Saliva Proteomics as Non-Invasive Application for Biomarker Studies

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Abstract

Nowadays, saliva being a source of broad-spectrum biomolecules (mainly proteins, lipids, hormones, and nucleic acids that originated from various local/systemic sources) holds a promising future among diagnostic samples. Compared to blood, the use of saliva is advantageous because sample collection and processing are easy, minimally invasive, low cost, and better tolerated by individuals. Saliva proteome analysis can therefore give valuable contributions in understanding the pathophysiology of diseases and provide a foundation for the recognition of potential protein markers. A pathophysiological condition caused by an ascent to a high altitude named hypobaric hypoxia occurs due to deficiency of oxygen at the tissue level caused by lower atmospheric pressure of oxygen. Although few reports have documented the effect of exposure to hypobaric hypoxia on plasma and tissue proteome, salivary proteome-based studies remain uninvestigated. Therefore, identification of molecular signatures having key roles in hypobaric hypoxia by analyzing the salivary proteome founds promising. Through salivary proteome, a few proteins such as alpha-enolase, cystatins, apoptosis inducible factor 2, prolactin inducible protein, carbonic anhydrase 6, phospholipid transfer protein (PLTP), interleukin 1 receptor antagonist (IL1R1), albumin, alpha-1 acid glycoprotein, and alpha-1 antitrypsin were found to be evolved as biomarkers for hypobaric hypoxia. In conclusion, these studies provided the proofs of concept for translating salivary proteins into a non-invasive putative diagnostic panel for assessing hypobaric hypoxia.

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10.1 Saliva: A Novel Informative Sample

Saliva is an oral fluid that originates from major salivary glands such as parotid, sublingual, and submandibular and minor salivary glands such as labial, buccal, lingual, and palatal glands (Fig. 10.1) (Carranza et al. 2005; Forde et al. 2006; Yoshizawa et al. 2013).

Some of the salivary components may not have originated from salivary glands as saliva also contains fluids from oral mucosal cells, upper respiratory secretions, and gastro-intestinal reflux (Mager et al. 2005; Zhang et al. 2010). Salivary glands are enveloped by capillaries and highly permeable for exchanging blood based molecules into saliva freely (Fig. 10.2) (Haeckel and Hanecke 1996). Also, blood based compounds move from plasma to saliva involving several processes (Yoshizawa et al. 2013; Yamaguchi et al. 2005). These processes include a) ultra-filtration of molecules such as water, ions, catecholamines, and steroids through gap junctions between secretory cells; b) selective transport (actively or passively)

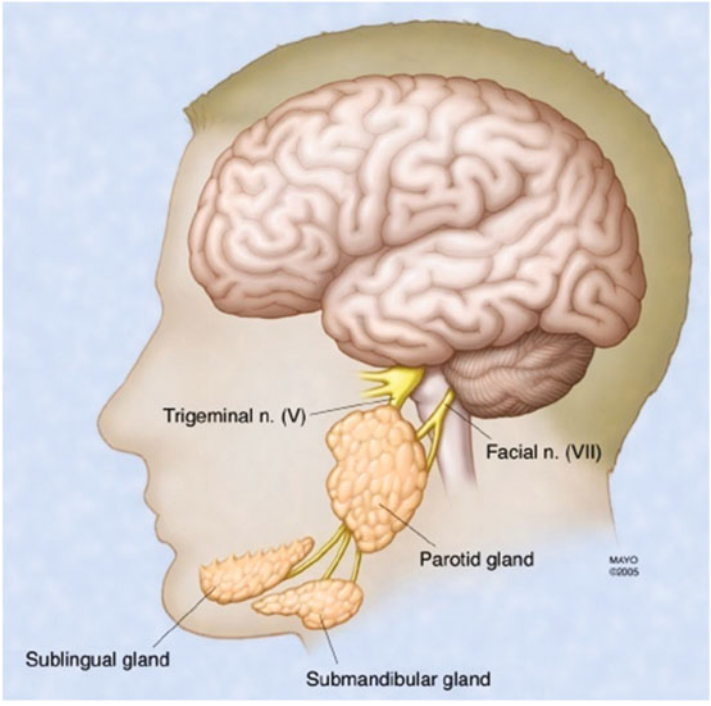

Fig. 10.1 Major salivary glands. (Adapted from Forde et al. 2006)

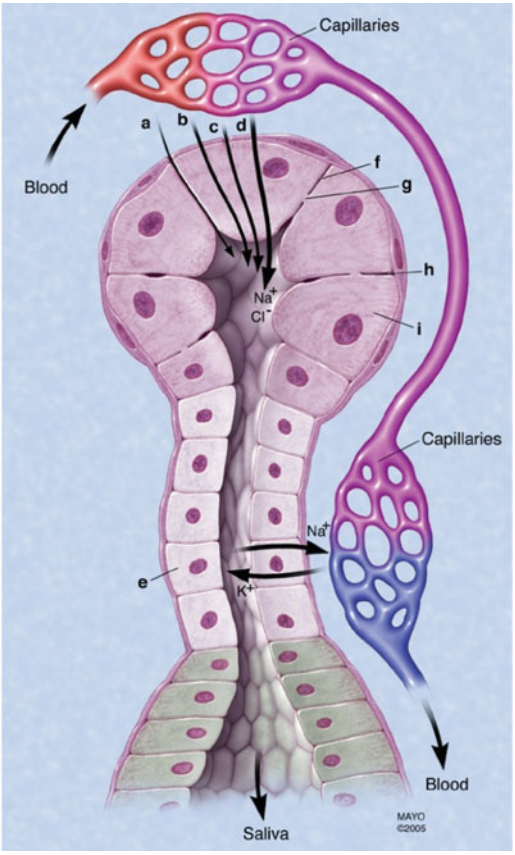

Fig. 10.2 Mechanism of molecular transport from blood to saliva through capillaries. (Adapted from Haeckel and Hanecke 1996)

through cellular membranes; and c) transudation of albumin directly from cervical fluid into oral cavity (Chiappin et al. 2007).

Healthy individuals produce 500–1500 mL of saliva (slightly acidic clear fluid having pH = 6.0–7.0) per day (Chicharro et al. 1998; Edgar 1990; Humphrey and Williamson 2001; Van Nieuw Amerongen et al. 2004; Zalewska et al. 2000). Several pathophysiological conditions can modify the amount of saliva (Chicharro et al. 1998; Aps and Martens 2005; Walsh et al. 2004). Saliva contains a variety of compounds such as inorganic (water, ions); organic (non-proteins such as uric acid, bilirubin, creatine, glucose, lipids, amines, and lactate); proteins and hormones (catecholamines and steroids) (Chicharro et al. 1998; Actis et al. 2005; Agha-Hosseini et al. 2006; Cooke et al. 2003; Coufal et al. 2003; Diab-Ladki et al. 2003; Guan et al. 2004; Larsson et al. 1996; Lloyd et al. 1996; Nagler et al. 2002; Rehak et al. 2000; Zelles et al. 1995). These biomolecules being present in the saliva provide information from several organs and systems and raise the possibility of their use as disease biomarkers.

