Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Physiology

The many acid–base manifestations and consequences of hypoxia

Erik R Swenson[1,2,3]

Hypoxia evokes a spectrum of acid–base changes from alkalosis to acidosis and alters many responses to hypoxia at non-genomic and genomic levels, in part via altered hypoxia-inducible factor (HIF) metabolism. Healthy people at altitude and persons hyperventilating to non-hypoxic stimuli can raise arterial pH as high as 7.7. In these circumstances, alkalosis reduces sympathetic tone, blunts hypoxic pulmonary vasoconstriction and cerebral vasodilation, and increases hemoglobin-oxygen affinity. With severe hypoxia owing to profoundly low arterial O_2 content (hypoxemia) or poor perfusion (ischemia), metabolic and hypercapnic acidosis develops along with considerable lactate formation with pH falling to below 6.8. Although acidoses are considered deleterious to cell function and survival, they can be cytoprotective by various anti-inflammatory, anti-oxidant, and anti-apoptotic mechanisms. Attempts to correct acidosis under these circumstances concurrent with re-oxygenation efforts may be ill advised. This so-called 'pH paradox' or permissive acidosis may offer therapeutic possibilities. Rapidly growing cancers often outstrip their oxygen and nutrient delivery and metabolic waste disposal, thus limiting growth and metastatic potential. However, their excessive glycolysis and lactate formation may not necessarily represent oxygen insufficiency, but an attempt to provide sufficient amounts of small carbon intermediates to supply many synthetic pathways of cellular proliferation. In either case, there is expression and upregulation of many genes involved in acid–base homeostasis, in part by HIF-1α and HIF-2α signaling. Inhibition of these proteins or gene suppression may have important therapeutic application in cancer therapy.

Addresses
[1] Department of Medicine, University of Washington, USA
[2] Department of Physiology and Biophysics, University of Washington, USA
[3] Pulmonary, Critical Care and Sleep Medicine, Veterans Affairs Puget Sound Health Care System, S-111-PULM, 1660 South Columbian Way, Seattle, WA, 98108, USA

Corresponding author: Swenson, Erik R (eswenson@uw.edu)

Current Opinion in Physiology 2019, **7**:72–81

This review comes from a themed issue on **Hypoxia**

Edited by **Hesham Sadek** and **Gregg Semenza**

For a complete overview see the Issue and the Editorial

Available online 16th January 2019

https://doi.org/10.1016/j.cophys.2019.01.003

2468-8673/Published by Elsevier Ltd.

Introduction

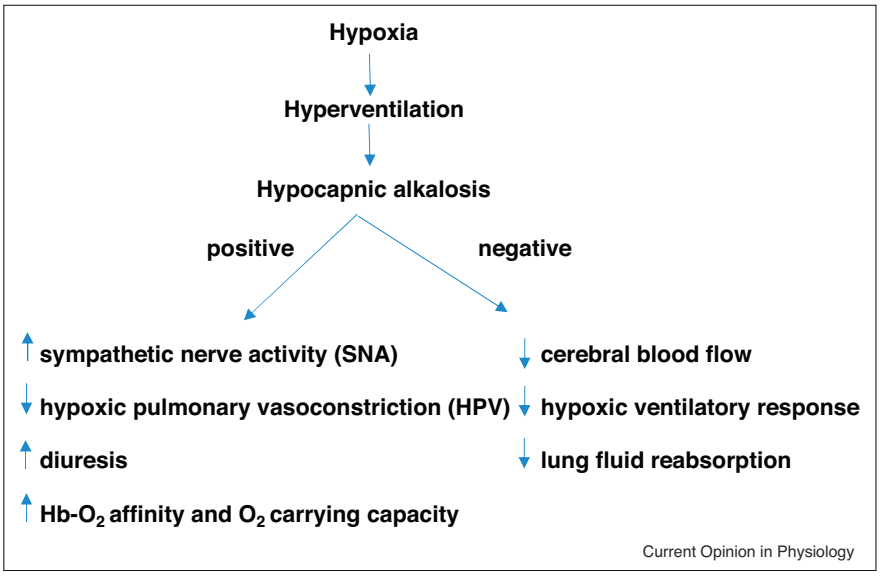
Depending upon its magnitude and causes, hypoxia evokes considerable effects on local and systemic acid–base status and organ/cellular function. These are mediated by pH changes and the reversible binding of protons to titratable amino acids of proteins, which affect their charge and secondary/tertiary structures, leading to altered activity of membrane receptors, channel proteins, enzymes, cell polarization, intracellular signaling events, and gene transcription and translation. Acid–base changes can alter hypoxia inducible factor (HIF-1α and 2α) metabolism, in hypoxia and normoxia. Thus, any understanding of responses to hypoxia without appreciating the accompanying acid–base changes will be less rigorous. I will discuss the association of hypoxia with different acid–base consequences, how these acid–base changes alter the magnitude and dynamic aspects of hypoxic responses, how HIF metabolism may be affected, how altered acid–base conditions may be deleterious or beneficial, and how the acid–base milieu might be manipulated to better treat cancer and hypoxic/ischemic injuries.

Hypoxia and respiratory alkalosis at high altitude and with early lung disease

High altitude hypoxia and lung diseases provoke hyperventilation (hypoxic ventilatory response or HVR) to raise arterial pO_2 and lower arterial/tissue pCO_2 and increase arterial/tissue pH. This is one of the most important compensatory responses for high altitude success and survival and possibly so in acute lung diseases. Healthy people at high altitude or persons hyperventilating to non-hypoxic stimuli can become quite alkalemic with arterial pH rising as high as 7.7 and $PaCO_2$ falling to as low 10 mmHg [1].

Respiratory alkalosis has many consequences for the responses to hypoxia in altitude adaptation beyond greater arterial oxygenation (Figure 1). The neural and CNS effects of hypoxia include increased sympathetic nervous activity (SNA), increased HVR, and increased cerebral blood flow. All are blunted by respiratory alkalosis and augmented by hypercapnic acidosis [2]. The alkalotic reduction of SNA decreases hypoxic pulmonary vasoconstriction (HPV), renal vasoconstriction, and sodium retention, responses which mitigate high altitude pulmonary edema (HAPE) and limit fluid retention. In contrast, hypercapnic hypoxia augments of the above hypoxic responses. Alveolar hypocapnia directly reduce pulmonary artery pressure and inhibit HPV [3]. Hypoxia reduces alveolar epithelial sodium and fluid reabsorption

Figure 1



Hypoxia at high altitude evokes hyperventilation and the generation of a hypocapnic acidosis with a variety of positive (beneficial) effects and negative effects on the sympathetic nervous system, pulmonary and cerebral hemodynamics, hypoxic ventilatory responsiveness, lung fluid balance, hemoglobin-oxygen affinity and blood oxygen content, and renal function.

by downregulation and internalization of apical Na^+–K^+ ATPase and basolateral epithelial sodium channels (ENaC), and this may be further aggravated by both hypocapnia and hypercapnia [4].

The responses of the pulmonary circulation and control of ventilation to hypoxia discussed above have only begun to be considered in the light of changes in HIF production at the vascular smooth muscle, endothelium, brain and chemoreceptors. The limited evidence from studies in mice with heterozygous HIF-1 deficiency reveals that HPV [5] and HVR [6] are blunted. Paradoxically, heterozygous HIF-2α deficiency increases HVR [7], thus pointing out the complexity of HIF expression and action with respect to the HIF subtype, organ, and function studied. Endothelial cell-specific deletion of HIF-2 alpha in mice prevents the development of hypoxia-induced hypertension, whereas specific deletion of HIF-1α in endothelial cells does not, nor does specific pulmonary vascular smooth muscle cell HIF-2α deletion [8]. HIF-1α also represses the expression of the cystic fibrosis transmembrane regulator (CFTR) in the intestine [9] and in lung [10], and this may contribute both to GI abnormalities and alveolar fluid clearance problems at high altitude.

