Effect of hypoenergetic feeding and high-carbohydrate refeeding on muscle tetanic tension, relaxation rate, and fatigue in slow- and fast-twitch muscles in rats1–3

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ABSTRACT Studies using nuclear magnetic resonance have shown that undernutrition affects muscle performance and energetics. It is unclear to what extent underfeeding and refeeding influence the availability of muscle glycogen, net glycogenolysis, skeletal muscle wasting, and recovery. We hypothesized that muscle performance is independent of muscle size and weight, is specific to muscle type, and is unrelated to muscle glycogen concentrations. Slow- and fast-twitch muscles were studied in three groups of adult male Wistar rats: well-fed controls, hypoenergetically fed (Hypo) rats, and rats refed for 4 d after the hypoenergetic diet. Glycogen concentrations and net glycogenolysis; serum glucose, insulin, and protein concentrations; and muscle weight, protein, and cross-sectional area were studied relative to the performance of both types of muscles. Our study controlled for muscle size, weight, and type and electrolyte-micronutrient deficiency. Undernutrition affected muscle performance in five ways. First, compared with controls, fatigue increased only in the soleus muscles of Hypo rats yet the maximal relaxation rate (MRR) decreased in both the soleus and extensor digitorum longus (EDL) muscles. Second, muscle glycogen concentrations did not significantly correlate with fatigue in either the soleus or the EDL although net glycogenolysis was significantly correlated with fatigue in the soleus (r = −0.64; P > 0.01 < 0.05). Third, lower glycogen concentrations did not hinder net glycogenolysis in the EDL of Hypo rats or the soleus of refed rats. Fourth, muscle weight, size, and protein were dissociated from function. Fifth, refeeding did not restore muscle endurance; however, the MRR of the soleus normalized. In conclusion, glycogen values and muscle performance did not correlate but net glycogenolysis correlated with fatigue in the soleus. Also, there was a dissociation between muscle weight, size, and protein and muscle function during hypoenergetic feeding and refeeding. Am J Clin Nutr 1997; 66:293–303.

KEY WORDS Muscle function, glycolysis, glycogen, malnutrition, glycogenolysis, muscle fatigue, body composition, slow-twitch muscle, fast-twitch muscle, soleus, extensor digitorum longus, rat

INTRODUCTION

Traditionally, the effects of malnutrition and feeding have been identified by assessment of wasting, restoration of total lean and fat body mass, and changes in plasma protein concentra
tions (1, 2). However, studies have suggested that changes in muscle performance may be a better predictor of nutrition-related surgical complications than is body composition (3, 4). Russell et al (5, 6), Pichard and Jeejeebhoy (7), and Christie and Hill (8) showed that electrically stimulated muscle function changes faster than standard nutrition-assessment variables such as anthropometric values and serum protein in response to nutritional manipulations. However, it is not clear from several studies (5, 6, 8, 9, 10) whether changes in performance were influenced by muscle size or weight, muscle glycogen and net glycogenolysis, or dietary intake of electrolytes and micronutrients.

We hypothesized that muscle performance is independent of muscle size and weight, is specific to muscle type, and is unrelated to muscle glycogen concentrations. Pichard et al (11) and Mijan de la Torre et al (12), in studies using 31P nuclear magnetic resonance (NMR) to assess stimulated muscle of hypoenergetically fed rats, found an increase in free ADP concentrations and altered respiration from ATP that suggested reduced mitochondrial activity. However, studies that explored the effect of hypoenergetic feeding and refeeding on muscle function did not control for intake of minerals and micronutrients and these nutrients would be deficient in rats fed restricted amounts of a commercial stock diet (a practice normally followed to produce underfed rats).

The role of minerals is important because attention to fluid balance and mineral intake is recognized as being especially important for optimal muscle performance (13). In addition, the response of different muscle fiber types has not been clearly differentiated. Therefore, in this study we used three liquid diets in which the electrolyte and micronutrient content was kept constant while the macronutrients were altered. Hence, we developed an energy-complete diet for well-fed controls, an energy-deficient diet (Hypo), and a high-carbohydrate energy-
complete diet for early refeeding. We compared the effects of hypoenergetic feeding and the effects of early refeeding with a diet complete in electrolytes and micronutrients on the weight, protein content, and performance [tetanic tension, maximal relaxation rate (MRR), and fatigue] of muscles of two different fiber types, namely, the soleus (mostly slow-twitch oxidative fibers) and the extensor digitorum longus (EDL) (mostly fast-twitch glycolytic fibers).

METHODS

Animals

Male adult Wistar rats (Charles River Laboratories, Montreal) were obtained for the study. On arrival from the supplier the rats were housed individually in an environmentally controlled area with an ambient temperature of 22 °C and a 12-h light-dark cycle. The rats were fed a commercial stock diet (Rodent Laboratory Chow; Ralston Purina, Toronto) and allowed to adapt to the animal facility for an average of ~2 wk before the start of the study. The rats weighed 350–380 g at entry into the study and were weighed daily thereafter.

Measurements of dietary intake, weight gain, and serum biochemical variables were taken. Muscle function, muscle glycogen, glycolytic metabolites were measured as described below.

