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MULTI-AUTHOR REVIEW

The exercising heart at altitude

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Abstract Maximal cardiac output is reduced in severe acute hypoxia but also in chronic hypoxia by mechanisms that remain poorly understood. In theory, the reduction of maximal cardiac output could result from: (1) a regulatory response from the central nervous system, (2) reduction of maximal pumping capacity of the heart due to insufficient coronary oxygen delivery prior to the achievement of the normoxic maximal cardiac output, or (3) reduced central command. In this review, we focus on the effects that acute and chronic hypoxia have on the pumping capacity of the heart, particularly on myocardial contractility and the molecular responses elicited by acute and chronic hypoxia in the cardiac myocytes. Special emphasis is put on the cardioprotective effects of chronic hypoxia. (Part of a multi-author review.)

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Introduction

Maintaining oxygen homeostasis is critical for survival and proper function of cells and organisms. During exercise, systemic O_2 demand is augmented due to increased metabolic rate in the active skeletal muscles, the respiratory muscles, and the heart. This increase in systemic O_2 demand is fulfilled by increasing systemic O_2 delivery and O_2 extraction. Systemic O_2 delivery depends on the arterial oxygen content and cardiac output. During submaximal exercise, systemic O_2 demand and O_2 delivery are well matched, and for a given metabolic rate (oxygen uptake: $\dot{V}O_2$), cardiac output increases with the reduction in arterial oxygen concentration (CaO_2) [1–5]. Conversely, cardiac output (Q) is reduced when the CaO_2 is increased either by raising blood hemoglobin concentration [2, 6–9] or with hyperoxia [5, 10]. Since hemoglobin (Hb) saturation is, in general, maintained close to its maximum even at maximal exercise, maximal cardiac output (Q_{max}) is the principal factor determining maximal O_2 delivery and, hence, exercise capacity at sea level [7, 11–14]. During exercise at altitude, SaO_2 falls as exercise intensity increases, and thus cardiac output is even more critical to assure appropriate O_2 delivery [15, 16]. With altitude acclimatization, [Hb] increases and, after few weeks of residence at altitude, [Hb] reaches values similar to those observed in altitude natives [17–19]. This increase is sufficient to offset the decrease in arterial O_2 saturation (SaO_2) caused by the reduced inspired PO_2 and thus restore arterial O_2 concentration to [17] or even above sea level values [20]. Consequently,

during submaximal exercise at a given absolute intensity, cardiac output is increased in acute hypoxia compared to the value observed in normoxia, so as to offset the reduction in CaO_2 and to maintain systemic O_2 delivery at the same level as in normoxia. In altitude acclimatized humans, cardiac output during submaximal exercise is similar to that observed at sea level [21].

The arterial blood pressure response to dynamic whole body exercise in acute hypoxia is similar (or slightly reduced) compared to that observed in normoxia [1, 15]. Thus, for a given cardiac output, the work of the left heart is essentially similar in normoxia and hypoxia. However, for a given absolute workload or VO_2, the work of the heart is increased during exercise in acute hypoxia due to need of a higher cardiac output to compensate for the reduction in CaO_2.

In chronic hypoxia, resting blood pressure and sympathetic nerve activity are increased [20, 22]. During exercise, mean arterial pressure is slightly higher in chronic as compared to acute hypoxia, implying that for a given cardiac output the work of the left heart is increased in chronic compared to acute hypoxia or normoxia [23, 24]. Nevertheless, for a given absolute exercise intensity or VO_2, in chronic hypoxia the work of the heart is lower than in acute hypoxia.