It is conceivable that blunting of many hypoxic responses by alkalosis in normal animals and humans may be related to alkalotic suppression of HIF-1α levels and activity [11], but this needs to be more formally investigated. In contrast, acidosis independent of hypoxia increases HIF-1α levels and its gene transcriptional and protein translation [12] in part, by inducing nucleolar sequestration of von Hippel Lindau (VHL) protein, a factor critical to the targeting of HIF-1α for destruction [13]. Other effects of acidosis include upregulation of 2-hydroxyglutarate in normoxia and even more so in hypoxia, which inhibits the prolyl-hydroxylases (PHD) that target HIFs for degradation [14].

Respiratory alkalosis and hypocapnia increase hemoglobin-oxygen affinity allowing greater lung oxygen uptake. In terms of tissue O_2 delivery, increased O_2 content is clearly favorable. Countering the hypocapnic left shift of the hemoglobin-O_2 dissociation curve, is a rise in 2,3-diphosphoglycerate (2,3-DPG) elicited by alkalosis that reduces hemoglobin-oxygen affinity in humans to enhance tissue off-loading [15]. While hypercapnic and metabolic acidosis inhibits erythropoietin (EPO) formation, there does not appear to be a potentiation of EPO production by respiratory alkalosis directly or in those with a high HVR [16].

Respiratory alkalosis enhances glycolysis and lactate formation by stimulating phosphofructokinase (PFK) activity, the rate limiting enzyme in glycolysis. Under some circumstances such as severe hypoxia or ischemia, lactate may be a preferred fuel by critical cells such as neurons [17•], but whether this extends to more physiological state of hypocapnic hypoxia at altitude is unknown. Respiratory alkalosis induces sodium, potassium, and bicarbonate losses that contribute in part to the diuretic phase of persons adapting well at altitude. This renal

response (a compensatory metabolic acidosis) has the important effect of returning arterial pH back toward normal and mitigating the effect of alkalosis on reducing the full strength of HVR in defense of arterial oxygenation [18].

Acidosis and hypoxia with exercise

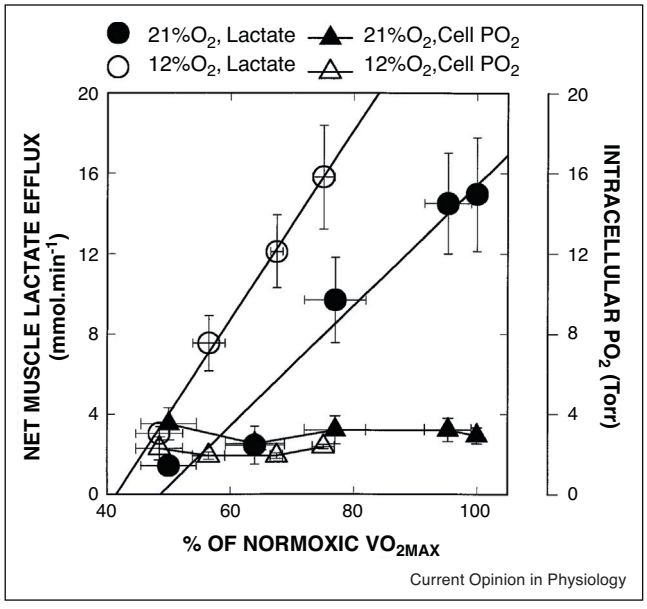
In heavy and maximal exercise 'lactic acidosis' does not appear to impose much limitation, despite winners crossing the finish line with pHs as low as 6.7 and lactates as high as 34 mM [19]. There may even be several benefits to the profound acidosis of heavy exercise. First, either hypercapnic or metabolic acidosis shifts the Hb–O_2 dissociation curve to the right to aid progressively more O_2 unloading. Intracellular acidosis helps to preserve muscle excitability when muscles become more depolarized due to rising extracellular potassium [20]. The generation of lactate acid and CO_2 in substantial amounts with heavy exercise (Figure 2), is not driven by any critical hypoxia at the respiring mitochondria [21] as is very commonly taught, but rather by ever-increasing sympathetic activity and rising catecholamine levels, acting to maintain a higher level of arterial blood pressure to support muscle perfusion and cerebral blood flow. Adrenergic activity also increases muscle sarcolemmal membrane Na^+/K^+ ATPase pump activity via local glycolytic production of ATP [22], which is necessary to counter the constant K^+ leak out of muscle with repetitive contractions [23] that can drive interstitial and arterial potassium as high as 13 mM and 8.0 mM, respectively.

Increased lactate generation may also support skeletal and cardiac muscle energetics, because its uptake into mitochondria by monocarboxylate transporter-1 (MCT-1) and oxidation in the Krebs cycle permits better maintenance of redox balance in cytosol and mitochondrial compartments. In addition, lactate itself may be an important signaling molecule for tissues under stress via changes in redox state [17•]. Acidosis and catecholamines help to limit the potential negative electro-physiological effects of exercise-induced hyperkalemia on the heart [24].

There has been a long-running debate whether lactate formation might also be beneficial since the reduction of pyruvate to lactate by lactate dehydrogenase is proton consuming and might limit the acidosis of exercise. The idea that lactate formation might reduce acidosis presupposes that acid production is the result of other H^+ producing reactions, such as ATP hydrolysis. However, with constancy of cellular ATP largely maintained, even at work rates generating lactate and lactic acidemia, any protons formed with ATP breakdown are consumed with ADP rephosphorylation, making it difficult to explain acidosis on this basis because it would require stoichiometric accumulation of ADP. With the complicated bookkeeping of all relevant buffers and H^+ producing/consuming reactions in exercising muscle, the idea that lactate formation reduces the total acid burden and is beneficial must remain speculative and a matter for lively debate [25•].

As mentioned above, while maximal exercise is not accompanied by any rate-limiting reduction in mitochondrial respiration and ATP generation during normoxic sea level exertion [21], nonetheless there is a progressive decline in muscle PO_2 sufficient to elicit elevations of both HIF-1α and HIF-2α. These HIF increases help to increase muscle capillarity and blood flow via vascular endothelial growth factor (VEGF)-mediated angiogenesis, upregulation and expression of vascular endothelial nitric oxide synthase, enhanced intermediary metabolism efficiency and glucose uptake [reviewed in Ref. 26]. A critical role of HIF-1α in training and endurance is evident in a mouse model of skeletal muscle selective knockout of HIF-1α, in which the transgenic mice showed greater muscle injury with intense exercise and reduced exercise capacity compared to wild type controls [27]. With training, there is a significant attenuation of the

Figure 2



Net muscle lactate efflux and intracellular PO_2 as a function of O_2 consumption (VO_2) in normoxia (breathing 21% O_2) and hypoxia (breathing 12% O_2) in healthy subjects performing single leg quadriceps exercise. Intracellular PO_2 was derived from phosphorus proton magnetic resonance spectroscopy measurement of myoglobin (Mb) saturation. The data show increasing lactate efflux as measured by venous-arterial lactate differences multiplied by thermodilution measured leg blood flow to maximum exercise output without a decline of intracellular PO2 fully adequate to support mitochondrial respiration. (for lactate efflux, $r = 0.97$ and 0.99 in normoxia and hypoxia, respectively, $P < 0.05$). VO_2 max equals maximum leg muscle oxygen consumption. McKenna *et al.* [23]. Reproduced with permission.

acute HIF-1 alpha response in muscle with exertion through both a reduction in the fall in muscle PO_2 and an increase in some of the negative regulators of HIFs, such as factor inhibiting HIF (FIH), sirtuin 6, and PHDs 2, and 3 [26]. The extent to which the accompanying myocyte intracellular acidosis and systemic metabolic acidosis of heavy exercise contributes to changes in HIF expression, and metabolism has not been studied.

