Cite this article as: Muradian K.K. & Fraifeld V.E. (2023). Embryogenesis of Longer-Lived Mammalian Species Occurs in a More Severe Hypoxic-Hypercapnic Environment, *AgingBio*, *1*, 1–4. doi: 10.59368/agingbio.20230018.

Research Paper

Embryogenesis of Longer-Lived Mammalian Species Occurs in a More Severe Hypoxic-Hypercapnic Environment

Khachik K. Muradian,[1,2,*] and Vadim E. Fraifeld[1,2,*]
[1]Department of the Biology of Aging and Life Span Extension, State Institute of Gerontology of National Academy of Medical Sciences of Ukraine, Kiev, Ukraine
[2]The Shraga Segal Department of Microbiology, Immunology and Genetics, Center for Multidisciplinary Research on Aging, Ben-Gurion University of the Negev, Beer-Sheva, Israel
[*]Corresponding authors: muradian1944@gmail.com; vadim.fraifeld@gmail.com
https://doi.org/10.59368/agingbio.20230018

In this study, we analyzed for the first time the relationships between species-specific longevity (maximum lifespan [MLS]) or gestation period (GP) and partial pressure of O_2 (Po_2), partial pressure of CO_2 (Pco_2), and pH in the blood of umbilical arteries (UAs) and veins in mammals. The results obtained allow to make four suggestions: (a) Embryonic development of placental mammals occurs in hypoxic-hypercapnic environment (HHE); (b) longer-lived and slower-developing species start their lives in more severe HHE; (c) mammalian longevity is associated with more efficient exchange of fetal blood CO_2 in the placenta; and (d) UA blood Pco_2 and pH, but not Po_2, display strong correlative links with MLS and GP, thus underpinning a special role of hypercapnia in development and longevity.

Introduction

It has long been known that embryonic development in multicellular animals occurs in low O_2 and high CO_2 environments[1,2]. In mammals, the partial pressure of O_2 (Po_2) in fetal blood and tissues is lower, whereas the partial pressure of CO_2 (Pco_2) is higher than in adult organisms. For example, in pregnant sheep, llamas, horses, and humans, umbilical artery (UA) blood Po_2 is around twice as low as in the uterine artery and several fold lower than in the maternal systemic arterial Po_2[3–6].

The occasional decline of Po_2 from the normal values is a frequent and hazardous event, often with clinical complications for the fetus and mother[7,8]. Natural selection should allegedly establish a wide safety window between the normal "hypoxic" values of Po_2 and critical levels of hypoxia. Nevertheless, Po_2 in a mammalian fetus is usually kept on the brink of life-threatening low levels. Suffice to mention that in humans, UA Po_2 is around five times lower than in the maternal artery[6,9]. Yet, it is believed that many late-onset diseases originate from the latent impairment generated during embryogenesis but manifested in the advanced ages. The list of such diseases includes cardiovascular disorders, hypertension, obesity, type II diabetes, neurological and cognitive disorders, and so on[10–12]. However, the question of how and why the hypoxic-hypercapnic environment (HHE) has become indispensable in embryogenesis and to what extent fetal hypoxia may impact gestation period (GP) and longevity has not yet been explored. Equally enigmatic and even less explored are the effects of elevated Pco_2 and associated hypercapnia on embryogenesis and longevity. The role of Pco_2 could be even more multifocal than Po_2 due to its direct effects not only on metabolic processes but also on the ubiquitous acid-base balance and pH through the well-known chain: $CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+$. In this comparative study, we demonstrate for the first time the strong correlative links between species-specific longevity, GP, and fetal blood Pco_2 and pH, but not Po_2, in placental mammals.

Materials and Methods

Data on Po_2, Pco_2, and pH in the blood of UAs and veins of mammalian species were collected from publications in the PubMed, PMC, and Google Scholar databases. Over a thousand articles were extracted and manually curated. After rigorous curation, the data for random sampling of all mammals available in the literature (10 species, mostly animal husbandry or model organisms), with a clear description of the subjects and methods of measurement, were included in the analysis. To somewhat compensate for the relative scarcity of species, the data of different experiments (n) on the same species were used. The following species were included in the analysis: humans (n = 9), rhesus monkey (n = 9), horse (n = 5), cow (n = 3), llama (n = 3), pig (n = 4), sheep (n = 5), goat (n = 6), dog (n = 6), and guinea pig (n = 3), totaling 51 entries (**Supplemental Table**). The GP, maximum lifespan (MLS), and adult body mass (BM) records for these species were taken from the AnAge database[13]. Three-dimensional (3D) relationships between MLS, Po_2, and Pco_2 of UA or umbilical vein (UV) blood were analyzed by the quadratic 3D contour plots. Pairwise correlative relations were assessed by Spearman's nonparametric coefficient of correlation or Pearson's parametric coefficient of correlation. The parametric and nonparametric estimates brought about nonconflicting results.

