Key glycolytic metabolites in paralyzed skeletal muscle are altered 7 days after spinal cord injury in mice

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1. ABSTRACT

Spinal cord injury (SCI) leads to rapid losses in muscle mass due to immobilization and loss of communication with the central nervous system. SCI is also associated with an oxidative-to-glycolytic fiber-type transition which likely results in altered muscle metabolic function. How paralysis affects the levels of major muscle metabolites is not well-described. **Purpose:** to identify changes in metabolite levels in paralyzed muscle at 7 and 28 d following a complete SCI. **Methods:** Female C57BL6 mice aged 20 weeks underwent sham or complete SCI surgeries. The sham group (Sham) was sacrificed at 7 d and SCI animals were sacrificed at 7 d (7d SCI) or 28 d (28d SCI) post-surgery (n=5/group). Gastrocnemius muscles were removed at sacrifice and flash frozen. Primary untargeted metabolomics analysis was performed on the muscle samples using GC-TOF mass spectroscopy (West Coast Metabolomics, NIH). Statistical analyses of mass spectroscopy peaks was completed using Metaboanalyst 4.0, Metabox and R Software. Protein expression was determined using western blotting. **Results:** A principle components analysis identified muscle metabolites at 7 d SCI as a distinct cluster when compared to Sham and 28 d SCI. Metabolomic profiling identified 203 metabolites, with 83 being identifiable with BinBaseIDs. Of the known metabolites, 19 had ANOVAs with p values <0.05 and 8 remained after false discovery rate exclusion: lactate, glucose, maltose, oxoproline, sorbitol, tryptophan, maltotriose and pyruvate. Because glucose, lactate and pyruvate are key substrates of glycolysis, the expression of key glycolytic proteins was probed. GLUT4 levels were upregulated (p=0.06) in 7 d SCI animals compared to Sham and 28 d SCI animals. There was a strong trend (p=0.07) for reduced pyruvate kinase expression in 7 d SCI animals compared to Sham and 28 d SCI animals while pyruvate dehydrogenase was greatly reduced in 28 d SCI compared to 7 d SCI (p<0.05). The level of lactate dehydrogenase approached statistical reductions (p=0.09) at 28 d. **Conclusions:** Paralysis following SCI leads to reductions in glucose, lactate and pyruvate at 7 d post-injury with levels recovering by 28 d. Reductions in levels of these are seen despite elevations in the expression of GLUT4 expression at 7 d, suggesting SCI leads to a disruption in glucose handling and glycolytic functioning in paralyzed muscle in the acute timeframe after injury.

2. PURPOSE

This study compared the state of muscle metabolite levels at two distinct timepoints after SCI: at 7 d post-paralysis when muscle loss is rapid and there are large-scale changes in programs for protein catabolism, and at 28 d post-paralysis, a timepoint when muscle atrophy has slowed or ended.

3. METHODS

- 20 week-old female C57BL/6 mice (n=15; 5/group) were purchased from Charles River and were given a sham SCI (laminectomy only) or a laminectomy followed by a complete T9 spinal cord transection. Sham animals were sacrificed at 7 d post-surgery and SCI animals were sacrificed at 7 and 28 d post-SCI.
- The left gastrocnemius muscles were sent to West Coast Metabolomics for primary metabolite analysis while the right gastrocnemius was used for western blotting.
- Metabolomics data were analyzed using Metaboanalyst 4.0, Metabox and R software, with figures being generated with Metaboanalyst 4.0.
- Changes in protein expression were tested using one-way ANOVAs with Tukey's post hoc

4. RESULTS

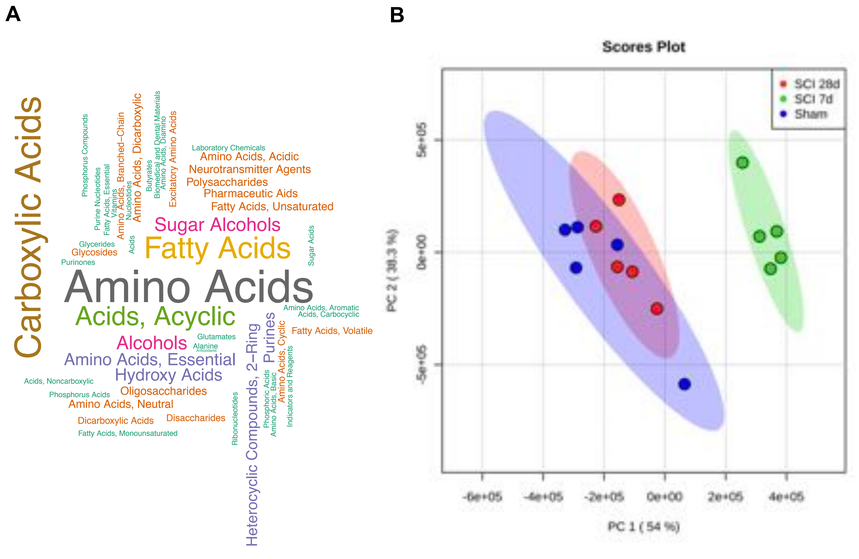
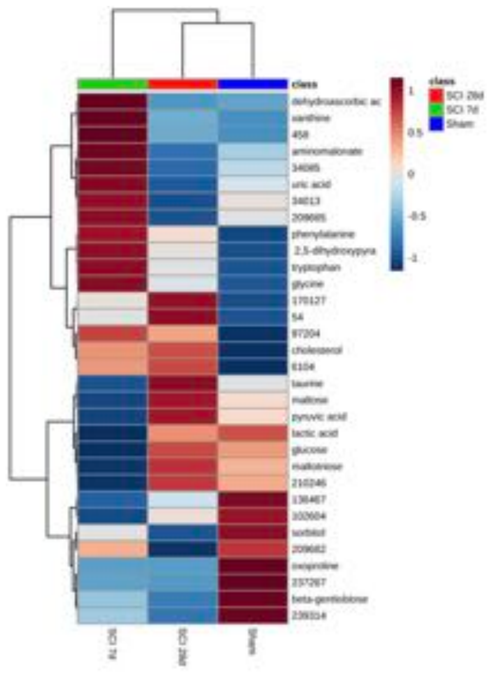


