Effect of exercise on proglycogen and macroglycogen content in skeletal muscles of pigs with the Rendement Napole mutation

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Muscle glycogen content and its rate of degradation before and after slaughter play an important role for quality of pork. The dominant Rendement Napole (RN) mutation is common in Hampshire pigs and causes an increase in glycogen stores of approximately 70% in skeletal muscles. The RN mutation is a missense mutation (ie, R225Q) in the PRKAG3 gene, which encodes a muscle-specific isoform of the regulatory γ subunit of adenosine monophosphate–activated protein kinase (AMPK). The RN mutation has beneficial effects for lean meat content but detrimental effects for water-holding capacity and processing yield.

It is believed that AMPK plays an important role as a regulator of carbohydrate and fat metabolism in mammalian cells. Muscle glycogen content is an important energy source that is used during physical exercise, and high glycogen content is associated with optimal performance. Glycogen is found in 2 forms in skeletal muscle; the forms are characterized by the number of glucose units and their solubility in acid. Macroglycogen (MG) is an acid-soluble compound; it has a high ratio of glucose units to protein content and a molecular weight of approximately 10,000 kd. Proglycogen (PG) is an acid-insoluble compound; it has a low ratio of glucose units to protein content and a molecular weight of approximately 400 kd.

Humans and horses differ in their rate of degradation of MG and PG during exercise, depending on the intensity and duration of exercise. In addition, MG and PG are resynthesized at differing rates after exercise.

When total glycogen content increases in muscle after exercise because of a high carbohydrate intake, there appears to be greater synthesis of the MG fraction. To our knowledge, the influence of the RN mutation on MG and PG concentrations in muscle of pigs and on glycogen degradation and resynthesis in connection with physical stress has not been studied. Because pigs with the RN mutation synthesize more glycogen than do clinically normal pigs, we hypothesized that a great portion of glycogen in pigs with the RN mutation is stored as MG.

The objectives of the study reported here were to investigate MG and PG concentrations before exercise, after exercise on a treadmill, and during recovery after exercise in pigs that were and were not carriers of the RN mutation. Furthermore, it was of interest to determine whether pigs with the RN mutation could perform treadmill exercise for a longer period as a result of increased glycogen stores in muscle.

Two exercise tests were used in the study. The purpose of the first test was to allow all pigs to perform the same amount of work to study whether PG and MG degradation and response of blood lactate concentrations differed between groups. The purpose of the sec-
ond test was to allow all pigs to run until fatigued to study whether exercise performance differed between groups as a result of differences in muscle glycogen stores. In addition, glycogen degradation within various types of muscle fibers was evaluated by the use of histochemical analysis.

Materials and Methods

Animals—Eight healthy female purebred Hampshire pigs were purchased from a breeder. Each pig weighed approximately 20 kg. Use of DNA testing revealed that 5 pigs were carriers of the RN– mutation and 3 were not carriers (ie, rn+/rn–). The pigs were housed at the Department of Large Animal Clinical Sciences, Swedish University of Agricultural Sciences. Pigs were housed in 2 pens (4 pigs/pen); each pen had a concrete floor and straw bedding. The pigs were provided ad libitum access to a commercial diet formulated for finisher pigs and had unlimited access to water. The pigs were inspected daily to determine their state of health. All pigs remained healthy throughout the study, except for 1 pig with the RN– mutation. That pig was euthanatized because of lameness before the study was completed. The experimental design was approved by the Ethical Committee for Animal Experiments, Uppsala, Sweden.

Experimental design—The study was conducted during an 11-week period. Initially, there was a 2-week period of acclimation for the pigs during which blood samples were obtained for routine hematologic and biochemical analyses. The pigs were also acclimated to exercising on the treadmill. Pigs were allowed to walk on the treadmill for a few minutes on 3 separate days before the first exercise test was conducted.

Exercise tests—The first exercise test was performed during the third week of the study. Mean weight of the pigs at that time was 27 kg (range, 24 to 35 kg). Each of the 8 pigs ran on the treadmill. Two pigs ran on the treadmill concurrently. Speed of the treadmill was 1.5 m/s, and pigs ran a distance of approximately 800 m. Because of a problem monitoring the distance, the actual distances run by the 4 pairs of pigs were 878, 724, 801, and 807 m, respectively. The second exercise test was performed 8 weeks after the first exercise test. One pig was euthanatized because of lameness in the interval between tests. Mean weight of the remaining pigs at the time of the second test was 63 kg (range, 53 to 78 kg). Each pig ran separately on the treadmill at a speed of 1.5 m/s. Exercise was discontinued when the pig could not maintain its place on the treadmill despite gentle, humane encouragement.