To evaluate a possible impact of BM on correlations of umbilical blood parameters with MLS, we used partial correlation analysis, which allows for eliminating the covariation effects. To ensure linear relationships, the MLS and BW values were log-transformed. Only coefficients with $p < 0.05$ were considered statistically significant and taken into discussion. All statistical analyses and figure drawing were performed by the Microsoft "Statistica-64" program.

Results and Discussion

The MLS values of the analyzed species varied within an order of magnitude (from 12 y in guinea pigs to 122.5 y in humans), and GP varied around 5 times (from 68 days in guinea pigs to 337 days in horses). As seen in **Figure 1A**, UA Pco_2 highly correlates with logMLS or GP, so the higher the UA Pco_2, the longer the MLS or GP. It should be taken into account that these correlations could be driven by BM rather than MLS. For example, Lorenzini et al. (2005) showed that the replicative capacity of cultured fibroblasts derived from 11 mammalian species correlates primarily with their BM but not longevity[14]. Similarly, as was shown in 15 rodent species, telomerase activity coevolves with BM but not MLS[15,16]. In our dataset, the UA Pco_2 significantly correlates not only with logMLS (**Fig. 1A**) but also, almost to the same degree, with logBM ($r = 0.69$; $p = 0.029$). Yet, the partial correlation analysis revealed that controlling for logBM did not significantly affect the correlation between logMLS and UA Pco_2 ($r = 0.69$; $p < 0.05$). A similar effect was observed for logBM and UA Pco_2 when logMLS was controlled ($r = 0.68$; $p < 0.05$). The results obtained suggest that the impact of UA Pco_2 on mammalian longevity is independent of BM.

The CO_2 clearance from CO_2-enriched blood of UA occurs in the placenta via the UA-capillary-UV system. Then, CO_2-depleted blood returns back to the fetus via UV. Thus, the difference in Pco_2 in UA and UV could obviously be indicative of the efficiency of fetal CO_2 diffusion and removal. Fairly good positive correlations of ΔPco_2 (UA Pco_2 – UV Pco_2) with MLS or GP suggest that longer-lived and slower-developing mammalian species possess a more efficient system of fetal trans-capillary CO_2 outflow (**Fig. 1B**).

Highly significant correlations of UA blood pH with MLS or GP were also observed (**Fig. 1C**). Because of the inverse relations between Pco_2 and pH, the negative correlations of pH with MLS and GP could at least partly be the consequences of the higher Pco_2 in longer-lived and slower-developing species.

In contrast to the significant correlations of UA Pco_2 and pH with MLS or GP, the corresponding correlations for Po_2 were insignificant (data not shown). Moreover, the same trend in adult mammalians has previously been reported by us, indicating that MLS correlates with systemic arterial blood Pco_2 and related HCO_3^- and pH, but not with Po_2[17].

On the whole, the results of pairwise correlative analysis were further strengthened by the 3D contour plots of the MLS dependence on Pco_2 and Po_2 in UA. It should, however, be stressed that the 3D analysis highlights the complicated relationships between biological variables more adequately than linear models. Indeed, as seen in **Figure 2**, not just a high UA Pco_2, but rather the combination of higher UA Pco_2 with lower UA Po_2 is typical for the longer-lived mammals (red area). Specifically, in our sampling, the longer-lived mammals have UA Pco_2 higher than 50 mm Hg and UA Po_2 lower than 25 mm Hg. Accordingly, the shorter-lived species (green area) have UA Pco_2 lower than 50 mm Hg and UA Po_2 covering the entire range, including values that are higher than 25 mm Hg. It means that fetuses of long-lived species develop more severe HHE.

