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Three weeks of early-onset exercise prolongs obesity resistance in DIO rats after exercise cessation

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Patterson CM, Dunn-Meynell AA, Levin BE. Three weeks of early-onset exercise prolongs obesity resistance in DIO rats after exercise cessation. *Am J Physiol Regul Integr Comp Physiol* 294: R290–R301, 2008. First published November 7, 2007; doi:10.1152/ajpregu.00661.2007.—We assessed the effect of early-onset exercise as a means of preventing childhood obesity using juvenile male rats selectively bred to develop diet-induced obesity (DIO) or to be diet resistant (DR) when fed a 31% fat high-energy diet. Voluntary wheel running begun at 36 days of age selectively reduced adiposity in DIO vs. DR rats. Other 4-wk-old DIO rats fed a high-energy diet and exercised (Ex) for 13 wk increased their core temperature, gained 22% less body weight, and had 39% lighter fat pads compared with sedentary (Sed) rats. When wheels were removed after 6 wk (6 wk Ex/7 wk Sed), rats gained less body weight over the next 7 wk than Sed rats and still had comparable adipose pad weights to 13-wk-exercised rats. In fact, only 3 wk of exercise sufficed to prevent obesity for 10 wk after wheel removal. Terminally, the 6-wk-Ex/7-wk-Sed rats had a 55% increase in arcuate nucleus proopiomelanocortin mRNA expression vs. Sed rats, suggesting that this contributed to their sustained obesity resistance. Finally, when Sed rats were calorically restricted for 6 wk to weight match them to Ex rats (6 wk Rstr/7 wk Al), they increased their intake and body weight when fed ad libitum and, after 7 wk more, had higher leptin levels and adiposity than Sed rats. Thus, early-onset exercise may favorably alter, while early caloric restriction may unfavorably influence, the development of the hypothalamic pathways controlling energy homeostasis during brain development.

diet-induced obesity; hypothalamus; caloric restriction; development; leptin; proopiomelanocortin

THERE IS AN OBESITY EPIDEMIC in much of the developed world (2, 3, 18, 29, 30, 57, 58). This is particularly a problem among the juvenile population where the prevalence of obesity is increasing at an alarming rate (55, 61, 70). The majority of dietary treatments for human obesity have failed such that many individuals regain lost body weight in the months or years following treatment (31, 67, 72). Similarly, obese rodents that have been weight-reduced through caloric restriction quickly regain lost weight and adiposity when allowed to eat ad libitum (26, 38, 42, 46, 49). One reason for this recidivism is that weight loss in both humans and rodents is accompanied by a reduction in resting metabolic rate (15, 34, 35, 49). This may, in part, be due to the lowering of plasma leptin and insulin levels, which occurs following acute and chronic negative energy balance (50, 71). In rats, a reduction in these hormones causes an increase in hypothalamic arcuate nucleus (ARC) mRNA expression of anabolic peptides [e.g., neuropeptide Y (NPY) and agouti-related peptide (AgRP)] (7, 68) and a decrease in catabolic peptides [e.g., proopiomelanocortin (POMC)] (13, 14, 23, 54). These neuronal changes result in a strong metabolic drive to seek and ingest food to replace lost energy stores.

Exercise has long been known to affect energy balance, and some humans who successfully maintain weight loss for several years self-report high levels of physical activity (8, 17, 66, 73). In some instances, such self-reported exercise is associated with a normalization of resting energy expenditure in weight-reduced individuals (74). Because of the powerful forces opposing prolonged weight loss, it is likely that prevention of obesity, particularly in children would be a preferred means of stemming the obesity epidemic. While multiple studies have examined the effects of exercise on energy homeostasis in adult rodents (6, 37, 51), only a few have examined early-onset exercise in juvenile animals and explored the possibility that this might prevent the development of obesity (25, 32, 33, 52, 64, 76). Neurons in the ARC, which express POMC, NPY, and AgRP are involved in the regulation of energy homeostasis (63). While axons from these neurons reach their targets in the paraventricular nucleus (PVN) by the end of the third postnatal week (9), we reasoned that it was still possible that modifications of these pathways might continue well into the juvenile period. Thus, we postulated that early-onset postweaning exercise might positively alter hypothalamic development such that it would prevent the onset of adult obesity in rats genetically prone to become obese.

Here we demonstrate that postweaning voluntary wheel running selectively reduces obesity in juvenile rats that are bred to develop diet-induced obesity (DIO) vs. those bred to be diet resistant (DR). We also found that limited exposure to early-onset exercise has a sustained effect on preventing DIO rats from becoming obese, even when they ceased exercising and continued to eat a diet relatively high in fat (31%) and calories [high-energy (HE) diet]. Conversely, we found that postweaning caloric restriction increased the propensity of the DIO rats to become obese as adults.

METHODS

Animals and diet. Our protocols were approved by and animal usage was in compliance with the Institutional Animal Care and Usage Committee of the East Orange Veterans Affairs Medical Center and the guidelines of the American Physiological Society (1). Male rats bred selectively to express the DR and DIO traits (43), when fed a diet moderately high in fat and calories (HE diet), were raised in our in-house colony and used for all studies. To avoid potential litter

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effects, 6–8 litters were culled to 10 pups per dam on postnatal *day 2* (P2) and weaned at P28 onto Purina rat chow or the HE diet and water ad libitum, unless otherwise noted. Purina rat chow (prod. no. 5001) contains 3.30 kcal/g with 23.4% as protein, 4.5% as fat, and 72.1% as carbohydrate, which is primarily in the form of complex polysaccharide (45). The HE diet is a defined diet (cat. no. C11024F, Research Diets, New Brunswick, NJ) containing 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 (45). All rats were individually housed from weaning at 23–24°C on a reversed 12:12-h light-dark cycle (lights off at 0900). During the course of all experiments, cumulative food intake and body weight measurements were obtained on a weekly basis.

