Thyroid hormone influences muscle mechanics in carp (Cyprinus carpio) independently from SERCA activity

James, RS, Little, AG, Tallis, J & Seebacher, F

Original citation:
James, RS, Little, AG, Tallis, J & Seebacher, F 2016, 'Thyroid hormone influences muscle mechanics in carp (Cyprinus carpio) independently from SERCA activity' *Journal of Experimental Biology*, vol 219, pp. 2806-2808
https://dx.doi.org/10.1242/jeb.143529

DOI 10.1242/jeb.143529
ISSN 0022-0949
ESSN 1477-9145

Publisher: Company of Biologists

© 2016. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2016) 219, 2806-2808 doi:10.1242/jeb.143529

SHORT COMMUNICATION

Thyroid hormone influences muscle mechanics in carp (*Cyprinus carpio*) independently from SERCA activity

Rob S. James[1,*], Alexander G. Little[2], Jason Tallis[1] and Frank Seebacher[2]

ABSTRACT

Thyroid hormone is a key regulator of metabolism, and in zebrafish, hypothyroidism decreases sustained and burst swimming performance. These effects are accompanied by decreases in both metabolic scope and the activity of sarco-endoplasmic reticulum ATPase (SERCA) in zebrafish. Our aim was to determine whether thyroid hormone affects skeletal muscle contractile function directly and whether these effects are mediated by influencing SERCA activity. We show that hypothyroidism reduces sustained locomotor performance but not sprint performance in carp (*Cyprinus carpio*). We accept our hypothesis that hypothyroidism reduces force production in isolated skeletal muscle, when compared with the thyroid hormone T_2, but we reject the hypothesis that this effect is mediated by influencing SERCA activity. Blocking SERCA activity with thapsigargin reduced muscle fatigue resistance, but hypothyroidism had no effect on fatigue. Hence, thyroid hormone plays a role in determining isolated skeletal muscle mechanics, but its effects are more likely to be mediated by mechanisms other than affecting SERCA activity.

KEY WORDS: Calcium, Fatigue, Force, Hypothyroidism, Stress

INTRODUCTION

Thyroid hormone plays a key role in the regulation of energy metabolism, including effects on metabolic rate, thermogenesis and energy balance (Mullur et al., 2014). In skeletal muscle, thyroid hormone can modulate muscle fibre type and mitochondrial function (Lombardi et al., 2015; Salvatore et al., 2014). Hypothyroidism has been associated with below-normal skeletal muscle force generation and relaxation rate in human patients (Duyff et al., 2000; Khaleeli and Edwards, 1984). We have recently shown that in zebrafish (*Danio rerio*), thyroid hormone regulates thermal acclimation of metabolism and locomotor performance (Little and Seebacher, 2013; Little et al., 2013). Chemically induced hypothyroidism in *D. rerio* decreased critical sustained swim speed (U_{crit}), burst swimming performance, metabolic scope and lactate dehydrogenase activity (Little and Seebacher, 2013; Little et al., 2013). Hypothyroidism in zebrafish also decreased the activity of the sarco-endoplasmic reticulum ATPase (SERCA), an enzyme that is instrumental in re-sequestering calcium into the sarcoplasmic reticulum and thereby facilitating muscle relaxation (Berchtold et al., 2000; Little et al., 2013). These data raise the question of whether thyroid hormone influences locomotor performance via its effect on muscle function directly or by some other mechanism, such as regulating energy metabolism. Muscle performance is affected by rates of intrinsic muscle calcium cycling dynamics (Berchtold et al., 2000; Seebacher et al., 2012), and by ATP supply to support the activities of myosin ATPase and SERCA (Allen et al., 2008). The aim of this study was to determine whether isolated muscle mechanics in carp (*Cyprinus carpio*) is influenced by hypothyroidism. Reduced SERCA activity in this species leads to decreased skeletal muscle fatigue resistance, indicating that SERCA activity is important in maintaining calcium stores to facilitate sustained performance (Seebacher et al., 2012). Hence, if thyroid hormone influences SERCA activity, it thereby also modulates muscle contractile function and, as a result, swimming performance. We tested the hypothesis that hypothyroidism reduces sustained and sprint swimming performance, which is paralleled by reductions in muscle force production and fatigue resistance. Additionally, we hypothesised that the effects of hypothyroidism are similar to those of pharmacological reductions in SERCA activity, if the action of thyroid hormone is mediated by its influence on SERCA.