All in all, placental mammals, humans included, start their existence and develop in HHE. Whatever the case, HHE effects are apparently not just the sum of reactions to hypoxia and hypercapnia but rather the result of intricate confrontation and/or cooperation, presumably between hypoxia-inducible factor-1 (HIF-1) and hypothetical hypercapnia-inducible factor (HcIF)[18,19]. Acting mostly through HIF-1, lower oxygenation in hypoxia may decrease the fetal metabolic rate and thereby extend GP[20,21]. The same hypometabolic effect could be expected for hypercapnia and hypothetical HcIF[19,22]. Moreover, although one should be careful in the interpretation of correlations, positive links between Pco_2 and MLS could underpin the special role of hypercapnia in longevity.

Mammalian clades differ in types of placenta, which presumably may determine the level of fetal HHE. For example, primates have a hemochorial placenta that deeply penetrates into the uterine endometrium, while ungulates have a shallow placenta of epitheliochorial type[23]. This, however, could not explain quite strong correlations between UA Pco_2, exchange of fetal blood CO_2, UA pH, and longevity. Indeed, the shortest-lived species in our set, guinea pigs, have the same placental type as humans and rhesus monkeys, the first- and third-longest-lived species in our set. Along with this, horses, the second-longest-lived species, have another type of placenta, an epitheliochorial one (**Supplemental Table**).

It remains poorly understood when and why HHE became an indispensable attribute of fetal development. We do not exclude

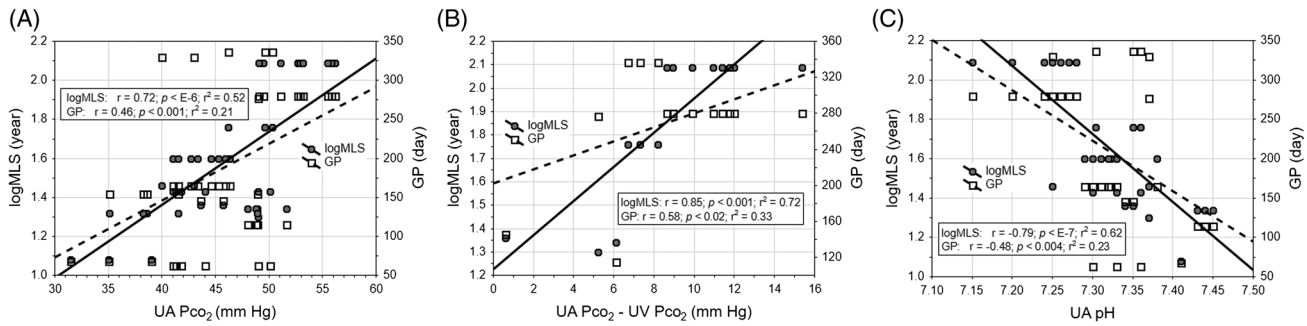

Figure 1. Relationships of mammalian maximum lifespan (logMLS, left ordinate) or gestation period (GP, right ordinate) with **(A)** the umbilical artery (UA) partial pressure of CO_2 (Pco_2), **(B)** the difference of Pco_2 (ΔPco_2) in UA and umbilical vein (UV) (UA Pco_2 – UV Pco_2), and **(C)** the UA pH.

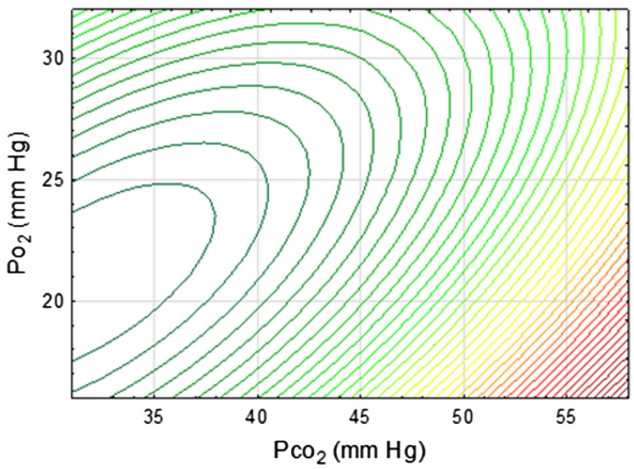

Figure 2. Three-dimensional (3D) quadratic contour plots for the dependence of logMLS on Pco_2 and the partial pressure of O_2 (Po_2) in the UA. The 3D quadratic response surface of the logMLS dependence on Pco_2 and Po_2 in the UA was statistically significant ($r^2 = 0.77$; $F = 56.3$; $p < E - 25$).

that fetal HHE is a remnant of the adaptive reactions in response to the lower atmospheric O_2 and higher CO_2 at the time of life or multicellularity origin[24]. Such adaptive traits could be fixed in the genome and preserved in the program of embryogenesis, as some other primordial features do.

