Effect of long-term caloric restriction and exercise on muscle bioenergetics and force development in rats

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Horská, Alena, Larry J. Brant, Donald K. Ingram, Richard G. Hansford, George S. Roth, and Richard G. S. Spencer. Effect of long-term caloric restriction and exercise on muscle bioenergetics and force development in rats. Am. J. Physiol. 276 (Endocrinol. Metab. 39): E766–E773, 1999.—We evaluated the hypothesis that long-term caloric restriction and exercise would have beneficial effects on muscle bioenergetics and performance in the rat. By themselves, each of these interventions is known to increase longevity, and bioenergetic improvements are thought to be important in this phenomenon. Accordingly, we investigated rats that underwent long-term caloric restriction and were sedentary, ad libitum-fed rats permitted to exercise by daily spontaneous wheel running (AE), and the combination of the dietary and exercise interventions (RE). Ad libitum-fed, sedentary rats comprised the control group. 31P NMR spectra of the gastrocnemius muscle (GM) were collected in vivo at rest and during two periods of electrical stimulation. Neither caloric restriction nor exercise affected the ratio of phosphocreatine to ATP or pH at rest. During the first stimulation and after recovery, the RE group had a significantly smaller decline in pH than did the other groups (P < 0.05). During the second period of stimulation, the decrease in pH was much smaller in all groups than during the first stimulation, with no differences observed among the groups. The combination of caloric restriction and exercise resulted in a significant attenuation in the decline in developed force during the second period of stimulation (P < 0.05). A biochemical correlate of this was a significantly higher concentration of citrate synthase in the GM samples from the RE rats (32.7 ± 5.4 umol·min⁻¹·g⁻¹) compared with the AE rats (17.6 ± 5.7 umol·min⁻¹·g⁻¹; P < 0.05). Our experiments thus demonstrated a synergistic effect of long-term caloric restriction and free exercise on muscle bioenergetics during electrical stimulation.

skeletal muscle; energy metabolism; nuclear magnetic resonance spectroscopy

LONG-TERM CALORIC restriction is known to extend life span across a wide range of species (11). This nutritional manipulation retards the age-related decline in numerous physiological processes as well as delays or reduces the incidence of many age-related diseases. Although the effects of caloric restriction are well established, the mechanisms for its antiaging effects have not been identified, although several hypotheses have been proposed (11, 26). Caloric restriction does not appear to retard aging processes by slowing metabolic rate; lean body mass eventually adjusts to the reduced caloric intake so that the weight-adjusted caloric intake is the same in caloric-restricted and in ad libitum-fed animals (25). Caloric-restricted rats metabolize carbohydrates at the same rate per unit body mass as ad libitum-fed animals, but at lower plasma glucose and insulin levels (10, 24). Based on these and related studies, it has been hypothesized that reduced energy intake modulates the aging process by altering the characteristics of metabolic fuel use (24). Recently, it has been suggested that there are synergistic effects of moderate caloric restriction and moderate voluntary exercise (17).

Both acute hypocaloric intake (1, 9, 32, 35) and exercise (16, 18) have a profound effect on metabolism of high-energy phosphates in muscle. However, although changes in fuel utilization resulting from long-term caloric restriction have been well documented, the direct bioenergetic consequences have not been studied. Furthermore, little is known about the combined effects of long-term dietary restriction and exercise on energy metabolism. We therefore designed the present study to test if long-term caloric restriction and free exercise alone, and the combination of both interventions, will have a beneficial effect on high-energy phosphate metabolism in skeletal muscle of rats. Accordingly, we employed 31P NMR spectroscopy to evaluate relationships between metabolism and muscle force noninvasively and with high temporal resolution.

METHODS

Animals

Male Wistar rats between the ages of 14 and 17 mo were studied. The rats were obtained at weaning from the breeding colony at the Gerontology Research Center of the National Institute on Aging. The animals were kept in conditions of an artificial light cycle (from 10:00 PM to 10:00 AM), constant temperature of 22 ± 2°C, and controlled humidity of 70%. The rats were fed NIH-07 chow daily, at 20 g/d. All rats received adequate access to water. At 3 mo of age, 40 rats were randomly assigned to 4 groups: ad libitum fed sedentary (AS), ad libitum fed with free exercise (AE), caloric restricted sedentary (RS), and caloric restricted with free exercise (RE). At 3 mo of age, AE and RE rats were given free access to a running wheel attached to their home cage. The distance run in the wheel was monitored continuously. From 5 mo of age, rats in the RS and RE groups were placed on caloric restriction. The AS group constituted the diet control group. Food intake was measured weekly. Food intake was weighed before it was placed in...
the floor hopper on the cage, and visible pieces of food were retrieved from the floors of the cages and weighed as spillage. During a given week, the AE group was given the same amount of food as was consumed by the AS group during the previous week while the RS and RE groups received 30% less than this amount. The number of rats used for the current study was as follows: n = 7 in AS, n = 8 in AE, n = 9 in RS, and n = 10 in RE.