Diet and feeding protocol

Rats were fed liquid diets made up of an amino acid mixture (Travasol Electrolyte Free; Baxter, Toronto), a lipid emulsion (Intralipid; Baxter), a glucose solution (Dextrose 50%; Baxter), vitamins (Multi-1000; Sabex Inc, Boucherville, Canada, and Barroca-C; Roche, Montreal), electrolytes, and trace elements (in injectable sterile bottles, Baxter) (Table 1). Animals were randomly assigned to two possible diets: a control diet complete in protein energy or a protein-reduced hypoenergetic diet that was given for 7 d. Rats in the Hypo group were either studied on the seventh day or were refed and studied over 4 d (RE1–RE4) during which they were fed a diet complete in protein energy. The control, hypoenergetic, and refeeding diets were isovolumic and complete in micronutrients: all rats were given a 120-mL dietary formula that contained the same amounts of vitamins, electrolytes, and trace elements (Table 1). Only chloride and acetate concentrations were slightly different between diets. This variation was necessary to ensure uniformity of micronutrients and other electrolytes between diets because many of the micronutrients and electrolytes were acetate- and chloride based and were already present to a small degree in the control diet before the micronutrients were added. The volume of 120 mL was sufficient to allow control, Hypo, and refed rats to consume ad libitum amounts.

The control rats were fed the liquid diet for 10 d. The energy density of the hypoenergetic diet, however, was such that only 20% of the energy of the control diet was provided to the Hypo rats (Table 1). The control diet contained 49.4 g protein/L and the hypoenergetic diet 6.4 g/L. Rats were fed in metabolic cages with drip collectors and the exact volumes consumed were measured. Keeping the diets isovolumic allowed the reduction in energy intake to be achieved in the Hypo rats without reducing the time over which the diet was consumed.

In the control rats consuming the nutrient-complete liquid diet muscle function was studied on days 7, 8, and 10 of consumption. The Hypo and refed rats were studied according to a similar time frame: on day 7 (Hypo) and on days 8 (RE1), 9 (RE2), 10 (RE3), and 11 (RE4).

Controls

Hoshino et al (14) showed that the liquid diet given to controls in this study resulted in normal growth and normal serum biochemical variables. In the current study controls lost 5% of their starting weight by day 4 because of loss of solid fecal contents and adaptation to the diet. After this adjustment period the rats gained an average 3.74 g/d. This growth rate was comparable with the typical 3–4 g/d weight gain expected for rats of this size (15). It is also comparable with or superior to rates reported (16, 17) in rats fed liquid diets. Control rats ingested a mean (± SEM) 77.1 ± 1.26% of the total volume provided, which represented a mean (± SEM) energy intake of 325.10 ± 5.44 kJ/d (77.7 ± 1.27 kcal/d). The macronutrient composition of the control diet was similar to that of the commercial stock diet.

Hypo rats

The Hypo rats ate 73.22 ± 2.50 kJ/d (17.50 ± 0.55 kcal/d) and consumed 85.5 ± 2.7% of the total volume of diet provided. The rats were observed to be satisfied with their diet, with its large volume of liquid, and did not appear hyperactive.

Refed rats

Rats were refed with a diet that had the same total energy content as the control diet but the carbohydrate content was increased at the expense of protein content (Table 1). The objective was to carbohydrate-load the muscles of Hypo rats to maximize muscle glycogen. We should emphasize that despite the increased glucose content of the diet, the amount of protein was not limiting; rats were observed to consume a mean of 2.57 g protein/d during refeeding. For rats of this size, normal growth necessitates ingestion of 2.4–2.85 g digestible protein/d
(18–20), an amount provided in the refeeding diet as readily available amino acids. Mean (± SEM) energy intake measured over 4 d was 315.06 ± 6.27 KJ/d (75.29 ± 1.48 kcal/d). Again, the water, electrolyte, and micronutrient content was identical to that in the control diet. While the rats were consuming the refeeding diet, the slope of weight gain was significantly greater than that for the controls (Figure 1), indicating that the diet provided sufficient macronutrients for accelerated growth.

Serum electrolyte, calcium, phosphorus, and magnesium concentrations remained constant and within the normal range in all groups of rats (controls, Hypo, and REl–RE4) throughout the study (Table 2).

Muscle-stimulation protocol

Exposure of muscle and stimulation conditions

The muscle-stimulation protocols were based on work by Russell et al (9) and Nishio and Jeejeebhoy (21). Rats were first anesthetized with an intraperitoneal injection of pentobarbital sodium (0.065 mg/g body wt). Supplemental doses equal to ≈14% of the initial dose were administered as required. When the animals were fully sedated the two hindlimbs were prepared according to protocols described elsewhere (21, 22). The sciatic nerve was exposed in the hip area and surrounded by two electrodes (Dantec 13L20 surface electrodes; Dantec Electromedical and Scientific Equipment, Ltd, Scarborough, Canada) embedded in plastic; this allowed the electrodes to be attached solidly by sewing together the two ends of the plastic sheath. The electrodes were in turn connected by means of a stimulus isolation unit (Grass SIU5: Grass Instrument Co, Quincy, MA) to a stimulator (Grass Stimulator S84: Grass Instrument Co).

