Data in brief 28 (2020) 105056

Contents lists available at ScienceDirect

Data in brief

journal homepage: www.elsevier.com/locate/dib

Data Article

RNA-seq data of soleus muscle tissue after spinal cord injury under conditions of inactivity and applied exercise

Julia H. Chariker [a, b, 1], Morgan Sharp [c, d, 1], Sujata Saraswat Ohri [c, d], Cynthia Gomes [e], Fiona Brabazon [c, d, 2], Kathryn A. Harman [c, f], Scott R. Whittemore [c, d, e], Jeffrey C. Petruska [c, d, e], David SK. Magnuson [c, d, e, **], Eric C. Rouchka [b, g, *]

[a] Department of Neuroscience Training, University of Louisville, 522 East Gray St., Louisville, KY, 40202, USA
[b] Kentucky Biomedical Research Infrastructure Network Bioinformatics Core, University of Louisville, 522 East Gray St., Louisville, KY, 40202, USA
[c] Kentucky Spinal Cord Injury Research Center, University of Louisville, 511 South Floyd St., Louisville, KY, 40202, USA
[d] Department of Neurological Surgery, University of Louisville, 220 Abraham Flexner Way, Suite 1500, Louisville, KY, 40202, USA
[e] Department of Anatomical Sciences and Neurobiology, University of Louisville, 511 South Floyd St., Louisville, KY, 40202, USA
[f] Department of Health & Sport Sciences, University of Louisville, 2100 South Floyd Street, Louisville, KY, 40208, USA
[g] Department of Computer and Engineering Science, Speed School of Engineering, University of Louisville, Duthie Center for Engineering, 2301 South 3rd St., Louisville, KY, 40292, USA

ARTICLE INFO

Article history:
Received 17 September 2019
Received in revised form 27 November 2019
Accepted 17 December 2019
Available online 31 December 2019

ABSTRACT

Reduced muscle mass and increased fatiguability are major complications after spinal cord injury (SCI), and often hinder the rehabilitation efforts of patients. Such detriments to the musculoskeletal system, and the concomitant reduction in level of activity, contribute to secondary complications such as cardiovascular disease, diabetes, bladder dysfunction and liver

* Corresponding author. Kentucky Biomedical Research Infrastructure Network Bioinformatics Core, University of Louisville, 522 East Gray St., Louisville, KY, 40202, USA.
** Corresponding author. Kentucky Spinal Cord Injury Research Center, University of Louisville, 511 South Floyd St., Louisville, KY, 40202, USA.
E-mail addresses: david.magnuson@louisville.edu (D.SK. Magnuson), eric.rouchka@louisville.edu (E.C. Rouchka).
[1] These authors contributed equally to this work.
[2] Current address: Wiley Publishing, Hoboken, NJ.

https://doi.org/10.1016/j.dib.2019.105056

Keywords:
Spinal cord injury
SCI
Soleus muscle
Inactivity
Exercise
Transcriptomics

damage. As a result of decreased weight-bearing capacity after SCI, muscles undergo morphological, metabolic, and contractile changes. Recent studies have shown that exercise after SCI decreases muscle wasting and reduces the burden of secondary complications. Here, we describe RNA sequencing data for detecting chronic transcriptomic changes in the rat soleus after SCI at two levels of injury severity, under conditions of restricted in-cage activity and two methods of applied exercise, swimming or shallow water walking. We demonstrate that the sequenced data are of good quality and show a high alignment rate to the *Rattus norvegicus* reference assembly (Rn6). The raw data, along with UCSC Genome Browser tracks created to facilitate exploration of gene expression, are available in the NCBI Gene Expression Omnibus (GEO; GSE129694).

Specifications Table

Subject	Cellular and Molecular Neuroscience
Specific subject area	Transcriptomic changes in the soleus muscle of spinal cord injured rats at two levels of severity under conditions of inactivity and applied exercise
Type of data	Transcriptomic data
How data were acquired	RNA sequencing (polyA enrichment; 75bp single end sequencing on an Illumina NextSeq 500)
Data format	Raw (fastq) Normalized count matrix (txt) UCSC Genome Browser tracks (bigwig)
Parameters for data collection	Female rats, housed in tiny (activity restricting) cages, were given a T2 spinal cord transection, a T2 spinal cord contusion, or no SCI. A subset of rats with spinal cord contusion were given 10 weeks of exercise rehabilitation post-SCI, consisting of swimming or shallow water walking.
Description of data collection	Animals were sacrificed at 8.5, 11.5, or 13.5 weeks post-SCI, depending on condition. All uninjured animals were sacrificed at a time point equivalent to 11.5 weeks post-SCI in the other animals. Soleus muscle tissue was extracted and processed using RNeasy Lipid Tissue Mini Kit (Qiagen) to isolate RNA. PolyA enriched samples were sequenced on an Illumina NextSeq 500 using the NextSeq 500/550 1X75 cycle High output kit (Illumina, Carlsbad, CA).
Data source location	University of Louisville, Louisville, KY, USA
Data accessibility	Raw data and processed data can be accessed at NCBI's Gene Expression Omnibus (GEO accession GSE129694) http://identifiers.org/geo:GSE129694