During exercise in acute hypoxia with a large muscle mass (i.e., at least the muscle mass of both lower extremities being recruited, i.e., upright bicycling exercise), the pumping capacity of the heart is apparently similar to that observed in normoxia [25]. In fact, at exhaustion, maximal cardiac output (Q_{max}) is the same in normoxia and in acute hypoxia [26]. However, in severe acute hypoxia (i.e., at altitudes above 4,500 m), Q_{max} is reduced and hence systemic O_2 delivery is severely affected because not only SaO_2 is reduced but also maximal cardiac output [15, 23]. Since the seminal study by Pugh, it is known that Q_{max} is reduced in chronic hypoxia [27]. This response is also observed in Andean natives living at 4,100 m [18] and in well-acclimatized lowlanders living at 5,260 m [21]. The mechanisms leading to the reduction in Q_{max} in acute and chronic hypoxia are poorly understood. In theory, the reduction of maximal cardiac output could result from: (1) a regulatory response from the central nervous system, i.e., hypoxia could blunt the cardiovascular drive from the CNS, (2) reduction of maximal pumping capacity of the heart due to insufficient coronary oxygen delivery prior to the achievement of the normoxic maximal cardiac output, or (3) reduced central command, i.e., as in hypoxia the maximal exercise intensity is lower in absolute terms, it is possible that a lower recruitment of motor units is paralleled by lower activation of the cardiovascular nuclei and, hence, stimulation of the heart to pump maximally. In this review, we will focus on the effect that acute and chronic hypoxia may have on the pumping capacity of the heart, particularly on myocardial contractility and the molecular responses elicited by acute and chronic hypoxia in the cardiac myocytes.

Reduction of maximal cardiac output in severe acute hypoxia

The mechanism responsible for the reduction in peak cardiac output in severe acute hypoxia is likely linked to low PaO_2, since maximal cardiac output is not reduced in acute [7] or chronic anemia [28]. In principle, this reduction in cardiac output may represent a failure of the cardiovascular system or may be the result of regulatory mechanisms aimed at protecting either the heart itself or, more importantly, the central nervous system, from hypoxic damage [29] due to the risk of increased desaturation at very high cardiac outputs [16, 30]. Based on the model of pulmonary gas exchange of Piiper and Scheid [31], it can be predicted that pulmonary gas exchange will be impaired as cardiac output increases, particularly when pulmonary gas exchange has to occur at PO_2 which falls in the steepest region of the downslope of the hemoglobin O_2 dissociation curve. An increase of cardiac output could impair pulmonary gas exchange by reducing the time available for alveolar-end capillary diffusion equilibration [31–34]. Under these circumstances, a further elevation in cardiac output might result in no increase or, even worse, a deterioration of systemic O_2 supply. If this hypothesis is true, maximal O_2 delivery in severe acute hypoxia should be attained at a lower maximal cardiac output than in normoxia. We have hypothesized that a down-regulation of maximal cardiac output is likely mediated by PaO_2, and presumably CaO_2 and SaO_2, sensing mechanisms that adjust the output drive from cardiovascular nuclei in the central nervous system [15, 20]. The cardioinhibitory effect of hypoxia could also have been mediated by activation of the peripheral chemoreceptors which, through the release of NO, may attenuate the activation of presympathetic vasomotor neurons at the rostral ventrolateral medulla during hypoxia [35]. Hypoxia can be sensed directly by sympathoexcitatory reticulospinal vasomotor neurons of the rostral ventrolateral reticular nucleus of the medulla [36], which initiate the integrated response to hypoxia by activating neurons distributed elsewhere in the CNS.

Another mechanism unrelated to the heart itself that could explain a reduction in maximal cardiac output is an impairment of venous return and, hence, ventricular filling pressure [37]. Several factors may influence venous return during exercise, such as central blood volume, body posture, cardiac aspirating effects, venous vascular tone (venous capacitance), mean arterial pressure, the muscle pump, the respiratory pump, and cardiac output itself [38]. The action

of the respiratory pump is likely similar at maximal exercise or slightly lower if maximal exercise ventilation at exhaustion is reduced, as observed during whole body exercise under severe acute hypoxia conditions [15, 17]. The action of the muscle pump increases with exercise intensity and exerts an important influence on venous return and cardiac output [38–42]. The action of the muscle pump may be blunted during exercise in severe acute hypoxia simply due to the lower maximal power output attained in hypoxia compared to normoxia. However, it is more likely that hypoxia first attenuates increases in cardiac output which limits muscle oxygen delivery and power output, and in turn, the muscle pump and ventricular filling.