Collection of muscle biopsy specimens and blood samples—On the day before each exercise test, pigs were anesthetized and muscle biopsy specimens were obtained from the right biceps femoris muscle. Anesthesia was achieved by administration of a combination of medetomidine and tiletamine-zolazepam. The mixture contained 1 mg of medetomidine/mL and 100 mg of tiletamine-zolazepam/mL and was administered IM at a dosage of 0.05 mL/kg. All muscle specimens were obtained from the middle part of the muscle at a depth of 3 to 4 cm by use of a needle-biopsy technique. Biopsy specimens were immediately frozen in liquid nitrogen and stored at −80°C until analyzed.

Immediately after each exercise test (within 15 minutes after completion of the first test and within 10 minutes after completion of the second test), anesthesia was administered locally and a biopsy specimen was obtained from the left biceps femoris muscle of each pig. The pigs were restrained at the front of the treadmill by use of a board to enable biopsy specimens to be collected. Twenty-four hours after the first exercise test and 3 hours after the second exercise test, the pigs were again anesthetized and another biopsy specimen was obtained from the right biceps femoris muscle of each pig.

Blood samples were collected from the cranial cava vein when pigs were anesthetized before the first exercise test. Blood samples were also collected within 15 minutes after completion of each exercise test. Blood samples were centrifuged immediately after collection, and plasma was harvested and used for analysis of lactate concentrations.

One day after completion of the second exercise test, the pigs were euthanatized. Euthanasia was accomplished by use of a captive bolt followed by exsanguination.

Analysis of PG and MG content—Muscle specimens were freeze-dried and dissected free from the admixture of blood, fat, and connective tissue by use of a dissection microscope. Freeze-dried muscle (1 to 2 mg) was immersed in 200 μL of ice-cold 1.5 M perchloric acid (PCA) and crushed with a glass rod. The mixture was allowed to incubate on ice for 20 minutes to permit extraction of MG into the PCA. The mixture was then centrifuged (1,500 × g at 4°C for 10 minutes), and 100 μL of supernatant was removed and used for analysis of MG content. An additional sample of supernatant was removed for analysis of free glucose concentrations. The remaining supernatant was discarded, but the pellet was saved and used for analysis of PG content.

Glycogen was hydrolyzed in the PG and MG sample tubes by the addition of 1M HCl. Tubes were sealed and heated to 100°C in a water bath for 2 hours. Glucose concentrations were then analyzed in all samples by use of a fluorometric technique. The free glucose concentration in the

Table 1—Mean ± SE values for body weight, distance run on the treadmill, and proglycogen (PG) and macroglycogen (MG) concentrations in biopsy specimens obtained from the biceps femoris muscles in pigs with the Rendem Napole (RN) mutation and noncarrier pigs before and after exercise tests conducted on a treadmill.

