LIPID STOCK-PILING IN THE GROWING OOCYTE – ARE ANDROGENS DRIVING LIPID DELIVERY TO SUSTAIN THE FUTURE EMBRYO OF THE EEL, *ANGUILLA AUSTRALIS*?

Forbes, E.L. and Lokman, P.M

Department of Zoology, University of Otago, 340 Great King Street, Dunedin 9016, New Zealand. Fax +64-34797584, e-mail: forer069@student.otago.ac.nz

Introduction:

As a prerequisite to producing viable healthy offspring, female fish need to provision their eggs with yolk proteins and lipids. Relatively little is known about the mechanisms that regulate lipid accumulation, despite key roles for lipids in (marine) teleosts in the context of egg quality. The liver is known to first package lipids in lipoprotein complexes made up chiefly of triacylglycerides (TAGs) and apo(lipo)proteins, especially apolipoprotein-B (apo-B). The inherently hydrophobic lipids can accordingly be easily transported through the blood stream for uptake elsewhere in the body. Lipid uptake is greatly dependent on activity of the enzyme lipoprotein lipase (LPL) – this enzyme, associated among others with adipose tissue, can catalyze the cleavage of free fatty acids from TAGs contained within very low density lipoproteins (VLDL) [1]**.** We recently identified a key role for the androgen 11-ketotestosterone (11-KT) in increasing LPL mRNA levels in the eel ovary [2]. Furthermore, incubation of eel ovarian tissue in the presence of VLDL and 11-KT resulted in histologically-detectable increases in accumulated lipids in the cytoplasm. Are the androgen-mediated increases in lipid accumulation associated with increases in lipid packagers, the apoproteins? To address this question, we artificially elevated the serum level of 11-KT in female eels in a time course experiment and measured hepatic transcript abundance of genes involved in lipid packaging, namely apob and microsomal triglyceride transfer protein, mttp. We also evaluated the effects of 11-KT treatment on expression of the VLDL receptor (vldlr) in the ovary.

Methods:

Experimental fish - Seventy eight eels were captured in fyke nets in Lake Ellesmere (South Island, New Zealand) and transported to Dunedin (Department of Zoology, University of Otago) where they were held in aquaria (6 eels / 200 L) at ambient autumn photoperiod and temperature for one week and allowed to acclimate. All eels, with the exception of six initial controls, were anesthetised (0.15% benzocaine) and implanted intraperitoneally with slow release pellets containing either cholesterol (placebo controls) or 1 mg of 11-KT. Eels were then separated into the treatment groups and placed back into aquaria until terminal sampling at 3 hrs, 9 hrs, 27 hrs, 81 hrs, 243 hrs and 729 hrs after implantation. At each sampling, six controls and six androgen-treated fish were euthanised (0.30% benzocaine). During sampling, length and total fish weight, as well as liver and ovary weights, were recorded, blood samples collected, and fragments of the liver and ovary were snap-frozen. Wild fish – twenty four eels in the previtellogenic (PV) or early vitellogenic (EV) stage were captured in fyke nets in Lake Ellesmere and immediately euthanised. Sampling techniques and tissue collection was identical to that for experimental fish. Analytical techniques - apob, mttp and vldlr transcript abundance were quantified using real-time PCR and serum 11-KT levels were measured using radioimmunoassay.

Results and Discussion:

Significant increases in hepatosomatic (60%) and gonadosomatic indices (300%) were seen in both 11-KT-treated and wild-caught migratory eels compared to their respective controls. The levels of circulating 11-KT in the serum were successfully raised to levels (150-200 ng/ml) crudely comparable to those found in wild caught-migratory animals (50-80 ng/ml) and maintained throughout the duration of the experiment. Changes in *apob* and *mttp* mRNA levels between control and 11-KT treated fish were evident by 3 h of treatment; 11-KT-treated fish had significantly higher transcript abundances for both genes, reflecting the differences seen between wild-caught feeding PV and migratory EV eels, respectively. In contrast, 11-KT treatment had no effect on *vldlr* mRNA levels, although there were significant differences between PV (8.64) and EV eels (14.30).

Conclusion:

This study demonstrates that 11-KT has the ability, at least in part, to increase the hepatic packaging of lipid into lipoproteins by increasing *apob* and *mttp* mRNA levels. This response to androgen is further reinforced by a notable increase in liver weight. However, 11-KT did not affect *vldlr* transcript abundance, suggesting that increases in accumulated lipids are probably not driven by increases in uptake of VLDL and its associated TAGs; instead, it reinforces the importance of LPL as a major mediator of such uptake.

References:

[1]ECKEL, R.H. 1989. Lipoprotein lipase. A multifunctional enzyme relevant to common metabolic diseases. N. Eng. J. Med**.,** 322(7).

[2]DIVERS, S.L., MCQUILLAN, H.J., MATSUBARA, H., TODO, T., LOKMAN, P.M. 2010. Effects of reproductive stage and 11-ketotestosterone on LPL mRNA levels in the ovary of the shortfinned eel. J. Lipid Res., 51.