A huge number of protein components are present in saliva (Hu et al. 2005; Huang 2004). These proteins have been identified using proteomics tools such as 2-DE coupled with MALDI-TOF/TOF followed by shotgun proteomics and LC-MS/MS (Castagnola et al. 2017). By using these techniques, most of the salivary proteins secreted in saliva from salivary glands were found to be proline-rich proteins (PRPs), mucins, cysteine-rich proteins (cystatins), and histidine-rich proteins (histatins). PRPs are highly polymorphic in nature with 50 different proteins encoded by different gene arrangements and post-secretory processing of only six genes (Carpenter 2013).

Secretion from each salivary gland differs in concentration of proteins and salts/ions (Hu et al. 2004; Kalk et al. 2002). For example, sublingual gland secretes mucin MUC5B and calgranulin, whereas submandibular gland secretes cystatin C. Human salivary proteins have functions related to immune defense in which a variety of proteins such as lactoferrin, lysozyme, immunoglobulins, agglutinins, and mucins participate in the protection of oral tissues and proteins like histatins and defensins possess bactericidal properties (Chiappin et al. 2007). Other functions of salivary proteins include inhibition of calcium precipitation by PRPs and statherins; taste perception by carbonic anhydrase; digestion of starch by amylase; endonuclease activity by Von Ebner minor gland proteins; and proteinase inhibition by cystatins (Amerongen and Veerman 2002).

Previously, blood/serum/plasma was frequently used as the source of biomarker but in many conditions, the blood sample collection could be problematic, expensive, and invasive. Comparatively, saliva offers various advantages over blood such as: (a) quick and easy method of sample collection: non-requirement of a trained professional and self-collection; (b) non-invasive: painless collection; (c) safe handling: diseases such as HIV cannot be transmitted through saliva samples; (d) easy storage and shipping as saliva does not clot like blood; and (e) cost-effective (Yoshizawa et al. 2013; Chiappin et al. 2007; Schafer et al. 2014; Kaczor-Urbanowicz et al. 2017; Lee and Wong 2009; Pfaffe et al. 2011; Campo et al. 2006). Recently, it gained attention as an effective strategy for screening, diagnosis, prognosis, and monitoring post-therapy status. And, it is imperative to explore potential of saliva based diagnostics as discussed in the following section.

10.2 Hypobaric Hypoxia: A Pathophysiological Condition

Ascent of an individual to high altitude, the parallel low atmospheric pressure of oxygen leads to decreased body's partial pressure of oxygen, thereby causing a pathophysiological condition named hypobaric hypoxia (HH) (Fig. 10.3). HH affects the ability of body to exchange oxygen between the lungs and the bloodstream, thereby disrupting the oxygen availability to the tissues. A significant population of world resides at an elevation of 10,000 ft. above sea level. This population consists of people who have lived there for generations and travelers who travel to high altitude intermittently (soldiers) or for short to moderate periods

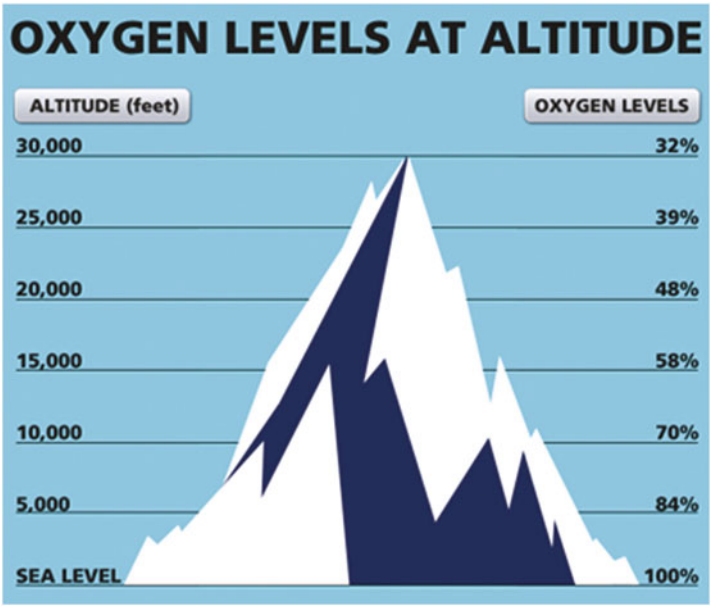

Fig. 10.3 Oxygen levels at different altitude elevations. (Adapted from http://www.altitude.org)

of time (e.g. mountain climbers) (Askew 2002). The people residing at high altitude for generations have already evolved various mechanisms to generate energy at high altitude (Hoppeler and Vogt 2001). Whereas travelers may experience AMS or potentially more serious impairments such as HACE and HAPE (Hackett and Roach 2001, 2004; San et al. 2013; Sharp and Bernaudin 2004; Wilson et al. 2009). These maladies develop over hours to days at high altitude and are known to be preventable but still remain common consequences of rapid ascent to high altitude.

10.3 Redox Stress: Molecular Responses in Hypobaric Hypoxia

The main consequence of hypobaric hypoxia is generation of reactive oxygen and nitrogen species (RONS). RONS, physiological modulators of cellular redox mechanism control wide range of physiological and pathophysiological processes (Bakonyi and Radak 2004). Hypoxia is caused by the limited availability of oxygen in mitochondria for reduction to H_2O at cytochrome oxidase and leads to generation of RONS. In order to produce energy (ATP), auto-oxidation of one or more mitochondrial complexes, such as the ubiquinone–ubiquinol redox couple results in accumulation of reducing equivalents (RONS) (Fig. 10.4). Despite the presence of antioxidant system, the levels of RONS generation can lead to oxidative stress (Askew 2002). These accumulated oxidants such as RONS cause imbalance between oxidative stressors and antioxidant capacity. Oxidants and antioxidants

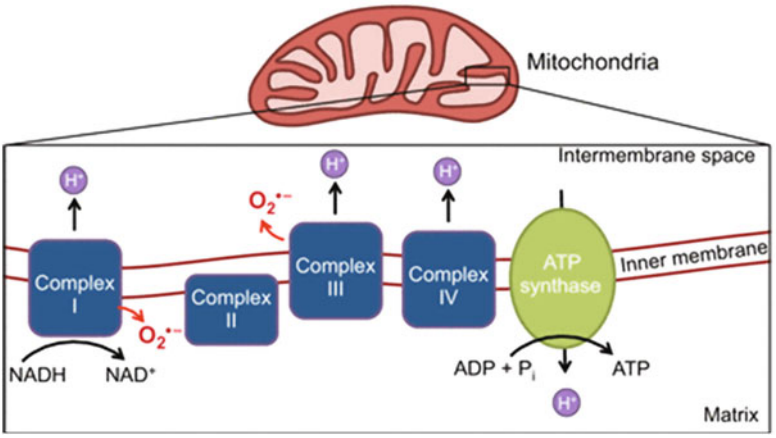

Fig. 10.4 Oxidants generation in mitochondria. (Adapted from Bigarella et al. 2014)

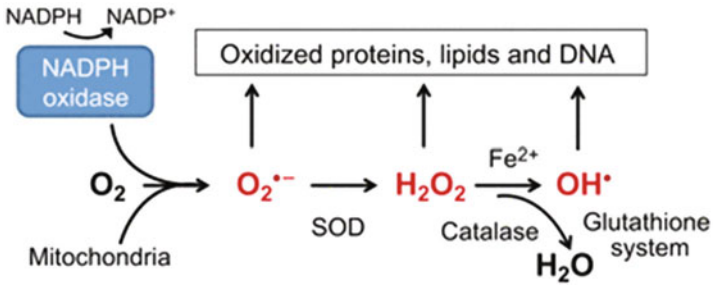

Fig. 10.5 Oxidative stress to biomolecules. (Adapted from Bigarella et al. 2014)

imbalance leads to damage in biomolecules such as lipids, proteins, and DNA (Fig. 10.5) (Dosek et al. 2007; Jefferson et al. 2004; Strapazzon et al. 2016).

