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Comprehensive Invited Review

Oxygen in metabolic dysfunction and its therapeutic relevance

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Abstract

Significance: In recent years, a number of studies have shown altered oxygen partial pressure at a tissue level in metabolic disorders, and some researchers have considered oxygen to be a (macro) nutrient. Oxygen availability may be compromised in obesity and several other metabolism-related pathological conditions, including sleep apnea-hypopnea syndrome, the metabolic syndrome (which is a set of conditions), type 2 diabetes, cardiovascular disease and cancer.

Recent Advances: Strategies designed to reduce adiposity and its accompanying disorders have been mainly centered on nutritional interventions and physical activity programs. However, novel therapies are needed since these approaches have not been sufficient to counteract the worldwide increasing rates of metabolic disorders. In this regard, intermittent hypoxia training and hyperoxia could be potential treatments through oxygen-related adaptations. Moreover, living at high altitude may have a protective effect against the development of abnormal metabolic conditions. In addition, oxygen delivery systems may be of therapeutic value for supplying the tissue-specific oxygen requirements.

Critical Issues: Precise *in vivo* methods to measure oxygenation are vital to disentangle some of the controversies related to this research area. Furthermore, it is evident that there is a growing need for novel *in vitro* models to study the potential pathways involved in metabolic dysfunction in order to find appropriate therapeutic targets.

Future directions: Based on the existing evidence, it is suggested that oxygen availability has a key role in obesity and related comorbidities. Oxygen should be considered in relation to potential therapeutic strategies in the treatment and prevention of metabolic disorders.

Keywords: oxygenation; metabolism; chronic disease; hypoxia; hyperoxia

I. Introduction

Oxygen is a member of the chalcogen group in the periodic table, and is a highly reactive nonmetal that readily reacts with other elements to form oxides. By mass, it is the third-most abundant element in the universe, after hydrogen and helium. At standard temperature and pressure, two atoms of the element bind to form dioxygen (O_2), a colorless, odorless, gaseous element (206). Two centuries ago, scientists began to realize that there was an element in the air that was essential for life; something that could be depleted with a flame enclosed in a chamber ("phlogiston theory"), with severe consequences for small rodents inside the chamber (215, 369). Joseph Priestley is credited with these experiments as he published first, but, at the same time Carl Wilhelm Scheele was undertaking similar studies (215). Indeed, both scientists had communicated the experiments with Antoine Lavoisier, who disproved the combustion theory when he discovered the chemical significance of the oxidation and named the element "oxygen" from the Greek roots ὀξύς oxys- "acid" and γενής -genes "producer" (206).

During the Hadean eon (4,600–3,800 million years ago), before life existed on earth, oxygen levels in the atmosphere were nearly zero (1 part in a million), and gradually in the subsequent 1,500 million years the first cells developed systems for energy metabolism under anoxic conditions (Figure 1A) (215, 369). The first form of aerobic respiration was possible due to the disproportionate amount of H_2O_2 in the atmosphere, early catalase enzymes leading to the avoidance of the harmful products as hydroxyl radicals. What is termed the "Great Oxidation event" was the transition that occurred around 2,500 million years ago from the anoxic environment of the early Earth to an "oxic" atmosphere, whereby there was a rise of oxygen in the atmosphere to up to 2% (215, 369).

Throughout evolution, living organisms have been required to adapt to changes in atmospheric concentrations of carbon dioxide, nitrogen and oxygen. Fluctuations in the levels of these elements have occurred multiple times throughout the evolution of the atmosphere of the Earth. Nearly 4,000 million years ago the Earth's atmosphere was composed of a ratio of CO_2:N_2:O_2 of 98:1.9:0, and today this ratio is 0.03:79:21 (35, 369). This progressive change from an atmosphere lacking oxygen to one relatively rich in the element has been accompanied by a substantial increase in the complexity of living organisms (Figure 1B) (35).

The increased presence of oxygen produces a more efficient energy supply by aerobic metabolism, this generating 16–18 times more adenosine triphosphate (ATP) per hexose sugar than anaerobic metabolism (369). The higher energy supply generated by aerobic metabolism allowed hundreds of new reactions, and therefore new metabolites, to emerge (206, 369). Once oxygen became more abundant aerobic respiring bacteria were able to thrive, including the ancestors of mitochondria (206), thereby increasing cellular complexity. The increasing content of oxygen in the atmosphere is paralleled by an increase in oxygen-rich protein domains such as the extracellular domains of

transmembrane proteins (2). Thus the compartmentalization of eukaryotic cells and the allocation of cellular respiration to mitochondria could have evolved as a mechanism to protect oxygen-rich protein domains (369). Through the development of complex compartmentalization, multiple processes including signaling and different biochemical conditions could also be controlled in different parts of the cell, which in turn, may have led to the emergence of multiple cell types within the same "greater" organism (with over 200 different cell types in the adult human body) (2, 101, 410).

Even though oxygen is essential for life in most organisms, its associated metabolic products may become toxic (369). The paramagnetic characteristic of O_2 renders a barrier to chemical reaction since organic donors have to experience a slow spin inversion to donate electrons. This spin restriction is avoided by the electronically excited singlet oxygen ($^1O_2$), making it vastly more reactive than ground state oxygen (206). Over 95% of all the oxygen we breathe is reduced by a very efficient system to $2H_2O$, but a small proportion produces the superoxide anion radical ($O_2{\cdot}-$) (293). Superoxide can be subject to dismutation producing hydrogen peroxide (H_2O_2), which can also react with reduced transition metals to form a hydroxyl radical (·OH) (376). These intermediates, reactive oxygen species (ROS), may lead to a serious threat to cells as they can interact easily with organic compounds damaging cellular components (316). ROS oxidize redox-reactive cysteine (Cys) residues of proteins producing reactive sulfenic acid (−SOH) which can form disulfide bonds with closely located cysteines (−S–S−) or suffer additional oxidation to sulfinic ($-SO_2H$) or sulfonic ($-SO_3H$) acid (316). For this reason, organisms should avoid their overproduction through oxygen reduction using paramagnetic transition metals and organic substances with reactive sites (206). Nevertheless, there are other enzymes responsible for minimizing the effects of an excess of ROS production such as catalases, glutathione peroxidases and superoxide dismutases. These enzymes involved in counteracting ROS toxicity are thought to have evolved before the rise in oxygen levels that allowed for aerobic respiration (206, 369).

Energy homeostasis is a key aspect of the metabolic regulation involved in the adaptation to different circumstances (410). Among the regulatory changes, some of the most important are increases in the capacity of tissues to carry out oxidative metabolism together with the availability of oxygen itself (105). Oxygen is just as important for organisms (aerobic) as the other nutrients that they oxidize to generate energy. The average human has an intake of 713 g of food (in a diet of 3,420 Kcal/day as an average energy consumption in developed countries — according to FAO) and 1,696 ml (g) fluid per day (396). Thus, the total weight of food and fluid intake is 2,409 g. Assuming an average food density of 1 g/cm^3 (fat has a lower density, while protein is usually greater) equivalent of that of water, a standard human consumes 2.4 l of nutrients per day.

On the other hand, a standard 70 kg-adult, when resting, averages 12 breaths per minute, with each inhalation (and exhalation) moving about 500 ml of air into the lungs (136). This means 6 l of inhaled air per minute for a standard 70 kg-person (86 ml/kg/min of air). More specifically, an average person inhales approximately 8,640 l of air gas mixture per day — despite variations with the physical activity level. This totals 1,810 l of pure oxygen per day. Making an analogy with the iceberg paradigm, 99.87% corresponds to oxygen while the rest corresponds to what we have commonly accepted as the food pyramid, representing a mere 0.13% of our daily consumption — considering the approximate volume of the components when they are to enter the body (Figure 1C). In terms of weight, the amount of classical nutrients (fluids and food) and oxygen (density 1.429 g/l at standard pressure and room temperature) consumed is similar (oxygen is 51.77% while classical nutrients and fluids represent 48.23% of the total weight).

The maintenance of an adequate supply of oxygen requires the coordinated operation of the three major systems involved in oxygen transport: respiratory, cardiovascular, and blood (299). Oxygen is recognized as a critical factor for respiration, and key metabolic processes and low levels of oxygen are characteristic of certain tissues under normal conditions as well as in pathological situations (385, 386). In this regard, the normal oxygen levels range from 14.5% pO_2 in the lung alveoli to 3–10% in most of the peripheral tissues (53).

This raises important questions about the use of the term “normoxia”, which is almost universally applied to refer to the highest oxygen availability at sea level (21% oxygen as inspired) as well as with reference to normal oxygen levels for each tissue. Some authors have proposed the use of “physiological hypoxia”, “physioxia”, or “physoxia” to define the oxygen concentration at which tissues respond to maintain their preferred oxygen level (242). In this sense, hypoxia-response elements could regulate cellular processes at various oxygen levels in different tissues, hindering the overall analysis of oxygen homeostasis. On the other hand, from a pathological point of view hypoxia could be considered when oxygen levels are below the physiological conditions for a given tissue. This detrimental hypoxia may be found in a wide variety of diseases, such as ischemia, cancer and obesity (92, 347, 385). Conversely, recent work has shown that mitochondrial disease models display tissue hyperoxia and that disease pathology can be reversed by normalization of excess oxygen (17, 169, 170).

Cellular responses to low oxygen levels are mediated by specific transcription factors, particularly by hypoxia inducible factor-1 (HIF-1). Two other key HIFs are evident, namely HIF-2 and HIF-3, but HIF-1 has received considerably more attention. HIF-1 is, an oxygen-labile DNA binding transcriptional activator responsible for the induction of the expression of multiple genes involved in glucose uptake and anaerobic metabolism, mitochondrial function, angiogenesis, inflammation,

proliferation and cell survival (35, 120, 348, 442). The activity of this transcription factor has been particularly studied in the context of cancer since hypoxia is a prominent characteristic of the tumor environment and is strongly associated with aggressive tumors (367). HIF-1 activates several hallmarks of cancer such as angiogenesis, cell proliferation, invasion and metastasis, and, therefore, has a crucial role in tumor survival and progression (302). Although the stabilization of the oxygen-responsive subunit of HIF-1, namely HIF-1α, usually involves a tissue hypoxic milieu (it is rapidly degraded in the presence of oxygen) due to decreased oxygen delivery and diffusion (occasionally combined with increased oxygen consumption), the stabilization of HIF-1α can also be triggered by non-hypoxic stimuli as discussed in the following sections.

The present review focuses on the complexity of oxygen delivery and sensing, the relevance of this element in the cellular stress antioxidant system, oxygen's role in metabolic disorders as well as oxygen-related therapeutic approaches. Over the last few decades, effort has been devoted to understanding the role of oxygen in metabolic disorders at a cellular level. The stabilization and consequent activity of HIF-1 in metabolically active tissues has been associated with metabolic diseases (120). However, the mechanism by which hypoxia pathways are involved in the progression of metabolic impairment is still unclear. Due to the considerable complexity of metabolic dysfunction and the side-effects of current treatments, there has been a growing interest in developing new therapeutic approaches for metabolic diseases. In this sense, a wide-variety of oxygen-related therapies have been studied — as we discuss in the present review.

II. Oxygen delivery

At sea level, 1 atmosphere (pressure unit) is equivalent to 760 mm of mercury (mmHg), and oxygen is 21% of dry air. Hence, the oxygen partial pressure (pO_2) of inspired air is around 160 mmHg at sea level. In the body, oxygen is transported by the blood to different organs producing a physiological distribution of the molecule in tissues (Figure 2). Thus, those tissues with lower than normal pO_2 can be considered as hypoxic from a molecular point of view (35).

In normal conditions, oxygen delivery by arterial blood flow to the capillaries in man is approximately 14 ml/kg/min, with 25% oxygen being exchanged between blood and tissues, the oxygen consumption being 3.5 ml/kg/min (172, 299). Changes in oxygen transport usually lead to hypoxic tissue environments. From a physiological standpoint this can be classified according to the origin in terms of cardiovascular, respiratory, blood or tissue defects (299): hypoperfusion, hypoxemia, anemia and histotoxicity. First, situations involving cardiovascular system defects usually cause extremely low blood flow or hypoperfusion (306). This defect can be local, as ischemic perfusion, or systemic, i.e. reduced cardiac output. For example, in obesity, white adipose tissue (WAT) experiences a certain

grade of hypoxia due to impaired perfusion (123). Also, in the inner regions of a tumor (53, 67, 286), and during acute myocardial infarction (343, 348), a perfusion deficit is experienced.

Secondly, conditions entailing the respiratory system, such as low inspired pO_2 (i.e. at high altitude) and breathing problems (i.e. pulmonary edema, ventilation-perfusion mismatch, apnea), lead to falls in arterial pO_2 producing hypoxemia (299). Concerning low inspired pO_2, high-altitude sickness is a shared term for the syndromes affecting non-acclimated travelers shortly after ascent to altitudes above 2500 m (recognized also above 1500 m), and encompasses the cerebral conditions of acute mountain sickness, high-altitude cerebral and pulmonary edema syndromes (22). Sleep apnea-hypopnea syndrome (SAHS) can be also classified in these respiratory problems which present hypoxemia and is associated with hypertension (hereafter arterial hypertension, unless otherwise stated), ischemia and metabolic dysfunction (79). Moreover, oxygen consumption is associated with resting metabolic rate through the metabolic equivalent classification or METs (172). This term refers to the oxygen consumption, where 1 MET is equivalent to 3.5 ml oxygen/kg/min, and it is used to quantify the physical activity intensity. Several studies have shown approximately 30% lower oxygen consumption, compared to the standard 1 MET, in both obese subjects with a mean BMI of 30 kg/m^2 (44) and extremely obese individuals with a mean BMI of 42 kg/m^2 (415). In a recently published bibliographic review, where this threshold is also discussed, the authors suggest an overestimation of resting oxygen consumption among coronary patients and the morbidly obese (104). Noteworthy is that this report provides information on lower oxygen consumption at rest in obesity and cardiovascular disease (CVD). In this regard, low oxygen consumption was associated with systemic inflammation in obese men with SAHS (223). The measurement of basal oxygen consumption is an easy to perform, non-invasive and affordable approach, which other than in the particular study, is not commonly used to differentiate between metabolically healthy individuals and those with metabolic disorders.

Thirdly, blood oxygen deficiency, or anemia, occurs when the concentration of hemoglobin, responsible for carrying oxygen through the blood to the tissues, and/or erythrocyte count, are lower than normal and insufficient to meet an individual's physiological needs (61). This means that fewer oxygen-binding sites are available to hold oxygen and is frequently caused by blood loss, iron and the deficiency of other nutrients' — vitamins A, B2, B9, and B12 — as well as inflammatory and infectious diseases (61). The adaptive response to anemia is erythrocytosis, an abnormal increase in the number of circulating erythrocytes (345). Erythrocytosis is defined by: (i) hemoglobin >18.5 g/100 ml in men, or 16.5 g/100 ml in women, for Caucasians; or (ii) hemoglobin or hematocrit >99th percentile of method-specific reference range for age, sex and altitude of residence; or (iii) hemoglobin >17 g/100 ml in men or 15 g/100 ml in women for Caucasians (or the equivalent in other

races and age groups) if associated with a documented and sustained increase of at least 2 g/100 ml from an individual's baseline value and cannot be attributed to the correction of iron deficiency (292). Finally, mitochondrial dysfunction associated with impaired oxygen utilization, or histotoxic hypoxia, could lead to reduced ATP production (1). This deficit is due to a disturbance of oxygen usage by cells and involves chemicals acting as poisons (cyanide, rotenone, antimycin A) as well as by inflammatory mediators (1, 200, 299). For example, rotenone — used as a broad-spectrum insecticide, piscicide, and pesticide, and naturally present in several plants — inhibits the complex I (NADH:ubiquinone oxidoreductase) of the mitochondrial respiratory chain in all cell types by inhibiting the ubiquinone-dependent oxidation of the mitochondrial NADH to NAD (353, 420). In cells, rotenone decreased the mitochondrial membrane potential, increasing ROS production and shifting respiration to a more anaerobic state that develops lactic acidosis (310). In the case of rotenone intoxication in humans, the inhibition of the mitochondrial respiratory chain led to severe metabolic acidosis (pH 6.76), reduced level of consciousness, coma and respiratory depression leading to respiratory arrest (420). In this regard, cyanides are found in certain seeds and fruit stones, while hydrogen cyanide is produced by the combustion of certain materials under oxygen-deficient conditions, such as in combustion engines and in tobacco smoke. Cyanide is a potent inhibitor of the cytochrome c oxidase (mitochondrial complex IV), decreasing oxygen consumption at the cellular level (111). This disruption of electron transport also induces cellular redox imbalance and excessive ROS production (116). On the other hand, antimycin A — a secondary metabolite produced by *Streptomyces* bacteria and the active ingredient in Fintrol, a chemical piscicide — is a mitochondrial complex III inhibitor that completely inhibits oxygen consumption in the respiratory chain (171). Noteworthy, the expression of alternative oxidase (AOX) from the ascidian *Ciona intestinalis*, confers resistance to antimycin A and cyanide, bypassing the cytochrome segment of the respiratory chain (116, 135, 375). AOX also inhibits the mitochondrial membrane hyperpolarization, and the superoxide overproduction induced by acute hypoxia in pulmonary artery smooth muscle cells, avoiding the subsequent pulmonary vasoconstriction in mice (366). In this regard, a chemically modified RNA encoding a humanized AOX has been recently generated as a therapeutic route (115). Lastly, inflammatory mediators such as nitric oxide and oxygen radicals have been suggested to play a role in the impairment of ATP production, resulting in a form of histotoxic hypoxia (1, 224).

III. Oxygen sensing

Organisms need oxygen sensing mechanisms to allow a fast response to changes in pO_2, thereby maintaining intracellular oxygen homeostasis. Oxygen sensor systems are able to sense oxygen concentrations, initiating intracellular signaling cascades in response to altered pO_2 (94). The

chemoreceptors of the central nervous system are located on the ventrolateral medulla, while the peripheral chemoreceptors are located in the carotid and aortic bodies. The specialized glomus cells in the carotid body sense even minor changes in arterial blood oxygen tension, eliciting afferent signals in the carotid sinus nerve (215). As Pittman has explained in detail, the aortic bodies are responsible for the cardiovascular response to respiratory-linked chemical factors in the arterial blood. On the other hand, the carotid bodies sense when the arterial pO_2 falls below 50 mm Hg, and consequently peripheral chemoreceptors increase ventilation as a stimulatory response to hypoxia. While the carotid bodies are not sensitive to hypoperfusion or anemia because they have high blood flow, aortic bodies are sensitive as their perfusion is lower. The inability of the respiratory chemoreceptors to sense changes in pO_2 elevation above 50–60 mmHg leads to a ventilatory unresponsiveness in hyperoxia (299). In addition, other stimuli such as plasma glucose and blood osmolality also trigger the carotid body, serving as a polymodal sensor involved in metabolic homeostasis (215). Besides these specialized chemoreceptors, each tissue has its own oxygen sensor and threshold to low pO_2, depending on its normal pO_2 (94).

At a cellular level, the adaptive response to low oxygen availability is primarily regulated through HIF-1, as noted above, a master regulator which mediates the transcriptional activity of multiple genes (45, 442). It is known that HIF-1 binding is associated mainly at genes with increased expression; however, HIF-1 downregulates gene expression indirectly by regulating transcriptional repressors and microRNAs (345). As noted earlier, HIF-1 is a heterodimer of a constitutively expressed HIF-1β subunit and an oxygen-regulated HIF-1α subunit, which under normoxic (normal oxygen concentration) conditions is hydroxylated at specific proline residues by prolyl hydroxylase domain proteins (Figure 3), which use oxygen and α-ketoglutarate as substrates and contain Fe^{+2} in their catalytic center (346). Hydroxylated HIF-1α interacts with the von Hippel-Lindau (VHL) protein, the substrate-recognition subunit of the E3 ubiquitin-protein ligase that targets HIF-1α for proteasomal degradation (77). Under hypoxic conditions, hydroxylation is inhibited and HIF-1α remains stable (128). The HIF-1α subunit contains two transactivation domains: an amino-terminal transactivation domain that lies within the oxygen-dependent degradation domain, and a carboxy-terminal transactivation domain. The cAMP response element-binding (CREB)-binding protein (CBP) and p300, two transcriptional co-activators, interact with the carboxy-terminal transactivation domain of HIF-1α to induce transcription of HIF-1 target genes (128, 183).

