Chronic exercise lowers the defended body weight gain and adiposity in diet-induced obese rats

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Levin, Barry E., and Ambrose A. Dunn-Meynell. Chronic exercise lowers the defended body weight gain and adiposity in diet-induced obese rats. Am J Physiol Regul Integr Comp Physiol 286: R771–R778, 2004. First published December 24, 2003; 10.1152/ajpregu.00650.2003.—The effects of running wheel exercise and caloric restriction on the regulation of body weight, adiposity, and hypothalamic neuropeptide expression were compared in diet-induced obese male rats over 6 wk. Compared with sedentary controls, exercising rats had reduced body weight gain (24%), visceral (4 fat pads; 36%) and carcass (leptin; 35%) adiposity but not insulin levels. Hypothalamic arcuate nucleus (ARC) proopiomelanocortin (POMC) mRNA expression was 25% lower, but ARC neuropeptide Y (NPY), agouti-related peptide, dorsomedial nucleus (DMN) NPY, and paraventricular nucleus (PVN) corticotropin-releasing hormone (CRH) expression was comparable to controls. Sedentary rats calorically restricted to 85% of control body weight reduced their visceral adiposity (24%), leptin (64%), and insulin (21%) levels. ARC NPY (23%) and DMN NPY (60%) were increased, while ARC POMC (40%) and PVN CRH (14%) were decreased. Calorically restricted exercising rats ran half as much as ad libitum-fed exercising rats and had less visceral obesity than comparably restricted sedentary rats. When sedentary restricted rats were refed after 4 wk, they increased intake and regained the weight gain and adiposity of sedentary controls. While refed exercising rats and sedentary rats ate comparable amounts, refed exercising rats regained weight and adiposity only to the level of ad libitum-fed exercising rats. Thus exercise lowers the defended level of weight gain and adiposity without a compensatory increase in intake and with a very different profile of hypothalamic neuropeptide expression from calorically restricted rats. This may be due to exercise-related factors other than plasma insulin and leptin.

THE RECIDIVISM RATE in the long-term treatment of human obesity is high, and most lost weight is replaced over the ensuing months and years (15, 39). Similarly, weight-reduced rats quickly regain lost weight when allowed free access to food (14, 19, 22, 26). Such regain in humans may be partly attributable to the chronic reduction in resting metabolic rate associated with such weight loss (16, 17). In rats, long-term weight loss is also associated with reduced metabolic rate (8) and an increase in the hypothalamic arcuate nucleus (ARC) mRNA expression of anabolic neuropeptides such as neuropeptide Y (NPY) (4, 41) and a decrease in peptides such as proopiomelanocortin (POMC), a precursor of the catabolic melanocortin agonist α-melanocyte-stimulating hormone (α-MSH) (6, 7, 10, 32, 40). This combination is associated with a powerful, centrally mediated drive to restore lost energy stores by increasing energy intake and reducing energy expenditure.

The addition of high levels of physical activity to caloric restriction appears to be a critical factor for successful long-term weight loss maintenance in some humans (42). However, very little is known about the effects of exercise and the combination of exercise and caloric restriction on the defended body weight relative to the central neuropeptides involved in the regulation of energy homeostasis. Here we used a rat model of diet-induced obesity (DIO) to investigate these interactions. We measured the mRNA expression of the signaling form of the leptin receptor (Lepr-b) and neuropeptides [NPY, POMC, agouti-related peptide (AgRP), corticotropin-releasing hormone (CRH)] involved in the regulation of energy homeostasis as indexes of changes induced in these critical control mechanisms by caloric restriction and exercise.

METHODS

Animals and diet. Animal usage was in compliance with the animal care committee of the East Orange Veterans Affairs Medical Center and the guidelines of the American Physiological Society (1). The study began with 42 male selectively bred DIO rats from our in-house colony. These rats were bred from the outbred Sprague-Dawley strain for their propensity to develop DIO when fed a diet relatively high in fat and energy content [high-energy (HE) diet] (23). Rats were fed Purina rat chow from weaning and then placed on HE diet for 8 wk, beginning at 10 wk of age. Food and water were available ad libitum during this time, and rats were housed individually in shoebox cages on a 12:12-h light-dark schedule with lights out at 1800 and lights on at 0600. Purina rat chow (no. 5001) contains 3.30 kcal/g with 23.4% protein, 4.47 kcal/g with 21% of the metabolizable energy content as protein, 31% as fat, and 48% as carbohydrate, 50% of which is sucrose (25).

