Intermittent Hypoxia and Health: From Evolutionary Aspects to Mitochondria Rejuvenation

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Abstract

Mitochondrial aging manifests as gradual depletion of energy reserves at cellular and systemic levels, as well as lowered stress resistance. Vital functional state of mitochondria is essential to reduce burden of age-dependent degenerative diseases and prolong health span. Two mitochondria-rejuvenating interventions: intermittent hypoxic training (IHT) and extended morning fasting (EMF), as engineered derivates of naturally occurred intermittent oxygen restriction (IOR) and intermittent calorie restriction (ICR), have been already in clinical practice. IHT and EMF utilize the familiar developmental and adaptational genetic programs, evolutionarily "preinstalled" in all aerobic organisms. Both ICR and IOR employ a common mitochondria-rejuvenating pathway, the mitoptosis – a selective elimination of the mitochondria that excessively produce reactive oxygen species in the cells. Mitoptosis is a natural process that maintains quality of mitochondria in the female germinal cells during early embryogenesis and can be stimulated and maintained by IOR and ICR also in postmitotic cells of adult 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 health span in aerobes. Furthermore, ICR and IOR influence 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 erythropoetin, growth hormone, heat shock protein 70, and other functional proteins. In addition, the IOR specifically intensifies stem cells-dependent tissue repair. The synergistic application of IOR- and ICR-based protocols, accompanied by nutrigenomical adjustment and individualized nutraceutical supplementation, brings multiple health benefits and alleviation or cure in numerous chronic degenerative and age-related diseases. Further development of engineered ICR and IOR protocols should help their advanced clinical implementation and user-friendly, self-help applications.

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L. Xi, T.V. Serebrovskaya (eds.), *Intermittent Hypoxia and Human Diseases*,
DOI 10.1007/978-1-4471-2906-6_21,

Abbreviations

ADCR	Alternate day calorie restriction
ADF	Alternate day fasting
ATP	Adenosine triphosphate
BMI	Body mass index
BW	Bowhead whale (*Balaena mysticetus*)
CNS	Central nervous system
CR	Calorie restriction
EMF	Extended morning fasting
EPO	Erythropoetin
GH	Growth hormone
HIF-1	Hypoxia-inducible factor-1
HSP70	Heat shock protein 70
ICR	Intermittent caloric restriction
IHT	Intermittent hypoxic therapy/training
IOR	Intermittent oxygen restriction
MSC	Mesenchymal stem cells
mtDNA	Mitochondrial DNA
NO	Nitric oxide
nuDNA	Nuclear DNA
OSA	Obstructive sleep apnea
OXPHOS	Oxidative phosphorylation
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SC	Stem cells
SOD	Superoxide dismutase

21.1 Introduction

Complementary to the multilevel definition (The WHO world report 2008 http://www.who.int/whr/2008/en/index.html), the biological meaning of wide-ranging term "health" is related to the individual's healthy life span, as well as to reproductive health span, both of which could be measured and compared among individuals and species. It is generally agreed that the vital cellular and, ultimately, mitochondrial functional fitness constitute the fundament of biological health in aerobes.

This chapter aims to elucidate the role of intermittent hypoxia in health maintenance, focusing on its utility as a multifunctional tool of a natural mitochondria-rejuvenative strategy and to discuss its current derivative clinical use. The interrelated evolutionary strategies and metabolic pathways that underlie exceptionally lengthy and healthy life span of some mammals will be explored. It is neither a systematic review nor a summary of controlled randomized clinical studies; it is rather an eclectic attempt to clarify the whole picture by "connecting the dots," as well as an unfolding reflection of author's thoughts and experiences, accumulated during more than two decades of practicing therapeutic hypoxia.

Commonly, it is agreed that an efficient preventive and therapeutic strategy aiming to prolong healthy life span, slow down aging process, and reduce age-related pathology in humans shall address a better preservation and continuous rejuvenation of mitochondrial populations in the postmitotic cells [14, 26]. Mitochondria carry multiple functions, different from ATP production [136]. These include participation in apoptosis and cellular proliferation, generation, and transmission of the 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 listed functions, reactive oxygen species (ROS) and reactive nitrogen species (RNS) modulate vitally important physiological cellular activities, hence the importance of integrity of the mitochondrial DNA.

On the other hand, the oxidative mutational damage to nuclear genome (nuDNA) and particularly to the mitochondrial genome (mtDNA) is believed to be the culprit of aging-related genomic instability that underlies degenerative disease and frailty of old age. ROS and RNS are constantly produced in oxidative phosphorylation (OXPHOS) in the mitochondria, inducing mutational deletions in the mtDNA [69]. Suppressing the mitochondrial ROS production prolongs healthy life span in numerous studies on different species [45, 110].

Currently applied mitochondria-supportive interventions confined to attempts in slowing down ROS/RNS-induced damage, either by dietary supplementation with antioxidants [74] or by engineered overexpression of genes encoding endogenous antioxidant enzymes (e.g., SOD, catalase, glutathione-peroxidase). However, the antioxidative supplementation provided, until now, controversial results [43], whereas the engineered enhancement of antioxidant enzyme-encoding genes is still far away from practical use.

In the meantime, the analysis of exceptional longevity phenomenon, found in some animals, is also worthwhile if we want to evaluate the late-life-onset interventions aiming to postpone aging and alleviate age-related pathology in humans [96]. Elucidating constituting pathways may help increase effectiveness of current therapies and outline prospective interventions, either behavioral, or straightforwardly pharmaceutical, or genomic.

21.2 The Challenge of Bowhead Whales

Extraordinary longevity of bowhead whales (BWs, *Balaena mysticetus*), as well as their remarkable resistance to cancer, attracted attention recently. These diving mammals have at least two key denominators: (1) they occupy ecological niche in rather unproductive environment that offers season-dependent nutrition and have relatively few predators (killer whales and humans), and (2) they continuously experience significant oscillations of cellular O_2 and CO_2 tensions (diving

hypoxia - hypercapnia), combined with seasonal (in winter months) severe calorie restriction or total fasting.

21.2.1 Enhanced Longevity and Low Cancer Morbidity

George et al. [37] conducted aspartic acid racemization measurements of the eye lenses of 48 BWs harvested between 1978 and 1996 in order to estimate the whale's age at the time of death. It was found that four animals were older than 100 years, and one was estimated to be 211 years old. The method has an accuracy range of about 16%, which means this whale could have been from 177 to 245 years old. Amazingly, one of the 100+ males was killed during mating. The oldest living person with a verified birth certificate was a 122-year-old French woman, Jeanne Calment, who died in 1997. Elephants have lived to 70 years in captivity, so BW appears to hold "the longevity record" for mammals.