This oxidative damage to biomolecules is nearly similar to the stress associated with cancer, neurological, pulmonary, and cardiovascular diseases (Reuter et al. 2010).

In literature, the increased production of oxidants has been documented in blood, tissue, urine, and breath samples of rats and humans in response to hypoxia (Strapazzon et al. 2016; Chang et al. 1989; Irarrazaval et al. 2017; Magalhaes et al. 1985; Maiti et al. 2006; Radak et al. 1994; Ribon et al. 2016; Siervo et al. 2014; Singh et al. 2013; Yoshikawa et al. 1982).

In vivo, aerobic cells develop antioxidant system to regulate the effects of RONS. This system contains mitochondrial, cytosolic, and extra-cellular superoxide dismutase (SOD). SOD converts reactive superoxide ions to H_2O_2 which is then decomposed into water by the action of glutathione system and catalase. Previously, high altitude related studies measured the content of glutathione, SOD, and GPX (Askew 2002; Bakonyi and Radak 2004; Radak et al. 1994, 1997; Nakanishi et al.

1995; Imai et al. 1995; Ilavazhagan et al. 2001; Joanny et al. 2001). And, it appeared that the capacity of antioxidant system was found to be decreasing at high altitude. These changes in redox parameters are responsible behind physiological and pathophysiological changes in the body.

10.4 Use of Biological Fluids Such as Plasma/Serum in Search of Potential Protein Markers

In hypobaric hypoxia related molecular studies, particularly proteomics, biological fluids such as blood/plasma/serum have been used among clinicians and biological scientists in search of potential protein markers for hypobaric hypoxia and acclimatization. Multiple scattered plasma proteins have been identified in samples from patients suffering from high altitude illnesses such as AMS, HAPE, and HACE (Lu et al. 2018; van Patot et al. 2005; Droma et al. 1996; Sartori et al. 1999; Charu et al. 2006; Berger et al. 2009; Barker et al. 2016; Ahmad et al. 2011, 2015; Zhang et al. 2013; Sikri and Bhattachar 2017).

Another important aspect is that the redox imbalance could also trigger healthy cellular processes that aid in acclimatization to hypobaric hypoxia. Protein markers have been studied in rats and humans to understand such cellular processes in acclimatized state (Ahmad et al. 2013, 2014, 2015; Siervo et al. 2014; Luks et al. 2017; Levett et al. 2011; Hartmann et al. 2000).

Newer strategies that are completely non-invasive in nature such as study of saliva have been implicated for diagnostics purposes. Thus, we move on to newer studies for exploration and improvement.

10.5 Saliva as a Diagnostic Fluid in Translational Studies

Previous findings have described that chronic diseases such as cancer, cardiovascular, diabetes, pulmonary, and neurological diseases are associated with continued oxidative stress (Reuter et al. 2010). In translational research, saliva has been explored for the detection of oral cancer (de Jong et al. 2010; Hu et al. 2007a; Gallo et al. 2016), Sjogren's syndrome (Giusti et al. 2007; Hu et al. 2007b; Peluso et al. 2007), breast cancer (Streckfus et al. 2008), lung cancer (Xiao et al. 2012), and systemic disorders such as hepatitis, HIV, and HCV (Elsana et al. 1998; Yaari et al. 2006; Hodinka et al. 1998). A previous study by Shen Hu et al. provided a proof for exploring salivary proteins in oral cancer and revealed thioredoxin as a salivary biomarker for human oral cancer (Hu et al. 2007a). Other researchers, Ebbing P. de Jong et al. reported myosin and actin as promising salivary biomarkers for distinguishing between pre-malignant and malignant oral lesions (de Jong et al. 2010). Another recent study done by Eva Csosz et al. highlighted the importance of identification of population tailored biomarkers and reported oral squamous cell carcinoma (OSCC) biomarkers in a Hungarian population. And, S100A9 and IL-6 were shown to be candidate biomarkers for OSCC (Csosz et al. 2017). In a study

performed by G. Peluso et al. in Sjogren's syndrome (SS), saliva collected from patients with primary SS revealed higher levels of alpha-defensin 1 and the presence of beta-defensin 2 which could be potential markers of oral inflammation in SS patients group (Peluso et al. 2007). Omer Deutsch et al. observed profilin and CA-I as biomarker candidates for Sjögren's syndrome following high-abundance protein depletion (Deutsch et al. 2015). In HIV and HCV, rapid point-of-care HIV tests utilize oral fluids to rapidly provide test results to patients (Hodinka et al. 1998; Fernandez Rodriguez et al. 1994). In cases of non-oral cancers such as lung and breast cancers, various researchers suggested modifications in the salivary proteome and provided proof for candidate biomarkers (Streckfus et al. 2008; Xiao et al. 2012; Bigler et al. 2009; Streckfus and Bigler 2016). Another example of its use for determining hormone levels, including estradiol, progesterone and testosterone, DHEA, and cortisol (Groschl 2008).

10.6 Saliva in Response to Hypobaric Hypoxia

In the context of events related to hypobaric hypoxia, studies on saliva have been performed due to its diagnostic potential. An initial study, observed in 1990s, suggested an increase in salivary flow rate and low concentration of potassium in response to acute hypobaric hypoxia exposure (Pilardeau et al. 1990). Another researcher, McLean reported decreased levels of aldosterone in response to both ACTH and renin–angiotensin stimulation at high altitude (McLean et al. 1989). Additionally, a rise in the salivary activities of aminotransferases was observed while HH exposure (Mominzadeh et al. 2014). A recent study by Woods DR et al. reported an alteration in the levels of salivary cortisol and suggested that an elevated cortisol levels may contribute to fluid retention associated with acute mountain sickness (Woods et al. 2012).

