Skeletal and cardiac muscle protein turnover during cold acclimation in young rats

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McAllister, T. A., J. R. Thompson, and S. E. Samuels. Skeletal and cardiac muscle protein turnover during cold acclimation in young rats. Am. J. Physiol. Regulatory Integrative Comp. Physiol. 278: R705–R711, 2000.—The effect of long-term cold exposure on skeletal and cardiac muscle protein turnover was investigated in young growing animals. Two groups of 36 male 28-day-old rats were maintained at either 5°C (cold) or 25°C (control). Rates of protein synthesis and degradation were measured in vivo on days 5, 10, 15, and 20. Protein mass by day 20 was ~28% lower in skeletal muscle (gastrocnemius and soleus) and ~24% higher in heart in cold compared with control rats (P < 0.05). In skeletal muscle, the fractional rates of protein synthesis (k_{syn}) and degradation (k_{deg}) were not significantly different between cold and control rats, although k_{syn} was lower (approximately −26%) in cold rats on day 5; consequently to the lower protein mass, the absolute rates of protein synthesis (approximately −21%; P < 0.05) and degradation (approximately −13%; P < 0.1) were lower in cold compared with control rats. In heart, overall, k_{syn} (approximately +12%; P < 0.1) and k_{deg} (approximately +22%; P < 0.05) were higher in cold compared with control rats; consequently, the absolute rates of synthesis (approximately +44%) and degradation (approximately +54%) were higher in cold compared with control rats (P < 0.05). Plasma triiodothyronine concentration was higher (P < 0.05) in cold compared with control rats. These data indicate that long-term cold acclimation in skeletal muscle is associated with the establishment of a new homeostasis in protein turnover with decreased protein mass and normal fractional rates of protein turnover. In heart, unlike skeletal muscle, rates of protein turnover did not appear to immediately return to normal as increased rates of protein turnover were observed beyond day 5. These data also indicate that increased rates of protein turnover in skeletal muscle are unlikely to contribute to increased metabolic heat production during cold acclimation.

protein synthesis; protein degradation; cold exposure; environment

SURVIVAL OF HOMEOTHERMIC animals subjected to cold environmental temperatures is dependent on the animal’s ability to make the necessary physiological and metabolic adaptations. There is increased heat production that is fueled by increased food intake and/or mobilization of body reserves (13). This often results in decreased body weight gain, nitrogen balance, and skeletal muscle accretion (13, 16, 22). However, not all tissues are depleted in response to cold. There is also an attendant increase in the size of the heart (8, 9, 16), which likely reflects the higher workload of heart in support of the increased thermogenesis.

Protein turnover may play a role in increasing metabolic heat production during cold exposure. Protein turnover contributes to ~20% of thermoneutral heat production (14), so we and others have speculated that cold exposure might stimulate increased rates of protein turnover (5, 16, 19). We previously found that the fractional rates of protein synthesis (k_{syn}) and degradation (k_{deg}) in heart increased dramatically, but that the k_{syn} actually decreased in skeletal muscle in young rats exposed to cold (5°C) for 2–5 days (16). This indicated that increased rates of protein turnover in skeletal muscle are unlikely to contribute to increased metabolic heat production during short-term cold exposure.

Skeletal and cardiac muscle protein turnover during short-term cold exposure may be different from that during long-term acclimation. Prolonged decreased skeletal muscle k_{syn} would promote an ever-decreasing skeletal muscle size and its contribution to metabolic heat production. Reported increased fractional rates of protein turnover in the heart may not be sustainable (16). Thus acclimation to cold may involve establishing a new homeostasis in protein metabolic parameters to ensure long-term survival. There is little understanding of how longer-term cold exposure affects protein turnover in skeletal and cardiac muscle. In calves exposed to moderately cold temperatures for 21 days, Scott et al. (19) found no difference in the k_{syn} in muscle and heart in contrast to what we found in rats exposed to cold (5°C) for 2–5 days (16). The discrepancy between researchers may reflect the duration of cold exposure, but species differences or the intensity of cold exposure cannot be excluded.

There is a lack of research exploring protein metabolism in skeletal and cardiac muscle during both short- and long-term cold exposure. Such studies are required to understand the mechanisms by which animals acclimate to cold environmental temperatures. Our objective, therefore, was to determine protein mass and the rates of in vivo protein synthesis and degradation in skeletal muscle and heart during long-term cold exposure in rats.
MATERIALS AND METHODS

Animals and diet. Studies were carried out in accordance with the guidelines of the Canadian Council of Animal Care. Male Sprague-Dawley rats, ~21 days of age, were obtained from Biosciences Animal Services, University of Alberta, (Edmonton, Canada). Rats were housed in individual wire mesh cages in rooms that were maintained at 25°C on a 12:12-h light-dark cycle (lights on at 0800). Animals were given free access to a purified diet containing 20% casein (1) and water throughout the experiment. Animals were given 7 days to adjust to their new diet and environment before treatments were imposed.