Of 130 harvested BWs examined between 1980 and 1989, only one exemplar had a benign tumor, found in the liver. According to Philo et al. [93], "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" [27]. Similarly, a single benign tumor was observed in 55 slaughtered pilot whales in Newfoundland [20], and only two benign tumors (0.1%) were reported in 2000 baleen whales hunted in South African waters [121]. Only three cases of cancers (0.7%) were found during the postmortem examination of 422 odontocetes from British waters [60]. Among few cancerous tumors ever discovered in baleen whales, there were no metastatic ones and those found were small and encapsulated [39].

21.2.2 Life Span, Cancer, and Mitochondria

All metazoans face the problem of controlling cancer, which is a by-product of one of the major evolutionary events, the advent of multicellularity [77]. Theoretically, the chance of malignant transformation is proportional to the number of cells multiplied to the life span of the organism [92]. So humans have much higher cancer-control capacity than mice (about two thirds of wild mice, kept in a laboratory setting, naturally die from cancer). Prevention and suppression of malignancy in constantly proliferating tissues (e.g., epithelial, liver, bone marrow) become progressively more difficult as body size increases, requiring the accelerated recruitment of additional controls that are supposed to operate efficiently during initiation, promotion, and progression – at all three levels of cancerous genome evolution in the host. Therefore, BWs that can weight 2,000 times more and live twice longer than humans obviously have much better cancer control.

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 [89]. 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, stable metabolic conditions, the damaged, partially deleted mtDNA copies acquire replicative advantage and increase their number more rapidly than the intact and less damaged ones, thus progressively escalating accumulative ROS burden [83].

What biological mechanisms provide such an extraordinary combination of increased cancer resistance and extended health span in BW? Here, I hypothesize that the intermittent hypoxia in the form of continuous, lifelong oscillations of tensions of O_2 and CO_2 and the general pattern of oxygen metabolism, together with habitual intermittent calorie restriction, are the factors synergizing in this phenomenon.

21.2.3 Peculiarities of Bowhead Whale Physiology

In their natural habitats, BWs continuously undergo the intermittent hypoxia, or *intermittent oxygen restriction* (*IOR*), which is characterized by oscillating systemic hypercapnic hypoxia that all mammals are normally exposed to during embryonic and prenatal period. IOR is dubbed to emphasize its deep interrelation with the established term: *intermittent calorie restriction* (*ICR*). The behavioral IOR in BW may induce and maintain a phenotypic adaptation to intermittent hypoxia or a lifelong phenomenon of hypoxic preconditioning that is well known to reduce oxidative stress and prevent apoptotic and necrotic damage caused by acute hypoxia reoxygenation [78, 102].

21.2.4 Intermittent Calorie Restriction (ICR) and Fat-Based Oxidative Metabolism

Bowhead whales 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 depletion of stored nutrients, particularly fat, during winter months, followed by summer periods of great abundance. This pattern of ICR makes BW 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 natural mechanism of mitochondrial as well as cellular rejuvenation and cancer suppression [28, 72, 90].

As all baleen whales, BW thrives on fat- and protein-rich zooplankton. Fat-based OXPHOS has distinctive advantages compared to glucose-dependent OXPHOS (the later prevails in mitochondrial energy pathways in terrestrial herbivores). Marine mammals do not drink seawater; instead, they produce it metabolically (oxidation of 1 g fat gives 1.07 g water). Remarkably, 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 [48]. 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 this 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 [12, 84, 91]. Ketone bodies, consisting of acetoacetate and β-hydroxybutyrate, originate from fat metabolism 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 [124]. 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, which increases ATP production. Remarkably, the ketone body-induced boost in the ATP production is accomplished with diminished oxygen consumption [104]. In addition to increasing ATP production while sinking oxygen consumption, ketone metabolism also lessens production of free radicals, which suppresses tissue inflammation provoked by ROS [30]. It is noteworthy that compared to oxidation of fat acids and ketones, glucose oxidation in mitochondria results in significantly higher ROS production [103].

Conversely, physiological hypoglycemia selectively induces mitochondria-triggered apoptosis of malignant cells, while mitochondria of normal cells easily tolerate even deeper hypoglycemia [57]. Thus, fat-derived ketone bodies are not only a more efficient metabolic fuel than glucose, but also provide mitochondria-protective, anti-inflammatory, and antineoplastic effects.

Bowheads grow to about 8 m during their first year then they grow very slowly after weaning. Affluent, protein- and fat-rich nutrition during weaning and growing period, followed by a lifelong, rhythmically predictable, season-dependent ICR results in downregulation of longevity-modulating genes daf-2 and daf-16 [33, 53], a highly conserved genomic response found in yeasts, *C. elegans*, mice, and men. Hsu et al. [53] have found that in adulthood, only daf-2-deficient *C. elegans* are both longer lived and resistant to oxidative stress. It is noteworthy that ICR induce daf-2 product deficiency.

21.2.5 Genomocentristic Viewpoint

In contrast to often prevailing cellulocentristic picture of an organism, the following argumentation employs the evolutionary-based genomocentristic viewpoint. Would it be a mistake to believe that bodies, cells, and cellular organelles can be logically viewed as molecular machines that are designed, assembled, used, and maintained by their genomes with the single purpose of enabling transfer of derivative genome copies into the next generations? [24]. The theory of integration of anaerobic cells and aerobic protomitochondria and their genomes via endocytosis, which happened about two billion years ago and consequently evolved into mutual symbiosis, is widely accepted. The sequence of evolutionary coadaptative steps created the multitude of aerobic species. According to Dawkins' "The Selfish Gene" theory, "adaptations are the phenotypic tools through which genes secure their propagation."

The mutual cooperation and cross-talk between huge nuclear genome (about 3.3 Gb in size) and tiny mitochondrial genomes (16.6 Kb) in human cells, is similar to that one of 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.

