Effect of Energy Restriction on Muscle Function and Calcium Stimulated Protease Activity in Recreationally Active Women

Sabina C. Parkes, Angelo N. Belcastro, Linda J. McCargar, and Donald McKenzie

Catalogue Data

Key words: substrate oxidation, protein metabolism, contractile protein profiles, muscle biopsy, calpain
Mots-clés: oxydation de substrat, métabolisme des protéines, profil des protéines contractiles, biopsie musculaire, calpain

Abstract/Resumé
The purpose of this study was to investigate whether changes in substrate oxidation that are caused by energy restriction influenced muscle function and skeletal muscle calcium stimulated protease activity in female athletes. Endurance athletes were randomly assigned to maintenance energy (100% kcal) or energy restricted (75% kcal) diet treatment groups for 14 days while maintaining regular activity. Body weight significantly decreased in the 75% diet group (−1.7 ± 0.3 kg; p < .05), while fat oxidation increased (p < .05). Minimal changes in quadriceps function (assessed using the Kin/Com isokinetic dynamometer) were observed following diet treatment, except selected loss of muscle function in the 75% diet group at a movement velocity of 120 deg/s. These results suggest that increased fat oxidation that is induced by an acute energy restriction does not promote loss of general muscle function and activation of calcium-sensitive muscle proteases.

Sabina C. Parkes and Linda J. McCargar are with the Department of Agricultural Food and Nutritional Science at the University of Alberta, 4-10 Agriculture Forestry Centre, Edmonton, AB T6G 2P5, Canada. Angelo N. Belcastro is with the Faculty of Health Sciences at the University of Western Ontario, London, ON, N6A 3K7, Canada. Donald McKenzie is with the Allan McGavain Sports Medicine Center at the University of British Columbia, Vancouver, BC, V6T 1Z4, Canada.
Cette étude analyse les effets d'une variation de l'oxydation des substrats, comme c'est le cas au cours d'une période de restriction calorique, sur les fonctions musculaires et l'activité d'une protéase activée au calcium dans le muscle squelette d'athlètes féminins. Des athlètes d'endurance ne changeant rien à leur activité habituelle sont assignées aléatoirement à l'un des deux groupes suivants: régime standard de 14 jours (100% des kcal) ou régime restrictif d'une même durée (75% des kcal). La masse corporelle du groupe restreint diminue significativement (-1,7 ± 0,3 kg; p < 0,05) et l'oxydation de leurs graisses augmente (p < 0,05). Les variations des fonctions musculaires du quadriceps du groupe restreint (évaluées au moyen du dynamomètre isokinétique de marque Kin/Com) sont peu importantes à l'exception d'une perte de fonction précise à une vitesse de mouvement de 120 degrés par seconde. Les deux groupes ne montrent pas de variation dans les activités analogues à celle de la calpaine. Les résultats indiquent qu'une augmentation de l'oxydation des graisses, occasionnée par une brève période de restriction énergétique, n'entraîne pas de perte globale de fonction musculaire ni n'active les protéases musculaires sensibles au calcium.

**Introduction**

Energy restriction causes changes in substrate oxidation, which may alter muscle structure and function (Butterfield, 1987) through selective breakdown of functional muscle proteins (Dahlmann et al., 1986). Calcium activated neutral protease, or calpain, is involved in selectively degrading contractile and cytoskeletal proteins, which results from substrate oxidation changes that are induced by energy restriction or exercise (Belcastro et al., 1994; Kettellhut et al., 1994). Energy restriction, which results in increased fat oxidation, elevates calpain activity. For example, calpain activity increased by 29, 13, and 20%, respectively, in rats who were either fasted or placed on 50 or 75% diet restriction for 2 days (Murray et al., 1991). Similarly, calpain activity increased in rats as a result of exercise, presumably due to increased fat oxidation (Belcastro, 1993). This increase was believed to be caused by enhanced calcium sensitivity of the enzyme as well as increased susceptibility of myofibrillar substrate protein to calpain action with prolonged, level running (Belcastro, 1993). Researchers have not determined whether an energy deficit in the form of combined caloric restriction and exercise comparably affects human muscle function and calcium-stimulated protease activity.