Experimental design. After adaptation, 36 rats were transferred to a 10°C cold room. After 1 day, the temperature of the cold room was lowered to 5°C. A control group of 36 rats remained in a room at 25°C. Although cold exposure causes an increase in food intake, a cold group pair fed to the intake of control rats was not included because the rats would not survive. Food intake and body weight were measured every 1–2 days. On days 6, 5, 10, 15, 20, and 25, six rats from each group were killed by cervical dislocation. Protein turnover in soleus, gastrocnemius, and heart muscles and plasma triiodothyronine (T3) status were measured on days 5, 10, 15, and 20. Gastrocnemius was chosen because it is of mixed fiber type, representative of whole body musculature; soleus was used because it is of predominantly red fiber type (11).

A preliminary experiment was conducted to characterize the specific radioactivity time course in skeletal muscle in rats housed at 5 and 25°C after a flooding dose of [3H]phenylalanine. This was done to ensure the phenylalanine specific radioactivity remained at plateau during radiolabel incorporation. Animals, diet, and treatments in the preliminary study were as above. Two groups of 20 rats were housed at either 25 or 5°C. On day 10, rats were killed by cervical dislocation, and [3H]phenylalanine specific radioactivity was measured in soleus and gastrocnemius 5, 10, 15, and 20 min after radiolabel injection.

Measurement of protein turnover in vivo. Protein synthesis was measured using the flooding-dose method of Garlick et al. (7). Tissue protein mass and rates of growth

Body weight and food intake. Body weight and food intake of rats exposed to 5°C was higher (P < 0.05) than rats housed at 25°C. These animals weighed less 0.05) than control animals throughout the experimental period. Food intake of rats exposed to 5°C was higher (P < 0.05) than that of rats exposed to 25°C throughout the study; on average, food intake was 40% higher.

Tissue protein mass and rates of growth. Tissue protein masses are shown in Fig. 3, and rates of growth (protein deposition) are shown in Table 1. Over the course of the experiment, the protein mass of cold and control rats increased. However, protein masses of soleus and gastrocnemius muscles were lower (P < 0.05) in cold compared with control rats; on average, they were 21 and 13% lower, respectively; by day 20,

Blood sampling and handling. Whole blood was obtained from each rat by severing the subclavian artery and vein and collecting blood in evacuated tubes coated with heparin. Samples were mixed and immediately centrifuged at 3,500 g for 15 min at 5°C. Plasma was removed and stored at –50°C until analyzed.

Analyses. For the analysis of protein synthesis, tissue samples were prepared in a similar manner to that reported by Garlick et al. (7). Free and bound phenylalanine specific radioactivities were measured after transformation to β-phenylethylamine as previously described (12). Protein content of tissues was determined by the method of Lowry et al. (10). Total plasma concentration of T3 was determined using a Coat-a-count RIA kit obtained from Diagnostic Products (Los Angeles, CA).

Data analyses. The effects of treatment, day, and treatment times day interaction were tested using a two-way ANOVA (17). When no treatment times day interaction was present, statistical comparisons were made only between the two treatment groups, i.e., main effects means. In the experiment, there was no treatment times day interaction; thus, statistical comparisons were made between the two overall treatment group means (i.e., control vs. control). Variability of data is expressed as means ± SE. Differences were considered statistically significant at P < 0.05.

RESULTS

Body weight and food intake. Body weight and food intake of rats are shown in Figs. 1 and 2. Animals housed at 5°C immediately began to grow more slowly than rats housed at 25°C. These animals weighed less (P < 0.05) than control animals throughout the experimental period. Food intake of rats exposed to 5°C was higher (P < 0.05) than that of rats exposed to 25°C throughout the study; on average, food intake was 40% higher.