21.3 Evolutionary Preservation of mtDNA

Since uncorrected accumulation of mtDNA mutations would, within a very small number of generations, become incompatible with survival, it is hypothesized that there should exist some common mechanisms for preservation of innate, wild-type mtDNA and selection against harmful, ROS-enhancing mtDNA deletions. According to Allen [2], the mtDNA evolutionary maintenance mechanism relies on the repressed oxidative function of female germ line mitochondria (promitochondria). The obligatory matrilineal mitochondria inheritance is found in the vast majority of species. They delegate to egg cells to contain, transmit, and preserve from oxidative mutational damage the germinal line promitochondria, which do not enter postmitotic oxidative phosphorylation.

mtDNA of oxidatively 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 [127]. This leads to accelerated mutations and deletions in mtDNA, involving the genes coding for respiratory chain proteins, and also may disturb the continuous fission and fusion of mitochondria, followed by their enlargement. Larger mitochondria are slowly autophagocytosed and undergo further oxidative damage, as well as produce more ROS [118]. As the oxidative mtDNA damage gradually progresses, the proportion of deleted mtDNA to wild-type mtDNA (heteroplasmy) increases [59]. Critically damaged mitochondria undergo *mitoptosis* (self-destruction of deleted and "worn-out" mitochondria) [111] and *mitophagy*; whereas the less damaged mtDNAs multiply continuously and more rapidly than wild-type mtDNAs, thus achieving selective replicative advantage. This manifests as clonal expansion of deleted mtDNA – a phenomenon consistently found both in inherited and acquired mitochondrial diseases [59, 86, 117].

In addition, 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 [113]. Another mechanism underlying cellular senescence is telomere erosion [47]. It is found that higher level of ROS accelerates telomere shortening and triggers senescence [126].

Energy demand and increased functional activity stimulate mitochondrial biogenesis in the postmitotic cells. The primary messenger NO and thyroid and steroid hormones, as well as mitochondria-specific nutrients and cofactors (L-carnitine, alpha-lipoic acid, taurine, coenzyme Q10, etc.), may stimulate and support mitochondrial proliferation nonspecifically, irrespective of the degree of mutational burden presented in a particular clone of mtDNA.

This is a common clinical situation seen in many patients suffering from inborn or acquired mitochondrial disorders. Patients are stabilized when supplemented with large amounts of mitochondrial nutrients, but usually experience an immediate exacerbation of the disease, as soon as supplementation balance is changed. Additionally, the excessive antioxidative supplementation may suppress signaling functions of mitochondrial ROS and hinder adaptive response to exercise [43], consequently diminishing quality of life. Thus, mitochondrial supplementation alone does not prevent and probably may even support the clonal expansion of mutated, deleted mtDNA.

21.4 Natural Selection of Better Quality Mitochondria

Natural mtDNA selection and purification mechanism is presented by the follicular atresia [25] that is common in all vertebrates**.** This phenomenon is suggested to be responsible for maintenance of germ line mtDNA quality in vertebrates. Follicular atresia, executed via apoptotic and/or necrotic elimination of about 90% of germinal cells in the ovaries of early female embryos, presents an efficient "quality control" tool [65]. It eliminates the majority of ROS-producing mitochondria in the female germinal cell lines, thus preventing them from entering future generations, which would definitely reduce offspring evolutionary fitness. For the same reason, the mitochondria of a sperm cell undergo annihilation in the egg cell immediately after fertilization, since to start a new life with foreign, oxidatively damaged mitochondria (to succeed in competition, winner sperm cells have to sacrifice them) would be a great disadvantage that was sorted off at the earlier evolutionary stages.

The clonal expansion of mutated and partially deleted mtDNA copies that is found in more intensively ROS-producing mitochondria correlates with advance of senescence and aging. It is discovered that with *ad libitum* available nutrition and uninterrupted supply of sea level O_2 (21%, 160 torr), the damaged, deleted mtDNA enjoys replicative advantage over wild-type, nondeleted mtDNA, which ultimately accelerates senescence [83]. This phenomenon employs the chemokinetic advantages of replication of shorter mtDNA molecules in the postmitotic mitochondria reproduction cycle: smaller molecules need shorter time to make their own copies (Fig. 21.1).

Clonal expansion of mutated mtDNA ultimately increases oxidative stress, thus accelerating senescence. Uninterrupted oxygen supply and affluent nutrition help mutated mitochondria outcompete the wild-type mitochondria for room and resources, thus further increasing oxidative stress. This vicious circle underlies advent of age-related pathology (Fig. 21.2).

However, there is evidence that nuclear genome is capable to indirectly maintain better quality of subordinated mitogenome not only in gametes but also in somatic cells of adult animals, such as bowhead whales, which may, at least partially, explain their significantly increased healthy life span. This would be typically achieved as a beneficial "side effect" of behavioral adaptation, described as the "extended phenotype" [23]. Such evolutionary-conserved strategies as adaptation to intermittent hypoxia and ICR provide survival in hostile environments characterized by predictable, rhythmic fluctuations in the availability of oxygen and nutrients.

Notably, similar strategy makes possible the extraordinary life span extension in plants that grow in hostile, oscillating in temperature and altitude environments. Thus, Bristlecone pines (*Pinus longaeva and Pinus aristata*) are among the oldest living organisms on earth. Bristlecone pines grow in Californian mountain isolated groves at and just below the tree line. Because of cold temperatures, dry soils, high winds, and short growing seasons, the trees grow very slowly. The wood is very dense and resinous, and thus resistant to invasion by insects, fungi, and other potential pests. The oldest living tree is 4,765 years old (http://www.nps.gov/brca/naturescience/

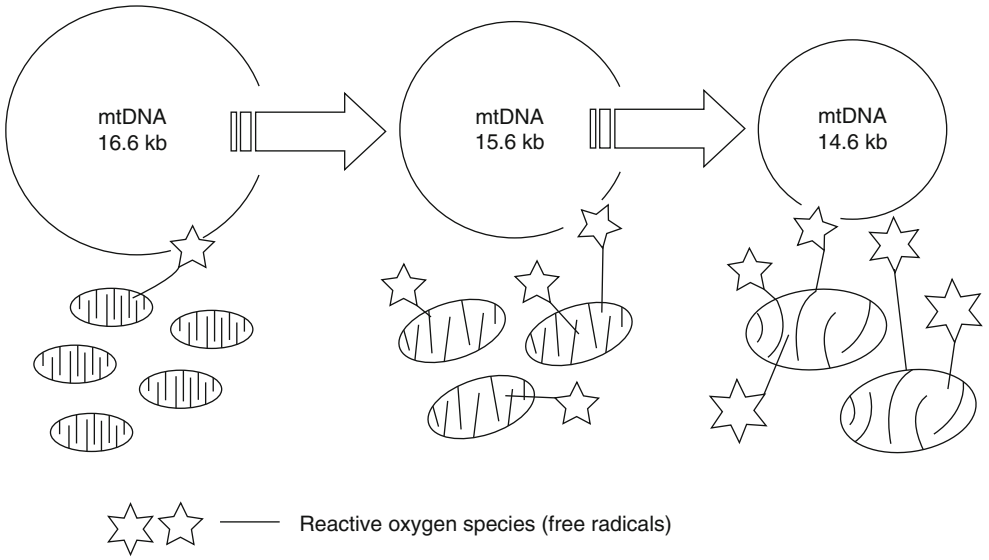

Fig. 21.1 mtDNA mutations/deletions produce inefficient, more ROS-producing mitochondria. mtDNA (*ring-shaped* molecule, approximate size in kb.) suffers about a magnitude higher oxidative mutational damage compared to nuDNA. During insufficient self-repair, the mutated segment of mtDNA ring would be excised/deleted and free ends merged together. The mtDNA loses 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 large and sluggish; they do not fuse and abundantly produce of ROS

