Chapter 9
Metabolic Control: Immune Control?

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9.1 The Essence of Metabolism

Metabolic challenges under the condition of space have been reported from the very beginning of human spaceflight as by the effects on the muscular and skeletal system. The causes and the consequences for metabolism, which includes "construction" (anabolism) and "destruction" (catabolism) of energy depots and tissues on the organic level, respectively, are not well understood because of their complex orchestrated network of endo-, auto-, and paracrine pathways in the regulation of the cell metabolic functions. Such metabolic and inflammatory causes are, for example, considered to be strongly contributing to the degeneration of the musculoskeletal system, as observed during spaceflights (Smith et al. 2015).

All metabolic changes result from substrate and enzyme interactions at the cellular and subcellular levels. Here, the recurrent pathways use "downstream" products of carbohydrate-, fat-, and protein-metabolism to finally confluence into the high-energetic reduction equivalents nicotinamide adenine dinucleotide (NADH/H^+) and flavin adenine dinucleotide ($FADH_2$). Together with oxygen, they are converted into the ubiquitary cellular source of energy, adenosine triphosphate (ATP) in the mitochondria. To produce ATP, products of intermediate metabolism enter the Krebs cycle and deliver electrons for reduction equivalents NADH/H^+ and $FADH_2$. Finally, these equivalents are oxidized by oxygen while delivering energy for the creation of the proton gradient over the inner mitochondrial membrane. The establishment of the proton gradient is regulated by four distinct enzymes (mitochondrial "complexes 1-4"), located in the inner membrane and known as the *electron transport chain*. The backflow of protons into the mitochondrial matrix is used by ATP-synthase (mitochondrial complex 5) for ATP synthesis (Mitchell 1961). For this

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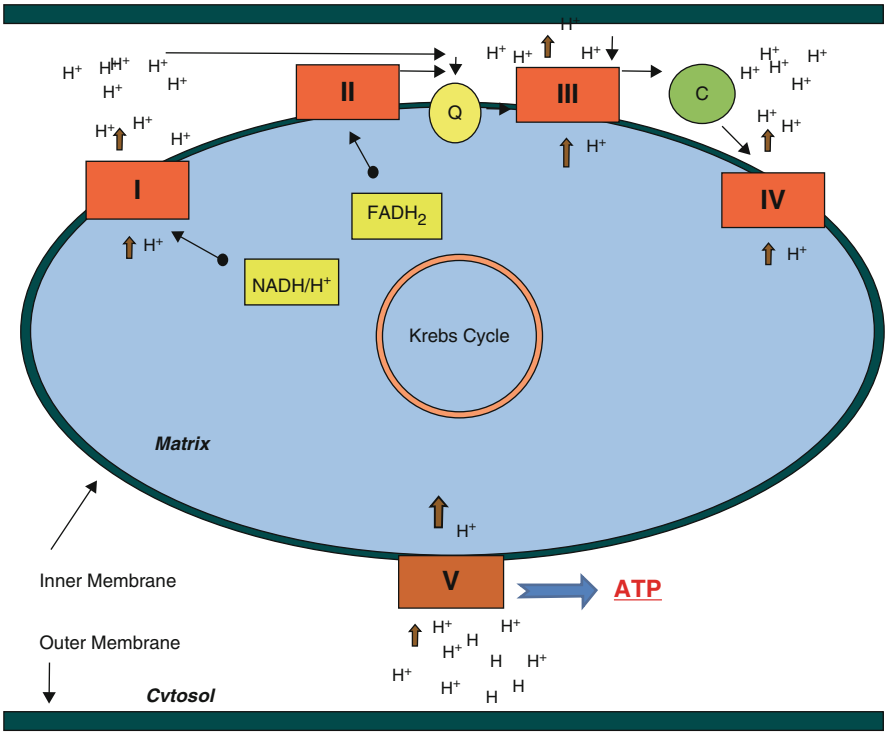

Fig. 9.1 Overview of ATP-(adenosine triphosphate) synthesis in mitochondria: cytochrome c (Cyt), nicotinamide adenine dinucleotide (NADH/H+), flavin adenine dinucleotide ($FADH_2$), and Ubichinon (Q). Enzymes of electron transport chain (*I*, *II*, *III*, *IV*) and ATP-synthase (*V*): I-NADH-Q-oxidoreduktase; II-succinatdehydrogenase; III-Q-cytochrom-c-oxidoreduktase; IV- cytochrom-c-oxidase; V-ATP-synthase. H+: protons

reason, and besides many other physiological functions (Galluzzi et al. 2012), mitochondria are considered the metabolic "heart" of the cells, tissues, and organs [for an overview, see Fig. 9.1].