Hypoxia, metabolic acidosis and respiratory acidosis in critical illness

With severe hypoxia either in the setting of profound arterial hemoglobin desaturation or anemia and reduced O_2 content, poor perfusion (ischemia) at the global or local level with microvascular flow dysregulation, or primary mitochondrial dysfunction (as in sepsis), metabolic and hypercapnic acidosis can develop along with impressive lactate formation. Under these situations the pH may fall well below 7.0. Although no doubt exists that the disorders causing severe acidoses can be life-threatening, the conventional wisdom that acidosis itself is harmful does not stand up to scrutiny. More interestingly it may be an attempt by the organism refined by evolution to mitigate injury and enhance survival.

Both hypercapnic and metabolic acidoses may be cytoprotective by various anti-inflammatory, anti-oxidant, anti-apoptotic mechanisms which limit total hypoxic or ischemic reperfusion injury. The traditional reasons given to treat severe acidemia ($pH < 7.1–7.2$) in cardiogenic, hypoxic, and septic shock states are to reverse depressed catecholamine and insulin sensitivity, and raise the threshold for cardiac arrhythmias. There have been several randomized trials in patients with arterial pH between 6.8 and 7.10 using sodium bicarbonate against equal amounts of sodium as NaCl in diabetic ketoacidosis and various forms of lactic acidosis. The amounts given ranged from 100 to 200 mEq over 15–30 min, rates roughly equivalent to or greater than the ongoing rates of acid production in these situations. The data in aggregate are consistent in showing no meaningful clinical benefit or even in improving acid–base status to any significant degree [1]. Any purported benefit, such as a transient rise in blood pressure, may simply be due to the volume expanding effects of the accompanying sodium administration. In a more recent study of bicarbonate administration in critically ill patients with metabolic acidosis ($pH < 7.30$) to keep pH above 7.30 showed that compared to no bicarbonate administration, there was no survival benefit and only a modest reduction in the need for renal replacement therapy. This last finding may be the result of how bicarbonate reduces acidemia and hyperkalemia, two of several indications to begin dialysis [28•].

The failure of bicarbonate has multiple causes and any purported benefits may be outweighed by disadvantages, such as hyperosmolality, hypernatremia, hypokalemia, hypocalcemia, and post-therapeutic metabolic alkalosis. While important, more alarming are the deleterious effects of alkalization on O_2 delivery, cytokine, and oxidant radical formation, and increasing the likelihood of cell death in marginal areas ahead of resolution of the primary cause [29•]. This 'pH paradox' is not entirely understood. One explanation is as cells become critically hypoxic, they are unable to maintain low intracellular concentrations by outward calcium flux indirectly tied to active maintenance of a large transmembrane sodium gradient. An acidic pH inhibits calcium entry into cells and thereby slows the eventual critical rise in intracellular concentration, which as it rises above its low submicromolar concentrations, several metabolic pathways and enzymes are stimulated.

These include those that activate degradative proteases, lipases, and nucleases, causing cell necrosis, but are inhibited by acidic pH. Other benefits include blunting of oxidant NO and ROS formation [30], and inhibition of pro-inflammatory cytokine production [31]. Acidosis blocks many apoptotic pathways and enhances the signaling of several anti-apoptotic mediators [32,33] and attenuates mitochondrial permeability transition pore opening that release cytochrome C3, a pro-apoptotic signaling molecule [34]. Lastly, as with the high metabolic demand of exercise, in critical illness with compromised perfusion and oxygen delivery, lactate may be a preferred fuel for mitochondrial oxidation [17•] and reduce activity of pro-inflammatory immune cells [33].

This knowledge leads to the idea of delaying the correction of acidosis to permit reestablishment of oxygenation first, or the administration of drugs that limit the ability of cells to export protons or carbon dioxide. The first strategy involves initiating reperfusion of an ischemic organ with acidic perfusates as low as 6.4. In small mammal models of myocardial infarction caused by 30–60 min ischemic periods, reperfusion with oxygenated acidic fluid for several minutes delays intracellular pH normalization, improves phosphocreatine recovery, decreases LDH release and almost halves infarct size [35,36,37•]. Blocking membrane sodium–hydrogen exchange (NHE) by caroporide, is cardioprotective in ventricular fibrillation by attenuating intracellular calcium overload and is enhanced when co-administered with an inhibitor of the sodium-bicarbonate symporter [38]. Lastly, acetazolamide and other carbonic anhydrase (CA) inhibitors at doses capable of generating a combined metabolic and respiratory acidosis by renal and red cell CA inhibition are protective in models of cerebrovascular occlusion [39], ischemic liver injury [40] and coronary artery occlusion [37•]. These results should encourage the limited use of alkalinizing agents, except in a few situations such as hyperkalemia and toxic ingestions, in which renal clearance can be accelerated [41].

Local and systemic hypercapnia may also develop with hypoxic ischemia and with severe ventilatory failure. In poorly perfused areas, CO_2 retention results from insufficient clearance and CO_2 generation as accumulating H^+ is buffered by tissue bicarbonate stores [42]. As with metabolic acidosis, the clinical reflex until most recently was to increase ventilation, but the evidence for harm of hypercapnia has always been weak. The safety of permissive hypercapnia-purposefully limiting tidal volume and pressure and accepting subsequent hypercapnic acidosis in status asthmaticus [43] resulted in no pneumothoraxes or hemodynamic compromise, and a mortality reduction from 20% to almost 0, even with $PaCO_2$s exceeding 100 mmHg. In the large ARDSNet trial of 6 versus 12 ml/kg tidal volumes [44], controlling for other variables predictive of mortality, moderate levels of hypercapnic acidosis (pH 7.15–7.35 $PaCO_2$: 45–65 mmHg) lowered the odds ratio of death significantly in the larger tidal volume group [45]. Hypercapnic acidosis is well tolerated if perfusion and arterial oxygenation are assured [46]. The ability to withstand hypercapnic acidosis depends upon strong neuro-endocrine responses that maintain cardiac output despite direct negative inotropic and vasodilating effects of CO_2 and active intracellular pH defense mechanisms that export H^+ (Na/H exchange and H^+ translocating ATPase) out of the cell [47].