Direct effects of acute hypoxia on the heart

Myocardial hypoxia may be caused by a mismatch between myocardial O_2 demand and delivery (in general caused by insufficient coronary blood flow) and/or a reduction in arterial blood oxygen partial pressure. Although, ischemia (i.e., reduced tissue blood flow) causes hypoxia (reduced PO_2), both conditions are clearly different [43], since ischemia also causes a decrease in both supply of substrates and removal of metabolites. The effects of ischemia are usually more severe than hypoxia and typically include lactic acidosis due to anaerobic glycolysis, diminished mitochondrial energy production, and cell death [44]. Hypoxia elicits both direct and indirect effects on the heart, which are mediated by neurohumoral mechanisms.

A complete absence of oxygen (anoxia) ablates ATP synthesis leading to cell death by induction of apoptosis [45–47]. However, cells exposed to hypoxia may be able to maintain normal ATP synthesis and survive [48, 49]. Mammalian cells respond to hypoxia by activating transcription factors and, in particular, hypoxia-inducible factors, or HIFs [50, 51]. HIFs bind to hypoxia-responsive elements and consensus sequences in the promoter region of more than 100 genes, which activates the transcription of genes that allow the cell to adapt to and survive in the hypoxic environment [52]. Genes regulated by HIFs include glucose transporters that allow the cells to efficiently import glucose to continue generating ATP despite reduced nutrient availability [53], and genes that reorganize the microenvironment to facilitate oxygenation, such as vascular endothelial growth factor, which stimulates formation of new blood vessels [54].

Effects of acute hypoxia on myocardial contractility

The myocardium has a rather low anaerobic capacity and ATP turnover is very dependent on oxygenation [55]. Myocardial oxygen consumption increases linearly with heart work [56], and the increase in heart rate accounts for 50–70% of the increase in myocardial oxygen consumption during exercise [57]. Since myocardial oxygen extraction fraction at resting state is already high (70–80%) [58], the approximately sixfold increase in left ventricle myocardial oxygen demand in the transition from resting to heavy exercise is met principally by augmenting coronary blood flow (approximately fivefold), as hemoglobin concentration and oxygen extraction increase only modestly and only for exercise intensities above 70% of VO_{2max} [57, 59].

It has been demonstrated that when oxygen supply is critically reduced, causing a myocardial O_2-delivery-VO_2 mismatch, such that the oxygen demand exceeds the O_2 supply, the energy demands of myocardial contraction is reduced to match the diminished myocardial O_2 delivery [60]. Although hypoxia is thought to elicit a negative influence on myocardial contractility, several neural and humoral changes act conjointly to increase myocardial contractility in hypoxia, namely the increase in sympathetic activity and the release of apelin. In contrast, adenosine reduces myocardial contractility, whilst the production of nitric oxide may reduce or increase contractility depending on the micro-environmental circumstances.

Sympathetic activation

Activation of peripheral chemoreceptors causes positive inotropic effects in a working heart–brainstem preparation of the rat by a mechanism that is sympathetically mediated and attenuated by β-adrenoceptor blockade with atenolol [61]. This activation requires L-glutamate and ATP in the neurotransmission of the sympatho-excitatory component of the chemoreflex in the commissural nucleus tractus solitarii of awake rats and in the working heart–brainstem preparation [62]. The β-adrenergic inotropic effect is mediated by (protein kinase A) PKA-dependent phosphorylation of several proteins, such as L-type Ca^{2+} channels (which increases sarcolemmal Ca^{2+} entry), phospholamban (increases sarcoplasmic reticulum (SR) Ca^{2+} uptake and Ca^{2+} loading), and likely cardiac troponin I (cTnI) [63]. PKA-dependent cTnI phosphorylation increases cross-bridge cycling rate and maximum unloaded shortening velocity (V_{max}), which contributes to the lusitropic effects (acceleration of relaxation) of β-adrenergic stimulation [64, 65]. An increased shortening velocity also contributes to positive inotropy, particularly in the auxotonically contracting heart [63], since the power output of cardiac muscle is determined by the product of force and velocity [66].

