Supplemental Material

Sensibaugh *et al.*

Undergraduate performance in solving
ill-defined biochemistry problems

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The Lorrat

Hypothesize

During one of your many hikes on top of Sandia Mountain (10,600 ft.), you come across a rapidly scurrying mammal that you have never seen before. Intrigued, you ask the folks at the Southwestern Museum of Biology what is known about the little animal. They tell you that this furry high-altitude creature, the lorrat, has been well-studied and is extremely unusual in its physiology for several reasons:

- It is an incredible athlete; it can run for hours without tiring.
- It is able to remain slim (reduction in adipose tissue) even when fed large portions of high-fat food in captivity.
- It stays active all winter, and its body temperature remains high even during its sleeping hours, even during very cold periods, which is unlike other small mammals.

While the lorrat's physiological abilities are well-known, the biochemical mechanisms for these have not yet been studied. This is where you come in…

What are your <u>BIOCHEMICAL</u> hypotheses to explain the Lorrat's unique physiological abilities?

Literature

Field researchers have not observed any unusual changes in the lorrat population over the past three years, either in numbers or in habitat. Lorrats are typically awake for about 20 hours daily, and are extremely active during most of that time. The lorrat's diet consists mainly of pinon seeds and small insects, for which it forages constantly. Therefore, lorrats must continually change their feeding location, leaving little food for other small mammals.

Before designing experiments to test your hypothesis, you search the literature about lorrat metabolism, and find that it focuses on phosphoenolpyruvate carboxykinase (PEPCK). The PEPCK enzyme has a role in carbohydrate, lipid, and protein metabolism, as shown in the exhibit below.
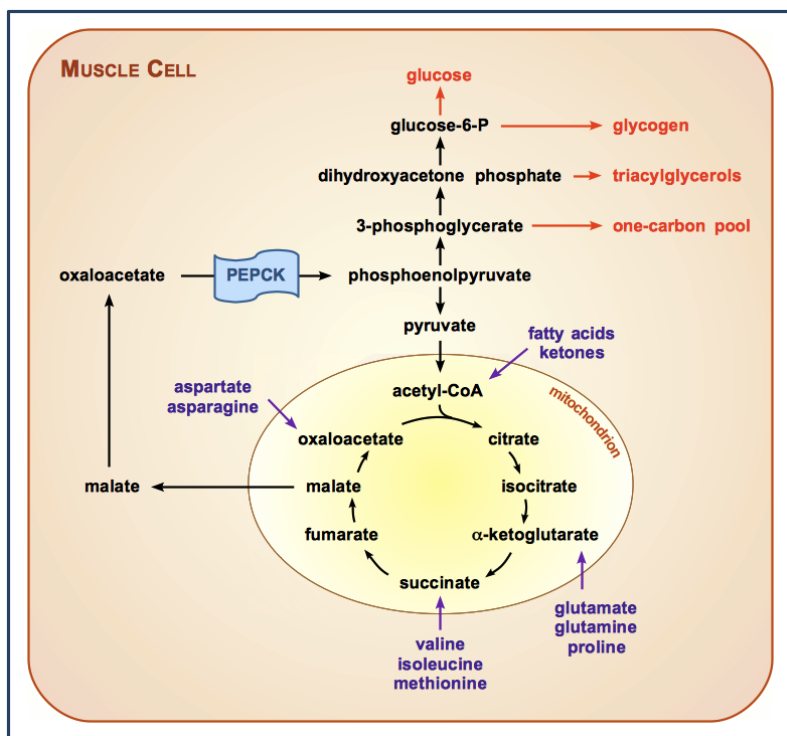
Adapted from Yang *et al.* (2009) *J BiolChem*, *284*(40), 27025-27029.

Previous studies have demonstrated greater muscle PEPCK activity in lorrats than in other small mammals.

Together with other evidence, the literature suggests that the unique physiological abilities of the lorrat may be the result of PEPCK upregulation, which could be due to a number of factors such as gene copy number, transcription, translation, enzyme activity, or other regulatory mechanisms.