Value of the Data

- Nearly 17,000 new cases of SCI occur each year with devastating consequences on quality of life for the individuals involved. Multiple pathologies, including cardiovascular disease, diabetes, liver damage and metabolic dysfunction, result from both denervation and a plegia-induced decrease in weight-bearing activity post-SCI [1,2]. The soleus muscle experiences deleterious changes when denervated since it functions as an important anti-gravity postural muscle [3,4]. In rats, and other animals, well-documented physical changes occur in the soleus after spinal cord transection and contusion injuries, including a change in muscle fiber composition from slow to fast twitch fibers [5–7]. These data provide insight into transcriptomic changes in the soleus that occurs with different injury severities and exercise rehabilitation of two different types, swimming and shallow water walking post-SCI.
- Researchers interested in understanding the molecular response to SCI associated with prolonged inactivity and post-SCI exercise rehabilitation will find these data a valuable resource.
- Transcriptomic changes associated with mechanisms such as response to oxidative stress and hypoxia, provide molecular insight into the effects of exercise that challenges the cardiovascular system (swimming) or involves dynamic body weight support (shallow water walking). This information will allow researchers to manipulate and study the expression of specific genes in hindlimb muscles such as the soleus, yielding further insight into the effects of these two forms of exercise rehabilitation.

- These data may also provide insight into the underlying mechanisms of other pathologies of the soleus muscle including atrophy, neuropathy and compartment syndrome.
- This dataset is part of a larger study measuring the systemic transcriptional response to spinal cord injury, including dorsal root ganglion [8] and liver [9], all of which are included as part of a GEO superseries (GSE129704) [10].

1. Data

Twenty raw sequencing files are available in a compressed fastq format (fastq.gz). The files represent five experimental groups with four replicates each (see Experimental design and SCI below). Raw sequencing data were input to FastQC [11] for quality assessment. All samples were deemed of high quality. In Fig. 1a, the Phred quality score/base is displayed for a representative sample from each experimental group. With the exception of the last base in all samples, the 25th percentile of quality scores is at or above a Phred score of 30, reflecting 99.9% accuracy in base calling. The gradual drop in quality at the end of the sequence is a common phenomenon with Illumina's approach of sequencing by synthesis [12]. Sequencing generated a mean of 39.4 ± 8.9 (S.D.) million reads/sample (range: 28.3 to 52.6 million). Table 1 displays the number of raw reads generated and successfully aligned to the *Rattus norvegicus* reference assembly (rn6) for each of the samples. The alignment rate for uniquely mapped and multi-mapped reads combined ranged from 93 to 98% with a mean of 97 across the 20 samples.

Along with the raw data files, a gene matrix of raw read counts for 24,613 loci is provided in a compressed text file format (txt.gz). A principal component analysis (PCA) using normalized read counts was performed to look at the between- and within-group variation among the no SCI, CONT SCI, and CONT SCI + SWIM/SWW samples (Fig. 1b). The separation between experimental groups improves with CONT SCI replicate 3, CONT SCI + SWIM replicate 2, and CONT SCI + SWW replicate 2 removed (Fig. 1c). A third PCA analysis examined variation across samples at two levels of injury severity (Fig. 1d).

Gene expression was examined to confirm that high level activity was found for genes relevant to skeletal muscle activity. In Fig. 2, mean expression across CONT SCI samples is displayed for highly expressed genes found within four Gene Ontology [13] categories: response to oxidative stress (GO:0006979), response to hypoxia (GO:0001666), fatty acid catabolic process (GO:0009062), and glucose metabolic process (GO:0006006).

UCSC Genome Browser expression tracks are available in binary (bigwig) format for each of the twenty samples. The tracks were created to facilitate exploration of gene expression across samples. Fig. 3 displays the UCSC Genome Browser expression tracks for the CONT SCI samples positioned at *Tpm1*, a gene known to be involved in regulation of striated muscle contraction.

2. Experimental design, materials, and methods

2.1. Experimental design and SCI

The experimental design is illustrated in Fig. 4. Prior to the study, 20 animals were randomly assigned to five groups: no injury (No SCI, 4 replicates), T2 transection injury (TX SCI, 4 replicates), T2 contusion injury (CONT SCI, 4 replicates), T2 contusion injury followed by swimming exercise rehabilitation (CONT SCI + SWIM, 4 replicates), and T2 contusion injury followed by shallow water walking exercise rehabilitation (CONT SCI + SWW, 4 replicates).