Researchers have investigated how dietary restriction affects muscle function by looking at how energy restriction influences VO$_{2}$max. Ross et al. (1995a) suggested that a combined diet and exercise-induced weight-loss program for obese women resulted in improved muscle function, as indicated by increased VO$_{2}$max (expressed in absolute terms). However, this increased, peak VO$_{2}$ may reflect training effect, as subjects may have been sedentary prior to the study and thus improved their training status with a diet and exercise program. Furthermore, comparing Ross et al.'s (1995a) results to those of the present study is difficult since subject groups were different (i.e., obese versus athletic women, respectively).

Improved muscle function also occurs when energy restriction is combined with resistance training. Ross et al. (1995b) found that obese women who were placed on a diet that created a 1,000 kcal deficit and who also participated in a resistance weight training program 3 days/week for 16 weeks improved upper- and lower-body strength. Therefore, these researchers suggested that combining energy restriction and weight training does not compromise but rather improves muscle function.
In contrast, other researchers have found that decreased muscle function results from combined diet and exercise programs. This was indicated by decreased VO\textsubscript{max} (Ingjer and Sundgot-Borgen, 1991; Mendez et al., 1984; Walberg et al., 1988) following an endurance exercise program combined with a very low-energy diet. Specifically, Ingjer and Sundgot-Borgen (1991), who investigated the effects of 2-month, self-imposed, caloric restriction combined with rigorous exercise, reported a loss of 9.4% (p < .001) body weight and significantly decreased VO\textsubscript{max} and running speed relative to controls (p < .001). The mechanism(s) for this decreased performance may have been a selective decrease in muscle function (Beals and Manore, 1994), perhaps induced by selective protein degradation. However, this has not been experimentally tested. We contend that calpain is involved in this loss of potential muscle function since some of the sarcomeric proteins (especially Z-line proteins) are substrates for calpain (Kettelhut et al., 1994). Thus, we hypothesise that increased calpain activity that is induced by fat-oxidation changes resulting from combined energy restriction and exercise may alter muscle function.

Female endurance athletes provide a special population for investigating these issues since these individuals habitually restrict food intake and exercise excessively (Davis and Cowles, 1989). Also, although eating disorders have been documented in athletes, few researchers have examined physiological effects of restricted energy intake on athletes. Therefore, we examined how short-term, energy restriction affected female athletes. The purpose of this study was to do the following:

1. Demonstrate increased fat oxidation with a 25% dietary energy deficit in female endurance athletes
2. Measure how the energy restriction affected muscle function
3. Measure alterations in skeletal muscle calcium activated neutral protease activity (calpain)

**Methods**

**SUBJECTS**

We recruited 14 healthy endurance-trained athletes to participate in the study. Inclusion criteria included eumenorrhea; not following a therapeutic diet regimen, which was assessed using a 4-day, diet record; body fat between 16–26%; VO\textsubscript{max} > 42 ml/kg/min; endurance athletes; running distance per week > 30 km combined with participation in another endurance sport for at least 3 hr; and not taking anti-inflammatory medication. Training history was established in two ways. Subjects' exercise frequency, duration, and type were documented through interviews. Also, each subject's training status was assessed with a VO\textsubscript{max} test, which was conducted on a bicycle ergometer (Mijnhard KEM-3 bicycle ergometer) because it was more available at the time of the study and subjects had regular cycling and running experience. After a 20-min warm-up at approximately 55% of VO\textsubscript{max}, ergometer resistance was increased such that subjects approached exhaustion after another 3-4 min. Using the classical criterion, plateaued oxygen uptake at increasing workloads, combined with a respiratory exchange ratio above 1.1 and nearly maximal heart rate (> 97% of maximal heart rate), ensured that maximal oxygen uptake was achieved.
Athletes received written and verbal explanations of the experimental procedures. Informed consent was obtained from each participant in accordance with the university’s Clinical Screening Committee for Research and Other Studies Involving Human Subjects and with the American College of Sports Medicine policy statements.