After 8 wk on HE diet, rats were switched back to chow for 2 wk (weeks −2 to 0). At that time, they were randomized by body weight and divided into six groups of seven rats each. 1) Sedentary-ad lib rats remained sedentary with ad libitum chow intake for 6 wk (weeks 0–6). 2) Exercise-ad lib rats had a running wheel (MimMitter) placed in their home cages and had chow available ad libitum for 6 wk (weeks 0–6). 3) Exercise-restrict 6 wk rats had running wheels in their home cages for 6 wk with their food intake restricted so as to bring their body weight to 85% of their baseline level. This was accomplished by reducing food intake to 12, 16, and 20 g/day at 4-day intervals and then readjusting to 22 g/day (72.6 kcal/day) in all rats to maintain body weights at an average for the group of 85% of the group baseline. For all restricted rats, food was provided at 2 h before dark onset each day (1600; weeks 0–6). 4) Sedentary-restrict 6 wk rats

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remained sedentary but had their food intake restricted to the same intake as the exercise-restrict 6 wk rats (72.6 kcal/day; weeks 0–6). This turned out to be the same amount of food (22 g/day) as the exercise-restrict 6 wk rats. 5) Exercise-restrict 4 wk rats were treated in the same way as the exercise-restrict 6 wk rats for 4 wk (weeks 0–4), after which time they were allowed ad libitum access to chow and continuous access to the running wheels (weeks 4–6). 6) Sedentary-restrict 4 wk rats remained sedentary but had their food intake restricted for 4 wk (weeks 0–4) so that their body weights matched those of the exercise-restrict 6 wk rats. At 4 wk, they were allowed ad libitum access to chow for 2 wk (weeks 4–6). At the end of week 6, wheels were removed from cages, and all rats were killed by decapitation 1 day later between 0800 and 1200. This was done so that brain parameters would not reflect the effects of exercise at the time of death. Food intake and body weight were measured during the last 24 h of wheel access and the terminal 24 h after wheel removal. After decapitation, brains were quickly removed, frozen on dry ice, and stored at −80°C. Epididymal, retroperitoneal, perirenal, and mesenteric fat pads were removed and weighed as visceral fat pads. During the course of the experiment, cumulative food intake and body weight were monitored every 3–4 days. At weekly intervals, blood was collected by tail nip for assay of plasma leptin and insulin levels.

**Measurement of wheel running.** Wheel running data were collected in 1-h time bins remotely by a computerized system (MiniMitter) continuously during the entire 6-wk experimental period.

**Plasma leptin and insulin levels.** Insulin and leptin levels were measured using radioimmunoassay kits (Linco).

**In situ hybridization.** The mRNA for Lepr-b and for NPY, AgRP, and POMC was assayed in serial 15-μm sections taken through the rostrocaudal extent of the ARC, dorsomedial (DMN), and ventromedial (VMN) hypothalamic nuclei. Assay for CRH was carried out in serial section through the rostrocaudal extent of the paraventricular nucleus (PVN). Sections were processed for in situ hybridization by minor modifications of previously described methods (21, 27). Briefly, cRNA was synthesized and radiolabeled from a 400-bp probe corresponding to the intracellular domain of the long form of the leptin receptor (Lepr-b; amino acids 930–1063), NPY (511 bp), AgRP (348 bp), CRH (455 bp), and POMC (923 bp). The probes were hydrolyzed in 0.5 M NaOH for 15–30 min. The probes were then subjected to our standard method for in situ hybridization (18, 22). On completion of hybridization, slides were opposed to SB-5 X-ray film (Kodak, Rochester, NY) for 24–48 h (NPY, POMC, AgRP, CRH) or for 3 wk (Lepr-b). The resulting autoradiograms were read by an experimentally “blinded” observer using computer-assisted densitometry (Drexel Univ., Philadelphia, PA) (29). Areal measures (mm²) (NPY, AgRP, POMC, CRH) or optical density (Lepr-b) measures were read through the entire rostrocaudal extent of the hypothalamus in the ARC, VMN, DMN, and PVN. The outlines of the autoradiographic images of NPY, AgRP, POMC, and CRH expression were well defined. These images were quantitated using our standard method by which a line is drawn around the exposed image of each nucleus and the area within the boundaries of the outlined anatomic structure is measured (29). The Lepr-b images were less well defined than those for the neuropeptide mRNA such that no distinct borders for a given hypothalamic nucleus could be clearly discerned. For these images, the brain section used to generate the individual autoradiographic image was stained with cresyl violet, and the digitized image of that histological section was superimposed on the digitized autoradiographic image. A line was drawn around the anatomic boundaries of the ARC, VMN, and DMN on the histological image, and the optical density was read from the superimposed autoradiographic image. The three highest readings for a given probe were averaged for each animal.