2.2. Animals

All animal procedures were performed in accordance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council, 1996) and the University of Louisville Institutional Animal Care and Use Committee.

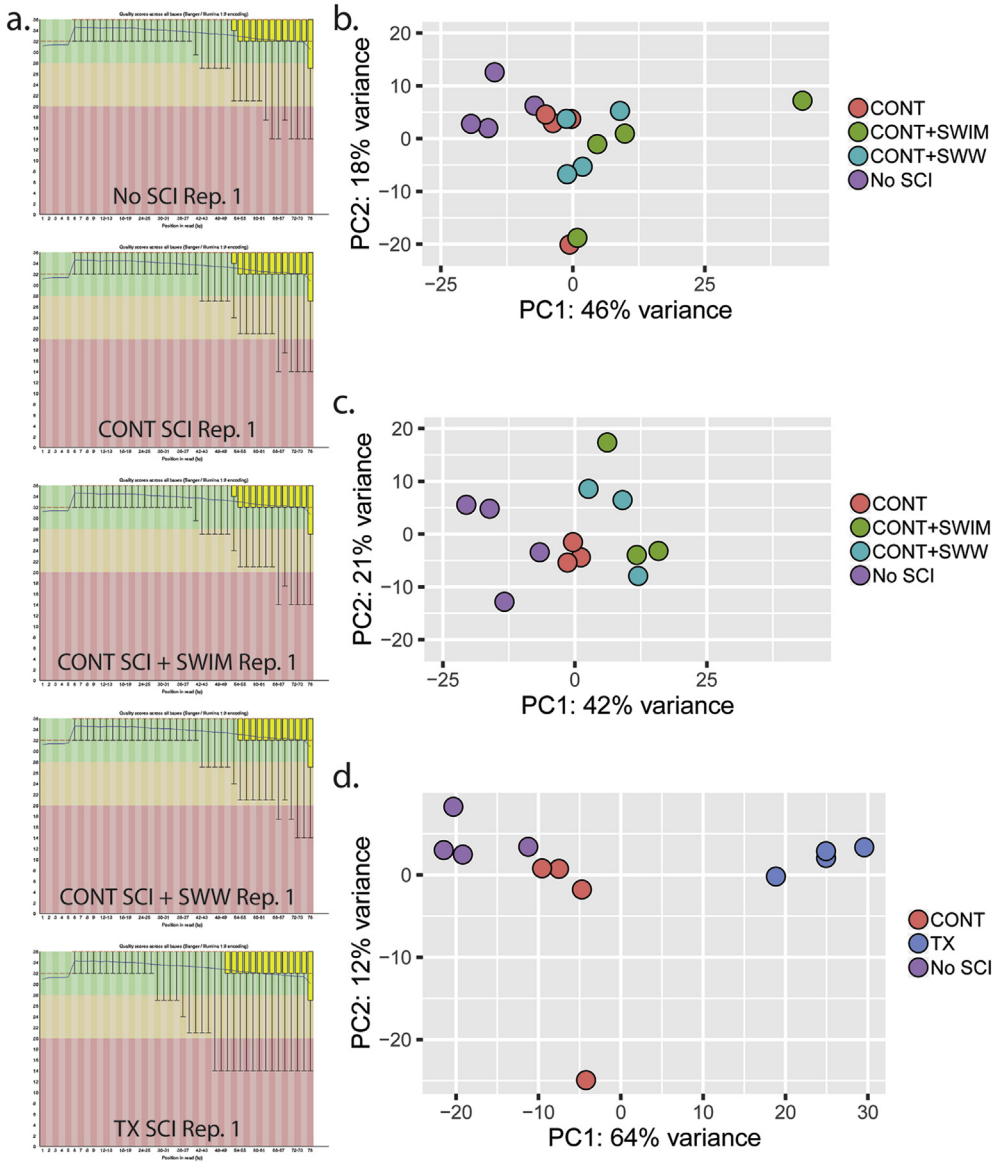

Fig. 1. Quality control analysis. Phred quality scores per base for one representative sample from each experimental group (a). On the Y-axis a Phred score of 30 indicates 99.9% accuracy in base calling. Phred scores above 28 (green) are considered very good quality. Scores between 20 and 28 (orange) are considered reasonable quality. Scores below 20 (red) are considered poor quality. The yellow box represents the inter-quartile range (25–75%). The lower and upper whiskers represent the 10th and 90th percentiles respectively. On the right, PCA plots for the comparison of SCI alone and SCI followed by exercise (b), the same experimental groups as in (b) with CONT SCI replicate 3, CONT SCI + SWIM replicate 2, and CONT SCI + SWW replicate 2 removed (c), and PCA focused solely on a comparison of injury severity (d).