Investigate

A staff member at the Southwestern Museum of Biology has introduced you to the field researchers. They have maintained their stock of lorrat tissue samples, and are excited to collaborate with you during your investigation. However, they will only provide the samples; you must design the experiments.

Given the hypothesis that **PEPCK is upregulated in lorrat muscle at the transcriptional level**, **briefly describe your proposed experimental design, with appropriate controls, to test this hypothesis. DO NOT simply name a technique, but rather explain the reasoning for your design and how the methods will provide supportive evidence.**

Evaluate

To lend support for the PEPCK transcriptional upregulation hypothesis, muscle tissues from both the lorrat and the common laboratory rat were first analyzed to establish preliminary indicators of increased PEPCK transcription and activity. Three characteristic parameters were compared: enzyme concentration, enzyme activity, and the Michaelis constant (Km).

Following homogenization and centrifugation of the tissue samples, the soluble fractions of each sample were used for enzyme analyses. Two different enzymes in the supernatant were assayed, PEPCK and aldolase.

Enzyme concentration was determined using a competitive inhibition enzyme immune assay. Enzyme activity was determined using standard substrates for the two different enzymes, with a unit of activity defined as micromoles of product formed per minute. Concentration and activity values were normalized per gram of muscle tissue used in the initial homogenate. The Km values were determined using standard Michaelis-Menten kinetics.

The results of these tests are shown below.

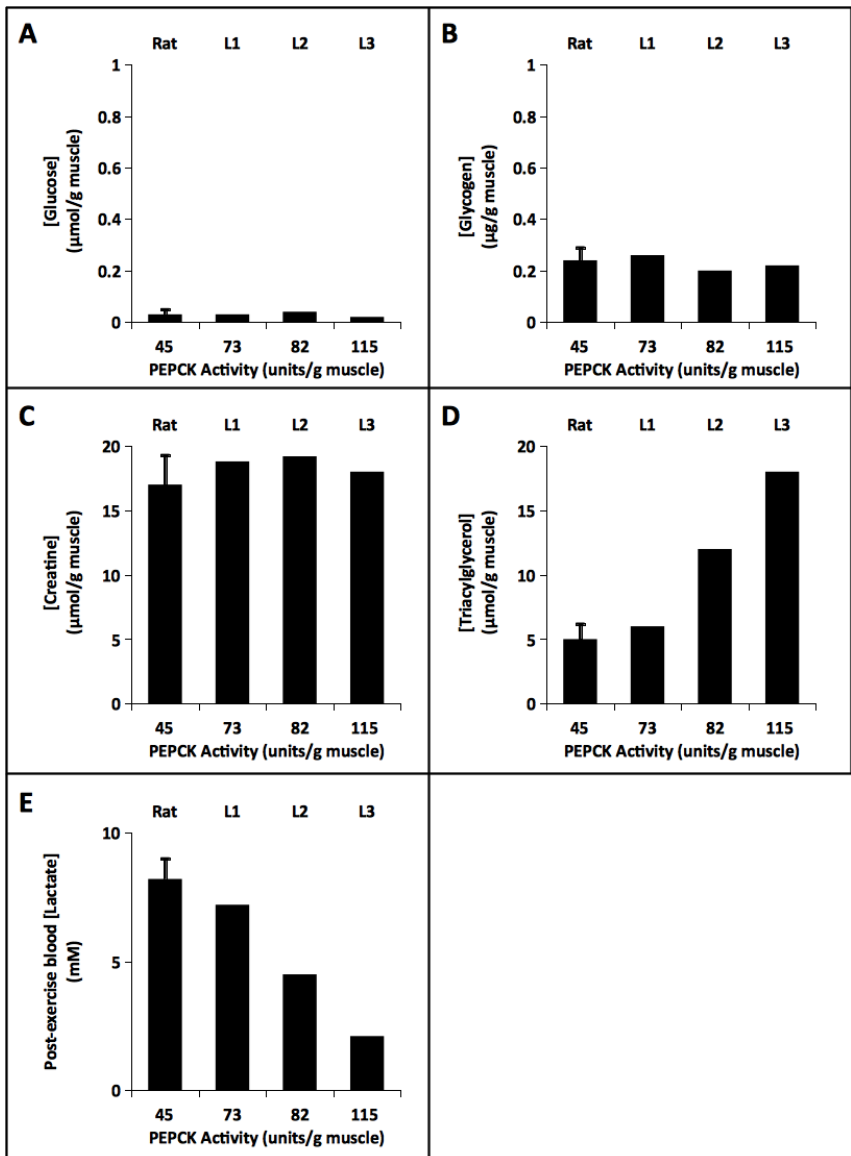| Protein Characteristic	Rat	Lorrat
[Aldolase] (ng/g tissue)	12	12
Aldolase activity (units/g tissue)	40	41
Aldolase Km (mM)	0.062	0.061
[PEPCK] (ng/g tissue)	15	30
PEPCK activity (units/g tissue)	45	90
PEPCK Km (mM)	0.012	0.013

