Single muscle fibre analysis of proteins important for glycogen metabolism in skeletal muscle from trained cyclists following varying bouts of exercise

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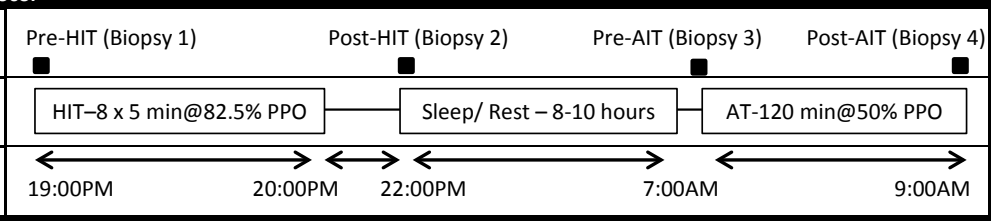
Background: Glycogen is an important fuel source, providing energy for contracting skeletal muscle. Humans possess an active protein/carbohydrate relationship within skeletal muscle. These proteins include anabolic enzymes glycogen synthase (GS) and branching enzyme (GBE), and catabolic enzymes glycogen phosphorylase (GP) and debranhcing enzyme (GDE). Skeletal muscle is heterogeneous, comprising slow-twitch (Type I) and fast-twitch (Type II) fibres, which are distinct in their metabolic and contractile properties. This characteristic is emphasized by the relative abundance of GS, GBE, GP and GDE proteins in rat fast and slow twitch muscle, with a bias for catabolic proteins in fast-twitch fibres. In the current study we used two exercise interventions (Table A) with differential demands on glycogen utilization, and single fibre western blotting to assess the abundance of glycogen-related proteins in specific fibres in endurance trained cyclists.

Methods: The study was approved by the Human Ethics Committee at RMIT University. Muscle biopsies were taken from the *vastus lateralis* of 3 trained cyclists using the Bergstrom biopsy technique. Following injection of 1% lidocaine (Xylocaine) into the skin/fascia, a small incision was made, and muscle samples were collected at four time points (Table A). Individual fibres were collected from freshly obtained tissue under paraffin oil, and prepared for western blotting analysis.

Results: Fibre type distribution varied between subjects (Table B). Following HIT, there was a decrease in GS, GBE, GP, but not GDE in Type I fibres, but only in GS in Type II fibres (Table C). Following AT only phos-GS and GBE and this was in Type I fibres only. This shows altered adaptions to the opposing exercise bouts.

Conclusion: Glycogen regulation is complex, and fibre type needs to be considered when understanding regulation. Any analyses conducted in whole muscle should therefore be interpreted with caution.

A. Study Protocol



B. Fibre type distribution

Subject (n= Total Fibres)	Type I Fibres (%)	Type II Fibres (%)	Hybrid Fibres (%)	VO_2 (L/min)	PPO (Watts)
A. (n=51)	53	35	12	5.18	403
B. (n=38)	79	21	-	5.45	472
C. (n=18)	50	39	11	4.93	408

C. Protein abundance

		Pre HIT, Biopsy 1		Post HIT, Biopsy 2		Pre AT, Biopsy 3		Post AT, Biopsy 4	
	Protein	Type I	**Type II**	Type I	**Type II**	Type I	Type II	Type I	**Type II**
Anabolic	GS	1.33 ± 0.1	**1.51 ± 0.5**	0.38 ± 0.04*	**0.64 ± 0.12***	0.82 ± 0.07	**1.17 ± 0.13**	1.15 ± 0.18	**0.87 ± 0.16**
	phos-GS	1.08 ± 0.12	**N/A**	1.21 ± 0.15	**1.03 ± 0.07**	0.75 ± 0.05	**0.82 ± 0.15**	1.02 ± 0.11^	**1.04 ± 0.01**
	GBE	1.13 ± 0.05	**0.85 ± 0.29**	0.96 ± 0.06*	**0.65 ± 0.06**	0.89 ± 0.06	**0.92 ± 0.03**	1.24 ± 0.15^	**0.97 ± 0.15**
Catabolic	**GP**	1.07 ± 0.08	**1.43 ± 0.98**	0.48 ± 0.12*	**1.99 ± 0.21**	0.9 ± 0.1	**1.94 ± 0.43**	1.16 ± 0.2	**2.25 ± 0.52**
	phos-GP	1.08 ± 0.13	**3.2 ± 1.4**	0.66 ± 0.19	**1.94 ± 0.46**	0.67 ± 0.16	**1.67 ± 0.61**	1.16 ± 0.26	**2.25 ± 0.88**
	GDE	0.97 ± 0.05	**1.39 ± 0.1**	0.95 ± 0.13	**2.05 ± 0.16**	0.9 ± 0.07	**1.59 ± 0.09**	1.01 ± 0.1	**1.72 ± 0.13**

Table A. Study protocol outlining high intensity (HIT) or aerobic (AT) exercises and biopsy times. **B.** Fibre type distribution (%), VO_2 peak and Peak power output (PPO) for subjects. **C.** Fibre type specific abundance of glycogen proteins. Paired Student t-tests for Pre *vs* Post-HIT/AT in specific fibre type (*i.e.* Type I or Type II fibres compared), * $p < 0.05$ different from Pre-HIT, and ^ $p < 0.05$ different from Pre-AT. n = 2-22, n = 3.