Experiment 1: effect of voluntary wheel running on body weight, food intake, insulin, and adiposity in juvenile DR vs. DIO rats. DR ($n = 16$) and DIO ($n = 16$) rats were weaned onto chow and randomized by body weight and genotype into four groups of eight rats each at P36. DR sedentary (DR Sed) and DIO Sed rats remained sedentary from P36-P57. DR exercise (DR Ex) and DIO Ex rats had free access to a running wheel placed in their home cages from P36 to P50 and remained sedentary from P50 to P57. At P58, food was removed 2 h prior to dark onset, and rats were decapitated in the 4 h after dark onset. Following decapitation, trunk blood was collected for insulin radioimmunoassay. Epididymal, retroperitoneal, perirenal, and mesenteric adipose depots were removed and weighed.

Experiment 2: effect of postweaning exercise and caloric restriction on body weight, fat mass, food intake, insulin, and leptin. DIO rats ($n = 40$) were weaned onto HE diet and randomized by litter and body weight into five groups of eight rats each: *1*) Sed rats remained sedentary with ad libitum access to HE diet for 13 wk; *2*) Ex rats had free access to a running wheel with ad libitum access to HE diet for 13 wk; *3*) 6-wk-Ex/7-wk-Sed rats had free access to a running wheel for 6 wk after which their wheels were removed and they remained sedentary for an additional 7 wk with ad libitum access to the HE diet; *4*) restricted (Rstr) rats remained sedentary and were food restricted to ~85% of the daily caloric intake of SED animals for 13 wk. For all Rstr rats, food was provided 2 h prior to dark onset on a daily basis, and *5*) 6-wk-Restr/7-wk-ad libitum (6-wk-Restr/7-wk-Al) rats were food restricted to ~85% of the daily caloric intake of SED animals for the initial 6 wk and were then allowed to eat ad libitum for an additional 7 wk. Prior to weaning, Sed and Ex rats were implanted intraperitoneally with PDT-4000 E-Mitter activity/temperature transponders (Minimitter) under light isoflourane anesthesia. Approximately 0.5 ml of tail blood was collected via tail nip prior to the initiation of the experiment and again following the 6th and 13th wk for plasma leptin and insulin assays. At the termination of the experiment, wheels were locked 4 h prior to dark onset, food was removed 2 h prior to dark onset, and rats were decapitated in the 4 h after dark onset. Following decapitation, trunk blood was collected and brains were quickly removed, frozen on dry ice, and stored at −80°C until further analysis by quantitative PCR (QPCR). Epididymal, retroperitoneal, perirenal, mesenteric, and inguinal adipose depots were removed and weighed. Brains of 6-wk-Restr/7-wk-Al rats were not analyzed by QPCR.

Experiment 3: determination of the minimal amount of postweaning exercise required for sustained reduction in body weight gain, food intake, and adiposity. An additional set of DIO rats ($n = 18$) was weaned onto the HE diet and were randomized by litter and body weight into three groups of six rats each: *1*) Sed rats remained sedentary with ad libitum access to HE diet for 13 wk; *2*) 3-wk-Ex/10-wk-Sed rats had free access to running wheels for 3 wk and then remained sedentary for 10 wk more; *3*) 2-wk-Ex/11-wk-Sed rats had 2 wk of wheel exposure and remained sedentary for 11 wk more. Approximately 0.5 ml of tail blood were collected via tail nip prior to the initiation of the experiment for plasma leptin and insulin radioimmunoassay. Terminally, all rats were weighed and killed as described above. Epididymal, retroperitoneal, perirenal, mesenteric, and inguinal adipose depots were removed and weighed. Fat pad weights were not measured for 2-wk-Ex/11-wk-Sed rats.

Experiment 4: effect of 3-wk postweaning exercise on hypothalamic neuropeptide receptor and growth factor mRNA expression. Additional male DIO rats ($n = 24$) were weaned onto the HE diet and randomized by litter and body weight into three groups of eight rats each: *1*) Sed rats remained sedentary for 3 wk; *2*) Ex rats had free access to a running wheel for 3 wk; and *3*) Rstr rats remained sedentary and were food restricted to 85% of the daily caloric intake of the Sed animals for 3 wk. Following 3 wk, all rats were killed and brains were collected for QPCR as described in *experiment 2*.

Measurement of wheel running, core temperature, and motor activity. Running wheel, core temperature (°C), and motor activity (arbitrary units) data were collected continuously at 5-min intervals remotely by a computerized system (MiniMitter) using transponders placed in the peritoneal cavity, which transmitted to a receiver placed under the individual cages. Data were acquired and stored using Vital View software (MiniMitter).

Plasma insulin and leptin levels. Blood was collected in EDTA-coated tubes and plasma insulin and leptin levels were analyzed by radioimmunoassay utilizing antibodies authentic to rat insulin and leptin (Linco, St. Charles, MO).

Hypothalamic neuropeptide and receptor mRNA assays by real-time QPCR. Frozen brains were cut on a cryostat at −12°C into 300-μm serial sections centered on both the hypothalamic PVN and the central portion of the ARC at the level of the compact zone of the dorsomedial hypothalamic nucleus (DMN). These latter sections also contained the midpoint of the ventromedial nucleus (VMN) and the lateral hypothalamus. Sections were placed in RNA Later (Ambion) until micropunched. Punches of the PVN, ARC, VMN, DMN, and lateral hypothalmus were collected by modifications of the method of Palkovits (56) by placing cut sections on the base of a stereotaxic frame to which a syringe with a hypodermic needle, specifically designed to the dimensions of each nucleus, was attached. Punches were made under microscopic guidance and placed in RNA Later (Ambion). Micropunched brain nuclei were then processed using QPCR as previously described (19).