In molecular omics-based studies, preferably proteomics, only limited studies have been performed so far. Jain et al. have reported significantly altered proteins such as alpha-enolase, cystatin SN, apoptosis inducing factor 2 (AIF-2), cystatin S, carbonic anhydrase 6 (CA6), and prolactin inducible protein (PIP) and plausible pathways involving these proteins such as inflammation, impaired glycolysis, and respiratory alkalosis during HH exposure (Jain et al. 2018). Another study by Jain et al. suggested proteins such as albumin, carbonic anhydrase 6, prolactin inducible protein, alpha-enolase, phospholipid transfer protein (PLTP), alpha-1 acid glycoprotein, interleukin 1 receptor antagonist (IL1RA), and alpha-1 antitrypsin as protein candidates for assessing hypobaric hypoxia (Jain et al. 2020).

10.7 Proteins Evolved as Biomarkers for Hypobaric Hypoxia

10.7.1 Alpha-Enolase

Alpha-enolase is a multifunctional enzyme known to be involved in various processes such as allergic responses, growth control as well as glycolysis other than inflammatory hypoxic tolerance. Earlier studies suggested in hypoxic situations, expression of alpha-enolase got differentially modulated and provides protection to cells so as to acclimatize to low oxygen levels through increased anaerobic metabolism (Aaronson et al. 1995; Sedoris et al. 2010). Alpha-enolase has several interacting partners such as glyceraldehyde 3-phosphate dehydrogenase, phosphoglycerate kinase, and pyruvate kinase. Alpha-enolase has earlier been observed in various studies and well-established with hypoxia induced physiological changes (Kim et al. 2006; Semenza et al. 1996), suggesting its role as potential protein marker if carefully evaluated and utilized (Mikuriya et al. 2007; Xu et al. 2005).

10.7.2 Cystatins

Salivary cystatins such as cystatin S and SN were observed in whole human saliva. Cystatin S was known to be present in three forms: mono-phosphorylated, di-phosphorylated, and non-phosphorylated (Isemura et al. 1991) and involved in the mineral balance of the tooth. And cystatin-SN was found to inhibit the human lysosomalcathepsins B, H, and L in vitro (Baron et al. 1999). Recent studies reported salivary cystatins as prospective biomarkers of oral diseases and diabetes (Rudney et al. 2009; Bencharit et al. 2013). Cystatins are not elevated in normal conditions.

10.7.3 Apoptosis Inducing Factor 2 (AIF2)

AIF 2, an oxidoreductase acts as a caspase-independent mitochondrial effector of apoptotic cell death and plays a role in mediating apoptosis response. AIF 2 was over-expressed in response to hypobaric hypoxia, thereby confirming its induction by hypoxia (Greijer and van der Wall 2004).

10.7.4 Prolactin Inducible Protein (PIP)

PIP, an extra-parotid glycoprotein associated with secretory cell differentiation was found to be decreased in hypobaric hypoxia. Earlier, it was observed that decreased levels of salivary PIP were found in patients with bleeding oral cavities (Huang 2004).

10.7.5 Carbonic Anhydrase 6 (CA6)

CA6, an enzyme involved in respiratory alkalosis, could be considered as potential marker (Taylor 2011). As per earlier reports, buffering capacity of saliva plays an important in oral homeostasis and the bicarbonate buffer is the main buffer that contributes to the salivary buffering capacity (Bardow et al. 2000; Peres et al. 2010). Other findings suggested an ascent to high altitude was known to be associated with decreased concentration of bicarbonate and hydrogen ions, increased pH and thus, resulting in respiratory alkalosis (Goldfarb-Rumyantzev and Alper 2014). The increased expression of carbonic anhydrase 6 in this study suggested its probable role in conferring acclimatization to hypobaric hypoxia by neutralizing pH through bicarbonate balance.

10.7.6 Phospholipid Transfer Protein (PLTP)

PLTP, a regulator of lipid metabolism is known to play an important role in oxidative stress. Recent research suggested that PLTP modulates BBB integrity, possibly through its ability to transfer vitamin E, and modulate cerebro-vascular oxidative stress (Zhou et al. 2014). Another study suggested regulation of vitamin E bioavailability in lipoproteins through PLTP in order to protect circulating lipoproteins from oxidative stress (Jiang et al. 2002). Also, a finding suggested that this protein showed enhanced expression in hypoxia stimulus in case of emphysema (Jiang et al. 1998).

10.7.7 Interleukin 1 Receptor Antagonist (IL1R1)

Interleukin 1 receptor antagonist (IL1R1) is an upstream regulator of the pathway and a cytokine widely known to be associated with inflammation and expressed by activated mononuclear cells. The levels of circulating IL1RA in the plasma were increased in response to hypoxia suggesting the reason for its decreased level in salivary secretions and as a potential target for anti-inflammatory therapy (Fritzsching et al. 2015).

10.7.8 Albumin, Alpha-1 Acid Glycoprotein, and Alpha-1 Antitrypsin

These downstream regulators of acute phase response signaling also showed similar patterns as IL1R1. According to previous research, albumin was observed to suppress VEGF via alteration of HIF/HRE pathway (Katavetin et al. 2008). Albumin also provides protection against hypoxia induced injuries (Strubelt et al. 1994). Another protein, alpha-1 acid glycoprotein is known to provide protection against hypoxia by inhibiting inflammation and apoptosis, thereby could be given exogenously as therapeutics (de Vries et al. 2004; Hochepied et al. 2003; Van Molle et al.

1997). Similar to other downstream regulators of the pathway, alpha-1 antitrypsin also has anti-inflammatory properties (Ahmad et al. 2013).

10.8 Concluding Remarks

In conclusion, these studies provided the proof of concept for translating salivary proteins into a non-invasive putative diagnostic panel for assessing hypobaric hypoxia. This panel has the potential to be used in future to diagnose individuals affected by hypobaric hypoxia.

References

Aaronson RM, Graven KK, Tucci M, McDonald RJ, Farber HW (1995) Non-neuronal enolase is an endothelial hypoxic stress protein. J Biol Chem 270:27752–27757

Actis AB, Perovic NR, Defago D, Beccacece C, Eynard AR (2005) Fatty acid profile of human saliva: a possible indicator of dietary fat intake. Arch Oral Biol 50:1–6

Agha-Hosseini F, Dizgah IM, Amirkhani S (2006) The composition of unstimulated whole saliva of healthy dental students. J Contemp Dent Pract 7:104–111

Ahmad Y, Shukla D, Garg I, Sharma NK, Saxena S, Malhotra V, Bhargava K (2011) Identification of haptoglobin and apolipoprotein AI as biomarkers for high altitude pulmonary edema. Funct Integr Genomics 11:407

Ahmad Y, Sharma NK, Garg I, Ahmad MF, Sharma M, Bhargava K (2013) An insight into the changes in human plasma proteome on adaptation to hypobaric hypoxia. PLoS One 8:e67548

Ahmad Y, Sharma NK, Ahmad MF, Sharma M, Garg I, Bhargava K (2014) Proteomic identification of novel differentiation plasma protein markers in hypobaric hypoxia-induced rat model. PLoS One 9:e98027

Ahmad Y, Sharma NK, Ahmad MF, Sharma M, Garg I, Srivastava M, Bhargava K (2015) The proteome of hypobaric induced hypoxic lung: insights from temporal proteomic profiling for biomarker discovery. Sci Rep 5:10681

