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GLYCOGEN RESYNTHESIS AND RECOVERY FROM EXERCISE: EFFECTS OF VERY-LONG-CHAIN ACYL-COENZYME, A DEHYDROGENASE DEFICIENCY

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Abstract:

Disorders of fatty acid metabolism pose a variety of problems including hypoglycemia, muscle weakness, muscle cramping, rhabdomyolysis, and, in the most severe cases, death. Individuals with a deficiency of very-long-chain acyl-CoA dehydrogenase (VLCAD), the enzyme initiating the oxidation of very-long-chain fatty acids, are particularly prone to exercised-induced declines in muscle function because of the importance of very-long-chain fatty acids to energy metabolism in exercise. The purpose of this project was to study muscle glycogen resynthesis in the recovery from high intensity exercise in VLCAD deficient mice. Recovery of skeletal muscle function (gastrocnemius) following exercise is not as rapid in VLCAD deficient mice as recovery in non-deficient mice. Based upon previous reports, recovery of muscle from exercise is, at least in part, related to glycogen resynthesis. It was hypothesized that muscle glycogen levels as well as liver glycogen levels would be significantly depressed 24 hours after exercise in VLCAD deficient exercised mice compared to non-exercised VLCAD deficient and exercised and non-exercised non-deficient mice. Twenty VLCAD deficient mice (knockout mice) and 20 non-deficient 129 Sv/C57BL6 mice were used in this project. Ten from each group exercised on a motor-driven treadmill to exhaustion using high intensity interval exercise while an additional 10 animals from each group served as non-exercised controls. The exercised mice were allowed to recover for 24 hours and then anesthetized using sodium pentobarbital (80 mg·Kg⁻¹). The right and left gastrocnemius muscles, the vastus lateralis muscle, the heart, and the liver were removed and frozen and the glycogen content subsequently determined. The resynthesis of glycogen was only significantly (p < 0.05) effected in the vastus lateralis muscle and the liver. Resynthesis was significantly reduced in the vastus muscle of VLCAD deficient mice and enhanced in the vastus muscle of non-deficient mice. It was concluded that VLCAD deficiency does delay recovery of muscle glycogen after exercise and that the delay may be affected by muscle fiber type or patterns of recruitment.

Introduction:

Glycogen utilization, both oxidatively and non-oxidatively begins after the first 30 seconds of exercise. For the first 10 to 15 minutes of prolonged exercise, muscle glycogen is the primary, but not exclusive-energy source. As exercise proceeds, fatty acids become energetically more important and muscle glycogen and blood glucose less important to total energy production. In an animal with an inability to utilize very-long-chain fatty acids, muscle glycogen and blood glucose likely remain primary energy sources. Indeed, it is not uncommon to observe muscle and liver glycogen depletion after both short-term high intensity and prolonged exercise even in animals capable of using fatty acids.

Muscle glycogen depletion parallels the perception of fatigue and significant stores of muscle glycogen are needed for optimal performance (1). At the conclusion of exercise, muscle, heart and liver glycogen stores must be resynthesized. The depletion of muscle glycogen provides a strong drive for its own resynthesis and skeletal muscle glycogen resynthesis takes precedence metabolically over liver glycogen resynthesis. Glycogen resynthesis is assumed to be under negative feedback control (9). When muscle glycogen stores are depleted, there is an increase in the rate of blood glucose transport into the muscle and an increased capacity to dispose of glucose by converting it to glycogen (3). An inverse relationship exists between serum free fatty acid (FFA) concentration and muscle glycogen content. A low glycogen content stimulates glycogen synthase activity, and high levels of FFA regulate the amount of glycogen in the muscle when glycogen stores are filling (7).

