Glycogen synthesis and metabolism of lactic acid after exercise

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The metabolism of an organism or muscle after exercise is characterized by a prolonged elevation in oxygen consumption. The initial interpretation of this phenomenon given by Hill et al. (10) was that the elevated rate of postexercise oxygen consumption represented the oxidation of a portion (3/5) of the lactic acid produced during exercise to provide the energy necessary for the reconversion of the remaining lactate (2/5) to glycogen. Since then generally acknowledged hypotheses concerning the metabolic processes underlying elevated rates of postexercise O\textsubscript{2} consumption have retained this essential lactic acid feature of the original Hill hypothesis (12, 15). The purpose of this investigation was to determine if significant glycogen synthesis occurs during the postexercise period of elevated O\textsubscript{2} consumption held to insure that they could run 1 hr on the motor-driven treadmill at 18 m/min up a 10% grade. Additionally, animals were handled several times daily to familiarize them with the procedure. To minimize diurnal effects, animals were run to exhaustion in the morning after an overnight fast. Exercised animals were fasted only 10 hr so that the total time of the fast plus the run approximated the 12-hr control fast period of nonexercised animals. Maximum performance during exhaustive runs was insured by the use of electrical as well as airjet stimulation.

In order to induce a large lactic acid postexercise O\textsubscript{2} consumption component, the exercise regimen leading to fatigue was designed to result in significant glycogen depletion and lactacidemia. Previous research (1, 13) indicated that prolonged exercise at a constant pace results in glycogen depletion, but less than maximal blood lactate concentrations. On the other hand, short, very intense exercise bouts have been associated with high lactate concentrations (6). Therefore, it was decided to combine the two exercise regimens. In this study the exercise protocol used was a 1-hr run at 18 m/min on a 10% grade to reduce glycogen and then a 2 m/min increase in speed every 2.5 min to produce a finishing sprint and anaerobic glycolysis. Fatigue was determined to be that point at which animals were no longer able to keep pace with the treadmill even in response to the unpleasant stimuli.

At the appropriate time called for by the experimental protocol, rats were killed by a blow to the head and decapitated. Animals were bled over a large powder funnel lined with a layer of cheesecloth. Hair and other debris were trapped on the cheesecloth, and the blood trickled directly into a preweighed centrifuge tube which contained 5 ml of 70% perchlorate. Immediately after 1–4 ml of blood were collected by bleeding, the abdominal cavity of the rat was opened, and the liver was dissected free and immersed in liquid N\textsubscript{2}. After the removal of the liver, the hindlegs were

METHODS

A total of 83 female Holtzman rats was used in the two phases of this study. These animals were selected from a total of over 100 rats on the basis of their ability to run on a motor-driven treadmill. Female animals were used exclusively because of their predisposition to run. At the time
excised, skinned, and immersed into ice-cold saline. Rough dissection was performed on muscle immersed in ice-cold saline. Large pieces of fascia and fat were dissected free from the muscle chunk samples during their transfer to liquid \( \text{N}_2 \). Both liver and muscle samples were stored at \(-18\,\text{C}\) until analysis.

All analyses were performed at least in duplicate. Blood, muscle, and liver lactate concentrations were determined according to the enzymatic method of Hohorst (11). Liver and muscle sample powders for acid extraction were prepared by crushing and grinding at liquid \( \text{N}_2 \) temperatures. Blood glucose concentration was determined by the glucose oxidase technique (Worthington Biochemicals, Freehold, N.J.) Assays were performed on neutralized blood acid-extracted samples (11) and incubations were conducted in a phosphate-buffered medium, pH 7.0.

Tissue glycogen was isolated as described by Good et al. (9). Frozen muscle and liver samples were digested in hot 30% KOH. Glycogen was precipitated with 95% ethanol in the cold and was washed by resuspension and precipitation. The glycogen concentrations of the washed and resuspended residues were estimated by the colorimetric method of Dubois et al. (8).