Apelin

Apelin is a highly conserved 77 amino acid prepropeptide expressed in the endothelium of heart, kidney, and lung, whilst its G-protein-coupled receptor [the apelin-angiotension receptor-like 1 (APJ)] is expressed by myocardial

cells and some vascular smooth muscle cells [67–70]. Hypoxia, at least in part via HIF pathways, is a stimulus for the expression of apelin-APJ in heart and lung endothelial cells, leading to an increase in extracellular apelin protein content [71, 72]. Apelin has a potent positive inotropic effect and modulates systemic vascular resistance through nitric oxide-dependent signaling [73, 74]. Enhanced contractility can be a result of increased availability of Ca^{2+}, and/or increased Ca^{2+} responsiveness of the myofilaments, or both. The inotropic effect of apelin is in part mediated via an enhanced myofilament sensitivity to Ca^{2+} as apelin enhanced the activity of the sarcolemmal Na^+/H^+ exchanger NHE with consequent intracellular alkalinization, without affecting $[Ca^{2+}]$ transient amplitude [74]. But it has also been shown that apelin increases in force development are accompanied by increases in amplitudes of $[Ca^{2+}]_i$ transients [75]. Apelin is also a potent angiogenic factor required for normal vascular embryonic development [76], and could also have a role in hypoxia-induced capillarisation in heart submitted to chronic hypoxia [77]. Moreover, apelin (at 10 nM) increases conduction velocity in monolayers of cultured neonatal rat cardiac myocytes [74]. In vivo, apelin administration to rodents has a clear acute inotropic effect [78]. Administered chronically, it reduces left ventricular preload and afterload and increases contractile reserve without evidence of hypertrophy [78]. In addition, activation of the apelin pathway elicits arterial and venous vasodilation via a nitric oxide-dependent mechanism [70, 79, 80]. The precise effect that apelin may have in the hypoxic exercising human heart remains to be determined.

Nitric oxide

Hypoxia elicits the release of nitric oxide (NO) from the endothelium but also intra-cytoplasmatically, by direct and indirect mechanisms. NO is an ubiquitous intra- and intercellular signaling molecule principally generated by a family of NO synthases (NOSs), which catalyze the conversion of the amino-acid L-arginine to L-citrulline in a reaction that requires O_2 and cofactors. In addition, both deoxyhemoglobin [81] and deoxymyoglobin [82] have a nitrite reductase function which, under allosteric control, leads to the formation of NO from nitrite. Nitrite reduction by hemoglobin reaches maximal activity at the hemoglobin P_{50} (PO_2, at which hemoglobin saturation is 50%, i.e., close to a PO_2 value of 25 mmHg) [83]. This effect is likely more accentuated during intense exercise accompanied by lactic acidosis. Nitrite reduction is potentiated by protons, due to the fact that protons enhance the formation of nitrous acid increasing the reaction rate to a much greater extent than the nitrite reductase slowing effect attributable to the Bohr effect [83]. When in the cardiomyocyte oxygen concentration decreases to a value around the P_{50} of myoglobin (3.1 μM), myoglobin deoxygenates and reduces existing nitrite (present at a rather high concentration in the cardiomyocytes) to NO [84]. The nitrite reductase activity of myoglobin is enhanced at higher levels of hypoxia [83] and also when tissue pH drops [84].