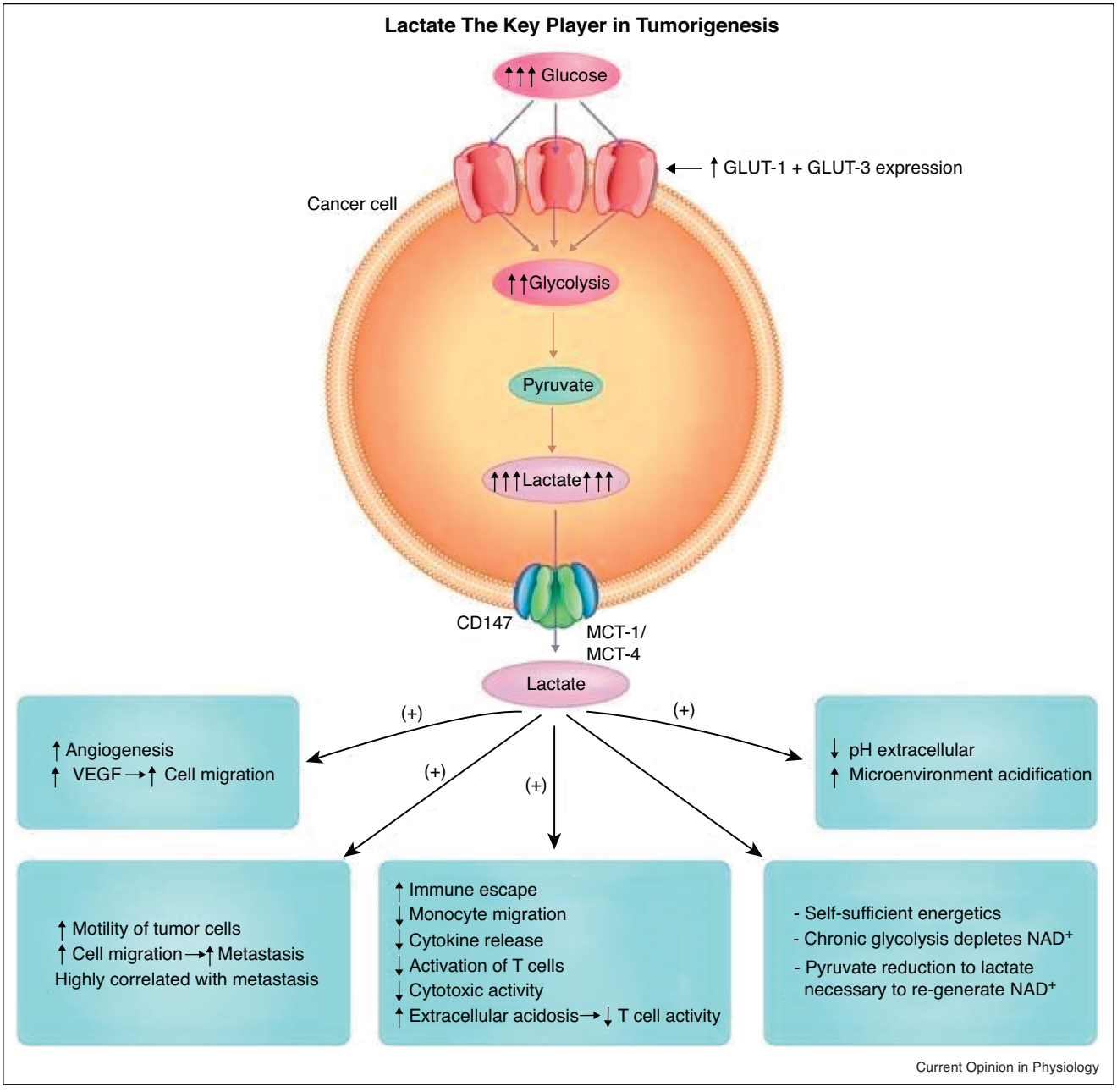
With growing acceptance of permissive hypercapnia and 'pH paradox' paradigms, deliberate imposition of hypercapnia (therapeutic hypercapnia) by ventilation with CO_2-enriched gas mixtures ranging from 10 to 25% was found to be protective in isolated perfused lungs subjected to ischemia-reperfusion, infectious and oxidant injuries [reviewed in Ref. 47]. The favorable results on alveolar edema, compliance, gas exchange and histology were replicated in live animals even when inspired carbon dioxide as high as 10–12% was given to lower arterial pH into the 7.00–7.15 range and $PaCO_2$ as high as 80–90 mmHg [47]. Despite the preponderant evidence for salutary anti-inflammatory, immunomodulatory and antioxidant effects of hypercapnic acidosis [47,48], concern remains that therapeutic hypercapnia might blunt desirable host defense mechanisms necessary for dealing with infection-related ARDS or on infections developing during their need for mechanical ventilation [49–52].

Hypoxia, acidosis, and malignancy

Rapid growth of malignant cancer cells can lead to hypoxia that upregulates hypoxia inducible factor (HIF)-mediated gene expression to ensure adequate nutrient and energy provision to sustain continued neoplastic growth. The ability to limit the accumulation of large amounts of acidifying waste products (carbon dioxide and lactic acid), features prominently in this regard even in tumor regions that are well oxygenated. Acidosis induces HIF-1α [13,53], but this does not extend to all genes targeted by it [52]. As mentioned previously, this effect of acidosis is in part mediated by redistribution of VHL [13] and is also important for the stabilization of HIF-1α in formerly ischemic tissues soon after reperfusion [54] that may permit cells close to death to survive. With long-term exposure of cancer cells to acidosis, there is a reprogramming toward glutamine metabolism, triggered by the need to reduce the production of protons from glycolysis and further maintained by the NAD(+)-dependent increase in sirtuin-1 deacetylase activity to ensure intracellular pH homeostasis. A consecutive increase in HIF-2α activity promotes the expression of various transporters and enzymes supporting the reductive and oxidative glutamine metabolism, whereas a reduction in functional HI-1α expression reduces glycolysis [55].

Otto Warburg in his classic studies in the 1920s observed that tumors rely heavily on glycolysis even in the presence of sufficient oxygen and grow in a more acidic milieu not generally tolerated by normal cells. Why tumor metabolism is so heavily dependent upon glycolysis has been conventionally explained by a need to generate sufficient ATP in hypoxia. However, more compelling and consistent with the high rate of glycolysis even in the presence of oxygen, despite its inefficiency in ATP generation, is that a high production rate of 3–4 carbon products of glycolysis (lactate) and Krebs cycle intermediates are necessary as substrates for nucleotide, amino acid, and lipid production [56] and other numerous advantages to cancer cells in their growth and escape from immune surveillance [57•] as shown in Figure 3. Measurements of extracellular pH (pHe) and intracellular pH (pHi) have shown that the ECF of tumors that becomes acidic (6.0–6.8 versus 7.2–7.4 in normal cells) [58,59] due to high lactic acid concentration (5–10 mM) and CO_2 tensions of 60–80 mmHg [60]. Despite the combined metabolic and respiratory acidoses, intracellular tumor pH is much more alkaline and nearly in the range of normal cells (pHi of 7.1–7.3) [61]. HIF-dependent gene transcription facilitates high rates of acid export and/or bicarbonate uptake. To support high rates of glycolysis, membrane glucose transporters (1 and 3) and hexokinase 1/2 are upregulated to increase glucose uptake [62]. The switch to lactic acid production and suppression of aerobic glycolysis is brought about by upregulation of lactate dehydrogenase (LDH) that increases the flux from pyruvate to lactate, and of pyruvate dehydrogenase kinase (PDK) to block pyruvate uptake into the mitochondria [63]. To maintain high rates of lactic acid export [64], several monocarboxylate transporters (MCT) are inserted into the membrane (Figure 3). In addition, proton export via NHE-1 is present in malignant cells [65] and may be upregulated by HIF-1 [66]. Other membrane bound proton translocating ATPases (vacuolar H^+-ATPase and H^+–K^+-ATPase) are expressed in some tumor cells [67–69], activity of which are enhanced by intracellular CA II shown in Figure 4.