Besides well-known lethal effects of poisons such as cyanides that block the respiratory chain, there is only very little knowledge about clinically applicable pharmacological substances that affect mitochondrial function directly and exclusively. In contrast, side effects of commonly used drugs on mitochondria are better established, but to the best of our knowledge, until now, *mito-drugs* do not exist (Parikh et al. 2009). However, manipulation of cellular metabolism on the mitochondrial level could be a unique and direct pathway to control and manipulate cell functions and thereby modulate organ-functioning under stressful situations, like lowered oxygen content in the living atmosphere (hypoxia), microgravity, disease states of individuals including inflammatory processes and altered nutritional supply, as all those are related to the challenges man has to face to during exploration-class space missions in the future.

9.2 Homeostasis, Oxygen, and Metabolic Derangements

Understanding the cellular, the organs' and the entire organisms' metabolic adaptation to environmental changes (stressors) in space is inherently multidisciplinary and complex and the metabolic adaptation during long-term space and exploration-class missions needs to be understood. Especially, the effects of gradual G forces as on Moon or Mars together with the effects of lowered oxygen tension (hypoxia) are a matter of concern. These additional environmental stressors will affect the space crew further, since reduced oxygen content is considered to be implemented on such missions and in future habitat designs for various operational and technical reasons.

Both, changes in gravity and living atmospheres can become key elements affecting the cells' metabolic states (Heer et al. 2001). Thus "metabolic control" has become more critical during such missions since it can mitigate unfavorable changes of the cells energy metabolism, the homeostasis. Homeostasis (Greek: ὁμοιοστάσις-balance) is the property of a system in which input and output variables are regulated in a way that internal conditions remain stable and tissue-specific requirements can be realized on a cellular level. Here, there are many actuating variables, like the pH, electrolyte distribution, water distribution, membrane potential, and temperature, which have to be adjusted exactly by energy-consuming biochemical reactions to enable homeostasis also of the immune cells. Mitochondria are the cellular components providing the energy for maintaining homeostasis. The function of these organelles is related to the use of oxygen since more than 90 % of the whole bodies' oxygen consumption takes place in the mitochondria (Ernster and Schatz 1981). During basal metabolism, the oxygen yield is almost complete; experiments have shown that oxygen consumption in Complex 4 (cytochrom-c-oxidase, the actual place of oxygen consumption) cannot be increased more than 16–40 % (Gnaiger and Kuznetsov 2002; Boveris and Britton 1973; Gnaiger et al. 1995; Nolana et al. 2010). In contrast, increasing evidence demonstrates that, during critical situations like systemic inflammatory response syndrome (SIRS), additional donation of oxygen can boost the immune response and further aggravate potential disease states (Strewe et al. 2015a; Zangl et al. 2014; Marconi et al. 2014; Saugstad 2005; Deulofeut et al. 2006; Deuber and Terhaar 2011; Kallet and Matthay 2013; Pagano and Barazzone-Argiroffo 2003; Deng et al. 2000; Garner et al. 1989; Rodríguez-González et al. 2014). This can be well explained by evolution of life on Earth since adaptation mechanisms were predominant to low oxygen concentrations (Hochachka 1998; Fisher and Burggren 2007; Kasting et al. 2003) while hyperoxic conditions probably did never exist in Earth history (Kasting et al. 2003) [see also Chap. 1]. To date, a good demonstration for such adaptation to lower oxygen levels is the intrauterine development of each individual life. Every fetus is subjected to oxygen partial pressures far below the reference areas after birth, though enough oxygen and energy are provided to enable the development of all organs. During those most complex steps of life-development, arterial partial pressures are low and remain between 18 and 26 mmHg, which corresponds to approximately 25 % the worth

adults have (Martin et al. 2010). So the evading question remains, if and how hypoxic environments together with gravitational changes enable mitochondria to maintain energy supply for the (immune-)cellular homeostasis, and where a potential threshold of lowered oxygen tension acceptance will be identified and defined for such missions?

9.3 Mitochondria and Immune Control

Mitochondria play multiple roles and have a critical impact on the regulation of innate and adaptive immune responses. They are important in their functions as bioenergetic organelles – as stated above – and in their biosynthetic functions, and also as immune cell signaling elements (Weinberg et al. 2015).