MATERIALS AND METHODS

Study animals

Carp [*Cyprinus carpio* (Linnaeus 1758)] were obtained from Australian Koi Farm (Bringelly, NSW, Australia). Fish were separated into control [mean±s.e.m. standard length (SL)=60.4±1.7 mm] and hypothyroid treatment groups. Within the hypothyroid group, fish were further divided into three treatment groups: (1) fish supplemented daily with 10 nmol l^{-1} 3,3′,5-triiodo-L-thyronine (T_3; Sigma-Aldrich, Castle Hill, NSW, Australia) (SL=59.4±1.4 mm); (2) fish supplemented daily with 10 nmol l^{-1} 3,3′-diiodo-L-thyronine (T_2; Sigma-Aldrich) (SL=62.5±1.5 mm); and (3) fish given the ethanol vehicle (SL=62.8±1.6 mm). We conducted the supplementation treatments to verify that any effect of the hypothyroidism-inducing drugs were in fact due to reductions in active thyroid hormone, assuming that muscle tissue concentrations of T_2 and T_3 would be similar to those found in a previous study using this same treatment regime (Little et al., 2013). There were five to six fish per plastic tank (645×423×276 mm) that were continuously filtered and aerated. We induced hypothyroidism by maintaining tank water at 0.3 mmol l^{-1} propylthiouracil (PTU; Sigma-Aldrich) which inhibits thyroid hormone production, and 5 µmol l^{-1} iopanoic acid (Thermofisher Scientific, Sydney, NSW, Australia) to inhibit deiodinase activity (Little et al., 2013). All fish were acclimated to 18°C, and treated as detailed above, for 1 month before experimentation. Animals were fed commercial koi carp diet daily, but were starved for 24 h before experimentation. All procedures had the approval of the University of Sydney Animal Ethics Committee (approval number L04/10-2009/2/5158).

[1]Research Centre for Applied Biological and Exercise Sciences, Coventry University, Coventry CV1 5FB, UK. [2]School of Life and Environmental Sciences A08, University of Sydney, Sydney, NSW 2006, Australia.

*Author for correspondence (r.james@coventry.ac.uk)

R.S.J., 0000-0002-3969-8261

Received 20 May 2016; Accepted 29 June 2016

Journal of Experimental Biology

Journal of Experimental Biology (2016) 219, 2806-2808 doi:10.1242/jeb.143529

Swimming performance and muscle mechanics

Sustained swimming performance was measured as the critical sustained swimming speed (U_{crit}; Brett, 1964; Hammer, 1995). U_{crit} and sprint swimming speeds were measured according to published protocols (Seebacher et al., 2012). Sample sizes for U_{crit} measurements were: control=7 fish; hypothyroid=9; T_2 supplementation=8; T_3 supplementation=7; and for measurements of maximal sprint swimming speed: control=7 fish; hypothyroid=6; T_2=8; T_3=6.

Fish used to determine biomechanics of isolated muscle were euthanized via a blow to the head, transection of the spinal cord and pithing. Sample sizes were N=8 fish each for control, hypothyroid and T_2 supplementation treatments, and N=7 for the T_3 supplementation group. Mechanics of isolated caudal muscle fibre bundles were determined according to published protocols (Seebacher et al., 2012), using isometric measurements of muscle performance to determine the maximum tetanic force produced per muscle cross-sectional area (stress) and the rates of activation (between first stimulus and half maximal force) and relaxation (from last stimulus to half maximal force) of tetanic force. Fatigue resistance was determined by subjecting the muscle preparation to a series of tetani, each of 300 ms stimulation duration, at a rate of one tetanus per second for 25 s. We obtained two muscle preparations from each fish, one of which was exposed to fish Ringer's solution that contained 10 µmol l^{-1} of the SERCA blocker thapsigargin (Sigma-Aldrich) (Kurebayashi and Ogawa, 2001; Galli et al., 2006; Seebacher et al., 2012), and the other was kept in Ringer's solution without thapsigargin (control). We used the thapsigargin treatment to test the hypothesis that the effects of hypothyroidism parallel those of SERCA inhibition.

