BEHAVIORAL STRESS AND SKELETAL MUSCLE GLYCOGEN METABOLISM IN YOUNG BULLS

J. M. McVeigh and P. V. Tarrant

Dunsinea Research Centre, An Foras Taluntais, Co Dublin, Ireland

and

M. G. Harrington

University College, Belfield, Dublin 4, Ireland

Summary

Friesian bulls were penned with an established group of bulls for 6 h. This mixing procedure normally resulted in an intense behavioral interaction between the established herd and each of the newly introduced experimental animals. Muscle needle biopsy samples and blood samples were taken before stress and at intervals during the recovery period. Stressed bulls showed increases over controls in body temperature (P<.001), heart rate (P<.001), serum nonesterified fatty acids (P<.05) and plasma creatine kinase (P<.01). Muscle glycogen content fell during stress to 41% of the value for the control animals (P<.001). On the first day of the recovery period, the glycogen content increased slightly, to 45% of the mean control value, and on d 2 it increased substantially, to 70%. A significant difference existed between experimental and control groups until d 7 of the recovery period. Blood glucose did not differ significantly between the control and experimental groups immediately after stress or during the recovery period. Muscle glucose-6-phosphate was lower in the experimental animals immediately after stress (P<.01) and also on d 1 of recovery (P<.05). Plasma immunoreactive insulin remained close to 40 μunits/ml in both groups, except on the day after stress, when it was below (P<.05) the prestress value. The results show that substantial muscle glycogen breakdown occurred during the period of behavioral stress and that recovery to resting values was a comparatively slow process. It was concluded that the inherently lower blood glucose concentration and insulin activity in cattle than in nonruminant species may reduce glucose availability in cattle and thereby delay muscle glycogen recovery.

(Key Words: Behavioral Stress, Friesian Bulls, Muscle Biopsy Samples, Glycogenesis, Glycogenolysis, Plasma Insulin.)

Introduction

Glycogenolysis in skeletal muscle is activated by physiological stress, including heavy muscular work. In cattle, muscle glycogen deficiency at slaughter results in dark-cutting beef. This problem is of commercial importance in many major beef-producing countries (Tarrant, 1980). Dark-cutting is considered to result from exposure to various forms of stress during the preslaughter period (Hedrick, 1958; Lawrie, 1958) including mixed penning (Grandin, 1978). However, little is known about the rate and extent of glycogen breakdown and recovery in stressed cattle. One reason for this has been the experimental limitation imposed by post-mortem sampling.

In the present study, the Bergström percutaneous muscle biopsy needle was used to monitor muscle metabolism over extended periods in individual cattle. The Bergström needle was previously found to be acceptable for repeated muscle sampling in cattle (Tarrant and McVeigh, 1979). The object of the research was to determine changes in muscle glycogen and related metabolites in response to a common stress situation in cattle.
Materials and Methods

Animals. The experimental animals were 12 Friesian bulls aged 12 to 16 mo and weighing 355 to 453 kg. They consumed a concentrate barley diet (Keane, 1979) ad libitum from 6 mo of age. Throughout the experiment, they were individually tied in stalls and were familiarized with some of the handling and sampling procedures during a 7- to 10-d period before the start of the experiment, in order to reduce or eliminate the possibility of stressing by these procedures (Tarrant and McVeigh, 1979).

Experimental Design. Resting muscle and blood samples were taken twice, at 7 and 4 d before the imposition of experimental stress. The experimental animals were then randomly divided into two groups of six each, a control group and a stress group. The latter animals were stressed on d 0 by being put, two or three at a time, into a pen with an established herd of 10 adult Friesian bulls with which they had no previous contact. The animals were allowed to mix freely for 6 h. Muscle and blood samples were taken immediately after the stress period and at appropriate intervals during a 17-d recovery period.

Sampling Procedure. At each sampling time, muscle and corresponding blood samples were taken. Before the animal was removed from its stall, heart rate was measured by stethoscope for 1 min, and the rate obtained during the final 15 s was used. A blood sample was then taken from the jugular vein by puncture. The animal was moved (100 to 200 m) to a cattle restraining chute, where rectal temperature was measured before muscle sampling. Samples were taken from the M. longissimus by a percutaneous needle biopsy technique (Tarrant and McVeigh, 1979).

The sampling techniques and preparative procedures for blood fractions and muscle biopsy specimens have been described (Tarrant and McVeigh, 1979).

Biochemical Analysis. Blood glucose, serum nonesterified fatty acids (NEFA) and plasma creatine kinase were assayed as reported by Tarrant and McVeigh (1979). A unit of creatine kinase is the enzyme activity that causes the reaction of 1 μmol of substrate/min at 25 C.

Insulin was measured in plasma stored frozen at −27 C for up to 24 mo. Lengthy storage of frozen plasma does not affect the immunoreactive insulin (IRI) content (Trenkle, 1972). Samples were analyzed by radioimmunoassay with 125I-insulin. The radioimmunoassay kit used antisera to porcine insulin and was designed for measurement of human insulin. The percentage recovery of bovine insulin was estimated with crystalline bovine insulin dissolved by alkalization with NaOH and assayed at pH 7.5, and was found to be 101 ± 5.7%. The mean coefficient of variation for 50 triplicate assays of bovine plasma was 9.5%.