**Statistics.** Body weight gain, food intake, and plasma leptin and insulin data were assessed by two-way ANOVA for repeated measures. Terminal measures, including in situ hybridization data, were compared by two-way ANOVA and, where significant intergroup differences were found, by post hoc Scheffé comparisons.

**RESULTS**

All DIO rats in the study were switched to low-fat chow for 2 wk (weeks −2 and −1) after being on HE diet for 8 wk. During this time, they continued to gain weight from 436 ± 5 to 464 ± 5 g (P = 0.001, Fig. 1), but their leptin levels fell 28% from 11.3 ± 0.4 to 8.1 ± 0.4 ng/ml (P = 0.001; Fig. 2). This suggests that the switch from moderate- to low-fat diet resulted in a reduction in total carcass adiposity. In addition, plasma insulin levels fell 48% from 2.08 ± 0.12 to 1.09 ± 0.11 ng/ml (P = 0.001; Fig. 3) during the first 2 wk after this diet switch. All of this occurred with no change in food intake (Fig. 4).

**Effects of exercise.** At week 0, rats were randomized by body weight and placed in their respective treatment groups for 6 wk. When allowed ad libitum access to chow, exercise-ad lib rats reduced their cumulative body weight gain over the 6-wk period (weeks 0–6) by 33% compared with sedentary-ad lib rats (Fig. 1, Table 1). This reduced body weight gain was associated with a 36% reduction in the total weight of four visceral fat pads (mesenteric, retroperitoneal, perirenal, and epididymal pads) and 23% reduction in these fat pad weights as a function of body weight (Table 1). It is also likely that total
carcass adiposity was reduced because exercise-ad lib rats had 35% lower plasma leptin levels than sedentary-ad lib rats (Fig. 2; Table 1). Approximately 80% of the running took place during the dark cycle (Fig. 5A). Interestingly, despite a highly variable amount of running among rats (Fig. 5; Table 1), there were no correlations between the cumulative number of revolutions run and the final body weight, weight gained, or visceral fat pad weights. However, there were strong correlations between the amount of energy consumed and the body weight gain (r = 0.95; P < 0.01), visceral adipose depot weights (r = 0.9; P < 0.005), and terminal leptin levels (r = 0.87; P = 0.03).

During the 6-wk period after introduction of the running wheels, exercising rats increased their caloric intake above sedentary-ad lib rats transiently from week 3 to week 4 but decreased intake again over the last 2 wk (Fig. 4). Cumulatively, their intake did not differ over the 6-wk period from that of sedentary-ad lib rats (Table 1). Also, there was no correlation between the amount of running and caloric intake over 6 wk. Because exercising rats gained less weight during this period, their feed efficiency [amount of weight gained (g)/caloric intake (kcal)] over this period was only 79% of the sedentary-ad lib rats (Table 1).

Neuropeptide and Lepr-b mRNA expression was assessed 24 h after running wheels had been removed. During this time there was no significant change in either body weight or 24-h food intake compared with the previous 24 h when wheels were available (data not shown). The exercise-induced reductions in carcass composition, food intake, and plasma hormones were associated with a 25% decrease in ARC POMC mRNA expression compared with sedentary-ad lib rats (Fig. 6). While there was a tendency for exercise to increase DMN NPY expression, there was no significant effect of exercise on these levels or on the expression of ARC NPY or AgRP or PVN CRH mRNA (Fig. 6). Neither did exercise or any other manipulation in these studies alter hypothalamic Lepr-b expression (Table 2).

