Trends in
Cell Biology

CellPress

Forum

Oxidative phosphorylation system and cell culture media

M. Pilar Bayona-Bafaluy,[1,2,3,4] Julio Montoya,[1,2,3] and Eduardo Ruiz-Pesini[1,2,3,*]

Traditional culture media do not resemble the metabolic composition of human blood. The concentration of different metabolites in these media influences mitochondrial biogenesis and oxidative phosphorylation (OXPHOS) function. This knowledge is essential for the interpretation of results obtained from cellular models used for the study of OXPHOS function.

Oxidative phosphorylation (OXPHOS) disorders are an important group of genetic diseases. New sequencing protocols reveal many candidate mutations as potential etiologic factors. The confirmation of their pathogenicity is an important issue. Many cell types have been used as models to study these disorders. An essential part of the cell model is the culture medium. However, the composition of culture media is very different from that of human plasma (see Figure 2 in [1]) and affects OXPHOS function and mitochondrial biogenesis.

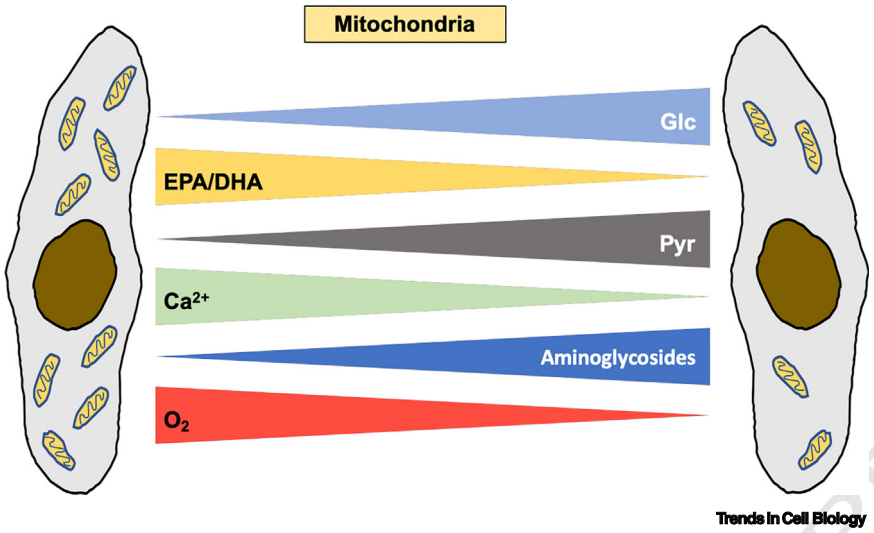
The most used culture media for mammalian cells are Dulbecco's Modified Eagle's Medium (DMEM) and Roswell Park Memorial Institute (RPMI)-1640 medium [2]. The most frequent glucose concentration is 25 mM in DMEM and 11 mM in RPMI-1640 [2]. However, the recommended reference range for plasma glucose is between 4 and 5.4 mM. An increase in glucose concentration of the culture medium from 5.5 to 30 mM significantly decreased mitochondrial inner membrane potential, oxygen consumption, mitochondrial DNA (mtDNA) copy number, and the levels of the mitochondrial transcription factor A (*TFAM*) mRNA in **HepG2** (see Glossary) cells. However, after reducing the glucose concentration, cells become more dependent on mitochondrial metabolism to obtain energy [3]. Thus, when glucose 25 mM was substituted by glucose-free/galactose 10 mM in the culture medium, **HeLa** and **U2OS** cells increased respiration and activities and levels of respiratory **supercomplexes**. In **143B cybrids**, the incubation in glucose-free/galactose 5 mM medium provoked an increase in mtDNA amount and mRNA levels for mtDNA-encoded genes, such as *MT-CO1* and *MT-ND5*. A decrease in glucose concentration from 25 to 2.75 mM caused an increase in the oxygen consumption, respiratory complex I activity, respiratory complex IV (CIV) p.MT-CO1 subunit amount, and mtDNA levels in **SH-SY5Y** cybrids. A reduction from 25 to 1 mM increased oxygen consumption in U2OS cells. Growing HepG2 cells in the absence of glucose showed an increase in CIV activity, mtDNA-encoded proteins, and mRNA and mtDNA amount versus those same cells growing at glucose 25 mM. Similar results can also be observed in human primary cells, such as fibroblasts [4]. Therefore, high glucose concentrations reduce mitochondrial biogenesis (Figure 1). Thus, under oxidative metabolic conditions, cells with mutations in OXPHOS-related genes show a deficit of OXPHOS function, which is not observed in wild type cells. However, under very high glucose concentrations, OXPHOS function is suppressed in wild type cells and it may be that there are no differences in OXPHOS function with cells carrying mutations in OXPHOS-related genes.