In phase 2 of this study (isotope infusion) 28 rats were trained to run as described above. The 20 best runners were then randomly assigned to either an exhaustive exercise group or to a group of pair-fasted controls. All animals were fasted overnight as described above, and the selected animals were exercised to fatigue also as described above. Sodium \( \text{t-L} -1\text{H}^\text{C} \)-lactate-\( \text{t-L}^\text{H}^\text{C} \)-lactate was injected simultaneously with 1 \( \mu \text{C} \) of the labeled lactate (0.152 \( \mu \text{C/mg} \)) brought up to 0.5 ml volume with saline. It was estimated that this quantity of lactate would increase the resting blood lactate pool by about 3%. Immediately after the injection of isotope, animals were sealed in separate glass metabolic chambers. In this apparatus air was drawn via a vacuum past the experimental animals at the rate of approximately 800 ml/min and was bubbled through a measured quantity of the organic \( \text{CO}_2 \) trap ethanolamine/methylcellusolve in the ratio of 1:2. The amount of labeled \( \text{CO}_2 \) production was determined for the first six, consecutive 10-min periods after exercise and for the 2nd hr after exercise. Following \( \text{CO}_2 \) collection, aliquots of trapping solution were counted in duplicate on a Nuclear-Chicago Unilux II counter using Bray's scintillation solution (2). The rate of disintegration for each sample was calculated on the University Univac 1108 computer.

Statistical significance of the results for each group was assessed by means of Dunnett's \( t \) test using the .05 level as the criterion for significance. Additionally, since the assumption of homoscedasticity implied in the Dunnett's test was not always met due to large differences between control and treatment values, on several variables Fisher's \( t \) tests were done comparing a control mean with one treatment mean. In these instances Satterthwait's correction for unequal variances was used to determine degrees of freedom. Unless specified, all tests were two tailed.

**RESULTS**

**Lactate concentrations.** Resting blood lactate values for fasted control rats and nonfasted, nonexercised controls (Fig. 1) appear to be significantly lower than published values for blood lactate in resting rats (4, 18). At the end of exercise blood lactate concentration was greatly increased with a peak apparently being delayed into recovery with the highest values for blood lactate being observed in the 5-min recovery group (Fig. 1). The postexercise lactate values correspond to the high blood lactate values reported by others for rats (18). Blood lactate concentration returned to resting levels within 15 min of recovery. Thereafter, blood lactate concentration continued to decline and after 1 hr of recovery was significantly below the control values (\( P < .05 \)).

The values reported for resting muscle lactate concentration (Fig. 1) appear to be somewhat higher than previously published data on rats (4). Muscle lactate in 12-hr fasted controls was significantly lower than that in the nonfasted group. As with blood lactate, the concentration of muscle lactate was significantly elevated as the result of exercise and returned to or below control values within 30 min of exercise. Postexercise values for muscle lactate correspond to published values for muscle lactate in rats (4) and humans (6) after maximal exercise.

The concentration of lactate found in the liver (Fig. 1) was significantly lower in the 12-hr fasted group than in the nonfasted controls. The mean concentration of lactate found in the livers of animals sacrificed at exhaustion was significantly higher than control values. However, compared to the large excursions in lactate contents of blood and muscle resulting from exercise, the relative elevation in liver lactate was minor with no delayed peak apparent in recovery. The general pattern of decline in liver lactate was, however, similar to that observed for blood and muscle. After the initial elevation due to exercise, liver lactate concentration declined in recovery. A comparison of the 12-hr fasted control and the 1-hr recovery groups indicates the decline in liver lactate below the control values to be statistically significant (\( P < .01 \)).

![Fig. 1. Tissue lactate concentrations (mean ± SE) as a function of time after exhaustive exercise in 10- to 12-hr fasted rats (closed symbols). Dotted line is to visually connect pre- and postexercise values. Open symbols in rest and recovery are nonfasted, nonexercised controls, and 36-hr fasted, nonexercised controls, respectively. \( n = \) at least 6 animals at each point.](http://ajplegacy.physiology.orgelsius/ Downloaded from http://ajplegacy.physiology.org/ by 10.220.23.247 on April 30, 2017)
Glycogen concentrations. Fasting had the expected effect of reducing the liver glycogen concentration in nonexercised control animals (Fig. 2). Compared to nonexercised controls, however, little glycogen remained in the livers of rats after exhaustive exercise. In the immediate postexercise period there was no indication that significant glycogen synthesis had occurred. The concentration of liver glycogen after 24 hr of recovery from exhaustive exercise was apparently no different from the immediate postexercise values and was also not indicative of significant glycogen synthesis. The mean concentration of liver glycogen in the 36-hr fasted group was slightly greater than that of the 24-hr recovery group, but compared to the nonexercised, 12-hr fasted control values, the concentration of liver glycogen after 36 hr of food withholding was negligible.

