Lactate and glucose metabolism in mouse (Mus musculus) and reptile (Anolis carolinensis) skeletal muscle
S. J. Wickler and T. T. Gleeson

You might find this additional information useful...

Medline items on this article's topics can be found at http://highwire.stanford.edu/lists/artbytopic.dtl
on the following topics:
  Biochemistry .. Metabolism
  Biochemistry .. Glycogen
  Biochemistry .. Glucose Metabolism
  Physiology .. Lacertilia
  Physiology .. Mammalia
  Physiology .. Mice

Additional material and information about American Journal of Physiology - Regulatory, Integrative and Comparative Physiology can be found at:
http://www.the-aps.org/publications/ajpregu

This information is current as of March 16, 2005.
Lactate and glucose metabolism in mouse (Mus musculus) and reptile (Anolis carolinensis) skeletal muscle

STEVEN J. WICKLER AND TODD T. GLEESON
Department of Animal and Veterinary Science, California State Polytechnic University, Pomona, California 91768; and Department of Environmental, Population, and Organismic Biology, University of Colorado, Boulder, Colorado 80309

Wickler, Steven J., and Todd T. Gleeson. Lactate and glucose metabolism in mouse (Mus musculus) and reptile (Anolis carolinensis) skeletal muscle. Am. J. Physiol. 264 (Regulatory Integrative Comp. Physiol. 33): R487-R491, 1993.—The reliance on anaerobic metabolism during exercise has been the subject of a growing body of literature in activity metabolism. Prior studies have demonstrated that lizards rely more on postexercise lactate to regenerate depleted glycogen stores than do many mammals. These studies prompted an in vitro comparison between the metabolic mechanisms for the handling of lactate and glucose in the muscles of a small mammal and lizard. Hindlimb muscles of Mus and Anolis were stimulated to fatigue and then incubated in the presence of 15 mM lactate and either 5.5 (mice) or 8.5 (anoles) mM glucose. Oxidation rates of lactate and glucose were seven to eight times higher in mice. Built species oxidized more lactate than glucose (8 to 9 times). However, anole muscle showed a preference for lactate as a substrate for glycolysis, incorporating 1.5 times as much lactate (expressed in glucose equivalents) as glucose. In contradistinction, mice incorporated 2.8 times as much glucose into glycogen as lactate. The quantitative differences in metabolic scope of mammals and reptiles are accompanied by fundamental differences in the capacity and patterns of skeletal muscle metabolism of lactate and glucose.

gluconeogenesis; glucogenesis; carbohydrate metabolism; anaerobic metabolism; exercise

THE RELIANCE on anaerobic metabolism during exercise in lizards has been the subject of a growing body of literature in activity metabolism. When reptiles are compared with mammals, it is evident that reptiles produce a greater fraction of ATP during vigorous activity through anaerobic means, sometimes as much as 90% (2). Such a dependence entails an accumulation of lactic acid and significant acid-base disruption, which can persist for hours (10, 11). Emphasis on anaerobic energy production during activity also results in the depletion of muscle glycogen. Given that activity is supported by anaerobic metabolism, and that anaerobic metabolism in turn is supported by muscle glycogen stores, depleted muscle stores of glycogen may be expected to compromise subsequent bouts of activity. It would seem reasonable therefore that animals that emphasize anaerobic metabolism during activity would also possess metabolic strategies that facilitate rapid recovery from anaerobically supported exercise. Such strategies might include mechanisms for rapid reduction of metabolic acids and replenishment of muscle glycogen stores. Although blood lactate generally returns to preexercise levels more slowly in reptiles than in mammals, muscle glycogen does indeed return more rapidly [see review by Gleeson (10)]. The different patterns of lactate and glycogen metabolism in mammals and reptiles suggest that lactate metabolism is different in the two groups in the postexercise state. This difference has prompted a number of studies examining the metabolic fates of lactate.