HIF-1α transcriptional activity is negatively regulated in an oxygen-dependent manner by a factor inhibiting HIF-1 (FIH)-dependent asparaginyl hydroxylation, which blocks the interaction between HIF-1 and the coactivators CBP and p300 (183, 347). Thus, in hypoxic conditions this hydroxylation is also reduced, allowing the interaction of HIF-1 with coactivators, thereby leading to the

transactivation of target genes (128). The HIF-1α and HIF-1β proteins both contain basic helix-loop-helix motifs that bind DNA in the hypoxia-response elements (HREs: 5'–A/GCGTG–3') and, finally, cause subunit dimerization (45, 77). Additionally, HIF-1α activity may be classified by the type of interplay between itself and target genes or transcription factors (Table 1). Further complexity is generated by the existence of multiple HIF isoforms, with HIF-2 recently receiving more attention (70). The HIF-2α subunit has a similar amino acid sequence compared to the isoform 1, but acting in a different manner on phenotype upon inactivation (254). HIF-2 function is essential for modulating ventilatory sensitivity to hypoxia, erythropoiesis and vascularization (151, 345).

The main adaptive molecular response to hypoxia is mediated through HIF-1-transcriptional control. A considerable number of molecular functions are associated with HIF-1, which can be classified in Gene Ontology (GO) terms (Table 2). Inferred from direct assay (biochemical or cell biological assay), and beyond oxygen homeostasis (158) and the response to hypoxia (29, 102, 139, 211, 421, 430), HIF-1is associated with neuronal survival under conditions of oxidative stress (288), vascular endothelial growth factor production (102, 177) and transcriptional regulation (29, 193, 207, 210, 212, 252, 349, 351, 408, 421). However, other regulatory mechanisms appear to be involved in the hypoxic response, such as non-coding RNA and alternative splicing (314). When oxygen is available, most cells produce ATP via oxidative phosphorylation, but in hypoxic environments there is a shift to anaerobic metabolism for cellular energy production (12, 229, 442). Apart from increasing the expression of genes encoding glycolytic enzymes, HIF-1 leads to the reduction in mitochondrial oxygen consumption via pyruvate dehydrogenase kinase I (PDK-1), B-cell lymphoma 2 interacting protein 3 (BNIP3), and Beclin-1 and autophagy protein 5 (ATG5) among others (344). This results from the PDK-1-mediated inhibition of the Krebs cycle (286), BNIP3-regulated reduction of mitochondrial biogenesis as well as Beclin-1 and ATG5-induced autophagy (356). Regarding transcriptional activation, HIF-1 mediates cellular responses to hypoxia by regulating glucose uptake and anaerobic metabolism, mitochondrial function, angiogenesis and inflammation, as well as cell proliferation and survival (35, 241, 348, 442). To identify the functional annotations associated with the genes directly regulated by HIF-1α or interacting with it (listed in table 1), Web-based Gene Set Analysis Toolkit (WebGestalt) was used to cluster these genes according to GO term categories of biological process and molecular function (Figure 4).

WebGestalt runs an over-representation analysis using the GO functional database categories of biological process and molecular function for a certain organism — in this case humans. Each GO annotation describes the function of a particular gene and includes an evidence code to indicate how the annotation to a particular term is supported. As explained by the GoConsortium, evidence codes fall into six general categories: experimental, phylogenetic and computational evidence, author

statements, curatorial statements and automatically generated annotations. Then, WebGestalt enrichment analysis statistically evaluates the over- (or under-) representation of a known fraction of genes in a particular GO term, or pathway, found among the set of input genes (244). Hence, if a statistically significant number of genes from the known set are present in the gene list, it may indicate that the gene function (GO term) or pathway plays a role in the biological condition under study (184). Among the enriched categories identified by gene clustering (Benjamini-Hochberg FDR<0.05; top 10 categories), the biological processes with the highest gene content per GO branch include response to oxygen levels, ROS, metabolic process and regulation of vasculature development (Figure 4A), while the enriched categories for molecular function comprise the binding of protein tyrosine kinase, growth factor receptor and chaperone (Figure 4B) highlighting the role of HIF-1α in oxidative and metabolic pathways.

IV. Crosstalk between inflammation and hypoxia pathways

Even though most studies on HIF-1 activity have been conducted under hypoxic stress, this transcription factor has also been found to be up-regulated in inflammatory and oxidative conditions, such as arthritis, diabetes and obesity (92). In this context, the activation of HIF-1could be triggered by non-hypoxic stimuli such as LPS (31, 271), cytokines (6, 134, 204, 398, 413), growth factors and vascular hormones (143, 321, 389, 434). For example, ROS could lead to a stabilization of HIF-1α under normoxia (34, 41, 258, 309, 321). Furthermore, a ChIP assay revealed that the activation of HIF-1 led to a transcriptional regulation in adipocytes treated with a conditioned medium from LPS-activated macrophages (224). In this sense, the phosphatidylinositol 3-kinase/AKT (PI3K/AKT) pathway is activated via several stimuli such as growth factors, cytokines and stress conditions (168, 428), and is involved in numerous cellular functions including proliferation, adhesion, migration, invasion, metabolism, and survival (181).

A. Non-canonical activation of HIF-1

The mechanistic target of rapamycin kinase (mTOR, also known as mammalian target of rapamycin), a well-known phosphorylation target of the PI3K/AKT pathway (181, 443), is involved in the onset and progression of diabetes, cancer and ageing, for example (443). Furthermore, HIF-1α levels are also regulated by different signaling cascades — the phosphatidylinositol 3-kinase (PI3K) and the mitogen-activated protein kinase (MAPK) cascades (24). Hence, the PI3K target AKT has been suggested to either activate or inactivate HIF-1α stabilization, translation or coactivator recruitment by several downstream proteins such as glycogen synthase kinase-3 (GSK3) and mTOR, among others (186). For instance, the AKT target GSK3 is known to directly phosphorylate HIF-1α, thereby

contributing to destabilization in response to long-term hypoxia (186). mTOR is a central regulator of many core metabolic processes leading to anabolic mechanisms including through HIF-1 (74, 411, 425). Moreover, several studies have demonstrated a mTOR-dependent activation of HIF-1 in an oxygen-independent manner (143, 224, 271, 347, 389, 411). The molecular basis of this non-canonical activation of HIF-1 is related to the activation of the PI3K/AKT/mTOR pathway, known to increase HIF-1α protein levels by increased translation (201, 271, 389, 434) even in normoxic conditions (77, 120, 164, 181, 258) (Figure 3).

In an oxygen-independent mechanism, an enhancement of *HIF1A* gene transcription has been suggested through the activation of protein kinase C (PKC) (77, 284, 426), which might occur by stimulating specific transcriptional regulatory elements, such as Sp1, to bind the *HIF1A* promoter (31, 77, 284). PKC regulates a wide variety of cellular functions including cell proliferation, cell death, gene transcription and translation, altered cell shape and migration, regulation of ion channels and receptors, regulation of cell-cell contact and secretion, and is, in turn, activated by diacylglycerol and calcium ions through a variety of signals such as hormones (adrenalin and angiotensin), growth factors (epidermal growth factor and insulin), and neurotransmitters (dopamine and endorphin) (253).

In addition, mTOR is also involved in the modulation of oxidative stress in hypoxia through the thioredoxin interacting protein (TXNIP) (419). This protein is induced by, and promotes, cellular oxidative stress by inhibiting thioredoxin — an antioxidant enzyme — reducing capacity and is in turn inversely regulated by ROS levels (8, 368). In hypoxic conditions, *TXNIP* gene expression is regulated in a biphasic manner whereby *TXNIP* shows an initial rapid down-regulation that may serve as an adaptive mechanism to increase glucose uptake under conditions of compromised oxidative phosphorylation, followed by an increase under prolonged hypoxia (419). This initial decrease of TXNIP in response to hypoxia results in enhanced insulin-stimulated AKT and downstream signaling in human myotubes (124). Thus, TXNIP is involved in the regulation of metabolic homeostasis thereby further linking oxidative stress and hypoxia pathways.

Moreover, the existing evidence indicates that nitric oxide (NO) influences HIF-1 signaling. However, complex mechanisms involving both positive and negative regulation of HIF signaling by NO have been described (40). NO can affect HIF-1 activation at multiple levels via several mechanisms (28). Briefly, regulatory capacities depend on NO concentration: lower NO leads to HIF-1α degradation, but high NO levels stabilize HIF-1 in normoxia, mimicking the hypoxia response (28, 280).

B. HIF and NF-κB crosstalk

Many of the stimuli that induce HIF-1 in normoxia are known to activate other transcription factors such as nuclear factor κB (NF-κB) (398). The activation of the canonical NF-κB pathway triggers a

rapid and reversible inflammatory and immune response, while a slower and irreversible developmental response classically occurs through the non-canonical pathway (359). The NF-κB family of transcription factors exist either as homo- or heterodimers and consists of five members — p50, p52, p65 (RelA), c-Rel, and RelB — which share an N-terminal Rel homology domain responsible for DNA binding to κB sites within the promoters/enhancers of target genes, regulating transcriptional activity (142). Some dimers are more prevalent than others and are mainly sequestered in the inactive form in the cytoplasm, inhibited by members of the IκB family (inhibitor of NF-κB) (398). When the signaling cascade is activated, IκB proteins are phosphorylated, ubiquitinated and degraded through the proteasome, resulting in NF-κB release and translocation into the nucleus (74). The transactivation domain necessary for the positive regulation of gene expression is present only in p65, c-Rel, and RelB, while p50 and p52 may repress transcription unless associated with a transactivation domain-containing NF-κB family member or other proteins capable of coactivator recruitment (142). The NF-κB transcription factor is known for its central role in the immune response (canonical pathway), especially in inflammatory processes present in cancer, muscular dystrophy, obesity, insulin resistance, and atherosclerosis (20, 142). NF-κB is involved in immune cell differentiation and maturation processes by the activation of the non-canonical pathway (359), known for coordinating metabolic stress responses to overnutrition (165).

Moreover, several studies demonstrate crosstalk between the NF-κB and HIF-1 signaling pathways and direct targets of both transcription factors (74). Direct targets of the NF-κB and HIF-1 transcription factors obtained from the MatBase Matrix Family Library were used for query (Version 8.3, Genomatix Software GmbH, Munich). Then, we compared NF-κB direct targets (of the five family members) with those of HIF-1 (α and β), 292 and 233 genes respectively, and found 78 overlapping genes (Figure 5A). The list of shared genes directly regulated or interacting with NF-κB and HIF-1 were further analyzed in WebGestalt to evaluate the enriched functional annotations. Among the top enriched GO term categories of biological process were reactive nitrogen species (RNS) and ROS metabolic processes, response to oxygen levels and regulation of vasculature development (Figure 5B), suggesting a contribution of these genes regulated by both transcription factors in oxygen and oxidative stress-related pathways. Furthermore, NF-κB activation could lead to transactivation of HIF-1 target genes in normoxia (224, 398).

V. Cellular stress and antioxidant defense

The antioxidant system is responsible for managing the ROS naturally present in cells, which are involved in a number of physiological processes — through growth factor signaling, inflammation and/or hypoxia, or immune responses (125, 440) — that produce desired cellular responses (including

activation, cell survival, proliferation, stress adaptation, cell motility, vasodilation, and angiogenesis), lately named by Sies and Jones as oxidative eustress (154, 352, 362). However, large quantities of ROS can lead to cellular damage in lipids, membranes, proteins and DNA, contributing to the development of metabolic diseases or cell death — also defined as oxidative distress (313, 362). The major endogenous sources of ROS are the mitochondrial respiratory chain, NADPH oxidases, monoamine oxidase (MAO), nitric oxide synthases (NOS), the Fenton reaction, cytochrome P450 oxidases, xanthine oxidoreductase, peroxidases, and peroxisomal β-oxidation (56, 113, 179, 362).

Mitochondria are a major source of cellular ROS as superoxide and hydrogen peroxide are mainly produced by these organelles, due to either a reduced NADH pool, or a high protonmotive force and a reduced coenzyme Q pool (260). Mitochondrial sodium import and interaction with phospholipids have a role in attenuating potential ROS production and injury upon cardiac reperfusion while promoting an adaptive ROS production during acute hypoxia (146). Mitochondrial oxidative phosphorylation is uncoupled when protons — translocated to the intermembrane space by respiratory complexes of the electron transport chain — return to the mitochondrial matrix independently of ATP synthase (proton leak) thereby generating heat instead of ATP (192). The process of proton leak increases the respiration rate and is a mechanism for energy dissipation (36). Mitochondrial uncoupling proteins (UCPs) are involved in the proton leak and in the control of mitochondrial ROS production. The adaptive thermogenesis that occurs in brown adipose tissue (BAT) is unambiguously mediated by UCP1; however, the functional role of the other UCPs, UCP2 and UCP3, has not been clearly established (36, 192). UCP2 has been associated with the control of ROS production (13) and to the modulation of insulin sensitivity (322). In WAT of mice fed a high-fat diet (HFD) for 15 weeks, *Ucp2* expression was decreased and HIF-1α was induced in normoxia (202). Also, *Ucp2* decreased in WAT and BAT of mice expressing a stable HIF-1α (176). Recently, a ChIP assay performed on adipocytes in a pro-inflammatory medium revealed that HIF-1α suppressed the enrichment at *Ucp2*, which was actually down-regulated in pro-inflammatory conditions (224). In hyperglycemia, pancreatic β-cells increase both superoxide and UCP2 production, leading to a decrease in ATP production and, consequently, to reduced ATP-dependent insulin secretion (36, 192, 322). On the other hand, UCP3 appears to be involved in promoting fatty acid oxidation, preventing mitochondrial damage from lipotoxicity (36). Thus, UCP1 mediates adaptive thermogenesis in BAT, while a role for UCP2 and UCP3 in the control of cellular redox status has been shown (36, 192). In this sense, a cardioprotective role of UCP-mediated uncoupling by means of reducing ROS production has been demonstrated (4, 327). Thereby, mild mitochondrial uncoupling is a well-known cytoprotective strategy under conditions of oxidative stress, including obesity, diabetes and ischemia-reperfusion injury (4, 36, 37, 192, 327).

Recently, NAD/NADH and NADP/NADPH pools have been also identified as cellular redox homeostasis regulators (196, 427). Similarly, redoxins (including peroxiredoxins, thioredoxins and glutaredoxins) have been characterized as electron donors, acting as redox catalysts (43, 137, 153, 362). Moreover, the Nrf2-Keap1 pathway is considered very relevant as a transcriptional antioxidant response (93, 362, 440). The intracellular distribution of these molecules is highly compartmentalized, maintaining cellular redox status in a wide range of subcellular organelles (137, 427). Therefore, ROS levels must be perfectly regulated in the cell environment.

Some rapid modifications in metabolic flux can be determined by the redox state within cells; when this situation is maintained over time, the oxidative stress triggers mitochondrial dysfunction (293, 313). Thus, it is well established that the production of ROS is both necessary, and at the same time potentially hazardous, for normal cell function. Modulation of the different antioxidant systems could cause either beneficial or adverse effects, depending on the context (56, 110, 336). Several studies showed that a high antioxidant intake increased blood pressure, prevented statin-induced c-HDL elevation, enhanced the oxidative stress and blocked the enhanced insulin sensitivity produced by acute exercise, increased the risk of breast cancer and raised mortality (401). Other studies have confirmed that a high intake of antioxidants do not show any beneficial effects in cancer patients (333).

Moreover, many cell stressors (free fatty acids, hypoxia, high glucose) that lead to ROS generation and the subsequent oxidative stress (110, 313) could induce endoplasmic reticulum (ER) stress (129, 318), affecting protein synthesis, folding and transport, and several cellular signaling processes — mainly those related with calcium (126). Likewise, many characteristics of metabolic disease that induce inflammatory signaling could also induce ER stress (129). The ER stress and downstream activation of the molecular pathways managing the unfolded protein response (UPR) seem to be closely related to inflammation, and indicate a conserved mechanism whereby ER stress is intimately connected to host-cell defense (129, 318). ROS are upstream of the UPR, but not necessarily upstream of ER stress, suggesting that ROS may also be involved in solving an early stage of ER dysfunction by activating the UPR (278). Also, hypoxia-derived ROS is known to specifically activate UPR pathway-promoting energy and redox homeostasis, enhancing cellular survival (218).

Under normal conditions, the three canonical UPR sensors — PKR-like eukaryotic initiating factor alpha kinase (PERK), activating transcription factor-6 (ATF6) and inositol requiring enzyme 1 (IRE1) — are capped by BiP/GRP78 chaperone and remain inactive (126, 154, 278, 334). When proteins are misfolded or an overload of protein synthesis machinery prevails, proteins accumulate in the ER and BiP dissociates, allowing oligomerization of PERK and IRE1, translocation of ATF6 to Golgi and ER signaling (126, 278). Hence, a number of pathways are involved in the UPR initiation, reflecting the extremely important role of ER stress and the complexity of the cellular response. Specifically,

hypoxia induces protein misfolding as a consequence of the need for oxygen to form disulphide linkages, which leads to ER stress and the activation of UPR (80). Moreover, UPR downstream pathways are linked to a wide range of processes. One such pathway involves IRE1 as an ER stress sensor, which increases the production and secretion of inflammatory cytokines as a downstream consequence (156). However, all three branches of UPR are at some level involved in inflammatory signaling (278).

If proper ER function is not achieved, or if the stress continues, the UPR may also initiate apoptotic pathways through the activation of C/EBP homologous protein (CHOP) downstream of IRE1 and PERK pathways (80, 334). ER stress can trigger autophagy, an essential homeostatic process whereby the cell breaks down its own components to help maintain a balance between the synthesis, degradation and subsequent recycling of cellular products, most likely via PERK and IRE1α pathways (80, 334). Recent reports have shown that the failure of autophagy-dependent control of immune-cell homeostasis can contribute to inflammation and insulin resistance (281).

Finally, the aging process has also been associated with the accumulation of cell damage over a lifetime, promoted mostly by increased mitochondrial dysfunction-derived ROS (36, 56). The free radical theory of aging proposes that free radicals produce oxidative damage to cellular components, with the accumulation over time contributing to aging (36, 336, 409). Despite an increase in overall lifespan, age-related diseases such as neurodegenerative disorders, diabetes and CVD are major causes of mortality and morbidity worldwide (336). However, studies in both *C. elegans* and *Drosophila* refute the free radical theory of aging due to a lack of association between increased ROS and lifespan, and indeed free radicals could even lengthen it (50, 336, 409). Antioxidants play a critical role neutralizing ROS, but the association with lifespan is complex.

VI. Metabolic dysfunction

A close interaction between AT, liver, pancreatic islets, muscle and immune cells generates an environment for continuous and dynamic interactions between immune and metabolic responses (155). This coordinated regulation of metabolic and immune responses is beneficial in certain conditions, since the organism needs to organize and redistribute its energy resources to block anabolic signaling pathways (38). However, this network becomes detrimental in the presence of continuous nutrient overload (281). Currently, the nutritional and lifestyle habits in Western countries (also known as the Occident world) strongly promote metabolic excess and have established a worldwide problem with chronic metabolic diseases.

The metabolic dysfunction is characterized by a wide variety of disorders commonly found in metabolically active tissues including the pancreas, arteries, liver, fat and skeletal muscle, to name key

examples (Figure 6). Although explained in more detail below, hyperlipemia is associated with hypercholesterolemia and lipotoxicity in several tissues and leads to the excessive accumulation of lipids in the abdominal cavity (visceral adiposity). Atherosclerosis is also related to hyperlipemia and involves immune cell infiltration in the atherosclerotic plaque, strongly linked to hypertension. Likewise, the metabolically altered WAT is infiltrated with macrophages. Thus, metabolically impaired tissues produce pro-inflammatory molecules able to promote insulin resistance. At the same time, hyperglycemia, as a result of metabolic dysfunction in pancreatic β-cells, leads to impaired insulin secretion and diabetes. Although not included in current diagnostic criteria of the metabolic syndrome, non-alcoholic fatty liver disease (liver steatosis) is a trait of this syndrome from a pathophysiological point of view, and a determinant of the development and progression of metabolic disorders.

The immune response triggered in metabolic disorders involves the integration of many complex signals in different cells and organs, and is characterized by a low-grade chronic inflammation, usually called metaflammation (157), and which refers to metabolically triggered inflammation. This condition is mainly initiated by an excess of nutrients that favors the storage of energy, disrupting metabolic homeostasis (318). Individual tissues and cells are effectors of the immune response when an inflammatory state is chronically established. In turn, responsiveness to certain inflammatory mediators (vasoactive amines, vasoactive peptides, complement components, lipid mediators, cytokines, chemokines and proteolytic enzymes) is almost ubiquitous, but these have distinct effects in different cell types (243).