The rats were placed on a dry base of silicone elastomer (Sylgard 184; Dow Corning, Elizabeth Town, KY) that overlay a double-walled 1.3-cm methacrylic acid box. Between the walls circulated warm water that kept the core temperature of the rats at 37 °C. Use of a heat lamp assisted in keeping body temperature at 37 °C and muscle temperature at about 33 °C as measured by a surface thermometer (model BAT-12: Physitemp Instruments Inc, Clifton, NJ). A nail was driven through the knee joint and pushed into the silicone base to ensure stability of the proximal tendon. The nail was tied securely with a 1–0 silk suture to a steel post that had been incorporated into the methacrylic acid box. The animals’ extremities were secured to the base with pins.

An open-mask method was used to administer to the rats a gas mixture containing 60% oxygen and 40% nitrogen. A previous study that followed a protocol similar to that used in this study found that the blood gas and pH in animals prepared in this way were normal in all nutritional states (11).

Measurement technique

The muscle tendon was connected to a transducer loaded with a yellow spring (Grass FT03; Grass Instrument Co) with a maximum work range of 0.05 kg and a displacement rate of 20 mm/kg. The transducer was connected to an analogue-digital converter (Labmaster; Scientific Solutions, Solon, OH) in a microcomputer with a 80386 processor (Intel, Folsom, CA) running at 33 MHz. The analogue-digital board was capable of converting the analog signal at 100 kHz. The digital output was transferred by direct memory access to the random access memory of the computer and stored on a disk. A customized program was used to display data graphically and the digital data were analyzed to obtain tension and MRR.

Stimulation protocol

Initially, it was determined that 20 V resulted in maximal stimulation by using square waves 75 μs in duration. Using this voltage we determined the optimal length of the muscle needed for a maximal twitch by using a method described previously (21). The stimulation protocols, which are described in Table 3, were then applied to the isolated soleus and EDL muscles. The measured tensions in both muscles were corrected for

![Figure 1](https://academic.oup.com/jnci/article-abstract/66/2/293/4655683/1)  
**Figure 1.** Weight of rats fed a hypoenergetic diet (Hypo; n = 11), refed a high-carbohydrate diet, and weighed on day 8 (n = 14), day 9 (n = 17), day 10 (n = 16), and day 11 (n = 11) compared with control rats (CN; n = 18) fed a high-energy diet over 10 d.
TABLE 2
Serum electrolytes, phosphate, and bilirubin in six groups of rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Hypo</th>
<th>RE1</th>
<th>RE2</th>
<th>RE3</th>
<th>RE4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mean n = 15)</td>
<td>(mean n = 6)</td>
<td>(mean n = 8)</td>
<td>(mean n = 13)</td>
<td>(mean n = 11)</td>
<td>(mean n = 6)</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>132.9 ± 2.5</td>
<td>142.3 ± 1.3</td>
<td>138.0 ± 2.0</td>
<td>139.0 ± 1.5</td>
<td>136.3 ± 3.0</td>
<td>141.3 ± 2.6</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>54.1 ± 0.1</td>
<td>5.1 ± 0.1</td>
<td>5.7 ± 0.3</td>
<td>5.5 ± 0.3</td>
<td>6.0 ± 0.3</td>
<td>6.1 ± 0.2</td>
</tr>
<tr>
<td>Chloride (mmol/L)</td>
<td>99.3 ± 1.4</td>
<td>105.5 ± 1.1</td>
<td>103.7 ± 2.0</td>
<td>100.7 ± 0.8</td>
<td>99.5 ± 1.7</td>
<td>101.8 ± 1.9</td>
</tr>
<tr>
<td>Calcium (mmol/L)</td>
<td>2.24 ± 0.07</td>
<td>2.31 ± 0.08</td>
<td>2.31 ± 0.08</td>
<td>2.27 ± 0.06</td>
<td>2.25 ± 0.10</td>
<td>2.42 ± 0.07</td>
</tr>
<tr>
<td>Magnesium (mmol/L)</td>
<td>1.02 ± 0.02</td>
<td>1.24 ± 0.04</td>
<td>1.02 ± 0.05</td>
<td>1.07 ± 0.04</td>
<td>1.08 ± 0.07</td>
<td>1.00 ± 0.04</td>
</tr>
<tr>
<td>Phosphate (mmol/L)</td>
<td>3.14 ± 0.34</td>
<td>3.28 ± 0.33</td>
<td>2.89 ± 0.29</td>
<td>2.95 ± 0.15</td>
<td>3.00 ± 0.30</td>
<td>3.35 ± 0.24</td>
</tr>
<tr>
<td>Bilirubin (µmol/L)</td>
<td>6.5 ± 0.3</td>
<td>6.8 ± 0.5</td>
<td>7.7 ± 0.8</td>
<td>7.1 ± 0.53</td>
<td>8.2 ± 0.54</td>
<td>6.7 ± 1.0</td>
</tr>
</tbody>
</table>

\(^1 \text{SEM. There were no significant differences among the groups (by ANOVA).}

\(^2 \text{Hypo, hypoenergetically fed rats; and RE1-RE4, rats refed for days 1–4, respectively, after a hypoenergetic diet.}

Bioassays of muscles

After the stimulation procedures samples for muscle biopsies were obtained by freeze clamping the isolated muscle type with forceps cooled in liquid nitrogen. The muscles were then lyophilized and stored at −70°C. Total protein in the samples was determined with the biuret test. Muscle glycogen in stimulated and unstimulated muscle was measured by using the method of Harris et al (25). The difference between glycogen content of unstimulated muscle and glycogen in muscle after 40 trains of stimulation (lasting 0.67 min) was referred to as net glycogenolysis and is reported in Table 4.

