regenerative potential during aging, does not equally affect all the cells within the stem cell pool remains enigmatic. However, it is worth reporting that the investigators highlighted both the sensitivity and the plasticity of MuSCs.[6] Indeed, young MuSCs exposed to an aged environment undergo defective changes while enhanced GSH synthesis and metabolism can revert the altered phenotype of the aged/defective subpopulation. These findings can shed new light on the design of possible therapeutic approaches aimed to repristinate or support MuSC functions in their regenerative role not only in the aged muscle but also in pathologic conditions in which oxy-inflammatory changes contribute to muscle degeneration.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. Larsson, L., Degens, H., Li, M., Salviati, L., Lee, Y.I., Thompson, W., Kirkland, J.L., and Sandri, M. (2019). Sarcopenia: Aging-Related Loss of Muscle Mass and Function. Physiol. Rev. *99*, 427–511. https://doi.org/10.1152/physrev.00061.2017.

2. Forcina, L., Franceschi, C., and Musarò, A. (2022). The hormetic and hermetic role of IL-6. Ageing Res. Rev. *80*, 101697. https://doi.org/10.1016/j.arr.2022.101697.

3. Rocheteau, P., Gayraud-Morel, B., Siegl-Cachedenier, I., Blasco, M.A., and Tajbakhsh, S. (2012). A Subpopulation of Adult Skeletal Muscle Stem Cells Retains All Template DNA Strands after Cell Division. Cell *148*, 112–125. https://doi.org/10.1016/j.cell.2011.11.049.

4. Tierney, M.T., and Sacco, A. (2016). Satellite Cell Heterogeneity in Skeletal Muscle Homeostasis. Trends Cell Biol. *26*, 434–444. https://doi.org/10.1016/j.tcb.2016.02.004.

5. Sousa-Victor, P., García-Prat, L., and Muñoz-Cánoves, P. (2022). Control of satellite cell function in muscle regeneration and its disruption in ageing. Nat. Rev. Mol. Cell Biol. *23*, 204–226. https://doi.org/10.1038/s41580-021-00421-2.

6. Benjamin, D.I., Brett, J.O., and Rando, T. (2023). Multiomics reveals glutathione metabolism as a driver of bimodality during stem cell aging. Cell Met *35*, 472–486.

7. Baldelli, S., Ciccarone, F., Limongi, D., Checconi, P., Palamara, A.T., and Ciriolo, M.R. (2019). Glutathione and Nitric Oxide: Key Team Players in Use and Disuse of Skeletal Muscle. Nutrients *11*, 2318. https://doi.org/10.3390/nu11102318.

8. Sharifi-Rad, M., Anil Kumar, N.V., Zucca, P., Varoni, E.M., Dini, L., Panzarini, E., Rajkovic, J., Tsouh Fokou, P.V., Azzini, E., Peluso, I., et al. (2020). Lifestyle, Oxidative Stress, and Antioxidants: Back and Forth in the Pathophysiology of Chronic Diseases. Front. Physiol. *11*, 694. https://doi.org/10.3389/fphys.2020.00694.

9. Miller, C.J., Gounder, S.S., Kannan, S., Goutam, K., Muthusamy, V.R., Firpo, M.A., Symons, J.D., Paine, R., Hoidal, J.R., and Rajasekaran, N.S. (2012). Disruption of Nrf2/ARE signaling impairs antioxidant mechanisms and promotes cell degradation pathways in aged skeletal muscle. Biochim. Biophys. Acta *1822*, 1038–1050. https://doi.org/10.1016/j.bbadis.2012.02.007.

10. Bellezza, I., Giambanco, I., Minelli, A., and Donato, R. (2018). Nrf2-Keap1 signaling in oxidative and reductive stress. Biochim. Biophys. Acta. Mol. Cell Res. *1865*, 721–733. https://doi.org/10.1016/j.bbamcr.2018.02.010.