STUDY DESIGN

Prior to the 2-week, experimental, dietary period, we took several baseline measurements for all subjects. This included assessing anthropometrics, substrate oxidation, calpain-like activity, and muscle function. For the first 4 days, all subjects consumed a maintenance energy diet, determined by the Harris-Benedict equation plus an activity factor of 1.82 for heavy activity (Harris and Benedict, 1919). During this time, we made adjustments to prevent weight change. Subjects were then randomly placed on a maintenance energy (100% kcal) or energy restricted (75% kcal) diet for 14 days. We chose this time period because fat oxidation (McCargar et al., 1989) and calpain activity (Belcastro et al., 1994) increase in less than 2 weeks with an energy restriction. At the end of the experimental diet period, postdiet measurements (identical to baseline assessments) were taken by the same examiner. During the 14-day period, subjects ingested meals at the University of British Columbia. All food was individually weighed and prepared for each subject. Subjects consumed all of the prepared food and were instructed not to ingest any other food except for noncaloric beverages. Compliance was monitored through daily weight measurements and contact with subjects. Throughout the study, subjects also recorded exercise participation. From these records, energy expenditure was evaluated using the Weight Loss Programmer Software (Version 3.1, Ohio Distinctive Software, Cleveland OH, 1992).

ANTHROPOMETRICS

Height, weight, and body fat were assessed pre- and postdiet for all subjects. Seven skinfold sites, which included chest, axilla, triceps, biceps, subscapular, suprailiac, and front thigh, were measured. Body density was calculated from skinfold values using a generalised regression equation derived from women varying in age and body composition (Jackson et al., 1980). Body fat percentage was then determined through Siri’s equation (Siri, 1961).

SUBSTRATE OXIDATION

We evaluated substrate oxidation by determining resting energy expenditure (REE) and resting and postprandial respiratory quotient (RQ) using indirect calorimetry. We used the MMC Delta Trac metabolic cart (Sensormedics, Anaheim, CA) to determine REE and RQ values.

We asked subjects to fast for 12 hr before reporting to the laboratory and to discontinue all physical activity for 24 hr prior to testing. REE and RQ measurements were taken for an additional 30 min with subjects lying supine in a quiet, dimly lit room. Briefly, each subject’s head was enclosed in a plastic canopy with a flexible seal at the neck. This system prevents discomfort associated with nose clips and mouthpieces. Subjects inhaled room air through an opening in the
canopy. Exhaled air was drawn by slight suction at a constant, low rate to the metabolic monitor mixing chamber, where it was analysed for oxygen and carbon dioxide.

Postprandial energy expenditure and RQ were monitored for approximately 2 1/2 hr after a test breakfast, which consisted of 12% protein, 60% carbohydrate, and 28% fat. This test breakfast provided 30% of maintenance energy intake for all subjects prediet (corresponding to 746 ± 23 kcal and 776 ± 28 kcal for the 100% and 75% diet groups, respectively) and 30% of daily energy intake for the diet group postdiet (corresponding to 746 ± 23 kcal and 578 ± 31 kcal for the 100% and 75% diet groups, respectively). The first 5-min measurement was taken 30 min postbreakfast and every 30 min thereafter for 2 1/2 hr. McCargar et al.'s (1989) results suggest that this data collection schedule was appropriate for measuring REE and RQ in this experiment. Substrate oxidation measurements for 10 subjects were measured during the follicular phase of the menstrual cycle, because resting energy expenditure is elevated in the periovulatory phase of the menstrual cycle (Herring et al., 1992). Four subjects (two subjects in the 100% diet group, two in the 75% group) were not assessed during the follicular phase due to scheduling difficulties.