Amerongen AV, Veerman EC (2002) Saliva—the defender of the oral cavity. Oral Dis 8:12–22

Aps JK, Martens LC (2005) Review: the physiology of saliva and transfer of drugs into saliva. Forensic Sci Int 150:119–131

Askew EW (2002) Work at high altitude and oxidative stress: antioxidant nutrients. Toxicology 180:107–119

Bakonyi T, Radak Z (2004) High altitude and free radicals. J Sports Sci Med 3:64–69

Bardow A, Moe D, Nyvad B, Nauntofte B (2000) The buffer capacity and buffer systems of human whole saliva measured without loss of CO2. Arch Oral Biol 45(1):1–12. https://doi.org/10.1016/s0003-9969(99)00119-3. PMID: 10669087

Barker KR, Conroy AL, Hawkes M, Murphy H, Pandey P, Kain KC (2016) Biomarkers of hypoxia, endothelial and circulatory dysfunction among climbers in Nepal with AMS and HAPE: a prospective case–control study. J Travel Med 23(3):taw005

Baron A, DeCarlo A, Featherstone J (1999) Functional aspects of the human salivary cystatins in the oral environment. Oral Dis 5:234–240

Bencharit S, Baxter SS, Carlson J, Byrd WC, Mayo MV, Border MB, Kohltfarber H, Urrutia E, Howard-Williams EL, Offenbacher S, Wu MC, Buse JB (2013) Salivary proteins associated with hyperglycemia in diabetes: a proteomic analysis. Mol Biosyst 9:2785–2797

Berger MM, Dehnert C, Bailey DM, Luks AM, Menold E, Castell C, Schendler G, Faoro V, Mairbäurl H, Bärtsch P (2009) Transpulmonary plasma ET-1 and nitrite differences in high altitude pulmonary hypertension. High Alt Med Biol 10:17–24

Bigarella CL, Liang R, Ghaffari S (2014) Stem cells and the impact of ROS signaling. Development 141:4206–4218

Bigler LR, Streckfus CF, Dubinsky WP (2009) Salivary biomarkers for the detection of malignant tumors that are remote from the oral cavity. Clin Lab Med 29:71–85

Campo J, Perea MA, del Romero J, Cano J, Hernando V, Bascones A (2006) Oral transmission of HIV, reality or fiction? An update. Oral Dis 12:219–228

Carpenter GH (2013) The secretion, components, and properties of saliva. Annu Rev Food Sci Technol 4:267–276

Carranza M, Ferraris ME, Galizzi M (2005) Structural and morphometrical study in glandular parenchyma from alcoholic sialosis. J Oral Pathol Med 34:374–379

Castagnola M, Scarano E, Passali GC, Messana I, Cabras T, Iavarone F, Di Cintio G, Fiorita A, De Corso E, Paludetti G (2017) Salivary biomarkers and proteomics: future diagnostic and clinical utilities. Acta Otorhinolaryngol Ital 37:94–101

Chang SW, Stelzner TJ, Weil JV, Voelkel NF (1989) Hypoxia increases plasma glutathione disulfide in rats. Lung 167:269–276

Charu R, Stobdan T, Ram R, Khan A, Pasha MQ, Norboo T, Afrin F (2006) Susceptibility to high altitude pulmonary oedema: role of ACE and ET-1 polymorphisms. Thorax 61:1011–1012

Chiappin S, Antonelli G, Gatti R, De Palo EF (2007) Saliva specimen: a new laboratory tool for diagnostic and basic investigation. Clin Chim Acta 383:30–40

Chicharro JL, Lucia A, Perez M, Vaquero AF, Urena R (1998) Saliva composition and exercise. Sports Med 26:17–27

Cooke M, Leeves N, White C (2003) Time profile of putrescine, cadaverine, indole and skatole in human saliva. Arch Oral Biol 48:323–327

Coufal P, Zuska J, van de Goor T, Smith V, Gas B (2003) Separation of twenty underivatized essential amino acids by capillary zone electrophoresis with contactless conductivity detection. Electrophoresis 24:671–677

Csosz E, Labiscsak P, Kallo G, Markus B, Emri M, Szabo A, Tar I, Tozser J, Kiss C, Marton I (2017) Proteomics investigation of OSCC-specific salivary biomarkers in a Hungarian population highlights the importance of identification of population-tailored biomarkers. PLoS One 12: e0177282

Deutsch O, Krief G, Konttinen YT, Zaks B, Wong DT, Aframian DJ, Palmon A (2015) Identification of Sjogren's syndrome oral fluid biomarker candidates following high-abundance protein depletion. Rheumatology (Oxford) 54:884–890

Diab-Ladki R, Pellat B, Chahine R (2003) Decrease in the total antioxidant activity of saliva in patients with periodontal diseases. Clin Oral Investig 7:103–107

Dosek A, Ohno H, Acs Z, Taylor AW, Radak Z (2007) High altitude and oxidative stress. Respir Physiol Neurobiol 158:128–131

Droma Y, Hayano T, Takabayashi Y, Koizumi T, Kubo K, Kobayashi T, Sekiguchi M (1996) Endothelin-1 and interleukin-8 in high altitude pulmonary oedema. Eur Respir J 9:1947–1949

Edgar WM (1990) Saliva and dental health. Clinical implications of saliva: report of a consensus meeting. Br Dent J 169:96–98

Elsana S, Sikuler E, Yaari A, Shemer-Avni Y, Abu-Shakra M, Buskila D, Katzman P, Naggan L, Margalith M (1998) HCV antibodies in saliva and urine. J Med Virol 55:24–27

Fernandez Rodriguez E, Carcaba Fernandez V, Rodriguez Junquera M, Alfonso Megido J, Garcia Amorin Z, Garcia Alonso S (1994) Detection of HIV antibodies in saliva using a rapid diagnostic immunoenzyme assay. Rev Clin Esp 194:523–525

Forde MD, Koka S, Eckert SE, Carr AB, Wong DT (2006) Systemic assessments utilizing saliva: Part 1 general considerations and current assessments. Int J Prosthodont 19:43–52

Fritzsching B, Zhou-Suckow Z, Trojanek JB, Schubert SC, Schatterny J, Hirtz S, Agrawal R, Muley T, Kahn N, Sticht C, Gunkel N, Welte T, Randell SH, Langer F, Schnabel P, Herth FJ, Mall MA (2015) Hypoxic epithelial necrosis triggers neutrophilic inflammation via IL-1 receptor signaling in cystic fibrosis lung disease. Am J Respir Crit Care Med 191:902–913

Gallo C, Ciavarella D, Santarelli A, Ranieri E, Colella G, Lo Muzio L, Lo Russo L (2016) Potential salivary proteomic markers of Oral squamous cell carcinoma. Cancer Genomics Proteomics 13: 55–61

Giusti L, Baldini C, Bazzichi L, Ciregia F, Tonazzini I, Mascia G, Giannaccini G, Bombardieri S, Lucacchini A (2007) Proteome analysis of whole saliva: a new tool for rheumatic diseases—the example of Sjogren's syndrome. Proteomics 7:1634–1643