Female Sprague Dawley rats of body weight 235–249 g (~8–9 weeks old) were obtained from Sprague Dawley, Inc. (Indianapolis, IN). Females were chosen because, in our experience, they have fewer post-surgical complications and exhibit greater motivation for the exercises. Prior to injury, all rats were

Table 1
Sequencing and alignment summary.

Sample ID	Experimental Group	Input	Number Uniquely Mapped Reads	Percent Uniquely Mapped Reads	Number Multi-mapped Reads	Percent Multi-mapped Reads
No SCI_Soleus, Rep. 1	No SCI	34,461,817	30,234,805	87.73%	3,219,090	9.34%
No SCI_Soleus, Rep. 2	No SCI	52,659,350	45,929,571	87.22%	5,330,524	10.12%
No SCI_Soleus, Rep. 3	No SCI	45,215,114	39,486,401	87.33%	4,631,202	10.24%
No SCI_Soleus, Rep. 4	No SCI	43,282,634	37,737,326	87.19%	4,403,769	10.17%
Contusion SCI_Soleus, Rep. 1	CONT SCI	29,674,153	25,954,487	87.46%	3,064,891	10.33%
Contusion SCI_Soleus, Rep. 2	CONT SCI	31,390,027	27,525,546	87.69%	3,175,265	10.12%
Contusion SCI_Soleus, Rep. 3	CONT SCI	33,185,693	28,881,605	87.03%	3,477,562	10.48%
Contusion SCI_Soleus, Rep. 4	CONT SCI	49,832,639	42,928,996	86.15%	5,210,955	10.46%
Contusion SCI + SWIM_Soleus, Rep. 1	CONT SCI + SWIM	36,938,790	32,264,532	87.35%	3,802,398	10.29%
Contusion SCI + SWIM_Soleus, Rep. 2	CONT SCI + SWIM	28,309,521	24,919,998	88.03%	2,781,084	9.82%
Contusion SCI + SWIM_Soleus, Rep. 3	CONT SCI + SWIM	29,319,512	25,698,024	87.65%	2,953,908	10.07%
Contusion SCI + SWIM_Soleus, Rep. 4	CONT SCI + SWIM	41,954,615	36,611,960	87.27%	4,268,234	10.17%
Contusion SCI + SWW_Soleus, Rep. 1	CONT SCI + SWW	29,577,126	26,027,663	88.00%	2,942,085	9.95%
Contusion SCI + SWW_Soleus, Rep. 2	CONT SCI + SWW	30,996,221	27,218,787	87.81%	3,104,595	10.02%
Contusion SCI + SWW_Soleus, Rep. 3	CONT SCI + SWW	28,425,718	24,857,936	87.45%	2,928,521	10.30%
Contusion SCI + SWW_Soleus, Rep. 4	CONT SCI + SWW	49,312,820	42,369,781	85.92%	5,261,967	10.67%
Complete transection SCI_Soleus, Rep. 1	TX SCI	47,542,690	40,964,957	86.16%	5,485,777	11.54%
Complete transection SCI_Soleus, Rep. 2	TX SCI	49,780,271	42,618,175	85.61%	5,883,275	11.82%
Complete transection SCI_Soleus, Rep. 3	TX SCI	45,638,101	37,359,474	81.86%	5,058,596	11.08%
Complete transection SCI_Soleus, Rep. 4	TX SCI	50,668,364	43,767,911	86.38%	5,584,869	11.02%

Note: spinal cord injury (SCI), contusion (CONT), transection (TX), shallow water walking (SWW), swimming (SWIM).