How do the parameters of interest compare across the two species?

Integrate

The previous data confirmed increased expression of PEPCK in the lorrat muscle as compared to the white laboratory rat, with an equivalent increase in enzyme activity but without any alteration of Km. To further probe whether this increased expression of PEPCK might be responsible for the unusual physiology of the lorrat, several common metabolites were quantified.

To investigate the metabolism of the lorrat, ten rat biopsies and three lorrat biopsies (L1, L2, and L3) of muscle tissue were evaluated for concentrations of glucose (A), glycogen (B), creatine (C), and triacylglycerol (D). Blood samples were also taken after 30 minutes of exercise to measure lactate levels (E). Means and standard deviations were calculated for the rat measurements. The PEPCK activities for the samples were measured as previously described.

The data are reported in the five panels of the exhibit below.

A — [Glucose] (µmol/g muscle) vs PEPCK Activity (units/g muscle): Rat (45), L1 (73), L2 (82), L3 (115)

B — [Glycogen] (µg/g muscle) vs PEPCK Activity (units/g muscle): Rat (45), L1 (73), L2 (82), L3 (115)

C — [Creatine] (µmol/g muscle) vs PEPCK Activity (units/g muscle): Rat (45), L1 (73), L2 (82), L3 (115)

D — [Triacylglycerol] (µmol/g muscle) vs PEPCK Activity (units/g muscle): Rat (45), L1 (73), L2 (82), L3 (115)

E — Post-exercise blood [Lactate] (mM) vs PEPCK Activity (units/g muscle): Rat (45), L1 (73), L2 (82), L3 (115)

How do you interpret the data collected throughout this investigation, to explain any role that PEPCK might have in the lorrat achieving its unique physiological abilities?

Be sure to address the results of the previous protein assays, these new metabolic assays, and any other relevant information.

Reflect

After graduating with your biochemistry degree, you take another enjoyable hike in the Sandia Mountains (secretly hoping to see another lorrat). You recall all that you learned about the lorrat, especially the savory moment when you were the first person to discover its increased levels of muscle PEPCK.

Though PEPCK has been previously described as primarily a gluconeogenic enzyme, in the lorrat muscle it acts mainly to generate high levels of DHAP. This leads to high levels of muscle triacylglycerols, which are then used by the muscle as fuel for aerobic catabolism, allowing the lorrat to run for a long time, while generating little to no lactate.

Your mind wanders back and forth between the lorrat itself and the investigation. Looking back, please answer the following questions:

1) Were you able to meet each of the tasks required in this case?

2) What aspects of your undergraduate education helped you the most for solving this case?

3) In one sentence or less, describe any personal relevance of working through this case study.

