APPENDIX A: Data Appendix

Table 3. Animal codes, dates of tissue collection, and method of storage.

Animal Code	Date Sacrificed	Tissue Storage
3	03-Mar-00	no sucrose
4	24-Apr-00	sucrose
5	25-Apr-00	sucrose
6	27-Apr-00	sucrose
8	03-May-00	sucrose
9	05-May-00	sucrose
10	09-May-00	sucrose
11	10-May-00	sucrose
12	11-May-00	sucrose
17	17-May-00	no sucrose
18	19-Jan-00	no sucrose
19	25-Jan-00	no sucrose
20	26-Jan-00	no sucrose
21	01-Dec-99	sucrose
22	02-Dec-99	sucrose
23	23-Feb-00	sucrose
24	02-Feb-00	no sucrose
25	21-Jan-00	perchloric acid
26	27-Jan-00	perchloric acid
27	01-Mar-00	perchloric acid
28	03-Mar-00	perchloric acid

Table 3. Animal codes, dates of tissue collection, and method of storage. No sucrose: isolated SR was not stored in sucrose in order to measure glycogen associated with the SR. Sucrose: isolated SR was stored in sucrose so that Ca^{2+} uptake measurements could be made. Perchloric acid: the whole muscle was homogenized in a perchloric acid solution so that whole muscle glycogen could be measured.

Whole Muscle Glycogen (mmol/kg wet wt.)

Control	Replication		
Animal	**1**	**2**	**Average**
25	46.6	45.3	45.96
26	44.10	44.80	44.45
27	38.3	38.3	38.30
28	44.6	45.3	44.95
		Mean	43.42
		SEM	1.73

Fatigue	Replication		
Animal	**1**	**2**	**Average**
25	8.2	9.7	8.95
26	14.2	13.1	13.65
27	8.4	8.5	8.45
28	7	10.6	8.80
		Mean	9.96
		SEM	1.23
		% Control	22.9
		t-Test	0.0002

Table 4. Whole muscle glycogen concentration.

SR Glycogen Concentration
(ug/mg SR Protein)

Control	Replication		
Animal	**1**	**2**	**Average**
3	274.25	279.69	276.97
17	633.38	670.51	651.95
18	335.64	324.38	330.01
19	529.81	535.59	532.70
20	507.36	489.98	498.67
24	119.48	113.92	116.70
		Mean	401.17
		SEM	79.81

Fatigue	Replication		
Animal	**1**	**2**	**Average**
3	13.96	19.23	16.60
17	13.81	13.81	13.81
18	21.48	21.48	21.48
19	22.67	22.67	22.67
20	28.99	28.99	28.99
24	19.23	19.23	19.23
		Mean	20.46
		SEM	2.16
		% Control	5.1
		t-Test	0.0025

Table 5. SR glycogen concentration.

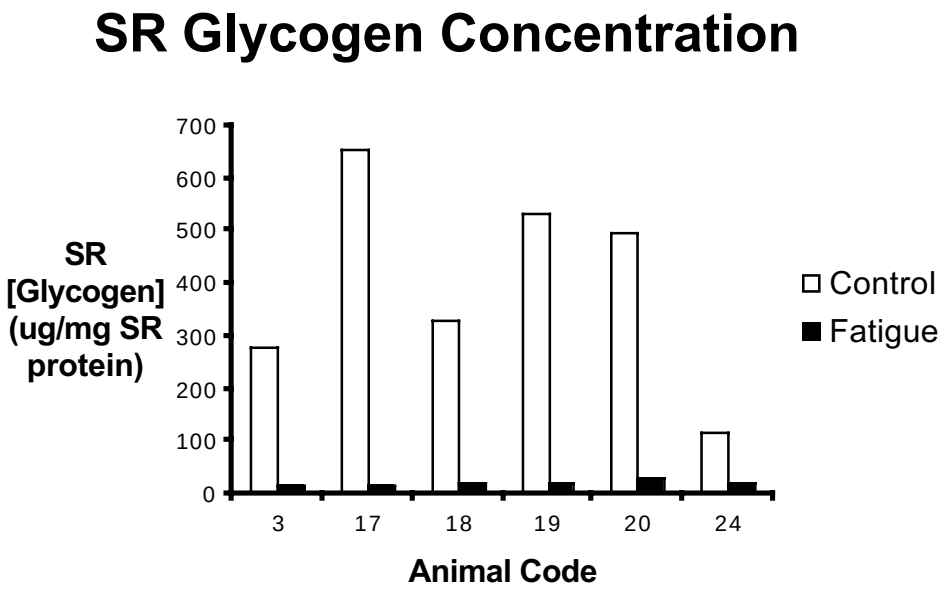


Figure 7. SR glycogen concentration.