Human plasma contains between 9.3 and 59.7 μM of pyruvate, however, 1000 μM pyruvate is found in DMEM and RPMI-1640. These supraphysiological concentrations of pyruvate stabilize hypoxia-inducible factor 1α (HIF1α) in normoxia. In **BT549** cells grown in different culture media, HIF1α levels were positively correlated with supplemented pyruvate concentrations [5]. Interestingly, by suppressing mitochondrial biogenesis, the HIF pathway reduces mitochondrial number in the cancer cell [6].

The fatty acid profile of cells maintained in culture shows substantial differences with the human tissues, with a 2.5-fold decrease in polyunsaturated fatty acids (PUFA). Human cells lack the ability to make their own PUFA, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and need to gain them from the environment. Classical culture methods use media with 10% fetal bovine serum (FBS), that is the only exogenous source of lipids. FBS has a low level of lipids and, at 10% of media, provides 1% of the PUFA available to cells in the body [7]. This is due to the fact that, in bovines, microorganisms in the rumen hydrogenate high proportions of the dietary PUFA. EPA/DHA increase mitochondrial inner membrane potential and ATP production, and upregulate genes encoding regulatory factors for

Glossary

143B: human osteosarcoma cells.
BT549: human breast cancer cells.
Cybrids: cell lines produced by the fusion of cells without mitochondrial DNA (rho^0 cells) with cytoplasts (enucleated cells) or platelets (cell fragments) harboring mitochondria and mitochondrial DNA but not nucleus or nuclear DNA.
Gentamycin: aminoglycoside antibiotic.
HeLa: human cervix cancer cells.
HepG2: human liver cancer cells.
Kanamycin: aminoglycoside antibiotic.
L6: rat skeletal muscle cells.
MCF7: human breast cancer cells.
MCF10A: human breast cancer cells.
MCF12A: human breast cancer cells.
MDA-MB-231: human breast cancer cells.
SH-SY5Y: human neuroblastoma cells.
Streptomycin: aminoglycoside antibiotic. Frequently used in cell culture protocols, usually in combination with penicillin.
Supercomplexes: assemblies of different respiratory complexes.
U2OS: human osteosarcoma cells.



Q1 Figure 1. Effect of different compounds from the cell culture media on mitochondrial biogenesis and oxidative phosphorylation (OXPHOS) function. Brown, grey, and yellow colors of the cells indicate nucleus, cytosol, and mitochondria, respectively. Glucose (Glc), pyruvate (Pyr), and aminoglycoside antibiotics reduce mitochondrial biogenesis and OXPHOS. However, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), calcium ($Ca2^{+}$), and oxygen (O_2) increase mitochondrial biogenesis and OXPHOS. The concentration of these compounds in the cell culture medium is represented by the thickness of the line.

mitochondrial biogenesis [peroxisome proliferator-activated receptor gamma coactivator 1-alpha (*PPARGC1A*)] and oxidative metabolism [nuclear respiratory factor 1 (*NRF1*), *TFAM*). They also upregulate genes for mitochondrial proteins, in particular, components of the OXPHOS system, such as *COX4*, an nDNA-encoded gene for a CIV subunit, elevate mitochondrial RNA and protein expression levels, and increase mtDNA content [8]. Therefore, EPA and DHA modulate mitochondrial biogenesis.

Ionized calcium levels in human plasma are between 1.11 and 1.32 mM. However, its concentration in DMEM and RPMI-1640 are 1.8 and 0.42 mM, respectively [2]. In skeletal muscle, endurance exercise induces an increase in mitochondrial mass, which is mediated by the increase in intracellular calcium levels during fiber contraction. Treating **L6** muscle cells with agents that increase calcium leads to an increase in mitochondrial protein content. This effect is controlled through the activation of PPARGC1A. The effect of calcium on the expression of mitochondrial proteins is not confined to muscle and it has also been described in other human and mouse cells [9]. Therefore, the low calcium concentration present in the RPMI-1640 culture medium could negatively impact mitochondrial biogenesis.

The principal role of O_2 in mammalian physiology is as the terminal acceptor in the electron transport chain [10]. One of the most striking discrepancies between routine mammalian cell culture and the *in vivo* environment is the oxygen tensions to which cells are exposed. The air in a humidified CO_2 cell culture incubator contains 140 mm Hg O_2 but the oxygen tensions to which human organs are exposed are from 5 to 100 mm Hg [10]. However, the O_2 concentration around cells growing as adherent monolayers at the bottom of the media column may differ from that of headspace gas [11]. Thus, the consumption of O_2 by cultured cells, depending on cell density, coupled to the poor solubility of O_2 in the culture medium, could provoke pericellular hypoxia by consuming O_2 faster than it can diffuse [10].

Antibiotics were introduced into culture media to reduce the frequency of microbial contamination. Two aminoglycoside antibiotics that inhibit bacterial protein synthesis, **streptomycin** 100 µg/ml and **gentamycin** 5 µg/ml, are frequently used in cell culture protocols. Although considered selective for prokaryotic ribosome, most aminoglycosides also bind to the mitochondrial ribosome. Interestingly, another aminoglycoside, **kanamycin** 25 µg/ml, significantly reduced basal and maximal respiration, mitochondrial membrane potential, and ATP levels of **MCF10A** cells [12]. Moreover, gentamycin 50 µg/ml inhibited mitochondrial membrane potential and upregulated the HIF1α expression of **MCF7**, **MCF12A**, and **MDA-MB-231** breast cancer cell lines [13].