<table>
<thead>
<tr>
<th>Exercise test</th>
<th>Group</th>
<th>Weight (kg)</th>
<th>Distance run (m)</th>
<th>Before exercise</th>
<th>10 minutes after exercise</th>
<th>Hours after exercise*</th>
<th>MG (mmol/kg of dry weight)</th>
<th>Before exercise</th>
<th>Immediately after exercise</th>
<th>Hours after exercise*</th>
<th>PG (mmol/kg of dry weight)</th>
<th>Before exercise</th>
<th>Immediately after exercise</th>
<th>Hours after exercise*</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>RN carriers (5)</td>
<td>28 ± 2</td>
<td>801 ± 34</td>
<td>330 ± 19</td>
<td>220 ± 16</td>
<td>321 ± 15</td>
<td>283 ± 13</td>
<td>266 ± 8</td>
<td>250 ± 11</td>
<td>231 ± 47</td>
<td>198 ± 17</td>
<td>232 ± 26</td>
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<tr>
<td></td>
<td>Noncarriers (3)</td>
<td>27 ± 3</td>
<td>805 ± 2</td>
<td>162 ± 51</td>
<td>61 ± 16</td>
<td>131 ± 21</td>
<td>196 ± 6</td>
<td>192 ± 10</td>
<td>72 ± 3</td>
<td>162 ± 10</td>
<td>176 ± 11</td>
<td>49 ± 23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second</td>
<td>RN carriers (4)</td>
<td>66 ± 5</td>
<td>991 ± 156</td>
<td>347 ± 26</td>
<td>226 ± 27</td>
<td>309 ± 20</td>
<td>195 ± 6</td>
<td>192 ± 10</td>
<td>72 ± 3</td>
<td>182 ± 47</td>
<td>188 ± 17</td>
<td>210 ± 26</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Noncarriers (3)</td>
<td>65 ± 4</td>
<td>1,022 ± 208</td>
<td>189 ± 461</td>
<td>83 ± 24</td>
<td>98 ± 20</td>
<td>176 ± 11</td>
<td>182 ± 10</td>
<td>72 ± 3</td>
<td>176 ± 23</td>
<td>190 ± 11</td>
<td>50 ± 23</td>
<td></td>
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</tr>
</tbody>
</table>

Values in parentheses are number of pigs in each group.
*Biopsy specimen was obtained 24 hours after completion of the first exercise test and 3 hours after completion of the second exercise test. Within an exercise test, value differs significantly (P < 0.05) from the corresponding value for the pigs with the RN– mutation.
supernatant was subtracted from results for the heated MG sample to provide only the MG fraction. Total glycogen was calculated as the sum of the PG and MG fractions.

Analysis of lactate concentrations—Plasma lactate content was analyzed by use of a lactate analyzer.

Histochemical analysis—Serial cross-sections of muscle biopsy specimens were incubated with acid and alkaline solutions and then stained to reveal myosin-ATPase to enable us to identify type I, IIA, and IIB fibers. A semiquantitative estimate of glycogen content in muscle fibers was obtained from sections stained with periodic acid-Schiff (PAS). Fibers were classified as having high or low glycogen content, and a pattern of glycogen depletion was determined for each biopsy specimen.

Statistical analysis—An unpaired t test was used to compare values between the groups, whereas a paired t test was used to compare values within each group. Significance was set at values of P < 0.05. Results were reported as mean ± SE.

Results
First exercise test—Mean ± SE values for body weight, distance run, and MG and PG concentrations in muscle specimens were summarized (Table 1). Total glycogen concentrations (MG plus PG) before the first test were significantly higher in pigs with the RN− mutation (612 ± 30 mmol/kg of dry weight), compared with noncarrier pigs (393 ± 1 mmol/kg of dry weight). The PG fraction before exercise was slightly higher in pigs with the RN− mutation than in noncarrier pigs. Degradation was observed in the MG fraction to a similar degree in both groups of pigs after exercise (pigs with the RN− mutation, 0.14 ± 0.03 mmol/kg of dry weight/m; noncarrier pigs, 0.13 ± 0.03 mmol/kg of dry weight/m). No significant changes were detected in the PG fraction after exercise. Resynthesis of MG after 24 hours of recovery from exercise was not different between groups.

Before exercise, plasma lactate concentrations were 1.9 ± 0.4 mmol/L in noncarrier pigs and 2.1 ± 0.4 mmol/L in pigs with the RN− mutation. After the first exercise test, plasma lactate concentrations increased significantly in all pigs, and the mean concentration was 13.2 ± 2.5 mmol/L in noncarrier pigs, which did not differ significantly from the mean concentration (16.8 ± 1.5 mmol/L) in pigs with the RN− mutation. After 24 hours of recovery from exercise, plasma lactate concentrations had decreased in all pigs to a concentration similar to that detected before exercise (noncarrier pigs, 2.5 ± 0.6 mmol/L; pigs with the RN− mutation, 1.5 ± 0.4 mmol/L).