Blood biochemistry

Before any surgical intervention and muscle-function analysis, blood samples were taken from the retroorbital vein for determination of serum glucose and insulin concentrations. Serum insulin concentrations were determined by the immunoassay laboratory of Toronto Hospital with use of the technique described by Livesey et al (26). Serum glucose was determined by the biochemistry laboratory of Toronto Hospital with use of an Olympus AU 800 analyzer (Olympus Co, Dallas) and a method described by Zilva and Pannall (27).

With use of a 5-mL syringe and a 22-gauge needle, 3–5 mL blood was also taken from the inferior vena cava for measurement of poststimulation biochemical variables. The blood from both sources was centrifuged for 20 min at 3000 rpm and room temperature and the serum extracted and stored at −20°C. The serum was used to determine total protein (27) and transferrin concentrations (immunorhibometric method; Ames Co, Etobicoke, Canada), which were measured on a Hitachi 717 instrument (Boehringer-Mannheim, Montreal) at Toronto Western Hospital. Bilirubin concentrations and serum electrolytes (sodium, chloride, potassium, phosphate, and calcium) were measured at the biochemistry laboratory of Toronto Hospital with an Olympus AU 800 analyzer according to methods described by Tietz (28, 29) and Kuffer and Degiampietro (30).

Ethics

The dietary protocol, surgical procedure for muscle isolation, and muscle-stimulation protocols were approved by the Animal Care Committee of the University of Toronto. This committee’s mandate is based on guidelines of the Canadian Council on Animal Care. While in the holding facility the rats were cared for by a fully trained team of animal care technicians and were housed according to standards accepted by the Canadian Council on Animal Care.

Statistics

The data are expressed as mean ± SEM. Analysis of variance (ANOVA) was conducted among either four groups (control, Hypo, RE1, and RE4) or six groups (control, Hypo, RE1, RE2, RE3, and RE4) of rats. If ANOVA results were significant (P < 0.05) then unpaired comparisons were made between the different experimental groups by using Duncan’s multiple-range test for unplanned comparisons (31). Differences for glycogen values in unstimulated muscle were considered significant at P < 0.05. For all other comparisons differences were considered significant at P < 0.01.
TABLE 4  
Net glycogenolysis and postfatigue muscle glycogen concentration in the soleus and extensor digitorum longus (EDL) muscles in four groups of rats.

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Control (n = 8)</th>
<th>Hypo (n = 7)</th>
<th>RE1 (n = 6)</th>
<th>RE4 (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Net glycogenolysis</td>
<td>25.06 ± 0.94</td>
<td>4.44 ± 2.89“</td>
<td>12.44 ± 6.89“</td>
<td>19.83 ± 2.94“</td>
</tr>
<tr>
<td>EDL</td>
<td>67.50 ± 4.61</td>
<td>95.28 ± 9.67“</td>
<td>103.50 ± 6.17“</td>
<td>76.89 ± 8.17“</td>
</tr>
<tr>
<td>Postfatigue glycogen concentration†</td>
<td>86.83 ± 6.05</td>
<td>65.67 ± 2.78“</td>
<td>87.50 ± 9.61“</td>
<td>61.57 ± 6.30“</td>
</tr>
<tr>
<td>EDL</td>
<td>89.22 ± 5.50 [7]</td>
<td>54.61 ± 8.00 [7]“</td>
<td>125.94 ± 20.05 [5]“</td>
<td>87.94 ± 10.28“</td>
</tr>
</tbody>
</table>

1 ± SEM; n in brackets. Hypo, rats hypoenergetically fed for 7 d; RE1 and RE4, rats on the first and fourth day of refeeding after a hypoenergetic diet.
2 Significantly different from controls, P < 0.01 (by ANOVA and Duncan’s multiple-range test for unplanned comparisons).
3 Significantly different from Hypo rats, P < 0.01.
4 Muscle glycogen values were measured in muscles that were freeze-clamped on the 40th tetanic contraction.

RESULTS

Muscle weight, cross-sectional area, and protein

The weight of the soleus and EDL in control rats did not change significantly over the 10 d of the study and therefore data from those animals were pooled. The dry weights of both the soleus and EDL (Table 5) fell significantly (P < 0.01), by 10.0% and 16.76%, respectively, in Hypo rats. Protein content also fell (P < 0.01), by 16.1% and 13.0%, respectively, in the soleus and EDL. Muscle cross-sectional area decreased, by 14% and 13%, respectively, in the soleus and EDL (P < 0.01). The cross-sectional areas, dry weights, and protein contents of the soleus and EDL remained significantly below control values over the 4 d of refeeding. Only one measurement, namely, cross-sectional area in the soleus of RE4 rats, was not significantly below control values. Therefore, on day 4, two of three variables were below control values. In particular, protein content, which is commonly used as an index of nutritional repletion, was subnormal in RE4 rats.

