*British Journal of Medicine & Medical Research*
*3(2): 210-215, 2013*

**SCIENCEDOMAIN** *international*
www.sciencedomain.org

# Renewal versus Retention: Isotopic Composition of Intestinal Epithelium and Eye Lens

**Yuri K. Doronin[1*], Daria Yu. Bednik[1], Andrei A. Ivanov[2] and Elena N. Kalistratova[1]**

*[1]M.V. Lomonosov State University, Biology Department, Leninskie Gory, 1-12, Moscow, 119991, Russian Federation.*
*[2]V. I. Vernadsky Institute of Geochemistry and Analytical Chemistry, Russian Academy of Sciences, Kosygin St. 19, Moscow 117975, Russian Federation.*

***Authors' contributions***

*This work was carried out in collaboration between all authors. Author YKD designed this study, managed the literature searches, performed the statistical analysis, wrote the first draft of the manuscript. Author AAI managed samples preparation for mass spectrometric measurements. Authors DYB and ENK managed the animals care, performed the dissection and sampling. All authors read and approved the final manuscript.*

***Research Article***

***Received 30th May 2012***
***Accepted 23rd November 2012***
***Published 19th January 2013***

## ABSTRACT

**Aims:** To compare isotopic signatures of contrasting (due to the structure and metabolism) organs in mice of two contrasting ages.
**Study Design:** Cross-sectional study.
**Place and Duration of Study:** Biology Department of Moscow State University; Institute of Geochemistry and Analytical Chemistry, Russian Academy of Sciences; 2007–2011.
**Methodology:** Mass spectrometric measurements of carbon and nitrogen isotopic ratios of jejunal epithelium and lens in 1- and 22-mo mice fed a monotonous diet.
**Results:** The lenses are enriched in carbon and nitrogen as compared with intestinal epithelium (by 5.5% and 4.5% in 1-mo mice and 8.3% and 6% in 22-mo mice, respectively). The $^{15}N$ content is also higher in lenses than in intestinal epithelium (8.97‰ vs. 7.62‰ in 1-mo mice, and 7.40‰ vs. 6.58‰ in 22-mo mice). The $^{13}C$ content of lenses exceeds that of intestinal epithelium in 1-mo mice (-20.27‰ vs. -21.69‰),

**Corresponding author: Email: ljh_rjycn@mail.ru;*

although $^{13}C$ content is equal in 22-mo mice (-22.56‰ vs. -22.67‰). $^{15}N$ content is depleted in the intestinal epithelium of 22-mo mice (-1.04‰), whereas $^{13}C$ depletion (-0.98‰) is non-significant. $^{13}C$ and $^{15}N$ content in lenses is also significantly decreased in 22-mo mice (-2.29‰ and -1.57‰).

**Conclusion:** The intestinal epithelium represents a structure with short-term isotopic memory lasting a few days, whereas the events of the organism's entire lifetime are retained in lens isotopic memory. The difference of the parameters measured is evidently determined by structural contrast, metabolic rate, and rejuvenation modes of the tissues. The $^{15}N$ depletion in both the intestinal epithelium and lenses, as well as $^{13}C$ depletion in lenses of 22-mo mice might be considered as a sign of ageing. In contrast, the depletion of $^{15}N$ in lenses of 22-mo mice should be considered primarily as a result of dilution of breastmilk isotopic signature that probably obscures age-related alterations of the organ. Comparison of isotopic compositions of these contrasting organs may be useful for physiological and ecological determinations.

*Keywords: $\delta^{13}C$; $\delta^{15}N$; intestinal epithelium; lens; young and old mice.*

## 1. INTRODUCTION

The isotopic composition of tissues and organs of heterotrophs reflects but is non identical to the isotopic composition of food consumed [1,2,3,4 and many others]. Dietary signature is altered to varying degrees in organ-specific biochemical conversions of absorbed nutrients and, in particular, depends on the rate and mode of organ renewal.

Here we present mass spectrometric data on two contrasting organs (due to their morphological and physiological features), that have been obtained from animals of two different ages.

*Intestinal epithelium* is composed of highly metabolically active cells that absorb the products of enzymatic digestion from the gut lumen and transfer them to the blood. The cellular replacement of jejunal epithelium is very fast and is estimated at 41 - 66 h in mice [5].