Effects of chronic caloric restriction. The reduction in body weight to 85% of that of sedentary-ad lib rats was accomplished over 5 days by restricting intake to 81% (72.6 kcal/day) of the group baseline (90 kcal/day; Table 1). Sedentary-restrict rats had a 24% reduction in their visceral fat
Table 1. *Physiological and biological parameters in sedentary and exercising rats during 6 wk of combinations of restricted or ad libitum food intake*

<table>
<thead>
<tr>
<th></th>
<th>Sedentary-ad lib</th>
<th>Exercise-ad lib</th>
<th>Sedentary-restrict 6 wk</th>
<th>Exercise-restrict 6 wk</th>
<th>Sedentary-restrict 4 wk</th>
<th>Exercise-restrict 4 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final body weight, g</td>
<td>513±14*</td>
<td>484±12*</td>
<td>431±10†</td>
<td>418±14†</td>
<td>500±15*</td>
<td>489±10*</td>
</tr>
<tr>
<td>Total body weight change, g</td>
<td>75.0±4.7*</td>
<td>50.1±2.3†</td>
<td>-7.2±11.1†</td>
<td>-23.3±11.3†</td>
<td>69±4*</td>
<td>47±5†</td>
</tr>
<tr>
<td>Final 2 wk intake, kcal/day</td>
<td>90.1±1.8*</td>
<td>86.7±2.0*</td>
<td>72.6</td>
<td>72.6</td>
<td>126±4.5†</td>
<td>126±3.1†</td>
</tr>
<tr>
<td>Total feed efficiency, g/kcal×1,000</td>
<td>23.1±1.6*</td>
<td>18.4±1.2†</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Final 2 wk feed efficiency, g/kcal×1,000</td>
<td>28.0±3.3*</td>
<td>20.4±2.3†</td>
<td>ND</td>
<td>ND</td>
<td>128±6†</td>
<td>113±4§</td>
</tr>
<tr>
<td>Total fat pads, g</td>
<td>23.7±3.2*</td>
<td>15.1±1.5†</td>
<td>12.9±1.5†</td>
<td>10.0±1.3†</td>
<td>20.2±1.8*</td>
<td>15.2±1.3†</td>
</tr>
<tr>
<td>Total fat pads/body weight, %</td>
<td>3.92±0.31*</td>
<td>3.00±0.22†</td>
<td>2.97±0.21†</td>
<td>2.29±0.21†</td>
<td>3.95±0.37*</td>
<td>3.05±0.22†</td>
</tr>
<tr>
<td>Liver, g</td>
<td>18.8±0.9*</td>
<td>17.7±0.6*</td>
<td>15.8±1.0*</td>
<td>14.6±0.7*</td>
<td>19.0±1.0*</td>
<td>19.3±0.7*</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>10.1±1.0*</td>
<td>6.6±0.6*</td>
<td>4.1±0.5†</td>
<td>3.7±0.5†</td>
<td>9.3±0.7*</td>
<td>6.2±0.6†</td>
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<tr>
<td>Insulin, ng/ml</td>
<td>0.97±0.20*</td>
<td>0.87±0.21†</td>
<td>0.49±0.11†</td>
<td>0.75±0.25*</td>
<td>1.18±0.23*</td>
<td>1.30±0.21†</td>
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<tr>
<td>Run restrict 4 wk, total revolutions</td>
<td>ND</td>
<td>28,817±2,697*</td>
<td>ND</td>
<td>15,439±1,914†</td>
<td>ND</td>
<td>18,099±2,700†</td>
</tr>
<tr>
<td>Run final 2 wk, total revolutions</td>
<td>ND</td>
<td>14,628±1,520*</td>
<td>ND</td>
<td>7,249±1,701†</td>
<td>ND</td>
<td>7,954±1,521†</td>
</tr>
<tr>
<td>Total run, total revolutions</td>
<td>ND</td>
<td>43,445±3,431*</td>
<td>ND</td>
<td>22,688±2,059†</td>
<td>ND</td>
<td>26,053±2,191†</td>
</tr>
</tbody>
</table>

Values are means ± SE. See *Animals and diet* for description of 6 groups. Final body weight and total body weight change are for the entire 6-wk period beginning at week 0 (see legend of Fig. 1). Final 2-wk intake is average daily intake for rats during the last 2 wk of ad libitum feeding (note that sedentary-restrict 6 wk rats and exercise-restrict 6 wk rats were on a fixed intake). Total feed efficiency and final 2 wk feed efficiency are total body weight gain (g)/total caloric intake (kcal) × 1,000 over the entire 6-wk and during the final 2-wk periods, respectively. Total fat pads are total weight of the retroperitoneal, mesenteric, perirenal, and epididymal fat pads. Total fat pads/body weight are percentage of the total fat pad weight (g)/final body weight (g). Run restrict 4 wk and Run final 2 wk are total number of revolutions run during the 4 wk of restricted caloric intake and the final 2 wk of running, respectively. Total run is the total number of revolutions run during the entire 6 wk. ND, not done. Comparable datasets with different superscripts differ from each other (*P ≤ 0.05) by post hoc t-test after intergroup differences were found by ANOVA.

