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Data Article

American locust (*Schistocerca americana*) post-exercise lactate fate dataset

Scott D. Kirkton*, Samantha K. Tyler

Department of Biological Sciences, Union College, Schenectady 12308, NY, United States

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ABSTRACT

The fate of lactate after exercise varies between animal groups. In ectothermic vertebrates, lactate is primarily converted to glycogen in the muscle. In mammals, lactate is intramuscularly oxidized or converted to glycogen in the muscle and/or liver. In addition, the rate at which post-exercise lactate pools are depleted varies between taxa. Grasshoppers are unique among insects in that they produce lactic acid during locomotion. However, it is unclear where the lactate is processed. Furthermore, insects have extremely efficient oxygen delivery systems which may enhance lactate removal compared to an ectothermic vertebrate. We fluorometrically measured lactate levels in the American locust (*Schistocerca americana*) during recovery from jumping. Male grasshoppers were forced to jump for five minutes in a large gloved box at 35 °C. After jumping, individuals were either immediately sampled for lactate or allowed to recover in insolation at 35 °C for different time periods (15, 30, or 60 min) before being processed for lactate. Lactate was measured in the hemolymph, jumping muscle, and abdomen of each grasshopper. A control group was isolated overnight but not jumped to provide pre-jumping lactate levels. During recovery, hemolymph and abdominal lactate levels remain virtually the same whereas muscle lactate levels decrease, suggesting that grasshoppers process lactate intramuscularly. Compared to mammals, ectothermic vertebrates have higher intramuscular lactate and lower blood lactate values after exhaustive activity [reviewed in 1]. Furthermore,

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* Corresponding author.
E-mail address: kirktons@union.edu (S.D. Kirkton).

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small mammals process and remove most blood lactate in approximately 20 min, while ectothermic vertebrates require multiple hours to recover [1]. Since grasshoppers have significantly more lactate in their muscle than hemolymph and their hemolymph lactates remain elevated at least one hour after recovery, grasshopper post-exercise lactate metabolism is more similar to ectothermic vertebrates than mammals.

Specifications Table

Subject	Animal Physiology
Specific subject area	Comparative locomotory physiology of insects
Type of data	Graph
How data were acquired	Molecular Devices Spectramax M3 microplate reader
Data format	Analyzed Means ± SEM
Parameters for data collection	Male *Schistocerca americana* grasshoppers were forced to jump for five minutes and either immediately processed for lactate measurements or allowed to recover for 15, 30, or 60 min before being processed for lactate.
Description of data collection	Jumping muscle, abdomen, and haemolymph lactate measurements
Data source location	Union College Schenectady, New York United States of America
Data accessibility	With the article
Related research article	S. D. Kirkton, A. A. Yazdani, Chronic electrical stimulation reduces reliance on anaerobic metabolism in locust jumping muscle, Comparative Biochemistry and Physiology, Part A. https://doi.org/10.1016/j.cbpa.2021.110954

Value of the Data

- These data provide insight into post-exercise lactate metabolism of an insect.
- These data allow comparative physiologists to compare anaerobic metabolism recovery of grasshoppers with well-known vertebrate data.
- These data provide a starting point for further analyses of lactate processing mechanisms in insects.
- These data may assist a variety of ecological and behavioral researchers studying agriculturally relevant plague species to better understand locust locomotory performance and muscle physiology.

1. Data Description

Male *S. americana* grasshoppers were isolated overnight in a 35 °C container. The next morning, individuals were forced to jump for five minutes in a large gloved box at 35 °C. After jumping, individuals were either immediately sampled for lactate or allowed to recover in insolation at 35 °C for different time periods (15, 30, or 60 min) before being processed for lactate. Lactate was measured in the hemolymph, jumping muscle, and abdomen of each grasshopper (Table 1). A repeated measures ANOVA showed that there were significant effects of recovery time ($F_{4,35}$=8.15, $p < 0.001$), location in the body ($F_{2,34}$=80.0, $p < 0.001$), and the interaction of location*recovery time ($F_{8,68}$=3.13, $p < 0.005$). During sixty minutes of recovery, muscle lactate levels decreased from 9.6 to 4.7 umol/g, while hemolymph and abdominal lactate levels were relatively unchanged. This decrease suggests that grasshoppers may process lactate intramuscularly rather than release it into the hemolymph to be processed in other tissues.

Table 1
Lactate values measured in the jumping muscle, hemolymph, and abdomen at rest and at different time points during recovery after five minutes of exhaustive jumping.