At the time of NMR experimentation, rats were anesthetized with an intramuscular injection of ketamine/xylazine (10:1, 100 mg ketamine/kg). The rats were placed on an isothermal pad to prevent heat loss during experimentation. Two platinum electrodes were placed percutaneously in the proximal parts of the gastrocnemius muscle (GM) and the Achilles tendon of the right hindleg, respectively. Pulses for supramaximal electrical stimulation of the GM were delivered from a Grass S11 (Quincy, MA) stimulator with a Grass SIU 5 stimulus isolation unit. The voltage applied was 120% of that required to initiate full muscle activity. The muscle was stimulated for 8 min with 500-ms trains of rectangular pulses each of duration 0.2 ms, with an interval between the pulses of 20 ms and an interval between the trains of 2 s. After an 8-min rest period, the GM was again stimulated for 8 min with the same stimulation parameters except for a decreased pulse interval of 10 ms. The second stimulation was followed by a 12-min recovery period. A ligature was attached to the foot and was connected to a Grass FT 10 force transducer to measure developed force throughout the protocol. The force transducer was placed outside of the magnet bore. Force levels in both periods of stimulation were expressed relative to initial force. At the end of the experiment, temperature was measured with a rectal thermocouple probe.

31P NMR Spectroscopy

31P NMR spectra were obtained with a 1.9 T, 31-cm Bruker Biospec spectrometer. A homebuilt single-tuned two-turn elliptical surface coil measuring 1.2 cm was used for spin excitation and signal reception. Magnetic field homogeneity was adjusted by shimming on water to proton line widths of <40 Hz. An adiabatic half-passage sin/cos pulse (5) of 2-ms duration was applied to achieve uniform excitation over the sensitive volume of the coil. Spectra were acquired with a repetition time of 2 s over a period of 2 min. Spectral width was 2,000 Hz, and 2,048 data points were acquired.

All NMR data were processed with NMR1 software (Tripos, St. Louis, MO). The free induction decays were corrected for DC offset, and a line broadening of 10 Hz was applied. After Fourier transformation and phasing of the spectra, manual baseline correction was performed using a polynomial fit. The resulting resonance lines were fit to a Lorentzian shape and integrated. For comparison of relative concentrations of Pi, PCR, and ATP at rest with published data, the signal intensities were corrected for saturation factors using spin-lattice relaxation time values of $T_1$ (Pi) = 4.2 s, $T_1$ (PCR) = 6.6 s, $T_2$ (Pi-ATP) = 2.2 s, and a value for the forward creatine kinase reaction rate constant of 0.2 s$^{-1}$ in accordance with previous studies (14).

Intracellular pH was calculated from the relative chemical shift of the Pi signal according to Meyer et al. (28). If the Pi signal was not visible, as was generally the case in spectra collected at rest and after the initial 2 min of recovery after stimulation, pH was estimated from the chemical shift of γ-ATP (30). This measurement was carefully calibrated by relating the chemical shifts of γ-ATP to the chemical shifts of Pi in those spectra where Pi was detectable, incorporating a range of pH values between 6.8 and 7.1.

Enzymatic Assays

Citrate synthase and 2-oxoglutarate dehydrogenase assays were performed in the GM from 17-mo-old rats from AE (n = 3) and RE (n = 5) groups. The rats were killed by decapitation, and the GM was excised and stored frozen at −80°C until required for assay. Whole muscle samples were then pulverized in liquid nitrogen using a mortar and pestle, weighed, and homogenized again in a medium comprised of 0.05 M potassium phosphate (pH = 7.4), 1 mM EDTA, 2 mM MgCl$_2$, 2 mM ADP, and 0.5 mM dithiothreitol, using a Dounce homogenizer (Thomas Scientific, Swedesboro, NJ). Homogenates were centrifuged at 100 g for 3 min. Citrate synthase and 2-oxoglutarate dehydrogenase activities were measured according to established procedures (12, 13).