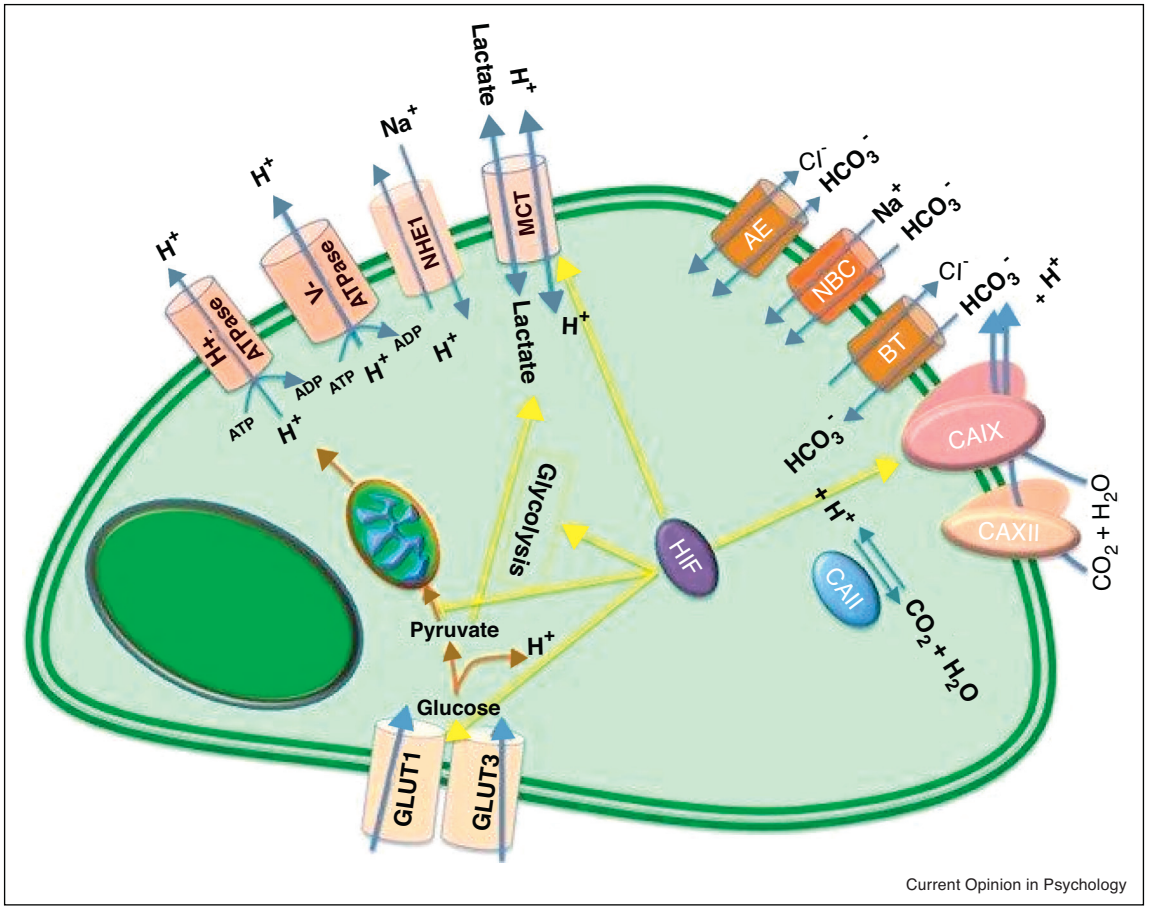
Figure 3



Lactate is necessary for all the major steps in carcinogenesis; Lactate increases the expression of vascular endothelial growth factor (VEGF) stimulating angiogenesis, increases motility and migration of cancer cells. Lactate is directly involved in the 'immune escape' by decreasing monocyte migration and decreased activation of T cells as well as cytokine release and cytotoxic activity. Lactate increases extracellular acidosis of tumor microenvironment decreasing capacity of T-cell to export lactate, thus decreasing T-cell activity. Finally, lactate is necessary for the self-sufficiency of cancer cells by replenishing cytosolic levels of nicotinamide adenine dinucleotide (NAD+) and regulating the status of the equilibrium of the cytoplasmic redox pair (NADH/NAD+) for continuation of glycolysis. San-Millán and Brooks [57•]. Reproduced with permission.

To maintain favorable intracellular pCO_2, carbon dioxide diffuses across the cell membrane via aquaporin-1 serving as a gas channel [70] and this activity is enhanced by intracellular CA II [71]. Aquaporin-1 and other aquaporins are richly expressed on tumors [72], are upregulated by HIF-1, [73,74] and are correlated with worse tumor prognosis and treatment failure [75]. In addition, tumor cells richly express two isoforms of membrane-bound carbonic anhydrases, CA IX and CA XII [76] that also promote CO_2 and H^+ export (Figure 4) and are the only

Figure 4



Major pH regulators in a cancer cell. After glucose uptake by specific transporters (GLUT1 and GLUT3), glucose is converted to pyruvate, generating 2 ATP per glucose and proton. In accordance with the Pasteur effect, in the presence of oxygen, pyruvate is oxidized to HCO_3^-, generating 36 additional ATP per glucose; in the absence of oxygen pyruvate is reduced to lactate, which is exported to the extracellular space. However, as Warburg proposed glycolysis is potent in cancer cells. Notably both processes produce protons (H+), which cause acidification of the extracellular space. This figure represents the main proteins that regulate intracellular and extracellular pH in tumors, including: monocarboxylate transporters (MCTs), which transport lactic acid and other monocarboxylates formed by the glycolytic degradation of glucose; the plasma membrane proton pump vacuolar ATPase (V-ATPase); Na^+/H^+ exchangers (NHEs); anion exchangers (AEs); carbonic anhydrases (CAII, CAIX, and CAXII); Na^+/HCO_3^- co-transporters (NBCs), and HCO_3^--transporters (BTs). Damaghi *et al*. [61]. Reproduced with permission.

isozymes upregulated in hypoxia via HIF-1 [77]. Their expression is a strong prognostic predictor of poorer outcome in most cancers [78].

CA IX consists of a large extracellular part, containing a unique N-terminal proteoglycan-like region (PG), and a carbonic anhydrase domain which accelerates CO_{23} intro-conversion 10^5–10^6-fold over the uncatalyzed reactions [79]. The PG domain appears to play some role in cell adhesion characteristics of malignant cells leading them to more easily separate from the primary tumor and metastasize. Its rich basic amino acid content provides buffering capacity for more optimal catalysis in the acidic extracellular environment of tumors [80]. Additionally, this domain by virtue of its many H^+-titratable amino acids, itself helps to augment lactic acid export by concentrating protons and acting as a 'proton antenna' [76,81] to augment the outward flux of lactic acid by MCT-1 and 4.

Given profound changes in tumor acid–base milieu and the role of pH in tumor survival and growth, altering the acid–base status has been of interest in treatment [82]. The marked extracellular acidosis appears to promote migration and metastasis of cancer cells by disrupting normal cell–matrix interactions that act to contain cells in stable or controlled growth patterns. Additionally, host defense against malignant cells involving macrophages, lymphocytes and natural killer cells is reduced with acidosis [83•,84]. Sodium bicarbonate to increase tumor pHe *in vivo* inhibits the metastatic spread and extends survival in animal models of cancer [85,86].

Pharmacologic therapy directed at lowering pHi of tumors has shown promising results in animal models. Acetazolamide and methazolamide, two clinically available non-selective CA inhibitors, suppressed growth and invasiveness of tumors *in vivo* and in culture [87]. The far greater expression of CA IX and CA XII in tumors and virtual lack of expression in normal tissues has generated a synthetic effort to target these membrane-bound CAs selectively. Because of their extracellular orientation, a specific hydrophilic CA IX sulfonamide inhibitor, SLC-0111, is now in phase II clinical trials in glioblastoma [88,89•]. Small interfering mRNA against CA IX reduced growth of a CA-IX bearing human cancer cells [90]. Inhibition of other membrane proteins contributing to pHi defense in malignant cells, such as NHE, bicarbonate transporters, H^+-ATPases, and MCT (Figure 4), give encouraging results in tumor models [82].