The pattern of response observed with muscle glycogen concentration (Fig. 2) was similar to that in liver glycogen. Both the withholding of food overnight prior to exercise and forced exercise significantly reduced the concentration of muscle glycogen. As in the case of liver glycogen, there was no evidence of muscle glycogen synthesis or replenishment during the immediate postexercise period. The mean concentration of liver glycogen after 24 hr of food withholding was negligible.

Blood glucose concentration. Mean values for 12-hr fasted and nonfasted control animals were identical, but the degree of hypoglycemia observed in rats after exhaustion was surprising (Fig. 3). Recovery to values equal to or in excess of nonexercise control values was, however, achieved within 15 min of recovery. After the rapid recovery of blood glucose to control values, which occurred simultaneously with the removal of blood lactate (Fig. 1), blood glucose showed an apparent decrease in concentration after 15 min of recovery. This trend was maintained throughout the period observed as the level of blood glucose after 24 hr of recovery was significantly lower than the 15-min recovery value (P < .05). The 36-hr fast and 24-hr recovery groups were not significantly different.

Lactate-1-14C infusion. In both the exercise exhausted and pair-fasted control groups, the onset of labeled CO2 evolution was rapid after lactate infusion (Fig. 4). The pattern followed was for 15–20% of the label infused to appear as CO2 in the first 10-min collection period. The greatest evolution of label occurred invariably during the second 10-min collection period when another 20–25% of that infused appeared as CO2. Following the second collection period, an exponential decrease in the production of labeled CO2 was apparent.

In each of the three initial collection periods, significantly more of the labeled carbon appeared as CO2 in the pair-fasted controls than in the exercise-exhausted animals. The difference at the end of 30 min amounted to approximately 8% of the total labeled carbon infused. The rates of labeled CO2 production during the last 90 min of collection period when another 20–25% of that infused appeared as CO2. Following the second collection period, an exponential decrease in the production of labeled CO2 was apparent.

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DISCUSSION

The data reported here contradict the lactacid theory of postexercise 

\( \text{O}_2 \) consumption. Not only is there no apparent 
glycogen synthesis from lactic acid after exercise, glycogen 

is apparently not synthesized from any other carbon source 
cither. The primary fate of lactic acid after exercise appears 
to be oxidative.

The data reported here on rats with regard to glycogen 

replenishment in skeletal muscle after exercise are in ac-

cordance with those published on humans. Hultman (13) 

has observed no glycogen replenishment in human skeletal 

muscle after 3 days of recovery from exercise should there 

be food withholding or only protein and fat feeding after 

exercise. It would appear that the hypothesis of significant 
glycogen synthesis immediately after exercise in humans 
is unfounded also. The replenishment of glycogen stores after 
prolonged severe exercise probably depends on the presence 
of dietary carbohydrate.

If during recovery from exercise lactic acid were recon-

verted to glycogen, little of the isotope injected in our experi-

ments should have appeared as \( \text{CO}_2 \). On the other hand, 

if the primary pathway for lactic acid during recovery from 
exercise was to oxidation, the majority of the isotopically 
labeled carbon of the lactate infused would appear as \( ^{14}\text{CO}_2 \). 

Since 70–90% of the isotope invariably appeared as \( \text{CO}_2 \) 

and since there was no glycogen replenishment apparent, we 

conclude that glycogen synthesis is not a dominant process 
during the immediate recovery period. Given the high 
circulating levels of catecholamines resulting from exercise 
and the effects of catecholamines on glycogen degradation, 
our conclusion regarding the improbability of glycogen 
synthesis after exercise seems reasonable.