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![Fig. 1. Effect of cold acclimation on body weight of rats; n = 6–36 rats/time point. Points represent means ± SE. *Cold (5°C) rats were significantly different from control (25°C) rats (t-test).](image-url)
protein masses of soleus and gastrocnemius muscles were 29 and 26% lower than in control rats. In rats exposed to 5°C, \( k_{\text{growth}} \) over the experiment was lower in soleus (−30%; \( P < 0.05 \)) and gastrocnemius (−21%; \( P < 0.10 \)) compared with control rats. The greatest difference appeared to occur on day 5, when \( k_{\text{growth}} \) was 46% (\( P < 0.05 \)) and 31% (\( P < 0.10 \)) lower in soleus and gastrocnemius muscles, respectively. However, this does not mean that protein deposition in cold-exposed rats beyond day 5 will become closer to control values. In cold-exposed rats, protein mass was lower; thus, the absolute rates of growth beyond day 5 will be lower by definition, so protein masses continued to diverge. Indeed, skeletal muscle protein deposition (absolute rate of growth) was lower (\( P < 0.05 \)) in cold-exposed rats compared with controls throughout the period of cold exposure.

Table 1. Tissue rates of growth in rats during cold acclimation

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<tr>
<th>Table 1. Tissue rates of growth in rats during cold acclimation</th>
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<tr>
<td><strong>Days</strong></td>
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<tr>
<td><strong>Fractional rates of growth, %/day</strong></td>
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<td>Soleus</td>
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<td>Gastrocnemius</td>
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<td>Heart</td>
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<tr>
<th><strong>Absolute rates of growth, mg protein deposited/day</strong></th>
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<tbody>
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<td>Soleus</td>
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<td></td>
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<tr>
<td>Gastrocnemius</td>
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<td>Heart</td>
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There were 4–6 animals/group. \( P \), probability of an overall treatment effect (cold vs. control; ANOVA). NS, nonsignificant.

0.10) compared with control rats. The greatest difference appeared to occur on day 5, when \( k_{\text{growth}} \) was 46% (\( P < 0.05 \)) and 31% (\( P < 0.10 \)) lower in soleus and gastrocnemius muscles, respectively. However, this does not mean that protein deposition in cold-exposed rats beyond day 5 will become closer to control values. In cold-exposed rats, protein mass was lower; thus, the absolute rates of growth beyond day 5 will be lower by definition, so protein masses continued to diverge. Indeed, skeletal muscle protein deposition (absolute rate of growth) was lower (\( P < 0.05 \)) in cold-exposed rats compared with controls throughout the period of cold exposure.

Protein mass of the heart averaged 28% higher (\( P < 0.05 \)) in cold compared with control rats. In rats exposed to 5°C, \( k_{\text{growth}} \) was not different from controls. Again, this does not imply that protein deposition was not different; heart protein deposition (absolute rate of growth) was significantly higher (approximately +22%; \( P < 0.05 \)) in cold-exposed rats because of the increased protein mass.

Specific radioactivity time course. The specific radioactivity time course changes of soleus and gastrocnemius muscles in rats housed at 25 and 5°C were determined. In both tissues and treatments, the phenylalanine specific radioactivity reached a plateau within 5 min, which was maintained for at least 20 min (data not shown).

Skeletal muscle protein turnover. Protein synthesis and degradation data for soleus and gastrocnemius muscles are shown in Figs. 4 and 5, respectively. Rates...
of protein degradation determined in vivo are indirect measures, i.e., they are determined as the difference between synthesis and growth. Thus there are more inherent errors associated with the determination, so results must be interpreted with additional caution. In rats exposed to 5°C, \( k_{\text{syn}} \) over the entire period of cold acclimation was not different from control rats. However, on day 5, \( k_{\text{syn}} \) was 30% (\( P < 0.05 \)) and 22% (\( P > 0.05 \)) lower in soleus and gastrocnemius muscles, respectively, compared with control rats. The absolute rate of protein synthesis in soleus was lower (\( P < 0.05 \)) and in gastrocnemius tended to be lower (\( P < 0.10 \)) in cold compared with control rats. The \( k_{\text{deg}} \) was not different between cold and control rats. The absolute rate of protein degradation was not different in cold compared with control rats, although it tended to be lower (approximately −20%; \( P < 0.1 \)) in soleus muscle.

Cardiac muscle protein turnover. Protein turnover data for heart are shown in Fig. 6. In contrast to skeletal muscle, heart \( k_{\text{syn}} \) tended to be higher (approximately +12%; \( P < 0.10 \)) in cold compared with control rats; \( k_{\text{deg}} \) was on average 22% higher (\( P < 0.05 \)) in cold conditions.

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**Fig. 4.** In vivo fractional and absolute rates of protein synthesis in soleus (A) and gastrocnemius (B) muscles in rats during cold acclimation; \( n = 4–6 \) rats/time point for each treatment. Values represent means ± SE. \( P \), probability of an overall treatment effect (cold vs. control; ANOVA). NS, not significant.