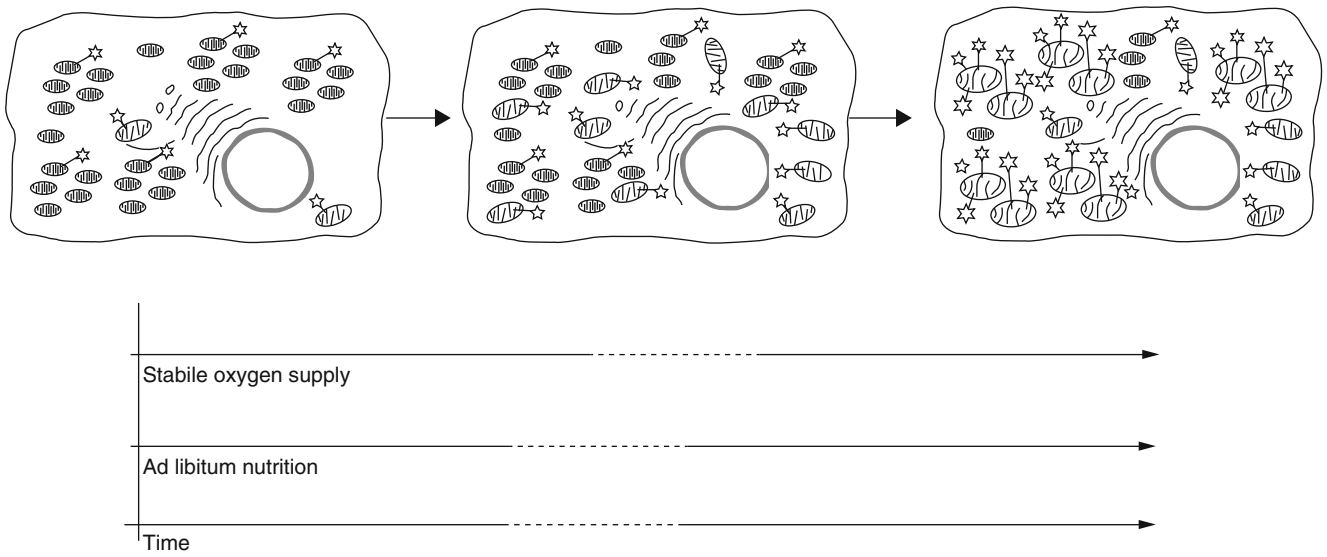

Fig. 21.2 Affluent and uninterrupted supply of oxygen and calories accelerates mtDNA deletions and clonal expansion of mutated mtDNA. Stabile oxygen supply and *ad libitum* nutrition help mutated mitochondria outcompete the wild-type mitochondria for room and resources. Mutated mitochondria multiply faster because their deleted mtDNA molecules are shorter. This vicious circle brings up the basal ROS output, which accelerates cellular senescence and development of age-related pathology

bristleconepine.htm). An even older tree was discovered in Sweden. Scientists from Umeaa University discovered it on Fulu Mountain in Dalarna province while carrying out a census of tree species there in 2004. A tree believed to be the oldest on the planet – nearly 10,000 years old (http://www.onlineweblibrary.com/blog/?p=511).

21.5 Intermittent Hypoxia in Ontogenesis

Combined hypoxia-hypercapnia is a primary physiological state in a developing mammalian embryo and is essential to support its growth [31, 108]. The corresponding redox potential of embryonic tissues differs significantly from that of

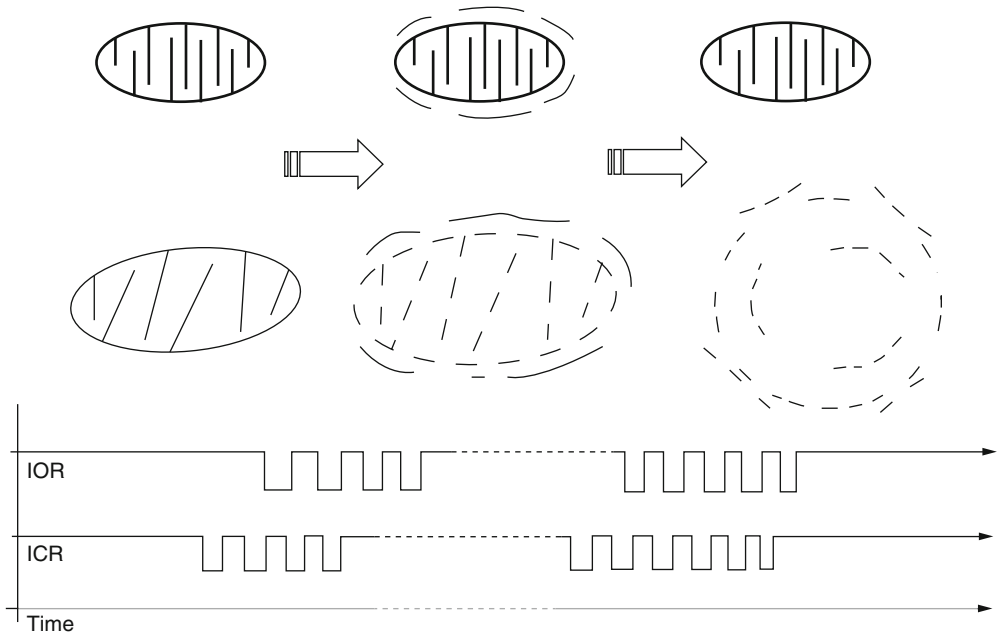

Fig. 21.3 Mitoptosis as a physiological response to hypoxia-reoxygenation and intermittent calorie restriction. Oscillations of oxygen and nutrients (intermittent oxygen restriction, *IOR* and intermittent calorie restriction, *ICR*) stimulate impulse ROS production in mitochondria, consequently overloading mitochondrial antioxidative defense. Wild-type mitochondria (*above*) respond with increased production of antioxidative enzymes and survive. Mutated mitochondria (*below*) are more vulnerable to oxidative stress; they would be selectively eliminated in mitoptosis

newborn and adult and is a necessary condition for growth and development. On the other hand, the gradual increase of cellular oxygen tension during the later phases of fetal development correlates with differentiation and maturation of tissues and organs [54, 55]. Yet, the first observations of life under increased oxygen partial pressure by J. Priestley in 1775 revealed that: "Oxygen might burn the candle of life too quickly, and soon exhaust the animal power within."