In most mammals lactate formed during exercise is oxidized to CO₂ and water. Whereas in vitro studies have shown that mammal muscle may have modest abilities to synthesize glycogen from lactate (8), most mammals do not appear to utilize this capacity in vivo. Generally <20% of postexercise lactate is used to regenerate glucose or glycogen, whereas most of the remainder is oxidized (4, 17). The case in humans is less clear, because reports of both significant (1, 16) and insignificant (21) lactate incorporation into glycogen exist. In contrast, fish (19), amphibians (26), and reptiles (12) appear to retain a large fraction of their postexercise lactate as a substrate for glycogen resynthesis.

Although the demonstration of such a recovery strategy in ectothermic vertebrates is quickly becoming widespread, the locations of and the mechanisms for lactate metabolism are less well known. In lizards skeletal muscle has been shown to have the capability to resynthesize glycogen from lactate (9). In vivo studies using radiolabeled substrates in iguanid lizards (12) indicate that, in contrast to dogs and rats, 50% of the lactate removed after exercise is used to synthesize glucose and glycogen. Only 16% of the lactate was oxidized, and lactate, rather than glucose, was used as the preferable substrate for glycogen replenishment. It also has been shown that it is the oxidative fiber types of lizards that possess the greater capacities for gluconeogenesis and glycogen replenishment (9, 13).

The above studies prompted an in vitro comparison between the metabolic mechanisms for the handling of lactate and glucose in the muscles of a small mammal and lizard. Whole animal studies do not allow the role of skeletal muscle to be directly assessed. Although hindlimb perfusion experiments in mammals have provided a direct analysis of skeletal muscle recovery, comparison with the reptile literature is difficult. In the present study we have attempted to demonstrate the capacities of reptilian muscle for glucose and lactate metabolism and contrast those capacities with those of mouse muscle under physiologically equivalent conditions in vitro.

MATERIALS AND METHODS

Animals. Adult, male anoles (Anolis carolinensis) were obtained from a commercial supplier (Buck’s, La Place, LA) collected in March 1991. In Boulder, animals were housed individually in plastic, shoe-box aquariums with solid tops containing an open Petri dish of water to elevate humidity. Room temperature was on a fluctuating cycle, with a high of 32°C during the day and a low of 20°C during the night. Photoperiod was set to 14:10-h light–dark cycle. Cardboard screens were inserted between aquariums to prevent animals from seeing one another because this impacts Anolis glucose metabolism (25). Food,
cose (either 8.5 or 5.55 mM for lizard and mouse, respectively). Sodium also contained representative values for postexercise glu-
ose oxida tion in CO₂ traps was counted in 1-ml aliquots mixed with 2 
radioactive counts appeared in the CO₂ traps did not decrease (or increase) in either species during the serial sampling, indicating that at least for this variable tissue viability was maintained over the 60-min measurement period. To see if larger tissues were diffusion limited, oxidation rates were re-
gressed against mass. Neither mouse nor lizard muscle demonstrated a decrease in mass-specific oxidation with increasing mass. The rate of glucose oxidation was sig-
nificantly lower in anoles than mice muscle (0.69 ± 0.006 vs. 0.507 ± 0.006 μmol glucose oxidized g⁻¹ h⁻¹, respectively). Muscles from both animals had higher ox-
significantly different (n = 29 for each substrate). However, for purposes of comparison with anoles, data were combined for both 
substrate incorporation into glycogen (Fig. 2). Anole muscle incorporated less substrate into glycogen than did mouse muscle when glucose was the substrate (0.352 ± 0.044 vs. 3.95 ± 0.75 μmol incorporated into}

**RESULTS**

Analysis of paired data for the EDL and soleus from mice indicated no significant differences between muscles for all parameters measured except for oxidation of lactate. The EDL had a higher rate of lactate oxidation than the soleus (4.96 ± 0.43 vs. 3.78 ± 0.63 μmol glucose oxidized g⁻¹ h⁻¹, P = 0.046). However, for purposes of comparison with anoles, data were combined for both soleus and EDL.