Concluding Remarks

Here, we analyzed the links of MLS or GS with Po_2, Pco_2, and pH in the blood of UAs and UVs in mammals. The results of this pilot study allow to make four suggestions: (a) Embryonic development of mammals occurs in HHE; (b) longer-lived and slower-developing species start their lives in more severe HHE; (c) longer-lived species are characterized by more efficient fetal blood O_2/CO_2 exchange in the placenta; and (d) UA blood Pco_2 and pH, but not Po_2, display strong correlative links with MLS and GP. To our knowledge, this is the first study of that kind and therefore cannot answer all rising issues. It rather provokes new questions and explores new ideas. There are no satisfactory explanations why HHE is needed for embryogenesis at all. Are there HHE regimes that could be more optimal for embryogenesis than natural ones? Is it possible to extend the health span by maintaining pregnant or adult animals in artificial HHE? Despite the long history of the problem and obvious successes in embryology, these and many other related issues remain completely unexplored. Definitely, further research is necessary to fill in the gaps and create a stronger and wider basis for further findings.

Acknowledgments

K.K.M. was awarded Emergency Fellowships for Ukrainian researchers from The Israel Academy of Sciences and Humanities. The authors appreciate the assistance of Dr. E. David and Dr. E. Rudnitsky in preparing this article.

Conflict of Interest

No conflict of interest is declared.

Supplementary Materials

Supplemental information can be found online at https://doi.org/10.59368/agingbio.20230018.

Accepted November 28, 2023
Published December 15, 2023

References

1. Baron D.H., & Meschia G. (1954). A comparative study of the exchange of the respiratory gases across the placenta. *Cold Spring Harb. Symp. Quant. Biol.* **19**, 93–101. PMID: 13291191; doi: 10.1101/sqb.1954.019.01.013.
2. Coleman A.J. (1967). Absence of harmful effect of maternal hypocapnia in babies delivered at caesarean section. *Lancet* **289**(7494), 813–814. PMID: 4164298; doi: 10.1016/S0140-6736(67)92778-X.
3. Comline R.S., & Silver M. (1970). PO2, PCO2 and pH levels in the umbilical and uterine blood of the mare and ewe. *J. Physiol.* **209**(3), 587–608. PMID: 5499798; doi: 10.1113/jphysiol.1970.sp009181.
4. Wilkening R.B., & Meschia G. (1992). Current topic: Comparative physiology of placental oxygen transport. *Placenta* **13**(1), 1–15. PMID: 1502133; doi: 10.1016/0143-4004(92)90002-B.
5. Riquelme R.A., Sánchez G., Liberona L., Sanhueza E.M., Giussani D.A., Blanco C.E., … Llanos A.J. (2002). Nitric oxide plays a role in the regulation of adrenal blood flow and adrenocorticomedullary functions in the llama fetus. *J. Physiol.* **544**(1), 267–276. PMID: 12356897; doi: 10.1113/jphysiol.2002.018325.
6. Kotaska K., Urinovska R., Klapkova E., Prusa R., Rob L., & Binder T. (2010). Re-evaluation of cord blood arterial and venous reference ranges for pH, pO2, pCO2, according to spontaneous or cesarean delivery. *J. Clin. Lab. Anal.* **24**(5), 300–304. PMID: 20872563; doi: 10.1002/jcla.20405.
7. Siragher E., & Sferruzzi-Perri A.N. (2021). Placental hypoxia: What have we learnt from small animal models? *Placenta* **113**, 29–47. PMID: 34074553; doi: 10.1016/j.placenta.2021.03.018.
8. Sebastiani G., Navarro-Tapia E., Almeida-Toledano L., Serra-Delgado M., Paltrinieri A.L., García-Algar Ó., & Andreu-Fernández V. (2022). Effects of antioxidant intake on fetal development and maternal/neonatal health during pregnancy. *Antioxidants (Basel)* **11**(4), 648. PMID: 35453333; doi: 10.3390/antiox11040648.
9. Baena-García L., Coll-Risco I., Ocón-Hernández O., Romero-Gallardo L., Acosta-Manzano P., May L., & Aparicio V.A. (2020). Association of objectively measured physical fitness during pregnancy with maternal and neonatal outcomes. The GESTAFIT Project. *PLoS One* **15**(2), e0229079. PMID: 32069319; doi: 10.1371/journal.pone.0229079.
10. Barker D.J. (1993). Fetal origins of coronary heart disease. *Br. Heart J.* **69**(3), 195–196. PMID: 8461215; doi: 10.1136/hrt.69.3.195.
11. Bateson P., Barker D., Clutton-Brock T., Deb D., D'Udine B., Foley R.A., … Sultan S.E. (2004). Developmental plasticity and human health. *Nature* **430**(6998), 419–421. PMID: 15269759; doi: 10.1038/nature02725.
12. Gluckman P.D., Hanson M.A., Cooper C., & Thornburg K.L. (2008). Effect of in utero and early-life conditions on adult health and disease. *N. Engl J. Med.* **359**(1), 61–73. PMID: 18596274; doi: 10.1056/NEJMra0708473.
13. Tacutu R., Thornton D., Johnson E., Budovsky A., Barardo D., Craig T., … de Magalhães J.P. (2018). Human ageing genomic resources: New and updated databases. *Nucleic Acids Res.* **46**, D1083–D1090. PMID: 29121237; doi: 10.1093/nar/gkx1042.
14. Lorenzini A., Tresini M., Austad S.N., & Cristofalo V.J. (2005). Cellular replicative capacity correlates primarily with species body mass not longevity. *Mech. Ageing Dev.* **126**(10), 1130–1133. PMID: 15993927; doi: 10.1016/j.mad.2005.05.004.
15. Seluanov A., Chen Z., Hine C., Sasahara T.H., Ribeiro A.A., Catania K.C., … Gorbunova V. (2007). Telomerase activity coevolves