It would appear that there are two major pathways by 

which lactate could appear as \( \text{CO}_2 \). First, of course, is the 

possibility of direct oxidation via conversion to pyruvate 

and entry into the Krebs cycle. Skeletal muscle (15, 17), 

heart, kidney, and liver (16) are capable of such functions. 

Since carbon-1 of the lactic acid is the first to be oxidized, 
the rapid onset of labeled \( \text{CO}_2 \) evolution can be taken to 
indicate that such a mechanism was operating. Second, 
any lactate that was sequestered by the liver, converted to 
glucose, and released into the blood would appear as \( \text{CO}_2 \) 
if oxidized by any of the body tissues. Since the incorpora-
tion of label into glucose was not measured in our experi-
ments, we are unable at this time to make any precise 
quantitative estimate of the route of label oxidation. In this 
regard, however, with intact dogs in mild exercise, Depocas 
et al. (5) have demonstrated that 74% of the lactate formed 
during exercise is promptly converted to \( \text{CO}_2 \) and that 
about 10% of the plasma glucose is derived from lactic acid 
during both rest and activity. With rabbits at rest Drury 
and Wick (7) observed that 80–90% of infused lactate is 
oxidized and that only 4–5% of the isotope is incorporated 
into glucose and glycogen. Our data are consistent with 
these observations (5, 7).

The initially lower rate of labeled \( \text{CO}_2 \) production in 
exercised compared to control animals was not expected. 
The lower rate of labeled \( \text{CO}_2 \) productions probably re-

sulted from isotope dilution and a greater total lactate pool 
size in exercised animals. However, if the results were due 
solely to a difference in total lactate pool size, then later on 
in recovery the production of labeled \( \text{CO}_2 \) in the exercised 
animals should have exceeded that of the controls. That did 
not happen, after 30 min of recovery the rates of labeled 
\( \text{CO}_2 \) production were the same in both treatments. The 
data are, therefore, consistent with the hypothesis of some 
carbon retention, even glucose or glycogen synthesis after 
exercise. However, since the quantitative glycogen deter-

minations in recovery give no evidence for glycogen syn-
thesis, a more likely explanation is that exercise resulted in 
an increased lactate-pyruvate recycling via the dicarboxylic 
acid cycle.

The findings of this study indicating glycogen depletion, 
hypoglycemia, and lactacidemia after exercise are in con-
trast to previous studies (1, 13) that have observed pro-
longed exercise to result in glycogen depletion and low 
lactate levels. A possible explanation is that our results were 
induced by the exercise task. Conceivably, the duration of 
the run could have induced the hypoglycemia and dimin-
ished liver and muscle glycogen stores to a relatively low 
level. However, as the exercise task called for animals to 
finish with a sprint, and since the circulating level of epi-
nephrine was undoubtedly high, the culmination of exercise 
might have resulted in the anaerobic glycolysis of most of 
the remaining muscle glycogen.

The gradient between muscle and blood lactate concen-

trations during both rest and exercise was greater than 

expected on the basis of previous work (4, 6). There exists 
the possibility that our data on muscle lactate concentration 
were affected by anaerobic glycolysis in hindleg muscles 
during dissection or as the result of the struggle after de-
capitation. That these mechanisms may have been in effect 
may be indicated by the substantial muscle lactate concen-

trations found in the exhausted, hypoglycemic animals 60 

min into recovery (Fig. 1). The possibility of significant 
anaerobic glycolysis in skeletal muscle during isolation 
would also serve to alter the quantitative data on muscle 
glycogen concentration (Fig. 2). However, it would seem 
that our data on blood and liver lactate concentrations 
(Fig. 1), blood glucose concentration (Fig. 3), and liver 
glycogen concentration (Fig. 2), where there was less oppor-
tunity for anaerobic glycolysis after death, support our 
contention that the carbohydrate depletion and lactaci-
demia observed were largely due to the exercise task.

The data reported here support the conclusion (3) that 
excess postexercise \( \text{O}_2 \) consumption cannot be taken as 
representative of the anaerobic metabolism that occurs 
during exercise. Further, with this realization, it becomes 
increasingly difficult to determine which portion of excess 
postexercise \( \text{O}_2 \) consumption should be included in calcula-
tions of gross or net body efficiencies.

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References