Immune responses to cellular stress through NF-κB signaling such as the production of antimicrobial factors, phagocytosis, leukocyte recruitment, and adaptive immunity could also involve HIF-1 activity (74, 92, 241). For instance, phagocytes lacking HIF-1α are unable to eliminate bacterial loads (92). In hypoxic and inflamed tissues, HIF-1α also stimulates ATP generation in myeloid cells to increase antibacterial activity, and to prevent neutrophil apoptosis (90, 92, 241). Moreover, after pathogen phagocytosis, oxygen is needed in the production of ROS — a process called respiratory burst — to lyse and kill microbes (90). The resident macrophages constitute 10–15% of the cellular content of most tissues and are responsible for monitoring the tissues, primarily in relation to host defense and the removal of apoptotic bodies. When tissues are under stress or dysfunction, these resident macrophages are activated and a pro-inflammatory response is initiated (166). Therefore, the immune response has a physiological role in restoring tissue homeostasis, and an inability to resolve the dysfunctional condition establishes a mild chronic inflammatory disease state (341). Metabolic organs under stress conditions dysregulate the production of classical cytokines — particularly tumor necrosis factor alpha (TNF-α), interleukin 1β (IL-1β) and interleukin 6 (IL-6) — and of proteins involved in

vascular hemostasis and angiogenesis such as plasminogen activator inhibitor 1 (PAI-1). The production of factors involved in glucose homeostasis, an example being adiponectin (ADIPOQ), and in immune cell recruitment, such as monocyte chemotactic protein 1 (CCL-2, also known as MCP-1) (377) — which are closely related to MetS, CVD and type 2 diabetes (T2D) (255) — is also dysregulated. Despite the fact that IL-6 is generally considered to participate in pro-inflammatory signaling, genetic inhibition of *Il6* expression has been reported to lead to insulin resistance and liver inflammation in mice, and IL-6R blocking drug-therapy increased body weight and resulted in dyslipidemia in humans, suggesting a dual role of this molecule in metabolism (340). In this sense, the IL-6 produced by skeletal muscle seems to have beneficial effects on metabolism energy sensing during exercise as (311); nevertheless, the chronic elevation of IL-6 could contribute to the development of insulin resistance (91).

A. Metabolic disorders and methylation

In recent years, epigenetic regulation of gene expression has become evident, leading to increasing scientific interest in metabolic disease (48, 332). In this regard, DNA methylation is one of the most widely studied epigenetic modifications, and has been used as a biomarker in metabolic diseases such as obesity, CVD and insulin resistance, since it controls the cell phenotype by altering the production of regulatory proteins (48, 235, 246, 332). The methylation of CpG islands in the promoter region of the long interspersed nucleotide element 1 (*LINE1*) retrotransposon, the only active, autonomous transposable element in humans, has been widely used as a marker of global DNA methylation (404). As a general rule, higher levels of DNA methylation used to be related to lower gene expression (320). However, in some metabolic disorders, both DNA hypo- and hypermethylation are present. For instance, previous studies found that *LINE1* promoter methylation (measured in blood cells unless stated otherwise) was inversely associated with BMI and HOMA-IR in healthy women (300), with body fat mass in healthy young adults (234), with CVD in overweight and diabetic subjects (54), with myocardial infarction risk in a case-control cohort (132) and with metabolic risk markers in the WAT of MetS subjects (392). In contrast, it was positively associated with weight loss (107) and insulin sensitivity (232) in blood cells from obese subjects. However, other authors suggested that there is no association (88, 436), or even a positive connection (52, 269, 319, 393), between *LINE1* methylation and metabolic markers. The disparities in the direction of the outcomes might be related to differences in gender and race/ethnicity, as previously evidenced (436).

The gene methylation pattern of pro-inflammatory molecules was previously linked with metabolism-related pathological conditions. For example, *IL6* methylation was increased 6 months after weight loss (269) and 12 months after bariatric surgery (190). However, it was also positively correlated with

obesity features in MetS subjects (52), and with women's obesity (261), but not significantly when associated with lifestyle (437). Moreover, *TNF* methylation increased a year after bariatric surgery (190). Despite that, other studies on *TNF* methylation have shown a decrease after a weight loss intervention (47, 71), a negative correlation with waist circumference in healthy young individuals (234), a positive association with adiposity (145), and no association with BMI (261).

Regarding *SERPINE1* methylation, a positive association with weight loss (269) and waist circumference (52) was found, as well as a negative association with obesity and CVD markers in MetS subjects (220). Besides, lower *TNF* promoter methylation was previously correlated with higher circulating TNF-α levels (145). In addition, aging is commonly associated with higher systemic levels of pro-inflammatory factors, such as CRP, IL-6 and TNF-α (294). Previous studies have described a link between DNA methylation and the aging process (65, 127, 223, 231, 329, 370). Nevertheless, aging-induced differential methylation occurs mostly without changes in gene expression (370).

Furthermore, resting oxygen consumption was associated with a hypomethylation in a CpG island in the promoter region of *IL6* as well as an increase in circulating IL-6 in obese subjects with SAHS (223). Concerning SAHS, DNA methylation patterns were associated with sleep severity in adults (64). In children with SAHS, an epigenetic dysregulation of vascular function is suggested through endothelial NOS hypermethylation (185), and of inflammation through the hypermethylation of the forkhead box P3 (FOXP3) gene in those individuals with higher systemic inflammation (188). However, a prospective study designed to evaluate the epigenetic mechanism involved in SAHS (233) showed that FOXP3 methylation, or its expression, is not altered in adults with OSA, whatever their inflammatory status (337). In this sense, it could be hypothesized that in the context of metabolic disorders, beyond transcriptional regulation, low oxygen consumption and hypoxia might be associated with levels of pro-inflammatory cytokines via epigenetic marks (Figure 7).

At a molecular level, DNA methylation marks can be removed by an active demethylation mechanism involving a family of DNA hydroxylases (TET proteins), or a passive demethylation process (inhibition of the maintenance methyltransferase, DNMT1), during cell division (208). In the active mechanism, the enzymes removing methyl groups from DNA and histones are included in the superfamily of 2-oxoglutarate-dependent dioxygenases, enzymes that are dependent on molecular oxygen as a co-substrate and ferrous (Fe^{+2}) iron as a catalyzing cofactor (330). Therefore, by this mechanism hypoxia could induce hypermethylation. Moreover, methylation is modified by ROS with important implications in the pathogenesis of metabolic disorders such as CVD, obesity and diabetes (83, 186). For example, ROS directly affect methylation by oxidation or hydroxylation of nucleotides, as well as indirectly, by means of modifying the activity and recruitment of methyltransferases and TET proteins (186). On the other hand, the hypoxic phenotype is at least partially mediated by DNA

methylation alterations, depending on both the modulation of the universal methyl donor S-adenosylmethionine availability and the regulation of enzymes involved in DNA methylation and demethylation by HIF-1 and 2 (49). Thus, many epigenetic regulators are affected by hypoxia and ROS, but their effects might be tissue-specific and could involve many other physiopathological aspects.

B. Metabolic syndrome

The metabolic syndrome (MetS) is a cluster of interconnected factors with a common low-grade, chronic pro-inflammatory state and is highly prevalent in Western countries, where more than 25% of adults are said to suffer from the condition (277). These factors are hyperglycemia, elevated blood pressure, hypertriglyceridemia, low levels of high-density lipoprotein cholesterol levels (c-HDL), and central adiposity; the latter can be measured by waist circumference and body mass index (BMI) (131). Many international organizations and expert groups have defined the MetS (Table 3). Nevertheless, a major problem with some definitions is apparent in terms of the applicability to different ethnic groups, especially when trying to define central adiposity according to waist circumference cut-offs (5). As a result, the prevalence of MetS varies and depends on the criteria used in different definitions, as well as the composition (sex, age, race and ethnicity) of the population studied. Despite this variance, there is enough evidence indicating that MetS is a risk factor for CVD and T2D (257, 417).

Recently, other disturbances have been related to the MetS, such as respiratory disorders, liver disease, arthritis, fertility disorders, psychological disturbances and cancer (57, 318). A link between MetS and respiratory disorders has been observed in several studies, not only sleep apnea but also lung function impairment, pulmonary hypertension and asthma (15). Sleep apnea, or SAHS, is characterized by recurrent episodes of apnea-hypopnea due to the occlusion of the upper airways with pharyngeal soft tissue, resulting in intermittent hypoxemia (79, 84). The carotid body senses the lack of oxygen and activates the sympathetic nervous system, clearing the airways and producing reoxygenation (346). This sequence is repeated with each apnea event, leading to oxidative stress and inflammation (79, 250). In this sense, hypoxia-reoxygenation in SAHS contributes to ischemia-reperfusion injury (79). SAHS is associated with hypertension (89), insulin resistance (381) and obesity (331). In the general population, SAHS is evident in approximately 6-13% of adults; however, this proportion is dramatically enhanced in those with MetS, around 60% MetS patients exhibiting the condition (86).

Another metabolic disorder closely related to MetS and SAHS is non-alcoholic fatty liver disease (NAFLD), which is characterized by an accumulation of triglycerides in hepatocytes (250, 390). The severe progression of NALFD is steatohepatitis, which presents ballooning degeneration and inflammation, and is a major cause of cirrhosis and hepatocellular carcinoma (250). The high

prevalence of NAFLD — up to 30% — is associated with the MetS (78, 109, 371). Finally, other disorders related to the MetS should be mentioned. About 10% of all cancer deaths among non-smokers are related to obesity; specifically breast, colon, endometrium, kidney and esophagus cancer are each associated with obesity and a sedentary lifestyle (57, 140). On the other hand, metabolic disorders have been associated with psychological features such as depression and neurodegenerative pathologies (Alzheimer's and Parkinson's diseases), since both pathologies share a dysregulation of inflammation and oxidative stress (156).

It is emphasized that recent studies indicate that increased adiposity does not always translate into metabolic dysfunction (121), and between 10 and 34% of obese individuals are considered metabolically healthy obese (MHO) since the excess in body weight is not accompanied by other metabolic disturbances (32). Despite the lack of relevant comorbidities and MetS clustering in MHO subjects, a similar risk of CVD and all-cause mortality has been observed (264), along with the risk of T2D (32), to that for unhealthy obese. However, there is a lack of information on the underlying mechanisms that make a difference between the healthy and unhealthy obese (259).

1. Obesity

The worldwide epidemic of obesity represents one of the greatest threats to global human health: world data from 2016 indicate that over 650 million adults are obese (13% world population), and over 1,300 million are overweight (39%). Moreover, the obesity epidemic is also affecting children and adolescents as the prevalence of childhood (5-19 years old) overweight/obesity is now over 18% (378). The complex physiopathological processes leading to obesity reflect environmental and genetic interactions, since not all individuals exposed to an environmental factor develop the same grade of obesity (140). The mutual gene-environmental interactions result in multi-factorial obese phenotypes (147).

The main feature of obesity is the excessive accumulation of lipid in white adipocytes as a result of a positive energy balance associated with overnutrition and sedentarism. WAT, as an endocrine organ, is responsible for the production of many secreted factors, both lipid and protein (adipokines),with systemic effects encompassing many different physiological and pathological functions (3, 16, 197, 209, 255). At the tissue level, obesity is characterized by a mild, chronic inflammatory state in metabolically active tissues including adipose, heart, liver and muscle, which induces a stress response characterized by increased levels of pro-inflammatory molecules (33). The immune cells present in WAT receive stress signals from the adipocytes, which are unable to store all the nutrients provided following overnutrition (9). Some chemotactic molecules are involved in the recruitment of immune

cells (MCP-1), while others are able to differentiate macrophages to the M1 phenotype (IL-6, TNF-α) which is generally considered pro-inflammatory (20, 157, 281, 318).

Several mechanisms have been proposed to explain the initial cause of the inflammatory processes during obesity, including oxidative and ER stress, and WAT hypoxia (117, 281, 318). The oxidative stress is associated with impaired mitochondrial capacity which could lead to a defect in oxygen consumption (60% lower in the WAT of obese individuals), regulated by WAT blood flow which is also lower in obese subjects (40% reduction) (122, 205). In this sense, an inability to preserve tissue perfusion might be related to the hyperplasia and hypertrophy of WAT. Notwithstanding the lower perfusion, some authors have reported a higher abdominal WAT pO_2 (4-34% increase) in obese subjects, while others showed lower abdominal WAT pO_2 (15-25% reduction) in obese/overweight subjects (68, 205). Moreover, a recent study reported a 25% lower pO_2 in subcutaneous abdominal WAT in unhealthy obese subjects — with prediabetes and high intrahepatic triglyceride levels — compared to MHO (12% reduction vs lean) and healthy lean subjects (68). On the other hand, the pO_2 of WAT was found to be lower in obese rodents (6% in lean *vs.* 2% in obese mice) (72, 121, 388). Importantly, obese WAT immunostaining with pimonidazole (a chemical marker of hypoxia) revealed the co-localization of hypoxic regions and infiltrated macrophages (318). The lowered oxygen consumption in (mouse) obese WAT leads to a metabolic switch from aerobic to anaerobic metabolism (203, 387). But the range of metabolic changes resulting from low oxygen availability in WAT extends well beyond the augmentation of glycolysis. Data from microarray studies have shown that over 1,000 genes are hypoxia-sensitive in human adipocytes (384, 385).

As noted above, WAT exhibits high levels of hypoxia in obese rodents (106, 155, 315). Mechanistic studies have shown that the activation of hypoxia signaling stimulates glucose transport in adipocytes through the GLUT1 facilitative glucose transporter (372, 387). However, hypoxia induces an impairment in insulin sensitivity in adipocytes, so that insulin-stimulated glucose uptake is compromised (317, 399, 432). Moreover, it is also known that the inhibition of hypoxia signaling in HFD mice improves WAT dysfunction and insulin resistance by enhancing insulin secretion, and reducing macrophage infiltration and inflammation (174, 176, 187, 287, 360, 373). Furthermore, some authors have suggested that HIF-1 overexpression is involved in the inhibition of cellular respiration in BAT, since it is accompanied by a decrease in thermogenesis (176). However, to date there is no conclusive data of whether the inflammatory, or the hypoxic, cascade occurs first in the molecular events in obesity and its related comorbidities (317). On the other hand, the genetic inhibition of oxygen sensing proteins that leads to a pseudohypoxia state, showed a preventive effect on WAT inflammation, insulin resistance and weight gain in HFD mice (312), as well as improved glucose

tolerance and less WAT macrophage infiltration (239); it also suppressed lipolysis and promoted benign WAT expansion (245).

2. Cardiovascular disease

The Global Burden of Disease study of 2015 estimated that there were 422.7 million cases of CVD, this being the most common cause of death worldwide with nearly 18 million people dying from the disorder (31% global deaths) (326). Coronary artery disease and cerebrovascular disease are the most common forms of CVD. The underlying pathological process of CVD is atherosclerosis which is a chronic inflammatory disorder in which cholesterol progressively accumulates in the large and medium-sized arteries, causing stenosis (138). This progression begins with endothelial activation and inflammation, that presumably originates from the high circulating levels of low-density lipoprotein cholesterol (c-LDL) which accumulates in the intima (the inner layer of the artery) (138). These c-LDL particles are prone to oxidation by enzymatic attack by myeloperoxidases, lipoxygenases and ROS, and this is a key factor in early atherogenesis. The accumulation of oxidized c-LDL also drives the recruitment of immune cells, which increases the pro-inflammatory response and provokes the formation of a fibrous cap. Over time this atherosclerotic plaque becomes a more complex lesion with a necrotic core, covered by a fibrotic layer (138, 154, 213). The plaque growth contributes to lumen stenosis and ischemia, which activates hypoxia signaling (200, 348). Moreover, thrombosis can be produced by a plaque rupture, and this material may block the lumen or become an embolus which clots in a distal point (138).

Atherosclerosis can be explained in similar molecular terms as obesity, as both involve a pro-inflammatory process, oxidative stress and hypoxia pathways. In endothelial cells, the activation of NF-κB induces the transcription of cell adhesion proteins such as intracellular cell adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1), as well as chemokines and cytokines — including IL-1β, IL-6, and TNF-α — involved in the atherogenic process (20, 114). Prolonged pro-inflammatory cytokine production leads to the generation of ROS and other oxidative stress molecules, which are major contributors in the development of cardiovascular-related traits such as ischemia/reperfusion injury, hypercholesterolemia and endothelial dysfunction (376). Moreover, the ER stress, also present in CVD, occurs in endothelial cells as a result of an excessive demand for protein synthesis and is aggravated by increased ROS and hyperlipidemia. (154). During the recruitment of macrophages to the atherosclerotic plaque, lipid transporters contribute to the removal of the excessive cholesterol accumulated in the vessel walls. This process triggers the UPR-dependent activation of inflammatory pathway (80, 278).

3. Diabetes

The number of people worldwide with T2D was more than 350 million in 2015 and is projected to rise to 439 million by 2030 (approximately 8% of the world's total adult population). Diabetes is a chronic degenerative disease characterized by insulin resistance, a pathological condition in which insulin becomes less effective at lowering blood glucose levels and which results in long-term complications affecting the eyes, kidneys and nervous system; it is also known to increase the risk of CVD and some types of cancer (63, 178, 262). The pro-inflammatory cytokines produced by metabolically impaired tissues, such as liver and AT, may promote insulin resistance in the tissues where they are produced or in distant tissues and organs, e.g. vessel walls, skeletal and cardiac muscle (85). Over-supply of nutrients — including glucose and fatty acids — as well as hypoxia are associated with β-cell damage, leading to impaired β-cell insulin secretion in T2D (110). Accumulating evidence points to a role for oxidative stress in both processes. Skeletal muscle is the largest insulin-sensitive organ in humans; consequently, insulin resistance in this tissue has a major impact on glucose homeostasis in the body as a whole. Impaired mitochondrial function might contribute to insulin resistance via altered metabolism of fatty acids, which, in turn, may lead to ROS generation (293).

It is known that β-cells have a very low level of antioxidant systems, and are therefore particularly vulnerable to oxidative stress, which is central in the development of insulin resistance (85, 110). In response, β-cells increase insulin production dramatically, which may generate ER stress (85, 283). Concretely, the IRE1 and PERK arms of UPR are involved in the ER stress associated with obesity and glucose intolerance (278, 283). Moreover, WAT hypoxia in obesity may lead to marked insulin resistance through multiple routes. For example, alterations in the production of adipokines linked to insulin sensitivity such as adiponectin (markedly reduced under hypoxia) could drive insulin resistance in WAT (383). Similarly, lactate release is increased under hypoxic conditions, and could lead to the induction of insulin resistance in skeletal muscle (384).

VII. Oxygen measurements

Given the critical importance of oxygen in aerobic organisms, it is essential to be able to monitor and measure its levels. In fact, some of the controversy and inconsistencies found in studies analyzing the role of oxygen in metabolism, seem to be related to the different measurement methods employed. In this context, most available techniques to directly measure oxygen levels are limited to the surface areas of a tissue. Thus, selecting the appropriate technique is based on applicability to the experimental model and the nature of the information pursued.

A. Electrochemical sensors

Oxygen polarographic (Clark) electrodes are considered as the reference method for measuring oxygen tension in permeabilized cells and isolated mitochondria (53, 99, 363), due to the following advantages: good reproducibility and accuracy, low deviation between each sensor, and the high detection resolution (99). These electrodes contain a noble metal (e.g. silver, gold, platinum) which reduces oxygen due to a negative polarizing voltage (53). While oxygen is reduced at the cathode surface, the amount of oxygen diffusing through the permeable membrane increases, closing the circuit and emitting a current proportional to the amount of oxygen at the measurement point (99, 335, 363). However, these electrochemical sensors have major drawbacks such as reliability, invasiveness and heterogeneity (53, 99). The reliability is challenged by the oxygen consumed by the electrode itself, although this mainly affects those situations in which there are either small samples or oxygen-deficient tissues (418). Hence, the measurement over time in a specific region is not possible and the tissue oxygen tension should be rather greater than the electrode oxygen consumption. Regarding the invasiveness, the use of a needle potentially damages a tissue. Finally, the sensor measures only one single point and does not reflect the heterogeneity of oxygen distribution in a tissue as a whole, thereby lowering the reproducibility between investigators and laboratories.

B. Optic fibers

Systems based on optic fibers are another method to measure tissue, and isolated mitochondria, oxygen levels using a fluorescent dye, usually ruthenium chloride or porphyrin platinum, in a sol-gel coating on the sensor tip (53, 99, 418). The oxygen quenches the light emitted by the fluorescent dye in tissues in proportion to the oxygen tension (53, 418). The advantages of this technique are: (i) the possibility of continuous measurement since the oxygen is not consumed during the process, (ii) the option to measure in multiple sites at the same time, (iii) the ability to work under highly different levels of oxygen and areas of application, as well as in hostile environmental conditions (418). However, this approach can be invasive and the fiber tip is fragile (99).

Nowadays, the Seahorse X and the OROBOROS oxygraphy-2K analyzers, which are optic fiber-based techniques, are among the preferred methods to measure oxygen consumption and for the study of mitochondria in cell cultures (87, 230). However, these techniques can only be applied in cell culture and isolated mitochondria assays, and still do not provide an *in vivo* analysis. In order to overcome the problems of the fiber tip and to measure in larger areas, a combination of the optical sensor with a dialysis technique has been developed to monitor tissue oxygen levels *in vivo*. The microdialysis catheter is inserted 6-8 cm lateral from the umbilicus to extract interstitial fluid as an artificial blood

vessel (122). The exchange of gases is measured by an optoelectronic unit in the catheter made of an oxygen-sensitive membrane connected to a computer for data collection (402).