Very-long-chain fatty acids, particularly palmitic acid, are a primary energy source for skeletal muscle and cardiac tissue especially during fasting and prolonged exercise (2). They are metabolized in the mitochondria through a process requiring activation of acyl coenzyme A (acyl-CoA) esters followed by transport across the mitochondrial membrane. The oxidation of activated fatty acids is carried out through the sequential activities of the enzymes of β-oxidation, the Krebs cycle, and the respiratory chain to generate ATP. The first step in β-oxidation is catalyzed...
by one of four chain length specific acyl-CoA dehydrogenase enzymes. For very-long-chain fatty acyl-CoA, the first step is catalyzed by very-long-chain acyl-CoA dehydrogenase (VLCAD) (4). A genetically linked deficiency or absence of this enzyme is associated with exercise intolerance characterized by cardiac and skeletal muscle myopathy, exercise-induced muscle pain, muscle weakness, hypoglycemia, myoglobinuria, fatigue, and recurrent episodes of rhabdomyolysis (4-6; 8). Although little is known about the responses of enzyme deficient humans or mice, it is likely that the inability to use very-long-chain fatty acids results in the increased utilization of carbohydrate to supply the energy demands of exercise.

Previous observations (unpublished) in our laboratory have determined that mice with this deficiency do not completely recover muscle function 24 hours following high intensity exercise, while non-deficient mice fully recover. These observations sparked interest in studying the muscle, liver, and cardiac glycogen content of the deficient and non-deficient mice twenty-four hours post-exercise. We hypothesized that the VLCAD deficient mice would not resynthesize glycogen following high intensity exercise as completely as non-VLCAD deficient mice.

**Methods and Procedures:**

**Animals.** Twenty-one VLCAD deficient mice (knockout mice) and 20 non-deficient 129 Sv/C57BL6 mice obtained from the Jackson Laboratories weighing between 10 – 25 g were housed 5 to 10 per cage in a room on a 12-hour light/dark cycle. All mice were fed solid food pellets and water *ad libitum*. All experiments were approved by the University of Arkansas Institutional Animal Care and Use Committee.

**Procedures.** Ten mice from each group were exercised on a motor driven treadmill to exhaustion using high intensity interval exercise while the additional animals from each group served as non-exercised controls. The groups were designated as VLCAD deficient, exercised (DE); VLCAD deficient, non-exercised (DNE); non-deficient, exercised (NDE); and non-deficient, non-exercised (NDNE). Each exercising mouse then ran at an initial speed of 16 m/min and 0% grade. The speed and grade were then increased at 6–min intervals to 24 m/min and 0% grade, 30 m/min at 2% grade, 35.5 m/min at 4% grade, 41 m/min at 6% grade, and 47 m/min at 8% grade. Exercise was terminated when the mouse could no longer keep up with the treadmill and when removed from the treadmill showed little movement. The mice were allowed to recover for 24 hours. At the end of the 24 hour recovery period, the mice were anesthetized using sodium pentobarbital (80mg/kg). The gastrocnemius muscles, the vastus lateralis muscles, the heart, and the liver were removed and immediately frozen using liquid nitrogen.

**Serum and Tissue Analyses.** The glycogen content of each of the tissues was determined using the phenol-sulfuric acid procedure. At the time of the surgical procedures, a blood sample was taken and the serum analyzed using o-toluidine to determine glucose concentration.

**Statistical Analysis.** The results were analyzed using ANOVA of data in a 2x2 factorial design. The Tukey procedure was used for post-hoc comparisons when indicated by a significant Fratio. All comparisons were made at the 0.05 level of significance.

**Results:**

**Descriptive Statistics.** The body weight, run times to exhaustion, and serum glucose concentrations are shown in Table 1. The body weight of the mice in the NDE group was significantly (p < 0.05) greater than that of the mice in the other three groups. The non-deficient mice ran approximately six minutes longer than the VLCAD deficient mice. However, the difference was not statistically significant. It was anticipated that the enzyme deficient mice, particularly the exercised mice, would have significantly lower serum glucose concentrations. Despite the highly elevated glucose concentration of the NDE mice, the difference was not significant.

**Tissue Analysis.** No differences were found in glycogen concentration in either the gastrocnemius muscle (Figure 1) or the heart (Figure 2). The ANOVA for the vastus lateralis muscle indicated a significant exercise state and deficiency interaction. The post hoc analysis showed that exercise caused a significant (p < 0.05) reduction in glycogen in VLCAD deficient mice but a significant (p < 0.05) increase in the non-deficient mice (Figure 3). In the liver (Figure 4), the only significant (p < 0.05) effect was that of exercise. Exercise caused an increase in liver glycogen but, as seen in Figure 4, the difference was largely due to the effects of exercise in the non-deficient mice.