NO mediates a number of hypoxic cell signaling responses including expression of hypoxia inducible factor 1 (HIF-1α) [85, 86], mitochondrial respiration and biogenesis [87–91], and angiogenesis [92, 93]. Rassaf et al. [82] recently reported that nitrite reduces myocardial oxygen consumption in response to hypoxia in wild-type but not in myoglobin knockout mice. NO also has effects on myocardial function which include the modulation of contractile function, energetics, substrate metabolism, cell growth, and survival [94, 95]. Endothelial nitric oxide synthase (eNOS or NOS3) is found in coronary and endocardial endothelial cells and cardiomyocytes [96, 97], whereas neuronal (nNOS or NOS1) is present in cardiac autonomic nerves and ganglia and cardiomyocytes [97–99]. eNOS and nNOS are expressed in distinct subcellular compartments in the cardiomyocyte [96, 99, 100] where they couple to distinct effector molecules [95]. The effect of NO is likely limited to the vicinity of its production site, since the diffusion distance of NO within cardiac myocytes is expected to be very short due to both a high cytoplasmic concentration of myoglobin (an NO scavenger) and an abundance of superoxide anions (particularly increased in acute hypoxia, but also in disease states), which can react with NO reducing its bioavailability [95]. Selective eNOS gene deletion ($eNOS^{-/-}$) enhances the inotropic response to β-adrenergic stimulation in vivo and in isolated hearts [101–103], but not in isolated LV myocytes [102, 104–106]. In agreement, the basal and isoproterenol-stimulated (inwards calcium current) I_{Ca} in LV myocytes from $eNOS^{-/-}$ mice do not differ from control mice [104, 105]. However, both contraction and isoproterenol-stimulated I_{Ca} are greater in LV myocytes from $nNOS^{-/-}$ mice both under basal conditions and in response to isoproterenol (a β-adrenergic agonist) [107, 108]. Thus, it seems that most of the physiological effects of eNOS-derived NO on myocardial contraction may be paracrine and require an intact endothelium. However, myocardial-specific overexpression of eNOS reduces β-adrenergic responses [109–112], suggesting that increased NO production in the heart may inhibit β-adrenergic inotropy, regardless of the localization (myocardial or endothelial) of its source [106]. Basal contraction is increased and relaxation (due to slower Ca^{2+} reuptake in the SR) is impaired in LV myocytes from $nNOS^{-/-}$ mice [106], implying that the negative inotropic and lusotropic effect is mostly mediated by NO produced by myocardial nNOS. In addition, deoxymyoglobin nitrite reductase elicits NO formation from nitrite, and this effect

is expected to be increased during maximal exercise under severe hypoxemia [83]. Moreover, it has been suggested that most of the NO produced in the hypoxic myocardium originates from the myoglobin nitrite reductase activity [83, 113].

The NO generated in the vicinity of the mitochondrion by deoxymyoglobin nitrite reductase activity inhibits mitochondrial respiration by binding to the heme a3/CuB center in cytochrome *c* oxidase [87]. This reduces myocardial $\dot{V}O_2$, aerobic ATP generation, and contractility [82]. Consequently, ROS production is also reduced. During maximal exercise in severe acute hypoxia, this mechanism could limit myocardial contractility and, hence, maximal cardiac output.

In contrast, NO may be implicated in the positive inotropic response to increased preload, i.e., the Frank–Starling response by NO-induced phosphorylation of thin filament protein troponin I (TnI) in its N-terminal [114], which amplifies the positive inotropic effects of stretch through increased crossbridge cycling [64], even if myofilament Ca^{2+} sensitivity is reduced by TnI phosphorylation [115].

Adenosine

Cardiac myocytes and endothelial cells produce and release adenosine in response to hypoxia or ischemia [116–120]. Adenosine may bind to four subtypes of cell surface adenosine receptors named A_1, A_{2A}, A_{2B}, and A_3 (A_1R, $A_{2A}R$, $A_{2B}R$, and A_3R, respectively). Adenosine receptors A_1 and A_{2A} are present in the cardiac myocytes. A_1 and A_3 receptors are negatively coupled to adenylyl cyclase, and A_2 subtypes are positively coupled to adenylyl cyclase [121]. Acting on A_1 receptors, adenosine elicits a negative dromotropic effect via blocking of the AV (atrioventricular) node, a negative chronotropic effect via depression of SA (sinoatrial) node and a negative inotropic effect [117, 122]. Systemic and local hypoxia causes endothelial cells to release adenosine, which acts back on endothelial A_1 receptors to induce vasodilatation [120]. Adenosine may also elicit vasodilation through the release of NO after binding to $A_{2A}R$ of smooth muscle and endothelial cells [123]. Activation of A_1R in cardiomyocytes reduces the contractile responsiveness of the myocardium to adrenergic stimulation [124]. In contrast, A_{2A}Rs have a direct positive inotropic effect [122, 125] by facilitating a greater response to adrenergic stimulation [122, 126] and an indirect effect by inhibiting the action of the A_1R [122, 127]. The anti-adrenergic action of A_1R is thought to be mediated through multiple signaling mechanisms involving a decrease in adenylyl cyclase activity [128], reduction in calcium transients [124, 127], and increased protein kinase C epsilon (PKCε) translocation [129].