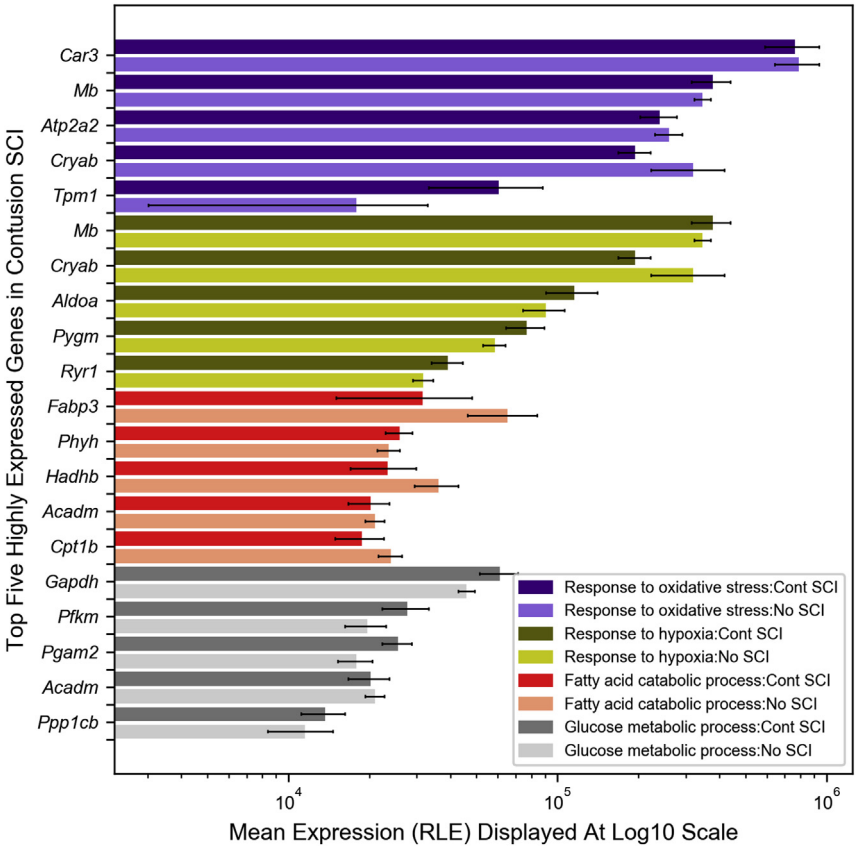

Fig. 2. Gene expression in four functional categories relevant to skeletal muscle activity. The five genes with highest mean expression across the four contusion injured (CONT SCI) samples are displayed for each category. Mean expression for No SCI is included as a comparison. Read counts are normalized using DESeq2's relative log expression (RLE).

doubly-housed in standard cages and maintained in a 12h-light/dark cycle throughout. Tap water and a standard rodent diet were available to all rats *ad libitum*.

All rats were initially gentled for two weeks, during which time they were introduced and acclimated to the testing and exercise facilities. After this period, animals were anaesthetized with a ketamine (50 mg/kg)/xylazine (0.024mg/kg)/acepromazine (0.005 mg/kg) cocktail (IP) and given glycopyrolate (0.08 mg/kg, IM) prior to SCI surgeries. For all injury groups (CONT and TX), after being anaesthetized with ketamine/xylazine supplemented as needed by isoflurane, a dorsal midline incision was made in the superficial muscle overlying the T1-T3 vertebrae. A single level laminectomy was then performed at the T2 vertebrae. Animals in the CONT groups received a moderately-severe contusion injury (25 g-cm) at the T2 spinal cord level using the NYU Impactor [14,15]. For animals in the TX injury group, a scalpel was used to deliver a complete TX of the spinal cord at T2. The muscle and skin overlying the injury was sutured in layers and antibiotic ointment was applied to the incision. Injured animals were monitored on heating pads until they recovered from the anesthesia. Rats were then doubly-housed in cages with ALPHA-dri® bedding (Shepherd's™ Specialty Paper, Milford, New Jersey) for the remainder of the study. Post-operative care consisted of daily injections of gentamicin sulfate for 7 days (20 mg/kg, S.C.), twice-daily injections of buprenorphine for 3 days (0.03 mg/kg, S.C., and as needed for pain management thereafter), and twice-daily 5 ml boluses of lactated ringers for three days (and as needed for hydration thereafter). Manual bladder expression was conducted three times a

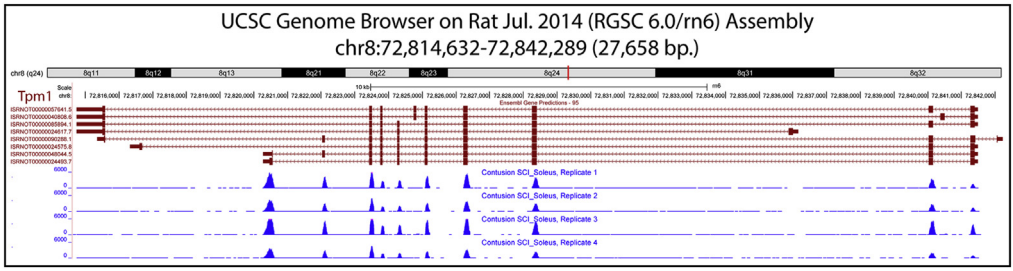

Fig. 3. UCSC Genome Browser gene expression tracks. Custom tracks display expression for *Tpm1* in four CONT SCI samples.