Statistics. Comparisons of body weight, food intake, fat pads, plasma hormones, area under the curve (AUC) for core temperature data, total number of running wheel revolutions, and QPCR data were carried out using one-way ANOVA. When significant differences were found, post hoc analysis was performed using Bonferroni corrections ($P \leq 0.05$). All data are expressed as means ± SE. Correlations between body weight, food intake, fat pad mass, and running wheel activity were carried out using Pearson's correlation coefficient.

RESULTS

Experiment 1: effect of voluntary wheel running on body weight, food intake, insulin, and adiposity in juvenile DR vs. DIO rats. At P36, DIO rats were ~65% heavier than DR rats (Table 1). When given voluntary access to a running wheel for 2 wk, beginning at ~5 wk of age, DIO rats ran 49% less than DR rats (Table 1). There was no effect of exercise on reducing body weight gain in either DIO or DR rats. Over the full 21-day period, DIO Sed rats had 40% greater food intake than DR Sed rats (Fig. 1*B*, Table 1). However, there was no effect of exercise on food intake in either DR or DIO rats. Terminally, DIO Sed rats had 61% greater visceral fat pad mass than DR Sed rats. Exercise had no effect on fat pad weights in DR rats. On the other hand, despite their greater food intake and reduced running activity, DIO Ex fat pads weighed 25% less and were 24% less, as a function of total body weight, than those of DIO Sed rats (Fig. 1*C*, Table 1). Overall, neither the exercised DIO nor DR rats displayed any correlations between

Table 1. *Experiment 1: effect of juvenile voluntary wheel running on body weight, food intake, insulin, and adiposity in DR vs. DIO rats*

	DR Sed	DR Ex	DIO Sed	DIO Ex
P36 body weight, g	87±5[a]	82±6[a]	140±5[b]	142±4[b]
P50 (2-wk) body weight gain, g	128+5[a]	116±5[a]	152±7[b]	150±4[b]
P57 body weight gain, g	177±8[a]	168±6[a]	210+12[b]	208±7[b]
Food intake, cumulative kcal/day	76±2[a]	76±3[a]	107±7[b]	103±5[b]
Average daily revolutions, 24 h		2,126±292[a]		1,043±162[b]
Total revolutions		36,141±4,960[a]		17,724±2,756[b]
Total fat pad weights, g	4.22±0.41[a]	3.14±0.23[a]	6.80±0.49[b]	5.09±0.38[a]
Total fat pads/body weight, %	2.38±0.18[a]	1.94±0.09[a]	2.80±0.28[b]	2.12±0.10[a]
Insulin, ng/ml	1.44±0.34[a]	1.67±0.30[a]	2.81±0.37[b]	2.53±0.41[b]

Values are means ± SE. At 36 days of age (P35), groups of 8 chow-fed, selectively bred, male, diet-resistant (DR) and diet-induced obese (DIO) rats were given access to a running wheel (DR Ex; DIO Ex) or left sedentary (DR Sed; DIO Sed) for 14 days. Following these 2 wk, the exercise wheels were removed for both groups of Ex rats, and all rats were sedentary for an additional 1 wk. Total fat pads equals the sum of epididymal, perirenal, retroperitoneal, mesenteric, and inguinal pads. [a,b]Parameters with differing superscripts differ from each other at the $P < 0.05$ level by post hoc Bonferroni adjustment after significant intergroup differences were found by 1-way ANOVA.

their body weight gain or fat mass with total running wheel activity. However, body weight ($r = 0.95$; $P = 0.001$) and fat mass ($r = 0.85$; $P = 0.001$) were both highly correlated with food intake. Terminally, DIO rats had higher insulin levels than DR rats, while there was no effect of prior exercise on insulin levels in either the DR or DIO rats (Table 1).

General characteristics of postweaning voluntary running wheel activity in DIO rats. DIO rats were begun on the HE diet at 4 wk of age and were exposed to a running wheel (Ex; $n = 8$) or remained sedentary (Sed; $n = 8$) for an 13-wk period. Running wheel activity during the postweaning period increased as a function of age. DIO rats gradually increased their spontaneous running wheel activity during the first 3 wk of wheel exposure. These rates plateaued around the 5th wk, during which time Ex rats were running ~36,000 revolutions/wk (39,000 m/wk) (Fig. 2*A*). DIO rats had a nocturnal cycle of running wheel activity, whereby 86% of running took place during the dark cycle (Fig. 2*A*). This circadian pattern also corresponded with food intake. During the 2nd wk of this study, Ex rats ate 95% and Sed rats consumed 91% of their daily intake during the dark cycle. Similar to adult DIO rats (37), juvenile DIO rats displayed wide individual variations in their amount of voluntary running wheel activity (Fig. 2*A*).

Experiment 2: effect of postweaning exercise and caloric restriction on body weight, fat mass, food intake, insulin, and leptin. After weaning onto the HE diet at 4 wk of age, Sed rats gained weight steadily over the ensuing 13 wk. By contrast, Ex rats reduced their cumulative body weight gain during the first 6 wk of exercise by 18% compared with the Sed rats (Fig. 3*A*; Table 2). This reduced body weight gain was associated with a 74% reduction in plasma leptin and a 44% reduction in plasma insulin levels, suggesting that 6 wk of exercise reduced total carcass adiposity (Fig. 3*C*; Table 2). Weekly food intake was similar in the Ex and Sed rats during the first 6 wk of postweaning exercise and this was not correlated with body weight gain or running wheel activity (Fig. 3*B*; Table 2). Because Ex rats gained less weight, their feed efficiency during this period was only 86% of the Sed rats (Table 2). When challenged with an overnight fast, both the Ex and Sed rats equally compensated during the following 11- and 24-h periods without change in running wheel activity in Ex rats (data not shown). Core temperature AUC was 86% higher during the 12-h dark phase and 95% higher during the 12-h light phase in Ex compared with the Sed rats when measured during the 3rd wk of postweaning exercise. These data suggest that they had an exercise-induced increase in thermogenesis (Fig. 2, *B* and *C*). The 12-h light phase increase in core temperature among the Ex rats was not due to increased activity since average 12-h light cycle activity did not differ between Ex and Sed rats and because average light cycle core temperatures were higher in Ex rats even during periods of inactivity (assessed during periods of the 12-h light cycle when the activity monitor failed to register any activity; Fig. 2*D*).