SR Calcium Uptake Rates
(ug/mg SR protein/min)

Control	Replication			
Animal	**1**	**2**	**3**	**Average**
4	2.35	2.41	2.45	2.40
5	2.54	2.73	2.54	2.60
6	2.89	2.76	2.67	2.77
8	3.43	3.4	4.01	3.61
9	3.39	3.4	3.4	3.40
10	3.78	4.01	3.91	3.90
11	2.28	2.38	2.29	2.32
12	2.93	2.82	2.94	2.90
			Mean	2.99
			SEM	0.21

Fatigue	Replication			
Animal	**1**	**2**	**3**	**Average**
4	1.82	1.9	1.91	1.88
5	2	1.91	1.81	1.91
6	2.5	2.51	2.53	2.51
8	3.2	3.39	3.33	3.31
9	3.01	2.89	2.83	2.91
10	3.21	2.68	2.85	2.91
11	2.19	2.07	2.12	2.13
12	1.63	1.83	1.85	1.77
			Mean	2.42
			SEM	0.205
			% Control	80.8
			t-Test	0.0011

Table 6. SR Ca^{2+} uptake rates.

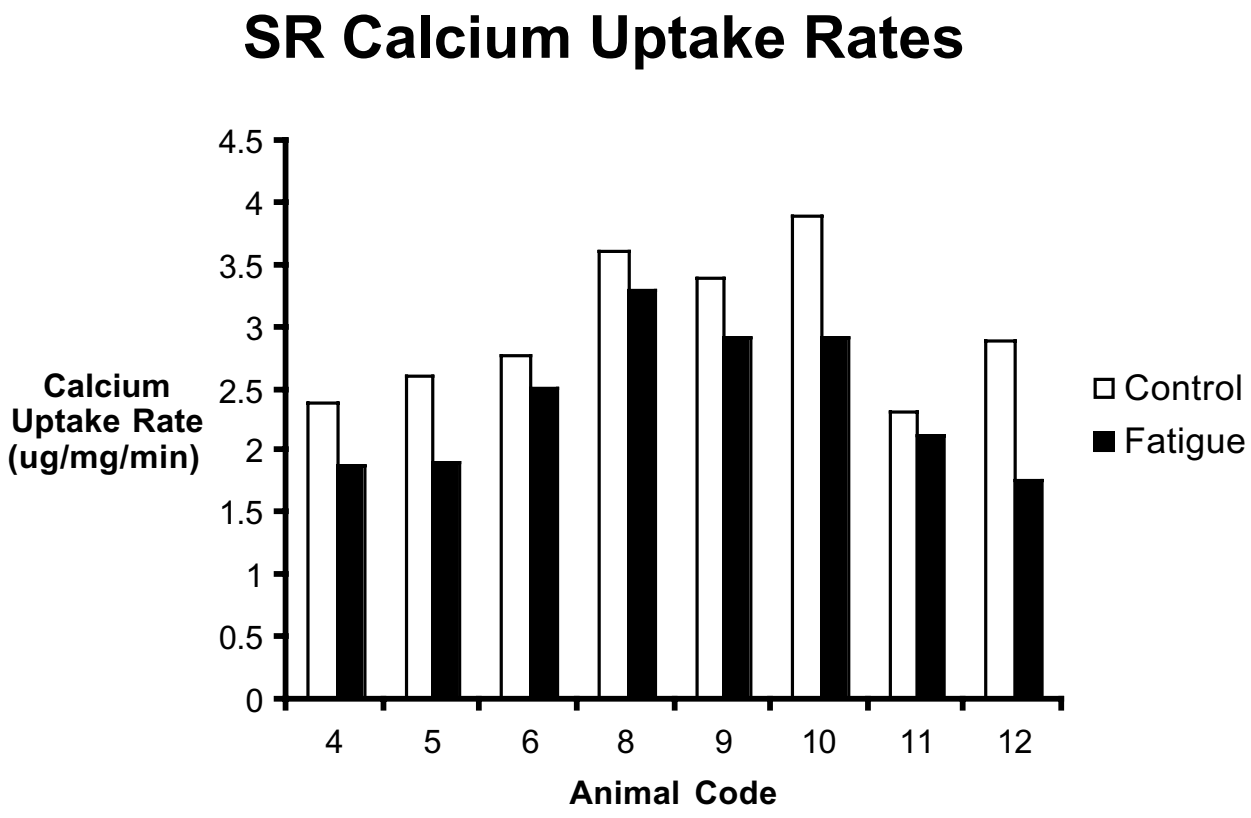
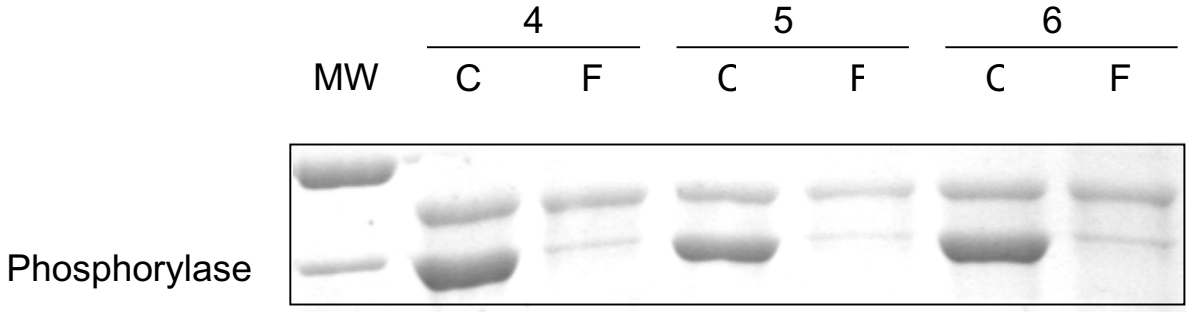


Figure 8. SR calcium uptakes.