C. Pulse oximetry

A frequently used method for hemoglobin oxygen saturation (sO_2) is near-infrared spectroscopy (pulse oximetry). This optical technique applies light transmission and absorption principles to dynamic changes in biological molecules bound to oxygen (99). Based on the Beer-Lambert law, the visible light in the near infrared region (700–3,000 nm) easily passes through biological tissues. This technique is unable to measure tissue pO_2, but provides peripheral sO_2 instead, which results from the balance between oxygen delivery and consumption (53).

D. Hypoxia imaging

Other methods may be used to measure relative oxygenation by the imaging of hypoxic areas, most often through labelling the oxygen content in a tissue. Electron paramagnetic resonance spectroscopy (EPR) offers the unique capability to detect unpaired electrons (53), which can be used to measure tissue oxygenation. ERP involves the use of an external probe (most commonly lithium phthalocyanine) consisting of either implantable paramagnetic particles, or soluble probe molecules (nitroxides) that physically interact with, but do not consume, oxygen (374). The interaction of two paramagnetic species, molecular oxygen and the probe, gives the signal that allows pO_2 to be determined (53, 374). The advantages of EPR comprise continuous readouts of the same sample, site specificity and accuracy (53). However, the limited range of detection (10–100mm Hg pO_2), high cost, lack of evidence against toxicity (implantation or injection of paramagnetic material) and the low sensitivity-safety ratio (10 mm from the surface) result in it not being commonly employed in clinical practice (53, 99). Moreover, this method requires imaging by positron emission tomography (PET) scanning, a technique based on the detection of anti-parallel 511 keV photons emitted during the annihilation of positrons with electrons.

PET radiotracers (compounds labelled with short-lived positron-emitting radionuclides) are injected or inhaled to reach the target to be detected with a ring-shaped array of photoelectric crystals by the PET scanner (53). Despite newer radiotracers having been developed, the limited sensitivity (anatomical resolution of 4-8 mm^3) and the cost make it difficult to apply this approach for indirect oxygen measurements (53, 99). In essence, magnetic resonance imaging (MRI) techniques using perfluorinated contrast agents (fluorine) could be used for quantifying tissue oxygen levels (335) with better resolution than PET imaging (53). MRI is based on the paramagnetic properties of oxygen dissolved molecules affecting the relaxation rate of the contrast agent, which is used to either monitor

the pO_2 levels or create an organ oxygen distribution map (99). However, this technique is high-priced and requires patient cooperation (53).

E. Indirect methods

Lactic acid (2-Hydroxypropanoic acid) is generated in tissues from pyruvic acid under anaerobic conditions. Lactate determination is an indirect measurement of oxygen availability that can be made by basic analytic methods (colorimetry, spectrophotometry, fluorometry, voltammetry, MRI and chromatography) or by biosensing techniques (electrochemical, electrochemiluminescence, fluorescence and microband lactate biosensors) (305). The authors of the aforementioned review (305) support the use of biosensing methods pointing out that they are better than traditional analytic methods, as they are very simple, sensitive, selective, disposable, automated and give rapid results. However, more simple, accurate, reliable and cheap lactate biosensors are needed. Thus, lactate biosensors are promising devices for clinical analysis of several metabolic disorders related to hypoxia. Lastly, indirect calorimetry is regarded as the gold standard to estimate resting energy expenditure, by measuring oxygen consumption and carbon dioxide production. Heat production from substrate oxidation is estimated from measures of oxygen consumption and carbon dioxide production using Weir's equation: 3 heat output (Kcal) = 3.9 × oxygen consumed (l)+1.11 x carbon dioxide produced (l; 1 Kcal =4.186 kJ), where in a balanced diet it is considered that 1 l of O_2 consumed equals to 4.825 Kcal (195, 325). The most common indirect calorimetry device is the metabolic cart, which captures exhaled gas using a canopy, facemask or mouthpiece with a nose clip connected to oxygen and carbon dioxide analyzers (195). These systems are relatively inexpensive and easy to use, but measurements can be influenced by subject anxiety and hyperventilation (325). Indications for indirect calorimetry can be clinical conditions altering resting energy expenditure, and patients with a lack of predictive equations for their particular situation.

VIII. *In vitro* experimental models of metabolic disease

The interplay between the different pathways together with gene-environment interactions make the study and treatment of metabolic diseases particularly difficult. Common metabolic disorders are polygenic, involving complex gene-gene and gene-environment interactions, producing multi-factorial phenotypes. For the correct study of every single disease, the cell biology and physiology, the interaction with other cell types and the specific milieu should each be well-understood. Thus, there is a profound need for adequate experimental models of each metabolic disease, including the pathways affected, in order to study the possible causes and potential therapeutic targets (7, 279, 295, 297, 403). Animal models for the study of the physiopathology of metabolic disorders represent an important tool

in research, as they provide information under relatively controlled conditions. However, such studies have some ethical and economic limitations (279, 295, 297). For this reason, *in vitro* models have emerged as the favored choice for many mechanistic investigations (279, 297).

A. 2D-Cell culture models

One example is adherent 2D cultures and these are defined by a cell monolayer attached to a plastic surface and have the main advantage of simple handling and low-cost maintenance. However, the major drawback is the lack of reproducibility of the *in vivo* tissue conditions (180). The absence of different cell-type interactions makes it necessary to develop new culturing techniques. In co-cultures, different cell types are grown together in the same environment allowing the study of cell-cell communication (290). Co-cultures can be divided into direct and indirect types, depending on the contact between the cultures (mixed or with a physical barrier) (180). The main short-coming of co-cultures is the inevitable inclusion of variables that require a higher experimental design than unique cell-type 2D cultures.

B. 3D-in vitro approaches

Novel methods of tissue engineering are critical for relating the results of *in vitro* studies to *in vivo* conditions. Spheroids and organoids have recently been developed as a 3D approach relying on spontaneous morphogenesis in cell aggregates (289). These three-dimensional tissue cultures are a fast-growing method for investigating cell biology that could be helpful in increasing the range of *in vitro* models in human medicine (99, 226). Spheroids are multicellular aggregates of microscopic tissues with a modifiable composition and biological properties which may be of considerable interest in diverse applications due to the micro-environment generated being similar to that of tissues *in vivo* (189, 226). Thus, this approach could be useful for mimicking the physiopathological conditions on the way to complex organ-like structures (99, 189). The study of spherical cellular aggregates provides insight into variations between the surface of the spheroid and its core (189). However, this also implies a serious limitation due to insufficient oxygen diffusion towards their cores (99).

C. Novel experimental models

Further along the direction of mimicking real tissues, organoids have been presented as an approach for organ-like culturing of *in vitro* studies and *in vivo* transplantation (226). Organoids are characterized by a group of organ-specific cells that are developed from stem cells or organ progenitors (99, 226). Hence, organoids are promising new tools in the arsenal for developing strategies for organ replacement, the modeling of many diseases, drug discovery, and safety screening studies (226, 289). However, the organoid approach faces important challenges, such as controlling the organoid micro-

environment including extracellular matrix composition, stiffness, and architecture and managing the cellular heterogeneity in each specific organoid system, as well as providing the appropriate oxygen and nutrient supply for each particular cell type within the organoid (99, 189, 226). Nevertheless, reliable and accurate animal models are still the best choice for bridging *in vitro* mechanistic investigations with clinical application, despite ethical and economical barriers.

IX. Therapeutic approaches

With regard to the clinical management of the MetS, the primary goal is to reduce the risk factors comprising the syndrome. The first emphasis is on the mitigation of the underlying modifiable factors (diet, habits, physical activity) through lifestyle changes. These low-risk treatments are based on lifestyle alterations such as dietary modification and exercise to produce an energy deficit as the first-line choice. When this approach is not enough, drug therapy is considered in order to maintain the cardiovascular and diabetes risk as low as possible. However, due to the side effects of current drug therapies, and the risks derived from surgical treatments, there has been a growing interest in developing novel therapeutic approaches for metabolic diseases. In this sense, new strategies applying genetic data (specific targets coming from pharmacogenetic and nutrigenetic screening), antibodies or vaccines against mediators (ghrelin, IL-6, IL-1, IL-1β), bioactive compounds, gene therapy, RNA interference technologies and oxygen therapy have all been considered (73, 85, 117, 119, 236). Oxygen-related therapies include a wide-variety of approaches, such as intermittent hypoxia (IH) and chronic hypobaric hypoxia (altitude), hyperoxia exposure and oxygen delivery strategies; these will be discussed next.

A. Intermittent hypoxia

Although the detrimental effects of severe hypoxia on a variety of physiological outcomes have been established, there are a number of intervention studies that employ IH due to some health-promoting effects as a result of adaptation. The protocols for IH commonly used in experimental research normally refer to normobaric hypoxia in cycles along time. However, the protocols vary in terms of the severity of hypoxia, duration of episodes, the number of hypoxia/reoxygenation cycles, and the pattern in both time and cumulative duration of exposure. Generally speaking, IH protocols considered to provide beneficial outcomes are defined by episodes of mild hypoxia (10–14% oxygen) that are short in duration (15 seconds to 4 minutes), small in number (around 10 episodes), and have a short length of exposure (up to 1 hour) — although these milder protocols may also have some inherent risks (236).

1. Functional principle

IH therapy, besides having been found in obese WAT to be probably linked to lower oxygen perfusion (121), has been suggested as a putative treatment for obesity and related risk factors (182, 249, 263, 308). Several beneficial metabolic responses to IH have been reported including reductions in body weight, cholesterol, glycemia and insulin resistance. IH protocols have been used as a treatment for different pathologies such as respiratory disorders, CVD, inflammation and neurodegeneration. The underlying mechanisms of the putative benefits of IH may involve the enhancement of angiogenesis, increased satiety through sympathetic activation, enhanced mitochondrial enzymatic activity and enhanced glucose uptake due to the translocation of glucose transporters (263, 394). Moreover, IH could improve cardiometabolic risk by reducing blood pressure via RNS production, which would lead to endothelial relaxation and adaptive responses for NO production (237).

The potential mechanisms of IH are summarized in Figure 8. In this context, IH could be considered as a therapeutic strategy to manage some metabolic-related disorders such as SAHS, possibly due to the reduction in the number of apneas by sustained hypercapnia, and CVD, associated with the adaptation to hypoxic stress and the molecular consequences of the remodeling of the cardiovascular system (263). In rodents, the PI3K/AKT pathway has been suggested to be involved in IH-induced cardioprotection (247). In this sense, current evidence suggests that regular exercise-induced adaptations to ROS handling, through redox signaling, including antioxidant and oxidative damage repair systems, contribute to the beneficial effects of regular exercise (311). The intermittent factor provides a preconditioning role to both exercise and hypoxia.

It should be noted that SAHS patients suffer from cycles of IH and hypercapnia, which differs from IH therapies due to the hypocapnia (365). In SAHS patients, hypoglossal nerve activity could be a therapeutic target as this nerve is responsible for a decrease in pharyngeal dilator muscle tone and ultimately the collapse in apnea episodes; it has been suggested that IH might benefit SAHS patients through reduction in hypoglossal activity (237). Furthermore, IH could have a neuroprotective effect via increased angiogenesis and ROS-dependent endothelial adaptation, as well as ischemia-preconditioning (236). Another way to benefit from a hypoxic stimulus without undergoing the detrimental effects of a prolonged exposure to hypoxia is IH training, consisting of physical activity under hypoxic conditions (for short periods) remaining at normoxic conditions for the rest of the time, which has been suggested as a weight loss approach (394). Obesity, in turn, is the most important risk factor for SAHS development, and both conditions would benefit from weight loss and physical activity (11, 391). Finally, it has been suggested that IH training induces specific molecular adaptations at the muscle level which are not achieved by exercising under normoxic conditions (394).

2. Experimental evidence

Regarding the specific effects of IH on the MetS and its components, several studies of obese subjects treated with normobaric IH training found greater weight loss (191, 266), decreased body fat (414), improved cardiometabolic fitness (118) and lowered systolic pressure (191) over a medium-term period (4-8 weeks). Also, in healthy young men IH training for 4 weeks increased lean mass (18), attenuated the acute exercise-induced lipid peroxidation (19), decreased triglyceride levels and improved glucose tolerance (141). Another trial reported that pre-diabetic subjects (males and females) exposed to IH training for 3 weeks improved glucose tolerance and blood insulin (post-oral glucose tolerance test) (356), as well as respiratory and cardiovascular parameters (355). In short-term trials (3-5 nonconsecutive days), T2D males who followed a continuous exercise program of sixty minutes in hypoxic conditions improved insulin sensitivity (227, 228). In healthy males exposed to 10 successive days of IH training, a decreased postprandial glucose response and reduced cholesterol levels were observed (76). Finally, an investigation conducted in obese men treated with normobaric hypoxia for 10 consecutive nights showed an improvement in insulin sensitivity (199), although this only involved a small sample size.

On the other hand, a study performed in well-trained healthy men with normobaric IH high-intensity training for 4 weeks found an impairment in insulin sensitivity compared with training in normoxia (198). The level of specific adipokines was not changed following an IH training protocol of 13 weeks in obese men with SAHS (118), which could be in line with a recent short-term study (3 sessions) performed in healthy men exposed to IH (without exercise) that showed an improvement in respiratory plasticity in an inflammation-independent manner (23). Also, a study performed with healthy men under 10 days of normobaric hypoxia treatment (1 h/day, without exercise) found no changes in inflammatory markers (307). Moreover, over a longer period of time (8 months), no differences were shown in body weight and metabolic markers between obese individuals (males and females) in IH training and exercising under normoxic conditions (108).

Nevertheless, further studies are needed to identify the hypoxic protocol that best provides the amelioration of metabolic disorders by means of changing the hypoxia intensity/amount of oxygen, normo- or hypobaric pressure, number and duration of episodes, combination with exercise, and total protocol length in terms of the number of exposure days (236, 249). Besides, it seems likely that the responses to exercise may vary across individuals and clinical conditions (82). Hence, a comprehensive examination of the metabolic responses to IH should be undertaken for both healthy subjects and those suffering from the MetS and associated comorbidities. The selection of the proper protocol will depend on the appropriate identification of biomarkers of the pathological features.

B. Hypobaric hypoxia

Studies of short-term exposure to environmental altitude, or as simulated with hypobaric hypoxia, have suggested a positive effect on insulin sensitivity and appetite reduction (422), and seem to be similar to those of normobaric IH (263). Certain studies have reported beneficial effects of a short-term geographical altitude exposure for subjects that already have MetS (130, 133, 267, 342).

1. Potential benefits

The available evidence shows that hypobaric hypoxia may have potential effects on metabolic disorders (148, 182) since it has been associated with weight loss and appetite reduction (217, 400), higher arterial sO_2 (303), lower adiposity and increased serum adiponectin levels (272), along with improved lipid metabolism (133). Chronic exposure to altitude has been associated with a lower prevalence of metabolic disorders in permanent populations living at very high- to extreme altitudes (Figure 9) (21, 81, 221, 222, 405, 406, 423, 424). Moreover, research conducted in Tibetans (358) showed that BMI decreased with increasing altitude (for each 1,000 m ascent the BMI was reduced by 1.43 kg/m^2). Also, previous studies have reported lower fasting glycemia (55, 216) and better glucose tolerance (296) at high altitude. However, some of these studies are descriptive and just reported prevalence, and hence the cause-effect relationship is still unclear. Moreover, the underlying mechanisms of the inverse association between altitude and glycemia remain uncertain (422).

Despite some studies reporting less ischemic heart disease, inconclusive data concerning blood pressure are evident as a higher prevalence of hypertension was associated with the very high altitudes (over 3,000 m) in highlanders of India (275); however, the evidence seems to support lower blood pressures in subjects who reside at altitude (10, 148, 285). In addition, living at high altitude reduces the mortality rate from ischemia (95), stroke (96) and coronary heart disease (96, 97), while the mortality from chronic obstructive pulmonary disease increases (95). Despite most studies being adjusted for multiple factors, exposure to altitude could influence the central nervous system by a regulatory role on appetite — at least in short-time exposures. Nevertheless, the direct effect of prolonged altitude exposure on appetite remains unknown. Basal metabolic rate and sympathetic activation in highlanders seems to be similar to those living on the coast, even if normalized to fat-free mass (424). Leptin and noradrenaline could be influencing the changes in energy expenditure and food intake at high altitude, since they increase energy expenditure via sympathetic nerve activity, even in acclimatized subjects (148, 285, 406). In addition, leptin levels were increased in participants who lost weight at very high altitude compared to those at sea level (285). Finally, the beneficial effects of ROS production, oxidative eustress — mainly through cellular signaling (362) — might explain the effect of altitude on metabolic disorders. In this sense, controlled ROS production due to altitude could exert

several beneficial effects (394) through oxidative eustress and the physiological adaptation needed to breathe an adequate amount of oxygen at several hundred meters above sea level.

2. Scientific rationale

As noted earlier, the highest concentration of oxygen occurs at sea level, where the pO_2 is approximately 160 mmHg (21 kPa), corresponding to 21% oxygen. The arterial pO_2 is approximately 13 kPa, with almost 100% of oxygen being available at 0 meters of altitude (Baillie lab, University of Edinburgh). This atmospheric pO_2 means roughly 98% of arterial sO_2. For every 500 m in elevation the arterial pO_2 is reduced by 1 kPa, at the same time the arterial sO_2 decreases by around 1% and the oxygen availability falls by 7%. A reduction in arterial pO_2 by more than 1 kPa, and/or an arterial sO_2 below 95%, is considered hypoxemia (270). Therefore, we could assume that those subjects who permanently live at moderately high altitude (above 500 m) experience a certain degree of hypoxemia. Chronic hypobaric hypoxia exposure is more pronounced in studies including participants living above 1,500 m, which means 3 kPa less than those at sea level and an arterial sO_2 of 77%. This situation leads to various physiological changes following adaptation to chronic hypoxemia.

The available evidence suggests a potential preventive effect of living at altitude, but other possible explanations apart from reduced oxygen availability cannot be dismissed, since at high altitude there is lower pressure, temperature and humidity (Figure 10) (240, 412). In this regard, previous studies have found higher rates of obesity at higher temperatures (395, 431). Moreover, other factors such as genetic polymorphisms for the adaptation to very high and extreme altitudes could be involved in the lower prevalence of metabolic disorders, at least in Andean, Ethiopian and Tibetan highlanders (30, 159, 397). In this respect, some of the genetic variants that could contribute to human adaptation to altitude are linked to hypoxic adaptation (159), while others are related to the antioxidant system and lung function (397). Antioxidant adaptation is needed as hypobaric hypoxia is known to induce oxidative stress, which in turn contributes to endothelial damage and vascular remodeling (291). It is emphasized that we should be cautious in generalizing the effects on highlanders to populations living at moderate to high altitudes.

The emergence of COVID-19 infection in 2020 has aroused interest in relation to geographic factors in the transmission of the causative virus (SARS-CoV-2). In the midst of the crisis some authors have observed that an epidemiological trend has emerged in relation to high altitude populations exhibiting attenuated rates of transmission with limited COVID-19 infection severity (175). However, the potential contribution of ethnic genetic variations in the expression of angiotensin-converting enzyme 2 (ACE2), a protein recently associated with COVID-19 pathogenesis and mortality, has been

recognized. Thus, further studies are needed to analyze the potential protective effect of altitude against COVID-19 infection, especially in relation to altitude adaptation polymorphisms in highlanders.

C. Hyperoxia

Oxygen therapy, originally pioneered by Thomas Beddoes in Bristol (UK) at the end of the 18th century, has been used in clinical practice for the treatment of various disorders such as chronic obstructive pulmonary disease, ulcers in diabetic patients and cerebral ischemia, in addition to acutely in medical emergencies, such as for resuscitation and anaphylaxis, as well as for the treatment of chronic lung disease (117, 386). There are two different hyperoxia treatments depending on the pressure: hyperbaric oxygen therapy (HBOT) and normobaric oxygen therapy (NBOT). The administration of 100% oxygen at normal pressure (1 atmosphere absolute, ATA) or NBOT is indicated at any state which produces hypoxemia, in order to recover blood oxygen levels. HBOT corresponds to 100% oxygen with an atmospheric pressure equal or greater than 1.4 (usually 2.4–2.8 ATA) and it is already approved by the US Food and Drug Administration (FDA) for medical use in specific situations (Table 4).