Summary

In conclusion, I have attempted to show the complex and inextricable links between hypoxia and acid–base alterations and how a fuller understanding of hypoxia in various circumstances can only be achieved by appreciating the myriad of effects of acid–base changes on hypoxic and ischemic responses as well as on other cellular functions. This is as relevant to high altitude adaptation and in exercise as it is to patients with critical illnesses and cancer. More investigation of the role that pH and acid–base status have in altering HIF metabolism in normoxia and hypoxia is needed. Although alkalosis helps to blunt some deleterious effects of hypoxia, it is becoming less clear that acidosis, both lactic acidosis and hypercapnic acidosis in critically ill patients, should be aggressively reversed owing to their cytoprotective effects. Lastly, insights into intracellular pH defense may lead to therapies that control cancer by impairing the impressive acid–base regulating abilities of malignant cells.

Conflict of interest statement

Nothing declared.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest

1. Effros RM, Swenson ER: **Acid-base balance**. In *Textbook of Respiratory Medicine,* edn 6. Edited by Mason RJ, Broaddus VC, Martin TR, Schraufnagel DE, Murray JF, Nadel JA.Philadelphia: Saunders Elsevier; 2016:113-133.

2. Fan JL, Burgess KR, Basnyat R, Thomas KN, Peebles KC, Lucas SJ *et al.*: **Influence of high altitude on cerebrovascular and ventilatory responsiveness to CO_2**. *J Physiol* 2010, **588**:539-549.

3. Dorrington KL, Balanos GM, Talbot NP, Robbins PA: **Extent to which pulmonary vascular responses to PCO_2 and PO_2 play a functional role within the healthy human lung**. *J Appl Physiol* 2010, **108**:1084-1096.

4. Mairbaeurl H, Huber F, Luetge A, Sahinoz M, Ermis-Kaya E, Swenson ER: **Hypocapnia aggravates hypoxic inhibition of ENaC in rat primary alveolar epithelial cells**. *Am J Respir Crit Care Med* 2018, **197**:A2273.

5. Shimoda LA, Laurie SS: **HIF and pulmonary vascular responses to hypoxia**. *J Appl Physiol* 2014, **116**:867-874.

6. Kline DD, Peng YJ, Manalo DJ, Semenza GL, Prabhakar NR: **Defective carotid body function and impaired ventilatory responses to chronic hypoxia in mice partially deficient for hypoxia-inducible factor 1 alpha**. *Proc Natl Acad Sci U S A* 2002, **99**:821-826.

7. Peng YJ, Nanduri J, Khan SA, Yuan G, Wang N, Kinsman B *et al.*: **Hypoxia-inducible factor 2α heterozygous-null mice exhibit exaggerated carotid body sensitivity to hypoxia, breathing instability and hypertension**. *Proc Natl Acad Sci U S A* 2011, **108**:3065-3070.

8. Tang H, Babicheva A, McDermott KM, Gu Y, Ayon RJ, Song S *et al.*: **Endothelial HIF-2α contributes to severe pulmonary hypertension due to endothelial-to-mesenchymal transition**. *Am J Physiol* 2018, **314**:L256-L275.

9. Zheng W, Kuhlicke J, Jäckel K, Eltzschig HK, Singh A, Sjöblom M *et al.*: **Hypoxia inducible factor-1 (HIF-1)-mediated repression of cystic fibrosis transmembrane conductance regulator (CFTR) in the intestinal epithelium**. *FASEB J* 2009, **23**:204-213.

10. Bartoszewska S, Kamysz W, Jakiela B, Sanak M, Króliczewski J, Bebok Z, Bartoszewski R, Collawn JF: **miR-200b Downregulates CFTR during hypoxia in human lung epithelial cells**. *Cell Mol Biol Lett* 2017, **22**:23-29.

11. Hulter HN, Krapf R: **Interrelationships among hypoxia-inducible factor biology and acid-base equilibrium**. *Semin Nephrol* 2006, **26**:454-465.

12. Filatova A, Seidel S, Böğürcü N, Gräf S, Garvalov BK, Acker T: **Acidosis acts through HSP90 in a PHD/VHL-independent manner to promote HIF function and stem cell maintenance in glioma**. *Cancer Res* 2016, **76**:5845-5856.

13. Mekhail K, Gunaratnam L, Bonicalzi ME, Lee S: **HIF activation by pH-dependent nucleolar sequestration of VHL**. *Nat Cell Biol* 2004, **6**:642-647.

14. Nadtochiy SM, Schafer X, Fu D, Nehrke K, Munger J, Brookes PS: **Acidic pH is a metabolic switch for 2-hydroxyglutarate generation and signaling**. *J Biol Chem* 2016, **291**:20188-20197.

15. Liu H, Zhang Y, Wu H, D'Alessandro A, Yegutkin GG, Song A *et al.*: **Beneficial role of erythrocyte adenosine A2B receptor-mediated AMP-activated protein kinase activation in high-altitude hypoxia**. *Circulation* 2016, **134**:405-421.

16. Chapman RF, Stray-Gundersen J, Levine BD: **Epo production at altitude in elite endurance athletes is not associated with the sea level hypoxic ventilatory response**. *J Sci Med Sport* 2010, **13**:624-630.

17. • Brooks GA: **The science and translation of lactate shuttle theory**. *Cell Metab* 2018, **27**:757-785.
This is a comprehensive review of the multiple aspects of lactate metabolism under stressful conditions and its physiological relevance to exercise and critical illness.

18. Swenson ER: **Carbonic anhydrase inhibitors and ventilation: a complex interplay of stimulation and suppression**. *Eur Respir J* 1998, **12**:1242-1247.

19. Nielsen HB: **pH after competitive rowing: the lower physiological range**. *Act Physiol Scand* 1999, **165**:113-114.

20. Cairns SP: **Lactic acid and exercise performance: culprit or friend?** *Sports Med* 2006, **36**:279-291.

21. Richardson RS, Noyszewski EA, Leigh JS, Wagner PD: **Lactate efflux from exercising human skeletal muscle: role of intracellular PO_2**. *J Appl Physiol* 1998, **85**:627-634.

22. Levy B, Gibot S, Franck P, Cravoisy A, Bollaert PE: **Relation between muscle Na^+K^+ ATPase activity and raised lactate concentrations in septic shock: a prospective study**. *Lancet* 2005, **365**:871-875.

23. McKenna MJ, Bangsbo J, Renaud JM: **Muscle K+, Na+, and Cl- disturbances and Na+-K+ pump inactivation: implications for fatigue**. *J Appl Physiol* 2008, **104**:288-295.

24. O'Neill M, Sears CE, Paterson DJ: **Interactive effects of K+, acid, norepinephrine, and ischemia on the heart: implications for exercise**. *J Appl Physiol* 1997, **82**:1046-1052.

25. • Ferguson BS, Rogatzki MJ, Goodwin ML, Kane DA, Rightmire Z, Gladden LB: **Lactate metabolism: historical context, prior misinterpretations, and current understanding**. *Eur J Appl Physiol* 2018, **118**:691-728.
This is a comprehensive review of the intermediary metabolism of lactate and arguments around its effects on the development of acidosis in ischemia, exercise, hypoxia and critical illness.

26. Lindholm ME, Rundqvist H: **Skeltal muscle hypoxia-inducible factor-1 and exercise**. *Exp Physiol* 2016, **101**:28-32.

27. Mason SD, Howlett RA, Kim MJ, Olfert IM, Hogan MC, McNulty W, Hickey RP, Wagner PD, Kahn CR, Giordano FJ, Johnson RS: **Loss of skeletal muscle HIF-1 alpha results in altered exercise tolerance**. *PLoS Biol* 2004, **2**:e288.

28. • Jaber S, Paugam C, Futier E, Lefrant JY, Lasocki S, Lescot T *et al.*: **Sodium bicarbonate therapy for patients with severe metabolic acidaemia in the intensive care unit (BICAR-ICU): a multicentre, open-label, randomised controlled, phase 3 trial**. *Lancet* 2018, **392**:31-40.
This is a recent large randomized trial of bicarbonate treatment of metabolic acidosis in acidotic critically ill patients, showing little benefit to this controversial practice.