Glycogen Phosphorylase Content Determined via SDS-PAGE



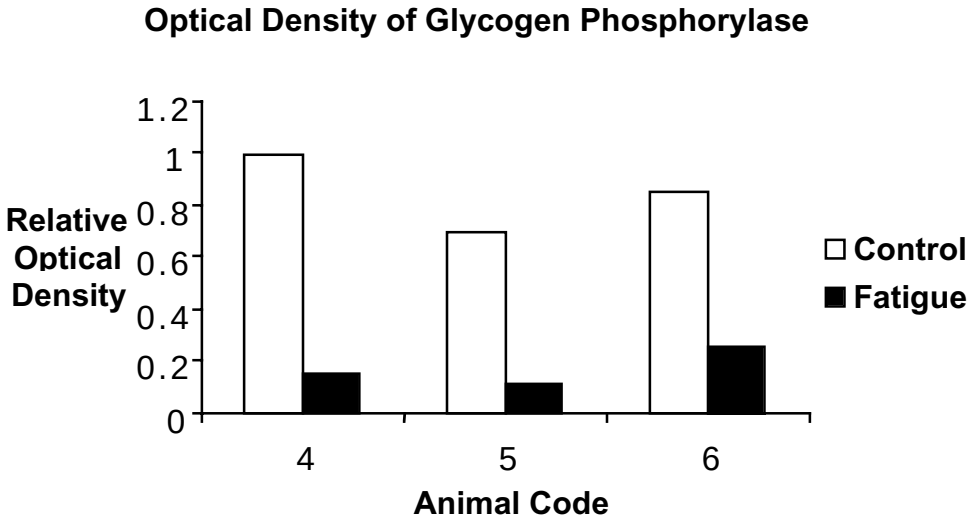
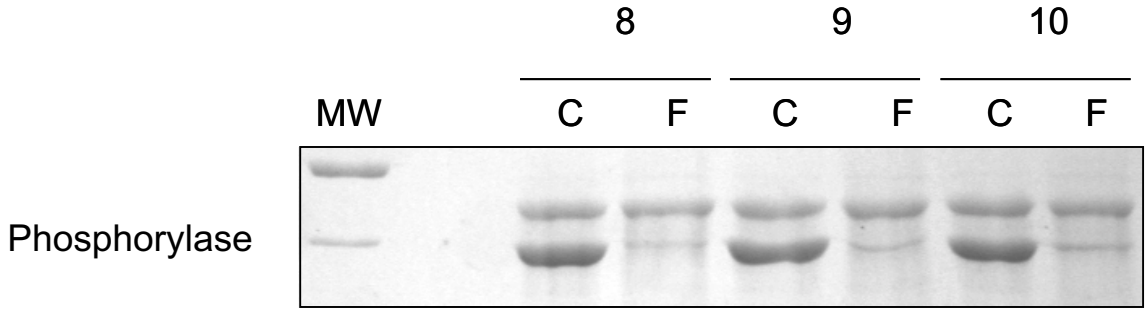


Figure 9. Glycogen phosphorylase content determined via SDS-PAGE.