Over the full 13 wk, Ex rats gained 22% less body weight than did Sed controls (Fig. 3*A*; Table 2). This reduction in weight gain was associated with a 51% reduction in plasma leptin and 54% reduction in plasma insulin levels (Fig. 3*C*; Table 2). Furthermore, 13 wk of postweaning exercise led to a 39% reduction in total fat pad mass and a 26% reduction in fat pad mass as a function of body weight (Fig. 3*D*; Table 2). Ex rats failed to increase their caloric intake to compensate for their increased energy expenditure either during or after exercise termination (Fig. 3*B*). As a result, their feed efficiency decreased to 68% of Sed rats during the final 7 wk (Table 2). Despite large variations in their amount of running activity, there were no correlations between the cumulative number of revolutions run and final body weight, body weight gained, or adiposity over the entire 13 wk of running wheel activity. However, there was a positive correlation between cumulative food intake and total running wheel activity ($r = 0.9$, $P = 0.02$). Postweaning exercise had no effect on linear growth since naso-anal length of rats chronically exercised postweaning was equal to Sed rats (Table 2).

Following 6 wk of postweaning exercise, 6-wk-Ex/7-wk-Sed rats had their running wheels removed and remained sedentary for 7 wk more. These 6-wk-Ex/7-wk-Sed rats did not alter their caloric intake following exercise termination (Fig. 3*B*; Table 2). They maintained comparable body weights to Ex rats for 5 wk despite their continued intake of the HE diet. However, by 7 wk after exercise cessation, 6-wk-Ex/7-wk-Sed rats did increase their body weight gain but not to the level of the Sed rats (Fig. 3*A*; Table 2). Although they did gain more weight than Ex rats after exercise termination, terminally 6-wk-Ex/7-wk-Sed leptin levels, total and relative fat pad weights were comparable to Ex rats but were 47%, 38%, and 30% lower than Sed rats, respectively (Fig. 3, *C* and *D*; Table

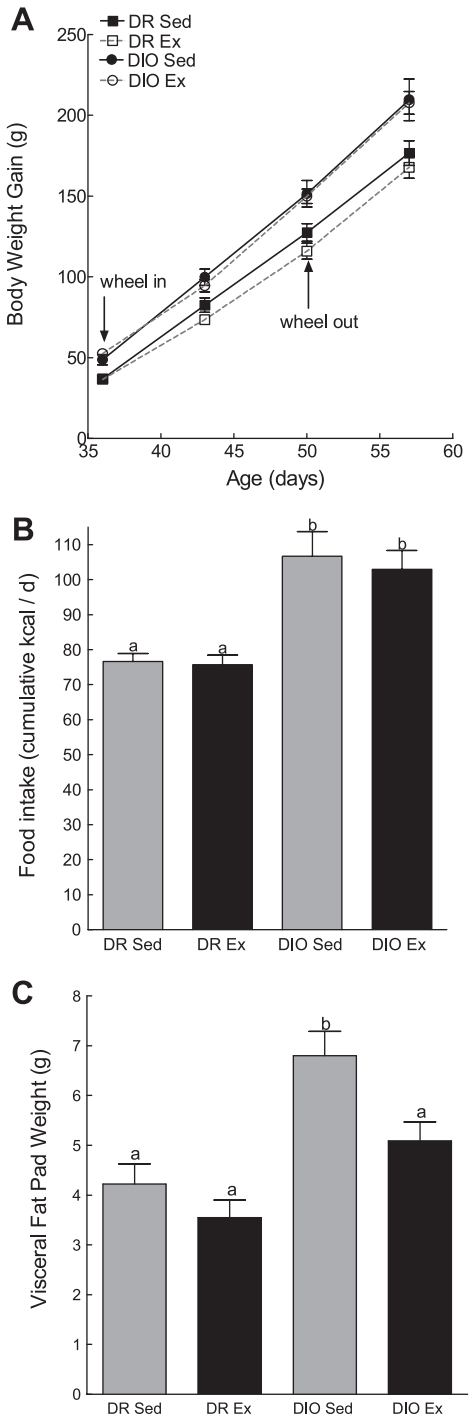

Fig. 1. At postnatal *day 36* (P36) chow-fed male diet-resistant (DR) and diet-induced obesity (DIO) rats were left sedentary (DR Sed; $n = 8$, DIO Sed; $n = 8$) or given free access to exercise on a running wheel (DR Ex; $n = 8$, DIO Ex; $n = 8$). Ex groups were allowed to exercise for 2 wk after which time their wheels were removed and all rats were allowed to remain sedentary for an additional 1 wk. *A*: body weight gain. *Wheel in*, when rats were given voluntary access to a running wheel. *Wheel out*, when wheels were removed from cages. *B*: cumulative food intake per day calculated over 21 days. *C*: terminal visceral fat pad weights are the sum of epididymal, retroperitoneal, perirenal, and mesenteric fat pads. Data are means ± SE. [a,b]Groups with differing superscript letters differ from each other at the $P \leq 0.05$ level by post hoc Bonferroni adjustment.

2). On the other hand, termination of Ex did cause a partial rebound in plasma insulin levels (Table 2).