Statistical analysis

We analysed all data with Bayesian inference using Markov chain Monte Carlo (MCMC) estimation in the package MCMCglmm, R version 2.22 (Hadfield, 2010). Significance was based on a P_{MCMC}-value of <0.1 as we are testing one-tailed directional hypotheses. For *post hoc* tests, we assessed significant differences between levels of the same factor by comparing the Bayesian 95% confidence intervals of the posterior means. We analysed U_{crit} and maximal sprint swimming speed with thyroid treatment (control, hypothyroid, supplementation with T_2 and T_3) as independent factors and fish standard length as a covariate. We analysed muscle mechanics data (tetanus stress, activation rate and relaxation rate) with thyroid treatment and thapsigargin treatment (treatment and control) as factors. Muscle fatigue was compared between treatments at tetanus numbers 5, 10, 15 and 20 with fish identity as a random factor to account for repeated measures of consecutive tetani within individuals. We calculated effect sizes for comparisons between multiple means for the thyroid treatments and for two means for the thapsigargin treatment according to Cohen (1992) using α=0.05. We determined the power of our comparisons in the package pwr 1.1-3 in R (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS AND DISCUSSION

Swimming performance

Similar to previous work on zebrafish (Little and Seebacher, 2013), the hypothyroid treatment significantly decreased U_{crit} compared with the other groups (P=0.0012; Fig. 1A). There were no differences between control, T_2 and T_3 groups, confirming that the effects of the hypothyroid treatment were in fact due to reductions in active thyroid hormone. In contrast to zebrafish, there was no significant effect of hypothyroid treatment on maximal sprint swimming performance (P=0.45; Fig. 1B). The effect of thyroid hormone on locomotor performance therefore appears to vary between species. However, the power (Table 1) for the comparisons of sprint speed between treatments was very low (assuming 0.8 as a guide for adequate power; Cohen, 1992), with a medium effect size (Cohen, 1992); therefore, conclusions from these data are not robust and should be confirmed with larger sample sizes.

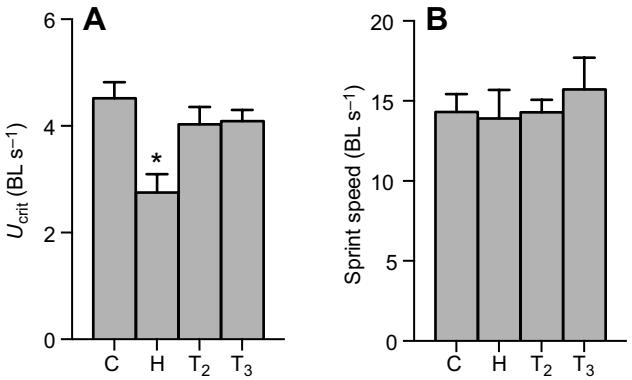

Fig. 1. Swimming performance of carp. (A) Critical sustained swimming speed (U_{crit}) was significantly lower in hypothyroid fish (H) compared with the control group (C) and the T_2 and T_3 supplemented groups (P=0.0012). The latter three groups did not differ from each other, showing that supplementation with both T_2 and T_3 reversed the effects of hypothyroidism. Sample sizes were: control=7 fish; hypothyroid=9; T_2=8; T_3=7. (B) Maximal sprint swimming speed was not significantly different between treatment groups (P=0.45; sample sizes: control=7; hypothyroid=6; T_2=8; T_3=6). BL, body lengths. Data represent means±s.e.m. Significant differences are indicated by an asterisk (*P_{MCMC}<0.1 for one-tailed directional hypotheses).

Muscle stress

We accept our hypothesis that hypothyroidism reduces muscle force production, and thyroid treatment had a significant effect on muscle stress (force per cross-sectional area of muscle; P=0.066). The lowest stress was in muscle from the hypothyroid treatment, which was significantly different from the T_2 supplementation treatment, but the other means did not differ (Fig. 2A). Muscle stress increased significantly following treatment with the SERCA blocker thapsigargin (P=0.071; Fig. 2B). Hence, we reject the hypothesis that the effect of thyroid hormone on muscle force production is mediated via its influence on SERCA activity. Other potential targets of thyroid hormone include a shift in expression of isoforms of myosin heavy chain and changes in substrate oxidation, resulting in mechanical and metabolic properties typical of faster muscle phenotype (Salvatore et al., 2014). There was no interaction between thyroid and thapsigargin treatments (P>0.34).