Effects of exercise plus caloric restriction. Despite comparable caloric intake, the body weights, visceral fat pad weights, and plasma leptin levels of restricted exercising rats did not differ statistically from restricted sedentary rats over 6 wk (Fig. 2; Table 1). However, visceral fat pad weights as a function of body weight were significantly lower (23%) than those of restricted sedentary rats. Food was provided at 2 h before dark onset in these calorically restricted rats. This altered intake pattern was associated with a gradual shift over 2 wk such that rats ran more during the light than dark phases (Fig. 5, B and C). In addition, restricted rats compensated for their decreased energy intake by running only 40–50% as much as exercising rats fed ad libitum (Table 1). While caloric restriction, with or without exercise, reduced body weight gain and carcass adiposity, it affected neither plasma insulin levels nor liver weights (Fig. 3; Table 1). The combination of exercise and caloric restriction had no additive effect on ARC NPY but did elevate PVN CRH mRNA and reduce DMN NPY expression back to the level of sedentary-ad lib rats (Fig. 6). While the addition of exercise increased POMC levels by 30% over caloric restriction alone, it did not return them to the level of sedentary-ad lib rats. Again, there were no effects on ARC AgRP or Lepr-b expression (Fig. 6; Table 2).

When restricted exercising rats were given ad libitum access to food after 4 wk, they brought their diurnal pattern of running back to that of exercise-ad lib rats over the next 2 wk (Fig. 5, A and C). However, they still ran only half as much as exercise-ad lib rats during this final 2 wk (Table 1). Thus the exercise-ad lib rats ended up running 80–90% more than the 4- and 6-wk restricted groups over the entire 6-wk study (Table
The exercising rats ate the same amount of food over 2 wk of ad libitum refeeding (Fig. 4) but gained less body weight and had 12% lower feed efficiency than the refeeding sedentary rats (Figs. 1 and 4; Table 1). Also, there were no correlations in refeeding exercising rats between the amount of weight gained and energy intake ($r = 0.37; P = 0.4$) or the amount of running ($r = 0.01; P = 0.98$). Nevertheless, their final body weight gain was comparable to that of exercise-ad lib rats (Fig. 1), as were their absolute and relative visceral fat pad weights and leptin levels (Fig. 2; Table 1). On the other hand, insulin levels were essentially unaffected by either exercise or food restriction over this period (Fig. 3). The return of these previously restricted exercising rats to the level of carcass adiposity of exercise-ad lib rats was associated with an ~45% increase of ARC NPY expression above sedentary-ad lib and exercise-ad lib rats (Fig. 6). PVN CRH expression returned to the level of both those groups. There were no additional effects of exercise in refeeding rats on either ARC POMC (which remained below both sedentary-ad lib and exercise-ad lib levels) or any other neuropeptides.

**DISCUSSION**

The current studies assessed the effects of two regimens that are frequently used in the treatment of obesity (exercise and dieting) on peripheral and central systems involved in energy homeostasis. Exercise alone lowered the defended levels of body weight gain and carcass adiposity, and exercise plus caloric restriction had an additive effect on lowering relative visceral adiposity compared with caloric restriction alone. As shown previously in exercising rats (12, 13), it is likely that our exercising rats also increased their facultative, resting, and exercise-induced energy expenditure. Yet exercising rats did not increase their energy intake or alter their expenditure sufficiently to fully compensate for their negative energy balance. Importantly, exercising rats not only failed to compensate for their lowered levels of weight gain and adiposity, but they also defended it against chronic caloric restriction. When calorically restricted exercising rats were given free access to food, they attained the same level of weight gain and adiposity as exercise-ad lib rats within 2 wk. On the other hand, restricted sedentary rats had the expected (14, 19, 20, 26) return to the higher levels of sedentary-ad lib rats when they were allowed free access to food. Therefore, these studies suggest that exercise resets the homeostatic balance between energy intake and expenditure toward defense of a lower level of weight gain and adiposity.