Serum biochemistry

Serum biochemical values did not differ over the 10 d of study in control rats and data from those animals were pooled (Table 2). The relative ratio of glucose (mmol/L) to insulin (mmol/L) in controls (Table 6) was 0.62 and was comparable to that in well-fed control rats in a study by Zorzano et al (32). In Hypo rats compared with controls there was a significant (P < 0.01) decline in serum insulin and glucose concentrations. Furthermore, the glucose-insulin ratio increased significantly (P < 0.01) to 0.160 in Hypo rats, indicating a disproportionate increase in glucose relative to insulin and a possible fall in glucose access to cells. On the first day of refeeding insulin concentrations rose and the glucose-insulin ratio fell to 0.060, which was not different from control values. On days 2, 3, and 4 of refeeding the glucose-insulin ratios were significantly more elevated than control values. Serum electrolytes, phosphate, bilirubin, and γ-glutamyl transferase values were not different among control, Hypo, and RE1–RE4 rats.

Muscle-function measurements

The muscle-function variables discussed below did not change significantly over the 10 d of the study in controls and data from those rats were pooled.

Maximal tetanic tension

The maximal tension of contraction [Po(N)] of the soleus and EDL muscles fell in Hypo rats (P < 0.01) (Table 7). Refeeding restored the Po(N) in the soleus. In the EDL, refeed-

TABLE 5  
Total muscle protein concentrations, cross-sectional areas, and dry weights of soleus and extensor digitorium longus muscles (EDL) of Wistar rats.

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Control</th>
<th>Hypo</th>
<th>RE1</th>
<th>RE2</th>
<th>RE3</th>
<th>RE4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein (mg)</td>
<td>0.136 ± 0.006 [14]</td>
<td>0.118 ± 0.003 [8]</td>
<td>0.126 ± 0.004 [8]</td>
<td>0.123 ± 0.003 [9]</td>
<td>0.125 ± 0.003 [10]</td>
<td>0.129 ± 0.004 [10]</td>
</tr>
<tr>
<td>Cross-sectional area (cm²)</td>
<td>48.41 ± 2.20</td>
<td>43.57 ± 1.57</td>
<td>42.16 ± 1.50</td>
<td>40.88 ± 1.27</td>
<td>42.16 ± 1.52</td>
<td>41.93 ± 1.37</td>
</tr>
<tr>
<td>Dry weight (mg)</td>
<td>39.49 ± 0.98 [12]</td>
<td>34.36 ± 0.86 [10]</td>
<td>35.77 ± 1.05 [10]</td>
<td>36.31 ± 0.87 [10]</td>
<td>35.40 ± 0.80 [10]</td>
<td>35.13 ± 0.94 [9]</td>
</tr>
<tr>
<td>EDL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein (mg)</td>
<td>0.130 ± 0.004 [10]</td>
<td>0.113 ± 0.004 [8]</td>
<td>0.122 ± 0.002 [8]</td>
<td>0.123 ± 0.003 [9]</td>
<td>0.121 ± 0.002 [9]</td>
<td>0.117 ± 0.003 [9]</td>
</tr>
<tr>
<td>Cross-sectional area (cm²)</td>
<td>45.12 ± 1.02</td>
<td>37.56 ± 0.72&quot;</td>
<td>39.14 ± 0.94&quot;</td>
<td>40.39 ± 1.01&quot;</td>
<td>38.88 ± 0.59&quot;</td>
<td>39.56 ± 0.62&quot;</td>
</tr>
</tbody>
</table>

1 ± SEM; n in brackets. Hypo, hypoenergetically fed rats; RE1–RE4, rats refed for 1–4 days after a hypoenergetic diet.
2 Per kilogram dry weight of muscle multiplied by the dry weight of muscle to provide a total protein value.
3 Significantly different from controls, P < 0.01 (by ANOVA and Duncan’s multiple-range test for unplanned comparisons).
TABLE 6
Serum insulin and glucose, and ratio of glucose to insulin concentration

<table>
<thead>
<tr>
<th>Rat group</th>
<th>Insulin (µmol/L)</th>
<th>Glucose (µmol/L)</th>
<th>Glucose:insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 10)</td>
<td>143.6 ± 10.9</td>
<td>8.54 ± 0.69</td>
<td>0.062 ± 0.006</td>
</tr>
<tr>
<td>Hypo (n = 8)</td>
<td>43.9 ± 5.5</td>
<td>6.38 ± 0.44</td>
<td>0.160 ± 0.202</td>
</tr>
<tr>
<td>RE1 (n = 8)</td>
<td>111.7 ± 10.8</td>
<td>8.38 ± 0.66</td>
<td>0.060 ± 0.008</td>
</tr>
<tr>
<td>RE2 (n = 10)</td>
<td>110.7 ± 37.6</td>
<td>7.03 ± 0.40</td>
<td>0.096 ± 0.005</td>
</tr>
<tr>
<td>RE3 (n = 10)</td>
<td>98.8 ± 12.4</td>
<td>7.70 ± 0.25</td>
<td>0.074 ± 0.009</td>
</tr>
<tr>
<td>RE4 (n = 9)</td>
<td>101.6 ± 13.9</td>
<td>7.35 ± 0.32</td>
<td>0.074 ± 0.007</td>
</tr>
</tbody>
</table>

1 Invalid values.

2 Significantly different from controls, P < 0.01.

3 Significantly different from controls and Hypo rats, P < 0.01 (by ANOVA and Duncan’s multiple-range test for unplanned comparisons).