To acknowledge the importance of the culture media, it has been reported that long-term culture under similar incubation conditions produces cells with similar composition, irrespective of their different origins [7]. However, the same pluripotent stem cell can be differentiated to very different cells simply using distinct differentiation media. Although physiologic media with metabolite concentrations similar to human plasma are now available [1], they are not the definitive solution, since many compounds can be rapidly consumed during the culture. In fact, Dulbecco's modification to produce DMEM focused on having constituents in excess to account for their depletion over time by cellular metabolism [11]. This can be particularly important for O_2. When more physiologic O_2 concentrations are used, pericellular hypoxia becomes a possibility [10] and the HIF pathway would reduce the mitochondrial mass in the cell [6].

To avoid the problems discussed here, we could look for other models, such as mouse models. However, animal models

are not without drawbacks and show fundamental differences to humans. For example, similar to traditional culture media, mouse plasma poorly reflects the metabolite composition of human plasma. As previously said, 'No model can faithfully capture the full complexity of conditions encountered in the human body. This truth effectively defines the term "model"' [1]. Recent advances in 3D and microfluidic cell cultures, along with improvements in physiological media, will help to solve the problem of the current culture media. While these improvements are being developed, knowing the potential influence of the culture media on cellular phenotypes will help us to better interpret the results of cell models for OXPHOS disorders.

Acknowledgments

This work was supported by grants (FIS-PI17/00021, PI17/00166) from Instituto de Salud Carlos III and European Regional Development Fund (FEDER); and Gobierno de Aragón (Grupos Consolidados B33) and FEDER 2014-2020 'Construyendo Europa desde Aragón'.

Declaration of interests

No interests are declared.

[1]Departamento de Bioquímica, Biología Molecular y Celular, Universidad de Zaragoza, 50009 and 50013 Zaragoza, Spain
[2]Instituto de Investigación Sanitaria (IIS) de Aragón, 50009 Zaragoza, Spain
[3]Centro de Investigaciones Biomédicas en Red de Enfermedades Raras (CIBERER), 28029 Madrid, Spain
[4]Instituto de Biocomputación y Física de Sistemas Complejos (BIFI), Universidad de Zaragoza, 50018 Zaragoza, Spain

*Correspondence:
eduruiz@unizar.es (E. Ruiz-Pesini).

https://doi.org/10.1016/j.tcb.2021.05.003

References

1. Cantor, J.R. (2019) The rise of physiologic media. *Trends Cell Biol.* 29, 854–861
2. McKee, T.J. and Komarova, S.V. (2017) Is it time to reinvent basic cell culture medium? *Am. J. Physiol. Cell Physiol.* 312, C624–C626
3. Emperador, S. *et al.* (2019) Ketogenic treatment reduces the percentage of a LHON heteroplasmic mutation and increases mtDNA amount of a LHON homoplasmic mutation. *Orphanet J. Rare Dis.* 14, 150
4. Pereira, S.P. *et al.* (2018) Metabolic and phenotypic characterization of human skin fibroblasts after forcing oxidative capacity. *Toxicol. Sci.* 164, 191–204
5. Vande Voorde, J. *et al.* (2019) Improving the metabolic fidelity of cancer models with a physiological cell culture medium. *Sci. Adv.* 5, eaau7314
6. Thomas, L.W. and Ashcroft, M. (2019) Exploring the molecular interface between hypoxia-inducible factor signalling and mitochondria. *Cell. Mol. Life Sci.* 76, 1759–1777
7. Else, P.L. (2020) The highly unnatural fatty acid profile of cells in culture. *Prog. Lipid Res.* 77, 101017
8. Lee, M.-S. *et al.* (2016) Effects of eicosapentaenoic acid and docosahexaenoic acid on mitochondrial DNA replication and PGC-1α gene expression in C2C12 muscle cells. *Prev. Nutr. Food Sci.* 21, 317–322
9. Diaz, F. and Moraes, C.T. (2008) Mitochondrial biogenesis and turnover. *Cell Calcium* 44, 24–35
10. Keeley, T.P. and Mann, G.E. (2019) Defining physiological normoxia for improved translation of cell physiology to animal models and humans. *Physiol. Rev.* 99, 161–234
11. Abbas, M. *et al.* (2021) Vertebrate cell culture as an experimental approach - limitations and solutions. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 254, 110570
12. Kalghatgi, S. *et al.* (2013) Bactericidal antibiotics induce mitochondrial dysfunction and oxidative damage in mammalian cells. *Sci. Transl. Med.* 5, 192ra85
13. Elliott, R.L. and Jiang, X.-P. (2019) The adverse effect of gentamicin on cell metabolism in three cultured mammary cell lines: "Are cell culture data skewed?". *PLoS One* 14, e0214586