One possible strategy to slow down the ongoing oxidative mtDNA damage in postmitotic cells could employ maintaining and/or constantly returning back to more economical, embryonic-type pattern of oxidative metabolism, with its hypoxia-resistant 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 proliferative potential [22, 35].

A complementary strategy, useful in selection of mtDNA clones for fitness, would be a periodical exposure of populations 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 apoptotic damage.

Oxygen oscillations boost mitochondrial ROS production that consequently stimulates enhanced enzymatic antioxidative defense in healthy mitochondria [1], whereas mutated mitochondria that are not able to endure ROS oscillation would be self-eliminated via *mitoptosis* [111]. Mitoptosis is not only a key mechanism behind germinal follicles atresia [67], but also plays an important role in the erythrocyte maturation cycle [36] and underlies apoptotic remodeling in normal growth, development, and tissue healing (Fig. 21.3).

One can assume that mitoptosis, being repeatedly induced by IOR and ICR in an adult organism, could continuously purify mitochondrial populations in postmitotic somatic cells from the constantly appearing, oxidatively damaged, ROS-producing mtDNA copies (Fig. 21.4). This would shift replicative advantage in favor of wild-type, nonmutated mtDNAs that are more efficient, significantly less ROS-producing, but replicate slower than mutated mtDNA copies [83, 132].

21.6 The IOR Stimulates Multiple Genome-Stabilizing and Cellular Stress-Reducing Mechanisms

It is recognized that within the physiological range, hypoxia is a universal challenge rapidly triggering multiple compensatory strategies that support genome integrity [7, 94]. Most eukaryotic cells maintain biological functions under hypoxia by switching energy source from fat acids to glucose and shutting down mitochondria. The shift is virtually instant and occurs simultaneously at the level of enzyme activity and gene expression. The first-line antioxidative defense is triggered by hypoxia-induced mitochondrial ROS production and employs the glucose metabolism alteration. The 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 [8]. The reversion to hypoaerobic metabolism is not limited to bioenergetic pathways, it stimulates expression of

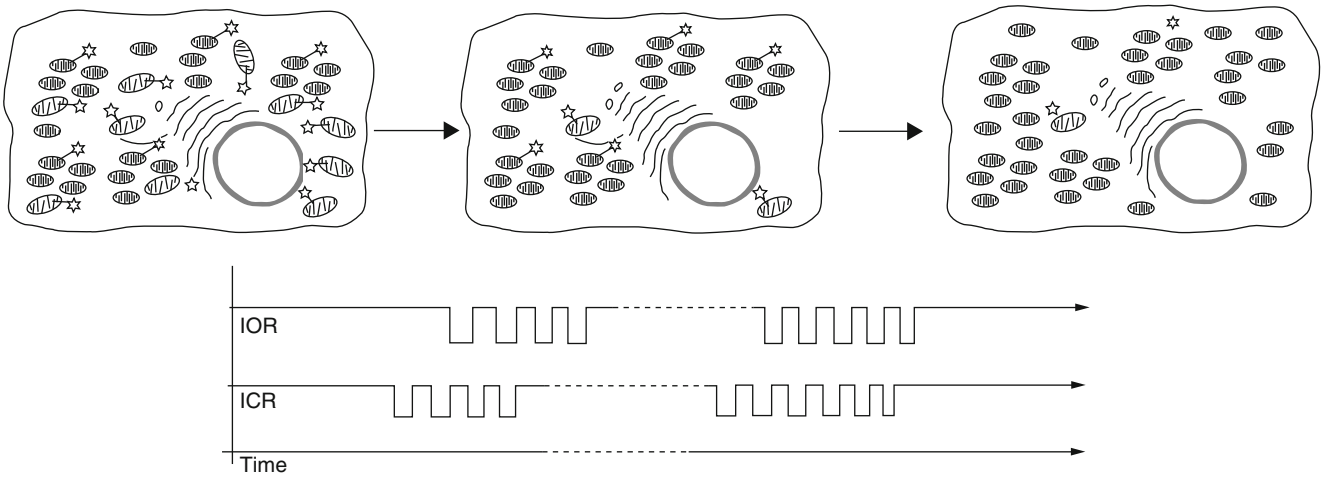

Fig. 21.4 Oscillations of oxygen partial pressure and nutrients availability selectively eliminate the most ROS-producing mitochondria. Multiple oscillations of availability of O_2 and nutrients purify postmitotic cells from mutated mitochondria via mitoptosis. In the absence of clonal competition of mutated mitochondria, wild-type mitochondria rapidly repopulate cells

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 mitochondrial maintenance energy expenses are reduced because of notably lesser proton leak [41].

The IOR in the various forms of physiological intermittent hypoxia evokes especially beneficial adaptations, not seen in continuous hypoxia. On the other hand, the obstructive sleep apnea (OSA) that obliterates physiological sleep presents a pathological IOR pattern, which consists of multiple uncontrolled anoxic episodes that induce systemic oxidative stress and chronic sympathetic overactivity [32].

There are multiple pathways, which constitute health beneficial effects, specific for the adaptation to intermittent physiological hypoxia, distinct from mitochondrial rejuvenation. Nevertheless, they all mediated by mitochondria-based ROS signaling.

For instance:

1. Adaptation to IOR elicits upregulation of cytoglobins (myoglobin and neuroglobin), which function as intracellular O_2 buffer and provide protection against RNS [116].
2. 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 [10].
3. IOR is more efficient than chronic hypoxia in stimulating activator protein-1 and HIF-1, the master proteins responsible for numerous adaptational pathways [94].
4. IOR efficiently stimulates erythropoietin (EPO) production [50]. EPO is not only the main regulator of erythropoiesis, but also provides multiple adaptogenic and protective effects, particularly in the CNS [29].
5. HSP70, one of the key members in the chaperons family, is also stimulated by IOR [133, 134]. It was demonstrated that lifelong overexpression of HSP70 in skeletal muscle provided protection against injury and facilitated successful recovery after damage in muscles of old mice [9].
6. IOR is shown to stimulate growth hormone and IGF-1 release, while chronic hypoxia suppresses both [128].
7. IOR stimulates increased production of endogenous antioxidative enzymes [135].
8. IOR modulates humoral and cellular immunity [38, 64].
9. IOR stimulates brain-derived growth factor (BDGF) and glial cell-derived growth factor (GDNF) that provide neuronal protection and regeneration [40].