As a comparison to the effects of exercise, 4-wk-old rats were restricted to 85% of the caloric intake of the HE diet of Sed rats (Rstr) rats for 13 wk. This effectively weight-matched Rstr rats to Ex rats over the first 6 wk (Fig. 3*A*; Table 2) and was associated with an 86% reduction in plasma leptin levels and a 92% reduction in plasma insulin levels compared with Sed rats and 48% and 84% lower leptin and insulin levels, respectively, than those in Ex rats (Fig. 3*C*; Table 2). However, despite their continued caloric restriction, Rstr rats began to gain more body weight than the Ex rats during the next 7 wk. Terminally, Rstr rats had final body weights which were between Sed and Ex rats. However, their relative fat pad mass was comparable to Sed rats and 38% higher than Ex rats, while their leptin and insulin levels did not differ significantly from either Sed or Ex rats (Table 2).

To rule out the possibility that either reduced body weight or adiposity associated with exercise during the early postweaning period alone might alter future weight gain and adiposity, rats were calorically restricted for 6 wk and then allowed to eat ad libitum for 7 wk (6 wk Rstr/7 wk Al). When allowed to eat ad libitum, they increased their intake by 27% compared with Sed rats over the following 7 wk (Fig. 3*B*; Table 2). Terminally the body weight gain and plasma insulin levels of the 6-wk-Restr/7-wk-Al rats were similar to the Sed rats. However, they were fatter than even the Sed rats. They had 33% higher plasma leptin levels, 35% higher fat pad mass, and 35% higher relative fat pad mass compared with Sed rats (Fig. 3, *C* and *D*; Table 2). These data demonstrate that early postweaning caloric restriction had a significant adverse effect on body weight and adiposity when animals were also fed a diet relatively high in fat and caloric density.

Effects of exercise and food restriction on hypothalamic neuropeptide, receptor, and growth factor mRNA expression. At 13 wk, Sed, Ex, 6-wk-Ex/7-wk-Sed, and Rstr rats were killed and their brains were removed at the onset of dark, 4 h after running wheels were locked. As above, 6-wk-Ex/7-wk-Sed rats failed to increase their food intake and, despite increased body weight, had reduced adiposity comparable to rats which exercised for a full 13 wk. This sustained reduction in adiposity, despite continued intake of the HE diet was associated with a highly selective 55% increase in ARC POMC mRNA expression and a 49% increase in VMN melanocortin-3 receptor (MC3R) mRNA expression compared with rats which remained sedentary for the full 13 wk (Table 3). On the other hand, rats which exercised for the full 13 wk had a 49% increase in DMN NPY compared with Sed rats (Table 3). Although short-term caloric restriction is associated with increased ARC NPY and decreased POMC expression (5, 36, 40), the 13 wk Rstr rats did not display such changes. In fact, rats that were caloricly restricted for the full 13 wk paradoxically had an even greater increase in ARC POMC than was seen in 6-wk-Ex/7-wk-Sed rats (Table 3). Also, the chronic reduction in plasma leptin levels caused by restriction was not associated with any change in the ARC expression of the long form of the leptin receptor-b. However, the Rstr rats had reduced expression of VMN MC3R, MC4R, leptin receptor-b, and DMN NPY compared with Sed rats. There were no other significant intergroup differences in any of the other variables assessed among these groups (Table 3).