Unanticipated metabolic plasticity in response to chronic hypoxia

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In this issue of *Cell Metabolism*, Midha et al. investigate the metabolic changes in mice after exposure to reduced oxygen tension for an acute or chronic duration. Their organ-specific findings may help explain physiological observations in humans living at high altitude but raise additional questions concerning pathological hypoxia after vascular damage or in cancer.

The textbook example of human adaptation to hypobaric hypoxia (such as travel to high altitudes where the atmosphere is thinner) is the induction of the erythropoietin gene by the hypoxia inducible factor (HIF) transcription factors and subsequent increase in red blood cell mass and oxygen carrying capacity. This adaptation helps the body extract more oxygen from the air and deliver more oxygen to tissues where it is used to maintain organ function. What is less clear is how physiology and metabolism also coordinately adapt to the low oxygen environment to maintain organismal function. To address these questions, Midha et al.[1] perform a comprehensive examination of animals exposed to hypoxia for tissue oxygenation, motor function, circulating metabolic hormones, body temperature, and metabolic activity of organs using positron emission tomography and stable isotope tracing. They measure these parameters after acute (8 h) up to chronic (3 weeks) exposure to 11% or 8% normobaric oxygen tensions. This in-depth approach gives insight into how these processes

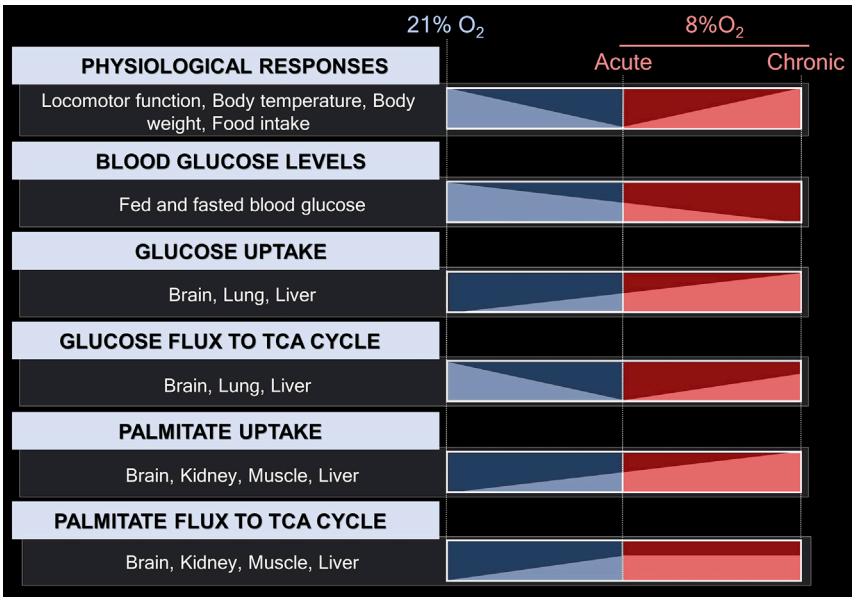

Figure 1. Overview of physiological changes in response to hypoxia

work together to provide robust adaptation and lead to surprising metabolic fuel decisions at the organ level.

Our most detailed understanding of metabolic adaptation to reduce oxygen tension comes from studies of cultured tumor or primary normal cells placed in reduced-oxygen environments. These studies have yielded significant mechanistic details about the hypoxia-induced changes in intermediate metabolism. One overarching theme is the attempt to bring oxygen supply and demand into balance, at least in part through the reduction of mitochondrial oxygen consumption. Processes such as the inhibition of pyruvate dehydrogenase (PDH) and reductive carboxylation of glutamine decrease mitochondrial activity and thereby conserve oxygen.[2,3] From the study by Midha et al., it appears that reduced motor function and decreased thermogenesis also reduce oxygen demand while increased hematocrit combines to bring oxygen supply and demand back in balance at the whole animal level.