**Substrate oxidation (Fig. 1).** Radioactive counts appearing in the CO₂ traps did not decrease (or increase) in either species during the serial sampling, indicating that at least for this variable tissue viability was maintained over the 60-min measurement period. To see if larger tissues were diffusion limited, oxidation rates were re-
gressed against mass. Neither mouse nor lizard muscle demonstrated a decrease in mass-specific oxidation with increasing mass. The rate of glucose oxidation was sig-
nificantly lower in anoles than mice muscle (0.69 ± 0.006 vs. 0.507 ± 0.006 μmol glucose oxidized g⁻¹ h⁻¹, respectively). Muscles from both animals had higher ox-
significantly lower in anoles than mice muscle (0.591 ± 0.052 vs. 4.82 ± 0.51 μmol glucose equivalents oxidized g⁻¹ h⁻¹, respectively).

**Substrate incorporation into glycogen (Fig. 2).** Anole muscle incorporated less substrate into glycogen than did mouse muscle when glucose was the substrate (0.352 ± 0.044 vs. 3.95 ± 0.75 μmol incorporated into...
CARBOHYDRATE METABOLISM IN MOUSE AND ANOLE MUSCLE

Fig. 2. Rates of substrate incorporation into muscle glycogen (μmol glucose equivalents·g⁻¹·h⁻¹) for anoles and mice for lactate (light stippled bar) and glucose (dark stippled bar). Values with different superscript letters are significantly (P < 0.05) different (n = 26 for each substrate).

glycogen·g⁻¹·h⁻¹), and this relationship was true for lactate as well (0.538 ± 0.168 vs. 1.40 ± 0.88 μmol glucose equivalents incorporated into glycogen·g⁻¹·h⁻¹). In a comparison of incorporation of substrates within species, there was no difference between glucose and lactate incorporation in anoles, but glucose incorporation was greater than lactate in mouse muscle.

Substrate preferences during oxidation (Fig. 3). To assess the relative substrate preference of muscle for either lactate or glucose oxidation, and to permit a between-species comparison, oxidation rates were expressed as a ratio of micromoles of lactate oxidized (expressed as glucose equivalents) relative to micromoles of glucose oxidized. In both species there was approximately a ninefold preference for oxidation of lactate, and this was not different between species.

Substrate preferences for glycogenesis (Fig. 4). To assess the relative substrate preference of muscle for glycogenesis using either lactate or glucose, and to permit a between-species comparison, substrate incorporation into glycogen was expressed as a ratio of micromoles of lactate (as glucose equivalents) into glycogen relative to micromoles of glycogen oxidized. In anoles lactate was used preferentially over glucose (~1.5:1), whereas in mice glucose was used preferentially (~3:1). Others (3) have determined that the rate of glycogen formation determined from lactate may underestimate glycogenesis from lactate by 35%. If calculations were adjusted to take into account this difference, mice would still demonstrate a preference for glucose and anoles would demonstrate a preference for lactate.

Citrate synthase. Citrate synthase activity of anole muscle averaged 11.4 ± 1.2 μmol of substrate converted·g muscle tissue⁻¹·min⁻¹ (n = 5), a value significantly lower than both of the skeletal muscle from the mouse. Mouse soleus averaged 59.7 ± 3.2 U/g muscle (n = 5), which was significantly higher than the EDL (42.8 ± 5.1 U/g muscle, n = 5).

DISCUSSION

Oxidation rates (Fig. 1) and rates of substrate incorporation into glycogen (Fig. 2) are higher in mice than in anoles. These rates are consistent with the higher mass-specific metabolism of mice, the temperatures of the incubation medium (39°C for mice, 30°C for anoles), and the lower mass-specific oxidative capacity as assessed by citrate synthase activity. Differences between the oxidative capacities of lizard and mammal muscle have also been correlated with differences in mitochondrial structure and densities (6, 7). These structural differences may correlate with the different metabolic strategies during recovery discussed below.

The ratio of lactate to glucose oxidation indicates a preference for lactate oxidation relative to glucose oxidation in both species by ~10:1 (Fig. 3). This preferential oxidation of lactate over glucose is supported by studies of both poikilothermic vertebrates [lizards (12, 14); toads (26); and salamanders (Wickler and Gleeson, unpublished data)] as well as mammals (20).

