Cellular adaptations in fat tissue of exercise-trained miniature swine: role of excess energy intake

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Cellular adaptations in fat tissue of exercise-trained miniature swine: role of excess energy intake. J. Appl. Physiol. 88: 881–887, 2000.—This study examined the influence of energy expenditure and energy intake on cellular mechanisms regulating adipose tissue metabolism. Two sets of female Yucatan miniature swine were assigned to restricted-fed sedentary, restricted-fed exercise-trained, full-fed sedentary, or full-fed exercise-trained groups. After 3 mo of treatment, adipocytes were isolated and adipocyte size, adenosine A1 receptor characteristics, and lipolytic sensitivity were measured. Swine were infused with epinephrine during which adipose tissue extracellular adenosine, plasma fatty acids, and plasma glycerol were measured. Results revealed that adipocytes isolated from restricted-fed exercised swine had a smaller diameter, a lower number of A1 receptors, and a greater sensitivity to lipolytic stimulation, compared with adipocytes from full-fed exercised swine. Extracellular adenosine levels were transiently increased on infusion of epinephrine in adipose tissue of restricted-fed exercised but not full-fed exercised swine. These results suggest a role for adenosine in explaining the discrepancy between in vitro and in vivo lipolysis findings and underscore the notion that excess energy intake of a low-fat diet would dampen the lipolytic sensitivity of adipocytes, even if adipocytes were isolated from exercise-trained animals. In vitro, we examined adipocyte lipolytic sensitivity to isoproterenol, epinephrine, and adenosine and adenosine A1 receptor characteristics. In vivo, we examined extracellular adenosine levels in adipose tissue and whole body lipolysis. Our results revealed that in vivo and in vitro exercise-induced changes in adipose tissue lipolytic sensitivity were negated by excess energy intake. The results also suggested that the presence of extracellular adenosine may account, in part, for the discrepancy often reported in the literature between in vivo and in vitro lipolytic responsiveness of adipose tissue.

MATERIALS AND METHODS

Pigs and diet. Six sets of female Yucatan miniature swine, four litters per set at age 10–12 wk, were used for this 3-mo study. Each littermate was assigned to one of four treatments: restricted-fed sedentary, restricted-fed exercised, full-fed sedentary, or full-fed exercised. Adipose tissue from male and female swine respond similarly to adenosine in vitro (5); female swine were used in this study because of their better compliance to the exercise regimen and their greater amount of subcutaneous adipose tissue compared with males. Swine were housed four to six swine per 18-m² pen at the Burley-Demeritt swine facility (Lee, NH). All swine were fed a miniature swine ration (Agway, Syracuse, NY) with a caloric density of 10.21 kJ/g for the nutritional needs of growing miniature swine. Full-fed swine were fed 73 g·kg body wt⁻¹·day⁻¹ in two meals; this was equivalent to the daily caloric intake of swine in our previous study (28). Restricted-fed swine were fed 53 g·kg body wt⁻¹·day⁻¹ in two meals; this was 25% less than the full-fed swine and
equivalent to the usual daily caloric intake of our swine. Restricted-fed swine were fed individually, but logistical constraints necessitated that full-fed swine be fed as pairs (sedentary and exerciser). Food was preweighed, and any leftovers were collected and weighed to determine actual food intake. Swine had free access to water except during the exercise sessions. Swine body weights were recorded at the beginning of each week. All procedures were approved by the University of New Hampshire Animal Care and Use Committee (Approval No. 930305).

Exercise. An endurance exercise regimen was employed as described by Carey and Sidmore (5). This regimen gradually adapted the swine to run on motor-driven treadmills with rubberized belts so that, by the end of 2 mo, they ran for 45 min/day, 5 days/wk, at 9 km/h under climate-controlled conditions. They continued to train for an additional month at this intensity.

Jugular catheter implantation. After 3 mo of exercise training or rest, swine were anesthetized with isoflurane and catheters were implanted into the left jugular vein. The surgical procedure of Moritz et al. (29) was followed, and the tubing was kept patent with a 60% polyvinylpyrrolidone solution in saline with 500 U heparin/ml.

Fat biopsy and adipocyte isolation. Concurrent with the jugular vein catheterization, an 8-g portion of adipose tissue from over the shoulder was removed surgically and transported to the laboratory in warm saline. Tissue was minced into 1-mm³ pieces, rinsed with warm saline, and dissociated with collagenase; adipocytes were then isolated as described previously (5).