Not only did exercise lower the defended body weight, but it also produced a very different pattern of hypothalamic neuropeptide expression than calorically restricted sedentary rats. States of negative energy balance and reduced plasma leptin and insulin levels are associated with a number of changes in hypothalamic neuropeptide expression (3, 18, 21, 29, 35, 36). Sedentary-restrict rats showed these expected changes, i.e., decreased ARC POMC and PVN CRH and increased ARC and DMN NPY mRNA expression. However, despite their net loss of visceral adipose stores and a 35% reduction in leptin levels, exercising rats had only decreased ARC POMC expression. While mRNA expression does not necessarily reflect changes in synaptic release of neuropep-
tides, reduced POMC mRNA suggests the possibility of lower α-MSH release at catabolic melanocortin receptors (9). If so, exercising rats should have increased their energy intake (32, 40) and/or decreased their resting or facultative energy expenditure (6, 7, 10). Because they maintained and defended a lower weight gain and adiposity, this suggests that isolated reduction of ARC POMC expression was insufficient to compensate for the loss of adiposity during exercise without the addition of raised ARC NPY (and/or AgRP) or decreased PVN CRH. In fact, the failure to make the appropriate adjustments in caloric intake appeared to be an important contributor to the exercise-induced decreases in body weight gain and adiposity. The amount of running was not a primary determinant because, despite wide variations in voluntary running, all exercising rats reached a comparably lower homeostatic balance. There were strong correlations between caloric intake and weight gain, visceral adipose mass, and leptin levels [an index of total carcass adiposity in DIO rats (23, 26, 34)]. However, there were no correlations between the amount of running and any of these parameters.

Table 2. Hypothalamic leptin receptor (Lepr-b) mRNA expression

<table>
<thead>
<tr>
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<th>Sedentary-ad lib</th>
<th>Exercise-ad lib</th>
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<th>Sedentary-restrict 4 wk</th>
<th>Exercise-restrict 4 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARC</td>
<td>0.182±0.005</td>
<td>0.174±0.011</td>
<td>0.176±0.013</td>
<td>0.180±0.005</td>
<td>0.171±0.006</td>
<td>0.197±0.017</td>
</tr>
<tr>
<td>VMN</td>
<td>0.183±0.006</td>
<td>0.176±0.008</td>
<td>0.169±0.015</td>
<td>0.168±0.006</td>
<td>0.172±0.004</td>
<td>0.175±0.006</td>
</tr>
<tr>
<td>DMN</td>
<td>0.168±0.006</td>
<td>0.164±0.007</td>
<td>0.170±0.011</td>
<td>0.177±0.009</td>
<td>0.177±0.006</td>
<td>0.172±0.007</td>
</tr>
</tbody>
</table>

Data are means ± SE optical densities (OD) for the exposure of autoradiograms generated by in situ hybridization in the arcuate (ARC), ventromedial (VMN), and dorsomedial (DMN) hypothalamic nuclei. There were no intergroup differences in any area. See Animals and diet for description of groups.

Fig. 6. Hypothalamic neuropeptide mRNA expression during exercise and/or food restriction. For groups described in the legend of Fig. 1, neuropeptide mRNA expression was assessed by in situ hybridization, and expressed are area (mm²) of exposed film for neuropeptide Y (NPY; A), agouti-related peptide (AgRP; B), and proopiomelanocortin (POMC; C) in the hypothalamic arcuate nucleus (ARC) and for dorsomedial nucleus (DMN) NPY (D) and paraventricular nucleus (PVN) corticotropin-releasing hormone (CRH; E). Data are means ± SE. Data bars with differing superscripts differ from each other by P ≤ 0.05 by post hoc t-test after repeated-measures ANOVA showed significant intergroup differences.
The combination of caloric restriction and exercise produced a greater reduction in relative visceral adiposity than caloric restriction alone. Because leptin levels in restricted exercising rats were comparable to those in restricted sedentary rats, the major effect of exercise plus caloric restriction may have been on visceral rather than subcutaneous fat depots. However, this assumption might not be valid because leptin levels may not be a good index of total adiposity during chronic caloric restriction (19). Because restricted exercising rats could not compensate for their negative energy balance by eating more, they reduced their energy expenditure by halfing the amount of running. However, this compensation was still inadequate to maintain visceral adiposity at the same level as restricted sedentary rats. Even if they did not fully compensate, the reduction in exercise clearly demonstrates that they were able to monitor their overall state of energy homeostasis. A central role in such monitoring is suggested by the fact that restricted exercising rats had increased ARC POMC and PVN CRH expression compared with restricted sedentary rats. This combined increase might explain their further reductions in adiposity because both are catabolic peptides that could raise non-exercise-induced energy expenditure (6, 7, 10, 11).

