, susceptible to viral infections and food intoxications; they also have decreased endurance.

On the other hand, the predictably oscillating availability of oxygen and nutrients activates multiple (from intracellular to behavioral level) "saving" strategies, which are not only free from these limitations, but induce increased genome stability and extend life span in diverse species. Both IOR and ICR synergistically stimulate multiple genome-stabilizing pathways. Concomitant application of IOR and ICR seem to significantly intensify DNA-and tissue repair.

ICR also may efficiently minimize latent threats that are presented by constantly appearing mutated genome copies, such as cancerous cellular clones. Under a severe calorie restriction or fasting first the alternated and mutated cells undergo apoptosis and autophagy, while survived healthy cells recover and proliferate in feasting times. It is found that tumors that have lost their self-eliminatory apoptic mechanisms still can undergo immune attacks, and if not destroyed by them, they would be encapsulated and stay dormant [156, 157]. These processes seem to be strongly facilitated by ICR.

Observing appropriate recovery and repair intervals in contrast to constant, uninterrupted functional load—drastically lowers risk of pathological outcomes. Thus, interval physical training in general is significantly safer and more efficient than continuous training [158, 159].

The oscillating character of the IOR and ICR, as well as their synergism seems to be crucial for observed effects. Species adapted to a rhythmic, oscillating pattern of accumulation and depletion of structural and energy reserves constantly exercise their storage and mobilization mechanisms, according to the universal principle "Use it, or loose it." Exercising energy-extracting and energy-storing systems, as well as continuous training of endogenous cellular antioxidative defense network provides a specific maintenance-and repair tool to slowing down aging process.

Conclusion

Exercising physiological functions, building up and regularly emptying the bodily reserves is a widely-known recommendation, which becomes more difficult to follow with each passing year of an individual's life, particularly when a person has no previous experience of regular exercising. Certainly, there is a constant demand for cost-efficient, naturally-based rejuvenative interventions that could be used in clinical settings, as well as incorporated into the most demanding and time-deficient lifestyles. The engineered derivatives of IOR and ICR seem to fulfill this requirement.

Nature offers a universal mitochondria-rejuvenating and tissue-regenerative strategy that modulates life span in evolutionary distanced species such as *C. elegans*, the naked mole rat and the bowhead whale, in correspondence with the cyclic availability of O_2 and nutrients. This natural strategy incorporates an affluent nutrition during postnatal development, followed by continuous ICR and IOR in adulthood. The underlying mechanisms and pathways synergistically modulate oxygen absorption, transportation and utilization, resulting in improved mitochondrial efficiency and reduction of basal oxidative stress. This in turn brings improved genome stability, postponed senescence and retarded development of age-related pathology, which ultimately increases healthy lifespan. While the underlying conserved evolutionary pathways have been found at all levels of biological organization, there is little

doubt that the same strategy is equally efficient in humans. Historically, different forms of ICR and IOR have an impressive account of empiric and evidence-based use in health and spiritual practices of various human cultures.

The efficient mitochondria-rejuvenating intervention in the form of IOR and ICR derivates IHT and ADF/EMF is already used successfully in clinical settings. It brings measurable reversal of biomarkers of aging, aging signs and age-related pathology, improved homeostatic and hormonal regulation, balanced humoral and cellular immunity, reduced chronic inflammation and markedly augmented non-specific stress resistance. The synergistic application of such protocols, accompanied by an individualized nutriceutical supplementation program, brings multiple anti-aging benefits and alleviation or cure in numerous chronic degenerative and age-related diseases.

Abbreviations

ADF	alternate day fasting
ADCR	alternate day calorie restriction
ATP	adenosine triphosphat
BW	bowhead whale (*Balena mysticetus*)
BMI	body mass index
CNS	central nervous system
EMF	extended morning fasting
EPO	erythropoetin
GH	growth hormone
HIF-1	Hypoxia inducible factor -1
ICR	intermittent caloric restriction
IHT	intermittent hypoxic therapy/training
IOR	intermittent oxygen restriction
MSC	mesenchimal stem cells
mtDNA	mitochondrial DNA
NMR	naked mole rat (*Heterocephalus glaber*)
NO	nitric oxide
nuDNA	nuclear DNA
OXPHOS	oxidative phosphorylation
OSA	obstructive sleep apnea
ROS	reactive oxygen species
RNS	reactive nitrogen species
SC	stem cells
SOD	superoxiddismutase

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