Membrane preparation and adenosine A₁ receptor binding assay. Approximately 3 ml of packed cells were used to prepare an adipocyte crude plasma membrane fraction, as described by Dong and Carey (8). The fraction was resuspended to 2 mg protein/ml and stored at –80°C until being assayed for A₁ receptor binding kinetics (8).

Adipocyte incubations. The remaining adipocytes were resuspended to a 5% solution (vol/vol) and used for in vitro lipolysis measurements. Duplicate aliquots of cells (600 µl) were pipetted into 7-ml polypropylene incubation vials and placed in a 38°C (swine body temperature) shaking water bath at 50 oscillations/min. Lipolysis was initiated with the addition of 1 unit of adenosine deaminase and one of eight levels of phenylisopropyladenosine (PIA, 10⁻⁸ to 10⁻³ M) epinephrine, 10⁻⁶ M isoproterenol, or 10⁻⁶ M epinephrine plus one of eight levels of phenylisopropyladenosine (PIA, final concentration varied from 10⁻⁸ to 5 × 10⁻¹⁰ M). Final incubation volume was 750 µl. Vials were gassed with 95% O₂-5% CO₂ and capped and shaken at 90–100 oscillations/min at 38°C.

After 90 min, lipolysis was stopped by decanting vial contents into microcentrifuge tubes containing 56 µl of 7 M HClO₄. After remaining on ice for 60 min, the precipitated proteins were removed by centrifugation and the supernate was neutralized with 10 N KOH. Samples were adjusted to pH of 7 and frozen at –80°C until being assayed for glycerol and free fatty acids (Sigma Chemical, St. Louis, MO).

Statistical analyses. Data were analyzed using a two-way ANOVA, blocked for litter for the treatment effects of exercise and energy intake. When a significant F test was obtained, pairwise comparison was performed via Fisher’s least significant difference test. Student’s t-test was used to test for temporal changes in plasma free fatty acids, plasma glycerol, and extracellular adenosine. Significance was set at P < 0.05 unless otherwise indicated. Systat software (version 7.0.1) was used for all statistical analyses.

RESULTS

Food intake, body weight, and muscle enzyme activity. Restricted-fed swine consumed an average of 52 g·kg body wt⁻¹·day⁻¹, whereas full-fed swine consumed an average of 71 g·kg body wt⁻¹·day⁻¹ (Table 1). These diet intake values approximated the targeted energy consumption for the restricted-fed and full-fed swine at 53 and 73 g·kg body wt⁻¹·day⁻¹, respectively. Exercised restricted-fed swine consumed slightly less food than sedentary restricted-fed swine; exercised full-fed swine and sedentary full-fed swine were fed as pairs; therefore, it was not possible to determine the effect of exercise on food consumption in full-fed swine. Average body weights of swine in each group were
Energy intake, exercise training, and adipose tissue

Table 1. Food intake, body weight, and muscle citrate synthase activity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Food Intake, g/kg body wt (^{-1})day (^{-1})</th>
<th>Body Weight, kg</th>
<th>Average Gain, kg/week</th>
<th>Muscle Citrate Synthase, (\mu)mol \cdot \text{min}^{-1} \cdot \text{g tissue}^{-1}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Restricted-fed sedentary</td>
<td>52.3</td>
<td>10.1</td>
<td>14.3</td>
<td>20.2</td>
</tr>
<tr>
<td>Restricted-fed exercised</td>
<td>51.9</td>
<td>10.0</td>
<td>14.3</td>
<td>20.7</td>
</tr>
<tr>
<td>Full-fed sedentary</td>
<td>71.1</td>
<td>10.1</td>
<td>16.6</td>
<td>25.8</td>
</tr>
<tr>
<td>Full-fed exercised</td>
<td>71.1</td>
<td>10.2</td>
<td>16.3</td>
<td>24.7</td>
</tr>
<tr>
<td>Pooled SE</td>
<td>0.5</td>
<td>0.5</td>
<td>0.8</td>
<td>1.1</td>
</tr>
<tr>
<td>Statistical summary, P value</td>
<td>0.065</td>
<td>0.979</td>
<td>0.843</td>
<td>0.819</td>
</tr>
<tr>
<td></td>
<td>0.001</td>
<td>0.729</td>
<td>0.039</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>0.248</td>
<td>0.835</td>
<td>0.842</td>
<td>0.507</td>
</tr>
</tbody>
</table>