MUSCLE FUNCTION

All muscle function measurements were determined using an isokinetic dynamometer (Kin/Com, Model 5030, Med-ex Diagnostics of Canada Inc., Port Coquitlam, B.C.), which was connected online to a microcomputer that was programmed to calculate average and peak torque over the range of motion for concentric and eccentric contractions. Subjects were seated on the isokinetic dynamometer, with the hips at 80°, the back supported, and the pelvis stabilised on the bench with strapping. The Kin/Com centre of rotation was positioned opposite to the centre of the knee joint line. The resistance pad was positioned at a point on the lower leg that was 75% of the fibula length. Knee flexion and extension movements were measured at angular velocities of 30, 90, 120, and 180 deg/s (performed in random order) through a 65° range at a long muscle length (110–50° of knee flexion).

Before the test, submaximal extension-flexion movements at the specified movement velocity were performed four times so that subjects could familiarise themselves with the equipment. During testing, subjects performed four consecutive extension and flexion movements with maximal intensity at each movement velocity and received encouragement from the investigator. Peak and average torque values for each contraction type were used to describe muscle strength. Peak torque is the highest torque value obtained throughout the range of motion, while average torque is the value averaged throughout the same range. Although both peak and average torque values represent strength measurements, peak torque attained in a single, high-speed, velocity-specific test is often used as a relative index of maximum power and strength (Komi, 1986). For our test, the mean of the final three trials was used for statistical analysis; the first trial at each movement velocity is often variable since subjects are still becoming accustomed to the motion speed (Alexander, 1990).

Farrell and Richards (1986) reported that Kin/Com system measurements were repeatable (e.g., repeated loading and unloading of a strain gauge) and accurate to known weights (e.g., intraclass correlation coefficient [ICC] = .99) in static
testing. During dynamic testing, applied force throughout the trials resulted in an ICC = .95. Reliability of concentric and eccentric torque measurements on the Kin/Com can range from .93 to .98 for both slow (30 deg/s) and fast (180 deg/s) speeds among healthy, active subjects (Reitz et al., 1988; Snow and Johnson, 1988).

CALCIUM STIMULATED PROTEASE ACTIVITY FROM MUSCLE BIOPSY

Calpain-like activity was assessed in extracts that were obtained through a muscle biopsy (vastus lateralis), which was performed by a physician who had experience with this technique. The biopsy was performed 24 hr postexercise. Skin around the thigh area was first sterilised, and then the skin and subcutaneous tissues were infiltrated with 7-10 ml of 2% lidocaine without epinephrine. A 5–7 mm stab incision was made on the anterolateral surface of the thigh at the junction of the middle and distal third, since selecting this position is least likely to cause significant complications (Kirby et al., 1982). The biopsy needle was inserted perpendicular to the skin surface and advanced 2–5 cm into the vastus lateralis to the femur. The muscle sample was then removed, weighed, and frozen in precooled, isopentane stored in liquid nitrogen. This procedure provides sufficient tissue to prepare the required extracts and minimise bruising (Kirby et al., 1982).

Calpain-like activity was measured using a microplate assay (Belcastro et al., 1993). Approximately 50–100 mg of tissue was homogenised in buffer containing 25 mM KCl, 20 mM Tris (pH 7.5), 5 mM EGTA, and 5 mM DTT. The homogenate was then centrifuged at 22,000 rpm for 15 min (hermle 360Z, rotor VO2805) and the supernatant, the soluble particulate fraction, was transferred to an eppendorf tube and placed on ice. The remaining pellet was rehomogenised in buffer containing 0.35% Triton X-100, centrifuged, and stored on ice (particulate fraction). Then, 200 µl of either soluble or particulate fractions were added to 2 mg/ml casein, DTT, and Tris (pH 7.5) and incubated for 30 min at 37 °C. The assays were carried out with and without calcium. The amount of casein degraded was measured with Coomassie brilliant blue, where 1 unit of calpain-like activity = 0.1 ABS, measured at a wavelength of 595 nm. The coefficient of variation for determining calpain-like activity in this laboratory was 6%.

DATA ANALYSIS

Means and standard deviations of all pre- and postdiet variables and descriptive variables were determined using BMDP 9D statistical program (BMDP Statistical Software, PC 90, Los Angeles, CA, 1994). Baseline and poststudy parameters were analysed by performing a multiple analysis of variance through the Hotelling $T^2$-squared statistical test ($p < .05$). If differences existed between parameters, they were compared with a $2 \times 2$, group $\times$ time repeated measures ANOVA ($p < .05$).