**Fig. 5.** In vivo fractional and absolute rates of protein degradation in soleus (A) and gastrocnemius (B) muscles in rats during cold acclimation. Rates of protein degradation were determined as the difference between the rates of synthesis and growth; \( n = 4–6 \) rats/time point for each treatment. Values represent means ± SE. \( P \), probability of an overall treatment effect (cold vs. control; ANOVA).
rats. The absolute rates of protein synthesis (+44%) and degradation (+54%), on average, were higher in cold compared with control rats.

Plasma T3 concentration. Plasma T3 concentrations were higher (P, 0.05) in cold compared with control rats (Table 2).

DISCUSSION

Acclimation to cold environmental temperatures elicits changes in body weight, growth, food intake, and skeletal and cardiac muscle protein mass and turnover that could foster an animal's survival under these harsh environmental conditions for prolonged periods of time. This includes having a smaller-than-normal body size and skeletal muscle mass while maintaining normal rates of protein turnover and increasing heart mass. Furthermore, acclimation to cold is not furthered by increasing rates of skeletal muscle protein turnover to increase metabolic heat production.

Skeletal muscle and body weight growth. In the present study, short- and long-term exposure to cold resulted in poor body weight gain and skeletal muscle growth. This was despite a tremendous increase in food intake, which was expected (9, 16). This is not a new observation (9). It may be that the lower deposition of skeletal muscle is a simple consequence of substrates being shifted to heat production rather than to growth because of an induced energy deficit. It may also be part of a larger survival scheme to deliberately limit body size. In doing so, less food energy would be used for the maintenance and development of a larger size and more for heat production (16). To demonstrate this point, food intake on day 25 was 41% higher in cold rats. When expressed on a body weight basis, it was 62% higher; thus, there was 50% more food energy available for thermogenesis as a direct result of decreasing body size. Also, in our previous study, cold rats could not maintain normal growth despite their being given a more energy-dense diet (16). These all point to a deliberate strategy of reducing body size to effect relatively more metabolic heat production.

Cold acclimation appeared to be associated with a proportionally greater reduction in skeletal muscle mass. Cold-acclimated animals had a lower percentage of skeletal muscle mass than controls (data not shown). This change in body composition is unlikely to be a consequence of the animals simply being in an energy deficit, because decreased food intake alone would increase the percent of skeletal muscle (data not shown) and reduce that of body fat. This indicates that the reduction in skeletal muscle is specific and not merely nutrient limited.

Skeletal muscle protein turnover. In skeletal muscles, short-term cold exposure (2 and 5 days) in both our present and prior (16) research resulted in decreased $k_{syn}$. Because there were no changes in $k_{deg}$, reducing

Table 2. Plasma T3 concentrations in rats during cold acclimation

<table>
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<tr>
<th>Days</th>
<th>Control 25°C</th>
<th>Cold 5°C</th>
<th>P</th>
<th>Pooled SE</th>
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<tr>
<td></td>
<td>2.05</td>
<td>3.20</td>
<td></td>
<td>0.336</td>
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<tr>
<td>10</td>
<td>2.20</td>
<td>3.13</td>
<td></td>
<td>0.235</td>
</tr>
<tr>
<td>15</td>
<td>1.56</td>
<td>2.66</td>
<td></td>
<td>0.223</td>
</tr>
<tr>
<td>20</td>
<td>1.28</td>
<td>2.35</td>
<td></td>
<td>0.239</td>
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Units for triiodothyronine (T3) concentrations are ng/ml; n = 6 animals/group. P, probability of an overall treatment effect (cold vs. control; ANOVA).
k_{syn} was clearly the mechanism for initiating decreased skeletal muscle protein mass. In longer-term cold exposure (after day 5), k_{syn} returned to normal. This latter observation is in agreement with those made in calves exposed to cold for 21 days (19). Thus the apparent discrepancy in k_{syn} between Samuels et al. (16) and Scott et al. (19) can likely be explained by differences in the duration of cold exposure. Furthermore, results from both authors clearly do not support the hypothesis that increased protein turnover in skeletal muscle contribute to increased metabolic heat gain.

The observation during longer-term cold exposure (after day 5) that k_{syn} in skeletal muscle returned to normal control values makes sense. This does not mean that protein accretion will be the same as in control animals; it will still be lower in cold animals. In cold rats, skeletal muscle protein mass was lower; thus the absolute rates of protein synthesis and degradation and deposition would be lower by definition. As a consequence, the muscle protein mass will continue to diverge from non-cold-exposed (control) animals, even if k_{syn} and k_{deg} are not different form the control rats. If k_{syn} had remained lower, then the gap between the absolute rates of protein synthesis and degradation would widen and lead to an escalating divergence in skeletal mass.