**Muscle glycogen**

**Glycogen in unstimulated muscles**

Glycogen in muscles in the well-fed controls did not differ over the 10 d of study and data from those animals were pooled. Seven days of hypoenergetic feeding resulted in a significant drop in glycogen concentrations in both the soleus and EDL muscles (P < 0.05) (Figures 2 and 3). In the RE1 group glycogen in both muscles increased markedly with initiation of refeeding. The concentrations in the soleus fell again in RE2 and RE3 rats and then returned to control values in the RE4 group. There was, however, no correlation between glycogen concentrations and fatigue in the soleus.

In contrast, the EDL maintained similar glycogen concentrations from RE1 to RE3, after which the concentrations rose significantly (P < 0.05). In the EDL, as in the soleus, there was no correlation between glycogen concentrations and fatigue.

**Net glycogenolysis during stimulation**

Net glycogenolysis decreased in the soleus (P < 0.01) of Hypo rats compared with controls and was above normal in the EDL of Hypo rats. Refeeding increased net glycogenolysis in the soleus (Table 4) and restored it to control values after 4 d. In the EDL there was above-normal net glycogenolysis on the first day of refeeding: this normalized after 4 d of refeeding. Overall, there was a significant negative correlation between fatigue and net glycogenolysis in the soleus (r = −0.64; P < 0.01 < 0.05) (Figure 4); however, no correlation was found in the EDL.

**DISCUSSION**

In this study hypoenergetic feeding caused significant losses in muscle weight, muscle cross-sectional area, and protein in both the EDL and soleus muscles of rats. We assume, therefore, that glycolytic and oxidative fibers are equally susceptible to atrophy as a result of hypoenergetic feeding. Furthermore,
our study found that these muscle losses were not all reversed after 4 d of refeeding with a high-carbohydrate diet. Dry weights also remained below control values even after 4 d of refeeding. Refeeding did, however, result in a rapid increase in body weight over the 4 d but weights remained significantly below control values on day 4.

Despite the slow recovery of muscle weight, size, and protein concentrations, some aspects of muscle function did normalize with refeeding, depending on the muscle type. Furthermore, subnormal glycogen concentrations in the fast-twitch EDL muscle of Hypo rats did not appear to increase fatigability of the muscle. In contrast, a significant fall in glycogen concentrations in the soleus caused fatigue to rise. These findings support our initial hypothesis that the composition of muscle, the type of muscle, and muscle glycogen concentrations are independent of muscle function. We also found that the degree of net glycogenolysis was specific to muscle type and was significantly increased in the EDL of Hypo rats. This model of early recovery, rather than emphasizing full recuperation of body-composition variables, placed more focus on the effect of early refeeding on changes in muscle function relative to critical substrates such as muscle glycogen, serum glucose, and the influence of insulin.

Functional response of muscles

Single tetanic contraction of muscle

The response to hypoenergetic feeding in the muscles studied was clearly different for the single tetanic contraction corrected for wet weight [PhN]/g, which remained normal in
the soleus of Hypo rats but became significantly subnormal ($P < 0.01$) in the EDL. Hood et al (33) showed that a relation exists between tetanic contraction and oxygen consumption in isolated rat muscle. Our data in Hypo rats are consistent with this relation because the soleus corrected for weight [Po(N)/g] was able to maintain tetanic tension, probably because of a continued blood and oxygen supply, but the EDL was not. The reason for the fall in tetanic tension in the EDL was not investigated in this study. However, in view of the normal response of the soleus and the normal serum biochemical values in the Hypo rats, the reduced tension of the EDL was likely due to muscular factors rather than nerve activation or systemic factors such as acidosis, hypoxia, or electrolyte abnormalities. The loss of tension was not due to glycogen depletion because the reduced tension persisted in REI rats and coincided with above-normal glycogen concentrations. It was also not due to impaired net glycogenolysis.