with body mass not lifespan. *Aging Cell* **6**(1), 45–52. PMID: 17173545; doi: 10.1111/j.1474-9726.2006.00262.x.

16. Gorbunova V., & Seluanov A. (2009). Coevolution of telomerase activity and body mass in mammals: From mice to beavers. *Mech. Ageing Dev.* **130**(1–2), 3–9. PMID: 18387652; doi: 10.1016/j.mad.2008.02.008.
17. Muradian K. (2013). "Pull and push back" concepts of longevity and life span extension. *Biogerontology* **14**(6), 687–691. PMID: 24114506; doi: 10.1007/s10522-013-9472-1.
18. Fraifeld V.E., & Muradian Kh.K. (2021). Editorial. Hypoxic-hypercapnic environment as a model of hypometabolism, calorie restriction, hypoglycemia and non-medicamentous treatment of related pathology. *Diabetes Res. Metab.* **2**, 110. doi: 10.36879/DRM.20.000110.
19. Muradian Kh.K., & Fraifeld V.E. (2021). Hypercapnia inducible factor: A hypothesis. *Aging Longevity (A&L)* **2**(3), 27–31. doi: 10.47855/jal9020-2021-3-4.
20. Fajersztajn L., & Veras M.M. (2017). Hypoxia: From placental development to fetal programming. *Birth Defects Res.* **109**(17), 1377–1385. PMID: 29105382; doi: 10.1002/bdr2.1142.
21. Soares M.J., Iqbal K., & Kozai K. (2017). Hypoxia and placental development. *Birth Defects Res.* **109**(17), 1309–1329. PMID: 29105383; doi: 10.1002/bdr2.1135.
22. Tolstun D.A., Knyazer A., Tushynska T.V., Dubiley T.A., Bezrukov V.V., Fraifeld V.E., & Muradian K.K. (2020). Metabolic remodelling of mice by hypoxic-hypercapnic environment: Imitating the naked mole-rat. *Biogerontology* **21**(2), 143–153. PMID: 31667660; doi: 10.1007/s10522-019-09848-9.
23. Furukawa S., Kuroda Y., & Sugiyama A. (2014). A comparison of the histological structure of the placenta in experimental animals. *J. Toxicol. Pathol.* **27**(1), 11–18. PMID: 24791062; doi: 10.1293/tox.2013-0060.
24. Catling D.C., & Zahnle K.J. (2020). The Archean atmosphere. *Sci Adv.* **6**(9), eaax1420. PMID: 32133393; doi: 10.1126/sciadv.aax1420.