Biosynthetic functions include key steps of anaplerosis, which is the replenishment of lacking but needed components to realize reaction chains of metabolism. To create the "closed loop" of the citrat cycle (TCA, see figure 9.1), mitochondria have to deliver essential components like acetyl-Co-A, which can also further modify proteins (Hensley et al. 2013). Another molecule, which is substituted in an anaplerotical way, is α-ketoglutarate, also used for further immune-signaling (Wellen and Thompson 2012). Also, reactive oxygen species (ROS) are mostly generated inside mitochondria. ROS from mitochondria play a crucial role in the regulation of transcription via NF-kB (nuclear factor 'kappa-light-chain-enhancer' of activated B-cells), a specific transcription factor of almost all cell types in animals. Through the tight interaction between mitochondria and NF-kB, hundreds of immune genes that are involved in regulating cell growth, differentiation, development, and apoptosis, are regulated (Chandel et al. 2000, 2001). Further influences of mitochondria on immune cells beyond energy supply are the proper induction of antiviral signaling (Reikine et al. 2014), T-cell activation (Sena et al. 2013), CD 4+ T-cell differentiation (Berod et al. 2014), and regulation of CD 8+ T-cell memory formation (MacIver et al. 2011). There might be possible interactions between the antiviral immune functions and the energetic state of the mitochondria, especially under deviant oxygen conditions like hypoxia, which are not well understood today.

The role of mitochondria as *signaling elements* is based on the endosymbiotic theory, which postulates, that mitochondria and bacteria share the same origin (Nass and Nass 1963). New insights into the most severe forms of systemic inflammation, sepsis and SIRS, have helped to understand the pathology of the inflammation and the role of mitochondria and bacteria: The two clinical entities of sepsis (induced by bacterial components in blood) and SIRS (the immune system's monotonous-systemic answer to any kind of lesion) are triggered by activation of pattern recognition receptors (PRR) by the innate immune system (Takeuchi and Akira 2010). In such inflammatory condition of sepsis, PRR identify pathogen-associated molecular patterns (PAMPS) from bacteria as the molecular inductors of inflammation. During SIRS, however, damage-associated molecular patterns (DAMPS), directly liberated from damaged mitochondria, activate the innate immune response via

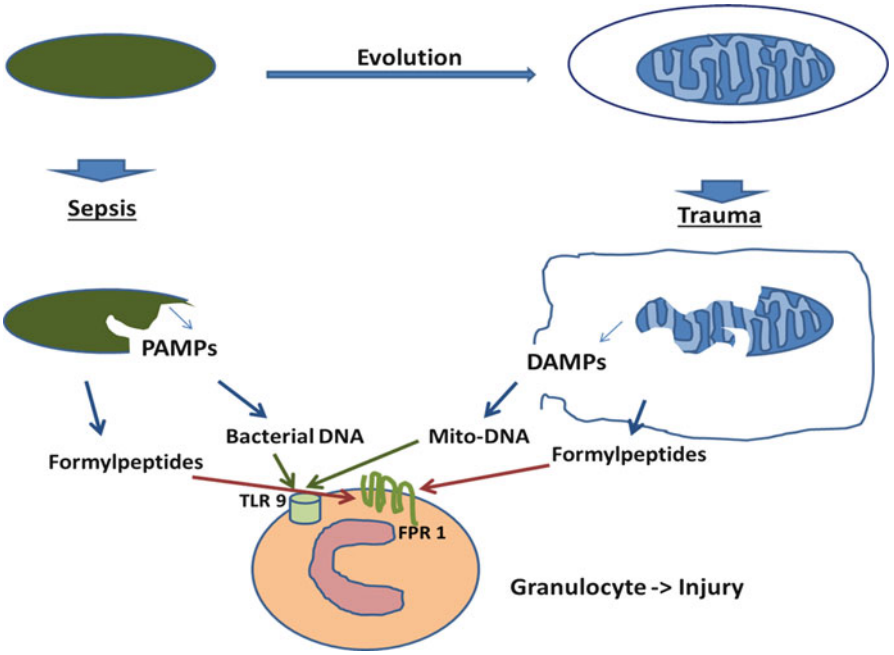

Fig. 9.2 PAMPs and DAMPs in the inflammatory response. Similar to the release of bacterial DNA (deoxyribonucleic acid) following sepsis, the mitochondrial DNA released by severe trauma can also act through the toll-like receptor-9 (TLR9) to activate neutrophils. Similarly, formylated peptides released from bacteria and mitochondria activate the formyl peptide receptor-1 (FPR1) and attract neutrophils by the process of chemotaxis to sites of inflammation and injury. In both cases, the outcome may be acute lung injury, which is part of the systemic inflammatory response syndrome (SIRS). *DAMPs* damage-associated molecular patterns, *PAMPs* pathogen-associated molecular patterns (redraw after: 2010 Nature Publishing Group (Calfee and Matthay 2010))

PRR (Vargas-Parada 2010). Both components, PAMPS from bacteria and DAMPS from mitochondria, confluence into a "crossover" activation of immune cells through the toll-like receptor-9 [TLR9] and formyl peptide receptor-1 [FPR1] on neutrophilic granulocytes (see Fig. 9.2), resulting in detrimental consequences for patients (Zhang et al. 2010)