Glycogen Phosphorylase Content Determined via SDS-PAGE



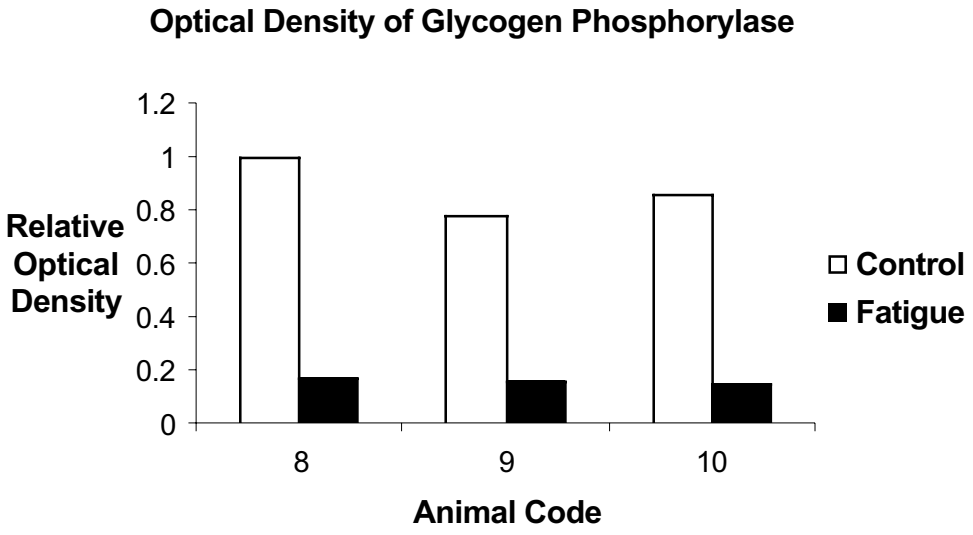
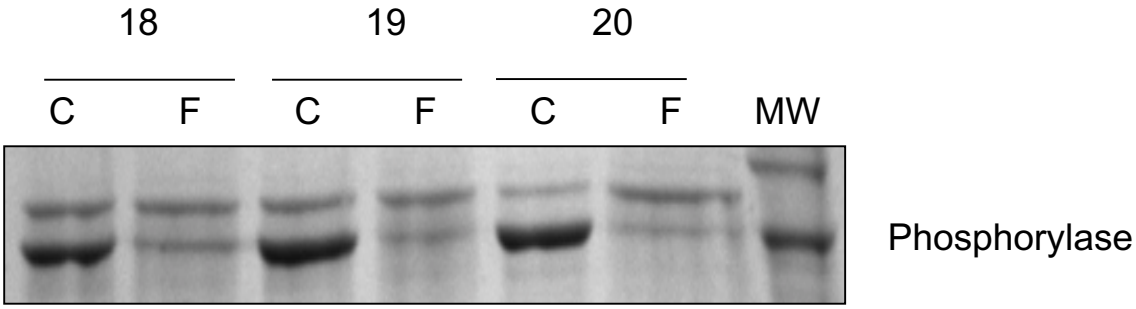


Figure 10. Glycogen phosphorylase content determined via SDS-PAGE.

Glycogen Phosphorylase Content Determined via SDS-PAGE



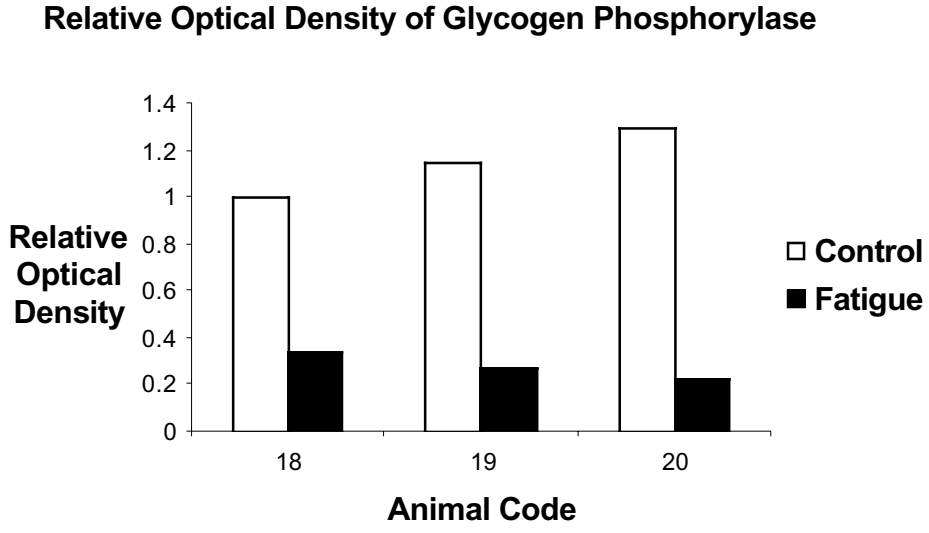
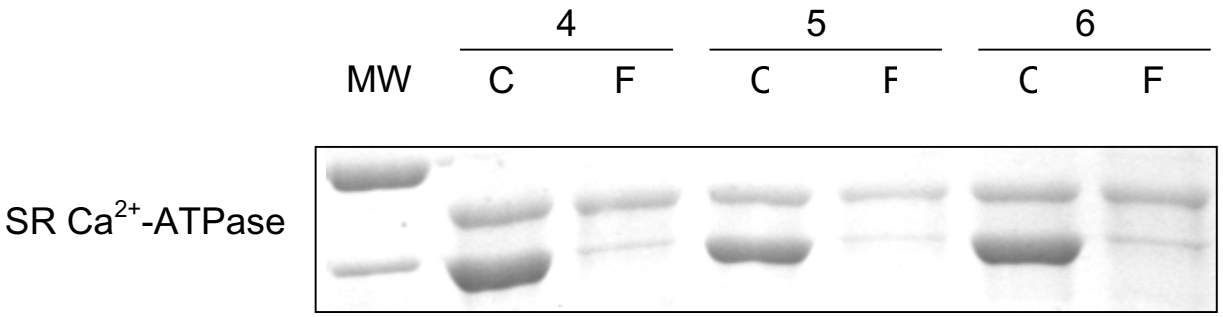


Figure 11. Glycogen phosphorylase content determined via SDS-PAGE.