Maintaining normal rates of protein turnover over long periods of time, whether in cold or normal conditions, is an advantage. Protein turnover provides a vital function. It removes and prevents the accumulation of abnormal and damaged proteins and peptides. During long periods of cold exposure, animals must forage for food and evade and defend themselves from predators. This requires a musculature that must be in good condition, which would be facilitated by maintaining normal rates of protein turnover. Also, animals in cold environments depend on their musculature for shivering thermogenesis, which would also be fostered by a musculature that was in optimal condition.

Cardiac muscle growth and protein turnover. In cardiac muscle, short-term cold exposure (2 and 5 days) in both our present and prior (16) research resulted in increased protein synthesis on both a fractional and absolute basis. In longer-term cold exposure, we observed in the present experiment that, after day 5, the fractional and absolute rates of protein synthesis and degradation in general remained higher in cold rats. However, there appeared to be a disproportionate increase in synthesis and degradation on day 20, but this may be anomalous because there were no disproportionate changes in heart protein mass or growth rate. If this were the case, then cardiac muscle protein turnover would be returning toward control values over the 20 days of cold exposure.

Overall, between days 5 and 20, the protein mass of the heart increased (because the absolute rates of protein synthesis were greater than degradation). Heart protein mass in cold rats was still diverging from control rats after 20 days. Obviously, heart protein mass cannot continue to diverge indefinitely, especially considering that body mass is becoming relatively smaller; this suggests that protein metabolism in heart had not fully acclimated to cold. This could explain why rates of protein synthesis and degradation remained high in cold-exposed rats. Maintaining higher-than-normal rates of protein turnover in heart would also put a strain on this tissue; returning rates of protein turnover to control values would aid in reducing the strain on the heart. This contention is supported by observations made in calves exposed to cold for 21 days (19) in which k_{syn} was not different from control animals. However, this difference could be attributed to the intensity of cold exposure; skeletal muscle protein mass and nitrogen balance were unchanged in the cold-exposed calves (19), implying less severe cold exposure. The high rates of protein turnover that we observed during cold acclimation could be also explained by the increase in contractile activity that the heart must make; increased rates of contractions increase protein turnover in skeletal muscle (20) to repair and/or replace damaged proteins, so the same may be true for heart. Observations beyond 20 days of cold exposure in rats would have to be made to resolve this.

Regulation. Acclimation of skeletal and cardiac muscle protein metabolism to cold must be controlled by hormones and/or other factors. Thyroid hormones would likely play a role. Plasma thyroid hormones are elevated with increased metabolic rate and during cold exposure (6). Increased concentrations of plasma T3 were observed throughout the period of cold exposure in the present study. In heart, increased plasma T3 levels increased both k_{syn} and k_{deg} (4), suggesting that this hormone may play a role in mediating the heart's response to cold.

In skeletal muscle, increased plasma T3 levels increased both k_{syn} and k_{deg} in rats (3, 4). In the present research, there was no measurable increase in skeletal muscle protein turnover, despite elevated plasma levels of T3. Thus other hormones and factors may be involved. Plasma concentrations of insulin, growth hormone, insulin-like growth factor I, and tissue amino acid concentrations are all depressed during cold exposure (18, 21), which inhibit skeletal muscle protein synthesis but foster degradation (20). This cacophony of factors could explain the decreased skeletal muscle k_{syn} but not k_{deg} because increased T3 and decreased insulin, etc., would promote higher rates of protein degradation. The factors regulating changes in protein metabolism during cold exposure require further attention.

Conclusion. Long-term cold exposure resulted in decreased skeletal muscle and increased cardiac muscle protein deposition. Cold acclimation in skeletal muscle was initiated by decreased k_{syn} followed by a return to normal; the transition occurred after ∼5 days. In heart, the majority of changes in protein metabolism also occurred during the first 5 days of cold exposure, but protein turnover was enhanced throughout the period of acclimation, suggesting acclimation may not yet be complete.
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Perspectives

We speculate that the lower rate of accretion in body and skeletal muscle protein mass is a part of a deliberate strategy to limit body size to make more effective use of substrates for heat production rather than for growth. This understanding of the role of skeletal and cardiac protein metabolism in the process of acclimation to cold environmental temperatures was dependent on making observations at multiple time points. The most dramatic changes in protein turnover occurred acutely, within 5 days, which initiated changes that would start the process of acclimation. The later time points revealed that reverting to “normal” fractional rates of protein turnover during longer-term cold exposure would allow for a slower rate of growth and at the same time aid in preserving skeletal muscle functional integrity. These short- and long-term adaptations are consistent with a strategy to ensure survival over long periods of exposure to cold environmental temperatures.

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