Results

Baseline characteristics for the two groups were similar (see Table 1). Subjects were recreationally active women who had participated in endurance sports, such as running, biking, and swimming. Subjects had participated in these sports for the past $9.4 \pm 1.0$ years and had competed in various community events. Eight subjects took oral contraceptives and were eumenorrheic in the past year. All subjects
Table 1 Baseline Characteristics According to the Dietary Subgroup

<table>
<thead>
<tr>
<th>Variable</th>
<th>All (N = 14)</th>
<th>100% diet (n = 7)</th>
<th>75% diet (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>SEM</td>
<td>M</td>
</tr>
<tr>
<td>Age (years)</td>
<td>27</td>
<td>2</td>
<td>27</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>167.2</td>
<td>1.6</td>
<td>164.5</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>61.8</td>
<td>1.9</td>
<td>58.5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.1</td>
<td>0.6</td>
<td>21.7</td>
</tr>
<tr>
<td>VO₂,max (ml/min/kg)</td>
<td>49.0</td>
<td>0.9</td>
<td>49.4</td>
</tr>
<tr>
<td>Years spent exercising</td>
<td>9.4</td>
<td>1.0</td>
<td>10.0</td>
</tr>
</tbody>
</table>

*Note. No significant differences. Comparisons made by Student’s unpaired t test.*

attained the specified criterion for the VO₂,max test. Throughout the 2-week, diet period, subjects in both groups consumed similar proportions of macronutrients. Energy intake for the 75% diet group (1,928 ± 103 kcal/day) was significantly lower (p < .0001) than that of the 100% diet group (2,487 ± 78 kcal/day), with both diets meeting the Canadian recommended nutrient intakes for all vitamins and minerals (Health and Welfare Canada, 1990). Women were involved primarily in running, cycling, and swimming throughout the study. Subjects’ exercise activities are listed in Table 2. Average time spent exercising each day was 99.9 ± 6.4 min/day, and average running distance/week was 37.1 ± 3.7 km (see Table 2). As anticipated, the 100% diet group’s pre- and postdiet anthropometric indices were not different (see Table 3). However, women in the 75% diet group had significantly lower values for body weight, BMI, sum of skinfolds, and percent body fat after diet treatment (p < .05; see Table 3). Estimated lean body mass did not change significantly pre- and postdiet and was not significantly different between the two groups.

Resting energy expenditure and predict resting RQ values did not differ between diet groups or pre- and postdiet for the 100% diet group. However, postdiet resting RQ value (.77 ± .02) of the 75% diet group were significantly lower than the predict value (.83 ± .03; p < .05) and postdiet resting RQ for the 100% diet group (.84 ± .02; p < .05). Postprandial RQ values for the 75% group decreased from the predict value at 30 min (−.07 ± .03, p < .05), 120 min (−.10 ± .02, p < .001), and 150 min (−.09 ± .03, p < .05). We did not find significant differences between the pre- and postdiet postprandial RQ values for the 100% diet group. Changes in postprandial RQ values for the 75% group were significantly larger than changes in the 100% diet group at 120 min (p < .001) and 150 min (p < .05; see Figure 1).

The overall muscle function was not different after the diet treatment. The torque values did not follow any specific trends, and few torque indicators changed significantly. Eccentric and concentric pre- and postdiet torque values changed for both diet groups. Eccentric average torque increased at a movement velocity of 90
Table 2  Activity Characteristics According to Dietary Subgroup

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All (N = 14)</th>
<th>100% diet (n = 7)</th>
<th>75% diet (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>SEM</td>
<td>M</td>
</tr>
<tr>
<td>Run distance per week (km/week)</td>
<td>37.1</td>
<td>3.7</td>
<td>42.4</td>
</tr>
<tr>
<td>Time spent exercising each day (min/day)</td>
<td>99.9</td>
<td>6.4</td>
<td>105.2</td>
</tr>
<tr>
<td>Mode of exercise performed</td>
<td>Refer to next two columns</td>
<td>Run, bike, swim, weights, field hockey, skiing, karate, aerobics</td>
<td>Run, bike, swim, weights, lacrosse, canoeing, weights, aerobics</td>
</tr>
<tr>
<td>Energy expenditure from exercise (kcal/day)</td>
<td>832</td>
<td>97</td>
<td>829</td>
</tr>
</tbody>
</table>