Fig. 1. A) Word cloud of annotated metabolites generated by Metabox. B) Principal components analysis of all detected compounds from sham muscle and muscle paralyzed at 7 or 28 days post-SCI.



Compound	ANOVA
lactic acid	0.0025
tryptophan	0.0031
maltose	0.0037
taurine	0.0075
sorbitol	0.0075
210246	0.0075
97204	0.0077
glucose	0.0081
458	0.0081
aminomalonate	0.0092
2,5-dihydroxypyrazine NIST	0.0122
170127	0.0140
136467	0.0148
maltotriose	0.0155
oxoproline	0.0207
6104	0.0221
pyruvic acid	0.0263
oleic acid	0.0305
glycine	0.0324
cholesterol	0.0324
102604	0.0324
34013	0.0339
34085	0.0344
54	0.0344
beta-gentiobiose	0.0347
phenylalanine	0.0365
dehydroascorbic acid	0.0365
239314	0.0388
209685	0.0404
237267	0.0464
uric acid	0.0465
209682	0.0493
xanthine	0.0498

Fig. 2. A heat map of compounds for which significant differences were identified between groups ($p < 0.05$; ANOVA). Compounds listed as numbers are unidentifiable molecules with no BinBase ID.

5. RESULTS CONT.

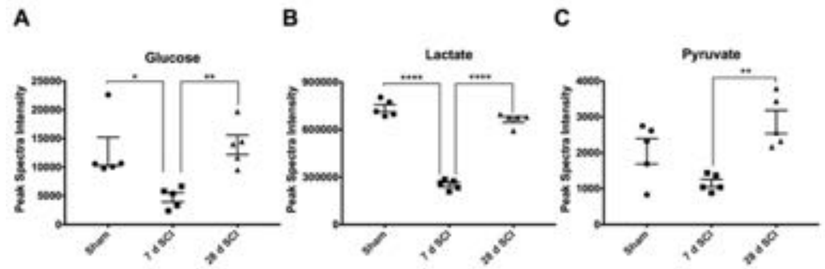


Fig. 3. Peak spectra intensities of A) glucose, B) lactate and C) pyruvate. (*) denotes group differences at p<0.05, (**) at p<0.01 and (****) at p<0.001. Data are presented as mean +/- SEM.

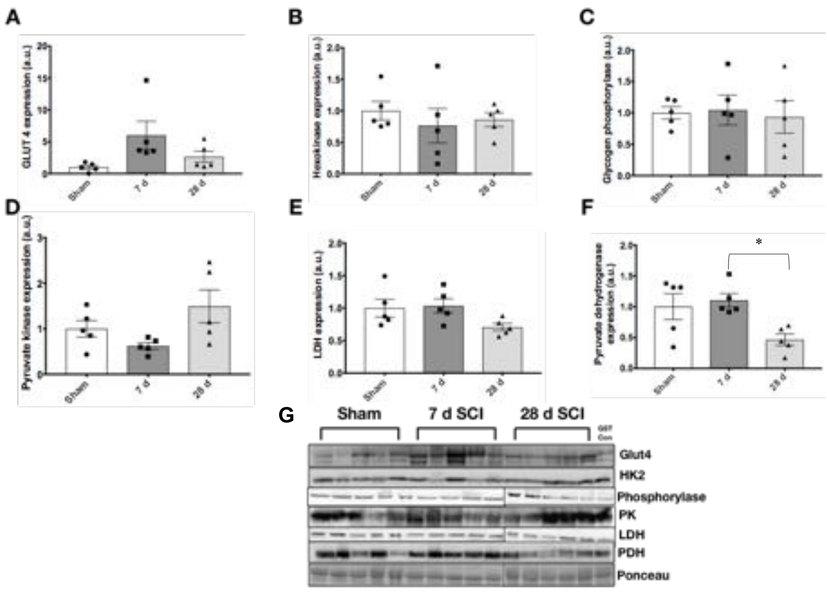


Fig. 4. Protein expression of key glycolytic enzymes at 7 and 28 d post-SCI. A) GLUT4, B) hexokinase 2, C) glycogen phosphorylase, D) pyruvate kinase, E) lactate dehydrogenase, F) pyruvate dehydrogenase and G) representative blots. All samples were normalized to whole-well Ponceau S stain densitometry and are shown relative to the 43 kDa band of the Ponceau S stain. (*) denotes group differences at p<0.05. Data are presented as mean +/- SEM.

6. CONCLUSIONS

- SCI resulted in altered levels of glucose and products of glycolysis at 7 d but not 28 d after SCI, despite elevations in GLUT4 protein levels at 7 d. The transient elevation in GLUT4 levels may be a response to low levels of intracellular glucose.
- Elevated levels of pyruvate and down-regulated PDH protein expression at 28 d post-SCI suggest disruption in the flow of products of glycolysis to the Krebs cycle.

7. ACKNOWLEDGMENTS

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