1. Current indications of HBOT

The European Committee for Hyperbaric Medicine (ECHM) evaluated the FDA indications, categorizing them according to the strength of recommendation and level of evidence, at a consensus conference in 2016 (238). HBOT is administered in a chamber or through an endotracheal tube, masks or head hoods, with a duration varying from 45 minutes for carbon monoxide poisoning, to several hours in decompression disorders (380). The indications approved by the FDA that are categorized by the ECHM as being strongly recommended — of critical importance for final outcome of the patient/quality of practice/future specific knowledge — with a moderate level of evidence, unless otherwise stated, are outlined hereunder grouped by the etiology of each disease.

Carbon monoxide poisoning, arterial gas embolism and decompression sickness are characterized by an alteration in blood gases. Carbon monoxide poisoning has acute toxic effects, as well as a high risk for delayed neuropsychological sequelae (256). Given these potentially life-threatening effects, the ECHM recommends HBOT in case carbon monoxide poisoning causes one or more of these conditions: loss of consciousness at or before admission, clinical neurological, cardiac, respiratory or psychological symptoms or signs — as well as in pregnant women (238). Recently, a clinical practice guideline also recommended HBOT for carbon monoxide poisoning (361). In this group of pathologies characterized by an alteration in blood gases, arterial gas embolism and decompression sickness are only supported by the ECHM in certain cases, with a moderate level of evidence (238).

Problematic wounds, anemia, radiation injuries, skin grafts, thermal wounds, traumatic injury and sudden deafness are defined by the presence of hypoxia, reduced irrigation of a tissue and insufficiency. When late radiation injuries occur, tissues suffer a progressive deterioration characterized by a reduction in the density of small blood vessels (reduced vascularity) and the replacement of normal tissue cells with dense fibrous tissue (fibrosis), until there is insufficient oxygen supplied to sustain normal function (25). HBOT promotes healing by increasing angiogenesis, cell proliferation and collagen formation, and prevents breakdown of irradiated tissue fields (25, 301). In traumatic brain injury, the brain and contiguous central nervous system structures suffer mechanical harm, with resultant ischemia, edema, compartment syndromes, and tissue necrosis — factors known to exacerbate secondary brain damage and ultimately to lead to neuron loss (39, 380). While surgery is a fundamental therapy for these injuries, the reduction of edema, protection from reperfusion damage, and enhanced wound healing are benefits of complementary HBOT (380). Lastly, for idiopathic sudden sensorineural hearing loss and tinnitus, treatment with HBOT remains recommended despite little knowledge on the mechanisms underlying the potential benefits (238, 364); but the benefits could be related to the etiology of the sudden deafness which has been suggested to result from a hypoxic event in the cochlear apparatus. Thus, HBOT might be able to reverse that oxygen deficit (27). The ECHM recommends HBOT for problematic wounds — moderate to low evidence, depending on the clinical aspects — and in particular cases of compromised skin graft and flaps — with a moderate to low level of evidence (238). Also, HBOT might help in the treatment of severe anemia and burn wounds, although with a low level of evidence (238).

Finally, infectious pathologies such as gas gangrene, intracranial abscess, necrotizing fasciitis and refractory osteomyelitis are suggested by the ECHM — with low evidence — to be treated with HBOT in conjunction with antibiotics and/or surgery to counteract the infectious agents (256, 380).

2. Clinical practice and experimental efficacy

In a recent meta-analysis of studies in critically ill patients in the first 24 h in ICU, it was concluded that hyperoxia treatment would lead to higher mortality (268). Furthermore, a meta-analysis with studies on acutely ill adults (66) concluded that a liberal oxygen therapy (above a peripheral sO_2 of 94-96%) increases mortality without improving other patient-important outcomes. Conversely, setting a "safe" peripheral sO_2 lower limit (avoiding a higher risk of death due to hypoxemia) is more difficult to establish. However, the authors support the conservative administration of oxygen therapy in patients, and this is in line with a recent clinical practice guideline (361), which recommends oxygen therapy only when peripheral sO_2 falls below 89% for patients at risk of hypercapnia, or 90–92% for patients with stroke or myocardial infarction. On the other hand, the latter recommends stopping

oxygen therapy when peripheral sO_2 reaches 96% because of increased mortality (around 1%) without any reduction in morbidity for higher values (disability, infection, length of stay, infarct size).

Therefore, and based on previous arguments, we think the guideline should be widened and thereby oxygen therapy should be started when peripheral sO_2 falls below 89% in patients at risk of oxygen-induced hypercapnia, or below 93% for other patients. Finally, oxygen therapy should be stopped when peripheral sO_2 achieves 96% in patients without hypercapnia and 92% in patients at risk of oxygen-induced hypercapnia.

Recent research has shown that oxygen therapy could improve other conditions such as wound healing, muscle regeneration, stroke recovery, traumatic brain injury, neurological damage, retrieval of surgical-related loss of cognitive function and migraine (26, 59, 103, 214, 301, 357, 379). However, excessive exposure to high oxygen — in time, or concentration — may lead to harmful effects. As discussed above, the main potential risk of oxygen therapy could be the imbalance between oxidative stress and antioxidant induction (282, 301, 364). After hours of higher oxygen exposure, the toxic effects of hyperoxia such as inflammation in the lungs, neurological damage, and ear and ocular injuries, could appear (301, 364).

Studies on hyperoxia treatment have provided evidence for an analogous beneficial effect to that found under acute hypoxia exposure on weight loss, glucose homeostasis and WAT inflammation (46, 117). Also nocturnal oxygen therapy improved exercise capacity, cardiac function, and cardiac sympathetic nerve activity in patients with heart failure and central sleep apnea (338, 382). Moreover, hyperoxia has been used in athletes as a support during training with an improvement in the oxygen transport capacity, lactate metabolism, power output and work tolerance (endurance) being shown (51). Hence, hyperoxia alone (117), or in combination with NO (276), has been suggested for the treatment of obesity and related disorders.

3. Mechanisms of action

Physiologically, hyperoxemia results in vasoconstriction in the brain, heart and skeletal muscle, which decreases blood flow (364). The combination of peripheral vasoconstriction and increased vascular resistance leads to higher blood pressure (39, 364). This is sensed by pressure-sensitive receptors (baroreceptors) and causes a reduction in heart rate through para-sympathetic activity (354, 364). Furthermore, in order to prevent hypertension, blood flow is reduced (39). Hyperoxia also decreases carbon dioxide transport from tissues since hyper-oxygenated blood weakens the affinity of carbon dioxide for hemoglobin (39, 75). The higher levels of tissue carbon dioxide together with the previously mentioned reduced blood flow lead to a hyperoxic hyperventilation phenomenon (39, 364). Finally, hyperoxemia increases ROS levels, leading to hyperventilation and hypocapnia, further

contributing to vasoconstriction (39, 75). The administration of HBOT is known to increase the dissolved oxygen fraction in comparison with that of NBOT (364). Also, the increased pressure may have additional benefits, but the specific mechanism is unclear.

The molecular basis of oxygen therapy has been proposed as an adaptive response to compensate for increased oxidative stress (301) with the levels of antioxidant enzymes being increased when oxygen is present (364). The combination of oxygen therapy and exercise has a good rationale, as the exercise-induced ROS generation is also a powerful stimulus to activate antioxidant enzymes. Thus, both intermittent exercise and hypoxia-induced ROS generation result in increased activity of enzymatic antioxidants, which then results in increased resistance to oxidative challenges, including a wide variety of oxidative stress-related diseases (311).

In marked contrast, hyperoxia increases the production of both ROS and nitrogen species (301, 354). Moreover, animal studies showed neovascularization which increases the recruitment and differentiation of progenitor cells that could improve wound healing (301). These progenitors under HBOT have shown oxidative stress resistance through growth factors, which also help to regenerate the tissue (364). In parallel, HBOT has been shown to have a potential inhibitory effect on adhesion and inflammatory molecules (117). Overall, it is possible that hyperoxia therapy could have antioxidant and anti-inflammatory effects in various organs.

D. Oxygen delivery systems

Whilst there are some investigations being conducted in relation to therapeutic approaches for metabolic diseases, the available strategies are not able to counteract the increasing rates of obesity and related disorders. Hence, novel therapies are needed, along with the personalization of treatments (119). In this sense, drug therapy against hypoxia signaling has been suggested to treat diverse disorders (200). Besides altered pO_2, obesity and associated comorbidities are characterized by increased oxidative stress and inflammation (117), which could be counteracted by novel agents (429). Therefore, the use of molecules with antioxidant activity could be a useful strategy to prevent the deleterious effects of ROS. Oxygenation strategies based on free or encapsulated oxygen carriers and oxygen-generating materials have been developed as tissue-engineered scaffolds to supply the tissue-specific oxygen-microenvironment requirements (99, 112).

1. Hemoglobin-based oxygen carriers

Hemoglobin-based oxygen carriers (HBOCs) have been developed as chemically modified hemoglobin (acellular) or encapsulated hemoglobin inside oxygen carriers (cellular) (98, 173). To improve the half-life and stability of HBOCs, strategies such as cross-linking, polymerizing, coating,

conjugating and complexation have been studied (99). The first generation of HBOCs was designed to avoid dissociation of hemoglobin tetramers into dimers by intramolecular or intermolecular crosslinked, polymer-conjugated and recombinant hemoglobin (173). Despite the substantial effort devoted on developing HBOCs, to date, acellular systems have been unsafe in humans. Especially concerning in relation to their use in clinical trials are induced hypertension, liver and pancreas damage, renal and neural toxicity and oxidative stress (42, 98, 298).

The second generation of HBOCs are based on co-assembly of hemoglobin with antioxidant enzymes — superoxide dismutase, catalase and rubrerythrin — to further stabilize and avoid inactive methemoglobin formation (173, 298). The cardiovascular system has been reported as the primary target of acellular HBOCs toxicity, with myocardial infarction, hypertension and death being the primary adverse events in clinical trials (339). Therefore, a third generation of HBOCs has been developed as cellular carriers by encapsulating hemoglobin inside several structures to protect tissue contact to HBOCs and avoid leakage, also prolonging the half-life in the circulation (298).

Generally, there are two main types of cellular HBOCs: liposome-encapsulated hemoglobin and polymer-encapsulated hemoglobin (173, 298). Although liposome-based strategies were found to have long vascular retention and stability (298), some defects still exist. For example, they are difficult to produce and expensive, and can activate the complement pathway and induce peroxidation (173, 298). On the other hand, polymer-encapsulated hemoglobin has generated greater interest due to the availability and lower price, broad variety and biocompatibility of polymers (173). Polyethylene glycol, poly(lactic acid), poly(glycolic acid) and their copolymers are the most applied polymers in the development of polymeric cellular HBOCs (298). Notwithstanding that some HBOCs have reached phase III clinical trials, reports of serious adverse events are the main reason for their being discontinued. Safety concerns about HBOCs-related vascular dysfunction characterized by hypertension, inflammation and oxidative stress have hindered further product development and licensing (42). Thus, further studies on cellular HBOCs are warranted.

2. Oxygen-releasing biomaterials

HBOCs have had less significant success in clinical trials than perfluorocarbons (PFCs), another artificial blood substitute that has been extensively studied. PFCs consist of fluorinated carbon chains which exhibit various properties that make them a highly suitable oxygen carrier for biological applications (98). These properties are biocompatibility with living tissues given by the strength of C-F bonds, structural stability due to hydro- and lipophobic and self-assemble features in aqueous solution, the low polarizability and miscibility with non-polar gases, enabling PFCs to dissolve O_2 by physically capturing the molecules (98). Since PFCs can easily dissolve oxygen in aqueous conditions,

PFC-containing gels are a promising solution for many of the limitations of current oxygen-related tissue engineering. PFCs have been used in a wide range of applications, including liquid ventilation, blood substitution, tissue preservation, wound healing, *in vitro* cell culture, and tissue engineering (439). PFCs were able to increase cell viability, promote cell differentiation, and maintain cell metabolism in various tissues and organs (98) and have been used as oxygen suppliers to preserve islets, brains, pancreas, hearts and kidneys (439). Nonetheless, their inability to provide a sustained release of oxygen may be a potential barrier for *in vivo* success (98).

Several PFC blood substitute products have been clinically tested and approved by different countries for specific applications (99). However, some products encountered setbacks in phase III clinical trials when patients experienced increased stroke risk and adverse neurological side effects (98). Hence currently, no PFC-based blood substitutes are approved for clinical use in any country — except Russia (439). Nevertheless, PFCs have overall shown greater clinical potential than other materials for oxygen delivery (98).

Alternate approaches have been experimentally used as oxygen-releasing biomaterials to provide adequate and sustained oxygen supply for engineered tissue both *in vitro* and *in vivo* by diffusion of entrapped, adsorbed oxygen or by chemical generation of oxygen (112). Some of these technologies are polymeric nanosponges, gas-filled microbubbles and microtanks for oxygen delivery. Experimental polymeric nanosponge-based formulations designed as oxygen delivery systems have been tested in cell cultures and in animal models (58, 100). In this regard, cyclodextrin nanosponges are biocompatible porous materials with adjustable release through ultrasound (439). Obtained by cross-linking a polymer with α- β- γ- cyclodextrins, these nanosponges have the capacity of encapsulating active molecules due to the cooperation of cyclodextrin cavities and cross-linker networks (58). With an average diameter of 400–550 nm, this material demonstrated an ability to reduce cell mortality during hypoxia and reoxygenation in a cardiac cell model *in vitro* (100) and possesses interesting potential for oxygen topical delivery in future medical applications (58). Nevertheless, the efficacy of the nanosponges delivering oxygen under hypoxic or anoxic conditions has not been examined yet (439).

Further, gas-filled microbubbles for oxygen delivery are biocompatible microparticles which showed a protective effect on rats with lung injuries through peritoneal perfusion (439). Another recent approach used polymeric hollow microspheres called microtanks, which can be hyperbarically loaded with oxygen, and these showed promising results as oxygen carriers in relation to proliferation and metabolic rates in cells treated with anoxia, while cell viability and survival were improved in hypoxia-treated cell cultures (98, 99).

3. Oxygen-generating materials

Oxygen-generating materials as peroxides and nano-structured particles, decompose in the biological environment to produce oxygen and, in some cases, byproducts (99). The poor stability of peroxides allows its decomposition to produce molecular oxygen when heated or when in contact with water (439). Oxygen generation by aqueous decomposition via the formation of hydrogen peroxide finally decomposes to produce molecular oxygen, and basic byproducts (439). Peroxides have been used to deliver oxygen to tissues and cells in several approaches such as *in vitro* cell culture and tissue preservation, where they have been shown to have the capacity to mitigate anaerobic glycolysis and preserve insulin release, increase cell survival and neovascularization (99, 112). Peroxides as oxygen delivery agents have low cost, easy storage, controlled generation of oxygen, and *in situ* release of oxygen (439). However, there is still no effective way to eliminate radicals generated during the decomposition of peroxides leading to cytotoxicity (112).

Materials at the nanoscale size are generally up to 100 nm in at least one dimension, a non-arbitrary threshold with physicochemical significance that makes a critical difference to their properties with respect to larger structures. In addition to size, the shape, elemental constitution and surface morphology strongly influence the reactivity of nanomaterials (50). These materials offer unique characteristics that offer considerable appeal for many types of application. The applications include nanomedicine, which is designed to overcome several issues as they provide nanosized solid structures that can target a treatment to a specific part of the body as well as preserving and masking a drug until the target is located. The administration of nano-structured particles has been shown to have therapeutic potential in nanomedicine due to better distribution and cellular uptake than other systems for delivering drugs; furthermore, the trans-excitation reactions make them able to take part in redox reactions (50, 328, 433).

Cerium oxide nanoparticles (CeO_2 NPs) are one of the most promising nanomaterials for antioxidant and anti-inflammatory pharmacological applications; they have been proposed for diverse therapies in pathological conditions such as neurodegenerative disorders, oxidative stress-related diseases, diabetes, chronic inflammation and cancer among others (50, 62, 150, 225, 429). The therapeutic potential is attributed first to the coexistence of two valence states (Ce^{3+} and Ce^{4+}) that provide the ability to mimic superoxide dismutase, behaving as efficient ROS scavengers (Ce^{3+} to Ce^{4+}) and changing to mimic catalase activity which reduces hydrogen peroxide releasing protons and O_2 (Ce^{4+} to the initial Ce^{+3}) (Figure 11). Thus, this self-regenerative property renders this nanomaterial as a very valuable tool for the pharmacological treatment of oxidative-related disorders(50).

Despite the large number of studies reporting beneficial effects of CeO_2 NPs, there is also research indicating toxic and pro-inflammatory responses. As a consequence, the net health effects of nanoceria

are still inconclusive, as several studies obtained contradictory findings about its biological activity (50, 429). Some authors have reported beneficial properties of CeO_2 NPs on *in vitro* macrophage inflammation (150), smoke-related cardiomyopathy (274), oxidative stress in mesenchymal-derived β-cells (435), as well as ROS protection in neurons (69). Animal studies showed diverse useful properties of CeO_2 NPs in the treatment of many redox dysregulated states. For example, reducing adipogenesis by a decrease in plasma insulin, leptin, glucose and triglycerides (324), reducing macular degeneration (194) and cardiac dysfunction (273), attenuating hypoxia-derived lung damage (14), and alleviating liver ROS toxicity (149). In contrast, other experiments indicated an inability to counteract inflammation in human monocytes (161, 162), or even cell death through apoptosis and autophagy on this cell type (160), and oxidative stress and inflammation in lung, liver, kidney, heart, spleen and brain of mice (265). Moreover, the CeO_2 NPs were used to induce cytotoxicity and oxidative damage in tumor cells (251, 328), while they are able to protect non-malignant cells from chemotherapy (328). Differences in biological targets (cell types and species), experimental design (preconditioning with inflammation/oxidants for treatment, or with the nanoparticles for prevention), nanoparticles (synthesis method, size, shape, chemical characteristics) and objectives of the studies could lead to these variations, making interpretation of the outcome and comparison between studies highly complex. Nevertheless, it has been suggested that the *in vitro* beneficial effects of these nanoparticles could differ due to diverse biochemical features, as lower pH was reported to lead them to behave as oxidants, thereby generating ROS (323). The different methods used to prepare the nanoparticles influences the relative proportion of surface charges (50). It is noted that the surface oxidation state of the CeO_2 NPs has been demonstrated to alter the accompanying enzyme-mimetic activity. Thus,Ce^{+3}charges at the nanoparticle surface are linked to the superoxide scavenging properties (144) and lower Ce^{+3}/Ce^{+4} ratios are less efficient (429).

X. Concluding remarks and future perspectives

The discovery of how cells sense and adapt to oxygen availability, for which William Kaelin, Jr., Sir Peter Ratcliffe, and Gregg Semenza were awarded the Nobel Prize in Physiology or Medicine in 2019, revealed that the HIF pathway has a key role in modulating oxygen-sensitive gene expression and further evidenced the important role of oxygen in body homeostasis. At an early stage, Semenza identified both the HRE at the 3'-end of the erythropoietin (*EPO*) gene and the transcription factor acting on this site induced by low oxygen levels, namely HIF (350). Thereafter, Ratcliffe showed that HIF-1α levels were regulated by changes in protein stability (304). Kaelin's focus on the VHL tumor suppressor led to the discovery of a link between the HIF response and VHL-linked tumorigenesis (163), that subsequently was associated with a role of VHL in oxygen-dependent HIF-1α degradation

(167). In the last two decades many other pieces of the puzzle of how oxygen is sensed and how cells respond to differing levels have been discovered and analyzed, which has led to recognition of the potential for pharmacological treatments and complementary therapies.

Some open questions for readers:

Should oxygen be considered as the 4th macronutrient (after proteins, lipids and carbohydrates) — or 5th if alcohol is included — as has recently been proposed (385, 386)? If so, how important is its purity (bearing in mind life in rural areas, as opposed to urban ones with contamination of the air through vehicle fumes, etc.)? Should the amount of antioxidants, and the percentage of macronutrients we consume, be correlated with our oxygen consumption? In our view, oxygen has to be considered as a very important factor in future lifestyle recommendations.

Will the measurement of oxygen consumption be included in strategies for the prevention of metabolic diseases, based on previous results (68, 223)? As mentioned above, measuring oxygen consumption is immediate, non-invasive and extremely cheap in comparison with many other measurements. Given the association of oxygen consumption and metabolic fitness, this additional measurement could be included in future intervention studies of metabolic disorders. If the efficacy in differentiating subjects at high metabolic risk and metabolically healthy individuals is proven, the measurement of oxygen consumption could be used in preventive care for metabolic diseases.

Should exposure to altitude be recommended for those genetically or phenotypically predisposed to develop the metabolic syndrome? Although high altitude living seems beneficial, very high and mainly extreme altitude have been linked with pernicious effects on metabolism. Therefore, which is the best altitude at which to live? Should altitude prescription be individualized, as with other therapies? Obviously, one cannot suggest living in a different city because of altitude, but perhaps a period in the mountains once or twice a year for shorter periods — as some sport professionals do — might be appropriate? Moreover, it would be interesting to study the potential benefit of locating residential institutions (care of the elderly, those with chronic disease) at high altitude for population-based prevention strategies of chronic diseases — especially those that are metabolism-related.