SR Ca^{2+}-ATPase Content Determined via SDS-PAGE



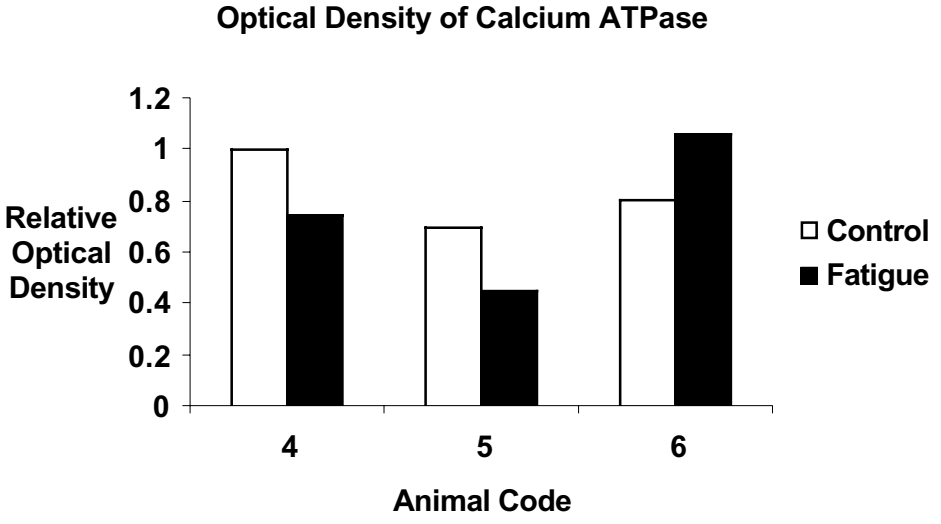
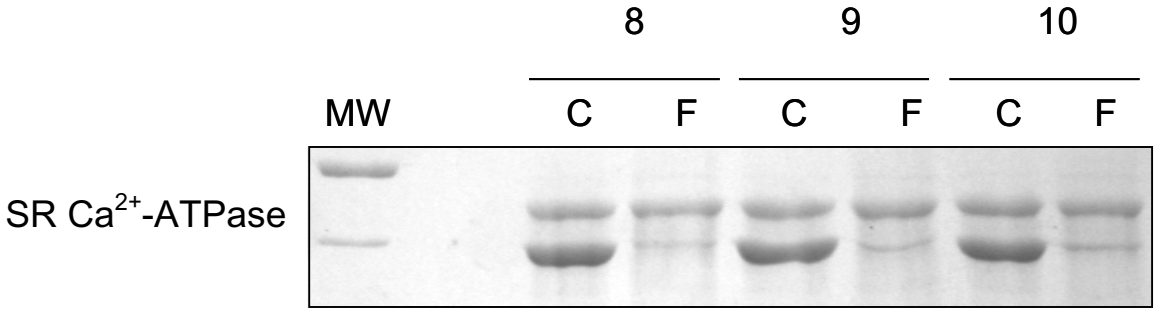


Figure 12. SR Ca^{2+}-ATPase content determined via SDS-PAGE.

SR Ca^{2+}-ATPase Content Determined via SDS-PAGE



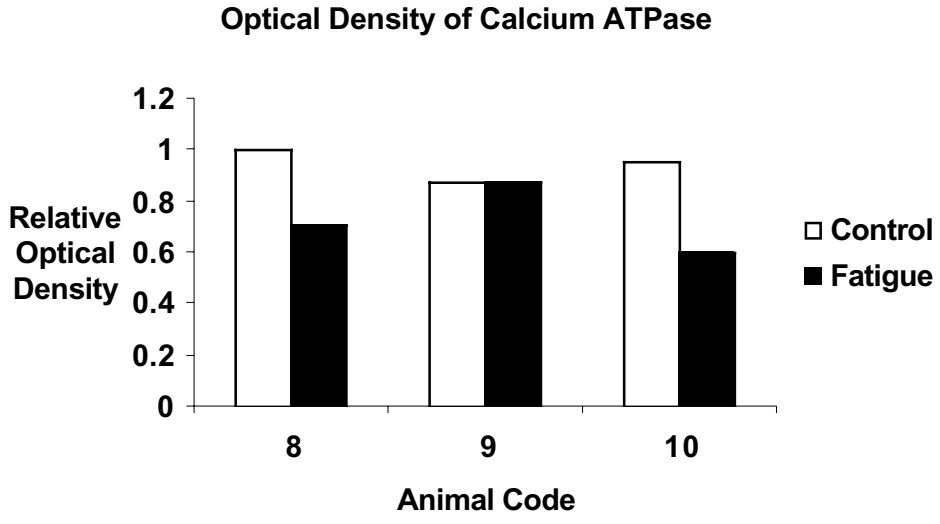
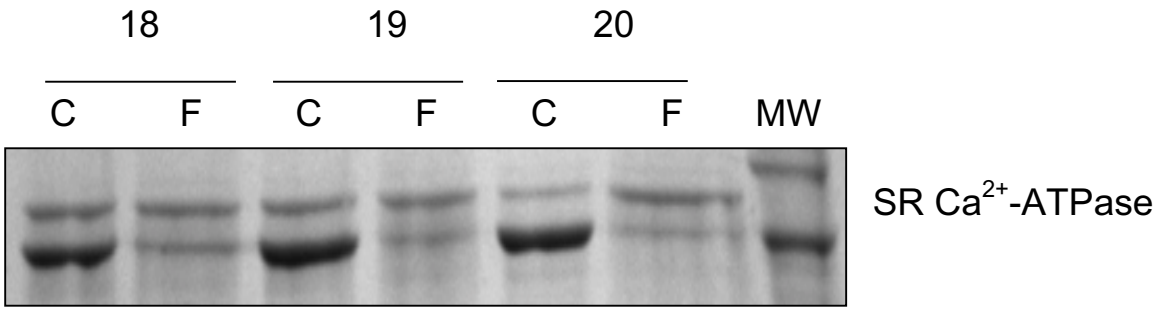