Thus, several neurohumoral factors convey in the hypoxic myocardium, some with positive and others with negative inotropic effects. However, the pumping capacity of the heart does not seem to be impaired in healthy humans, studied either under severe acute hypoxia [15, 130] or after acclimatization to high altitude [131, 132]. During exercise in acute hypoxia, the adrenergic response to maximal exercise is similar to that observed in normoxia [15, 17, 130, 133], indicating a similar positive inotropic stimulation and presumably a similar sympathetic vasoconstrictor drive at maximal exercise in acute hypoxia and normoxia. Perhaps higher levels of hypoxia than those tested so far could cause a negative inotropic effect in healthy humans. However, when the level of acute hypoxia exceeds approximately 6,000 m, the degree of hypoxemia reached during exercise may result in syncope due to severe hypoxia in the CNS [134], making it rather difficult to study the specific effect of very severe hypoxia on the exercising heart itself.

Repeated exposures to severe acute hypoxia induce myocardial adaptations which are cardioprotective

In the late 1950s, epidemiological observations indicated that the incidence of myocardial infarction is lower in people living at high altitude close or above 4,000 m [135]. Moreover, in 1977, Mortimer et al. [136] reported a progressive decline in mortality from coronary heart disease in men residing at altitudes between 914 and 2,135 m. Animal studies provided strong experimental support for hypoxia-induced cardioprotection [137, 138]. Later, Murry et al. [139] demonstrated that, in dogs, four cycles of 5-min ischemia separated by reperfusion markedly limited infarct size induced by subsequent prolonged ischemia. This phenomenon termed *ischemic preconditioning* is the most powerful form of in vivo protection limiting the infarct size other than early reperfusion [140, 141]. This adaptation occurs in a biphasic pattern with an early phase, which develops very quickly (within a few minutes from the exposure to the stimuli), and lasts only 1–2 h, and a late phase, which develops more slowly (needing 12–24 h) but lasts 3–4 days [141]. Early preconditioning is more potent than delayed preconditioning in reducing infarct size and depends on adenosine, opioids and, to a lesser degree, on bradykinin and prostaglandins released during ischemia [141, 142]. These molecules activate G-protein-coupled receptors, initiate activation of K(ATP) channel, generate oxygen-free radicals, and stimulate a series of protein kinases, among which PKC plays a central role [143]. Several stimuli may lead to PKC activation, namely mild oxidative conditions presumably linked to an increase of the Ca^{2+}/phospholipid-independent kinase activity [144]. Both hypoxia and hypoxia/reoxygenation causes a rapid activation of Src family tyrosine kinases, p60c-src and p59c-fyn, which are upstream mediators of MAP kinase

activation [145]. The Src family of tyrosine kinases is also involved in the ROS-mediated transactivation of receptor tyrosine kinase [145]. Src kinases are known to interact with many signaling proteins including PKC and phosphatidylinositol-3-kinase [146]. Mitochondrial ROS production, PTEN (phosphatase and tensin *homologue) oxidation, and AKT phosphorylation are impaired in mice heterozygous for a null allele at the locus encoding HIF-1α [147], which has partial deficiency of HIF-1α. In these mice, early preconditioning is abrogated, implying that HIF-1α is necessary for the early phase of ischemic preconditioning [147]. Repeated episodes of ischemia/reperfusion have been associated with a ROS-dependent reduction of PPARα gene expression [148].

Late preconditioning essentially depends on newly synthesized proteins, which comprise iNOS, COX-2, manganese superoxide dismutase, and possibly heat shock proteins [140, 141]. However, the cardioprotective effect of chronic hypoxia is blunted by concomitant hypercapnia induced via increased CO_2 levels in the inspired air, possibly by interacting with ROS signaling pathways [149].