Second exercise test—Mean values for body weight, distance run, and MG and PG concentrations in muscle specimens were summarized (Table 1). The pigs ran until fatigued, and large differences were seen among pigs in both groups. Mean ± SE total glycogen concentrations (MG plus PG) after exercise were significantly higher in pigs with the RN− mutation (543 ± 22 mmol/kg of dry weight), compared with the mean concentration for noncarrier pigs (367 ± 38 mmol/kg of dry weight). The PG fraction after exercise did not differ between pigs with the RN− mutation and noncarrier pigs. Degradation was observed in the MG fraction to a similar degree after exercise in pigs with the RN− mutation (0.13 ± 0.03 mmol/kg of dry weight/m) and noncarrier pigs (0.10 ± 0.01 mmol/kg of dry weight/m). No significant changes were seen in the PG fraction after exercise. Resynthesis of MG at 3 hours of recovery from exercise was significantly higher in pigs with the RN− mutation (83 mmol/kg of dry weight) than in noncarrier pigs (13 mmol/kg of dry weight).

Histochemical analysis of muscle specimens—No difference was seen between groups with regard to composition of fiber types. Mean ± SE value for all pigs was 23 ± 2% for type I, 12 ± 2% for type IIA, and 65 ± 2% for type IIB fibers.

Results of histochemical staining with PAS were evaluated (Figure 1). Examination of muscle specimens obtained before exercise revealed that all type IIA and IIB fibers were classified as having high staining intensity for glycogen. Type I fibers also had high staining intensity for glycogen, except for some type 1 fibers from pigs with the RN− mutation (29 ± 10%) and noncarrier pigs (17 ±
17%) that had low staining intensity for glycogen. After exercise, a higher percentage of fibers had low staining intensity for glycogen in pigs with the RN– mutation (type I, 78 ± 9%; type IIA, 41 ± 24%; and type IIB, 8 ± 4%) and noncarrier pigs (type I, 62 ± 3%; type IIA, 62 ± 3%; and type IIB, 4 ± 0%). Staining patterns did not differ between groups.

Discussion

The markedly higher total glycogen content in the biceps femoris muscle of pigs with the RN– mutation, compared with content of noncarrier pigs, is in agreement with several other studies14 in which investigators analyzed the longissimus dorsi muscle at slaughter. There were only 3 noncarrier pigs in the study reported here, but the total glycogen content in the biceps femoris muscle of these pigs was in good agreement with other observations.10,12

A novel result from our study was that the high amount of glycogen found in the pigs with the RN– mutation was mainly stored as MG. The pigs with the RN– mutation had almost twice as much MG before exercise, compared with values for the noncarrier pigs. Thus, the MG fraction was higher than the PG fraction in pigs with the RN– mutation, whereas the opposite pattern was seen for noncarrier pigs. The PG concentrations in the muscle of pigs with the RN– mutation and noncarrier pigs were similar to the values (223 to 242 mmol/kg of dry weight) obtained in the longissimus dorsi muscle of crossbred Danish pigs.11 In the pigs of that other study, the MG fraction (115 to 124 mmol/kg of dry weight) of noncarrier pigs was lower than the PG fraction, which is in agreement with results for noncarrier pigs of the study reported here. Concentrations of MG that are lower than those of PG have also been documented in muscle of healthy humans,10,11,12,23 horses,12,24 and rats.13 In those studies, resting total glycogen concentrations were between 300 and 500 mmol/kg of dry weight. A slightly higher fraction of MG than PG is found in horses when resting glycogen concentrations are as high as 500 to 600 mmol/kg of dry weight.10,11 This agrees with results for pigs with the RN– mutation in the study reported here, which had a high resting glycogen content of > 500 mmol/kg of dry weight and a high fraction stored as MG. When the PG fraction reaches approximately 200 to 250 mmol/kg of dry weight, it appears that the MG fraction is synthesized to a greater extent and therefore contributes more to the total glycogen fraction.

Analysis of results from the first exercise test revealed that glycogen, mainly from the MG fraction, was degraded to a similar extent in both groups of pigs. Analysis of results from the second exercise test revealed great differences among pigs in time until fatigue for pigs with the RN– mutation and noncarrier pigs. However, once again, the amount of glycogen degradation per meter did not differ between groups, and mainly MG was degraded. The total glycogen degradation per meter in the study reported here was in good agreement with results from another study12 in which Swedish Landrace pigs performed 10 minutes of exercise on a treadmill at a speed of 1.8 m/s.