Figure 13. SR Ca^{2+}-ATPase content determined via SDS-PAGE.

SR Ca^{2+}-ATPase Content Determined via SDS-PAGE



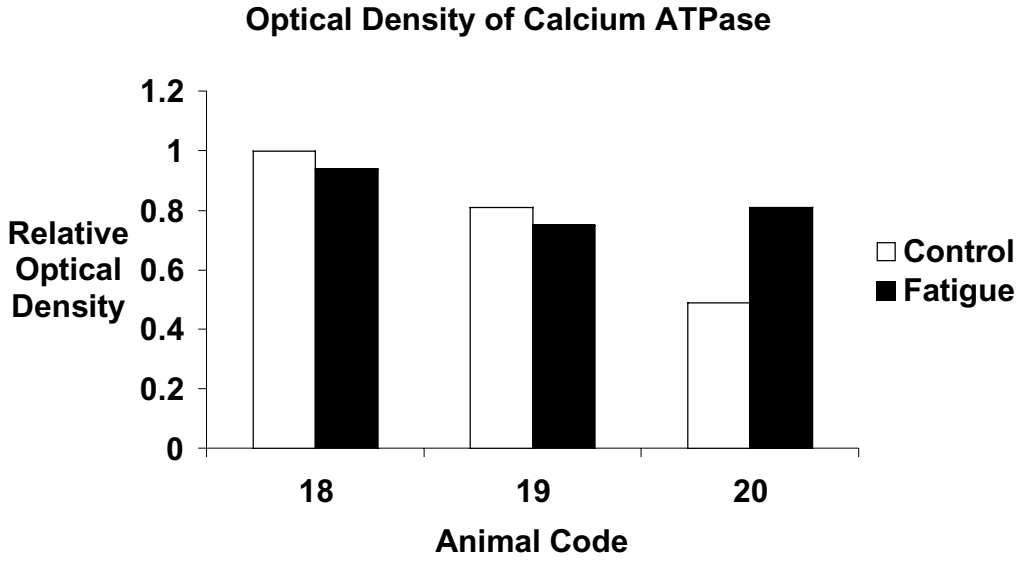


Figure 14. SR Ca^{2+}-ATPase content determined via SDS-PAGE.

PLP Content (ng/mg SR)

Control	Replication		
Animal	**1**	**2**	**Average**
3	417.10	419.38	418.24
18	547.41	565.61	556.51
19	765.65	766.58	766.12
21	1004.76	991.78	998.27
22	903.12	879.73	891.43
24	214.29	219.87	217.08
		Mean	641.27
		SEM	121.45

Fatigue	Replication		
Animal	**1**	**2**	**Average**
3	-12.14	39.61	13.74
18	-11.73	35.43	11.85
19	14.95	33.94	24.45
21	26.46	26.69	26.58
22	47.13	44.31	45.72
24	3.23	7.31	5.27
		Mean	21.27
		SEM	5.88
		% Control	3.3
		t-Test	0.0016

Table 7. PLP content.