Muscle glycogen was assayed in frozen biopsy samples stored in liquid N. The frozen tissue was extracted in HCl and the glycogen content was determined enzymically as described by Tarrant and McVeigh (1979). Subsequent research (McVeigh, 1981) established the necessity to include α-amylase (Type 1-A) with amyloglucosidase in the glycogen hydrolyzing medium. Use of this enzyme increased the recovery of muscle glycogen by 3.5%. In the light of this finding, the present results have been adjusted by this amount.

For glucose-6-phosphate assay, the frozen muscle sample was homogenized in ice-cold 6 M perchloric acid (19.25 ml/g wet tissue) and further disintegrated on a Potter S homogenizer. The filtrate was neutralized with 5 M K2CO3 and CO2 was removed under vacuum. Glucose-6-phosphate was determined with glucose-6-phosphate dehydrogenase and NADP+ (Lamprecht et al., 1974).

Statistical Analysis. An unpaired Student's t-test was used to test for statistical differences between the stressed and unstressed groups (intergroup test) and also to compare observations with control or resting values (i.e., d −7 and −4; intragroup test).

Results

The mixing stress was accompanied by a strong physiological response with major changes in all the physiological characteristics examined. The unaccustomed and intense physical activity associated with the mixing procedure was undoubtedly a major cause of these changes.

Rectal temperature (figure 1) immediately
The mean glycogen repletion rate over the first 7 d of the recovery period was 6.6 μmol·g⁻¹·d⁻¹.

The mean glucose-6-phosphate concentration in the unstressed group (n = 6 animals) throughout the experiment was 1.8 ± .21 μmol/g wet tissue (table 1). The effect of stress was to lower glucose-6-phosphate concentration. Immediately after the 6-h stress, glucose-6-phosphate was lower (P<.01) than in the unstressed group. The concentration was also low on d 1 (P<.05), but by d 2, there was no significant difference between the two groups.

There was no significant difference between the unstressed and stressed bulls in blood glucose (figure 5) or plasma IRI concentration (figure 6) during the recovery period. However, an intragroup test on the stressed animals confirmed that plasma IRI fell after stress and on d 1 was significantly lower than the resting value (P<.05). Mean values for the unstressed group throughout the entire experiment (n = 6 animals) were 3.27 ± .080 μmol/liter for blood glucose and 40 ± 4.3 μunits/ml for plasma insulin.
TABLE 1. MUSCLE GLUCOSE-6-PHOSPHATE AND PLASMA CREATINE KINASE CONCENTRATIONS IN STRESSED AND NONSTRESSED FRIESIAN BULLS

<table>
<thead>
<tr>
<th>Day</th>
<th>Glucose-6-phosphate b</th>
<th>Plasma creatine kinase c</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unstressed</td>
<td>Stressed</td>
</tr>
<tr>
<td>-7</td>
<td>1.5 ± 0.20(5)</td>
<td>2.2 ± 0.55(6)</td>
</tr>
<tr>
<td>-4</td>
<td>1.3 ± 0.22(3)</td>
<td>1.8 ± 0.33(5)</td>
</tr>
<tr>
<td>0</td>
<td>1.9 ± 0.09(5)</td>
<td>0.7 ± 0.19**(5)</td>
</tr>
<tr>
<td>1</td>
<td>2.5 ± 0.49(3)</td>
<td>1.1 ± 0.24*(6)</td>
</tr>
<tr>
<td>2</td>
<td>1.5 ± 0.33(4)</td>
<td>1.6 ± 0.48(6)</td>
</tr>
<tr>
<td>4</td>
<td>1.7 ± 0.31(5)</td>
<td>1.6 ± 0.27(5)</td>
</tr>
<tr>
<td>7</td>
<td>2.0 ± 0.32(5)</td>
<td>1.7 ± 0.22(6)</td>
</tr>
<tr>
<td>10</td>
<td>1.7 ± 0.12(5)</td>
<td>2.2 ± 0.33(6)</td>
</tr>
<tr>
<td>14</td>
<td>2.6 ± 0.74(4)</td>
<td>1.5 ± 0.48(5)</td>
</tr>
<tr>
<td>17</td>
<td>1.7 ± 0.23(5)</td>
<td>2.1 ± 0.27(5)</td>
</tr>
</tbody>
</table>

aValues listed are means ± SE; number in parenthesis is number of observations.

bμmol/g wet tissue.

cUnits/liter at 25 C.

*P<.05. **P<.01. Significance levels indicated by intergroup test.

Discussion

Resting muscle glycogen concentrations (94 ± 5.6 and 90 ± 4.0 μmol/g in the unstressed and stressed groups, respectively) were similar to values reported for humans (86 μmol/g), pigs (83 μmol/g) and horses (98 μmol/g; Hultman et al., 1971; Tarrant et al., 1972; Lindholm and Piehl, 1974). The present value is almost twice that reported for beef M. longissimus sampled shortly after slaughter (50 μmol/g; Bendall, 1973). The lower value reported by Bendall may reflect glycogen breakdown at slaughter. Also, the present results were obtained on barley-fed bulls. A carbohydrate-rich diet favors muscle glycogen deposition (Hultman et al., 1971) while castration has resulted in reduced muscle glycogen in several muscles of some mammalian species (Gillespie and Edgerton, 1970).