29. • Kalogeris T, Baines CP, Krenz M, Korthuis RJ: **Ischemia/reperfusion**. *Compr Physiol* 2016, **7**:113-170.
This is a comprehensive review of ischemia-reperfusion injury in its many manifestations and its treatment including the concept of delaying correction of acidosis.

30. Kumar S, Reusch HP, Ladilov YV: **Acidic preconditioning suppresses apoptosis and increases expression of Bcl-xL in coronary artery endothelial cells under simulated ischemia**. *J Cell Mol Med* 2008, **12**:1584-1592.

31. Bidani A, Heming TA: **Effects of bafilomycin A1 on functional capabilities of LPS-activated alveolar macrophages**. *J Leukoc Biol* 1995, **57**:275-281.

32. Flacke JP, Kumar S, Kostlin S, Reusch HP, Ladilov Y: **Acidic preconditioning protects endothelial cells against apoptosis by p38- and Akt-dependent BcL-xL overexpression**. *Apoptosis* 2009, **14**:90-96.

33. Kellum JA, Song M, Li J: **Lactic and hydrochloric acids induce different patterns of inflammatory response in LPS-stimulated RAW 264.7 cells**. *Am J Physiol* 2004, **286** R686-92.

34. Ovize M, Baxter GF, Di Lisa F, Ferdinandy P, Garcia-Dorado D *et al.*: **Post-conditioning and protection from reperfusion injury: where do we stand?** *Cardiovasc Res* 2010, **87**:406-423.

35. Inserte J, Barba I, Hernando V, Abellan A, Ruiz-Meana M, Rodriguez-Sinbovas *et al.*: **Effect of acidic reperfusion on prolongation of intracellular acidosis and myocardial salvage**. *Cardiovasc Res* 2008, **77**:782-790.

36. Cohen MV, Yang XM, Downey JM: **The pH hypothesis of postconditioning; staccato re- perfusion reintroduces oxygen and perpetuates myocardial acidosis**. *Circulation* 2007, **115**:1895-1903.

37. • Ciocci Pardo A, Díaz RG, González Arbeláez LF, Pérez NG, Swenson ER *et al.*: **Benzolamide perpetuates acidic conditions during reperfusion and reduces myocardial ischemia-reperfusion injury**. *J Appl Physiol* 2018, **125**:340-352.
This is a trial of carbonic anhydrase inhibition in the heart to pharmacologically slow the correction of acidosis during reperfusion after a period of ischemia similar in efficacy to other trials in cerebral, renal and hepatic ischemia-reperfusion injury.

38. Schafer C, Ladilov YV, Siegmund B, Piper HM: **Importance of bicarbonate transport of protection of cardiomyocytes against reoxygenation injury**. *Am J Physiol* 2000, **278**: H1457-H1463.

39. Wang X, Figueroa BE, Stavrovskaya IG, Zhang Y, Siriani AC *et al.*: **Methazolamide and melatonin inhibit mitochondrial cytochrome C release and neuroprotective in experimental models of ischemic injury**. *Stroke* 2009, **40**:1877-1885.

40. Bejaoui M, Pantazi E, De Luca V, Folch-Puy E, Serafin A *et al.*: **Acetazolamide protects steatotic liver grafts against cold ischemia reperfusion injury**. *J Pharmacol Exp Ther* 2015, **355**:191-198.

41. Swenson ER: **Metabolic acidosis**. *Respir Care* 2001, **46**:342-353.

42. Mallat J, Lemyze M, Tronchon L, Thevenin D: **Use of venous-to-arterial carbon dioxide tension difference to guide resuscitation therapy in septic shock**. *World J Crit Care Med* 2016, **5**:47-56.

43. Darioli R, Perret C: **Mechanically controlled hypoventilation in status asthmaticus**. *Am Rev Respir Dis* 1984, **129**:385-387.

44. Acute Respiratory Distress Syndrome Network: **Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome**. *N Engl J Med* 2000, **342**:1301-1308.

45. Kregenow DA, Rubenfeld GF, Hudson LD, Swenson ER: **Hypercapnia and mortality in acute lung injury**. *Crit Care Med* 2006, **34**:1-7.

46. Potkin R, Swenson ER: **Resuscitation form severe acute hypercarbia: determinants of tolerance and survival**. *Chest* 1992, **102**:1742-1745.

47. Kregenow DA, Swenson ER: **Hypercapnic acidosis: implications for permissive and therapeutic hypercapnia**. *Eur Respir J* 2002, **20**:6-11.

48. Swenson ER: **Hypercapnic acidosis and sepsis: sailing too close to the wind?** *Anesthesiology* 2010, **112**:269-271.

49. Nichol AD, O'Cronin DF, Naughton F, Hopkins N, Boylan J, McLoughlin P: **Hypercapnic acidosis reduces oxidative reactions in endotoxin-induced lung injury**. *Anesthesiology* 2010, **113**:116-125.

50. O'Croinin DF, Nichol AD, Hopkins N, Boylan J, O'Brien S, O'Connor C, Laffey JG, McLoughlin P: **Sustained hypercapnic acidosis during pulmonary infection increases bacterial load and worsens lung injury**. *Crit Care Med* 2008, **36**:2128-2135.

51. Vadász I, Hubmayr RD, Nin N, Sporn PH, Sznajder JI: **Hypercapnia: a nonpermissive environment for the lung**. *Am J Respir Cell Mol Biol* 2012, **46**:417-421.

52. Nin N, Angulo M, Briva A: **Effects of hypercapnia in acute respiratory distress syndrome**. *Ann Transl Med* 2018, **6**:37-42.

53. Mehhail K, Khacho M, Gunaratnam L, Lee S: **Oxygen sensing by H^+: implications for HIF and hypoxic cell memory**. *Cell Cycle* 2004, **3**:1027-1029.

54. Xu J, Peng Z, Li R, Dou T, Xu W, Gu G *et al.*: **Normoxic induction of cerebral HIF-1α by acetazolamide in rats: role of acidosis**. *Neurosci Lett* 2009, **451**:274-278.

55. Corbet C, Draoui N, Polet F, Pinto A, Drozak X, Riant O, Feron O: **The SIRT1/HIF-2α axis drives reductive glutamine metabolism under chronic acidosis and alters tumor response to therapy**. *Cancer Res* 2014, **74**:5507-5519.

56. Vander Heiden MG, Cantley LC, Thompson CB: **Understanding the Warburg effect: the metabolic requirements of cell proliferation**. *Science* 2009, **324**:1029-1033.

57. • San-Millán I, Brooks GA: **Reexamining cancer metabolism: lactate production for carcinogenesis could be the purpose and explanation of the Warburg effect**. *Carcinogenesis* 2017, **38**:119-133.
This is a comprehensive review of the multiple roles that lactate and its generation in cancer plays in promoting growth and metastasis as both a substrate and signaling molecule.

58. Kallinowski F, Schlenger KH, Runkel S, Kloes M, Stohrer M, Okunieff P *et al.*: **Blood flow, metabolism, cellular microenvironment and growth rate of human tumor xenografts**. *Cancer Res* 1989, **49**:3759-3764.

59. Volk T, Jahde E, Fortmeyer HP, Glusenklamp KH, Rajewsky MF: **pH in human tumor xenografts; effects of intravenous administration of glucose**. *Br J Cancer* 1993, **68**:492-500.

60. Gullino PM, Grantham FH, Smith SH, Haggerty AC: **Modifications of the acid-base status of the internal milieu of tumors**. *J Natl Cancer Inst* 1965, **34**:857-869.