Perhaps most surprising is the metabolic data of the different organs in the later figures. The authors use 2-deoxy-2-[^{18}F]fluoro-D-glucose and [^{18}F]fluoro-4-thia-palmitate PET scans to measure glucose and palmitate uptake, respectively. For many organs, acute changes in glucose uptake appear to mimic what is seen in cells treated with hypoxia *in vitro*: increased glucose uptake and increased glycolysis (Figure 1). However, muscle and brown adipose tissue show an interesting "glucose sparing" and decreased uptake. Data generated in parallel with stable ^{13}C glucose tracers confirm that this increased uptake is not oxidized in the TCA cycle, following the *in vitro* finding of decreased PDH activity and decreased glucose oxidation due to HIF-inducible inhibitory PDH kinases 1 and 3. However, in chronic hypoxia, glucose entry into the TCA cycle in the heart surpassed normoxic flux, whereas other organs showed varied responses. The data from the palmitate reveal a new phenomenon. While there is little change in uptake during acute hypoxia, there is significant palmitate uptake after chronic hypoxia. In the brain, muscle, and kidney, palmitate uptake increases at 3 weeks, and stable ^{13}C isotope studies confirm that this fatty acid is oxidized in the TCA cycle.

Increased fatty acid uptake and oxidation after 3 weeks of hypoxia may represent a new mechanism of metabolic adaptation that has not been appreciated in cells cultured *in vitro* as these experiments are typically performed over hours, not weeks. *In vitro* experiments have shown that HIIF2a elevation can be maintained for days in hypoxia, although the metabolic consequences of this have not been reported,[4] and the reported effects on palmitate metabolism could be HIF-independent. In VHL-defective cancers that constitutively express HIF target genes, fatty acids are diverted away from beta oxidation toward storage.[5] Beta-oxidation of fatty acids classically introduces carbons into an oxidative mitochondrial pathway through the production of acetyl-CoA. It will be interesting to see if tracer studies that follow this pathway *in vitro* in more detail can determine if this palmitate oxidation is through a canonical pathway, or perhaps some unrecognized mechanism.

These organismal results may also help to explain epidemiological data relating to decreased metabolic syndrome and obesity in humans living at high altitude in hypobaric hypoxia. Enhanced oxidation of fatty acids may serve as a sink that naturally keeps their levels in a healthier range. In the future, it will be interesting to take a wholistic view of humans dwelling at high altitude for years, where decreased inspired oxygen is combined with lower ambient temperatures and specific dietary lifestyles, as these environmental conditions may coordinately adapt organismal metabolism.

These results also raise questions about pathological hypoxia. Conditions such as vascular damage or malformed tumor vessels can lead to chronic hypoxia, although instability in red blood cell flux can also create acute hypoxia in tumors. Does chronic hypoxia in tumors cause a shift to more oxidation of fatty acids? If so, how might it impact clinical outcome or influence therapeutic decisions? Would it be possible to image tumors with radiolabeled palmitate? Or would dietary interventions such as a ketogenic diet have unintended consequences? The current work would appear to stimulate these avenues of investigation.

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REFERENCES

1. Midha, A.D., Zhou, Y., Queliconi, B.B., Barrios, A.M., Haribowo, A.G., Chew, B.T.L., Y., F.C.O., Blecha, J.E., VanBrocklin, H., Seo, Y., and H., J.I.. (2023). Organ-specific fuel rewiring

in acute and chronic hypoxia redistributes glucose and fatty acid metabolism. Cell Metabol. *35*, 504–516.

2. Metallo, C.M., Gameiro, P.A., Bell, E.L., Mattaini, K.R., Yang, J., Hiller, K., Jewell, C.M., Johnson, Z.R., Irvine, D.J., Guarente, L., et al. (2011). Reductive glutamine metabolism by IDH1 mediates lipogenesis under hypoxia. Nature *481*, 380–384. https://doi.org/10.1038/nature10602.

3. Papandreou, I., Cairns, R.A., Fontana, L., Lim, A.L., and Denko, N.C. (2006). HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. Cell Metab. *3*, 187–197. https://doi.org/10.1016/j.cmet.2006.01.012.

