consumption rate (OCR) and extracellular acidification rate (ECAR) were assessed using the Seahorse XFe96, mitochondrial network morphology was evaluated by confocal microscopy, and substrate oxidation was determined using Biolog Metabolic Phenotype Microarrays. Oxidative stress was evaluated by using fluorescent dyes.
Results: Up-regulation of OXPHOS resulted in increased sensitivity to classical mitochondrial toxicants, mitochondrial mass, respiration, and oxidation of Krebs cycle substrates. Adaptation to increased OXPHOS-reliance resulted in higher basal oxidative stress, while showing increased resistance to oxidants.
Conclusion: Manipulation of substrates in the cell culture medium remodels responses to oxidative stress, which is relevant in the context of pre-clinical studies involving redox agents.
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NC46

Microgravity induces cellular senescence in human keratinocytes

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Altered gravity would be expected to have profound impacts on different human organs and tissues, including the cardiovascular system, the skeletal tissue, the central nervous system, or muscle tissues in terms of an accelerated senescence process. Although it has been demonstrated that also the skin can be susceptible to a prolonged altered gravitational force, the knowledge about the effects of microgravity on the cutaneous tissue has been little investigated.
To directly study the effects of microgravity on human keratinocytes senescence, the cells were placed for 6, 24, and 48 hours on Random Positioning Machine (RPM) device which is able to simulate a decrease of earth gravity, and cellular senescence-related markers were analyzed. Results showed that microgravity was able to reduce cell proliferation and growth capacity. Cellular morphology analysis by Transmission Electron microscopy (TEM) demonstrated alteration in keratinocytes from 6 to 48 hours. In addition, microgravity exposure increased the expression of the senescence-associated protein (i.e. p21 and p16) and an increased expression of specific senescence cytokines (i.e. IL1α, IL 6, and IL8), suggesting the induction of the senescence-associated secretory phenotype (SASP). Finally, the electrophysiological approach shows that microgravity decreases the total membrane current of human keratinocytes, indicating a more generic effect on the cellular homeostasis system.
In conclusion, the current work brings new insights on the effects of altered gravity on human skin; in particular, our results demonstrated that microgravity deeply alters the characteristic of human skin cells promoting ASSP phenotype. Further analysis of the molecular mechanisms by which microgravity induces cellular senescence, will be investigated to appreciate the impact of the gravitational forces on human skin aging and for developing effective countermeasures.

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Antimicrobial peptides as possible new players in pollution-mediated skin damage

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Ozone (O_3) exposure has been reported to contribute to various cutaneous inflammatory conditions, such as eczema, psoriasis, rush etc. via a redox-inflammatory pathway. O_3 is too reactive to penetrate cutaneous tissue; it interacts with lipids present in the outermost layer of skin, resulting in formation of oxidized molecules and hydrogen peroxide (H_2O_2). Interestingly, several inflammatory skin pathologies demonstrate altered levels of antimicrobial peptides (AMPs). These small, cationic peptides are found in various cells, including keratinocytes, eccrine gland cells, and sebocytes. Classically, AMPs function as antimicrobial agents. Recent studies indicate that AMPs also play roles in inflammation, angiogenesis, and wound healing. Since altered levels of AMPs have been detected in pollution-associated skin pathologies, we hypothesized that exposure to O_3 could affect the levels of AMPs in the skin. We examined levels of AMPs using qRT-PCR, Western blotting, and immunofluorescence in vitro (human keratinocytes), ex vivo (human skin explants), and in vivo (human volunteer subjects exposed to O_3) and observed increased levels of all the measured AMPs upon O_3 exposure. In addition, in vitro studies have confirmed redox regulation of AMPs in keratinocytes. This novel finding suggests that targeting AMPs could be a possible defensive strategy to combat pollution-associated skin pathologies.

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Role of glyoxalases in wound healing process: an in vitro study

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Maintenance of skin integrity is crucial to avoid infections, and wound healing is a complex process that involves ROS overproduction and the activation of redox-related mechanisms. Altered wound repair is often observed in redox-imbalanced states, such as aging and diabetes (DB). In particular, DB-induced hyperglycemia promotes the build-up of methylglyoxal (MG), a pro-oxidant dicarbonyl compound that in vivo forms the so-called advanced glycation end-products (AGEs), that worsen cell damage and tissue dysfunctions. Accordingly, impaired MG detoxification, whose levels are mainly controlled by glyoxalase 1 and 2 (GLO1 and GLO2), is suspected to play a role in DB-related "difficult wounds". However, little information is available on the function of MG metabolism in the wound healing physiology. To this aim, human keratinocytes (HaCat) were scratched and collected in a time-dependent fashion for assessing: i) gene/protein expression and enzymatic activities of GLOs; ii) levels of GSH (co-factor of GLO1); iii) levels of receptors for AGE (RAGE). In addition, ROS production, mitochondrial functional integrity and redox-modulated cell signaling pathways were also evaluated. Our preliminary results indicate that MG metabolism and other endpoints are dynamically modulated in wound healing, and this suggests that modulators of MG scavenging might be used to co-adjuvate skin lesion repair.
Keywords: glyoxalases, methylglyoxal, RAGE, mitochondria, reactive oxygen species, redox-dependent transcription factors, glutathione, keratinocytes, wound healing

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NC49

NALP1 involvement in cigarette smoke induces cutaneous inflammation

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Cigarette smoke alters biological processes in the skin such as redox homeostasis and inflammation response that might be involved in promoting skin