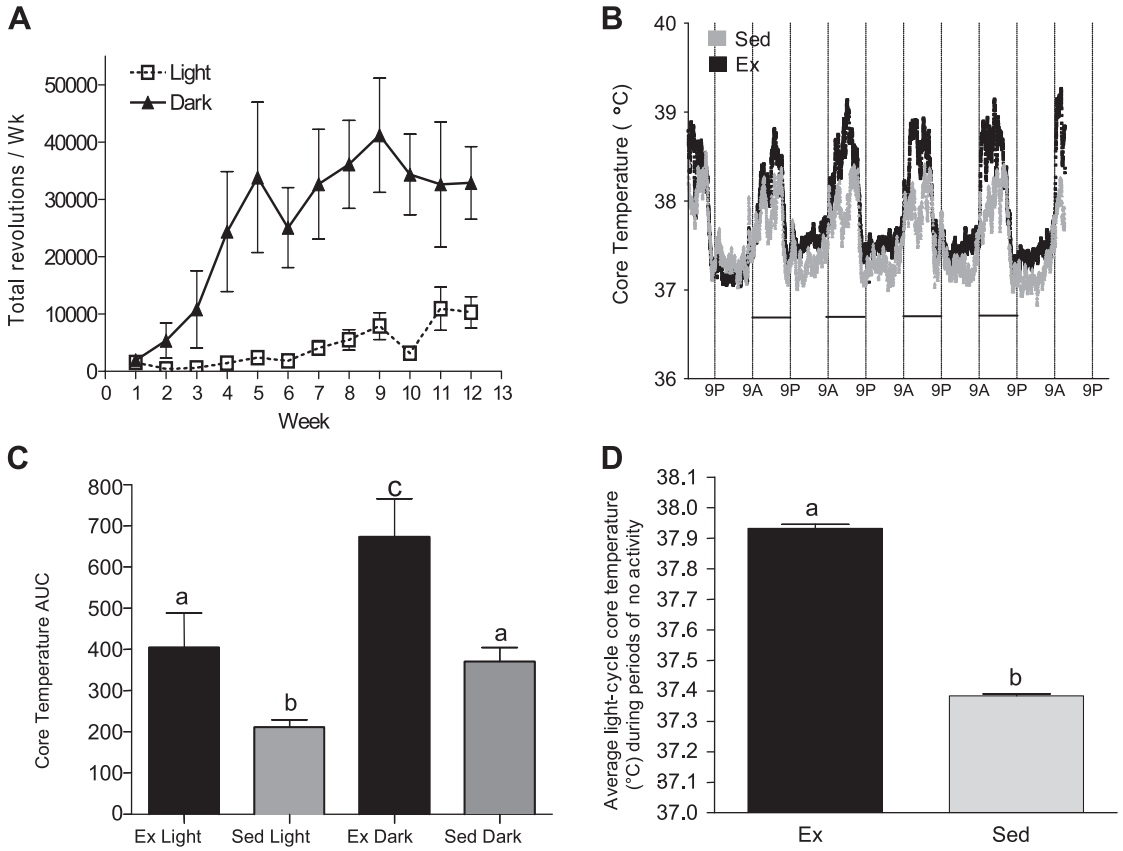

Fig. 2. At 4 wk of age, male DIO rats were weaned onto a high-energy (HE) diet and left sedentary (Sed; $n = 8$) or given free access to a running wheel (Ex; $n = 8$). PDT-4000 E-Mitter transponders (Minimitter) were implanted in the peritoneal cavity, and core temperatures were measured remotely. *A*: results of 13-wk running wheel activity of DIO rats given voluntary access to running wheels placed in their home cages. Running wheel activity is given as total revolutions/week during a 12:12-h light-dark period. *B*: core temperature of Sed and Ex rats over a 4-day period during the 3rd wk of postweaning exercise. Horizontal bars denote dark cycles. *C*: core temperatures area under the curve (AUC) of Ex and Sed rats during a 12:12-h light-dark period. *D*: core temperatures of Ex and Sed rats during periods of no activity. Light-cycle temperatures were averaged over periods during which readout from activity transponders was zero. Data are means ± SE. [a,b,c]Groups with differing superscript letters differ from each other at the $P \leq 0.05$ level by post hoc Bonferroni adjustment. Data represent rats from *experiment 2*.

Experiment 3: determination of the minimal amount of postweaning exercise required for sustained reduction in body weight gain, food intake, and adiposity. To determine the duration of early running wheel activity necessary to produce sustained suppression of weight gain and adiposity after exercise cessation, 4-wk-old DIO rats on the HE diet were exercised for either 2 wk (2 wk Ex/11 wk Sed) or 3 wk (3 wk Ex/10 wk Sed) postweaning and then remained Sed for 11 or 10 additional weeks, respectively (Fig. 4*A*; Table 4). Following both 2 wk or 3 wk of postweaning exercise, 2-wk-Ex/11-wk-Sed and 3-wk-Ex/10-wk-Sed rats had body weight gains that were comparable to Sed rats (Fig. 4*A*; Table 4). Rats exercised for only 2 wk remained at the body weight of Sed rats after exercise termination, while those exercised for 3 wk maintained a reduced body weight for 10 wk, despite continued intake of HE diet. Also, cumulative caloric intake did not differ among the groups during the last 10 wk of the study (Table 4). The persistent reduction in body weight gain in rats exercised for 3 wk was first seen 1 wk after becoming sedentary when they had a 14% reduction in body weight gain compared with the Sed rats (Table 4). After 10 wk of being sedentary, the 3-wk-Ex/10-wk-Sed rats had 14% lower body weight gain, 46% lower total fat pad mass, and 38% lower relative fat pad mass than Sed rats (Fig. 4, *A* and *B*; Table 4).

Experiment 4: effect of 3-wk postweaning exercise on hypothalamic neuropeptide, receptor, and growth factor expression. Since 3 wk of exercise appeared to be the minimal amount required to produce a sustained lowering of body weight and adiposity during an additional 10 wk of remaining sedentary on an HE diet, we expected exercise-induced changes in neuropeptide, receptor, and growth factor mRNA expression to provide an indication of factors that might be critical to these sustained effects. Rats exercised for 3 wk had 27% lower mRNA expression of ARC MC3R and 28% lower NPY1 receptor and 38% lower suppressor of cytokines signaling-3 (SOCS3) expression in the DMN compared with Sed controls (Table 5). On the other hand, postweaning caloric restriction for 3 wk was associated with 57%, 56%, and 38% lower VMN expression of MC3R, SOCS3, and brain-derived neurotrophic factor (BDNF), respectively, and 37% and 39% lower DMN expression of SOCS3 and BDNF compared with Sed rats, respectively (Table 5).

DISCUSSION

The present studies demonstrate that exercise-induced reduction in fat mass was selectively seen in DIO vs. DR rats and that only 3 wk (but not 2 wk) of voluntary exercise begun