Analysis of results from both exercise tests revealed that the high MG concentrations in the muscle of pigs with the RN– mutation did not seem to influence the rate of glycogenolysis during exercise because pigs with the RN– mutation responded similarly to the noncarrier pigs. Although the magnitude of change in the MG fraction did not appear to differ between the groups, the relative changes did. In noncarrier pigs, more than half of the total MG store was depleted, whereas only a third of the MG store was degraded in pigs with the RN– mutation.

The fact that no marked changes were seen in the PG fraction in the study reported here may have been related to the type of exercise used. Studies on humans22 and horses25 reveal a use of PG when exercise is of high intensity and short duration. A greater degradation from the MG fraction, compared with that for the PG fraction, in muscle has been observed in humans after running a marathon22 and in horses after endurance rides.11

In pigs with the RN– mutation and noncarrier pigs, a high percentage of type I and type IIA fibers but only a low percentage of type IIB fibers were depleted of glycogen after exercise (Figure 1). This indicates that these fibers had been recruited during exercise and that MG had been used as an energy source. These results agree with those of studies on humans22 and horses25 in which fiber recruitment during exercise appears to change from type I to type IIA followed by type IIB as the duration or intensity of exercise increases. Most of the type IIB fibers did not reveal any noticeable depletion of glycogen, which indicated that they had not been recruited during exercise. However, PAS stains are only semiquantitative, and there could also have been degradation of glycogen in these fibers without it being detected on the PAS-stained specimens, especially when resting glycogen concentrations are high in these fibers. Type I and type IIA muscle fibers in pigs have a high oxidative capacity, whereas most type IIB muscle fibers have a low oxidative capacity.21 Therefore, fatigue may develop quickly in type IIB fibers after recruitment because of the need for glycogenolysis with lactate production. The increased blood lactate concentrations observed after exercise in pigs with the RN– mutation and noncarrier pigs supported the hypothesis that type IIB fibers were recruited and that energy was released by anaerobic metabolism. This is in agreement with other studies22,23,24 in which increased blood lactate concentrations were found when pigs exercised on a treadmill at speeds between 1.5 and 2.0 m/s.

Muscle biopsy specimens in the study reported here contained as much as 69% type IIB fibers but only 12% type IIA fibers and 23% type I fibers. It is likely that type IIB fibers are most affected by the RN– mutation and consequently store large amounts of MG. Results of an electron microscopy study support this because accumulation of glycogen particles was mainly observed from pigs with the RN– mutation in muscles containing type IIB fibers. Furthermore, the RN– mutation increases oxidative capacity only in muscles such as the longissimus dorsi muscle that contain type IIB fibers, whereas there is no influence on the oxidative capacity in mus-
cles, such as the semispinalis capitis muscle, that contain mostly type I and IIA fibers. Thus, fibers that have a high oxidative capacity, such as type I and type IIA fibers, may be less affected by the RN– mutation. Because pigs with the RN– mutation had higher MG content than was detected in noncarrier pigs, it is likely that type IIB fibers contain more MG. However, it was not possible to detect this by use of the PAS stain because fibers that contain large amounts of glycogen will all be classified as having high staining intensity.

The RN– mutation in the PRKAG3 gene is associated with faster resynthesis of MG early during the recovery period after exercise in pigs with the RN– mutation. The β3 isoform of AMPK is the predominant isoform, especially in muscles containing type IIB fibers. The role of the AMPK system in eukaryotic cells is to maintain energy homeostasis. Activation of AMPK by exercise is considered to mediate stimulation of glucose transport.

Analysis of the results reported here suggests that the RN– mutation is not an activating or dominant-negative mutation that leads to impaired glycogen use during the type of exercise used in the study. Pigs with the RN– mutation used glycogen during exercise to the same extent as did the noncarrier pigs. The significant increase in rate of glycogen resynthesis after exercise in pigs with the RN– mutation suggests that it is an activating mutation that leads to an increase in glucose uptake and therefore a higher set point for glycogen storage in muscles. However, more studies on the rate of resynthesis of glycogen after exercise are needed to confirm this hypothesis.

The high muscle glycogen content in pigs with the RN– mutation is mainly attributable to the storage of MG. The RN– mutation does not impair glycogenolysis during exercise but may induce faster resynthesis of MG after exercise. Furthermore, the RN– mutation does not alter exercise performance in pigs.

References