Thus when given ad libitum food access, calorically restricted sedentary and exercising rats likely began with different baseline hypothalamic neuropeptides levels, and this might have contributed to their differing regain patterns. Sedentary rats quickly attained the weight gain and adiposity levels of sedentary-ad lib rats, while regain in exercising rats began to taper off between the first and second weeks of refeeding toward the lower levels of exercise-ad lib rats. Regaining sedentary rats reached their higher levels by a combination of increased food intake and increased feed efficiency (lowered energy expenditure). The regaining exercising rats had even lower food efficiency, but this was likely due to their added exercise-induced energy expenditure. Interestingly, they neither altered their running rates from prerestricton levels nor increased their intake sufficiently to attain the weight gain and adipose levels of sedentary-ad lib rats. This suggests that some internal set point caused them to balance the total amount of exercise and non-exercise-induced energy expenditure against their energy intake so that they reached only the homeostatic levels of exercise-ad lib rats. Possibly, given more time, regaining exercising rats might have risen to sedentary-ad lib levels. Certainly the fact that neuropeptide expression in both groups of regaining rats differed from their nonrestricted controls suggests that they had not yet reached a complete homeostatic balance. However, their clearly lower feed efficiency and the tapering off of weight and adipose regain, compared with the continued rise in sedentary rats, suggest that they were entering a plateau phase at the end of 2 wk. Our prior studies of regain after chronic caloric restriction in DIO rats suggest that they do not continue to gain more weight than nonrestricted controls once they enter such a plateau phase (19, 20, 22, 26, 31).

Although both play prominent roles in energy homeostasis (1a, 2, 3, 5), there was a striking lack of effect of exercise and/or caloric restriction on either ARC AgRP or Lepr-b mRNA expression. AgRP expression has variably been reported to be upregulated by both acute and chronic food restriction (2, 3) and Lepr-b mRNA expression is downregulated in the VMN but not the ARC or DMN by exposure to HE diet (24). On the other hand, as has been previously reported (3), DMN NPY mRNA expression was increased by chronic caloric restriction in our sedentary rats. Chronic exercise increases DMN NPY peptide levels (28), and we found a non-significant trend toward increased DMN NPY mRNA in our exercising rats. The lack of a significant increase may reflect the 24-h delay between death and the last running wheel exposure. Even with this delay, there were clear-cut differences in neuropeptide expression between each of the various groups of exercising rats and their respective sedentary controls. POMC levels were reduced in the exercise-ad lib group, ARC POMC and PVN CRH were higher and DMN NPY was lower in the 6-wk restricted exercise rats, and ARC NPY and PVN CRH mRNA levels were higher in refeeding exercising rats than each of their respective sedentary control groups. This suggests that the 24-h separation between running and death was not enough to ablate important alterations of peptide mRNA expression. One possible additional confound in the interpretation of the neuropeptide data is the fact that caloric restriction altered the running patterns of exercising rats. This food anticipatory activity (30, 33, 38) might well have had an independent effect on neuropeptide expression.

In conclusion, chronic exercise lowered the defended level of weight gain and carcass adiposity. Despite changes in hypothalamic neuropeptide expression that might be expected to drive them toward increased energy intake and/or reduced non-exercise-induced thermogenesis, exercising rats did not make the requisite adjustments to compensate for lost energy stores. Similarly, when exercise and caloric restriction were combined, rats became less viscerally obese than comparably restricted sedentary rats. Although exercising rats did reduce their running rates, this was not adequate to prevent added loss of visceral adipose tissue. In every case, exercising rats had differing complements of hypothalamic neuropeptide expression than their respective sedentary controls. Clearly, exercise produces a different set of regulatory signals from caloric restriction (18, 21, 29, 35, 36) or drugs that lower the defended level of weight gain and adiposity (22). Possibly the failure to fully compensate might be attributed to the fact that exercise failed to reduce leptin and insulin levels comparably to caloric restriction (14, 19, 22, 26). However, it is likely that additional, unidentified factors are associated with chronic exercise that lead to a lowered level of weight gain and adiposity as long as exercise is continued.

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GRANTS

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