*Note. No significant differences. Comparisons made by Student’s unpaired t test.*
*Calculated from daily exercise recording forms. *Main activities performed by the subjects.
*Calculated by taking average of all days of the study; determined by the Weight Loss Programmer (Version 3.1, Ohio Distinctive Software, Cleveland, OH, 1992).*

Table 3  Anthropometric Measures According to the Dietary Subgroup Before and After the Respective Diet Period

<table>
<thead>
<tr>
<th>Variable</th>
<th>100% diet (N = 7)</th>
<th>75% diet (N = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prediet</td>
<td>Postdiet</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>SEM</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>58.5</td>
<td>2.4</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>21.7</td>
<td>0.9</td>
</tr>
<tr>
<td>Sum of skinfolds (mm)</td>
<td>86.6</td>
<td>12.6</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>18.2</td>
<td>2.2</td>
</tr>
</tbody>
</table>

*Note. Comparisons made by a 2 × 2 RM ANOVA for each variable.*
*Significant difference between the change in the 100% group versus the change in the 75% group, p < .001. **Significant difference between the change in the 100% group versus the change in the 75% group, p < .01. ***Significant difference between the change in the 100% group versus the change in the 75% group, p < .05.*
Figure 1 — *Note.* Values are mean ± SEM. Comparisons made by a 2 × 2 RM ANOVA.

*Significant difference between pre- and postdiet for the 75% diet group, *p* < .05.

*Significant difference between pre- and postdiet for the 75% diet group, *p* < .001.

*Significant difference between 100% group and 75% group’s changes, *p* < .001.

*Significant difference between 100% group and 75% group’s changes, *p* < .05.

deg/s (36 ± 19 nm, *p* < .05) for the 100% diet group and 180 deg/s (19 ± 13 nm, *p* < .05) for the 75% diet group. Differences in the eccentric peak torque within or between each diet group were minimal. Concentric peak torque in the 75% diet group at a movement velocity of 120 deg/s was significantly lower (*p* < .05) than the predicted value, with torque decreasing by 10 ± 6 nm. This change in torque was significantly larger than that for the 100% diet group (*p* < .05). Concentric average torque at a movement velocity of 30 deg/s in the 100% diet group increased (11 ± 5 nm, *p* < .05).

Differences in calpain-like activity between the diet groups were minimal (see Figure 2). Furthermore, we did not find differences between prediet calpain-like activity (100% group: 28.82 ± 4.90 units/g wet weight; 75% group: 20.82 ± 2.49 units/g wet weight) and postdiet values (100% group: 34.84 ± 10.57 units/g wet weight; 75% group: 42.98 ± 12.49 units/g wet weight) within each diet group.

**Discussion**

Results from this experiment support the theory that imposed energy restriction causes increased fat oxidation, as shown by decreased postprandial RQ values. However, calpain-like activity and muscle function did not change with an imposed 25% energy restriction, which is consistent with Murray et al.’s (1991) results from studies involving rats, where 50% diet restriction did not cause a significant change in calpain activity. The fact that calpain-like activity and muscle function did not change with an imposed 25% energy restriction suggests that a severe energy restriction may be essential to activating calpain-like activity and
decreased muscle function in active females. Thus, further research should aim to investigate whether a more severe and lengthy energy restriction may be required to induce changes in muscle function and calpain-like activity or whether another myofibrillar proteolytic system may be responsible for the elevated muscle proteolysis found with energy restriction.

SUBSTRATE OXIDATION

Resting RQ value in the 75% diet group significantly decreased. Comparing this to the prediet value, we conclude that fat was utilised to a larger extent under resting conditions. This is consistent with results from Froidevaux et al. (1993), who found that resting RQ value decreased from .86 ± .03 to .77 ± .02 (Mean ± SD) in 10 moderately obese women who were placed on a diet and exercise program.