Statistical Analysis

The difference between group sample means (for body weight, daily distance run in the exercise wheel, core temperature, resting values of metabolite concentrations and pH, and muscle enzyme concentrations) was assessed with the two-tailed Student’s t-test. Two-way repeated-measures ANOVA (Statview; Abacus Concepts) and linear mixed-effects regression analysis (SAS, Cary, NC; see Ref. 21) were used to compare the values of relative metabolite concentrations, pH, and force across the two periods of stimulation. In ANOVA analysis, Bonferroni and Tukey comparisons were used to assess differences among the groups. Statistical significance was assessed at P < 0.05. Data are shown as means ± SD.

RESULTS

Physiological Parameters

At the time of experimentation, average body weights were greatest in the AS group and lowest in the RE group (Table 1). The AE and RS groups had intermediate body weights. Note that 30% caloric restriction and free exercise each had the same effect on the body weight, and the effects of the two interventions were additive. The rats in the RE group were more physically active than their ad libitum-fed counterparts over the duration of the study. Their daily distance run in the wheel, calculated as an average over the whole period rats were allowed to exercise, was >40% larger than in the AE rats (Table 1).

At rest, the core temperature in all rats was found to be in the normal range (35.8–37.6°C) with no differences among the groups. The differences in core temperature between the groups were not significant (Table 1).

Table 1. Body weight, average daily distance run in the wheel, and core temperature

<table>
<thead>
<tr>
<th></th>
<th>AS</th>
<th>AE</th>
<th>RS</th>
<th>RE</th>
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</thead>
<tbody>
<tr>
<td>n</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>605 ± 41*</td>
<td>510 ± 19</td>
<td>511 ± 18†</td>
<td>382 ± 38†</td>
</tr>
<tr>
<td>Daily distance</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>run in the</td>
<td>3.05 ± 1.07*</td>
<td>4.34 ± 1.15†</td>
<td></td>
<td></td>
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<tr>
<td>wheel, km</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Core temperature, °C</td>
<td>35.8 ± 0.7*</td>
<td>35.8 ± 0.8*</td>
<td>34.4 ± 0.5†</td>
<td>33.6 ± 0.6†</td>
</tr>
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</table>

Values are means ± SD; n, no. of rats. AS, ad libitum fed sedentary; AE, ad libitum fed with free exercise; RS, caloric restricted sedentary; RE, caloric restricted with free exercise. Core temperature was measured at the end of the NMR experiment, 45 min after onset of anesthesia. † P < 0.05 with respect to values marked * in the same row. †† P < 0.05 with respect to values marked † in the same row.
ences among the groups. At the end of the NMR experiment, the core temperature stayed in the normal range for the ad libitum-fed animals but was significantly lower in the caloric-restricted groups (Table 1).

31P NMR Spectroscopy of the GM at Rest

Typical 31P NMR spectra of the rat GM at rest and during electrical stimulation are shown in Fig. 1. At rest, there were no statistically significant differences in the relative concentrations of phosphocreatine (PCr) and ATP among the groups. The ratio of PCr to ATP was 5.3 ± 2.0 in AS, 4.4 ± 0.9 in AE, 4.3 ± 0.7 in RS, and 5.2 ± 1.5 in RE rats. In addition, no statistically significant differences in the resting values of intracellular pH were found among the groups: 7.04 ± 0.05 (AS), 7.00 ± 0.06 (AE), 7.05 ± 0.13 (RS), and 7.07 ± 0.06 (RE).

31P NMR Spectroscopy of the GM During Electrical Stimulation

Concentration of metabolites. ATP levels were unchanged during electrical stimulation of the muscle, except for a decrease in ATP signal intensity by ~20% on average in the AS group between minutes 3 and 6 of the second stimulation period (P < 0.05). However, during that time period, pH and the ratio of PCr to Pi were not significantly different from other groups. By the end of the second stimulation, the concentration of ATP was restored to the preexercise level.

Values of the ratio of PCr to Pi during the two stimulation periods and the first 2 min of recovery are shown in Fig. 2. The ratio of PCr to Pi dropped significantly at the beginning of each period of stimulation, with a steady state being reached after 2 min of stimulation. The PCr and Pi levels were restored within 4 min after cessation of stimulation in all four groups of rats.