In contrast, the bulk of the *lens* is composed of highly specialized lenticular epithelial cells consisting mainly of crystallines (approximately 90% of dry weight), losing their the nuclei and other intracellular organelles in course of differentiation. The lens grows throughout the whole lifetime because of new crystalline fiber formation. Molecular renewal of crystallines is nonexistant [6].

To our knowledge, isotope composition of intestinal epithelium and lens have scarcely been studied. Pelletier et al. [7] presented the carbon isotope ratios of duodenal mucosa of calves. In this structure $\delta^{13}C$ values did not differ from diet. Kaznacheev et al. [8] have shown $^{13}C$ depletion during the progression of cataracts in human beings.

Our goal was to describe the elemental and isotopic compositions of intestinal epithelium and lens across young and old mice. However, not only academic interest impelled to carry out this study. We believe that the discrimination of stable isotopes could be useful functional characteristic of normal and pathological changed tissues.

## 2. MATERIALS AND METHODS

Mice (strain C57BL/6J) were reared in standard conditions. Females were fed on a diet for rats, mice, and hamsters (GOST R50.258-92, "Laboratorkorm", Moscow) for three months prior to insemination and gestation. Litters were separated from their mothers at the age of 21 days after birth (a few days after weaning) and were fed *ad libitum* monotonously on the diet. The commercial food pellets include wheat, corn, sunflower oil, wheat bran, fish flour, meat-meal; metabolizable energy – 13000 kJ $kg^{-1}$, crude proteins – 19%, crude lipids – 5.0%, cellulose – 4.0%.

Fragments of intestinal epithelium and lenses of five 1-mo and five 22-mo females were used for analysis. Immediately after animal sacrifice (cervical vertebra dislocation), the proximal fragment of jejunum (approximately 3cm) was isolated and removed. The food and mucus particles were thoroughly removed under microscopic control. The mucosa was rinsed with a saline jet, and separated from submucosa by gentle spatula movement. The lens was extracted from the globes, cleaned out of the zonule remains, and washed in saline. The samples were dried at 60ºC for three days, and powdered using a mortar and pestle. One should notice both organs are not supplied directly with blood vessels, and samples contained no traces of blood.

The isotopic composition of carbon and nitrogen were measured using a Thermo-Finnigan DELTA V Plus mass spectrometer coupled to the elemental analyzer in continuous flow mode. Values are reported as $\delta^{13}C$ and $\delta^{15}N$ relative to VPDB and air nitrogen standards. Relative amounts of carbon and nitrogen (C% and N%) were measured simultaneously. The instrument precision (0.20‰ and 0.10‰ (1 SD) for $\delta^{13}C$ and $\delta^{15}N$, respectively) was assessed by repeated measurements of calibration standard.

## 3. RESULTS AND DISCUSSION

Lenses of 1- and 22-mo mice contained significantly more carbon and nitrogen than the diet (Student's *t* test, *P*<.000). Carbon content in intestinal epithelium of 1-mo mice and in diet did not differ significantly (*P* = .28), while % C in the intestinal epithelium of 22-mo mice was higher than in the diet (*P*<.01). The organs of 1- and 22-mo mice were also enriched in the stable isotopes of carbon and nitrogen relative to the diet (*P*<.000 for every comparison) (Table 1).

**Table 1. Summary of the data obtained for intestinal epithelium and lens in 1- and 22-mo mice**

| | Diet | Organs | 1-mo mice | 22-mo mice |
|---|---|---|---|---|
| C, % | 30.7±0.88* | Intestinal epithelium | 32.5±1.30 | 37.6±1.77 |
| | | Lens | 38.0±0.83 | 45.9±1.41 |
| $\delta^{13}C$, ‰ | -26.14±0.152 | Intestinal epithelium | -21.69±0.436 | -22.67±0.329 |
| | | Lens | -20.27±0.504 | -22.56±0.097 |
| N, % | 2.2±0.09 | Intestinal epithelium | 7.9±0.40 | 8.6±0.27 |
| | | Lens | 12.4±0.24 | 14.6±0.42 |
| $\delta^{15}N$, ‰ | 3.51±0.062 | Intestinal epithelium | 7.62±0.267 | 6.58±0.300 |
| | | Lens | 8.97±0.371 | 7.40±0.077 |

** Mean ± standard error of mean, n = 5.*

Carbon and nitrogen content are considerably higher in lens than in intestinal epithelium in animals of both ages ($P < .001$).