**Discussion and Conclusion:**

Interestingly, all glycogen levels except in the liver for DE mice were lower in concentration than DNE mice. However, the only statistically significant difference observed was in the vastus lateralis muscle. One reason for this may be the possibility that the vastus lateralis muscle is used more predominantly than the gastrocnemius muscle by mice while running. Another possibility is a fiber type effect on muscle glycogen recovery in the deficient animals. The vastus lateralis muscle has a distinct white component and a distinct red component. The gastrocnemius muscle on the other hand is a muscle of mixed fiber types. After exercise, one expects to see a supercompensation of muscle glycogen, but this was only seen in the NDE mice in the vastus lateralis muscle and the liver but not in the gastrocnemius. Therefore, the fact that the DE mice did not show supercompensation and did not even recover to the DNE resting levels of glycogen becomes striking and perhaps very important for future experiments.
Both DE and NDE mice showed an exercise effect on their liver glycogen levels, having a higher concentration than their non-exercised counterparts; however, the deficient mice did not show as large an exercise effect as the non-deficient mice. This is perhaps due to the prolonged recovery period of the vastus lateralis muscle of the DE. It is interesting that the glycogen concentrations of the mice in this group show an exercise effect since muscle glycogen resynthesis takes precedence metabolically. A possibility is that the vastus lateralis glycogen levels were far more depressed following exercise than the other tissues and therefore needed more time to recover and delayed the recovery of liver glycogen. This certainly is an area for further study specifically investigating the glycogen levels of DE mice 48 hours after exercise.

Since there was no significant difference in serum glucose levels between DE and NDE mice, one might expect complete recovery of glycogen in all tissues in both groups. However, this did not occur, indicating that glucose is not the only rate-limiting factor for glycogen resynthesis in the enzyme deficient mice. The serum glucose values in this study, however, must be viewed with some caution. The values were much higher for all groups in this study than have been observed in previous studies in our laboratory (unpublished).

In conclusion, VLCAD deficiency does delay exercise recovery in some tissues, particularly the vastus lateralis muscle, as evidenced by the decreased rate of glycogen resynthesis. Whether the reduced rate of resynthesis translates into a functional difference in the muscle is not clear. We have previously observed that the functional capacity of the gastrocnemius muscle is depressed 24 hours after this same exercise bout. Clearly, in that muscle it is unlikely that delayed glycogen resynthesis is the reason for the depressed function since this investigation confirms that glycogen resynthesis in the gastrocnemius muscle is not delayed by the VLCAD deficiency. Further research needs to be explored to determine the effect of muscle fiber type on both the resynthesis of muscle glycogen post-exercise and muscle function post-exercise. Similarly, blood glucose utilization in VLCAD deficient mice needs further study.

References:

Table 1. Descriptive characteristics of VLCAD deficient and non-deficient mice. Exercise consisted of high intensity intervals on a motor driven treadmill.

<table>
<thead>
<tr>
<th></th>
<th>Non-deficient</th>
<th></th>
<th>VLCAD deficient</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exercised</td>
<td>Not exercised</td>
<td>Exercised</td>
<td>Not exercised</td>
</tr>
<tr>
<td><strong>Body Weight (g)</strong></td>
<td>18.5 ± 1.6</td>
<td>14.8 ± 0.7</td>
<td>12.7 ± 0.3</td>
<td>13.2 ± 0.5</td>
</tr>
<tr>
<td><strong>Run Time (min)</strong></td>
<td>23.5 ± 2.7</td>
<td>17.7 ± 1.5</td>
<td>240 ± 9</td>
<td>241 ± 26</td>
</tr>
<tr>
<td><strong>Glucose (mg%)</strong></td>
<td>324 ± 36</td>
<td>228 ± 31</td>
<td>240 ± 9</td>
<td>241 ± 26</td>
</tr>
</tbody>
</table>

^ Significantly (p < 0.05) from all other groups.
Figure 1. Glycogen concentration of the gastrocnemius muscle twenty-four hours following high intensity exercise to exhaustion in VLCAD deficient and non-deficient mice.

Figure 2. Cardiac glycogen content twenty-four hours after exercise in VLCAD deficient and non-deficient mice.