Table 1. Effect sizes and power of statistical comparisons

	Thyroid treatment		Thapsigargin treatment	
	Effect size	Power	Effect size	Power
U_{crit}	0.70	0.83		
Sprint	0.23	0.13		
Stress	0.38	0.69	0.46	0.44
Activation rate	0.15	0.16	0.04	0.05
Relaxation rate	0.22	0.27	0.07	0.06
Fatigue (at 15th tetanus)	0.39	0.72	0.24	0.86

Journal of Experimental Biology (2016) 219, 2806-2808 doi:10.1242/jeb.143529

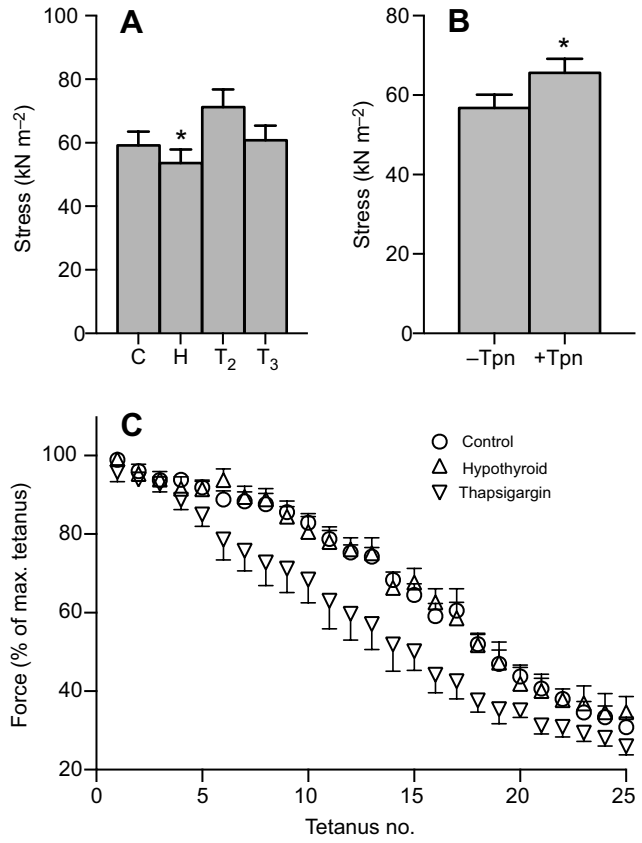

Fig. 2. Muscle stress (force per cross-sectional area) and fatigue resistance. (A) Muscle stress was significantly lower in hypothyroid fish compared with T_2 supplemented fish (P=0.066). Sample sizes for stress measurements were N=8 for each group, except for T_3, where N=7. (B) Thapsigargin (Tpn) significantly increased tetanic stress (P=0.071; data from all treatment groups were pooled). (C) Fatigue resistance (tetanus force as a percentage of the highest tetanus) decreased with thapsigargin treatment (P=0.010), but thyroid treatment did not have an effect on fatigue resistance. Control (open circles), hypothyroid (open triangles) and thapsigargin (inverted open triangle) are shown, and sample sizes were N=8 for each group. Data represent means±s.e.m. Significant differences are indicated by an asterisk (*P_{MCMC}<0.1 for one-tailed directional hypotheses).

Muscle activation and relaxation rates

There was no effect of thyroid treatment or thapsigargin treatment, or their interaction, on muscle tetanus activation rates (all P>0.25; Fig. S1A,B) or relaxation rates (all P>0.13; Fig. S1C,D). These data indicate that SERCA activity and resequestration of calcium into the sarcoplasmic reticulum do not constrain force production acutely during a single tetanus. However, statistical power was very low for all muscle activation and relaxation rate measurements (Table 1).

Muscle fatigue

Thyroid treatment did not affect muscle fatigue resistance (P=0.23; Fig. 2C). However, muscle fatigued significantly faster when SERCA was inhibited with thapsigargin (P=0.010; Fig. 2C). There was no interaction between thyroid and thapsigargin treatments (P=0.14). The data demonstrate the importance of effective calcium resequestration into the sarcoplasmic reticulum to reduce muscle fatigue (Allen et al., 2008; Seebacher et al., 2012). The discrepancy between the thyroid and thapsigargin treatments again indicates that thyroid hormone does not influence SERCA activity in this species. The power of the comparison between thyroid treatments was relatively high (Table 1), which lends confidence to this conclusion.