Values are means ± SE; \(n = 6\) swine. Full-fed swine were fed as pairs (sedentary plus exercised); values for food intake were calculated assuming equal food intake by each member of the pair. For full-fed sedentary and full-fed exercised (where \(n = 5\)), body wt at 4 wk: SE = 1.0, body wt at 8 wk: SE = 1.3, body wt at 12 wk: SE = 1.5. P values are from two-way ANOVA, blocked for litter.

similar at the start of the study, but, after 4 wk, there was a significant effect of diet on swine body weight (Table 1). Average weight gain for swine in the restricted-fed treatments was 1.46 kg/week, and there was no difference in the rate of gain for restricted-fed exercised vs. restricted-fed sedentary swine. Full-fed swine gained an average of 2.0 kg/week (Table 1).

Muscle citrate synthase activity was 47% greater in restricted-fed exercised vs. restricted-fed sedentary swine (11.18 vs. 7.64 \(\mu\)mol \cdot \text{min}^{-1} \cdot \text{g muscle}^{-1}\), respectively, \(P < 0.05\)) and 99% greater in full-fed exercised vs. full-fed sedentary swine (13.49 vs. 6.79 \(\mu\)mol \cdot \text{min}^{-1} \cdot \text{g muscle}^{-1}\), respectively, \(P < 0.05\)) (Table 1). Exercise caused an overall 72% increase in muscle citrate synthase activity; there was no significant effect of diet on muscle aerobic capacity.

Adipocyte size, lipolytic sensitivity, and \(A_1\) receptor characteristics. Exercise and diet treatment significantly influenced adipocyte size (Table 2). Restricted-fed exercised swine had the smallest diameter cells at 89 \(\mu\)m, and full-fed sedentary had the largest diameter cells at 114 \(\mu\)m. Exercise-trained swine within a dietary group had significantly smaller cells than their sedentary littersmates, and full-feeding increased adipocyte size, within both the exercise-trained and sedentary groups.

There was a significant effect of exercise on adipocyte lipolysis in response to stimulation by isoproterenol (Table 2). However, when adipocytes were lipolytically stimulated with epinephrine, both exercise and diet had significant treatment effects. In contrast to what was seen with isoproterenol, epinephrine-stimulated lipolysis was suppressed by \(\sim 50\%\) with full feeding in both the sedentary and exercise-trained groups, compared with their sedentary controls.

The dampening effect of overfeeding on adipocyte lipolytic sensitivity to epinephrine (Table 2) disappeared when the epinephrine concentration was raised from \(10^{-6}\) to \(10^{-3}\) M (Fig. 1). Lipolytic sensitivity at \(10^{-5}\) M epinephrine was similar to that at \(10^{-6}\) M isoproterenol. At concentrations of \(10^{-7}\) M epinephrine or lower, there was no significant difference in lipolytic response among the groups.

Although there was no significant treatment effect of exercise or diet on adipocyte sensitivity to adenosine (\(P = 0.205\) and 0.231, respectively), there were trends. The concentration of PIA needed for 50% inhibition of lipolysis was greatest for the restricted-fed exercised group.

Table 2. Adipocyte size, lipolytic sensitivity, and adenosine \(A_1\) receptor characteristics