Could hyperoxia or hypoxia be used to treat metabolic diseases? There is some indication that IH training could be beneficial for the adaptation of muscle to exercise in individuals suffering from the MetS and associated comorbidities. On the other hand, hyperoxia could be useful in clinical practice when peripheral sO_2 falls below 89% in patients at risk of oxygen-induced hypercapnia (or below 93% for other patients). In addition, oxygen therapy has been studied in a wide range of experimental conditions due to the potential adaptive response to increased oxidative stress. Nevertheless, an excess oxygen therapy may lead to detrimental effects. Thus, further studies are needed to evaluate the risks and benefits of treating with oxygen therapy. In this sense, alternative approaches have been

experimentally analyzed, such as oxygen-delivery systems (free or encapsulated oxygen carriers and oxygen-generating materials) to supply tissues with their specific oxygen requirements. Consequently, additional research on novel oxygen-based therapeutic approaches for metabolic diseases are warranted.

Therefore, there are still many unknowns, including: the exact role of HIF-2 and particularly HIF-3, the relationship of the gut microbiota, the link between neurodegenerative diseases and oxygen, and the influence of HIFs in metal transportation.

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Author Contributions

Conceptualization, A.L-P., J.A.M. and P.G-M.; Writing-Original Draft Preparation, A.L-P.; Review & Editing, A.L-P., P.T., J.A.M. and P.G-M.; Supervision, J.A.M. and P.G-M.; Funding Acquisition, J.A.M and A.L-P. All authors critically revised and approved the final version of the manuscript.

Author Disclosure Statement

The authors declare no conflicts of interest.

List of Abbreviations

ABCB1, adenosine triphosphate binding cassette subfamily B member 1

ABCB1, adenosine triphosphate binding cassette subfamily B member 1

ABCG2, adenosine triphosphate binding cassette subfamily G member 2 (Junior blood group)

ACAN, aggrecan

ADIPOQ, adiponectin

ADM, adrenomedullin

ADORA2A, adenosine A2a receptor

AFP, alpha fetoprotein

AGR2, anterior gradient 2, protein disulphide isomerase family member

AGT, angiotensinogen

AHA/NHLBI, American heart association/national heart, lung, and blood institute

AK1, adenylate kinase 1

AKT1, Akt serine/threonine kinase 1

ANG, angiogenin

ANGPT1, angiopoietin 1

ANGPT2, angiopoietin 2

ANGPTL4, angiopoietin like 4

AOX, alternative oxidase

APEX1, apurinic/apyrimidinic endodeoxyribonuclease 1

APOE, apolipoprotein E

AQP4, aquaporin 4

ARG1, arginase 1

ATA, atmosphere absolute

ATF6, activating transcription factor 6

ATG5, autophagy protein 5

ATP, adenosine triphosphate

ATPIII, adult treatment panel III

AURKA, aurora kinase A

AXL, Axl receptor tyrosine kinase

BAT, brown adipose tissue

BAX, B-cell lymphoma 2 associated X, apoptosis regulator

BBC3, B-cell lymphoma 2 binding component 3

BCL2, B-cell lymphoma 2 apoptosis regulator

BCL2L1, B-cell lymphoma 2 like 1

BHLHE40, basic helix-loop-helix family member e40

BHLHE41, basic helix-loop-helix family member e41

BIRC5, baculoviral inhibition of apoptosis repeat containing 5

BMI, body mass index

BNIP3, B-cell lymphoma 2 interacting protein 3

BNIP3L, B-cell lymphoma 2 interacting protein 3 like

BSG, basigin (Ok blood group)

BTG2, B-cell translocation gene family anti-proliferation factor 2

CA9, carbonic anhydrase 9

CALCRL, calcitonin receptor like receptor

CASP3, caspase 3

CBP p300, cAMP response element-binding (CREB)-binding protein and p300 complex

CCL2, C-C motif chemokine ligand 2 (MCP-1)

CCL4, C-C motif chemokine ligand 4

CCL5, C-C motif chemokine ligand 5

CCN1, cellular communication network factor 1

CCN2, cellular communication network factor 2

CCND1, cyclin D1

CCR1, C-C motif chemokine receptor 1

CCR5, C-C motif chemokine receptor 5 (gene/pseudogene)

CD44, cluster of differentiation 44 molecule (Indian blood group)

CDH1, cadherin 1

CDH2, cadherin 2

CDKN1A, cyclin dependent kinase inhibitor 1A

CEBPA, CCAAT enhancer binding protein alpha

CeO_2 NPs, Cerium oxide nanoparticles

c-HDL, high-density lipoprotein cholesterol levels

c-LDL, low-density lipoprotein cholesterol

COL1A1, collagen type I alpha 1 chain

COMMD1, copper metabolism domain containing 1

COPS5, constitutive photomorphogenesis 9 signalosome subunit 5

COX4I2, cytochrome c oxidase subunit 4I2

CP, ceruloplasmin

CPEB2, cytoplasmic polyadenylation element binding protein 2

CPT1A, carnitine palmitoyltransferase 1A

CREB3L1, cyclic adenosine monophosphate responsive element binding protein 3 like 1

CTNNB1, catenin beta 1

CTSD, cathepsin D

CUL2, cullin 2

CVD, cardiovascular disease

CXCL12, C-X-C motif chemokine ligand 12

CXCL8, C-X-C motif chemokine ligand 8

CXCR1, C-X-C motif chemokine receptor 1

CXCR2, C-X-C motif chemokine receptor 2

CXCR4, C-X-C motif chemokine receptor 4

CYBB, cytochrome b-245 beta chain

DCUN1D1, defective in cullin neddylation 1 domain containing 1

DDIT4, DNA damage inducible transcript 4

DLL4, delta like canonical Notch ligand 4

DNAJB1, DnaJ heat shock protein family (Hsp40) member B1

E2F7, E2 factor transcription factor 7

E2F8, E2 factor transcription factor 8

EAF2, eleven-nineteen lysine-rich leukemia associated factor 2

ECHM, European Committee for Hyperbaric Medicine

EDN1, endothelin 1

EDN2, endothelin 2

EDNRB, endothelin receptor type B

EGLN1, endoglucanase-9 family hypoxia inducible factor 1

EGLN3, endoglucanase-9 family hypoxia inducible factor 3

EIF4EBP1, eukaryotic translation initiation factor 4E binding protein 1

ELL, elongation factor for RNA polymerase II

ELOB, elongin B

ELOC, elongin C

ENTPD1, ectonucleoside triphosphate diphosphohydrolase 1

EPO, erythropoietin

EPR, electron paramagnetic resonance spectroscopy

ER, endoplasmic reticulum

ESR1, estrogen receptor 1

ESR2, estrogen receptor 2

ESRRG, estrogen related receptor gamma

EZH2, enhancer of zeste 2 polycomb repressive complex 2 subunit

FABP4, fatty acid binding protein 4

FAM162A, family with sequence similarity 162 member A

FASN, fatty acid synthase

FBXO32, F-box protein 32

FBXW7, F-box and tryptophan-aspartic acid (WD) repeat domain containing 7

FDA, Food and Drug Administration

FGF2, fibroblast growth factor 2

FIH, factor inhibiting HIF-1

FLT1, macrophage colony-stimulating factor related tyrosine kinase 1

FLT4, macrophage colony-stimulating factor related tyrosine kinase 4

FN1, fibronectin 1

FOXM1, forkhead box M1

FOXO3, forkhead box O3

FOXP3, Forkhead box P3

FTCD, formimidoyltransferase cyclodeaminase

GAPDH, glyceraldehyde-3-phosphate dehydrogenase

GO, Gene Ontology

HBOT, hyperbaric oxygen therapy

HDAC2, histone deacetylase 2

HDAC4, histone deacetylase 4

HDAC5, histone deacetylase 5

HDAC6, histone deacetylase 6

HDAC7, histone deacetylase 7

HEXIM1, hexamthylene bis-acetamide inducible 1 (HEXIM) - positive transcription elongation factor b (p-TEFb) complex subunit 1

HEY1, hes related family basic helix-loop-helix transcription factor with YRPW motif 1

HEY2, hes related family basic helix-loop-helix transcription factor with YRPW motif 2

HIF-1, hypoxia inducible factor-1

HIF1AN, hypoxia inducible factor 1 subunit alpha inhibitor

HIF3A, hypoxia inducible factor 3 subunit alpha

HK2, hexokinase 2

HMOX1, heme oxygenase 1

HNF4A, hepatocyte nuclear factor 4 alpha

HOTAIR, homeobox transcript antisense RNA

HR, hazard ratio

HRE, hypoxia-response element

HSP90AA1, heat shock protein 90 alpha family class A member 1

HSP90AB1, heat shock protein 90 alpha family class B member 1

HSPA1L, heat shock protein family A (Hsp70) member 1 like

HSPA4, heat shock protein family A (Hsp70) member 4

HSPA8, heat shock protein family A (Hsp70) member 8

IC, inferred by curator

ICAM-1, intracellular cell adhesion molecule 1

ID1, inhibitor of DNA binding 1, helix-loop-helix protein

IDA, inferred from direct assay

IDF, international diabetes federation

IDF-AHA/NHLBI, harmonized definition of IDF and AHA/NHLBI

IEP, inferred from expression pattern

IFNG, interferon gamma

IGF2, insulin like growth factor 2

IGFBP1, insulin like growth factor binding protein 1

IGFBP3, insulin like growth factor binding protein 3

IH, intermittent hypoxia

IKBKG, inhibitor of nuclear factor kappa B kinase regulatory subunit gamma

IL10, interleukin 10

IL1B, interleukin 1 beta (IL-1β)

IL22, interleukin 22

IL4, interleukin 4

IL6, interleukin 6 (IL-6)

IMP, inferred from mutant phenotype

INHA, inhibin subunit alpha

IRE1, inositol requiring enzyme 1

IRF1, interferon regulatory factor 1

JAG1, jagged canonical Notch ligand 1

JUN, Jun proto-oncogene, activating protein-1 transcription factor subunit

KAT2B, lysine acetyltransferase 2B

KAT5, lysine acetyltransferase 5

KDM3A, lysine demethylase 3A

KDM4B, lysine demethylase 4B

KDM4C, lysine demethylase 4C

KDR, kinase insert domain receptor

KITLG, Kit ligand

KLK3, kallikrein related peptidase 3

KRT18, keratin 18

KRT19, keratin 19

LDHA, lactate dehydrogenase A

LEP, leptin

LGALS3, galectin 3

LIFR, leukemia inhibitory factor receptor subunit alpha

LINC01139, long intergenic non-protein coding RNA 1139

LINE1, long interspersed nucleotide element 1

LOX, lysyl oxidase

LOXL2, lysyl oxidase like 2

LRRK2, leucine rich repeat kinase 2

MAFG, musculoaponeurotic fibrosarcoma basic leucine-zipper domain transcription factor G

MALAT1, metastasis associated lung adenocarcinoma transcript 1

MAPK1, mitogen-activated protein kinase 1

MAPK3, mitogen-activated protein kinase 3

MCL1, myeloid cell leukemia sequence 1 apoptosis regulator, B-cell lymphoma 2 family member

MCM3, minichromosome maintenance complex component 3

MCM7, minichromosome maintenance complex component 7

MCP-1, monocyte chemotactic protein 1

MDK, midkine

MDM2, mouse double minute 2 proto-oncogene

MetS, metabolic syndrome

MHO, metabolically healthy obese

MIF, macrophage migration inhibitory factor

MIR, microRNA

mmHg, millimeters of mercury

MMP1, matrix metallopeptidase 1

MMP14, matrix metallopeptidase 14

MMP2, matrix metallopeptidase 2

MMP28, matrix metallopeptidase 28

MMP3, matrix metallopeptidase 3

MMP9, matrix metallopeptidase 9

MRI, magnetic resonance imaging

MTA1, metastasis associated 1

MTDH, metadherin

MTF1, metal regulatory transcription factor 1

mTOR, mechanistic target of rapamycin kinase

MXI1, myelocytomatosis associated factor X interactor 1, dimerization protein

MYC, myelocytomatosis proto-oncogene, basic helix-loop-helix transcription factor

NAA10, N(alpha)-acetyltransferase 10, NatA catalytic subunit

NAFLD, non-alcoholic fatty liver disease

NBN, nibrin

NBOT, normobaric oxygen therapy

NCOA1, nuclear receptor coactivator 1

NCOA2, nuclear receptor coactivator 2

NDN, necdin, melanoma-associated antigen gene family member

NDRG1, N- myelocytomatosis downstream regulated 1

NEDD8, neural precursor cell expressed, developmentally down-regulated 8 ubiquitin like modifier

NEUROG3, neurogenin 3

NFAT5, nuclear factor of activated T cells 5

NFKB1, nuclear factor kappa B (NF-κB) subunit 1

NO, nitric oxide

NOS, nitric oxide synthase

NOS2, nitric oxide synthase 2

NOS3, nitric oxide synthase 3

NOTCH1, notch receptor 1

NOTCH3, notch receptor 3

NOTCH4, notch receptor 4

NOX4, NADPH oxidase 4

NQO1, NAD(P)H quinone dehydrogenase 1

NT5E, 5'-nucleotidase ecto

OCLN, occludin

OR, odds ratio

OS9, OS9 endoplasmic reticulum lectin

OTUD7B, OTU deubiquitinase 7B

PAI-1, plasminogen activator inhibitor 1

PARP1, poly (adenosine diphosphate-ribose) polymerase 1

PCGF2, polycomb group ring finger 2

PCNA, proliferating cell nuclear antigen

PDGFC, platelet derived growth factor C

PDK1, pyruvate dehydrogenase kinase I

PER1, period circadian regulator 1

PERK, protein kinase R-like like endoplasmic reticulum kinase

PET, positron emission tomography

PFKP, phosphofructokinase, platelet

PGF, placental growth factor

PGK1, phosphoglycerate kinase 1

PHD, prolyl hydroxylase domain proteins

PI3K, phosphatidylinositol 3-kinase

PIAS3, protein inhibitor of activated signal transducer and activator of transcription 3

PIN1, peptidylprolyl cis/trans isomerase, never in mitosis gene-interacting 1

PKC, protein kinase C

PKM, pyruvate kinase M1/2

PLAU, plasminogen activator, urokinase

PLAUR, plasminogen activator, urokinase receptor

PLD1, phospholipase D1

PLD2, phospholipase D2

PLIN2, perilipin 2

PNMT, phenylethanolamine N-methyltransferase

pO_2, oxygen partial pressure

POU5F1, pituitary specific 1-octamer transcription factor-uncoordinated 86 (Pit-Oct-Unc) class 5 homeobox 1

PPARA, peroxisome proliferator activated receptor alpha

PPARG, peroxisome proliferator activated receptor gamma

PRKDC, protein kinase, DNA-activated, catalytic subunit

PRKN, parkin ring-between-ring E3 ubiquitin protein ligase

PROM1, prominin 1

PROX1, prospero homeobox 1

PSMA7, proteasome 20S subunit alpha 7

PSMC3, proteasome 26S subunit, ATPase 3

PSMD10, proteasome 26S subunit, non-ATPase 10

PTBP1, polypyrimidine tract binding protein 1

PTGS2, prostaglandin-endoperoxide synthase 2

PTK6, protein tyrosine kinase 6

PTPRB, protein tyrosine phosphatase receptor type B

RAC1, Rac family small guanosine triphosphatase 1

RACGAP1, Rac guanosine triphosphatase activating protein 1

RACK1, receptor for activated C kinase 1

RB1, RB transcriptional corepressor 1

RBM38, RNA binding motif protein 38

RELA, reticuloendotheliosis viral homolog A proto-oncogene, NF-kB subunit

RHBDF1, rhomboid 5 homolog 1

RHOBTB3, Rho related broad complex-tramtrack-bric a brac domain containing 3

RNS, reactive nitrogen species

RORC, retinoic acid-related related orphan receptor C

ROS, reactive oxygen species

RPTOR, regulatory associated protein of MTOR complex 1

RUNX1, Runt-related transcription factor family transcription factor 1

RWDD3, ring finger and tryptophan-aspartic acid (WD) domain containing 3

SAHS, sleep apnea-hypopnea syndrome

SAT1, spermidine/spermine N1-acetyltransferase 1

SAT2, spermidine/spermine N1-acetyltransferase family member 2

SENP1, SUMO specific peptidase 1

SENP3, SUMO specific peptidase 3

SERPINE1, serpin family E member 1 (PAI-1)

SHC1, Schmidt-Ruppin homology 2 domain-containing adaptor protein 1

SLC2A1, solute carrier family 2 member 1

SLC2A3, solute carrier family 2 member 3

SLC2A4, solute carrier family 2 member 4

SMAD3, mothers against decapentaplegic homolog family member 3

SMAD4, mothers against decapentaplegic homolog family member 4

SMARCA2, switch/sucrose non-fermentable related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 2

SMARCA4, switch/sucrose non-fermentable related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4

SNAI1, snail family transcriptional repressor 1

SNAI2, snail family transcriptional repressor 2

sO_2, saturation of oxygen

SOCS3, suppressor of cytokine signaling 3

SOD2, superoxide dismutase 2

SOST, sclerostin

SOX2, sex-determining region Y-box transcription factor 2

SOX9, sex-determining region Y-box transcription factor 9

SP1, specificity protein 1 transcription factor

SP7, specificity protein 7 transcription factor

SPHK1, sphingosine kinase 1

SQSTM1, sequestosome 1

SRC, SRC proto-oncogene, non-receptor tyrosine kinase

SREBF1, sterol regulatory element binding transcription factor 1

STAT3, signal transducer and activator of transcription 3

STC1, stanniocalcin 1

STIM1, stromal interaction molecule 1

STUB1, stress-induced-phosphoprotein 1 homology and U-box containing protein 1

SUMO1, small ubiquitin like modifier 1

T2D, type 2 diabetes

TAS, traceable author statement

TERT, telomerase reverse transcriptase

TET1, tet methylcytosine dioxygenase 1

TF, transcription factor

TFRC, transferrin receptor

TGFB1, transforming growth factor beta 1

TGM2, transglutaminase 2

TH, tyrosine hydroxylase

TIMP1, tissue inhibitor of metallopeptidase 1

TLR4, toll like receptor 4

TNF, tumor necrosis factor

TNFAIP6, tumor necrosis factor alpha induced protein 6

TNFRSF12A, tumor necrosis factor receptor superfamily member 12A

TNF-α, tumor necrosis factor alpha

TP53, tumor protein p53

TP63, tumor protein p63

TRAF6, tumor necrosis factor receptor associated factor 6

TRIM63, tripartite motif containing 63

TSGA10, testis specific 10

TWIST1, twist family basic helix-loop-helix transcription factor 1

TXNIP, thioredoxin interacting protein

Ub, ubiquitin

UBXN7, ubiquitin regulatory X domain protein 7

UCPs, uncoupling proteins

UPR, unfolded protein response

USP19, ubiquitin specific peptidase 19

USP20, ubiquitin specific peptidase 20

USP7, ubiquitin specific peptidase 7

VCAM-1, vascular cell adhesion molecule 1

VEGFA, vascular endothelial growth factor A

VEGFC, vascular endothelial growth factor C

VHL, von Hippel-Lindau tumor suppressor

VHLL, von Hippel-Lindau like

VIM, vimentin

VLDLR, very low-density lipoprotein receptor

WAT, white adipose tissue

WC, waist circumference

WWOX, tryptophan-tryptophan (WW) domain containing oxidoreductase

XPO1, exportin 1

ZC3H12A, zinc finger cysteine-cysteine-cysteine-histidine (CCCH) type containing 12A

ZEB1, zinc finger E-box binding homeobox 1

ZEB2, zinc finger E-box binding homeobox 2

ZNF197, zinc finger protein 197

Table references

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Table 1. Major target genes and transcription factors associated to HIF-1α.