61. Damaghi M, Wojtkowiak JW, Gillies RJ: **pH sensing and regulation in cancer**. *Front Physiol* 2013, **4**:370-377.

62. Mathupala S, Ko YH, Pedersen PL: **Pivotal roles of mitochondria in cancer: Warburg and beyond and encouraging prospects for effective therapy**. *Biochim Biophys Acta* 2010, **1797**:1225-1230.

63. Swietach P, Hulikova A, Vaughan-Jones R, Harris AL: **New insights into the physiological role of carbonic anhydrase IX in tumor pH regulation**. *Oncogene* 2010, **29**:5609-5621.

64. Brahimi-Horn MC, Bellot G, Pouyssegur J: **Hypoxia and energetic tumor metabolism**. *Curr Opin Genet Dev* 2010, **14**:771-794.

65. Cardone RA, Casavola V, Reshkin SJ: **The role of disturbed pH dynamics and the Na+/H+ exchanger in metastasis**. *Nat Rev Cancer* 2005, **5**:786-795.

66. Shimoda LA, Fallon M, Pisarcik S, Wang J, Semenza GL: **HIF-1 regulates hypoxic induction of NHE1 expression and alkalization of intracellular pH in pulmonary arterial myocytes**. *Am J Physiol* 2006, **291**:L941-949.

67. Martínez-Zaguilán R, Raghunand N, Lynch RM, Bellamy W, Martinez GM, Rojas B *et al.*: **pH and drug resistance. Functional expression of plasmalemmal V-type H+-ATPase in drug resistant human breast carcinoma cell lines**. *Biochem Pharmacol* 1999, **57**:1037-1046.

68. Shimokawa O, Matsui H, Nagano Y, Kaneko T, Shibahara T, Nakahara A *et al.*: **Neoplastic transformation and induction of H+/K+-ATPase by N-methyl-N′-nitro-N-nitrosoguanidine in the gastric epithelial RGM-1 cell line**. *In Vitro Cell Dev Biol Anim* 2008, **44**:26-30.

69. Mullin JM, Gabello M, Murray LJ, Farrell CP, Bellows J, Wolov KR *et al.*: **Proton pump inhibitors: actions and reactions**. *Drug Discov Today* 2000, **14**:647-660.

70. Endeward V, Al-Samir S, Itel F, Gros G: **How does carbon dioxide permeate cell membranes? A discussion of concepts, results and methods**. *Front Physiol* 2014, **4**:382-387.

71. Vilas G, Krishnan D, Loganathan SK, Malhotra D, Liu L, Beggs MR, Gena P *et al.*: **Increased water flux induced by an aquaporin-1/carbonic anhydrase II interaction**. *Mol Biol Cell* 2015, **26**:1106-1118.

72. Nico B, Ribatti D: **Aquaporins in tumor growth and angiogenesis**. *Cancer Lett* 2010, **294**:135-138.

73. Wang YF, Fan ZK, Cao Y, Yu DS, Zhang YQ, Wang YS: **2-Methoxyestradiol inhibits the up-regulation of AQP4 and AQP1 expression after spinal cord injury**. *Brain Res* 2011, **1370**:220-226.

74. Higashida T, Peng C, Li J, Dornbos D 3rd, Teng K, Li X *et al.*: **Hypoxia-inducible factor-1alpha contributes to brain edema after stroke by regulating aquaporins and glycerol distribution in brain**. *Curr Neurovasc Res* 2011, **8**:44-51.

75. Machida Y, Ueda Y, Shimasaki M, Sato K, Sagawa M, Katsuda S, Sakuma T: **Relationship of aquaporin 1,3 and 5 expression in lung cancer cells to cellular differentiation, invasive growth and metastasis potential**. *Hum Pathol* 2011, **42**:669-678.

76. Ames S, Pastorekova S, Becker HM: **The proteoglycan-like domain of carbonic anhydrase IX mediates non-catalytic facilitation of lactate transport in cancer cells**. *Oncotarget* 2018, **9**:27940-27957.

77. Kaluz S, Kaluzová M, Liao SY, Lerman M, Stanbridge EJ: **Transcriptional control of the tumor- and hypoxia-marker carbonic anhydrase 9: a one transcription factor (HIF-1) show?** *Biochim Biophys Acta* 2009, **1795**:162-172.

78. Poulsen SA: **Carbonic anhydrase inhibition as a cancer therapy; a review of patent literature, 2007-2009**. *Expert Opin Ther Patents* 2010, **20**:795-806.

79. De Simone G, Supuran CT: **Carbonic anhydrase IX: biochemical and crystallographic characterization of a novel antitumor target**. *Biochim Biophys Acta* 2010, **1804**:404-409.

80. Innocenti A, Pastorekova S, Pastorek J, Scozzafava A, De Simone G, Supuran CT: **The proteo-glycan region of the tumor-associated carbonic anhydrase IX acts as an intrinsic buffer optimizing CO_2 hydration at acidic pH values characteristic of solid tumors**. *Bioorg Med Chem Lett* 2009, **19**:5825-5828.

81. Noor SI, Jamali S, Ames S, Langer S, Deitmer JW, Becker HM: **A surface proton antenna in carbonic anhydrase II supports lactate transport in cancer cells**. *eLife* 2018, **7**:e35176 http://dx.doi.org/10.7554/eLife.35176.

82. Parks SK, Chiche J, Pouyssegur J: **pH control mechanisms of tumor survival and growth**. *J Cell Physiol* 2011, **226**:299-308.

83. • Damgaci S, Ibrahim-Hashim A, Enriquez-Navas PM, Pilon-Thomas S, Guvenis A, Gillies RJ: **Hypoxia and acidosis: immune suppressors and therapeutic targets**. *Immunology* 2018, **154**:354-362.
An excellent review of tumor acidosis and how therapies may be directed at cancer cell acid–base regulation.

84. Corbet C, Feron O: **Tumour acidosis: from the passenger to the driver's seat**. *Nat Rev Cancer* 2017, **17**:577-593.

85. Robey IF, Bagett BK, Kirkpatrick ND, Roe DJ, Dosescu J, Sloane BF *et al.*: **Bicarbonate increases tumor pH and inhibits spontaneous metastases**. *Cancer Res* 2009, **69**:2260-2267.

86. Silva AS, Yunes J, Gillies RJ, Gatenby RA: **The potential role of systemic buffers in reducing intratumoral extracellular pH and acid-mediated invasion**. *Cancer Res* 2009, **69**:2677-2684.

87. Cianchi F, Vinci MC, Supuran CT, Peruzzi B, De Giuli P, Fasolis G *et al.*: **Selective inhibition of carbonic anhydrase IX decreases cell proliferation and induces ceramide-mediated apoptosis in human cancer cells**. *J Pharmacol Exp Ther* 2010, **334**:710-719.

88. Carta F, Vullo D, Osman SM, AlOthman Z, Supuran CT: **Synthesis and carbonic anhydrase inhibition of a series of SLC-0111 analogs**. *Bioorg Med Chem* 2017, **25**:2569-2576.

89. • Boyd NH, Walker K, Fried J, Hackney JR, McDonald PC, Benavides GA, Spina R *et al.*: **Addition of carbonic anhydrase 9 inhibitor SLC-0111 to temozolomide treatment delays glioblastoma growth in vivo**. *JCI Insight* 2017, **2**.
A first clinical trial of a selective inhibitor of CA IX in treatment of a highly resistant cancer showing safety and tumor regression.

90. Robertson N, Potter C, Harris AL: **Role of carbonic anhydrase IX in human tumor cell growth, survival and invasion**. *Cancer Res* 2004, **64**:6160-6165.