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cell Diameter, (\mu)m</th>
<th>Isoproterenol ((10^{-6}) M)</th>
<th>Epinephrine ((10^{-6}) M)</th>
<th>PIA (K_{i0.5}), nM</th>
<th>(K_A), nM</th>
<th>(B_{max}), fmol/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Restricted-fed sedentary</td>
<td>103</td>
<td>34.4</td>
<td>31.0</td>
<td>2.37</td>
<td>1.78</td>
<td>279</td>
</tr>
<tr>
<td>Restricted-fed exercised</td>
<td>89</td>
<td>51.8</td>
<td>53.0</td>
<td>2.68</td>
<td>0.72</td>
<td>280</td>
</tr>
<tr>
<td>Full-fed sedentary</td>
<td>114</td>
<td>21.6</td>
<td>15.9</td>
<td>1.90</td>
<td>1.21</td>
<td>310</td>
</tr>
<tr>
<td>Full-fed exercised</td>
<td>103</td>
<td>50.3</td>
<td>29.4</td>
<td>2.40</td>
<td>1.16</td>
<td>266</td>
</tr>
<tr>
<td>Pooled SE</td>
<td>4</td>
<td>8.9</td>
<td>4.7</td>
<td>0.28</td>
<td>0.62</td>
<td>34</td>
</tr>
<tr>
<td>Statistical summary, P value</td>
<td>0.004</td>
<td>0.025</td>
<td>0.006</td>
<td>0.205</td>
<td>0.398</td>
<td>0.069</td>
</tr>
<tr>
<td></td>
<td>0.006</td>
<td>0.447</td>
<td>0.003</td>
<td>0.231</td>
<td>0.931</td>
<td>0.201</td>
</tr>
<tr>
<td></td>
<td>0.721</td>
<td>0.552</td>
<td>0.789</td>
<td>0.754</td>
<td>0.442</td>
<td>0.449</td>
</tr>
</tbody>
</table>

Values are means for adipocyte diameter (\(n = 6\)), glycerol release (\(n = 6\)), concentration of phenylisopropyladenosine needed for 50% inhibition of lipolysis (PIA \(K_{i0.5}\) (\(n = 5\) or 6), and \(A_1\) receptor characteristics (\(n = 5\)). Adipocytes were isolated from restricted-fed vs. full-fed swine that were either exercise trained or sedentary. SA, adipocyte surface area; \(K_A\), ligand binding affinity; \(B_{max}\), no. of binding sites. \(^*\)For restricted-fed exercised treatment, \(n = 5\) and SE = 10.0. \(^\ddagger\)For restricted-fed exercised treatment, \(n = 5\) and SE = 5.3. \(^\dagger\)For restricted-fed exercised and restricted-fed sedentary treatments, \(n = 5\) and SE = 0.32. \(^\ddagger\)For restricted-fed exercised and restricted-fed sedentary treatments, \(n = 4\) and SE = 0.72. \(^*\)For restricted-fed exercised and restricted-fed sedentary treatments, \(n = 4\) and SE = 40. P values are from two-way ANOVA, blocked for litter.
group, the group that was most lipolytically sensitive to isoproterenol and epinephrine, whereas it was lowest for the full-fed sedentary group, the group that was the least lipolytically sensitive (Table 2). These trends were inversely related to the number of adenosine A1 receptors: the restricted-fed exercised group had the least number of receptors, whereas the full-fed sedentary group had the most. There was no difference in the binding affinity of PIA for the adenosine receptor among the four groups.

Adipose tissue extracellular adenosine levels. For the first 10 min after microdialysis probes were inserted in adipose tissue, extracellular adenosine levels averaged 685 nM (Fig. 2). This was apparently due to tissue trauma because, by 20 min, the levels dropped 78% to an average of 154 nM and, by 40 min, the levels had stabilized at 87 nM. There was no difference in extracellular adenosine levels among the four groups at the end of the 60-min baseline period.

The adenosine area under the curve (AUC) during each infusion period was calculated for each swine, after being corrected for baseline. The adenosine AUC for the epinephrine infusion was significantly influenced by diet, with the restricted-fed-exercised group significantly greater than the full-fed exercised group. The AUC for the epinephrine plus dipyridamole infusion was also influenced by diet, with the restricted-fed exercised group gradually rising during the infusion period, although this did not reach statistical significance (P = 0.073). There was no significant effect of exercise or diet on AUC when theophylline was added to the infusate.

Local inflammatory response due to probe. In a separate study of identical design, adipose tissue histology around the microdialysis probe insertion site was examined. At the conclusion of the in vivo challenge, the probe was removed and a 1-cm adipose tissue core surrounding the location of the probe was removed. A control tissue core was removed from a site 1 cm from the probe insertion site. Samples were placed in formalin and examined histologically using light microscopy. There was evidence of mild perivascular inflammation, with scattered neutrophils and macrophages, in 4 of 10 probe samples and 1 of 10 control samples.