Goldfarb-Rumyantzev AS, Alper SL (2014) Short-term responses of the kidney to high altitude in mountain climbers. Nephrol Dial Transplant 29(3):497–506. https://doi.org/10.1093/ndt/gft051. Epub 2013 Mar 22. PMID: 23525530; PMCID: PMC3938295

Greijer AE, van der Wall E (2004) The role of hypoxia inducible factor 1 (HIF-1) in hypoxia induced apoptosis. J Clin Pathol 57:1009–1014

Groschl M (2008) Current status of salivary hormone analysis. Clin Chem 54:1759–1769

Guan Y, Chu Q, Ye J (2004) Determination of uric acid in human saliva by capillary electrophoresis with electrochemical detection: potential application in fast diagnosis of gout. Anal Bioanal Chem 380:913–917

Hackett PH, Roach RC (2001) High-altitude illness. N Engl J Med 345:107–114

Hackett PH, Roach RC (2004) High altitude cerebral edema. High Alt Med Biol 5:136–146

Haeckel R, Hanecke P (1996) Application of saliva for drug monitoring. An in vivo model for transmembrane transport. Eur J Clin Chem Clin Biochem 34:171–191

Hartmann G, Tschöp M, Fischer R, Bidlingmaier C, Riepl R, Tschöp K, Hautmann H, Endres S, Toepfer M (2000) High altitude increases circulating interleukin-6, interleukin-1 receptor antagonist and C-reactive protein. Cytokine 12:246–252

Hochepied T, Berger FG, Baumann H, Libert C (2003) Alpha(1)-acid glycoprotein: an acute phase protein with inflammatory and immunomodulating properties. Cytokine Growth Factor Rev 14: 25–34

Hodinka RL, Nagashunmugam T, Malamud D (1998) Detection of human immunodeficiency virus antibodies in oral fluids. Clin Diagn Lab Immunol 5:419–426

Hoppeler H, Vogt M (2001) Muscle tissue adaptations to hypoxia. J Exp Biol 204:3133–3139

Hu S, Denny P, Denny P, Xie Y, Loo JA, Wolinsky LE, Li Y, McBride J, Ogorzalek Loo RR, Navazesh M, Wong DT (2004) Differentially expressed protein markers in human submandibular and sublingual secretions. Int J Oncol 25:1423–1430

Hu S, Xie Y, Ramachandran P, Ogorzalek Loo RR, Li Y, Loo JA, Wong DT (2005) Large-scale identification of proteins in human salivary proteome by liquid chromatography/mass spectrometry and two-dimensional gel electrophoresis-mass spectrometry. Proteomics 5:1714–1728

Hu S, Yu T, Xie Y, Yang Y, Li Y, Zhou X, Tsung S, Loo RR, Loo JR, Wong DT (2007a) Discovery of oral fluid biomarkers for human oral cancer by mass spectrometry. Cancer Genomics Proteomics 4:55–64

Hu S, Wang J, Meijer J, Ieong S, Xie Y, Yu T, Zhou H, Henry S, Vissink A, Pijpe J, Kallenberg C, Elashoff D, Loo JA, Wong DT (2007b) Salivary proteomic and genomic biomarkers for primary Sjogren's syndrome. Arthritis Rheum 56:3588–3600

Huang CM (2004) Comparative proteomic analysis of human whole saliva. Arch Oral Biol 49:951–962

Humphrey SP, Williamson RT (2001) A review of saliva: normal composition, flow, and function. J Prosthet Dent 85:162–169

Ilavazhagan G, Bansal A, Prasad D, Thomas P, Sharma SK, Kain AK, Kumar D, Selvamurthy W (2001) Effect of vitamin E supplementation on hypoxia-induced oxidative damage in male albino rats. Aviat Space Environ Med 72:899–903

Imai H, Kashiwazaki H, Suzuki T, Kabuto M, Himeno S, Watanabe C, Moji K, Kim SW, Rivera JO, Takemoto T (1995) Selenium levels and glutathione peroxidase activities in blood in an Andean high-altitude population. J Nutr Sci Vitaminol (Tokyo) 41:349–361

Irarrazaval S, Allard C, Campodonico J, Perez D, Strobel P, Vasquez L, Urquiaga I, Echeverria G, Leighton F (2017) Oxidative stress in acute hypobaric hypoxia. High Alt Med Biol 18:128–134

Isemura S, Saitoh E, Sanada K, Minakata K (1991) Identification of full-sized forms of salivary (S-type) cystatins (cystatin SN, cystatin SA, cystatin S, and two phosphorylated forms of cystatin S) in human whole saliva and determination of phosphorylation sites of cystatin S. J Biochem 110:648–654

Jain S, Ahmad Y, Bhargava K (2018) Salivary proteome patterns of individuals exposed to high altitude. Arch Oral Biol 96:104–112

Jain S, Paul S, Meena RN, Gangwar A, Panjwani U, Ahmad Y, Bhargava K (2020) Saliva panel of protein candidates: a comprehensive study for assessing high altitude acclimatization. Nitric Oxide 95:1–11

Jefferson JA, Simoni J, Escudero E, Hurtado ME, Swenson ER, Wesson DE, Schreiner GF, Schoene RB, Johnson RJ, Hurtado A (2004) Increased oxidative stress following acute and chronic high altitude exposure. High Alt Med Biol 5:61–69

Jiang XC, D'Armiento J, Mallampalli RK, Mar J, Yan SF, Lin M (1998) Expression of plasma phospholipid transfer protein mRNA in normal and emphysematous lungs and regulation by hypoxia. J Biol Chem 273:15714–15718

Jiang XC, Tall AR, Qin S, Lin M, Schneider M, Lalanne F, Deckert V, Desrumaux C, Athias A, Witztum JL, Lagrost L (2002) Phospholipid transfer protein deficiency protects circulating lipoproteins from oxidation due to the enhanced accumulation of vitamin E. J Biol Chem 277:31850–31856

Joanny P, Steinberg J, Robach P, Richalet JP, Gortan C, Gardette B, Jammes Y (2001) Operation Everest III (Comex'97): the effect of simulated sever hypobaric hypoxia on lipid peroxidation and antioxidant defence systems in human blood at rest and after maximal exercise. Resuscitation 49:307–314

de Jong EP, Xie H, Onsongo G, Stone MD, Chen XB, Kooren JA, Refsland EW, Griffin RJ, Ondrey FG, Wu B, Le CT, Rhodus NL, Carlis JV, Griffin TJ (2010) Quantitative proteomics reveals myosin and actin as promising saliva biomarkers for distinguishing pre-malignant and malignant oral lesions. PLoS One 5:e11148

Kaczor-Urbanowicz KE, Martin Carreras-Presas C, Aro K, Tu M, Garcia-Godoy F, Wong DT (2017) Saliva diagnostics—current views and directions. Exp Biol Med (Maywood) 242:459–472

