

“Examining sources of variation in HPG axis function among individuals and populations of the dark-eyed junco”

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SUPPLEMENTARY MATERIAL

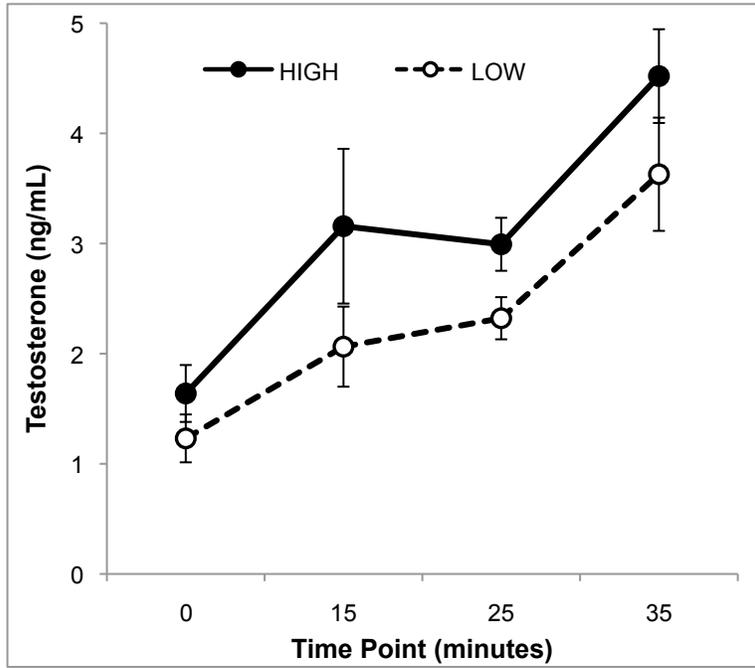
To examine dosage of intramuscular ovine luteinizing hormone injection (o-LH) and time course of testosterone (T) response, male Carolina juncos (different individuals from those included in the main study) were administered o-LH challenges in captivity. All birds were bled prior to injection (time point 0) and then injected intramuscularly with o-LH at one of two doses (5 μ g (HIGH) or 0.5 μ g (LOW) o-LH in 50 μ l phosphate buffered saline; N=10 males per dose). Each bird was sampled again at one or two of three total post-injection time points (15, 25 and 35 min) to achieve a total of N=6 blood samples per post-injection time point, per dose. T was assayed as described in the main study. Linear mixed models examined the effect of time point and dose (fixed factors) and individual (random repeated effect) on testosterone concentration.

Both dose and time point were significant predictors of T concentration (Figure S1; Table S1). Based upon these pilot data, we opted to use the HIGH o-LH dose in our main study. A prior study of the time course of T response to intramuscular injection of GnRH in juncos found that T levels peaked at 30 min, had begun to decline at 1 hour, and had returned to baseline by 2 hours (Jawor et al., 2006). Based on those findings as well as ours presented here, we opted to measure T response to both GnRH and o-LH challenges at 30 min in our main experiment.

Table S1

Factor	F	df	p
Random Effects			
Repeatability	216.6	1, 43	0.053
Residual Variance Component	0.54	1, 43	<0.001
Fixed Effects			
Time point (0, 15, 25, 35 minutes)	333.27	1, 42	<0.001
Dose (HIGH, LOW)	0.09	1, 42	0.042

Figure S1



Bibliography

Jawor, J.M., McGlothlin, J.W., Casto, J.M., Greives, T.J., Snajdr, E.A., Bentley, G.E., Ketterson, E.D., 2006. Seasonal and individual variation in response to GnRH challenge in male dark-eyed juncos (*Junco hyemalis*). *General and Comparative Endocrinology* 149, 182-189.