Scoring Rubrics: The Lorrat

	What are your top four <u>biochemical</u> hypotheses to explain the Lorrat's unique physiological abilities?	
Hypothesize Domain	10	Three hypotheses with rationales
	9	Three hypotheses
	8	Two hypotheses with rationales
	7	Two hypotheses about the following: Altered genetics/genetic processing of metabolic proteins Altered regulation of body temperature Altered cellular structure (more mitochondria) Oxygen transport/delivery (lung capacity, Hb, Mb, BPG) Nutritional deficiency Environment (infection or toxin) Trauma Cancer Autoimmune
	6	One hypothesis with rationale
	5	One hypothesis
	4	Unacceptable hypotheses: Teleological conceptions (outcomes) Increased/fast metabolism Increased energy needed for proliferation
	3	Pattern-matching
	2	Restating the case/problem (something functions differently)
	1	Off-topic
	0	No response

	Briefly describe your proposed experimental design, with appropriate controls, to test this hypothesis. DO NOT simply name a technique, but rather explain the reasoning for your design and how the methods will provide supportive evidence.	
Investigate Domain	10	As for 7, with three of the below
	9	As for 7, with two of the below
	8	As for 7, with one of the below: rationale = to detect transcription expected results interpretation of expected results
	7	All four of the following: Method Quantitative RT-PCR, luciferase/beta-gal reporter assay, electrophoresis/northern blot, hybridization techniques (cDNA microarray measuring hybridization of mRNA is theoretically logical) (Negative) Control – small mammal reference sample IV - differences in transcription DV – PEPCK mRNA in muscle tissue
	6	One of the above missing/incorrect
	5	Two of the above missing/incorrect
	4	Three of the above missing/incorrect
	3	Four of the above incorrect
	2	Proposal is not aligned with hypothesis (kinetics)
	1	Off-topic

	0	No response

	How do the parameters of interest compare across the two species?	
Evaluate Domain	10	As in 7, with specific activity AND value of aldolase control
	9	As in 7, with specific activity OR value of aldolase
	8	As in 7, with specific activity (same)
		OR value of aldolase (validity of result), but incorrect or vague
	7	All of the following:
		For Lorrat compared to control
		Increased [PEPCK]
		Increased PEPCK activity
		Equivalent Km
	6	One of the above missing/incorrect
	5	Two of the above missing/incorrect
	4	Three of the above missing/incorrect
	3	Four of the above incorrect
	2	Explain methods
	1	Off-topic
	0	No response

	How do you interpret the data collected throughout this investigation, to explain any role that PEPCK might have in the lorrat achieving its unique physiological abilities? Be sure to address the results of the previous protein assays, these new metabolic assays, and any other relevant information.	
Integrate Domain	10	All eight of the following:
		Increased muscle PEPCK expression/concentration
		$\rightarrow$ high PEPCK activity
		$\rightarrow$ high DHAP (some reference to glycerol)
		$\rightarrow$ high TAGs
		$\rightarrow$ aerobic catabolism
		$\rightarrow$ (a) low lactate
		$\rightarrow$ (b) high ATP yield
		$\rightarrow$ unique abilities
	9	Seven of the above
	8	Six of the above
	7	Five of the above
	6	Four of the above
	5	Three of the above
	4	Two of the above
	3	One of the above
	2	Incorrect interpretation
	1	Off-topic
	0	No response

Reflect Domain		Looking back, please answer the following questions: 1) Were you able to meet each of the tasks required in this case? 2) What aspects of your undergraduate education helped you the most for solving this case? 3) In one sentence or less, describe any personal relevance of working through this case study.
	10	As for 7, with three of the below
	9	As for 7, with two of the below

	8	As for 7, with one of the below:
		    Self-assessment is accurate
		    Describe method for improvement
		    Helped learn process not just facts

	7	Addressed all three of the following:
		    Self-assessment (do not accept "I hope so.")
		    Most helpful program aspect
		    Personal relevance is helped learn content, saw improvement over time, need for
		        future profession, etc.(it counts as long as it's addressed)