Kalk WW, Vissink A, Stegenga B, Bootsma H, Nieuw Amerongen AV, Kallenberg CG (2002) Sialometry and sialochemistry: a non-invasive approach for diagnosing Sjogren's syndrome. Ann Rheum Dis 61:137–144

Katavetin P, Inagi R, Miyata T, Tanaka T, Sassa R, Ingelfinger JR, Fujita T, Nangaku M (2008) Albumin suppresses vascular endothelial growth factor via alteration of hypoxia-inducible factor/hypoxia-responsive element pathway. Biochem Biophys Res Commun 367:305–310

Kim JW, Tchernyshyov I, Semenza GL, Dang CV (2006) HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. Cell Metab 3:177–185

Larsson B, Olivecrona G, Ericson T (1996) Lipids in human saliva. Arch Oral Biol 41:105–110

Lee YH, Wong DT (2009) Saliva: an emerging biofluid for early detection of diseases. Am J Dent 22:241–248

Levett DZ, Fernandez BO, Riley HL, Martin DS, Mitchell K, Leckstrom CA, Ince C, Whipp BJ, Mythen MG, Montgomery HE, Grocott MP, Feelisch M, For the Caudwell Extreme Everest Research, G (2011) The role of nitrogen oxides in human adaptation to hypoxia. Sci Rep 1:109

Lloyd JE, Broughton A, Selby C (1996) Salivary creatinine assays as a potential screen for renal disease. Ann Clin Biochem 33(Pt 5):428–431

Lu H, Wang R, Li W, Xie H, Wang C, Hao Y, Sun Y, Jia Z (2018) Plasma proteomic study of acute mountain sickness susceptible and resistant individuals. Sci Rep 8:1265

Luks AM, Levett D, Martin DS, Goss CH, Mitchell K, Fernandez BO, Feelisch M, Grocott MP, Swenson ER, the Caudwell Xtreme Everest, I. (2017) Changes in acute pulmonary vascular responsiveness to hypoxia during a progressive ascent to high altitude (5300 m). Exp Physiol 102:711–724

Magalhaes J, Ascensao A, Soares JM, Ferreira R, Neuparth MJ, Marques F, Duarte JA (1985) (2005) acute and severe hypobaric hypoxia increases oxidative stress and impairs mitochondrial function in mouse skeletal muscle. J Appl Physiol 99:1247–1253

Mager DL, Haffajee AD, Devlin PM, Norris CM, Posner MR, Goodson JM (2005) The salivary microbiota as a diagnostic indicator of oral cancer: a descriptive, non-randomized study of cancer-free and oral squamous cell carcinoma subjects. J Transl Med 3:27

Maiti P, Singh SB, Sharma AK, Muthuraju S, Banerjee PK, Ilavazhagan G (2006) Hypobaric hypoxia induces oxidative stress in rat brain. Neurochem Int 49:709–716

McLean CJ, Booth CW, Tattersall T, Few JD (1989) The effect of high altitude on saliva aldosterone and glucocorticoid concentrations. Eur J Appl Physiol Occup Physiol 58:341–347

Mikuriya K, Kuramitsu Y, Ryozawa S, Fujimoto M, Mori S, Oka M, Hamano K, Okita K, Sakaida I, Nakamura K (2007) Expression of glycolytic enzymes is increased in pancreatic cancerous tissues as evidenced by proteomic profiling by two-dimensional electrophoresis and liquid chromatography-mass spectrometry/mass spectrometry. Int J Oncol 30:849–855

Mominzadeh M, Mirzaii-Dizgah I, Mirzaii-Dizgah MR, Mirzaii-Dizgah MH (2014) Stimulated saliva aminotransaminase alteration after experiencing acute hypoxia training. Air Med J 33: 157–160

Nagler RM, Hershkovich O, Lischinsky S, Diamond E, Reznick AZ (2002) Saliva analysis in the clinical setting: revisiting an underused diagnostic tool. J Invest Med 50:214–225

Nakanishi K, Tajima F, Nakamura A, Yagura S, Ookawara T, Yamashita H, Suzuki K, Taniguchi N, Ohno H (1995) Effects of hypobaric hypoxia on antioxidant enzymes in rats. J Physiol 489(Pt 3):869–876

van Patot MCT, Leadbetter G, Keyes LE, Bendrick-Peart J, Beckey VE, Christians U, Hackett P (2005) Greater free plasma VEGF and lower soluble VEGF receptor-1 in acute mountain sickness. J Appl Physiol (1985) 98(5):1626–1629

Peluso G, De Santis M, Inzitari R, Fanali C, Cabras T, Messana I, Castagnola M, Ferraccioli GF (2007) Proteomic study of salivary peptides and proteins in patients with Sjogren's syndrome before and after pilocarpine treatment. Arthritis Rheum 56:2216–2222

Peres R, Camargo G, Mofatto L et al (2010) Association of polymorphisms in the carbonic anhydrase 6 gene with salivary buffer capacity, dental plaque pH, and caries index in children aged 7–9 years. Pharmacogenomics J 10:114–119. https://doi.org/10.1038/tpj.2009.37

Pfaffe T, Cooper-White J, Beyerlein P, Kostner K, Punyadeera C (2011) Diagnostic potential of saliva: current state and future applications. Clin Chem 57:675–687

Pilardeau P, Richalet JP, Bouissou P, Vaysse J, Larmignat P, Boom A (1990) Saliva flow and composition in humans exposed to acute altitude hypoxia. Eur J Appl Physiol Occup Physiol 59: 450–453

Radak Z, Lee K, Choi W, Sunoo S, Kizaki T, Oh-ishi S, Suzuki K, Taniguchi N, Ohno H, Asano K (1994) Oxidative stress induced by intermittent exposure at a simulated altitude of 4000 m decreases mitochondrial superoxide dismutase content in soleus muscle of rats. Eur J Appl Physiol Occup Physiol 69:392–395

Radak Z, Asano K, Lee KC, Ohno H, Nakamura A, Nakamoto H, Goto S (1997) High altitude training increases reactive carbonyl derivatives but not lipid peroxidation in skeletal muscle of rats. Free Radic Biol Med 22:1109–1114

Rehak NN, Cecco SA, Csako G (2000) Biochemical composition and electrolyte balance of "unstimulated" whole human saliva. Clin Chem Lab Med 38:335–343

Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB (2010) Oxidative stress, inflammation, and cancer: how are they linked? Free Radic Biol Med 49:1603–1616

Ribon A, Pialoux V, Saugy JJ, Rupp T, Faiss R, Debevec T, Millet GP (2016) Exposure to hypobaric hypoxia results in higher oxidative stress compared to normobaric hypoxia. Respir Physiol Neurobiol 223:23–27

Rudney JD, Staikov RK, Johnson JD (2009) Potential biomarkers of human salivary function: a modified proteomic approach. Arch Oral Biol 54:91–100

San T, Polat S, Cingi C, Eskiizmir G, Oghan F, Cakir B (2013) Effects of high altitude on sleep and respiratory system and theirs adaptations. ScientificWorldJournal 2013:241569