The 75% diet group also experienced a change in substrate oxidation after consuming a standard test breakfast. Subjects’ RQ significantly decreased 30, 120, and 150 min after they consumed the test breakfast, which suggests that fat oxidation increased post-consumption. These results are also consistent with Froidevaux et al.’s (1993) findings that postabsorptive RQ decreased from .81 ± .04 to .76 ± .03 in 10 moderately obese women who lost weight as a result of a diet and exercise program. Therefore, results obtained from the present study demonstrate that energy restriction created by diet and aerobic exercise promotes utilising fat as an energy source under resting conditions and postabsorptively. This is supported by the fact that energy restriction did not affect estimated lean body mass.

MUSCLE FUNCTION

Overall, muscle strength and performance were relatively unimpaired after 14 days of moderate, energy restriction. Significant changes in muscle function for both diet groups may have been due to variability that was introduced into measured torque values through body stabilisation, axial alignment, subject motivation, and subjects’ skill levels. Although care was taken to align knee axis with the exercise-arm axis of the machine, the exact positioning was not precise, and there was a tendency for a subject’s thigh to come forward during a test. Axial alignment,
therefore, likely accounts for a significant portion of the variance. In addition, the female subject groups’ increased muscle torque (postdiet) may have represented training effects. Pronk et al. (1992) found that combining resistance strength training and a very low-calorie diet results in increased strength. In our study, performance was relatively unimpaired after 14 days of an energy restriction.

Muscle function may not have changed in this study because energy restriction was not severe enough to induce such changes. Alternatively, the 14-day, imposed, energy restriction period may not have been long enough to induce changes in muscle function. Scott et al. (1992) supported these contentions by suggesting that muscle function did not change in 36 mildly obese (30–40% body fat) premenopausal women (ages 29–49) because the energy restriction (1,000 kcal/day) was not sufficiently severe or long in duration. Muscle function was assessed using a Cybex 340 isokinetic device during knee flexion and extension at a movement velocity of 60 deg/s (Scott et al., 1992). Thus, to induce negative changes in maximal physical performance measures in the present study, a greater degree of caloric restriction was required or a longer dietary period was needed, or both. In any case, both situations are undesirable recommendations.

In contrast to results from the present study, Ingjer and Sundgot-Borgen (1991) found that diet restriction did result in decreased performance in female elite athletes. The case-controlled study evaluated data that was systematically collected over 5 years from 33 endurance-trained female athletes. Self-imposed energy restriction resulted in a significant weight loss from only 7 of the 33 athletes. Results were compared to control subjects (n = 726), who belonged to the same national team as subjects in the study but did not restrict their energy intake. Diet restriction severity varied between athletes and was not documented or controlled by the researchers. VO_{2} max (expressed in l/min) significantly decreased, whereas relative VO_{2} max (expressed relative to body weight) did not change. Furthermore, running speed relative to controls decreased as a result of the 2-month, weight-reduction period. Thus, researchers suggested that a significant weight loss induced within a 2-month diet restriction may reduce endurance performance.

Ingjer and Sundgot-Borgen’s (1991) results may differ from those found in the present study since Ingjer and Sundgot-Borgen assessed whole body function (by evaluating aerobic capacity), whereas we investigated muscle function to relate it to changes at the myofibrillar level. Thus, different aspects of performance were evaluated in the two studies. Furthermore, Ingjer and Sundgot-Borgen’s (1991) conclusions are weakened by the fact that energy deficit was not recorded or kept consistent between experimental subjects. The authors did not comment on subjects’ average daily caloric intake but simply suggested that subjects were using pathogenic weight control methods (Ingjer and Sundgot-Borgen, 1991). Therefore, attributing diet effects to performance changes is difficult because the effects of energy restriction cannot be quantified. Thus, since Ingjer and Sundgot-Borgen (1991) evaluated different performance aspects and did not control energy restriction, their results may not apply to results found in the current study.

