A biologically based computational model for the hypothalamic-pituitary-thyroid (HPT) axis in *X. laevis* larvae

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Supplemental Information SI4

Measurement of Plasma Iodide

Plasma collections

All procedures were reviewed and approved by the institutional animal care and use committee (IACUC) at the Great Lakes Toxicology and Ecology Division. Whole blood was collected from larval *Xenopus laevis* at pro-metamorphic Nieuwkoop and Faber (NF; 1994) stages 54, 56 and 58 based on methods previously described (Haselman et al., 2020). Plasma samples from ~60–100 larvae per developmental stage were pooled into single 1.5 ml tubes and stored at –80ºC until further processing and analysis. Following euthanasia by a lethal dose of MS-222, whole blood was collected via heart puncture from adult *X. laevis* males (n = 3) and females (n = 3) using heparinized Natelson capillary tubes. Heparinized blood was transferred to 0.5 ml centrifuge tubes and spun at 10,000 x g for 5 min. A single plasma pool for adult *X. laevis* (mixed sex) was created by transferring equal volumes from all six samples. The plasma pool was then stored at –80ºC until further processing and analysis.

Sample processing

Plasma pools were thawed to room temperature, vortexed to mix and 50 µl were transferred to new 1.5 ml microcentrifuge tubes according to the following treatments and replication. Each plasma pool was processed for total iodine and fractionated iodine with technical duplication for each, resulting in a total of four aliquots being processed from each plasma sample/pool. For the total iodine samples, 450 µl of 2.5% TMAH was added to each,

briefly vortexed to mix and transferred to LC vials for analysis. For the fractionated samples, 1000 µl 5% TCA was added to each aliquot, samples were allowed to coagulate/precipitate at room temperature for 20 min, then centrifuged at 20,000 x g for 10 min to pellet the precipitated protein. The supernatant was removed and transferred directly to LC vials for analysis. 1000 µl 5% TCA was added to the protein pellets and vortexed to resuspend/liberate any free I^- pulled down with the protein pellet. These samples were then centrifuged at 20,000 x g for 10 min to pellet the protein; the second supernatant was transferred directly to LC vials for analysis. Finally, 50 µl ultrapure water was added to the leftover protein pellet and samples were vortexed and placed in a sonicating water bath to resuspend the protein; 450 µl 2.5% TMAH was added to the resuspended protein which further aided in resuspension and the complete 500 µl samples of resuspended protein were transferred to LC vials for analysis.

Instrumental analysis

Iodine was quantified in samples using direct injection into an ICP-MS (Agilent Model 7500CE, Agilent Technologies, Inc., Santa Clara, CA, USA) using an interfaced HPLC autosampler (Agilent Model 1100) and ultrapure water mobile phase. Mass 127 was monitored by the instrument to determine I^- abundance in each sample. Ion abundances were captured using ChemStation ICPMS chromatography software (Version B.03.02, Agilent Technologies) and raw data files were translated to MassHunter compatible files (MassHunter GC/MS Translator B.07.01 1805) so data processing could be performed in Agilent MassHunter software (version B.07.00). Absolute quantitation of I^- concentrations in samples was achieved by linear regression using external I^- standard series (0, 1, 5, 10, 25, 50 ppb) run in triplicate with each instrument run and prepared from a certified stock. The lower limit of quantitation (LLOQ) was 1 ppb.

Data processing and results

Sample concentrations were averaged across technical duplicates and dilution factors applied to determine the total, free and bound iodine concentrations in the initial 50 µl of plasma. As described in the methods above, two free iodide separations were performed per sample replicate to result in two supernatant analyses per sample. The sum of the two supernatant iodide concentrations represented the total free fraction in each sample. Summarized data consisting of the calculated molar iodine concentrations, determined from the means of technical duplicates, are reported in Table 1.

Table 1. Iodine concentrations in total and fractionated blood plasma from *X. laevis* larvae and adult.

Life stage	Gender	Total I (nM)	Free I^- (nM)	Bound I (nM)	*n* (technical replication)
Larvae (NF54)	Mixed	1418	851	206	2
Larvae (NF56)	Mixed	1108	822	144	2
Larvae (NF58)[a]	Mixed	1150	835	186	2
Larvae (NF58)[a]	Mixed	1467	1127	289	2
Larvae (NF58)[a]	Mixed	1448	1149	212	2
Adult	Mixed	425	391	144	2

[a]Common pool of larval NF58 plasma used across three instrument runs.

The results indicate that free plasma iodide levels in all three larval stages were very similar and were 2- to 3-fold higher than those in adults. The higher iodide levels in larvae may reflect the fact that larvae can take up iodide across the gills while uptake in adults is limited to dietary sources. For comparison, reported free iodide concentrations in human plasma range from 50–300 nM (Carrasco, 1993), while serum iodide concentrations in rats fed an iodine sufficient diet range from approximately 60–500 nM (Eng et al., 1999; Gilbert et al., 2013). The mean of all larval *X. laevis* free I^- concentrations in plasma is 956.8 nM.

References

Carrasco, N., 1993. Iodide transport in the thyroid gland. Biochimica et Biophysica Acta 1154(1), pp.65-82.

Eng, P.H.K., Cardona, G.R., Fang, S., Previti, M., Alex, S., Carrasco, N., Chin, W.W. and Braverman, L.E., 1999. Escape from the acute Woff-Chaikoff effect is associated with a decrease in thyroid sodium/iodide symporter messenger ribonucleotide acid and protein. Endocrinology, 140(8), pp. 3404-3410.

Gilbert, M.E., Hedge, J.M., Valentin-Blasini, B.C., Kannan, K., Tietge J., Zoeller, R.T., Crofton, K.M., Jarrett, J.M., and Fisher, J.W., 2013. An animal model of marginal iodine deficiency during development: The thyroid axis and neurodevelopmental outcome. Toxicological Sciences, 132(1), pp.177-195.

Haselman, J.T., Olker, J.H., Kosian, P.A., Korte, J.J., Swintek, J.A., Denny, J.S., Nichols, J.W., Tietge, J.E., Hornung, M.W. and Degitz, S.J., 2020. Targeted pathway-based in vivo testing using thyroperoxidase inhibition to evaluate plasma thyroxine as a surrogate metric of metamorphic success in model amphibian *Xenopus laevis*. Toxicological Sciences, 175(2), pp.236-250.

Nieuwkoop P. D., Faber J. (1994). Normal table of *Xenopus laevis* (Daudin): A Sytematical and Chronological Survey of the Development from the Fertilized Egg Till the End of Metamorphosis. Garland Publishing, New York.