Effects of chronic hypoxia on the heart

Chronic hypoxia elicits functional and structural changes in the heart which facilitate oxygen diffusion from the coronary capillaries to the myocardial mitochondria [150]. Chronic hypoxia also increases glycolytic and antioxidant capacities, and enhances mitochondrial respiratory function to sustain and to increase the efficiency of mitochondrial energy production, to preserve myocardial contractility [43, 151]. The main source of ATP in the heart is oxidative phosphorylation which by transfer of electrons through a series of acceptor cytochromes generates a proton gradient within the inner mitochondrial membrane. The potential energy of this gradient is used to synthesize ATP. In the fasting adult mammalian heart, ~60–80% of the ATP is obtained from fatty acid oxidation [152]. The rest of the heart's ATP is derived from glucose and lactate in nearly equal proportions [43]. After uptake, glucose is rapidly phosphorylated to glucose-6-P, and cardiomyocyte glucose utilization is limited by the rate of this reaction [153]. Hypoxia, through HIF-1α, induces the expression of genes encoding for glycolytic enzymes and glucose transport proteins [154]. Overexpressing hexokinase in cardiomyocytes increases ATP generation in hypoxic cardiomycytes, reducing the damage caused by severe hypoxia [155]. High altitude natives like the Himalayan Sherpas and the Andean Quechuas display enhanced myocardial glucose uptake [156]. A shift from fatty acid to glucose oxidation improves heart efficiency, since the oxidation of glucose in mammalian heart is 12–14% more O_2 efficient than the metabolism of free fatty acids [43]. Chronic hypoxia may also increase the capacity of the heart to uptake and oxidize lactate [157, 158]. Importantly, resting plasma lactate concentrations and lactate turnover are increased in humans adapted to chronic hypoxia [159]. Increased lactate levels have an inhibitory effect on lipolysis [160]. The latter could contribute to reduce heart O_2 uptake, due to the fact that free fatty acids exert an uncoupling effect on oxidative phosphorylation, [161]. In addition, the activity of β-hydroxy-acyl-CoA dehydrogenase (fatty acid β-oxidation enzyme) and CPT1 is reduced in chronic hypoxia [162, 163], likely due to down-regulation of PPARα [164], retinoid X receptor α (RXRα) [165], and their target genes [166]. Thus, with chronic hypoxia, heart energy metabolism becomes more like the fetal pattern, i.e., decreased fatty acid and increased carbohydrate utilization [43].

Excessive ROS production in hypoxic cells may be prevented by two mechanisms [167]. First, by expression of PDK1 [PDH (pyruvate dehydrogenase) kinase] which phosphorylates and inactivates PDH, the mitochondrial enzyme that converts pyruvate into acetyl-CoA. In combination with the hypoxia-induced expression of LDHA (lactate dehydrogenase A), which converts pyruvate into lactate, PDK1 reduces the delivery of acetyl-CoA to the tricarboxylic acid cycle, thus reducing the levels of NADH and $FADH_2$ delivered to the electron-transport chain. Second, the subunit composition of COX is altered in hypoxic cells by increased expression of the COX4-2 subunit, which optimizes COX activity under hypoxic conditions, and by increased degradation of the COX4-1 subunit, which optimizes COX activity under aerobic conditions [168]. Hypoxia-inducible factor 1 controls the metabolic adaptation of mammalian cells to hypoxia by activating transcription of the genes encoding PDK1, LDHA, COX4-2, and LON, a mitochondrial protease that is required for the degradation of COX4-1 (see, for review, [167]). This pattern of adaptation has been confirmed in rats submitted to severe chronic hypoxia whose myocardial mitochondrial mass has been found to be reduced [169].

