

**The formation of deep tissue injury triggered the release of injury-related molecules on a
rat SCI-PU model**

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1 **Introduction:** The diagnosis of deep tissue injury (DTI) is currently still vague¹ with
2 only subjective methods. Tools are missing for objectively sensing the DTI under the
3 intact skin, which hampers the development of evidence-based practice for early
4 diagnosis and treatment. A number of molecules²⁻⁹ have been reported as indicating
5 muscle injury because they are released during skeletal muscle damage and
6 subsequent inflammation¹⁰. Myoglobin (MB)^{2, 6}, heart-type fatty acid binding protein (H-
7 FABP)^{2, 8-9}, myosin⁴ and troponin-I (TnI)⁶ have shown significant increase after severe
8 skeletal muscle injury, while hydroxyproline (HP) is released during collagen break
9 down in tissue damage⁹. Common biomarkers for tissue injury related inflammation
10 are creatine kinase (CK)^{7-8, 11}, and α 1-acid-glycoprotein (α 1-AGP)¹². The objective of
11 this study was to examine the concentration of these molecules in blood and/or urine
12 on a SCI-DTI rat model¹³ during DTI formation to initially assess their potential as the
13 biomarkers indicating the onset of a DTI.

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15 **Methods:** Eleven adult female Sprague-Dawley rats (age 18.6 ± 1.7 weeks, weight
16 316.1 ± 18.8 g) were used after the approval by the IACUC of Northwestern University.
17 The surgical procedures and leg muscle loading protocol for the rat SCI-DTI model
18 were previously reported¹³. In addition, a cyclic loading protocol was used for one rat,
19 including leg muscle compression for 12h of 300mmHg followed by 12h release for 2
20 days, and the 3rd day as 12h of 200mmHg.

21 Blood and urine samples were collected before (baseline) and 12, 24, 36, 48, 72, 120
22 and 168 hours post compression to generate temporal profiles for concentration of
23 these molecules along the process of injury. Enzyme-linked immunosorbent assays
24 were used to measure concentration in serum and/or urine for MB, myosin, TnI, H-
25 FABP, α 1-AGP, HP, and CK.

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1 **Results:** All the tested molecules showed a substantial elevation of concentration in
2 serum and urine, apparently in response to the pressure injury (Fig. 1). The release
3 and clearance of 5 tested molecules in serum demonstrated relatively consistent
4 patterns. MB and H-FABP had the peak values at 24.0 ± 0.0 h post pressure application,
5 while myosin, TnI and $\alpha 1$ -AGP had their peaks at 40.0 ± 13.9 h, 44.0 ± 6.9 h and
6 60.0 ± 17.0 h post compression (Fig. 1 Top: left), respectively. The highest peak was
7 found in MB (1165.9 ± 83.0 ng/mL), while the lowest peak was found in H-FABP
8 (36.3 ± 10.8 ng/mL). The clearance of the molecules was the fastest for MB (18.0 ± 6.9 h)
9 and the slowest for $\alpha 1$ -AGP (118.0 ± 2.8 h) after the peak point.

10 The concentration of 3 molecules in urine for 7 rats is given in Fig. 1 (Top: right). A
11 change from 1.7 ± 0.7 ng/mL to 502.8 ± 435.7 ng/mL was found for MB, while the
12 elevation of urine HP was substantially less at 105.2 ± 43.9 ng/mL. Unlike the almost
13 unanimous pattern of the molecules in serum, elevation of MB concentration in urine
14 differed between individual rats on magnitude, as well as on the timing of the elevation,
15 possibly in relation to variation on renal function among individual animals to clear
16 these molecules. Histological analysis confirmed the massive deep muscle injury
17 induced by the compression as seen in previous publication¹³.

18 For the repeatedly loaded animal, each re-applied pressure triggered subsequent
19 release of molecules, and their final clearance took apparently longer time than that of
20 the single compression protocol.

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22 **DISCUSSIONS:** This study initially obtained the serum/urine concentration profile of
23 the tested molecules associated with the pressure induced DTI on a rat model. These
24 profiles demonstrated the sensitive response of these molecules to muscle injury in the
25 formation of a DTI. The rapid increase of the concentration of these molecules at the
26 time of compressive injury may provide the indication of a severe deep muscle injury.

1 The compression used in this experiment has previously been shown to cause DTI
2 and resultant PU formation¹³, therefore, the significant changes in these molecules in
3 the current study due to compression may be attributed to muscle tissue damage. On
4 the other hand, due to the inconsistent nature of the urinal concentrations of the tested
5 molecules compared to that of the serum concentrations, we speculated that serum
6 tests may be easier for early detection of a DTI since the output of these molecules in
7 urine would be dependent on renal activity. Conversely, urine samples can be
8 normalized by measuring renal output in future studies and the longer elevation time
9 may provide advantages in clinical application such as reduction in test frequency and
10 the ease to collect samples. Overall, based on the findings from this study, we can
11 surmise that these molecules present a potential for being the indicators for DTI and
12 PU formation in healthcare of SCI individuals.