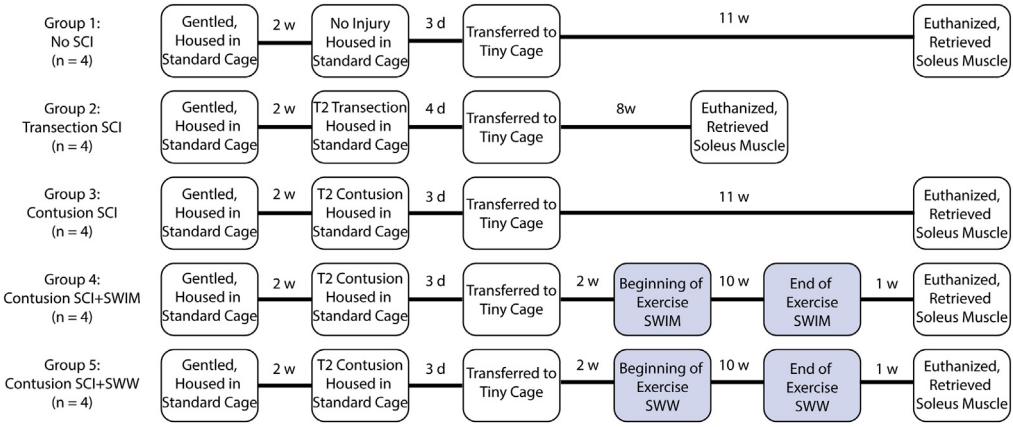

Fig. 4. Experimental design and time course.

day until reflexive voiding was re-established. Rats were maintained on a 12-h day/night light cycle throughout and had access to standard rat chow and water ad libitum. During the 2 week gentling and a 3 day recovery period, all animals were doubly-housed in standard cages, measuring 22″ x 12.5″ x 8″.

Three days after injury, all animals were removed from standard cages and doubly-housed in tiny cages (7.5″ x 8.5″ x 8.0″) to restrict in-cage activity for the duration of the study. Animals in the CONT SCI + SWIM and CONT SCI + SWW groups began exercising 14 days post-injury. Exercise sessions were conducted 5 consecutive days/week (Monday through Friday) for 10 weeks. Animals exercised for 30 minutes each day with 15 minutes of exercise in the morning and 15 minutes in the afternoon, separated by a minimum of 1 h. Each session consisted of three 5 min periods of exercise with breaks between the periods lasting approximately 20–25 minutes.

2.3. Tissue collection and RNA extraction

Animals were sacrificed with a ketamine overdose at 8.5, 11.5, or 13.5 weeks post-SCI, depending on condition (see Fig. 4). All uninjured animals were sacrificed at a time point equivalent to 11.5 weeks post-SCI in the other animals. These differences resulted from an effort to balance the requirements of the experimental design with the well-being of the animals. The animals undergoing a complete transection of the spinal cord required extensive care. Therefore, tissue was collected at the earliest time point, 8.5 weeks. The animals undergoing an exercise regimen required extra time for introduction to the exercise facility followed by a full 10 weeks of exercise training, resulting in tissue

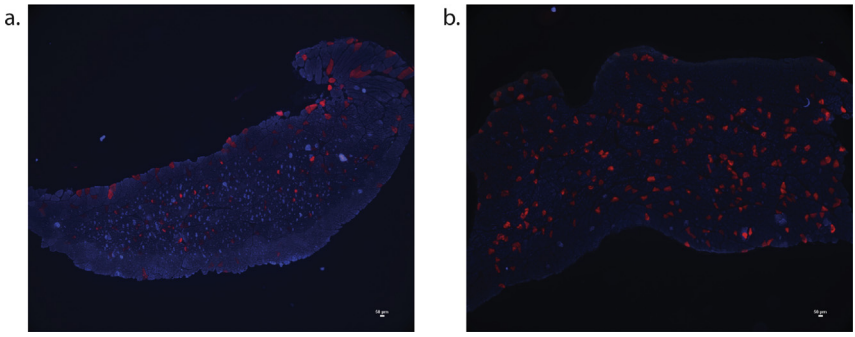

Fig. 5. Transformation of soleus muscle fibers after spinal cord injury. In an uninjured animal (a), Type I muscle fibers (blue) dominate the tissue. In an injured, unexercised animal (b), Type IIa muscle fibers (red) dominate, suggesting a muscle fiber transition from slow to fast. The scale in the lower portion of each image is 50 μm.

collection at 13.5 weeks. In all cases, tissue collection occurred well past the transition from acute to chronic SCI [16].

For all groups, tissue was collected by the same individual using the same methods. Hearts were arrested in diastole with an injection of 3 M KCl. Animals were perfused with PBS supplemented with 20% RNA*later* (Ambion, Life Technologies, Carlsbad, CA). Soleus muscle tissue was taken from each animal, and 200 mg of tissue was processed from each using RNeasy Lipid Tissue Mini Kit (Qiagen) to isolate RNA.

2.4. Immunohistochemistry

A comparison of soleus muscle tissue was made between animals with spinal cord injury and animals without injury to provide validation of phenotypical fiber changes occurring post-injury. In Fig. 5a, the soleus muscle in an uninjured animal shows the typical dominance of Type I (slow twitch, oxidative, fatigue resistant) muscle fibers in blue, whereas the soleus muscle fibers in an injured, unexercised animal in Fig. 5b, demonstrate a transition to Type IIa (fast twitch, glycolytic-oxidative, fatigable) muscle fibers in red.