4. Holmquist-Mengelbier, L., Fredlund, E., Lofstedt, T., Noguera, R., Navarro, S., Nilsson, H., Pietras, A., Vallon-Christersson, J., Borg, A., Gradin, K., et al. (2006). Recruitment of HIF-1alpha and HIF-2alpha to common target genes is differentially regulated in neuroblastoma: HIF-2alpha promotes an aggressive phenotype. Cancer Cell *10*, 413–423. https://doi.org/10.1016/j.ccr.2006.08.026.

5. Du, W., Zhang, L., Brett-Morris, A., Aguila, B., Kerner, J., Hoppel, C.L., Puchowicz, M., Serra, D., Herrero, L., Rini, B.I., et al. (2017). HIF drives lipid deposition and cancer in ccRCC via repression of fatty acid metabolism. Nat. Commun. *8*, 1769. https://doi.org/10.1038/s41467-017-01965-8.

Putting aging on ICE

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A recent report by Yang et al. in *Cell* demonstrates that faithful DNA double-strand breaks and repair cycles phenocopy many aspects of aging in mice. Whether this progeroid phenotype is caused by a loss of epigenetic information remains to be conclusively determined.

Aging is a complex phenotype characterized by an array of biological changes that lead to an increase in an organism's frailty and in the likelihood of its death. Conserved hallmarks of aging across taxa have been described,[1] including genomic instability, epigenetic alterations, senescent cell accumulation, and exhaustion of stem cells. However, there is still much debate about which hallmarks are primary drivers, secondary mediators, or simply downstream consequences of the aging process.

A recent paper by Yang and colleagues[2] attempts to test a unifying theory of aging, dubbed the "information theory of aging." Central to this theory is the notion that cellular identity is determined by a precise epigenomic landscape. As a by-product of cellular metabolism and exposure to external insults, DNA damage inevitably accumulates with time. Repair of DNA damage is coordinated by chromatin modifiers, which also play a key role in maintaining cellular epigenomes and, thus, cell identity. Based on this theory, the repeated relocalization of chromatin modifiers to DNA-damage sites over a lifetime leads to progressive loss of epigenomic identity, ultimately manifesting in what we know as aging at the organismal level. Crucially, the theory distinguishes itself from prior DNA-based theories of aging in that it posits that the loss of epigenomic, rather than genomic, information is the primary driver of aging.[2]

In order to test this theory, the authors use a mouse model expected to scramble epigenetic information while keeping genetic information intact, dubbed ICE for inducible changes to the epigenome.[2] In this model, mice carry 2 transgenes: (1) a ubiquitously expressed Cre recombinase localizing to the nucleus upon exposure to tamoxifen (TAM) and (2) a TAM-stabilized and nuclear-localized I-*Ppo*I endonuclease fusion protein transgene at the *Rosa26* locus, which can only be expressed once an upstream floxed stop cassette is excised by Cre. Using this system, transgenic mouse cells exposed to TAM should inducibly express nuclear I-*Ppo*I, which can cut 20 unique canonical sites in the mouse genome. To note, one of these sites is located within the 28S rDNA sequence, of which mice carry hundreds of genomic copies.[3] Since using I-*Ppo*I to induce double-stranded DNA breaks (DSBs) should create "sticky" 4-bp overhangs, these DSBs are expected to have low mutagenic potential.

The authors first characterized the ICE system using mouse embryonic fibroblasts (MEFs). After induction, ICE cells showed strong signs of dsDNA damage compared to controls, while displaying limited mutational load post-treatment, compatible with the notion that the ICE system increases the rate of mutationless DSBs. ICE MEFs showed increased estimated age based on a DNA-methylation (DNAme) epigenetic clock, increased expression of senescence-associated genes, and increased frequency of markers of cell senescence. The group tested the ICE system *in vivo* by inducing I-*Ppo*I expression in young adult mice and performed phenotypic characterization 1 and 10 months after induction. Consistent with the known progeroid impact of DNA damage,[4]