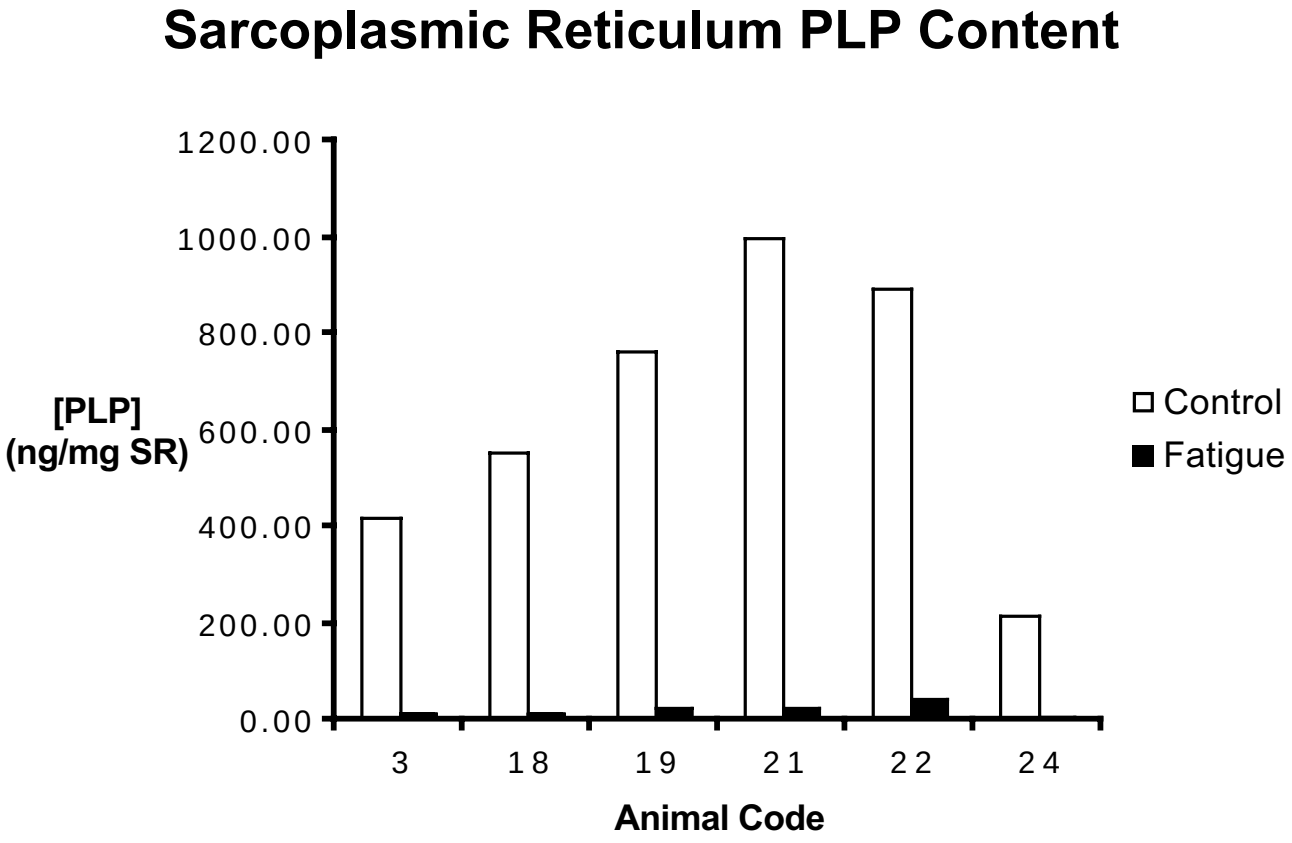


Figure 15. Sarcoplasmic reticulum PLP content.

Glycogen Phosphorylase Activity (nmol G-1-P/μg SR protein/min)

Control	Replication		
Animal	**1**	**2**	**Average**
4	3.22	3.08	3.15
5	3.33	3.75	3.54
6	2.24	2.24	2.24
8	2.88	2.94	2.91
9	2.16	2.27	2.22
10	2.74	2.74	2.74
18	2.57	2.54	2.56
19	3.06	2.98	3.02
		Mean	2.80
		SEM	0.16

Fatigue	Replication		
Animal	**1**	**2**	**Average**
4	0.148	0.148	0.148
5	0.143	0.164	0.154
6	0.153	0.148	0.151
8	0.061	0.061	0.061
9	0.055	0.05	0.053
10	0.072	0.077	0.075
18	0.189	0.182	0.186
19	0.096	0.088	0.092
		Mean	0.115
		SEM	0.018
		% Control	4.1
		t-Test	0.0000003

Table 8. Glycogen phosphorylase activity.