The principal pathway for lactate removal in the mouse is oxidation. In the present study, approximately three times as much lactate is oxidized by mouse muscle as is removed gluconeogenically (Figs. 1 and 2). This emphasis on oxidative lactate removal is consistent with data from intact rats and other mammals (8). Anole muscle demonstrates a different pattern; nearly equimolar amounts of lactate are removed gluconeogenically as are removed oxidatively (Figs. 1 and 2). The anole pattern is consistent with that of another iguanid lizard (10, 12, 13) and salamander muscle (Wickler and Gleeson, unpublished data).

The suggested advantages for such a strategy for lactate removal and glycogen replenishment are twofold and potentially complementary (9, 26). Arguing that a pathway for the oxidative removal of lactate would be linked to the metabolic rate of the animal, Withers et al. (26) suggest...
that the low metabolic rate of ectothermic vertebrates would make oxidative lactate removal a very slow process and delay the return to acid-base balance in the animal. Indeed, Coulsen (5) has used this rationale to predict that exhausted large dinosaurs, with low mass-specific rates, might have required days to recover by oxidative lactate removal. Use of lactate as a substrate for glycogen resynthesis, on the other hand, provides for a more rapid pathway for lactate removal and acid-base recovery not linked to resting metabolism. An adaptive advantage has also been suggested for a strategy that facilitates rapid glycogen replenishment in animals dependent on glycogen-supported metabolism during activity (9, 10). In mammals, with a greater scope for aerobic metabolism during exercise and therefore less reliance on muscle glycogen, there may be a greater emphasis on rapid lactate removal and acid-base balance independent of glycogen resynthesis.

The capacity of reptilian skeletal muscle to regenerate glycogen from lactate is a function of muscle fiber type (9, 13). Reptilian oxidative fibers (both slow and fast) show a fivefold higher rate of lactate incorporation into glycogen than fast-twitch glycolytic fibers. In mammals the incorporation rate of lactate into glycogen appears to be higher in fast-twitch fibers (both glycolytic and oxidative) than slow twitch oxidative fibers (18). In our study, we measured similar rates of lactate incorporation into glycogen for both the slow-twitch soleus and the fast-glycolytic EDL muscles, although Bonen et al. (3) were already able to measure significantly higher rates in the EDL muscle. The difference between this study and that of Bonen et al. may be due to differences in the treatment of the muscle before metabolism measurements. In our study, we attempted to mimic an immediate postexercise state, whereas Bonen et al. preincubated nonstimulated muscles in 0.5 mM palmitate (which would presumably elevate intracellular energy charge) before incubation with lactate or glucose. To test this hypothesis, we repeated incubations of both mouse (n = 6) and anole (n = 5) muscle using our incubation mediums with the 0.5 mM palmitate added 15 min before addition of labeled lactate. This treatment had no effect (P = 0.88) on glycogen deposition in the anole muscle but did affect mouse muscle. Palmitate increased incorporation of lactate into glycogen in both soleus (2 times) and EDL (3 times) and, further, the incorporation into the EDL was significantly higher than the soleus, findings consistent with Bonen et al. (3). Although preincubation of palmitate altered quantitative differences for mouse muscle, the qualitative differences between mouse and anole are maintained. Namely, mouse muscle shows higher oxidation rates of both lactate and glucose, and the muscle demonstrates a preferential use of glucose over lactate for glycogen replenishment.

The data on Anolis presented here are consistent with both advantages of rapid lactate removal and glycogen replenishment in reptiles. The quantitative differences in metabolic scope of mammals and reptiles are accompanied by fundamental differences in the capacity and patterns of skeletal muscle metabolism of lactate and glucose.

Thanks to Kristin Laubach and Frank van Breukelen for technical assistance.

This research was supported by the National Science Foundation Grant DEB 86 15603 to T. T. Gleeson, University of Colorado, and a California State Polytechnic University sabbatical leave.

Address for reprint requests: S. Wickler, Dept. of Animal and Veterinary Science, California State Polytechnic Univ., Pomona, CA 91768.

Received 21 November 1991; accepted in final form 8 September 1992.

REFERENCES