2.5. Library preparation and sequencing

1 μg of total RNA samples were used for poly-A enrichment. First and second strands were synthesised followed by 3′ end adenylation. Samples were barcoded with Illumina TrueSeq adapters. 1.8 pM of barcoded library was denatured, and sequencing was performed on the University of Louisville Genomics Core Facility Illumina NextSeq 500 using the NextSeq 500 1x75 cycle High output kit (Illumina, Carlsbad, CA).

2.6. RNA-seq data analysis

Across the 20 samples, sequencing produced just over 788 million single end reads with lengths between 74 and 76 bases. The quality of the reads was assessed using FastQC v.0.10.1 [11], which indicated no sequence trimming was necessary. The sequences were directly aligned to the *Rattus norvegicus* reference genome assembly (Rn6) using Star version 2.6 [17]. Read counts for gene regions were obtained with HTSeq (version 0.10.0) [18] using Ensembl annotations [19] (Rn6 version 93). The annotation file was parsed to exclude mitochondria genes in an effort to reduce non-relevant variation in subsequent steps of the analysis. The resulting annotation file extracted read counts for 24,613 gene locations.

A principal component analysis (PCA) was performed using the R programming language package ggplot2 [20] to examine within- and between-group variability of the samples. DESeq2's variance

stabilizing transformation [21,22] was applied to the raw counts prior to PCA to reduce the effect of genes with a high degree of variability on the spread of sample points.

Prior to examining gene expression, raw read counts were normalized to remove natural variation across samples arising from differences in tissue sampling and sequencing using DESeq2's default method, relative log expression (RLE) [21–23]. UCSC Genome Browser tracks were created to facilitate exploration of gene expression in each of the samples [24]. The tracks were created using methods and available utilities described on the UCSC Genome Browser website for converting sequencing alignment files in BAM format to BigWig format.

Acknowledgments

Support for JHC and ECR was provided by P20 GM103436 (Nigel Cooper, PI). Additional support was provided by the Leona and Harry Helmsley Charitable Trust (SSO, DSKM, MH, SRW, JHC, ECR), Commonwealth of Kentucky Challenge for Excellence (DSKM, MH, SRW), Norton Healthcare (SRW), and the NIH (NS094741 to JCP). We thank the University of Louisville Center for Genetics and Molecular Medicine (CGeMM) and the University of Louisville Genomics Core Facility, in particular Wolfgang Zacharias, Sabine Waigel, and Ashley Wise for performing the high-throughput sequencing experiments. We thank Christine Yarberry, Johnny Morehouse, and Darlene Burke for surgical and behavioral analyses assistance.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] M. Liu, P. Bose, G. Walter, F. Thompson, K. Vandenborne, A longitudinal study of skeletal muscle following spinal cord injury and locomotor training, Spinal Cord 46 (2008) 488–493, https://doi.org/10.1038/sj.sc.3102169.
[2] F. Ye, C. Baligand, J.E. Keener, R. Vohra, W. Lim, A. Ruhella, et al., Hindlimb muscle morphology and function in a new atrophy model combining spinal cord injury and cast immobilization, J. Neurotrauma 30 (2013) 227–235, https://doi.org/10.1089/neu.2012.2504.
[3] M.P. Galea, S.A. Dunlop, R. Marshall, J. Clark, L. Churilov, Early exercise after spinal cord injury ('Switch-On'): study protocol for a randomised controlled trial, Trials 16 (2015) 7, https://doi.org/10.1186/1745-6215-16-7.
[4] R.K. Shields, S. Dudley-Javoroski, A.E. Littmann, Postfatigue potentiation of the paralyzed soleus muscle: evidence for adaptation with long-term electrical stimulation training, J. Appl. Physiol. 101 (2006) 556–565, https://doi.org/10.1152/japplphysiol.00099.2006.
[5] K. Higashino, T. Matsuura, K. Suganuma, K. Yukata, T. Nishisho, N. Yasui, Early changes in muscle atrophy and muscle fiber type conversion after spinal cord transection and peripheral nerve transection in rats, J. NeuroEng. Rehabil. 10 (2013) 46, https://doi.org/10.1186/1743-0003-10-46.
[6] R.J. Talmadge, R.R. Roy, V.J. Caiozzo, V.R. Edgerton, Mechanical properties of rat soleus after long-term spinal cord transection, J. Appl. Physiol. 93 (2002) 1487–1497, https://doi.org/10.1152/japplphysiol.00053.2002.
[7] F. Ye, C. Baligand, J.E. Keener, R. Vohra, W. Lim, A. Ruhella, et al., Hindlimb muscle morphology and function in a new atrophy model combining spinal cord injury and cast immobilization, J. Neurotrauma 30 (2013) 227–235, https://doi.org/10.1089/neu.2012.2504.
[8] J.H. Chariker, C. Gomes, F. Brabazon, K.A. Harman, S.S. Ohri, D.S.K. Magnuson, et al., Transcriptome of dorsal root ganglia caudal to a spinal cord injury with modulated behavioral activity, Sci. Data 6 (2019) 83, https://doi.org/10.1038/s41597-019-0088-4.
[9] J.H. Chariker, S.S. Ohri, C. Gomes, F. Brabazon, K.A. Harman, K.M. DeVeau, et al., Activity/Exercise-induced changes in the liver transcriptome after chronic spinal cord injury, Sci. Data 6 (2019) 88, https://doi.org/10.1038/s41597-019-0087-5.
[10] Spinal Cord Injury, NCBI's Gene Expression Omnibus (GEO), 2019. http://identifiers.org/geo:GSE129704. (Accessed 28 August 2019).
[11] S. Andrews, FastQC: A Quality Control Tool for High Throughput Sequence Data, 2015. http://www.bioinformatics.babraham.ac.uk/projects/fastqc/. (Accessed 28 August 2019).
[12] C.W. Fuller, L.R. Middendorf, S.A. Benner, G.M. Church, T. Harris, X. Huang, et al., The challenges of sequencing by synthesis, Nat. Biotechnol. 27 (2009) 1013–1023, https://doi.org/10.1038/nbt.1585.
[13] Gene Ontology Consortium, The Gene Ontology (GO) database and informatics resource, Nucleic Acids Res. 32 (2004) D258–D261, https://doi.org/10.1093/nar/gkh036.
[14] K.M. DeVeau, K.A. Harman, J.W. Squair, A.V. Krassioukov, D.S.K. Magnuson, C.R. West, A comparison of passive hindlimb cycling and active upper-limb exercise provides new insights into systolic dysfunction after spinal cord injury, Am. J. Physiol. Heart Circ. Physiol. 313 (2017) H861–H870, https://doi.org/10.1152/ajpheart.00046.2017.