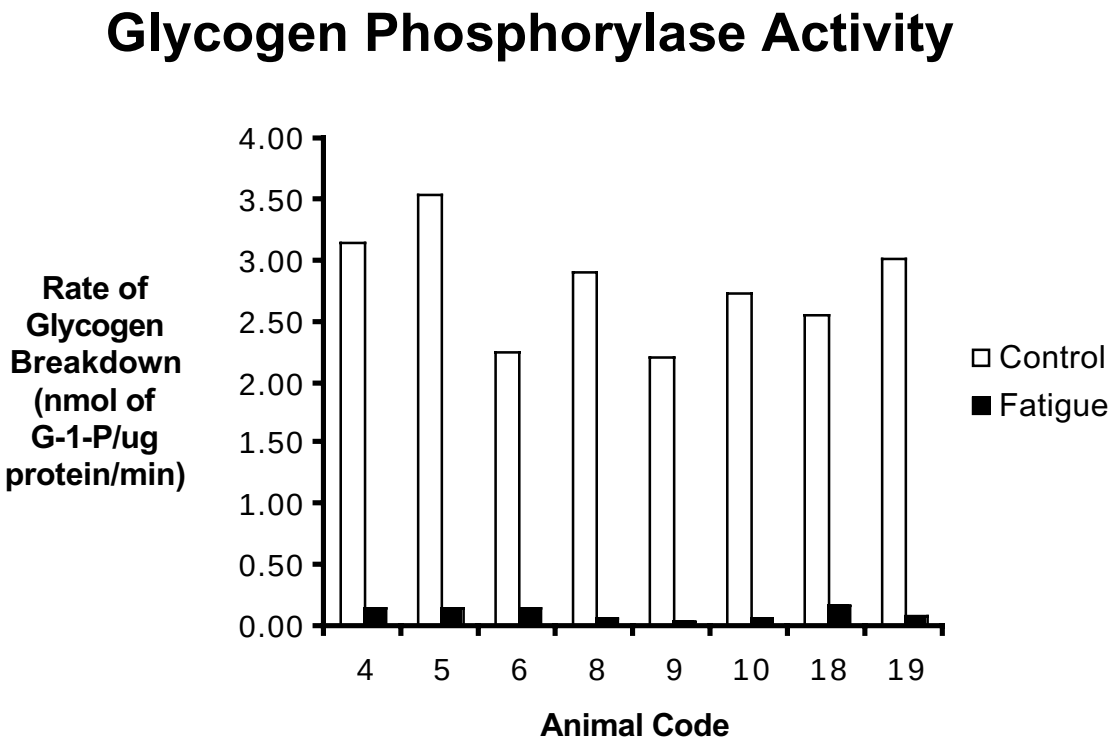


Figure 16. Glycogen phosphorylase activity.

APPENDIX B: Pilot Data

Pilot Data

Entman et al., (J Biol Chem, 255:6245, 1980) were some of the first to present a detailed description of a sarcoplasmic reticulum (SR)-glycogenolytic complex in skeletal muscle. Despite this, few studies report measurements of SR glycogen content and the effects of activity on SR glycogen are unknown. In this study, we examined glycogen content of two SR fractions prepared from rested muscles and from muscles subjected to repetitive stimulation. Heavy (HSR, 8,000-12,000g) and light (LSR, 12,000-49,000g) SR fractions were prepared from rat gastrocnemius-plantaris (GP) muscles. One GP was subjected to *in situ* stimulation (10Hz, 15min) while the contralateral served as the control. This protocol leads to a 45% decline in twitch force and a 75% reduction in whole-muscle glycogen. SR Glycogen was assessed using two different methods. Using an ethanol extraction, sulfuric acid hydrolysis and phenol determination, SR glycogen content, in rested muscles, was highly variable between animals, ranging from 58.6 to 178.5 ug/mg SR protein. There were no differences between the HSR and LSR fractions. Following stimulation, SR glycogen was significantly

reduced by 80-85% from 77.47±11.04 to 14.50±2.70 ug/mg in the HSR and from 123.51±19.93 to 19.08±6.56 ug/mg in the LSR. Using a glucoamylase digestion and fluorometric meaurement of β-NADP in an enzymatic system, SR glycogen, in rested muscles, was also highly variable, ranging from 142.63 to 698.34 μg/mg SR protein. There was a significant difference ($p \leq 0.05$) between rested HSR and LSR fractions using this assay 179.8 and 524.7 μg/mg SR protein, respectively. Following stimulation, HSR glycogen was significantly reduced by 83% from 179.8±14.2 to 30.4±2.0 ug/mg and LSR glycogen was reduced by 94% from 524.7±88.9 to 31.6±1.7 ug/mg. There was no difference in the activity-induced reduction in SR glycogen between fractions using either assay. These data indicate that there is considerable glycogen associated with skeletal muscle SR and that the glucoamylase-β-NADP assay is likely more sensitive than the sulfuric acid assay. They also suggest that repetitive activity leads to reductions in SR glycogen content. Supported by NIH AR 41727.

Curriculum Vitae

Simon Lees was born on May 2, 1974 in Chilliwack, British Columbia, Canada. He obtained an undergraduate degree in Exercise Science from the department of Human Kinetics at the University of British Columbia in 1998. He spent the next two years working on a Masters of Science in Muscle Physiology and Biochemistry at Virginia Polytechnic Institute and State University. During that time he received an outstanding student research award from the American Physiological Society for the presentation of his work at the 2000 Experimental Biology conference.

Simon Lees plans to continue his education and research at Virginia Polytechnic Institute and State University and complete a Ph. D. in Muscle Physiology and Biochemistry. He plans to pursue a career in academia, teaching at the university level and continuing with his research.