	6	Two of the above
	5	One of the above
	4	
	3	
	2	
	1	Off-topic
	0	No response

Qualitative Content Analysis Codes

Coded Segments	Prevalence
Table S1. Characterization of unsatisfactory responses for the Hypothesize domain	
Acceptable Segments	
Mechanistic hypotheses	
Oxygen transport or delivery	13%
Altered genetics or genetic processing of metabolic proteins	9%
Altered cellular structure	6%
Altered regulation of body temperature	2%
Nutritional supplement	1%
Total acceptable segments	31%
Unacceptable Segments	
Unmechanistic hypotheses	
Increased or fast metabolism	29%
Vague mechanism of lipid metabolism	16%
Vague mechanism of carbohydrate or CAC metabolism	4%
Inconsistent with given information	9%
Teleological conceptions	
Adapted to environment	4%
Efficient energy use	2%
Proliferation	1%
Other unacceptable hypothesis	5%
Total unacceptable segments	69%
(n responses = 39; n segments = 114; Values are rounded)	

Table S2. Characterization of unsatisfactory responses for the Investigate domain

Coded Segments	Prevalence
Acceptable Segments	
Experimental designaligns with hypothesis	
Appropriate dependent variable	8%
Appropriate method	7%
Appropriate negative control	6%
Appropriate independent variable	5%
Appropriate rationale	5%
Statement of expected results	3%
Interpretation of expected results	1%
Total acceptable segments	34%
Unacceptable Segments	
Experimental design does not align with hypothesis	
Inappropriate method	
Enzyme kinetics/activity	21%
Protein quantification	17%
Other	9%
Cell biology method	5%
Dietary induction	3%
Inappropriate negative control	7%
Inappropriatedependent variable	4%
Inappropriateindependent variable	1%
Total unacceptable segments	66%
(n responses = 39; n segments = 108; Values are rounded)	

Table S3. Characterization of unsatisfactory responses for the Evaluate domain

Coded Segments	Prevalence
Acceptable Segments	
Correct results	
Increased PEPCK activity	16%
Comparing Lorrat to control	15%
Increased PEPCK concentration	12%
Equivalent Km	7%
Specific activity is the same	3%
Importance of aldolase is to validate results	2%
Total acceptable segments	56%
Unacceptable Segments	
Extending response beyond Evaluate	
Addressing the Integrate domain	19%
Addressing the Hypothesize domain	4%
Incorrect results	
Incorrect/vague importance of aldolase	10%
Incorrect PEPCK Km	9%
Incorrect PEPCK concentration	1%
Explain methods	1%
Total unacceptable segments	44%

(n responses = 19; n segments = 99; Values are rounded)

Table S4. Characterization of unsatisfactory responses for the Integrate domain

Coded Segments	Prevalence
Acceptable Segments	
Plausible conclusions	
Low lactate due to aerobic catabolism	10%
Unique abilities arise from molecular phenomena	10%
Glucose, glycogen, and/or creatine conclusion(s)	4%
High ATP yield from aerobic catabolism	3%
Aerobic catabolism is occurring	1%
Correct results	
High triacylglycerol levels	17%
Glucose, glycogen, and/or creatine result(s)	9%
High PEPCK activity	6%
Increased muscle PEPCK expression/concentration	5%
High DHAP levels, or reference to glycerol	2%
Broad introductory statement	8%
Total acceptable segments	77%
Unacceptable Segments	
Unsubstantiated or incorrect conclusions	
Low lactate indicates lactate is being catabolized	4%
High TAG indicates fatty acids are converted into pyruvate	3%
High TAG indicates fatty acids are not being catabolized	2%
High TAG indicates altered fatty acid transport	2%
High TAG indicates dietary induction of PEPCK	1%
Results indicate metabolites affect PEPCK activity	3%
Results support the student's initial hypothesis	2%
Incorrect results	4%
Vague results	2%
Total unacceptable segments	23%

(n responses = 21; n segments = 98; Values are rounded)

Table S5. Characterization of unsatisfactory responses for the Reflect domain

Coded Segments	Prevalence
Acceptable Segments	
Incomplete response	
Addressed part 2 - most helpful program aspect	42%
Addressed part 1 - self-assessment	38%
Self-assessment is accurate	8%
Addressed part 3 - personal relevance	4%
Helped learn process not just facts	4%
Total acceptable segments	96%
Unacceptable Segments	
Thoughtless self-assessment	4%
Total unacceptable segments	4%

(n responses = 10; n segments = 24; Values are rounded)