[15] J.A. Gruner, A monitored contusion model of spinal cord injury in the rat, J. Neurotrauma 9 (1992) 123–128, https://doi.org/10.1089/neu.1992.9.123.
[16] N.D. James, K. Bartus, J. Grist, D.L.H. Bennett, S.B. McMahon, E.J. Bradbury, Conduction failure following spinal cord injury: functional and anatomical changes from acute to chronic stages, J. Neurosci. 31 (2011) 18543–18555, https://doi.org/10.1523/JNEUROSCI.4306-11.2011.
[17] A. Dobin, C.A. Davis, F. Schlesinger, J. Drenkow, C. Zaleski, S. Jha, et al., STAR: ultrafast universal RNA-seq aligner, Bioinformatics 29 (2013) 15–21, https://doi.org/10.1093/bioinformatics/bts635.
[18] S. Anders, P.T. Pyl, W. Huber, HTSeq—a Python framework to work with high-throughput sequencing data, Bioinformatics 31 (2015) 166–169, https://doi.org/10.1093/bioinformatics/btu638.
[19] P. Flicek, M.R. Amode, D. Barrell, K. Beal, K. Billis, S. Brent, et al., Ensembl 2014, Nucleic Acids Res. 42 (2013) D749–D755, https://doi.org/10.1093/nar/gkt1196.
[20] The R Foundation, The R Project for Statistical Computing, 2019. https://www.r-project.org/. (Accessed 28 August 2019).
[21] S. Anders, W. Huber, Differential expression analysis for sequence count data, Genome Biol. 11 (2010) R106, https://doi.org/10.1186/gb-2010-11-10-r106.
[22] S. Anders, D.J. McCarthy, Y. Chen, M. Okoniewski, G.K. Smyth, W. Huber, M.D. Robinson, Count-based differential expression analysis of RNA sequencing data using R and bioconductor, Nat. Protoc. 8 (2013) 1765–1786, https://doi.org/10.1038/nprot.2013.099.
[23] E. Maza, In Papyro comparison of TMM (edgeR), RLE (DESeq2), and MRN normalization methods for a simple two-conditions-without-replicates RNA-Seq experimental design, Front. Genet. 7 (2016) 164, https://doi.org/10.3389/fgene.2016.00164.
[24] W.J. Kent, C.W. Sugnet, T.S. Furey, K.M. Roskin, T.H. Pringle, A.M. Zahler, D. Haussler, The human genome browser at UCSC, Genome Res. 12 (2002) 996–1006, https://doi.org/10.1101/gr.229102.