Plasma free fatty acids and glycerol. Analysis of the plasma glycerol levels over the baseline period suggested an effect of diet (P < 0.055): overfed swine averaged 1.60 mg/100 ml glycerol, whereas restricted-fed swine averaged 1.20 mg/100 ml glycerol (Fig. 3). On epinephrine infusion, however, sedentary swine appeared more lipolytically sensitive than exercise-trained swine. Plasma glycerol levels were greater at 10, 20, and 30 min of epinephrine infusion in the...
restricted-fed sedentary and full-fed sedentary groups compared with their baseline values at 20 and 30 min in the full-fed exercised group compared with baseline and at 20 min in the restricted-fed exercised group compared with baseline. There were no differences among the groups in AUC for plasma glycerol during any of the infusion periods.

Unlike the plasma glycerol data, there was no significant effect of diet or exercise on plasma free fatty acid levels during the baseline period (Fig. 4). However, like the plasma glycerol response during the drug challenge, sedentary swine also showed a greater plasma free fatty acid response than the exercise-trained swine. Free fatty acid levels were greater at 20 and 30 min of epinephrine infusion in the restricted-fed sedentary and full-fed sedentary groups compared with baseline values and at 30 min only in the restricted-fed exercised group compared with baseline. There were no differences between the groups in the AUC for free fatty acids during any of the infusion periods.

**DISCUSSION**

The primary finding of this study confirms our hypothesis that excess energy intake, even if accompanied by exercise training, dampens the lipolytic sensitivity of adipocytes to β-agonists and adenosine at the cellular level. These cellular adaptations may be responsible for, or a consequence of, larger adipocytes and higher body weight of full-fed exercise-trained swine, compared with restricted-fed exercise-trained controls. In either case, the question arises: Must exercise be accompanied by energy restriction to be an effective mechanism for weight loss or weight maintenance?

It is commonly believed that an increase in energy expenditure will be compensated for by an increase in food intake. This implies that increasing physical activity is a poor strategy for losing weight. However, some researchers believe that energy expenditure and energy intake are weakly coupled (3, 20). Thus exercise may be a useful mechanism to achieve weight loss or weight maintenance, if dietary overindulgence is not practiced. This study illustrated that dietary overindulgence (which comes naturally to swine) attenuates the effects of exercise. For humans, such overindulgences include inappropriate food choices, a desire for self-reward after exercise, and misjudgments about the relative rates at which energy is expended or consumed (3).

Body weight is not necessarily an indicator of body fatness (11). In this study, restricted-fed exercised swine consuming the same amount of energy as their sedentary littermates had similar body weights. However, the smaller adipocyte of the restricted-fed exercised swine suggests that there was a difference in body composition between exercised and sedentary swine. Unfortunately, body composition was not measured in this study. In a study by Kraemer et al. (22), a disparity between body weight and body composition was documented. Overweight men subjected to caloric restriction for 12 wk lost the same amount of body weight as overweight men subjected to caloric restriction and exercise. However, the composition of that loss was significantly different: body fat decreased 3.6% with caloric restriction alone, whereas body fat decreased 8.4% with caloric restriction and exercise. This and other studies support the notion that body weight and body fat are not necessarily synonymous.

A secondary finding of this study is the potential role of extracellular adenosine in regulating lipolysis. Adenosine is a locally produced hormone with a half-life in the blood of 0.1 s (30). It is known to be taken up rapidly by endothelial cells (30, 32) and may be produced by parenchymal cells directly or from adenine nucleotide precursors either intracellularly (9) or extracellularly (47). In adipocytes, adenosine binds to the adenosine A₁ receptor and inhibits adenyl cyclase and thus lipolytic activity. In vitro and in vivo findings demonstrate that adenosine A₁ receptor downregulation can occur in response to elevated extracellular adenosine (12, 14, 26). In the present study, differences in extracellular adenosine between treatments were not observed under basal conditions but only on in vivo infusion of epinephrine or epinephrine plus dipyridamole in restricted-fed exercised swine. These transient rises in adenosine may, over time, be responsible for the observed downregulation of the A₁ receptor in the restricted-fed exercised group. The failure of theophylline infusion to further exacerbate the rise in extracellular adenosine may be due to the gradual desensitization to infused epinephrine; theophylline infusion occurred 60 min after the epinephrine infusion had begun. Houseknecht et al. (15) demonstrated that plasma free fatty acids and glycerol returned to baseline by 60 min in cows despite continued infusion of epinephrine; desensitization to epinephrine also has been demonstrated in vivo in humans (39). This limitation can be avoided in future studies by using a bolus injection of epinephrine rather than a continuous infusion or shortening the time frame of the experiment.