Chronic hypoxia increases cardiac tolerance to acute ischemia–reperfusion injury

Chronic hypoxia increases cardiac tolerance to acute ischemia–reperfusion injury, as evidenced by reduced myocardial infarction, improved recovery of contractile function, and limitation of ventricular arrhythmias [170–174], which persist for several weeks after resuming normoxic conditions [172, 175]. Chronic hypoxia increases myocardial tolerance to ischemia, and acute ischemic preconditioning increased the tolerance even further [176], although it has also been reported that the cardioprotective effects of chronic hypoxia and ischemic preconditioning

are not additive [177]. However, when the severity of hypoxia exceeds certain levels then adaptative responses are insufficient and/or inappropriate to elicit a healthy adaptation and, hence, pathological changes are elicited [178, 179]. For example, chronic hypoxia may result in pulmonary hypertension, increasing right ventricle afterload, and right heart hypertrophy, and lead to heart failure [179].

Chronic hypoxia and myocardial contractility

Chronic hypoxia may decrease myocardial contractility due to an alteration of intracellular Ca^{2+} ($[Ca^{2+}]_i$) homeostasis, such that the magnitude of the $[Ca^{2+}]_i$ transient in response to several inotropic factors is attenuated [180–182]. Chronic hypoxia ($F_IO_2 = 0.10$) is associated with reduced expression of and Ca^{2+} uptake by sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA), reduced release of Ca^{2+} via ryanodine receptors, and reduced extrusion of Ca^{2+} by Na^+/Ca^{2+} exchange in rats [183]. On the other hand, data collected during the 1998 CMRC's Chacaltaya Expedition have shown that chronic hypoxia increases sympathetic muscle nerve activity [20, 22] and cardiac norepinephrine spillover (a measure of cardiac sympathetic activity) (Kaijser, unpublished observations). Interestingly, myocardial hyperemia is limited by cardiac autonomic denervation [184] or by selective sympathetic denervation [185, 186] indicating that cardiac sympathetic overactivity could contribute to facilitate coronary vasodilation at rest and during exercise. Although circulating cathecholamines may also influence coronary blood flow during exercise [187], the weight of evidence is consistent with the concept that autonomic influences on the coronary circulation are principally neurally mediated [57]. In normoxia, β-adrenergic blockade causes a greater reduction of coronary flow than of myocardial oxygen consumption, resulting in increased oxygen extraction by the heart and demonstrating a direct feed-forward β-adrenergic vasodilator effect on the coronary vessels [185].

Sympathetic overactivity in chronic hypoxia is likely mediated by HIF-1, since it has been shown that the carotid bodies from mice that are heterozygous for a null (knockout) allele at the locus encoding HIF-1α appear histologically normal, but do not respond to continuous hypoxia or chronic hypoxia [188]. In contrast to wild-type littermates, when heterozygous-null mice are subjected to chronic intermittent hypoxia, they do not develop hypertension or increased levels of HIF-1, catecholamines, or ROS [188]. These findings led Semenza and co-workers to suggest the existence of a feed-forward mechanism in which chronic intermittent hypoxia-induced ROS activate HIF-1, which then promotes persistent oxidative stress, which may further amplify HIF-1 activation, with its consequent effects on gene expression [167, 188].

But is myocardial contractility impaired in altitude acclimatized humans? There is no single good measurement of myocardial contractility during maximal upright exercise in humans acclimatized to altitude. However, in series of studies with humans carried during the 1998 CMRC's Chacaltaya Expedition, it was shown that the maximal work attained by the heart during incremental exercise to exhaustion in healthy humans acclimatized to 5,260 m may be enhanced during isovolemic hemodilution despite reduced arterial CaO_2 and similar levels of hypoxia [189]. Likewise, by parasympathetic blockade with glycopyrrolate (a muscarinic blocker), it was shown that the heart is able to perform more work during maximal exercise in chronic hypoxia than that actually reached in the control condition (chronic hypoxia without glycopyrrolate), even when glycopyrrolate did not alter blood oxygenation [190]. Although some reduction in myocardial contractility may be present in chronic hypoxia, the experimental data obtained in Chacaltaya, Monte Rosa and during Operation Everest II, show that the reduction of maximal cardiac output observed in chronic hypoxia cannot be explained through changes in myocardial contractility or in the chronotropic response to exercise [1, 21, 23, 189–192]. The mechanisms and the rationals for the reduction of maximal cardiac output in chronic hypoxia remains to be deciphered.

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