Thus, the integrity and operational capability of mitochondria are of fundamental importance for immune functions: if homeostasis could not be balanced, malperformance of immune functions with insufficient reactions to pathogens can result. Further decrease of mitochondrial metabolism can result in increased ROS release with the result of direct cellular damage by liberated radicals. If malperformance of mitochondrial metabolism ensues, the breakdown of ATP-production and activation of apoptotic pathways with consecutive cell death would be the result (Wang and Youle 2009). In the case of total metabolic breakdown, direct induction of SIRS by mitochondria can occur. Therefore, both mitochondrial integrity and functionality are the basis of adequate immune answers. The oxygen thresholds for mitochondria to perform sufficient ATP production are not well

established; in vitro experiments showed good metabolic performance, even under hypoxic conditions (Gnaiger et al. 2000). The well-known records of mountain climbers in the Himalayas demonstrate that acclimatization and training enable life with 25 % of the above-mentioned values, though adverse effects on immune functions were observed depending on altitude and exposition time. Currently, interspace agency and polar institute research projects in the high Antarctic plateaus are conducted to investigate such effects in a systematic manner reflecting space-mission-relevant atmospheric conditions and exposition times (Pagel and Choukèr 2016).

9.4 Approaches and Benefit of Metabolic Control During Spaceflights

Obviously, there are many factors in the artificial environment of a spaceflight that can negatively affect the maintenance of homeostasis [see Chaps. 1 and 2]. If a fast, cheap, reversible, and safe method for the (down-)regulation of cellular metabolic activity at the mitochondrial level would exist, the below-mentioned problems could positively be influenced and also related immune responses be controlled, accordingly. The pathways of such an approach include the understanding of the metabolic control that can either include direct mitochondrial targeted drugs (such as adenosine) or the regulation by variation of the oxygen concentrations delivered to the mitochondria. Ultimately, the control of the immune cells' metabolisms and the reduction of the metabolic rate of the entire organism as such could lead to the induction of hibernation. Hibernation is an emerging scientific field for biology, human and life sciences in general and can become an interesting application for space. It is known, from animals and clinical studies in humans that some effects of "tissue hibernation" effects can be elicited by the preconditioning of organs. Preconditioning seems to have strong biological similarities to physiological states as elicited in hibernation and reduces tissue energy consumption and preserves the energy charge of the organ. Thereby, it evokes tolerance to further reduced nutritional supply as characterized by dampened expression of genes, the functions of which influence glucose metabolism, protein turnover, cell cycle, regulation, and ion-channel abundance. These features together mimic hibernation and hypoxia tolerance, suggesting the existence of a conserved endogenous genomic program of physiological adaptations to oxygen limitation that improve survival (Stenzel-Poore et al. 2003; Heldmeier et al. 2004).

Cells' metabolic states do inherently involve signaling through purines and their receptors. Adenosine is one of the key molecules that sense lack of oxygen and high-energy phosphates. Either cellular stress (hypoxia, reduction of tissue energy charge) can result in the production of adenosine and its binding to four different adenosine (A1, A2A, A2B, and A3) receptor sites and thereby regulate intracellular cAMP levels (Chouker et al. 2012; Abbracchio et al. 2009; Jinka et al. 2011). But also stress hormones (see Chap. 2), which are released in space, such as endocannabionoids (ECS) (Strewe et al. 2015b), are candidate ligands that can be involved

in cellular signaling related to metabolic control. Endocannabinoids are rapid-acting, lipid-signaling molecules that bind to endogenous endocannabinoid receptors. They play a critical role in the integration of adaptive responses of the organism to aversive environmental conditions including emotional and physical stress and are immune-regulatory (Hill et al. 2008; Dlugos et al. 2012). Moreover, endocannabinoid receptors are found on the mitochondrial membranes of cells, indicating a direct control of mitochondrial functions (Bénard et al. 2012).

9.5 Summary

The complexity of requirements during human spaceflights have led to developments in various scientific fields, especially in medicine. Knowledge regarding organ performance during critical situations, like degeneration of musculoskeletal system, severe illness, reduced nutritional support, and hypoxia is steadily increasing. A potential target point to influence such critical conditions is to modulate the highly preserved subcellular metabolism in mitochondria. Hypoxic conditions, stimulation with external and internal adenosine (or similar [ant]-agonists), and cannabinoids may help to reduce cellular metabolism and consecutively reduce resources and enable a higher mission success. The use of such pharmacological approaches can become a promising tool to mitigate immune- and metabolism-related risks and offer also new avenues to "metabolically shield" the human from the stressors that occur in such long-duration exploration missions.

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