HIF-1α activity	Gene symbol
Cooperates (TF)	E2F7; E2F8; JUN; MTF1; NFKB1; SMAD4; SP7
Interacts (TF)	BHLHE41; COPS5; CREB3L1; ELL; ELOB; ELOC; HDAC4; HDAC5; HDAC6; HDAC7; HIF1AN; HIF3A; HNF4A; IRF1; KAT2B; KAT5; KDM3A; KDM4C; MAFG; MDM2; MTA1; MYC; NCOA1; NCOA2; NEDD8; PCGF2; PER1; PIAS3; PRKDC; RB1; RELA; SMAD3; SMARCA2; SMARCA4; SP1; STAT3; TET1; TP53; TP63; TRAF6; VHL; ZNF197
Regulates (TF)	APEX1; BHLHE40; CCND1; CEBPA; CTNNB1; ESR1; ESR2; ESRRG; EZH2; FOXM1; FOXO3; HDAC2; HEY1; HEY2; ID1; KDM4B; LOXL2; MTDH; MXI1; NEUROG3; NFAT5; NOTCH1; PARP1; POU5F1; PPARA; PPARG; PROX1; RAC1; RORC; RUNX1; SNAI1; SNAI2; SOX2; SOX9; SREBF1; TWIST1; ZEB1; ZEB2
Interacts (gene)	COMMD1; CPEB2; CUL2; DCUN1D1; DNAJB1; EAF2; EGLN1; EGLN3; FBXW7; FTCD; HEXIM1; HSP90AA1; HSP90AB1; HSPA1L; HSPA4; HSPA8; IKBKG; LINC01139; LRRK2; MAPK3; MCM3; MCM7; NAA10; NBN; NDN; OS9; OTUD7B; PGK1; PIN1; PLD1; PLD2; PRKN; PSMA7; PSMC3; PSMD10; PTBP1; PTK6; RACGAP1; RACK1; RBM38; RHBDF1; RHOBTB3; RPTOR; RWDD3; SAT1; SAT2; SENP1; SENP3; SHC1; SQSTM1; SRC; STUB1; SUMO1; TSGA10; TXNIP; UBXN7; USP19; USP20; USP7; VHLL; WWOX; XPO1; ZC3H12A
Regulates (gene)	ABCB1; ABCG2; ACAN; ADM; ADORA2A; AFP; AGR2; AGT; AK1; AKT1; ANG; ANGPT1; ANGPT2; ANGPTL4; APOE; AQP4; ARG1; AURKA; AXL; BAX; BBC3; BCL2; BCL2L1; BIRC5; BNIP3; BNIP3L; BSG; BTG2; CA9; CALCRL; CASP3; CCL2; CCL4; CCL5; CCN1; CCN2; CCR1; CCR5; CD44; CDH1; CDH2; CDKN1A; COL1A1; COX4I2; CP; CPT1A; CTSD; CXCL12; CXCL8; CXCR1; CXCR2; CXCR4; CYBB; DDIT4; DLL4; EDN1; EDN2; EDNRB; EIF4EBP1; ENTPD1; EPO; FABP4; FAM162A; FASN; FBXO32; FGF2; FLT1; FLT4; FN1; GAPDH; HK2; HMOX1; HOTAIR; IFNG; IGF2; IGFBP1; IGFBP3; IL10; IL1B; IL22; IL4; IL6; INHA; JAG1; KDR; KITLG; KLK3; KRT18; KRT19; LDHA; LEP; LGALS3; LIFR; LOX; MALAT1; MAPK1; MCL1; MDK; MIF; MIR101-1; MIR107; MIR146A; MIR17; MIR182; MIR20A; MIR20B; MIR21; MIR210; MIR27A; MIR29C; MIR373; MIR503; MMP1; MMP14; MMP2; MMP28; MMP3; MMP9; NDRG1; NOS2; NOS3; NOTCH3; NOTCH4; NOX4; NQO1; NT5E; OCLN; PCNA; PDGFC; PFKP; PGF; PKM; PLAU; PLAUR; PLIN2; PNMT; PROM1; PTGS2; PTPRB; SERPINE1; SLC2A1; SLC2A3; SLC2A4; SOCS3; SOD2; SOST; SPHK1; STC1; STIM1; TERT; TFRC; TGFB1; TGM2; TH; TIMP1; TLR4; TNF; TNFAIP6; TNFRSF12A; TRIM63; VEGFA; VEGFC; VIM; VLDLR

HIF-1α cooperates with, interacts with or regulates proteins encoded by these genes. TF: transcription factor. HIF-1α also interacts with and regulates other genes. Data obtained from MatBase Matrix Family Library (Version 8.3, Genomatix Software GmbH, Munich).

Table 2. Human HIF-1 associated Gene Ontology terms.

GO term	Evidence
Axonal transport of mitochondrion	IMP (201)
Cellular response to hypoxia	IDA (133, 201, 411)
Cellular response to interleukin-1	IEP (168)
mRNA transcription by RNA polymerase II	IC (389)
Negative regulation of gene expression	IMP (422)
Negative regulation of oxidative stress-induced neuron intrinsic apoptotic signaling pathway	IDA (275)
Oxygen homeostasis	IDA (152)
Positive regulation of angiogenesis	IC (101)
Positive regulation of blood vessel endothelial cell migration	IMP (422)
Positive regulation of chemokine production	TAS (60)
Positive regulation of chemokine-mediated signaling pathway	IC (398)
Positive regulation of endothelial cell proliferation	IC (101)
Positive regulation of erythrocyte differentiation	IC (337)
Positive regulation of gene expression	IDA (240, 338)
Positive regulation of glycolytic process	IC (336)
Positive regulation of hormone biosynthetic process	IDA (337)
Positive regulation of nitric-oxide synthase activity	TAS (146)
Positive regulation of pri-miRNA transcription by RNA polymerase II	IMP (209)
Positive regulation of receptor biosynthetic process	IMP (398)
Positive regulation of transcription from RNA polymerase II promoter in response to hypoxia	IDA (200)
Positive regulation of transcription, DNA-templated	IDA (29, 197, 336)
Positive regulation of vascular endothelial growth factor production	IDA (101)
Positive regulation of vascular endothelial growth factor receptor signaling pathway	IC (101, 168)
Regulation of gene expression	IDA (202)
Regulation of transcription from RNA polymerase II promoter in response to oxidative stress	IDA (336, 390)

Regulation of transcription, DNA-templated	IDA (184, 402)
Regulation of transforming growth factor beta 2 production	IMP (419)
Response to hypoxia	IDA (29, 101, 402)
Response to iron ion	IEP (236)
Signal transduction	IMP (419)
Transcription by RNA polymerase II	IMP (313)
Vascular endothelial growth factor production	IDA (168)

Gene Ontology (GO) term and reference with evidence codes defined by the GO Consortium: Inferred from direct assay (IDA); inferred from expression pattern (IEP); inferred from mutant phenotype (IMP); inferred by curator (IC); traceable author statement (TAS). Data obtained from MatBase Matrix Family Library (Version 8.3, Genomatix Software GmbH, Munich).

Table 3. Criteria for definitions of the metabolic syndrome (MetS) in adults

	ATPIII (2001)	AHA/NHLBI (2004)	IDF (2005)	IDF-AHA/NHLBI (2009)
Criteria required	Any ≥ 3	Any ≥ 3	WC mandatory, plus ≥ 2	Any ≥ 3
Waist circumference	≥102 cm (men) ≥88 cm (women)	≥102 cm (men) ≥88 cm (women)	≥94 cm (men) ≥80 cm (women)	≥94 cm (men) * ≥80 cm (women) *
High-density lipoprotein	<40 mg/dl (men) < 50 mg/dl (women)	<40 mg/dl (men) < 50 mg/dl (women)	<40 mg/dl (men) < 50 mg/dl (women)	<40 mg/dl (men) < 50 mg/dl (women)
Triglycerides	≥150 mg/dl	≥150 mg/dl	≥150 mg/dl	≥150 mg/dl
Blood pressure	≥130/85 mmHg	≥130/85 mmHg	≥130/85 mmHg	≥130/85 mmHg
Blood glucose	≥110 mg/dl ‡	≥100 mg/dl	≥100 mg/dl	≥100 mg/dl

ATP III: Adult Treatment Panel III; AHA/NHLBI: American Heart Association/National Heart, Lung, and Blood Institute; IDF: International Diabetes Federation; IDF-AHA/NHLBI: Harmonized definition of IDF and AHA/NHLBI. *Waist circumference (WC) according to population and country-specific definitions, these values for Caucasian population. Any criterion is also met when there is a drug treatment specific for reducing the clinical condition. ‡ ATPIII has changed the cut-off for blood glucose in 2003 by matching with harmonized definition.

Table 4. Indications for Hyperbaric Oxygen Therapy

FDA approved uses of HBOT	Beneficial effect
Air or gas embolism	Resorption and elimination of the emboli
Carbon monoxide poisoning	Prevention of delayed neurological sequelae
Gas gangrene	Bacteriostatic properties
Crush injury	Counteract of cognitive impairments
Decompression sickness	Reduction in bubble size, correction of hypoxia
Arterial insufficiency	Improvement of oxygen tension around the wound
Severe anemia	Increase in blood oxygenation
Intracranial abscess	Prevention of anaerobic bacterial growth
Necrotizing infections	Reduction of tissue hypoxia
Osteomyelitis	Bacteriostatic effects
Delayed radiation injury	Promotion of wound healing
Compromised grafts and flaps	Enhance vascularization
Acute thermal burn injury	Reduction of edema, improved healing
Sudden hearing loss	Reverse hypoxia in the cochlear apparatus

FDA: Food and Drug Administration

Figure 1. The importance of oxygen. (A) Summary of oxygen accumulation during the history of the Earth. Estimated changes in the oxygen concentration in Earth's atmosphere over the last 4.000 million years. **(B)** Evolution of organisms (below) in time of their approximate appearance on Earth, which implies higher cellular complexity. **(C)** Iceberg paradigm: illustrative iceberg representation of the importance of considering oxygen as a nutrient. Iceberg tip contains the volume of classical nutrients included in the food pyramid: fluids and food. The underwater part represents the volume of oxygen inhaled by a standard human being. The expression "tip of the iceberg" illustrates a small part of a larger, unconsidered but vital nutrient. In terms of weight, the amount of fluids and food (48.23% of weight) and oxygen (51.77% of weight) consumed is similar.

Figure 2. Schematic illustration of human organ and tissue circulation and oxygenation. Upstream from the alveoli to downstream tissues. Normal values of blood flow distribution between organs at rest (percentage of cardiac output) and the partial pressure of oxygen (mmHg or equivalent percentage of total oxygen in the air). WAT: white adipose tissue.

Figure 3. Oxygen-dependent and independent regulation of HIF-1. In normal oxygen conditions (normoxia), HIF-1α is subject to oxygen-dependent prolyl hydroxylation by PHDs, which allows for ubiquitylation by the VHL complex. FIH asparaginyl hydroxylation of HIF-1α blocks the interaction between HIF-1 and the CBP/p300 complex. Polyubiquitylated HIF-1α is targeted for degradation by the proteasome. Under low oxygen tension (hypoxia), PHDs are unable to catalyze the hydroxylation at HIF-1α proline residues leading to its stabilization. FIH is incapable to hydroxylate an asparagine residue of HIF-1α under hypoxic conditions, allowing for CBP/p300 recruitment. The stable subunit is translocated to the nucleus, where it binds to HIF-1β. The heterodimer recognizes the HRE core consensus sequence in target genes, binds to coactivators as CBP/p300 and initiates transcription. Non-hypoxic stimuli (e.g. pro-inflammatory molecules) increase the transcription of the HIF-1α gene by a PKC-dependent pathway. The translation into HIF-1α protein is enhanced by the activation of the PI3K/mTOR pathway.

CBP p300: CREB-binding protein and p300 complex; FIH, factor inhibiting HIF-1; HREs: hypoxia-responsive elements; mTOR: mammalian target f rapamycin; PHD:

prolyl hydroxylase domain proteins; PI3K: phosphatidylinositol 3-kinase; PKC: protein kinase C; Ub: ubiquitin; VHL: von Hippel-Lindau protein.

Figure 4. Functional annotation clusters of HIF-1 regulated genes. Only the top 10 enriched categories are reported. Data obtained from Web-based Gene Set Analysis Toolkit (WebGestalt) and clustered according to GO term categories of **(A)** biological process and **(B)** molecular function. ROS: reactive oxygen species.

Figure 5. Functional annotation clusters of overlapping HIF-1 and NF-κB regulated genes. Only the top 10 enriched categories are reported. **(A)** Venn diagrams showing the overlap between HIF-1 and NF-κB regulated genes. **(B)** Analysis of overlapping HIF-1 and NF-κB regulated genes: GO term categories of biological process calculated by Web-based Gene Set Analysis Toolkit (WebGestalt). RNS: reactive nitrogen species; ROS: reactive oxygen species.

Figure 6. Overview of metabolic dysfunction. Based on epidemiological and biological data discussed in the text, the figure illustrates the dysfunction in metabolically active tissues associated with metabolic disorders.

Figure 7. Hypothesis of hypoxia-induced epigenetic regulation. The low oxygen consumption (air breathed) and tissue hypoxia might be associated with the levels of pro-inflammatory molecules via epigenetic marks. Specifically, DNA methylation changes in the promoter region of pro-inflammatory coding genes are proposed in this schematic diagram; however other epigenetic mechanisms cannot be excluded.

Figure 8. Schematic flow chart showing the potential mechanisms of intermittent hypoxia on cardiovascular, metabolic and neurodegenerative disorders. NO: nitric oxide; ROS: reactive oxygen species; EPO: erythropoietin. Adapted from Mateika, JH. and Komnenov D. 2017 and Quintero, P. *et al*. 2010.

Figure 9. Association between altitude and metabolic disorders. Studies are listed by increasing altitude level. m: meters; HR: Hazard ratio; OR: odds ratio; Diabetes T2: type 2 diabetes; c-HDL: high-density lipoprotein cholesterol.

Figure 10. Altitude levels and associated atmospheric conditions. Classification based on the balance between the benefits and risks of altitude. The cutoff point between high, very high and extreme altitude may differ among studies. This limit is set by the manifestation of the first symptoms of high-altitude sickness (includes cerebral syndromes of acute mountain sickness, high-altitude cerebral and pulmonary edema syndromes). The death zone is over 8,000 meters, where the availability of oxygen is insufficient to sustain human life for a prolonged time span.

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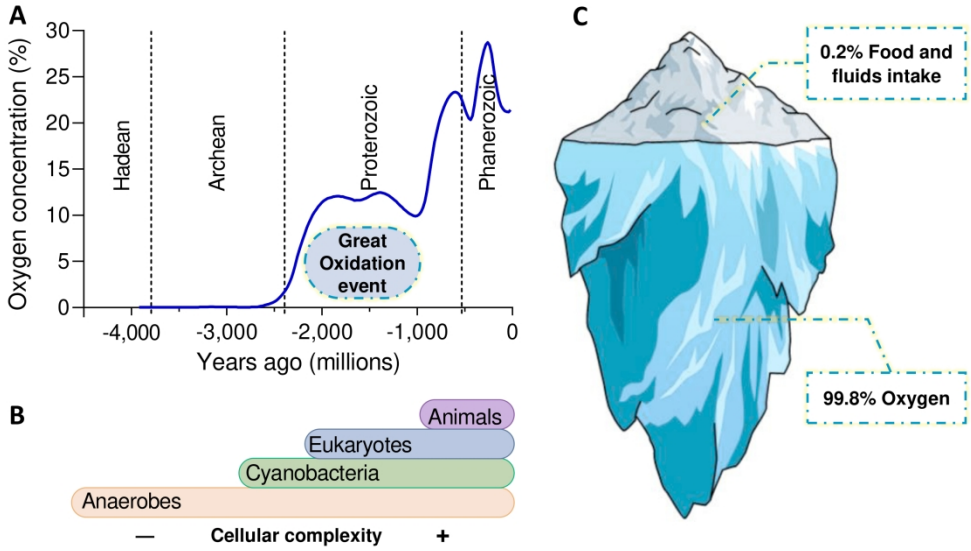

Figure 1. The importance of oxygen. (A) Summary of oxygen accumulation during the history of the Earth. Estimated changes in the oxygen concentration in Earth's atmosphere over the last 4.000 million years. (B) Evolution of organisms (below) in time of their approximate appearance on Earth, which implies higher cellular complexity. (C) Iceberg paradigm: illustrative iceberg representation of the importance of considering oxygen as a nutrient. Iceberg tip contains the volume of classical nutrients included in the food pyramid: fluids and food. The underwater part represents the volume of oxygen inhaled by a standard human being. The expression "tip of the iceberg" illustrates a small part of a larger, unconsidered but vital nutrient. In terms of weight, the amount of fluids and food (48.23% of weight) and oxygen (51.77% of weight) consumed is similar.

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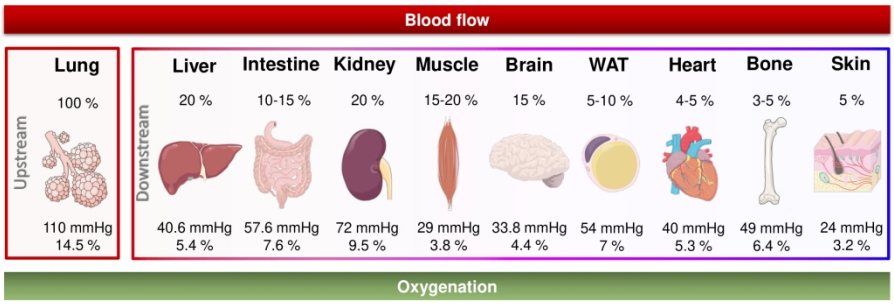

Figure 2. Schematic illustration of human organ and tissue circulation and oxygenation. Upstream from the alveoli to downstream tissues. Normal values of blood flow distribution between organs at rest (percentage of cardiac output) and the partial pressure of oxygen (mmHg or equivalent percentage of total oxygen in the air). WAT: white adipose tissue.

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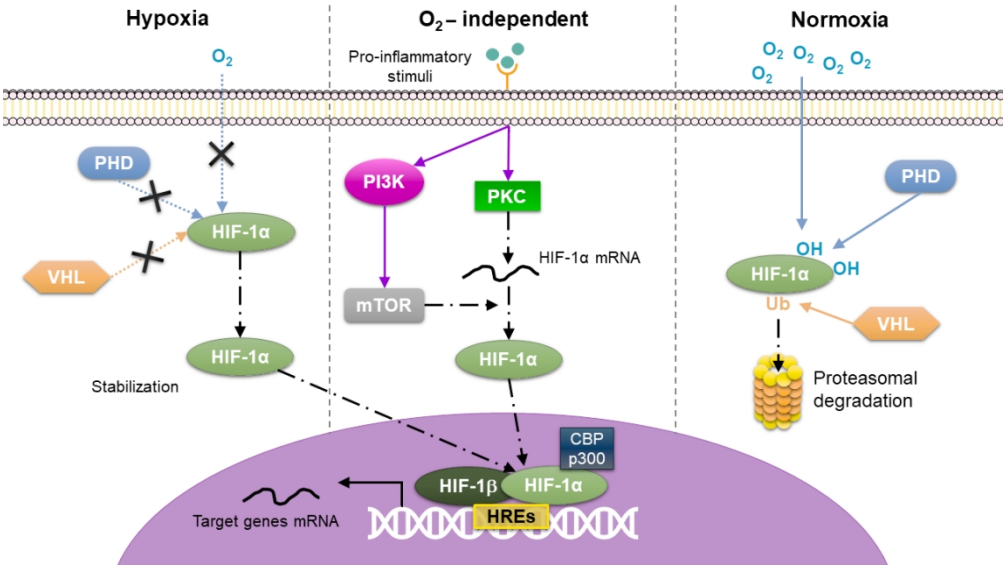

Figure 3. Oxygen-dependent and independent regulation of HIF-1. In normal oxygen conditions (normoxia), HIF-1α is subject to oxygen-dependent prolyl hydroxylation by PHDs, which allows for ubiquitylation by the VHL complex. FIH asparaginyl hydroxylation of HIF-1α blocks the interaction between HIF-1 and the CBP/p300 complex. Polyubiquitylated HIF-1α is targeted for degradation by the proteasome. Under low oxygen tension (hypoxia), PHDs are unable to catalyze the hydroxylation at HIF-1α proline residues leading to its stabilization. FIH is incapable to hydroxylate an asparagine residue of HIF-1α under hypoxic conditions, allowing for CBP/p300 recruitment. The stable subunit is translocated to the nucleus, where it binds to HIF-1β. The heterodimer recognizes the HRE core consensus sequence in target genes, binds to coactivators as CBP/p300 and initiates transcription. Non-hypoxic stimuli (e.g. pro-inflammatory molecules) increase the transcription of the HIF-1α gene by a PKC-dependent pathway. The translation into HIF-1α protein is enhanced by the activation of the PI3K/mTOR pathway.
CBP p300: CREB-binding protein and p300 complex; FIH, factor inhibiting HIF-1; HREs: hypoxia-responsive elements; mTOR: mammalian target f rapamycin; PHD: prolyl hydroxylase domain proteins; PI3K: phosphatidylinositol 3-kinase; PKC: protein kinase C; Ub: ubiquitin; VHL: von Hippel-Lindau protein.