Muscle function results that are obtained when an energy restriction is imposed in combination with exercise may be inconsistent due to differing exercise regimes implemented in each study. For example, endurance exercise in combination with energy restriction in the present study did not produce changes in muscle function, whereas other researchers found decreased muscle function (Ingjer and Sundgot-Borgen, 1991; Mendez et al., 1984; Walberg et al., 1988). In contrast,
combining resistance training and energy restriction resulted in improved muscle function (Ross et al., 1995b). The variable methods for assessing muscle function complicate comparing results between studies. However, from these studies, we can conclude that the effect of a combined diet and exercise program on muscle function may depend on the type of exercise stimulus imposed.

CALCIUM STIMULATED PROTEOLYSIS

Calpain-like activity did not change between pre- and postintervention. This may have been due to the conservative energy restriction. Murray et al.'s (1991) study, which involved putting rats into four diet groups (i.e., control rats, 50% diet restriction [DR] over 48 hr, 75% DR over 48 hr, fasting for 48 hr) supports these contentions. Calpain activity, expressed as units/g of muscle, significantly increased in the 75% dietary restricted and fasted group, compared to controls, while calpain activity did not change in the 50% dietary restricted group, compared to controls. Thus, Murray et al. (1991) demonstrated that calpain activity increased as severity of restriction increased. A 25% dietary restriction was not large enough to produce significant changes in calpain-like activity, as seen in the study by Murray et al. (1991).

The lack of change in calpain-like activity suggests that another proteolytic pathway may be responsible for the elevated muscle proteolysis found with an energy deficit. This is consistent with results from Kettelhut et al. (1994) who found that activation of the calcium dependent pathway involving calpain was not responsible for the increased muscle proteolysis observed during fasting. They suggested that the increase in muscle proteolysis during fasting was attributable to an enhancement of the energy-requiring process by finding an increase in the ATP-dependent proteolytic pathway in the extensor digitorum muscules of rats one day after food restriction. Thus, the energy restriction imposed on the female subjects in this study may have activated other pathways involved in muscle proteolysis other than calpain.

The lack of change in calpain-like activity may have also been due to subjects' differing exercise regimens and the site chosen for muscle biopsy. Differences in exercise intensity, time, frequency, and type may have caused the large variability in calpain-like activities, as female subjects were instructed to exercise "as usual." The variability associated with exercise intensity and duration has recently been suggested as a contributing factor, where calpain-like activity increased with increasing intensity (Belcastro et al., 1996). This intensity-dependent increased calpain activity may be dependent on total energy expired because intensity effect was not evident when exercise volume was decreased. Exercise type and frequency may also affect calpain activity, although the effect of these factors has not been documented. Thus, by controlling exercise intensity, duration, frequency, and type, a lower variability in calpain-like activity may have occurred.

The site chosen for the biopsy within the vastus lateralis may have also introduced variability into the calpain-like activity in both subject groups. Because fibre types are distributed unevenly in the vastus lateralis (e.g., superficial versus central), the imposed energy restriction may have induced changes at places that were different from the site chosen to assess activity. Thus, the varying exercise program and localised changes in calpain-like activity may have influenced changes in calpain-like activity measured in this study.
In addition, the lack of change in calpain-like activity may have been caused by the absence of different energy expenditures between diet groups. If calpain-like activity was solely determined by exercise duration (versus diet or diet combined with exercise), then the lack of change can be explained by the absence of significant differences in the amount of exercise that both groups performed. Further research should aim to identify the processes involved in muscle protein degradation in active females who are consuming an energy restricted diet.

Based on the results of this study, we conclude that a moderate energy restriction in active females does not impair muscle function. However, this group often has a high prevalence of more severe dieting practices, which are used to lose or maintain weight.

References


Acknowledgments
This research was supported by a grant from the Canadian Fitness and Lifestyle Research Institute, Ottawa, Ontario, Canada. The results of the present study do not constitute endorsement of the product by the authors or by the American College of Sports Medicine.

Received May 30, 1997; accepted in final form January 20, 1998.