	Individual	Muscle Lactate (umol/g^{-1})	Hemolymph Lactate (umol/ml^{-1})	Abdomen Lactate (umol/g^{-1})
Rest	1	3.29	0.88	0.78
Rest	2	0	0	0
Rest	3	4.95	0	0.75
Rest	4	1.32	0.14	0.35
Rest	5	0	0	0
Rest	6	0.63	0.48	0.82
Rest	7	4.34	0.45	0.93
Rest	8	2.07	0.42	0.85
Rest	**Mean ± SEM**	**2.07 ± 0.68**	**0.56 ± 0.14**	**0.56 ± 0.14**
Recovery Time after 5 Min of Exhaustive Jumping				
0 min	9	6.99	0.98	0.78
0 min	10	6.68	0.36	0.86
0 min	11	12.93	0	1.52
0 min	12	10.03	0.24	0.64
0 min	13	7.86	0.22	0.40
0 min	14	13.93	0.64	1.04
0 min	15	9.05	0.52	0.83
0 min	16	9.51	0.49	0.69
0 min	**Mean ± SEM**	**9.62 ± 0.93**	**0.43 ± 0.11**	**0.85 ± 0.12**
15 min	17	3.93	0.79	0.76
15 min	18	4.93	0.44	0.88
15 min	19	10.13	0	0.97
15 min	20	7.73	0.20	0.92
15 min	21	6.14	0	0.36
15 min	22	8.16	0.15	0.46
15 min	23	9.33	0.22	0.49
15 min	24	7.09	0.13	0.72
15 min	**Mean ± SEM**	**7.18 ± 0.75**	**0.24 ± 0.09**	**0.70 ± 0.08**
30 min	25	2.88	0.78	1.04
30 min	26	4.25	0.46	0.90
30 min	27	13.39	0.17	0.64
30 min	28	0	0	0.46
30 min	29	10.36	0.30	0.62
30 min	30	6.96	0.26	0.92
30 min	31	7.47	0.14	0.89
30 min	32	7.98	0.21	0.62
30 min	**Mean ± SEM**	**6.66 ± 1.50**	**0.29 ± 0.08**	**0.76 ± 0.07**
60 min	33	6.48	0	0.62
60 min	34	1.66	0.33	0.83
60 min	35	4.50	0.16	0.43
60 min	36	9.92	0.21	0.54
60 min	37	5.82	0.18	0.55
60 min	38	0.56	0.15	0.56
60 min	39	6.52	0.29	0.52
60 min	40	2.52	0.19	0.52
60 min	**Mean ± SEM**	**4.75 ± 1.09**	**0.19 ± 0.04**	**0.57 ± 0.04**

Even though muscle lactate levels decreased during recovery, the jumping muscle still accounted for the majority of lactate measured during recovery (Fig. 1). Immediately after jumping, 88.3% of all the measured lactate was in the muscle. After sixty minutes of recovery, muscle

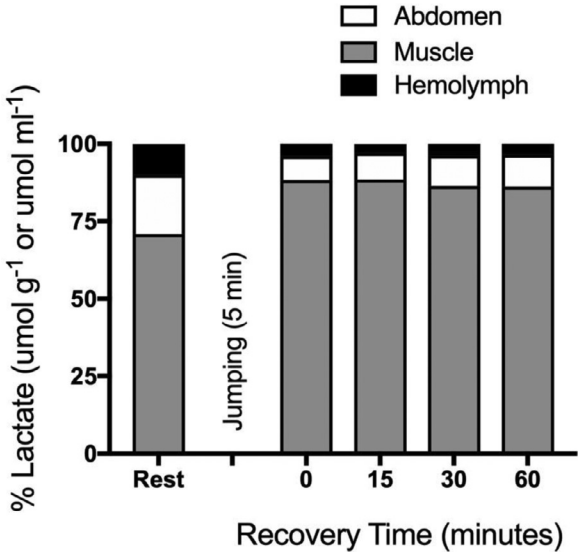

Fig. 1. Relative proportion of lactate measured in each body part (jumping muscle, hemolymph, and abdomen) of *S. americana* at rest and during recovery from five minutes of jumping. Immediately after jumping, the muscle had over 88.3% of the total lactate measured while the hemolymph (4.0%) and abdomen (7.8%) were much less significant. Even though muscle lactate decreases during recovery, the jumping muscles still had over 86.2% of all the measured lactate (hemolymph = 3.4%; abdomen = 10.4%) at the sixty-minute recovery point. Each group has a sample size of $n = 8$.

lactate still accounted for 86.2% of all measured lactate (Fig. 1). It should be noted that due to the handling necessary to remove the hemolymph, the pre-jumping values in the femur are higher than normally measured for resting grasshoppers (approximately 0 umol g^{-1} [2]).

When compared to vertebrate studies of lactate accumulation and recovery [reviewed in 1], grasshoppers show similar trends to ectothermic vertebrates (Fig. 2). After 2–10 min of maximal activity, ectothermic vertebrates have a greater proportion of lactate in their muscle than in their blood. Muscle lactate ranges from an average 83% in fish muscle [3–7] to an average of 63% in reptile muscle [8,9]. In contrast, small mammals have a greater proportion of their lactate found in their blood immediately following exhaustive exercise (averaging 63% in blood and only 37% in muscle [10,11]). Grasshoppers (this study) are similar to ectothermic vertebrates in having the majority of their lactate immediately following activity be located in the muscles (Fig. 2).