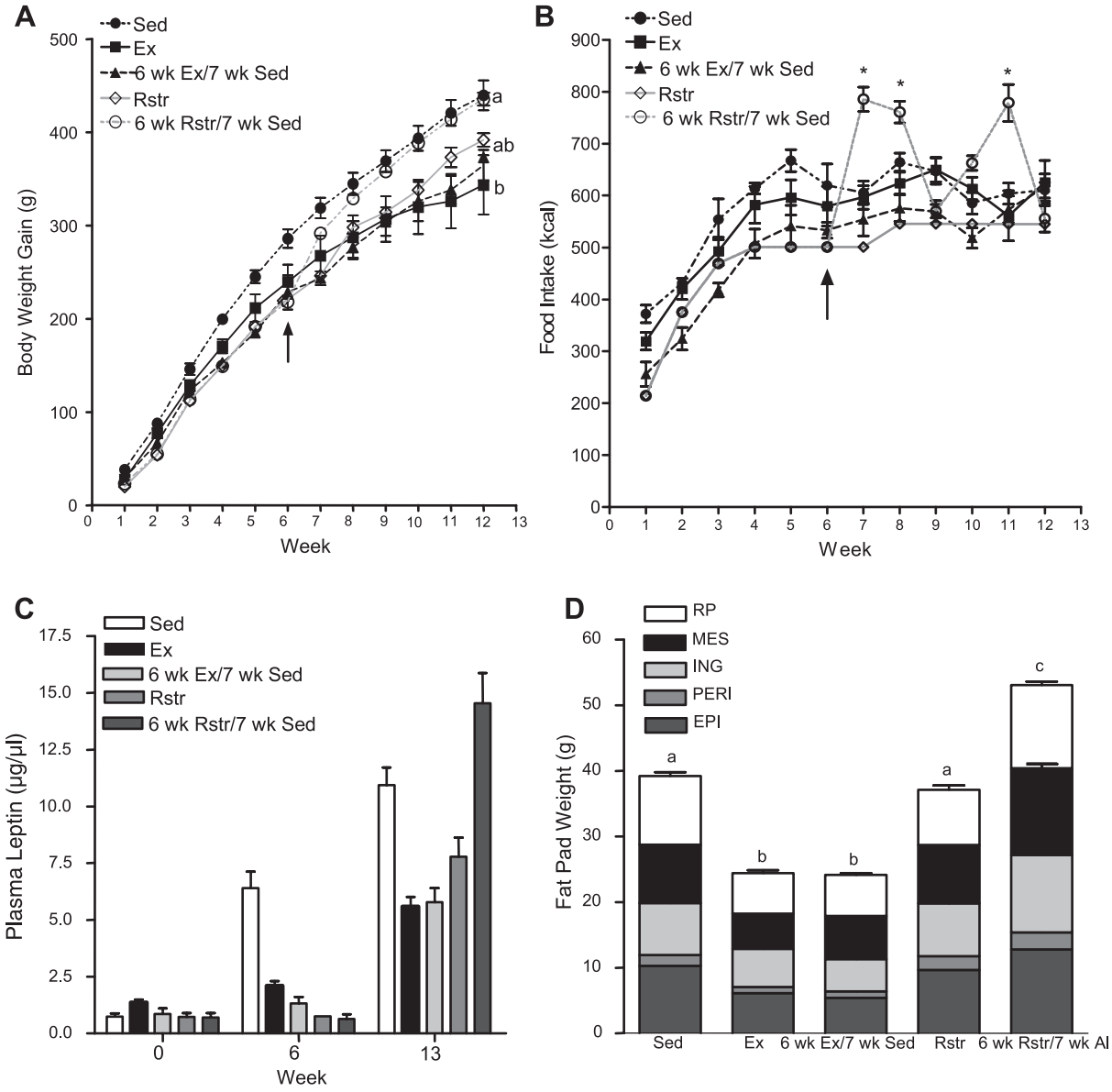

Fig. 3. At 4 wk of age, male DIO rats were weaned onto HE diet and given access to a running wheel (Ex; $n = 8$) or left sedentary (Sed; $n = 8$) for 13 wk. Another group of Ex rats had their wheels removed from their cages following 6 wk and were allowed to remain sedentary for an additional 7 wk (6 wk Ex/7 wk Sed; $n = 8$). Restricted (Rstr) DIO rats were limited to 85% of the daily caloric intake of the Sed animals for the full 13 wk ($n = 8$) or for the first 6 wk followed by 7 wk of ad libitum (Al) access to HE diet (6 wk Rstr/7 wk Al; $n = 8$). *A*: body weight gain. *B*: food intake. *$P < 0.05$ by post hoc Bonferroni test when ANOVA showed significant intergroup differences *C*: plasma leptin levels following 0, 6, and 13 wk of exercise. *D*: terminal fat pad weights (EPI, epididymal; RP, retroperitoneal; PERI, perirenal; MES, mesenteric; ING, inguinal) following 13 wk of exercise. Arrows denote termination of wheel running. [a,b,c]Groups with differing superscripts differ from each other at the $P \leq 0.05$ level by post hoc Bonferroni adjustment after significant intergroup differences were found by repeated-measures or 1-way ANOVA.

during the immediate postweaning period reduces body weight and adipose gain in selectively bred DIO rats for up to 10 wk after exercise cessation, despite their continued intake of HE diet. The selective effect of exercise to reduce adiposity in juvenile DIO rats is similar to what we previously found in adult rats (39) and in genetically obese vs. lean mice (51). It is likely that the selective effect of exercise on lowering adiposity in DIO vs. DR rats is due to the fact that DIO rats have more adipose mass to lose relative to DR rats. The ability of sustained exercise to prevent obesity appears to result from an increase in overall energy expenditure as indicated by raised core body temperature during both the dark and light phases (24, 25). In addition, similar to adult rats (6, 37, 39), exercising juvenile DIO rats failed to increase their caloric intake to compensate for their increased expenditure and reductions in adipose mass and plasma leptin levels compared with Sed rats. Also similar to adult rats (37) is the observation that energy intake and not the amount of wheel running is correlated with adiposity in juvenile rats. This suggests that the lack of caloric compensation during exercise is a main determinant of adipose loss.

The sustained suppression of adipose gain following only 3 wk of exercise is the most important difference between exercise begun in the early postweaning period and exercise

Table 2. *Experiment 2: effect of postweaning exercise and caloric restriction on body weight, fat mass, food intake, insulin, and leptin*