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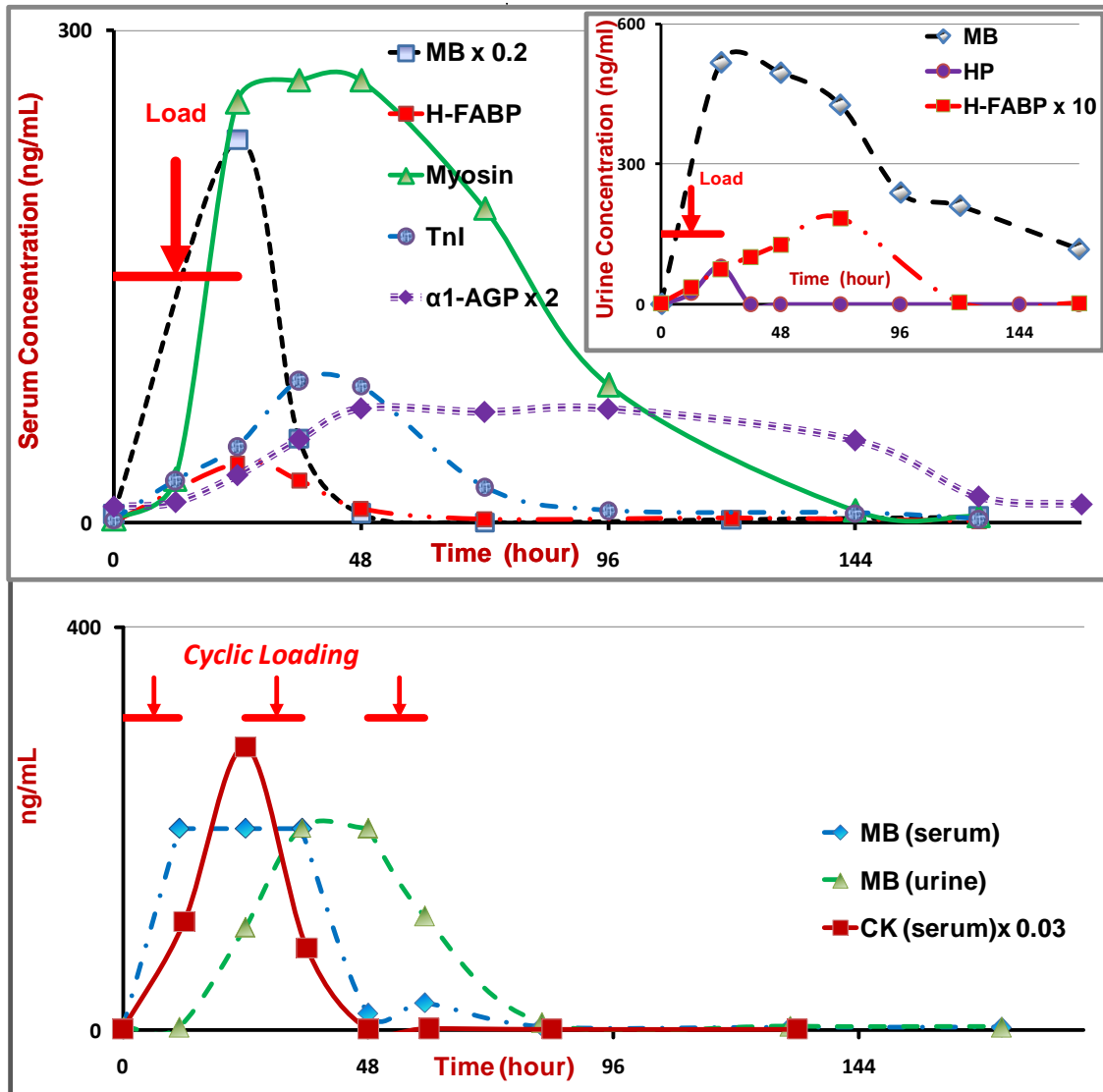
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1 Figure



2

3 **Fig. 1.** Top: The changes of myoglobin (MB), heart-type fatty acid binding protein (H-
4 FABP), myosin, troponin-I (Tnl) and α 1-acid-glycoprotein (α 1-AGP) in serum (left) and
5 changes of MB, H-FABP and hydroxyproline (HP) in urine (right) under a single loading
6 protocol (24hours, 400mmHg); Bottom: The changes of MB in both serum and urine,
7 and creatine kinase (CK) in serum were given for a cyclic loading protocol (12h of
8 300mmHg followed by 12h release for 2 days, and the 3rd day as 12h of 200mmHg).
9 Blood and urine samples were collected before (baseline) and 12, 24, 36, 48, 72, 120
10 and 168 hours post compression along the process of compression injury.

1 **Figure Legends:**

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3 **Fig. 1.** Top: The changes of myoglobin (MB), heart-type fatty acid binding protein (H-
4 FABP), myosin, troponin-I (TnI) and α 1-acid-glycoprotein (α 1-AGP) in serum (left) and
5 changes of MB, H-FABP and hydropoxyline (HP) in urine (right) under a single loading
6 protocol (24hours, 400mmHg); Bottom: The changes of MB in both serum and urine,
7 and creatine kinase (CK) in serum were given for a cyclic loading protocol (12h of
8 300mmHg followed by 12h release for 2 days, and the 3rd day as 12h of 200mmHg).
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