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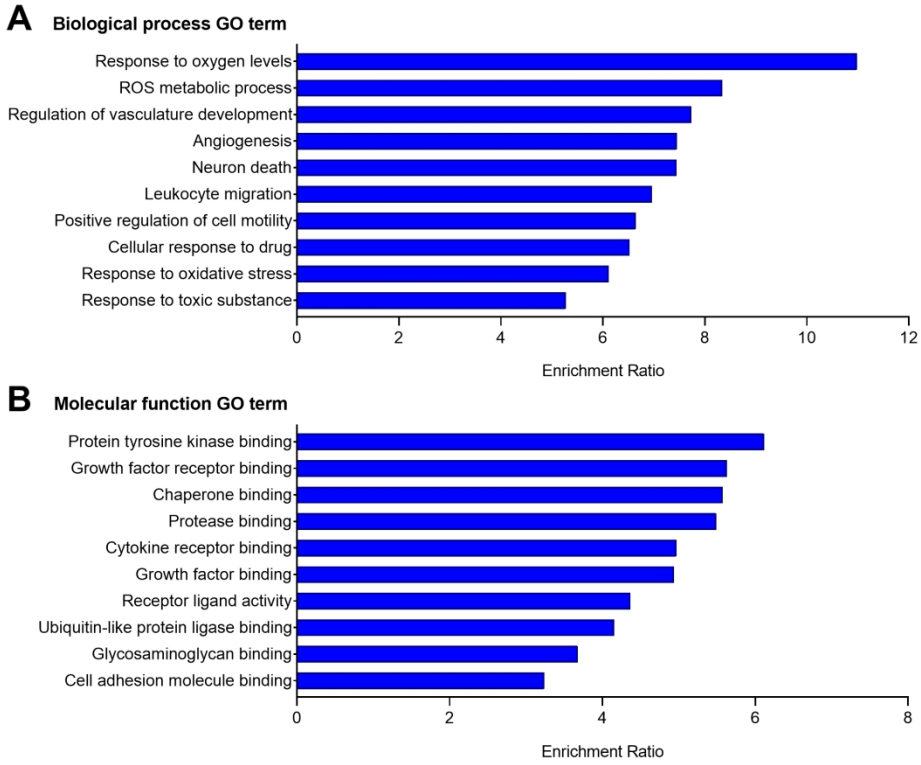

Figure 4. Functional annotation clusters of HIF-1 regulated genes. Only the top 10 enriched categories are reported. Data obtained from Web-based Gene Set Analysis Toolkit (WebGestalt) and clustered according to GO term categories of (A) biological process and (B) molecular function. ROS: reactive oxygen species.

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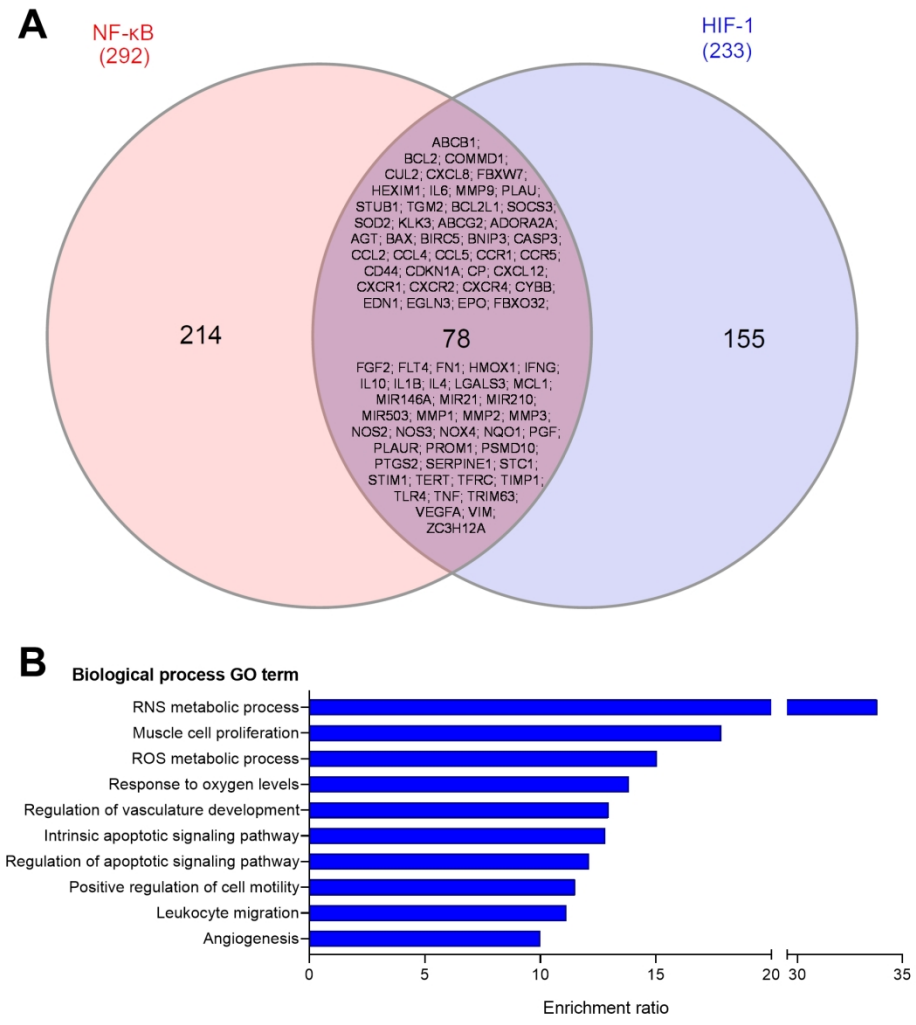

Figure 5. Functional annotation clusters of overlapping HIF-1 and NF-κB regulated genes. Only the top 10 enriched categories are reported. (A) Venn diagrams showing the overlap between HIF-1 and NF-κB regulated genes. (B) Analysis of overlapping HIF-1 and NF-κB regulated genes: GO term categories of biological process calculated by Web-based Gene Set Analysis Toolkit (WebGestalt). RNS: reactive nitrogen species; ROS: reactive oxygen species.

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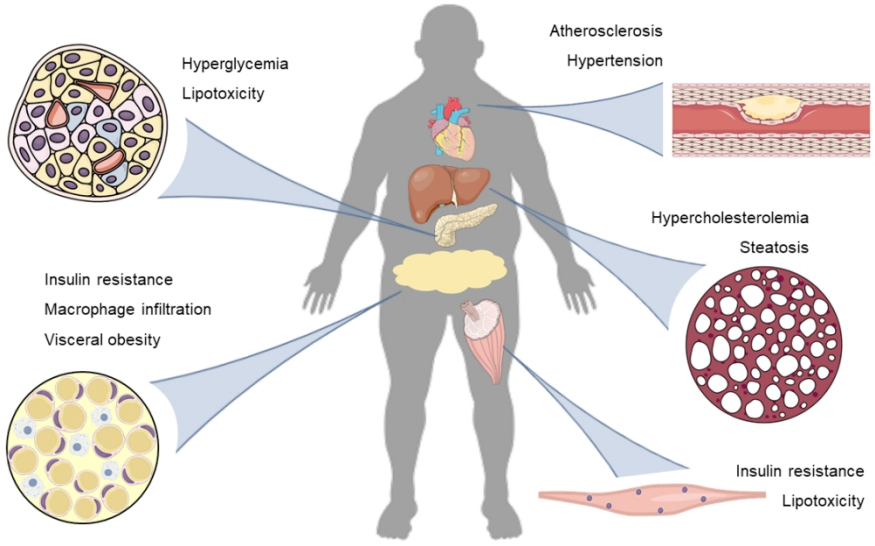

Figure 6. Overview of metabolic dysfunction. Based on epidemiological and biological data discussed in the text, the figure illustrates the dysfunction in metabolically active tissues associated with metabolic disorders.

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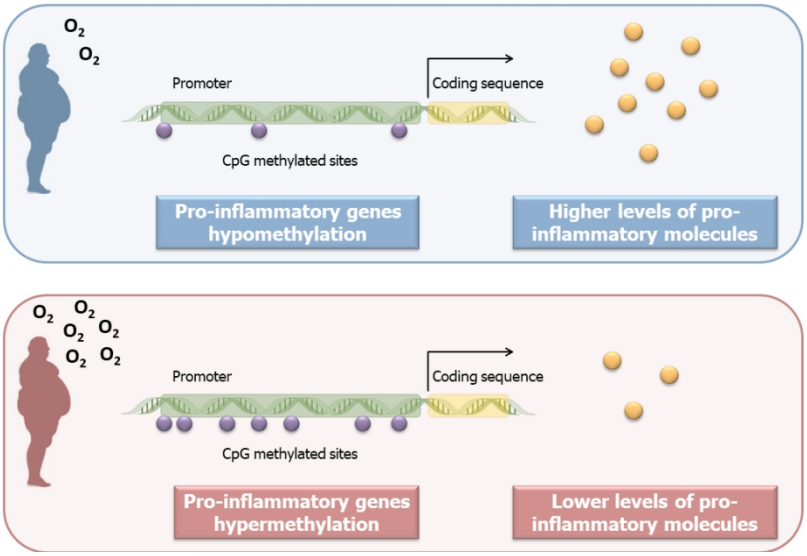

Figure 7. Hypothesis of hypoxia-induced epigenetic regulation. The low oxygen consumption (air breathed) and tissue hypoxia might be associated with the levels of pro-inflammatory molecules via epigenetic marks. Specifically, DNA methylation changes in the promoter region of pro-inflammatory coding genes are proposed in this schematic diagram; however other epigenetic mechanisms cannot be excluded.

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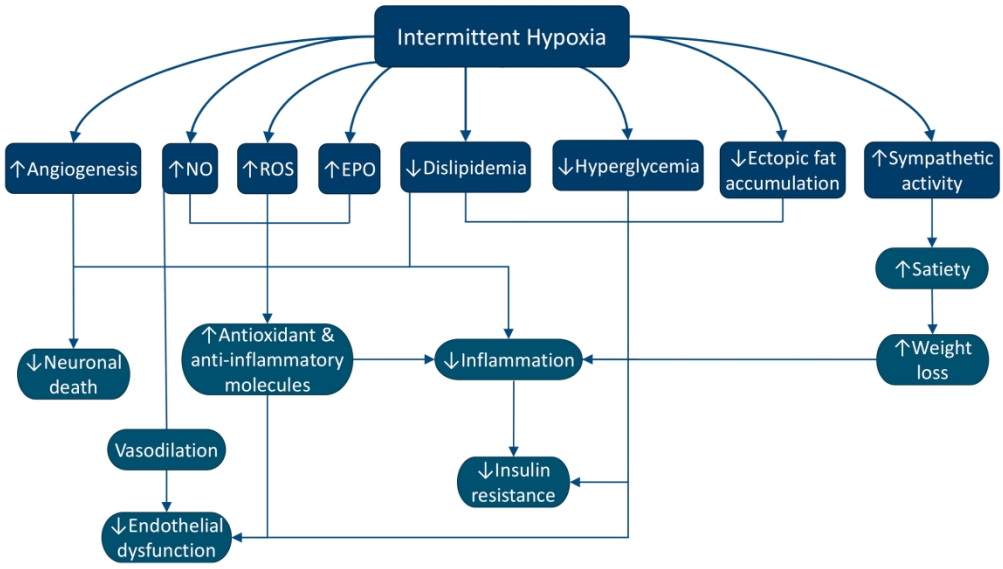

Figure 8. Schematic flow chart showing the potential mechanisms of intermittent hypoxia on cardiovascular, metabolic and neurodegenerative disorders. NO: nitric oxide; ROS: reactive oxygen species; EPO: erythropoietin. Adapted from Mateika, JH. and Komnenov D. 2017 and Quintero, P. et al. 2010.

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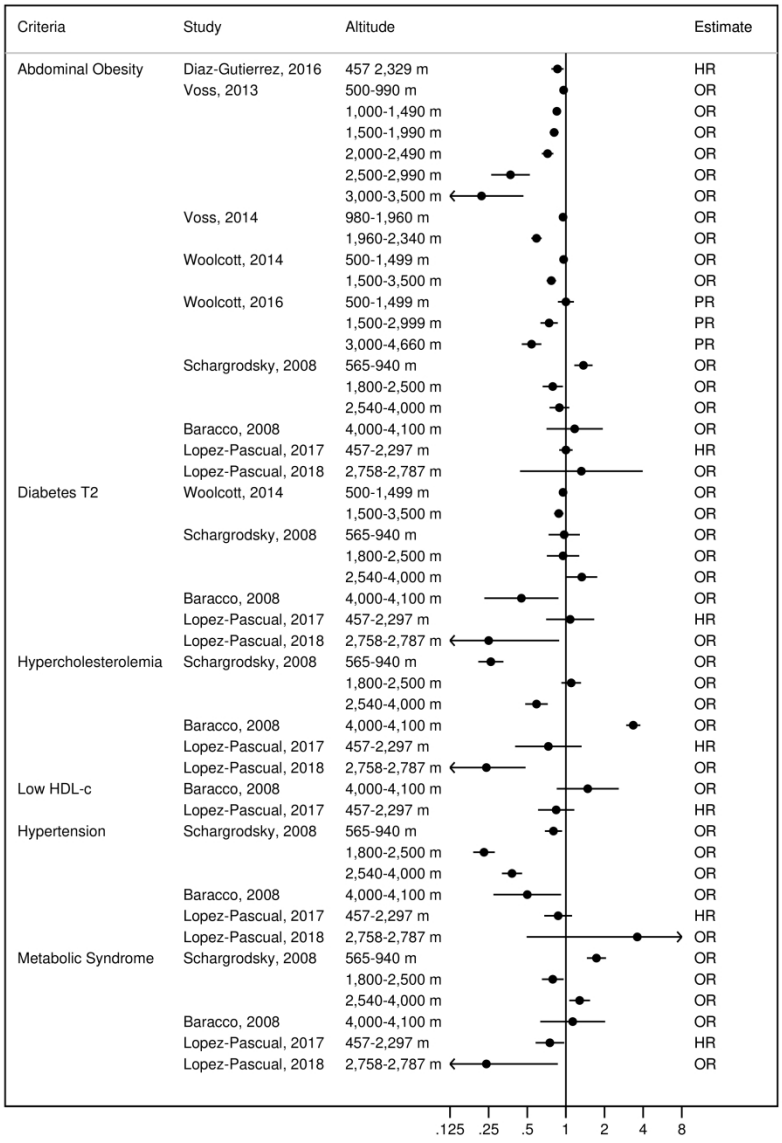

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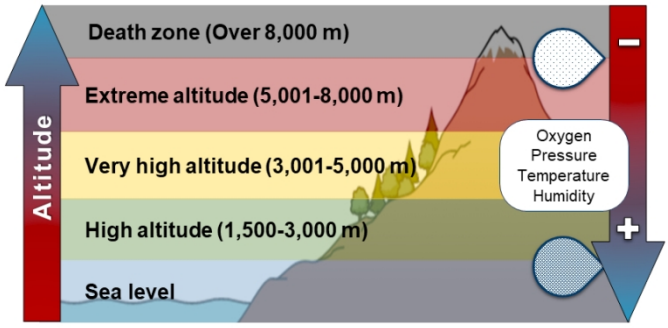

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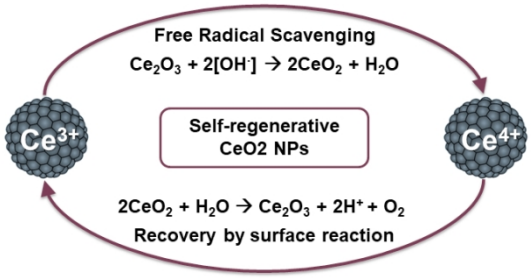

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338x190mm (96 x 96 DPI)

Comment to the Author

The manuscript has been considerably ameliorated. I am satisfied by the response and corrections of the authors. I believe that a suitable revised version of the manuscript is publishable. I found few minor issues that need to be addressed.

Thank you very much for all the comments made to improve the manuscript. We have uploaded the revised version including all the suggested changes.

Minor issues:

Page 8, line 36-47. "To address these cases of histotoxic hypoxia, the allotopic expression of an alternative oxidase (AOX) protein bypasses the cytochrome segment of the respiratory chain. This AOX feature confers resistance....." This sentence seems not clear and report redundant concepts, furthermore, since the authors referred to articles in which AOX has been expressed by allotopic and xenotopic techniques, in several *in vitro* (cells*)* and *in vivo* (mice) models, I would suggest to simply rephrase in: "Noteworthy, the expression of alternative oxidase (AOX) from the ascidian *Ciona intestinalis*, confers resistance to antimycin A and cyanide, bypassing the cytochrome segment of the respiratory chain (115, 134, 374). AOX also inhibits the mitochondrial membrane hyperpolarization, and the superoxide overproduction induced by acute hypoxia in pulmonary artery smooth muscle cells, avoiding the subsequent pulmonary vasoconstriction in mice (365). To this regard, a chemically modified RNA encoding a humanized AOX has been recently generated as a therapeutic route (PMID: 33664504)."

We appreciate the suggestion and have changed the relevant part of the article for a better understanding (page 8).

Page 8, line 28-29. Cyanide is not an uncoupling agent. It is an inhibitor of the mitochondrial complex IV (cytochrome c oxidase). Authors should remove "uncoupling agent".

We understand the reviewer's concern; "uncoupling agent" has been removed and the paragraph in question modified (page 8): "Cyanide is a potent inhibitor of the cytochrome c oxidase (mitochondrial complex IV)".

Page 86, line 22-47. Reference n. 365 is wrongly reporting multiple authors. It needs to be corrected.

Thank you for pointing this out. We have corrected the error.

Reviewer(s)' Comments to Author:

Reviewer: 2

Comments to the Author

(There are no comments.)

Reviewer: 1

Comments to the Author

(There are no comments.)

Reviewer: 3

Comments to the Author

Please correct:

"inhibits complex I of the mitochondrial respiratory chain in all cell types by blocking the mitochondrial NADPH dehydrogenase activity" is not a correct statement as complex 1 is an NADH:ubiquinone oxidoreductase.

We appreciate the reviewer's concern and have changed the sentence to (page 8): "inhibits the complex I (NADH:ubiquinone oxidoreductase) of the mitochondrial respiratory chain in all cell types by inhibiting the ubiquinone-dependent oxidation of mitochondrial NADH to NAD (353, 420)."

Cyanide prevents transport of electrons from cytochrome c to oxygen, the use of the term uncoupler is rather unspecific here.

We have removed "uncoupling agent" (page 8): "Cyanide is a potent inhibitor of the cytochrome c oxidase (mitochondrial complex IV)".

The HIF-2α subunit has a similar amino acid sequence compared to the isoform 1, but acting in a different manner on phenotypes upon inactivation (253). Correct your grammar.

Thank you for your comment. We have corrected the sentence (page 10): "The HIF-2α subunit has a similar amino acid sequence compared to the isoform 1, but acting in a different manner on phenotypes upon inactivation".

Reviewer: 6

Comments to the Author

(There are no comments.)

Reviewer: 8

Comments to the Author

The authors have properly considered the criticism, answered to the raised questions and added new information.

1. Although inclusion of oxygen among nutrients is controversial, we are all aware that oxygen is an essential molecule for all aerobic organisms and this is discussed in the text. Therefore, the authors might maintain the title of the last version of the manuscript which does not report the term "nutritional".

2. The number of references essentially depends on the policy of the journal while the choice is up to the authors.

3. Authors have clarified the figures about the quantitative estimation of inhaled air/oxygen in respiration by adding new information.

As suggested, we will maintain the title without the term "nutritional". Thank you for your comments.

Minor points: first sentence on page 5 "…average adult, when resting, averages 12 breaths..." might be improved avoiding repetitions. In addition, Ref 135 should be completed with the indication of the Publisher and the edition number.

Following your suggestion, we have changed the sentence to (page 5): "On the other hand, a standard 70 kg-adult, when resting, averages 12".

Regarding the reference 135, we have updated the information to the latest version of the book.

3. Regarding the relationship involving the relative intake of fluids + food and oxygen reported Fig. 1C, authors have indicated both volumes and weights for fluids + food and oxygen either in the text and in Fig. 1C.

Thank you for all your comments.

11-May-2021

Oxygen in metabolic dysfunction and its therapeutic relevance
Original submission: 21-Oct-2019
Final Revised Submission: 11-May-2021
Date of Acceptance:
11-May-2021

Dear Dr. González Muniesa:

****COMPREHENSIVE INVITED REVIEW****

It is a pleasure to accept your manuscript entitled "Oxygen in metabolic dysfunction and its therapeutic relevance" in its current form for publication in Antioxidants and Redox Signaling.

You should expect to receive galley proofs from the ARS production office (Chandhini.Arun@westchesterpubsvcs.com) within 8 weeks from now.

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Thank you for submitting your quality work to ARS. On behalf of the Editors of Antioxidants and Redox Signaling, we look forward to your continued contributions to the Journal.

Sincerely,
Chandan Sen
Editor-in-Chief, *Antioxidants and Redox Signaling*
cksen@iu.edu