Intracellular pH. Figure 3 shows the time course of intracellular pH. Early in the first stimulation period, pH decreased to a comparable extent in all groups. However, after 6 min of stimulation, pH decreased further to 6.77 ± 0.19 in the AS group, to 6.72 ± 0.25 in the AE group, and to 6.68 ± 0.17 in the RS group. In contrast, pH decreased to only 6.87 ± 0.05 in the RE group (P < 0.05 compared with AE and RS groups, P < 0.055 compared with the AS group). In the two groups of exercising rats, recovery of pH started during stimulation, after 4 and 6 min of stimulation in the RE and AE groups, respectively. Repeated-measures ANOVA revealed a significant difference in the time course of pH between the RE and the remaining groups during the first stimulation and 6 min of recovery. The average pH recovered fully in all groups by the end of the first rest period.

Fig. 1. Typical 31P NMR spectra of rat gastrocnemius muscle in ad libitum-fed rat (A) and in caloric-restricted, exercised rat (B). Spectra recorded at rest (bottom), at the end of the first stimulation (middle), and at the end of the second stimulation (top) are shown. Spectra were acquired with a surface coil, over a period of 2 min, with a repetition time of 2 s. Signals of Pi, phosphocreatine (PCr), and ATP were detected.
During the second stimulation, the average value of pH in the RE group was also the highest among the four groups, but the difference did not reach statistical significance. The overall decrease in pH in all groups was less than in the first period of stimulation, with the pH remaining above 6.85 throughout. At the end of the second stimulation, there was little difference in pH among the groups.

Measurement of Relative Force

Time course of relative force. The time course of relative force over the two periods of stimulation is shown in Fig. 4. The relative decrease in force output over the first period of stimulation was the same in all groups. At the end of the first stimulation, the average relative force developed was ~80% of the initial value in each group. At the beginning of the second stimulation, the average relative force levels were highest in the RE group. In addition, relative force was better maintained throughout the second stimulation period in this group; at the end of the second stimulation, relative force decreased by ~40% in the AS, AE, and RS groups but by only 18% in the RE group. A repeated-measures ANOVA comparison of the force values across the second stimulation revealed a significant difference between RE and the other three groups of rats.

Relationship between force and time, pH, dietary status, and exercise status

Using the linear mixed-effects regression model (21), force was expressed as a function of time, pH, exercise status, dietary status,
and a number of interaction terms, including pH × time, exercise status × time, and dietary status × time. The term exercise status was assigned the value 0 for the AS and RS groups and 1 for the AE and RE groups. Similarly, the term dietary status was assigned the value 0 for the AS and AE groups and 1 for the RS and RE groups. The significance of the terms was tested separately for each period of stimulation. The most important results are as follows.

In the first period of stimulation, the significant terms included time, pH, and their interaction term (pH × time). This indicates that the relative force was dependent only on the time of the stimulation and the intracellular pH and that the relationship between force and pH changed over time. In particular, at each time point of the stimulation, the relative force decreased with increasing acidosis of the muscle and, in addition, the force levels were lower as time of stimulation increased. The pattern of changes in the relative force was the same in all groups of animals.

In the second stimulation period, time, pH, dietary status, and exercise status were all significantly associated with relative force, as evident by the statistical significance of pH × time (P < 0.003), dietary status × time (P < 0.03), and exercise × time (P < 0.05). The significance of these interaction terms indicates that relative force changes differently over time between the combined RE and RS groups compared with the combined AE and AS groups and between the combined RE and AE groups compared with the combined RS and AS groups. Similarly as in the first stimulation period, relative force decreased with time but did not change with pH.

Enzymatic Assays

Based on the improved metabolic and functional performance observed in the RE group, we performed enzymatic analysis of the muscle from those animals, as well as of the muscle from animals in the AE group for comparison. Analysis of two enzymes of the tricarboxylic acid cycle, citrate synthase and 2-oxoglutarate dehydrogenase, was performed in 17-mo-old AE and RE animals (Table 2). A significantly higher level of citrate synthase was found in the GM of the RE group than in the AE group. The concentration of 2-oxoglutarate dehydrogenase was also higher in the RE rats than in the AE rats, but the difference was not statistically significant.

**DISCUSSION**

The present work provides the first noninvasive study of the effect of long-term caloric restriction alone and in combination with free exercise on the metabolism of high-energy phosphates and force development. We used NMR spectroscopy to assess the bioenergetics of GM at rest and under electrical stimulation. We monitored muscle fatigue by recording contraction force throughout stimulation. Biochemical assays demonstrated enzymatic adaptation of the muscle to lowered caloric intake in combination with free activity.