Sartori C, Vollenweider L, Löffler B-M, Delabays A, Nicod P, Bartsch P, Scherrer U (1999) Exaggerated endothelin release in high-altitude pulmonary edema. Circulation 99:2665–2668

Schafer CA, Schafer JJ, Yakob M, Lima P, Camargo P, Wong DT (2014) Saliva diagnostics: utilizing oral fluids to determine health status. Monogr Oral Sci 24:88–98

Sedoris KC, Thomas SD, Miller DM (2010) Hypoxia induces differential translation of enolase/MBP-1. BMC Cancer 10:157

Semenza GL, Jiang BH, Leung SW, Passantino R, Concordet JP, Maire P, Giallongo A (1996) Hypoxia response elements in the aldolase a, enolase 1, and lactate dehydrogenase a gene promoters contain essential binding sites for hypoxia-inducible factor 1. J Biol Chem 271: 32529–32537

Sharp FR, Bernaudin M (2004) HIF1 and oxygen sensing in the brain. Nat Rev Neurosci 5:437–448

Siervo M, Riley HL, Fernandez BO, Leckstrom CA, Martin DS, Mitchell K, Levett DZH, Montgomery HE, Mythen MG, Grocott MPW, Feelisch M, for the Caudwell Xtreme Everest Research, G (2014) Effects of prolonged exposure to hypobaric hypoxia on oxidative stress, inflammation and Gluco-insular regulation: the not-so-sweet Price for good regulation. PLoS One 9:e94915

Sikri G, Bhattachar S (2017) Comment on "Soluble urokinase-type plasminogen activator receptor plasma concentration may predict susceptibility to high altitude pulmonary edema". Mediators Inflamm 2017:8546027

Singh M, Thomas P, Shukla D, Tulsawani R, Saxena S, Bansal A (2013) Effect of subchronic hypobaric hypoxia on oxidative stress in rat heart. Appl Biochem Biotechnol 169:2405–2419

Strapazzon G, Malacrida S, Vezzoli A, Dal Cappello T, Falla M, Lochner P, Moretti S, Procter E, Brugger H, Mrakic-Sposta S (2016) Oxidative stress response to acute hypobaric hypoxia and its association with indirect measurement of increased intracranial pressure: a field study. Sci Rep 6:32426

Streckfus CF, Bigler L (2016) A catalogue of altered salivary proteins secondary to invasive ductal carcinoma: a novel in vivo paradigm to assess breast cancer progression. Sci Rep 6:30800

Streckfus CF, Mayorga-Wark O, Arreola D, Edwards C, Bigler L, Dubinsky WP (2008) Breast cancer related proteins are present in saliva and are modulated secondary to ductal carcinoma in situ of the breast. Cancer Invest 26:159–167

Strubelt O, Younes M, Li Y (1994) Protection by albumin against ischaemia- and hypoxia-induced hepatic injury. Pharmacol Toxicol 75:280–284

Taylor AT (2011) High-altitude illnesses: physiology, risk factors, prevention, and treatment. Rambam Maimonides Med J 2:e0022

Van Molle W, Libert C, Fiers W, Brouckaert P (1997) Alpha 1-acid glycoprotein and alpha 1-antitrypsin inhibit TNF-induced but not anti-Fas-induced apoptosis of hepatocytes in mice. J Immunol 159:3555–3564

Van Nieuw Amerongen A, Bolscher JG, Veerman EC (2004) Salivary proteins: protective and diagnostic value in cariology? Caries Res 38:247–253

de Vries B, Walter SJ, Wolfs TG, Hochepied T, Rabina J, Heeringa P, Parkkinen J, Libert C, Buurman WA (2004) Exogenous alpha-1-acid glycoprotein protects against renal ischemia-reperfusion injury by inhibition of inflammation and apoptosis. Transplantation 78:1116–1124

Walsh NP, Laing SJ, Oliver SJ, Montague JC, Walters R, Bilzon JL (2004) Saliva parameters as potential indices of hydration status during acute dehydration. Med Sci Sports Exerc 36:1535–1542

Wilson MH, Newman S, Imray CH (2009) The cerebral effects of ascent to high altitudes. Lancet Neurol 8:175–191

Woods DR, Davison A, Stacey M, Smith C, Hooper T, Neely D, Turner S, Peaston R, Mellor A (2012) The cortisol response to hypobaric hypoxia at rest and post-exercise. Horm Metab Res 44:302–305

Xiao H, Zhang L, Zhou H, Lee JM, Garon EB, Wong DT (2012) Proteomic analysis of human saliva from lung cancer patients using two-dimensional difference gel electrophoresis and mass spectrometry. Mol Cell Proteomics 11(M111):012112

Xu RH, Pelicano H, Zhou Y, Carew JS, Feng L, Bhalla KN, Keating MJ, Huang P (2005) Inhibition of glycolysis in cancer cells: a novel strategy to overcome drug resistance associated with mitochondrial respiratory defect and hypoxia. Cancer Res 65:613–621

Yaari A, Tovbin D, Zlotnick M, Mostoslavsky M, Shemer-Avni Y, Hanuka N, Burbea Z, Katzir Z, Storch S, Margalith M (2006) Detection of HCV salivary antibodies by a simple and rapid test. J Virol Methods 133:1–5

Yamaguchi M, Takada R, Kambe S, Hatakeyama T, Naitoh K, Yamazaki K, Kobayashi M (2005) Evaluation of time-course changes of gingival crevicular fluid glucose levels in diabetics. Biomed Microdevices 7:53–58

Yoshikawa T, Furukawa Y, Wakamatsu Y, Takemura S, Tanaka H, Kondo M (1982) Experimental hypoxia and lipid peroxide in rats. Biochem Med 27:207–213

Yoshizawa JM, Schafer CA, Schafer JJ, Farrell JJ, Paster BJ, Wong DT (2013) Salivary biomarkers: toward future clinical and diagnostic utilities. Clin Microbiol Rev 26:781–791

Zalewska A, Zwierz K, Zolkowski K, Gindzienski A (2000) Structure and biosynthesis of human salivary mucins. Acta Biochim Pol 47:1067–1079

Zelles T, Purushotham KR, Macauley SP, Oxford GE, Humphreys-Beher MG (1995) Saliva and growth factors: the fountain of youth resides in us all. J Dent Res 74:1826–1832

Zhang L, Farrell JJ, Zhou H, Elashoff D, Akin D, Park NH, Chia D, Wong DT (2010) Salivary transcriptomic biomarkers for detection of resectable pancreatic cancer. Gastroenterology 138(949–57):e1–e7

Zhang Y, Duan R, Cui W, Pan Z, Liu W, Long C, Wang Y, Wang H (2013) Proteomic identification of human serum biomarkers associated with high altitude pulmonary edema. Zhongguo Ying Yong Sheng Li Xue Za Zhi 29:501–507

Zhou T, He Q, Tong Y, Zhan R, Xu F, Fan D, Guo X, Han H, Qin S, Chui D (2014) Phospholipid transfer protein (PLTP) deficiency impaired blood-brain barrier integrity by increasing cerebrovascular oxidative stress. Biochem Biophys Res Commun 445:352–356