Additionally, Godt and Nosek (34), using skinned fast-twitch rabbit psoas fibers, showed that decreased muscle tension ($F_{\text{max}}$) resulted from increased hydrogen and inorganic phosphate and decreased affinity for ATP hydrolysis (reduced $\Delta G_{\text{ap}}$). Using $^{31}$P NMR to study the gastrocnemius muscle of rats (a muscle composed largely of fast-twitch fibers), we found previously a reduced pH and $\Delta G_{\text{ap}}$ in muscle from Hypo rats (11), which would lower the $F_{\text{max}}$. The inorganic phosphate, although unchanged, would be in a protonated form at a lower pH and thus contribute to the lower $F_{\text{max}}$, particularly in fast-twitch muscle (35). It is also worth considering that the elevated energy demand of this fast-twitch muscle (36) could possibly not be met in Hypo rats, thereby leading to some kind of metabolic failure resulting from a rapid depletion of phosphocreatine stores. Pichard et al (11) reported a significant decline in muscle phosphocreatine concentrations in Hypo rats. It is also known that the energy for a single tetanic contraction lasting $< 2$ s is derived from hydrolysis of ATP buffered by phosphocreatine reserves in the muscle (37). The lost tension in the EDL was, however, restored to normal by refeeding, thus confirming the nutritional origin of the reduced $F_{\text{max}}$ even though the muscle mass and protein content remained subnormal.

**Fatigability of muscle**

Fatigue, on the other hand, was increased in the soleus but not the EDL of Hypo rats. In controls the soleus, an oxidative muscle, lost only 29.7% of its initial force after 40 tetanic contractions. In contrast, the EDL, a muscle that relies mainly on glycolysis, lost 57.26% of its initial force after 40 tetanic contractions in controls. The soleus in Hypo rats showed increased fatigue and normal fatigability was not fully restored by refeeding. In contrast, the EDL, which was highly fatigable even in controls, did not show increased fatigue in Hypo rats. Fatigability of the muscles was assessed by measuring the percentage of initial tension lost after 40 repetitive tetanic contractions over 40 s. This test measures the ability to perform repetitive activity at a fast rate. One reason for inability to perform repetitive activity is lack of nerve response to a high rate of stimulation. Neuromuscular failure can in fact cause fatigue during sustained stimulation. However, the pattern of repetitive stimuli with rest periods of 0.5 s (soleus) and 0.7 s (EDL) between tetanic contractions precluded neuromuscular failure as a cause of fatigue.

With each tetanic contraction the energy for contraction is provided by ATP hydrolysis buffered by phosphocreatine. The high-energy substrates have to be restored during repeated tetanic contractions by a combination of anaerobic glycolysis and aerobic oxidation. The EDL in Hypo rats continued to use net glycogenolysis as a source of energy and did not show increased fatigue. This muscle was clearly not affected by the unusually high glucose-insulin ratio in Hypo rats. In contrast, the soleus in Hypo rats, which had reduced net glycogenolysis, did become fatigued, indicating that increased adaptation to fatty acids as a source of energy may reduce the ability of the muscle to sustain tension during repetitive contractions (38). This possibility was supported by a significant negative correlation between fatigue and net glycogenolysis in the stimulated soleus muscle.

**FIGURE 4.** Correlation between net glycogenolysis and fatigue in the soleus muscle ($n = 12$). The slope of the regression is significant ($r = -0.64$; $P > 0.01 < 0.05$; $n = 12$).
It is of interest that after glycogen depletion, long-distance runners (ultramarathoners), who rely on fatty acids as their main source of energy, can run at only one-half of their normal speed (39). It has been suggested that muscle function can be improved simply by infusing glucose and potassium (40), similar in concept to glycogen loading, a common practice in long-distance runners (41). In our study, however, glycogen depletion was not a factor in fatigue because even after the fatigue-inducing stimuli the soleus and EDL muscles in the Hypo rats retained 76% and 61%, respectively, of control glycogen concentrations. Moreover, no correlation was found between glycogen concentrations in the soleus muscle and fatigue nor could one be established between glycogen concentrations in the EDL and fatigue.

Others have also observed a lack of correlation between glycogen concentrations in muscle and fatigue (42–45). In contrast to the results in the EDL, it is also likely that the elevated glucose-insulin ratio may have been partly responsible for fatigue in the soleus of Hypo rats. The higher ratio indicates an unusual metabolism of glucose. This remains speculative, however, because glucose flux into the muscle was not measured in our study.

The cause of fatigue, if not based on glycogen concentration, can be considered alternatively within three contexts: a reduced release of Ca\(^{2+}\) from the sarcoplasmic reticulum, a reduced sensitivity of the myofibrils to Ca\(^{2+}\), and a reduced maximum Ca\(^{2+}\)-activated tension (46). In previous studies we showed that the gastrocnemius in Hypo rats had a reduced rate of rephosphorylation, increased free ADP concentrations, and reduced \(\Delta G_{\text{app}}\), all of which can alter the movement of calcium between the sarcoplasm and the sarcoplasmic reticulum. Consistent with abnormalities in Ca\(^{2+}\) kinetics in muscle in Hypo animals were the observations of O’Brien et al (47), who also observed impaired Ca\(^{2+}\) release from cardiac muscle in Hypo rats.

Another reason for altered Ca\(^{2+}\) kinetics is the reduction in myofibrillar sensitivity to Ca\(^{2+}\) due to reduced pH or increased inorganic phosphate (46). Reduced pH of muscle was unlikely to have been a factor in our experiment because in previous studies using 31P NMR spectroscopy to assess stimulated muscle (12), we showed that pH in the muscle of Hypo animals was comparable with that in controls. Finally, a reduction in the shortening speed of the fibers derived from a reduced Ca\(^{2+}\) activated tension may have occurred because of a reduced output of force for each cross-bridge or a decreased number of cross-bridges (46). This was not determined in our study.