**31P NMR Spectroscopy of the GM at Rest**

The GM from which the 31P NMR spectra were recorded is a mixed-fiber-type muscle. The signal was

![Graph](image_url)

**Table 2. Enzymatic assay of the gastrocnemius muscle**

<table>
<thead>
<tr>
<th></th>
<th>AE (µmol·min⁻¹·g wet wt⁻¹)</th>
<th>RE (µmol·min⁻¹·g wet wt⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Citrate synthase</td>
<td>17.6 ± 5.7</td>
<td>32.7 ± 5.4*</td>
</tr>
<tr>
<td>2-Oxoglutarate dehydrogenase</td>
<td>0.23 ± 0.16</td>
<td>0.35 ± 0.17</td>
</tr>
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Values are means ± SD; n, no. of rats. *P < 0.05, AE vs. RE.
obtained with a surface coil predominantly from the superficial part of the GM, which samples mainly types 2a and 2b fibers (19). In accordance with in vitro results (20, 28) and in vivo results (19), the spectra of the GM in all four groups of rats showed characteristics of type 2 fibers: very low P_i signal and ratio of PCr to ATP higher than four. Fiber types 2a and 2b can not be distinguished from normal ATP levels. This reflected a decrease in bioenergetic reserve of the muscle at rest. In contrast, our data showed that long-term caloric restriction alone or in combination with exercise in normal resting levels of high-energy phosphates.

31P NMR Spectroscopy of the GM

Concentration of metabolites. Although the ratio of PCr to P_i decreased significantly from the resting value during high-intensity electrical stimulation, there was no detectable decrease in ATP concentration in the muscle of rats that were subjected to either intervention. However, in the AS group, ATP levels decreased significantly during a 4-min period in the second stimulation. Thus, for a limited period of time, the production of ATP by oxidative phosphorylation was not sufficient to meet the energetic demand of the stimulated muscle in this group.

As previously noted, the ratio of PCr to P_i reached an approximate steady state 2 min after the onset of stimulation during both stimulation periods. This may be due to decreased actinomyosin ATPase activity and hence decreased energy requirements, as would be seen with failure of excitation-contraction coupling. This is consistent with the progressive decline in force development seen during both stimulation periods in all groups.

Intracellular pH. The decrease in pH after initiation of exercise or stimulation is consistent with lactate production accompanying an increase in glycolytic production of ATP (15). This occurs even under fully aerobic conditions (8). As the stimulation continues, aerobic pathways provide an increasing fraction of the energy supply for muscle contraction. Our pH results indicate that this process is initiated earliest in the RE rats, likely indicating a greater aerobic capacity of the GM in these animals. Although caloric restriction by 50% over the life span does not affect fiber-type composition (6), our results suggest that the combination of caloric restriction and exercise may further increase the oxidative capacity of the muscle fibers compared with control rats or rats subjected to caloric restriction only.

After cessation of muscle activation, glycolysis is inhibited and ATP is produced exclusively by oxidative phosphorylation (2). The lack of initial recovery of the cell, or recovery of potassium concentration (23).

Measurement of Relative Force

All force measurements were obtained simultaneously with metabolic measurements, permitting detailed statistical correlations to be made. Although all muscles of the lower leg contributed to the developed force, the GM constitutes the major part of the lower leg muscle. In addition, collecting data on the intact animal ensured that our results are comparable to those performed in human forearm or leg muscles (3, 7, 29, 31, 36, 38).

Although the pH and the relative concentration of phosphorylated metabolites were the same in all groups during the second stimulation, the GM of the RE rats demonstrated significantly less fatigue than in the other three groups of rats. A potential explanation for this observation is changes in the fiber-type composition in the RE rats; it is known that slow-twitch fibers (type 1) exhibit significantly less fatigue than glycolytic fibers (type 2). However, it is difficult to directly compare our results with published data on force production of muscles of different fiber types (27, 37), since those experiments were performed in isolated fibers, using different stimulation protocols and different methods of inducing changes in pH.

Enzymatic Assays

We sought to elucidate the biochemical basis for the contribution of diet restriction to the improved metabolic reserve and force production seen in the RE animals. To do so, we compared results for the RE and AE groups. Note that the AE, RS, and AS groups all
performed indistinguishably in terms of high-energy phosphate metabolism, pH, and function. A significantly higher concentration of citrate synthase and a trend toward a higher concentration of 2-oxoglutarate dehydrogenase in the muscle of the RE rats compared with the AE rats indicated a higher oxidative capacity of mitochondria per muscle fiber.

In conclusion, our study has demonstrated that, while long-term caloric restriction and exercise individually did not affect intracellular pH and developed force during electrical stimulation of the GM, the combination of caloric restriction and free exercise acted synergistically to decrease muscle fatigue and to improve muscle bioenergetics.

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