The C/N ratio for intestinal epithelium is higher than the C/N ratio for lenses in mice of both ages (4.1±0.21 and 4.4±0.28 versus 3.1±0.11 and 3.1±0.20 in 1-mo and 22-mo mice, correspondingly; the differences are significant, $P < .001$). The C/N value reflects the proteinaceous composition of the lens. The C/N ratio for intestinal epithelium specifies more portions of nitrogen-free compounds such as lipids.

Intestinal epithelium of 1-mo mice is depleted in $^{13}C$ as compared with the lens ($P = .001$). There was no difference in 22-mo mice ($P = .49$). $\delta^{15}N$ values for intestinal epithelium were also lower than the corresponding values for lenses for both 1- and 22-mo mice ($P < .001$).

Carbon content increased in organs with age ($P = .049$ for intestinal epithelium, and $P = .001$ for lens). N% does not change with age in intestinal epithelium ($P = .17$), but is significantly higher in lenses of 22-mo mice ($P = .002$).

The difference in $\delta^{13}C$ values of intestinal epithelium of 1-mo and 22-mo mice is non-significant ($P = .11$). However, the lenses of 22-mo mice are depleted in $^{13}C$ compared to lenses of 1-mo mice ($P = .01$). The heavy nitrogen isotope depletion in 22-mo mice is statistically significant ($P = .03$, intestinal epithelium; $P = .003$, lens). C/N of corresponding organs do not change with the mice age.

The $\delta^{13}C$ and $\delta^{15}N$ values for intestinal epithelium reflect the isotopic composition of cells that have existed no more than three days before samples were collected. In this regard, the depletion in $^{15}N$ should be associated with age-dependent alterations of enterocytes' metabolism. The details of this phenomenon are obscure. Perhaps, it is related to age-associated oxidant-induced alteration of proteins that initiates the cascade of events resulting in mucosal barrier dysfunction and inflammatory disorders of the gut [9,10].

The lens is a metabolically inert structure that is able to accumulate and retain any dietary alteration, including the inevitable isotopic signature of maternal milk. $\delta^{15}N$ and $\delta^{13}C$ differences between the corresponding tissues of dependent juveniles and their mothers in small-bodied species (red-backed vole, deer mice) varied from -0.8 to 1.9‰ and from -1.5 to 0.5‰, respectively [11,12]. One may expect the similar values of the isotopic signature of breast milk for mice due to the similarity of reproductive strategy ("income breeding") of these species. Corrected $\delta^{15}N$ and $\delta^{13}C$ for lenses of 1-mo mice obtained by subtracting the maximal values of milk isotopic signatures (1.9‰ and 0.5‰, see above) are equal to 7.07‰ and -20.77‰, respectively.

The lens mass of 22-mo mice is 2.6 fold greater than the lens mass of 1-mo mice [13]. Hence, the isotopic spike of breast milk should be diluted by 2.6 times in the lens of 22-mo mice, i.e. to be equal maximally to 0.7‰ for $^{15}N$ and 0.2‰ for $^{13}C$. According to this, $\delta^{15}N$ and $\delta^{13}C$ values without maternal milk contribution should be equal to 6.70‰ and -22.76‰, respectively.

Thus, the $^{13}C$ depletion in 22-mo mice can be explained not only by breast milk contribution but probably also by mice ageing. In particular, it might be the manifestation of age-related lens dysfunction (cataract) that is known for mice [14,15]. Cataract associated $^{13}C$ depletion of human lenses was reported in the early paper of Keznacheev et al. [8].

However we cannot assert the age-dependent $^{15}N$ depletion in lenses, due to the very small difference of corrected values for 1- and 22-mo mice. Inevitable case of breastfeeding obscures the following ontogenetic events.

## 4. CONCLUSION

The intestinal epithelium represents a structure with short-term isotopic memory lasting a few days, whereas the events of the organism's entire lifetime are retained in lens isotopic memory. The difference of the parameters measured is evidently determined by structural and metabolic differences of intestinal epithelium and lenses. The $^{15}N$ depletion in both the intestinal epithelium and lenses, as well as $^{13}C$ depletion in lenses of 22-mo mice might be considered as a sign of ageing. In contrast, the depletion of $^{15}N$ in lenses of 22-mo mice should be considered primarily as a result of dilution of breastmilk isotopic signature, that probably obscures age-related alterations of the organ. Comparison of isotopic compositions of these contrasting organs may be useful for physiological and ecological determinations.