Mixing was accompanied by a high degree of physical activity, which gradually lessened during the 6-h stress period. Physical exchanges between the newcomers (experimental animals) and the established group consisted of aggressive butting and pushing, and frequent mounting motivated by sexual impulse. This activity is associated with the reestablishment of group social order (Bouissou, 1980). Physical activity was probably accompanied by emotional arousal in response to a novel and threatening situation.

Mixing stress caused increases in body temperature (figure 1), heart rate (figure 2) and in blood constituents that respond to stress (table 1, figure 3). Temperature and heart rate are known to respond to strenuous muscular exercise, and cardiac acceleration also occurs in response to emotional excitement. Elevated plasma creatine kinase activity is associated with strenuous or unaccustomed muscular exercise (Berg and Haralambie, 1978), and also with adrenaline injection (McVeigh, 1981). Creatine kinase has been suggested as a "stress indicator" in athletes (Berg and Haralambie, 1978). Elevated serum NEFA indicates increased
beta-adrenergic activation of lipolysis. Increased NEFA content is associated with prolonged physical exercise and was also reported in sheep after minor surgical interference or adrenaline injection (Moseley and Axford, 1973). It was concluded from these measurements that mixed penning is an intense stressor with a high exercise component (confirmed by visual observation).

The probable cause of muscle glycogen breakdown was the increased intracellular energy demand during muscle contraction, combined with \( \beta \)-adrenergic activation of glycogenolysis. Activation of the adrenergic system during exercise is much stronger when accompanied by emotional stress than it is without the additional stress (Karki, 1956). The present experimental treatment included both exercise and emotional stress.

The slow rate of glycogen repletion was unexpected in view of the favorable conditions under which the animals were held, in relation to rest and nutrition, and also in view of relatively rapid rates of repletion reported for other species. The mean rate of muscle glycogen repletion for the first 2 d poststress was 14 \( \mu \)mol·g\(^{-1}\)·d\(^{-1}\) and for the first 7 d poststress was 6.6 \( \mu \)mol·g\(^{-1}\)·d\(^{-1}\). Recovery rates of about 118 \( \mu \)mol·g\(^{-1}\)·d\(^{-1}\) (Hultman, 1967), 95 \( \mu \)mol·g\(^{-1}\)·d\(^{-1}\) (Maehlum et al., 1978) and 58 \( \mu \)mol·g\(^{-1}\)·d\(^{-1}\) (MacDougall et al., 1977) were observed immediately after exercise in human subjects on normal or high carbohydrate dietary intake. High recovery rates were observed in rat muscle during the first 4 h after exercise; rates ranged from 190 to 343 \( \mu \)mol·g\(^{-1}\)·d\(^{-1}\), depending on the particular muscle studied (Terjung et al., 1974). These results were confirmed by Conlee et al. (1978).

There is approximately a 10-fold difference between the muscle glycogen repletion rates observed in the young bulls and those reported for humans and rats. Monin (1980) depleted muscle glycogen reserves in sheep by administering adrenaline and observed a recovery rate of 19 \( \mu \)mol·g\(^{-1}\)·d\(^{-1}\). This is close to the present value for cattle, suggesting that slow repletion rates may be characteristic of ruminants.

Glycogenesis in muscle is dependent on an adequate supply of the glycogen precursor, glucose-6-phosphate, the source of which is blood glucose. The entry of blood glucose into the muscle cell is stimulated by insulin (Krahl, 1961). The present results show that the mixing stress did not cause a substantial or long-term decrease in the concentration of these precursors (table 1, figure 5) or in that of insulin (figure 6) compared with the levels in the control animals. Small decreases were observed, however, in blood insulin and muscle glucose-6-phosphate contents during the 24-h period immediately after stress, signifying a reduction in the availability of glucose-6-phosphate. Glycogen recovery during this period was very slow (figure 4). Recovery of muscle glycogen during the subsequent week was not associated with below-normal concentrations of blood glucose, insulin or muscle glucose-6-phosphate.

Blood glucose content is inherently lower in cattle than in many nonruminant species (Spector, 1956). For example, the unstressed group of bulls had a mean blood glucose concentration of 3.27 ± 0.08 mmol/liter compared to a typical value in humans of 5.0 mmol/liter (Spector, 1956). Also, the activity of insulin in stimulating glucose uptake by muscle may be
inherently lower in ruminants than in non-ruminant animals (Reid, 1951, 1952; Prior and Christenson, 1978). These observations suggest that the availability of precursors for muscle glycogen synthesis may be lower in cattle than in many nonruminant species and provide a possible explanation for the comparatively slow rate of glycogen repletion reported here. A similar explanation may apply in the case of sheep, which also have slow muscle glycogen repletion rates (Monin, 1980) and low resting blood glucose content (Spector, 1956).

**Literature Cited**