Figure 3. Vastus lateralis muscle glycogen from VLCAD deficient and non-deficient mice twenty-four hours after high intensity exercise.

A Significantly (p < 0.05) different from the non-exercised VLCAD deficient mice.
B Significantly (p < 0.05) different from the non-exercised, non-deficient mice.
C Significantly (p < 0.05) different from the non-exercised VLCAD deficient mice.

Figure 4. Liver glycogen twenty-fours after high intensity exercise in VLCAD deficient and non-deficient mice.
Facility Comments:

Mr. Johnson's faculty mentor, Charles Riggs, has many positive remarks about Mr. Johnson's research. He writes:

I am pleased to have had the opportunity to work closely with Mr. Kyle Johnson over the past two and one half years both in the classroom and in the laboratory. During that time, I have had numerous opportunities to evaluate his academic performance and his professional promise. Needless to say, I have been very impressed with his capabilities and talents and, particularly, his willingness to work. That willingness is evidenced by his voluntary participation in research in my laboratory. The data utilized in the paper published here is a part of a larger project working with me and other students examining the consequences and potential benefits of exercise for people with genetically linked metabolic disorders. His project has focused on muscle glycogen resynthesis after exercise, a factor important in recovery from exercise and possibly important in preventing some of the negative effects of exercise in enzyme deficient individuals.

Mr. Johnson is clearly one of the top two or three undergraduate students I have had the opportunity to work with during my 24 years in higher education. He is highly motivated, self-disciplined, and very intelligent. He approaches his work with enthusiasm and perseverance and continually strives for excellence. Mr. Johnson has excelled in a rigorous course of study despite a busy extracurricular schedule. He has been a University cheerleader for the past three and one half years. He has been involved in several campus organizations and community charities. Even with all of these responsibilities, Mr. Johnson still volunteered to participate in my research. He is involved in data collection and analysis, but more importantly, he serves as a mentor to other graduate and undergraduate students participating in our research. I have been highly impressed by Kyle Johnson. He sets high goals and is very focused in his pursuit of those goals. His has been a quality project that provides important clues to the mystery of the muscular responses of enzyme deficient individuals to exercise while at the same time provoking several additional research questions.

Two other of Mr. Johnson's professors are equally enthusiastic about his work. Inza Fort writes:

I have had the privilege of teaching Mr. Johnson in one of my classes, KINS 3353, Mechanics of Human Movement, which is a detailed analysis of osteology, arthrology, and musculature as it pertains to movement and skill analysis. Out of a class of 30 students, Mr. Johnson achieved the highest point total. He is one of those students you love to have in class—attentive, questioning, analytical. Mr. Johnson has maintained a 3.94 GPA in Exercise Science. He became interested in research after taking Exercise Physiology and has spent many hours in the Human Performance Lab learning detailed research techniques under the mentorship of Dr. Charles Riggs. Assay analysis consumes many hours, and Mr. Johnson has dedicated himself to being thorough in his procedures. His protocol involved surgical procedures in mice so that muscle glycogen content could be analyzed after intense exercise. These procedures involved a high degree of precision in data collection. This project is important research with application for individuals with metabolism disorders, and the implications may include alterations in conventional thought about efficient exercise for those with enzyme deficiencies and metabolic disorders.

And Barry Brown says:

I have known Mr. Johnson for approximately 2 years, both as a student in two upper-level, science-based classes and as an undergraduate student involved in sophisticated research. Mr. Johnson has been working with Dr. Charles Riggs on a research project to determine the alteration in glycogen resynthesis in mice who demonstrate a deficiency in a very-long-chain fatty acid coenzyme (VLCAD). This is significant in that individuals deficient in this particular coenzyme A dehydrogenase may exhibit a reduction in muscle function especially at a young age. He has demonstrated a particular interest and affinity for this research methodology and has been a key player in the analytical process. The purpose of Mr. Johnson's research is to determine the role of glycogen resynthesis in muscle during the recovery from intense exercise in mice deficient in VLCAD. Little research has appeared in this area in our professional literature. With the knowledge obtained through Mr. Johnson's project, we may be able to explain hypoglycemic responses, muscle weakness, cramping, rhabdomyolysis and even death in individuals who suffer from this deficiency.