Conclusions

Hypothyroidism reduced both sprint performance and isolated muscle force production in carp. However, these reductions in performance were not mediated by SERCA. Thyroid treatment did not affect sustained swimming performance or isolated muscle fatigue resistance. Future studies need to investigate other potential targets of thyroid hormone, such as a shift in expression of isoforms of myosin heavy chain and changes in substrate oxidation.

Competing interests

The authors declare no competing or financial interests.

Author contributions

R.S.J. conducted experiments, analysed data and wrote the manuscript; A.G.L. conceived and conducted experiments; J.T. conducted experiments; and F.S. conceived the experiments, analysed data and wrote the manuscript.

Funding

This research was supported by an Australian Research Council grant to F.S. J.T. was funded by a Society of Experimental Biology Company of Biologists Travel Grant and Coventry University International Experience Mobility funding. R.S.J. was funded by a Coventry University Applied Research Fellowship.

Supplementary information

Supplementary information available online at
http://jeb.biologists.org/lookup/doi/10.1242/jeb.143529.supplemental

References

Allen, D. G., Lamb, G. D. and Westerblad, H. (2008). Skeletal muscle fatigue: cellular mechanisms. *Physiol. Rev.* **88**, 287-332.

Berchtold, M. W., Brinkmeier, H. and Müntener, M. (2000). Calcium ion in skeletal muscle: its crucial role for muscle function, plasticity, and disease. *Physiol. Rev.* **80**, 1215-1265.

Brett, J. R. (1964). The respiratory metabolism and swimming performance of young sockeye salmon. *J. Fish Res. Board Can.* **21**, 1183-1226.

Cohen, J. (1992). A power primer. *Psychol. Bull.* **112**, 155-159.

Duyff, R. F., Van den Bosch, J., Laman, D. M., Potter van Loon, B.-J. and Linssen, W. H. J. P. (2000). Neuromuscular findings in thyroid dysfunction: a prospective clinical and electrodiagnostic study. *J. Neurol. Neurosurg. Psychiatry* **68**, 750-755.

Galli, G. L. J., Taylor, E. W. and Shiels, H. A. (2006). Calcium flux in turtle ventricular myocytes. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **291**, R1781-R1789.

Hadfield, J. D. (2010). MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R Package. *J. Stat. Softw.* **33**, 1-22.

Hammer, C. (1995). Fatigue and exercise tests with fish. *Comp. Biochem. Physiol.* **112A**, 1-20.

Khaleeli, A. A. and Edwards, R. H. T. (1984). Effect of treatment on skeletal muscle dysfunction in hypothyroidism. *Clin. Sci.* **66**, 63-68.

Kurebayashi, N. and Ogawa, Y. (2001). Depletion of Ca^{2+} in the sarcoplasmic reticulum stimulates Ca^{2+} entry into mouse skeletal muscle fibres. *J. Physiol.* **533**, 185-199.

Little, A. G. and Seebacher, F. (2013). Thyroid hormone regulates muscle function during cold acclimation in zebrafish (*Danio rerio*). *J. Exp. Biol.* **216**, 3514-3521.

Little, A. G., Kunisue, T., Kannan, K. and Seebacher, F. (2013). Thyroid hormone actions are temperature-specific and regulate thermal acclimation in zebrafish (*Danio rerio*). *BMC Biol.* **11**, 26.

Lombardi, A., Moreno, M., de Lange, P., Iossa, S., Busiello, R. A. and Goglia, F. (2015). Regulation of skeletal muscle mitochondrial activity by thyroid hormones: focus on the 'old' triiodothyronine and the 'emerging' 3,5-diiodothyronine. *Front. Physiol.* **6**, 237.

Mullur, R., Liu, Y.-Y. and Brent, G. A. (2014). Thyroid hormone regulation of metabolism. *Physiol. Rev.* **94**, 355-382.

Salvatore, D., Simonides, W. S., Dentice, M., Zavacki, A. M. and Larsen, P. R. (2014). Thyroid hormones and skeletal muscle–new insights and potential implications. *Nat. Rev. Endocrinol.* **10**, 206-214.

Seebacher, F., Pollard, S. R. and James, R. S. (2012). How well do muscle biomechanics predict whole-animal locomotor performance? The role of Ca^{2+} handling. *J. Exp. Biol.* **215**, 1847-1853.