**Maximal MRR**

The responses of the muscles to underfeeding were similar with respect to MRR, which was slowed in both the soleus and EDL of Hypo rats. The MRR of the initial tetanic contraction provides us with useful information regarding whether ATP-dependent calcium transport is compromised in any way during starvation or refeeding. MRR after a tetanic contraction is related to cross-bridge detachment resulting from the pumping of Ca\(^{2+}\) back into the sarcoplasmic reticulum against a concentration gradient (48, 49). In the EDL and soleus of Hypo rats the MRR was slower, indicating that Ca\(^{2+}\) pumping was also slower. These observations are consistent with our previous findings (19). Four possible mechanisms could alter MRR. The first is a fall in \(\Delta G_{\text{app}}\), as shown by Dawson et al (48). Additionally, we showed previously that muscles in Hypo rats have a lower \(\Delta G_{\text{app}}\) (11, 12). The second possible mechanism is loss of myofibrillar integrity at Z lines (50). Indeed, our studies in humans found Z-line disruption with hypoenergetic consumption (51). The third possible mechanism is inhibition of calcium reentry into the sarcoplasmic reticulum related to the activity of glycogenolytic enzymes (52). This was unlikely because the slower MRR in the soleus and EDL of Hypo rats coincided with either slower, normal, or above-normal net glycogenolysis (Table 4), indicating a poor association.

Finally, a disruption of the sarcoplasmic reticulum–glycogenolytic complex as suggested by Enteman et al (53) may have been involved. Although there was a fall in glycogen concentrations and MRR in the muscles of Hypo rats, MRR remained slower in RE1 rats when glycogen concentrations increased dramatically in both muscles. Therefore, disruption of the sarcoplasmic reticulum-glycogenolytic complex was unlikely to have been a factor in the slower MRR in the muscle of Hypo rats. In Hypo rats the MRR in the EDL, similar to that in the soleus, was slower, indicating that calcium uptake by the sarcoplasmic reticulum appears to be an abnormality common to both types of muscles and fibers.

In conclusion, our data showed a clear dissociation between muscle weight, protein, and function and a lack of correlation between glycogen concentration and muscle fatigue. Therefore, simply increasing muscle glycogen did not improve function after hypoenergetic feeding, as we observed in the soleus in RE1 rats (Figure 2). We did observe that net glycogenolysis was not associated with glycogen concentrations in the EDL muscle and that above-normal net glycogenolysis in Hypo rats maintained normal muscle fatigue in the EDL. In contrast, we found a negative correlation between fatigue and net glycogenolysis in the soleus muscle. This confirmed our hypothesis that dissociation of muscle function from muscle composition and glycogen content depends on muscle type.

The study design was amenable to such findings for three important reasons. First, the control group received the same amounts of micronutrients as the Hypo and refed rats, thereby excluding the possibility that any discrepancies observed in muscle function may have been related to micronutrient deficiencies. Second, the short-term refeeding period (4 d) was used because it favored study of very rapid changes in function relative to muscle composition. Third, the accuracy of measurements of glycogen concentrations and ultimately of net glycogenolysis can be attributed to the protocol for isolating the two soleus and EDL muscles of the hindlimbs and freeze clamping the stimulated muscle while it was still in contraction. This technique greatly diminished the possibility that differences in glycogen concentrations between groups were overshadowed by a more-elevated intergroup variability. Furthermore, diurnal variations in glycogen concentrations were controlled for during glycogen sampling.

MRR fell in both the soleus and EDL muscles of Hypo rats. MRR depends on calcium reuptake by the sarcoplasmic reticulum and is clearly influenced by hypoenergetic feeding. Refeeding normalized MRR in the soleus. Interestingly, refeeding did not restore the MRR to control values in the EDL. The reason for this dual response was not resolved in this study. However, these observations together with our recent observations in cardiac muscle in Hypo animals indicate a need for
further studies of the effect of nutrition on muscle calcium kinetics. The effect of altered cell energetics (which we showed previously to be related to a reduced rate of oxidative phosphorylation) on calcium kinetics may play an important mechanistic part that needs further elucidation. Furthermore, the importance of an above-normal glucose-insulin ratio in Hypo and RE2–RE4 rats may have some bearing on the persistent fatigue observed in the soleus; however, we did not establish whether glucose access to the cell is restricted. Rofe et al (54) observed an elevated glucose-insulin response in patients with cancer and associated it with a likely decrease in pancreatic function. It is not clear whether this was the case in our study but it is an area that needs further study.

Our data are consistent with those from patients with Crohn disease that showed improved function when body protein values remained subnormal (5). This observation strongly supports the concept that it is possible to optimize muscle function despite loss of mass, a finding that is important to the length and objectives of nutritional support in patients with wasting.

This paper is dedicated to the memory of Allan Bruce-Robertson, to whom we are grateful for his breadth of knowledge and practical insights that were helpful in the resolution of some technical problems, for his years of dedication to research, and for his kind and gentle presence that will be greatly missed.

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