2. Experimental Design, Materials and Methods

Grasshoppers were reared in a colony at Union College. We selected adult male grasshoppers, recorded their body mass and removed their wings. The grasshoppers were isolated overnight in a 35 °C incubator. Grasshoppers were separated into groups based on post-jumping rest time (0, 15, 30, and 60 min; $n = 8$ for each group). These experimental groups were compared to a control group of grasshoppers that did not jump ($n = 8$). Each experimental grasshopper was placed in a 35 °C jumping chamber and manually stimulated with a paintbrush to continuously jump for 5 min.

At the designated time, the tissues and hemolymph were processed for lactate. First, hemolymph was sampled from a small incision between the head and thorax. A micropipette was used to extract approximately 15uL of hemolymph that was then diluted 1:10 with 0.6 M perchloric acid and kept on ice. This process took approximately one minute. The body of the grasshopper was then immersed in liquid nitrogen.

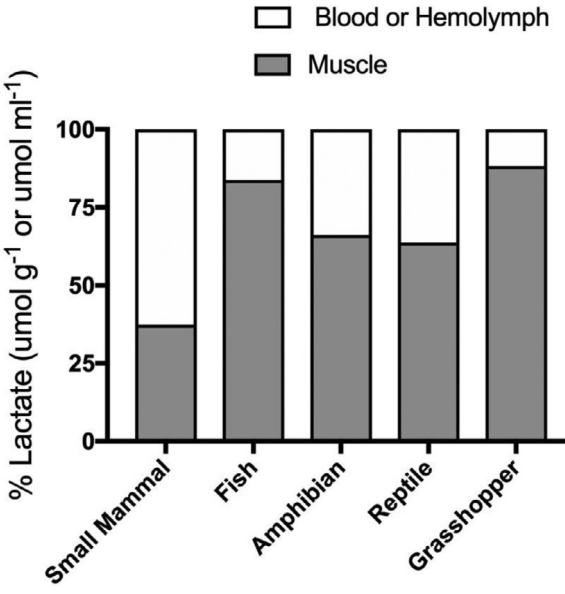

Fig. 2. Relative proportion of lactate measured in both the muscle and blood/hemolymph following 2–10 min of exhaustive activity varies with animal group [vertebrates reviewed in 1]. Ectothermic vertebrates had approximately twice the proportion of lactate found in their muscles (63–83%) after activity than smaller mammals (37%). Smaller mammals had a much higher proportion of their lactate found in the blood. Grasshoppers show trends similar to ectothermic vertebrates. Mean values were created from data that include small mammals (mice [10] and rat [11]); fish (rainbow trout [3], winter flounder [4], flathead sole [5], sea raven [6], skipjack tuna [7]); amphibians (leopard frog [12], bufonid toad [13]); and reptiles (green iguana [8], desert iguana [9]).

To prepare the tissues for sampling, the metathoracic femur was separated from the thorax and weighed. The abdomen was isolated and weighed as well. Each tissue was diluted 1:10 with 0.6 M $HClO_4$ and homogenized on ice using a motorized glass ground homogenizer.

The supernatant was transferred into a microcentrifuge tube and centrifuged for 5 min at 15,850 *g*. The resultant supernatant was transferred to clean tubes and diluted with KOH-MOPS (225 uL KOH-MOPS per 1 mL supernatant). This dilution was applied to the hemolymph-$HClO_4$ solution as well. These solutions were centrifuged for 1 min to separate the precipitate from the supernatant. Additional KOH-MOPS was then added to neutralize the solutions.

The supernatant was removed for a fluorometric assay [2] modeled after Passoneau and Lowry [14]. The lactate assay was conducted using a 96-well microplate (Greiner Bio-One, Monroe, NC). Each well was loaded with 20 µL of sample, 100 µL of NAD^+-tris-hydrazine buffer solution, and 80 µL of an LDH-water solution. The NAD^+ to NADH reduction was fluorometrically measured using a Molecular Devices Spectramax M3 microplate reader set to an excitation wavelength of 360 nm and an emission wavelength of 460 nm. Each standard or sample was run six times (three times with LDH and three times without LDH) to account for non-specific binding. Using a standard curve of known lactate concentrations, fluorescence values were converted into millimoles of lactate per kilogram of tissue [2]. The assay detection limit was 0.1 mM kg^{-1} of tissue. The data were analyzed using repeated measures ANOVA in JMP statistical software.

Ethics Statement

None necessary for insects.

CRediT Author Statement

All aspects of this project were shared equally.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships which have or could be perceived to have influenced the work reported in this article.

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