21.7 Hypoxia Facilitates Stem Cell-Based Tissue Repair

Remarkably, IOR modulates production and release of not only hematopoietic, but also stromal stem cells. Stromal or mesenchymal stem cells (MSC) can convert into specialized postmitotic cells (neurons, myocytes, cardiomyocytes, chondrocytes, and osteocytes) in damaged tissues [66]. 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 cell-dependent repair of tissues damaged with age [46].

Physiological hypoxia universally protects stem cells and stimulates release and homing of MSC [101]. It is found that MSC reside not only in the bone marrow but also in perivascular tissues [21]; thus, their activation by IOR seems to be a part of natural tissue-repair mechanism. At least in some occasions, MSC may donate wild-type mtDNA by fusion with alternated cells without actually transforming into them [114].

Therefore, intermittent hypoxia opens opportunity for enhanced MSC-dependent mitochondrial rejuvenation of the damaged postmitotic, nonreplaceable cells [87].

21.8 Protective Hypercapnic Hypoxia and Deleterious Hypocapnic Altitude Hypoxia

In diving animals, IOR is combined with intermittent hypercapnia. Compared to humans, diving mammals have increased basal CO_2 values but similar hypercapnia tolerance limit (37–60 vs. 45–60 torr, respectively) [11]. Physiological hypercapnia in vivo prevents damaging effects of ischemia or extreme hypoxia, which is investigated in models and used in clinic [70, 73]. 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 [125]. It is found that even moderately elevated pCO_2 directly suppresses mitochondrial ROS production [61]. This was shown in human blood phagocytes and alveolar macrophages, in the cells of 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 NADPH-oxidase activity. In addition, increased CO_2 efficiently scavenges peroxynitrite, which diminishes or prevents relevant nitration and oxidative damage, particularly in neurons [123].

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 pose significantly higher "price of adaptation" due to the 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 the Everest trail without supplementary oxygen suffer long-term CNS damage. The extent of this damage was proportional to the degree of altitude hypocapnia [34, 129]. It was found that continuous hypoxia caused accelerated mitochondrial damage, seen as accumulating lysosomal mitochondrial "junk" – lipofuscin [52].

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 [17]. The author examined cardiovascular, respiratory, neurological, immune, and endocrine systems of the subjects at different altitudes. The 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 high altitude and then moving to lowland areas for 4–7 years was still lower than those of lowlanders. Their immune and endocrine functions were suppressed as well. These changes indicated that environmental stresses at high altitude and particularly chronic hypoxia result in accelerated aging.

The underlying molecular mechanisms have been elucidated [56]. The authors verified that oxidative stress is increased in 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 around 3,500–4,000 m. These data indicate that chronic hypocapnic hypoxia at high altitude might cause oxidative distress, disadaptation, functional overload of mitochondria, and their accelerated structural degradation.

21.9 Adaptation to IOR Extends Health Span in Nondiving Animals

Whereas longevity-inducing and health-benefiting effects of ICR have been extensively demonstrated in numerous studies and in diverse species, there is also mounting data on similar effects of IOR. Experiments show that beneficial adaptations and extension of healthy life span may be induced by IOR in species that habitually live in normoxic and normocapnic atmosphere.

It was shown [51] that changes in the generation and destruction of free radicals could modulate *C. elegans* life span. The life spans under high and low oxygen partial pressures were shorter and longer, respectively, than those under normoxic conditions. Short-term exposure to high and low oxygen concentration also lengthens the life span. This is considered to be the result of an increase in enzymatic antioxidant defense induced by short-term oxidative stress, caused by oscillations of oxygen partial pressure.

Since the pioneer publication of Meerson [80], multiple aspects of adaptation to IOR were elucidated in detail. A number of animal studies show that beneficial effects of IOR would be achieved during short and/or multiple hypoxic exposures, varying from half an hour to several hours a day [85, 105]. IOR was also shown to directly prevent mtDNA deletions and mitochondrial structure damage in ischemia-reperfusion in vivo [88]. Milano et al. [82] focused on the difference in adaptation to continuous, compared with intermittent, hypoxia. Authors tested the hypothesis that repeated

brief reoxygenation episodes interspersed with chronic hypoxia improve myocardial tolerance to hypoxia-induced dysfunction. Three groups of male rats were exposed for 2 weeks to chronic hypoxia (10% O_2 and 90% N_2), intermittent hypoxia (same as chronic hypoxia, but 1 h/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 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 h daily exposure to room air, chronic and intermittent hypoxia induced different responses in animal homeostasis, markers of oxidative stress, and myocardial tolerance to reoxygenation. These 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.

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 the severe, uncontrolled, brief episodes of IOR, as occurs in obstructive sleep apnea (OSA). Alternatively, the adaptation to the mild, physiological, normo- or hypobaric IOR has been repeatedly demonstrated to prevent development of experimental hypertension and reduce blood pressure of hypertensive animals and human patients [107].

21.10 The Ontogenetic Basis of Therapeutic Intermittent Hypoxia

The physiological justification of the most efficient intermittent hypoxia protocols stems from the study of naturally occurring IOR (hypoxic cycles) in nonpregnant and pregnant mammalian uterus [15, 16]. The authors discovered series of rhythmical plummeting of oxygen tension in the uterus (−4 ± 2 torr from 6 to 8 torr baseline of pO_2, 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 the follicular atresia phenomenon. Similar spontaneous pO_2 oscillations were also found in the various tissues of adult mammals [44, 68].

The IOR protocols based on this discovery are currently known under the name of "intermittent hypoxic training/therapy" (IHT) [106]. The IHT efficiently induces hypoxic preconditioning or long-term adaptation to hypoxia in oxygen-sensitive organs [131, 135]. Hypoxic preconditioning or hypoxia adaptation presents a common physiological pathway that involves adaptive gene expression and synthesis of corresponding proteins; it modulates multitude of cellular functions both in health and disease. The mitochondria-produced ROS and RNS trigger adaptation in hypoxic preconditioning [75, 122]. The role of messenger nitric oxide (NO) that participates in hypoxia adaptation was suggested [76]. The NO-dependent protective mechanisms activated by IOR include stimulation of NO synthesis, dynamic NO storage, and restriction of NO overproduction. The availability of NO precursors and donors (arginine and ornithine) and negative feedback 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.

The unified positive effect of physiological IOR on the body is called cross adaptation (induction of nonspecific 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 [79].

21.11 Therapeutic Hypoxia in Clinic

Therapeutic hypoxia, physiologically optimized as designed intermittent hypoxia protocols (historically known as various forms of altitude training) that vary in periodicity, duration, and intensity of hypoxic challenge, is used for a long time in humans to accomplish particular aims, such as preacclimatize to high altitude and improve athletic performance [62].