The epinephrine-induced rise in extracellular adenosine also may account, in part, for the low-plasma free fatty acid and glycerol levels seen on epinephrine infusion in these swine. The in vivo findings, which
suggest low lipolytic sensitivity, contrast with in vitro findings in which adipocytes isolated from exercise-trained swine fed moderate amounts of feed have greater lipolytic sensitivity than adipocytes isolated from sedentary or overfed swine. This discrepancy between lipolysis in vitro and in vivo, noted by others (24), suggests the involvement of other physiological mechanisms present in intact adipose tissue that may regulate extracellular adenosine level. Such mechanisms may involve the rapid uptake of adenosine by endothelial cells, as observed in heart tissue (31, 33), or an increase in epinephrine-stimulated blood flow, as observed with exercise training in humans (40).

The plasma free fatty acid level in response to epinephrine infusion must be interpreted with caution. Free fatty acid levels represent the balance between release (primarily from adipose tissue), esterification (primarily by adipose tissue), and uptake (primarily by muscle); therefore, changes in free fatty acid levels may be the result of a change in release, esterification, uptake, or a combination of the three. The relationship between plasma free fatty acid concentration and lipolysis may be uncoupled by the physiological state, such as exercise, as elegantly demonstrated by Klein et al. (21).

The role of adenosine in regulating in vivo lipolysis in response to short-term fasting in humans was investigated by Peters et al. (34). Theophylline was infused at a dose sufficient to antagonize the adenosine receptor but not interfere with cAMP phosphodiesterase activity in subjects who were fasted for 14 or 86 h. In vivo lipolysis was measured by the rate of appearance of glycerol using d3-glycerol infusion. Theophylline infusion caused a mild increase in lipolysis in the 14-h fasted subjects and a marked increase in lipolysis in the 86-h fasted subjects, suggesting an increase in adenosine “activity” with fasting. However, these data can be viewed from another perspective: adenosine activity can be realized only when adenosine is present, and extracellular adenosine was not measured in this study. It is possible that the reverse adaptation that we observed with exercise was taking place with fasting: adenosine receptors may be upregulated in response to a lowered extracellular adenosine content. However, the greater number of receptors will only be called on if there is sufficient ligand to bind to them. Clearly, more work is warranted in this area.

Microdialysis has proven to be a very useful technique to ascertain adipose tissue extracellular metabolite concentrations (1, 2, 13, 35), including adenosine (27). The concentration of adenosine will be influenced by its rate of uptake and production by adipocytes and endothelial cells and its rate of delivery and removal via the microcirculation (23). One shortcoming of the present study is that adipose tissue blood flow was not measured; therefore, it is uncertain whether changes in adenosine levels reflect changes in adenosine production or removal. Resting adipose tissue blood flow in humans has been shown to increase with exercise training (40), and, conversely, adipose tissue blood flow plays an important role in regulating lipid metabolism (36). A second shortcoming is the mild inflammation around the probe and the possibility of leukocytes contributing to local adenosine production. Although this would not impact differences observed in the present study among groups, it may impact the extracellular adenosine values.

In summary, our results suggest that excess energy intake overrides the benefits of exercise training in adipose tissue of miniature swine. Furthermore, adenosine appears to play a role in regulating lipolysis in vivo. Future studies will be needed to examine the source of extracellular adenosine in adipose tissue and how adenosine production may be regulated.

This study represents the collective effort of many people; it would not have been possible without their help. I am most grateful to the following individuals from the University of New Hampshire (unless otherwise noted) for their contributions: Van Gould, animal technician, for adipose tissue biopsy and jugular vein surgery; Tom Oxford, swine barn manager, for care and feeding of the swine; Haven Hayes, technician, for assistance with surgeries, swine transportation, drug infusion studies, glycerol assays, and swine exercise; Jen LeClair, undergraduate student, for swine exercise; Jennifer Schuchman, Maureen Tanguay, and Eli Morse, undergraduate students, for plasma glycerol and free fatty acid assays; Joel Linden, Professor, and Toni Barbera, technician (University of Virginia), for adenosine assays; Ed Zambraski, Professor (Rutgers University), for advice on implanting jugular vein catheters, and Carroll Jones, veterinary pathologist, for adipose tissue histology.

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