## ACKNOWLEDGEMENTS

The authors are very grateful Dr. A.L. Buchachenko, voting member of the Russian Academy of Sciences, for his permanent attention to this work and for valuable and encouraging suggestions. We thank A.V. Tiunov, who performed the stable isotope analysis. We are sincerely grateful to an anonymous reviewer for benevolent comments which have greatly improved the manuscript.

## ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. DeNiro MJ, Epstein S. Influence of diet on the distribution of carbon isotopes in animals. Geochim Cosmochim Acta. 1978;42(5):495-506.
2. DeNiro MJ, Epstein S. Influence of diet on the distribution of nitrogen isotopes in animals. Geochim Cosmochim Acta. 1981;45(3):341-51.
3. Tieszen LL, Boutton TW, Tesdahl KG, Slade NA. Fractionation and turnover in animal tissue: Implication for δ13C analysis of diet. Oecologia. 1983;57(1-2):32-7.
4. Hobson KA, Alisauakas RT, Clark RG. Stable nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: implication for analyses of diet. Condor. 1993;95(2):388-94.
5. Cheng H, Bjerknes M. Cell production in mouse intestinal epithelium measured by stathmokinetic flow cytometry and coulter particle counting. Anat Rec. 1983;207(3):427-34.

6. Lynnerup N, Kjeldsen H, Heegaard S, Jacobsen C, Heinemeie J. Radiocarbon dating of the human eye lens crystallines reveal proteins without carbon turnover throughout life. P L o S One. 2008;3(1):e1529. doi:10.1371/journal.pone.0001529.
7. Pelletier G, Tyrrell HF, Chevalier R, Hillaire-Marcel C, Gagnon G. Stable isotope carbon content of various tissues in calves. Can J Anim Sci. 1984;64(Suppl.):124–26.
8. Kaznacheev VP, Doiltsyn EF, Gabuda SP, Rzavin AF, Malikov NG. Fractionation of carbon isotopes in human tissues with atherosclerosis. Bull Exp Biol Med. 1987;104(9):295-96. Russian.
9. DeMeo MT, Mutlu EA, Keshavarzian A, Tobin MC. Intestinal permeation and gastrointestinal disease. J Clin Gastroenterol. 2002;34(4):385–96.
10. Banan A, Zhang LJ, Shaikh M, Fields JZ, Farhadi A, Keshavarzian A. Novel effect of NF-kB activation: carbonylation and nitration injury to cytoskeleton and disruption of monolayer barrier in intestinal epithelium. Am J Physiol Cell Physiol. 2004;287:C1139–51. Accessed 2 June 2004. Available: http://ajpcell.physiology.org/.
11. Sare DTJ, Millar JS, Longstaffe FJ. Nitrogen- and carbon-isotope fractionation between mothers and offspring in red-backed voles (*Clethrionomys gapperi*). Can J Zool. 2005;83(5):712–6. Accessed 29 June 2005. Doi: 10.1139/Z05-054. Available: http://cjz.nrc.ca.
12. Miller JF, Millar JS, Longstaffe FJ. Stable nitrogen and carbon isotope discrimination between juveniles and adults in an income-breeding small mammal (*Peromyscus maniculatus*). Mamm Biol. 2011;76(5):563–9. Doi:10.1016/j.mambio.2011.02.006 Available: www.elsevier.de/mambio.
13. Rowe FP, Bradfield A, Qui RJ, Swinney T. Relationship between eye lens weight and age in the wild house mouse (*Mus musculus*). J Appl Ecol. 1985;22(1):55-61. Available: http://www.jstor.org/stable/2403326.
14. Pendergrass W, Penn Ph, Possin D, Wolf N. Accumulation of DNA, nuclear and mitochondrial debris, and ROS at sites of age-related cortical cataract in mice. Invest Ophthalmol Vis Sci. 2005;46(12):4661-70.
15. Baradia H, Nikahd N, Glasser A. Mouse lens stiffness measurements. Exp Eye Res. 2010;91(2):300-7. Accessed 11 June 2010.



*Peer-review history:*
*The peer review history for this paper can be accessed here:*
*http://www.sciencedomain.org/review-history.php?iid=177&id=12&aid=838*