Clinical experience shows that the IHT, as "engineered natural intervention" presents a feasible, compliant protocol that is effective in prevention, treatment, and rehabilitation of chronic degenerative diseases [119]. The IHT technology has been gradually developed during the last decades [5]. Ultimately, this intervention belongs to the tools of evolutionary medicine [120], which harnesses the process of adaptation to challenging environmental conditions or response to physical stimuli. A completed and sustained hypoxia adaptation, induced by IHT, provides multiple health benefits.

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 3–6 days a week. Throughout each session, a patient experiences controlled multiple hypoxia-reoxygenation episodes, and in the course of 2–6 weeks of treatment, a systemic, long-lasting hypoxia adaptation can be gradually induced.

During the last decades, the IHT was gradually progressing as a nonmedication treatment and revealing its notable preventative, curative, and rehabilitative potential. While theoretical basis of IHT has been consolidating through several decades of academic research in the former USSR, 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 the 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. In the light of accumulated evidence, the effectiveness and safety of IHT is unquestionable. The statistics of treatment of 46,723 patients (including 4,716 children) revealed good and satisfactory results in 75–95% of cases treated during a standard 2- to 3-week cure [42].

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

21.12 A Multiple-Modality Rejuvenative Intervention

The IHT influences 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 [6, 75]. 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 Type 2 diabetes, arterial hypertension, inflammatory diseases of joints and respiratory ways, allergy, gastrointestinal problems and autoimmune conditions, cancer, and neurodegenerative diseases. Figure 21.5 illustrates the influence of intermittent hypoxia on the evolution of mitochondria in human body.

The potential of IHT in future rejuvenative therapies has been discussed [63, 97]. In one study, using the biochemical parameters (levels of ROS 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 3–5 years [42].

21.13 Using Synergism of IOR and ICR in the Clinic

While a lifelong caloric restriction remains a golden standard in life-extending interventions, it is recently demonstrated that various forms of ICR can have even higher efficiency in inducing favorable gene-expression changes and corresponding health benefits. Anson et al. [3] 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 h and then permitted to free-feed. The intermittently fasting mice did not 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 disease. 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 occurred in the mice with an unrestricted diet. In addition, ICR decreases incidence and increases latency of mammary tumors in mice to a greater extent than does chronic caloric restriction does [19].

Human studies confirm the efficiency of ICR. A 6-month caloric restriction protocol resulted in improvement of biomarkers of longevity and oxidative stress in overweight subjects [49]. Similar results were obtained in obese diabetic patients [109]. Johnson and colleagues investigated the efficiency of alternate day calorie restriction (ADCR) protocol in asthma patients [58]. 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

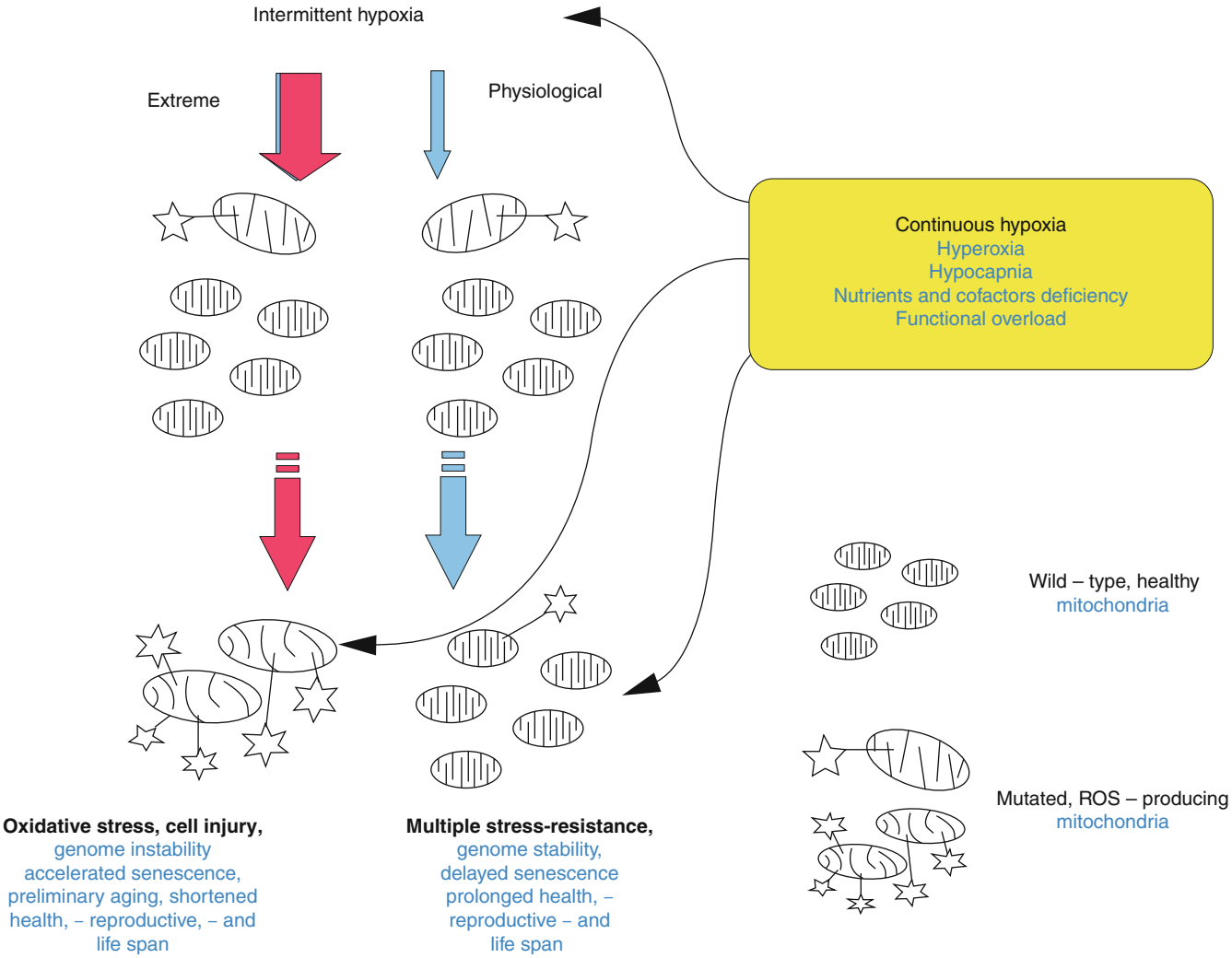

Fig. 21.5 Intermittent hypoxia governs evolution of mitochondria in postmitotic cells. *Left*: extreme intermittent hypoxia accelerates mtDNA mutation rate, ensuing clonal expansion of mutated, ROS-producing mitochondria; *Right*: physiological intermittent hypoxia facilitates multiplication of wild-type, healthy mitochondria, eliminating mutated, ROS-producing mitochondria. Note other important players

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 2 weeks of diet initiation. These changes persisted for the duration of the study. Levels of serum beta-hydroxybutyrate were increased, and leptin were decreased on the calorie restriction (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. [18], 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 the 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. It was also found that acute CR partially or completely reverses age-related alterations of liver, brain, and heart proteins [115].

CR also rapidly and reversibly mitigates biomarkers of aging in adult rhesus macaques and humans.

The IOR and ICR may synergistically modulate OXPHOS in the mitochondria and stimulate mitochondrial turnover and biogenesis. Similarly to ICR, the short-term IOR (as intermittent exposure to mountain altitude) induced clinical improvements in patients with metabolic syndrome and related conditions [71]. Current research together with earlier observations clearly indicates a spectacular similarity in mechanisms and outcomes of adaptation to ICR and IOR and justifies concomitant use of both. In our own practice, we have found that a combination of modified IHT and fasting shows synergism in the accelerated recovery of Type 2 diabetes patients and other degenerative diseases [95]. Earlier clinical research [4] has demonstrated that ICR in 5–7 days clinical setting induces a significant improvement in Type 2 diabetes 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) [99]. To achieve the optimum results from combining of IHT and EMF, it is necessary to individualize the intensity, dose, frequency, and timing between the applications of hypoxic stimuli and carefully monitor the patient's response. Fluctuations of blood oxygen saturation (SpO_2) and heart rhythm variability during an IHT session serve as a valuable indicator of responsiveness to treatment. An interesting case of functional and morphological brain recovery of an Alzheimer's-type dementia patient, achieved with the combined IHT–EMF protocol, was presented by the author recently [98].

Recently, Dr. B. Loeffler reported on the enhanced mitochondrial energy potential and increased up to 60% endogenous coenzyme Q10 production as a result of IHT course (Berlin, IPAM, personal communication and unpublished presentation). Coenzyme Q10 (ubiquinone) is a naturally occurring mitochondrial compound, an electron carrier in the mitochondrial respiratory chain. Q10 is one of the most important lipophilic antioxidants, preventing the generation of free radicals as well as oxidative modifications of proteins, lipids, and DNA. It can also regenerate the other powerful lipophilic antioxidant, alpha-tocopherol. Decreased levels of Q10 in humans are found in many diseases (e.g., cardiac disorders, neurodegenerative diseases, AIDS, cancer) associated with intensive generation of free radicals and their action on cells and tissues. Supplementation with cofactors (i.e., folic acid and B-group vitamins) only insignificantly increased mitochondria-dependent ubiquinone biosynthesis in the organism. Improved mitochondrial energy production, resulting from IHT course, may explain the observed effect on Q10.

Currently, the ultimate test of mitochondrial-oxidative stress theory of aging is under way [112]. Mitochondria-specific antioxidant SkQ, which selectively accumulates in mitochondria, applied in nanomole concentrations inhibited development of such age-related conditions such as osteoporosis, involution of thymus, cataract, retinopathy, some tumors, etc. SkQ1 has a strong therapeutic action on some 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 H_2O_2-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.

According to the theory of stress-induced premature senescence [13], sublethal doses of various noxious agents (such as environmental and behavioral stress, H_2O_2, 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 microinflammatory state, thereby participating in tissue aging. On the other hand, the same agents and interventions being applied in smaller doses and proper timing may induce increased nonspecific resistance to multiple stressors and increase healthy life span in various species via hormetic effect [100]. This is fully relevant to both IOR and ICR and their synergistic combination.

The oscillating character of the IOR and ICR, as well as their synergism, seems to be crucial for the observed effects. Oscillating stress/relaxation, damage/recovery interval patterns, in contrast to constant, uninterrupted functional load, diminishes risk of pathological outcome. Thus, interval physical training in general is significantly safer and more efficient than continuous aerobic training [81, 130]. 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 of "Use it or lose it." Exercising energy-accumulating and energy-mobilizing systems, as well as continuous training of endogenous cellular antioxidative defense network functions as is the evolutionarily developed maintenance and repair tool that slows down aging process and prolongs healthy life span.

21.14 Concluding Remarks

At the molecular level, any physiological activity causes a certain degree of functional damage and depletion of reserves, which would be repaired and, under favorable conditions, should be consequently supercompensated, thus increasing the amount of available cellular reserves. The same pathways and same cellular energy and structural reserves are depleted in the

normal aging; they may decline if not challenged and do not undergo continuous functional damage, repair, and supercompensation. On the other hand, they would degrade if overused and chronically underrepaired. In both cases, the mitochondria-modulated oxidative stress seems to be the culprit. It would not be a big exaggeration saying that we prematurely kill experimental animals, as well as ourselves, by *ad libitum* feeding. But it is also true for any aerobic organism, which is constantly and unlimitedly supplied with oxygen or, in contrary, being exposed to chronic hypoxia. The common "U" – or bell – curve scientifically validates the old wisdom: "Sola dosis facit venenum" (only the dose makes the poison; Paracelsus, 1538).

Nature offers a universal mitochondria-rejuvenating and tissue-regenerative approach that modulates life span in the evolutionary-distanced species, such as *C. elegans* and bowhead whale via cycling availability of O_2 and nutrients. This natural strategy incorporates affluent nutrition during postnatal development, followed by continuous ICR and IOR in adulthood. The underlying mechanisms and pathways synergistically influence oxygen absorption, transportation, and utilization, resulting in improved mitochondrial efficiency and reduction of basal oxidative stress level. This, in turn, results in improved genome stability, postponed senescence, and retarded development of age-related pathology, which ultimately increases healthy life span. While the underlying conserved evolutionary pathways have been found at all levels of aerobes 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.

Mitochondria-rejuvenating interventions, IHT and EMF, as engineered derivates of naturally occurred IOR and ICR, have been already in use in clinic. The synergistic application of such protocols, accompanied by individualized nutraceutical supplementation, brings multiple health benefits and alleviation or cure in numerous chronic degenerative and age-related diseases. Maintaining vital physiological functions and building up and regularly emptying the bodily reserves is a common advice, which becomes more difficult to follow with each passed year of an individual's life, particularly when a person does not exercise regularly. Certainly, there is a demand for the cost-efficient, naturally based rejuvenative interventions that could be used in clinical settings, as well as incorporated into modern demanding and challenging lifestyle. The engineered derivatives of IOR and ICR seem to fulfill this requirement.

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