	Sed	Ex	6 wk Ex/7 wk Sed	Rstr 13 wk	6 wk Rstr/7 wk Al
		Week 0			
Body weight, g	85±5	76±7	60±13	73±4	73±11
Leptin, ng/ml	0.74±0.1	1.1±0.2	0.89±0.3	0.89±0.0	0.73±0.2
Insulin ng/ml	1.2±0.2	0.74±0.1	0.68±0.1	0.91±0.2	0.96±0.2
		Weeks 1–6			
Body weight, 6 wk, g	350±10[a]	289±9[b]	270±9[b]	287±3[b]	281±3[b]
Body weight gain *weeks 1–6*, g	264±14[a]	217±7[b]	211±6[b]	214±3[b]	208±3[b]
Total intake *weeks 1–6*, kcal	3,258±63	2,785±100	2,580±25	2,561	2,561
Feed efficiency *weeks 1–6*, g/kcal × 1,000	88.9±3.2[ab]	77.1±4.7[a]	77.7±7.0[a]	96.6±2.0[b]	98.4±2.4[b]
Leptin, 6-wk, ng/ml	6.4±0.7[a]	1.7±0.2[b]	1.3±0.3[b]	0.89±0.1[c]	0.48±1.4[c]
Insulin, 6-wk, ng/ml	3.4±0.7[a]	1.9±0.4[b]	1.2±0.2[b]	0.30±0.1[c]	0.30±0.1[c]
		Weeks 7–13			
Final body weight, g	508±11[a]	412±8[b]	444±7[c]	465±3[c]	509±5[a]
Body weight gain *weeks 1–13*, g	423±14[a]	329±13[b]	384±18[ab]	392±7[ab]	436±7[a]
Total intake *weeks 7–13*, kcal	3,689±100[a]	3,649±90[a]	3,361±93[a]	3,227	4,674±66[c]
Feed efficiency *weeks 7–13*, g/kcal × 1,000	34.7±1.7[a]	23.8±2.0[b]	48.4±6.1[a]	48.9±1.0[a]	44.3±1.4[a]
Total fat pads, g	39.2±1.6[a]	23.9±1.2[b]	24.1±0.7[b]	37.1±2.1[a]	53.1±2.2[c]
Total fat pads/body weight, %	7.73±0.40[a]	5.79±0.19[b]	5.42±0.12[b]	7.98±0.44[a]	10.4±0.40[c]
Final leptin, ng/ml	10.9±0.8[a]	5.6±0.4[b]	5.8±0.6[b]	7.8±0.8[ab]	14.5±1.3[c]
Final insulin, ng/ml	6.8±1.1[a]	3.1±0.5[b]	5.6±0.2[ab]	4.8±0.8[ab]	7.2±1.0[a]
Nasoanal length, cm	24.0±0.4	23.0±0.5	ND	22.5±0.2	23.3±0.5

Values are means ± SE. At 4 wk of age, male DIO rats were weaned onto high-energy diet and randomized into 5 groups. Sedentary rats were left sedentary for 13 wk (Sed; $n = 8$). Exercised rats either had free access to a running wheel for 13 wk (Ex; $n = 8$) or were given access to a running wheel for the first 6 wk, and then wheels were removed and they remained sedentary for 7 wk more (6 wk Ex/7 wk Sed; $n = 8$). Restricted (Rstr) rats were restricted to 85% of the daily caloric intake of the Sed animals for the full 13 wk ($n = 8$) or for the first 6 wk followed by 7 wk of ad libitum (Al) access to high-energy diet (6 wk Rstr/7 wk Al; $n = 8$). Total fat pads equals the sum of epididymal, perirenal, retroperitoneal, mesenteric, and inguinal pads. Feed efficiency = body weight gain (g)/food intake (kcal) × 1,000. [a,b,c]Parameters with differing superscripts differ from each other at the $P < 0.05$ level by post hoc Bonferroni adjustment after significant intergroup differences were found by 1-way ANOVA.

begun in adult rats in which there is little or no sustained weight reduction after exercise cessation (4, 52, 65, 75). Shima et al. (64) did demonstrate a prolonged effect of early-onset exercise in CCK-A receptor-deficient, obesity-prone Otsuka Long-Evans Tokushima Fatty rats. However, those rats were fed a low-fat diet, began exercise at an older age (7 wk), and continued exercise for a longer period of time (8 wk) prior to cessation. Also, their rats gained more weight and adiposity than comparable rats that continued to exercise over the next 11 wk. Thus, our studies are the first to demonstrate that only 3 wk of postweaning exercise is required to completely prevent the development of obesity for up to 10 wk after exercise cessation in rats that are genetically predisposed to become obese, even when they are fed a moderate fat diet. Clearly, this sustained effect in DIO rats was not simply a function of reducing their body weight and adiposity during early development, since Sed rats of the same age that were weight matched to exercising rats for 6 wk rapidly increased their food intake and became even more obese than non-Rstr Sed rats when subsequently allowed to eat ad libitum. Thus, manipulations of energy homeostasis during the early postweaning period have important long-lasting effects on the subsequent regulation of energy homeostasis that last well into early adulthood.

One possible way in which such long-lasting effects might occur is through alterations in the central pathways involved in the regulation of energy balance. Because we (37) and others (6, 47) have demonstrated an effect of exercise on hypothalamic neuropeptide expression, we chose to examine the effect of the various perturbations used here on a variety of markers of hypothalamic functions relating to the control of food intake and body weight. Rats exercised for 13 wk had no substantial differences in their hypothalamic expression of various neuropeptide (e.g., ARC NPY, AgRP, or POMC) or receptor mRNAs despite the fact that their adiposity, leptin, and insulin levels were markedly lower than comparable Sed rats. The only exception was an increase in DMN NPY expression, which is similar to what has been reported in exercising adult rats (47) and adult DIO rats calorically restricted for 6 wk (37). While 13 wk of exercise produced few changes in hypothalamic mRNA expression, rats exercised for 6 wk and left sedentary for 7 wk had comparable decreases in adiposity, leptin, and insulin levels to exercising rats, but they had increased ARC POMC expression; exactly the opposite of what would have been predicted from their reduced leptin levels. Although the reason for this increase is unclear, if it were associated with an expected increased release of the anorectic peptide α-MSH, it could account for the failure of these rats to increase their food intake and body weight for a full 7 wk after exercise termination (13–15). Finally, another unexpected finding was that rats that were calorically restricted for 13 wk also had increased ARC POMC in association with reduced VMN MC3R and MC4R. Again, there is no obvious explanation for this increase, which could downregulate these receptors in response to increased α-MSH release (22). Clearly this increased POMC, along with a failure of these restricted rats to increase their ARC NPY or AgRP expression is contrary to what occurs in adult rats. It is possible that such apparently

Table 3. *Experiment 2: DIO rats fed high-energy diet and subjected to various regimens of exercise or food restriction for 13 wk*

	Sed	Ex	6 wk Ex/7 wk Sed	Rstr
		ARC		
CYC	0.94±0.29	0.54±0.13	0.52±0.14	0.10±0.04
NPY	0.77±0.12	0.92±0.16	1.0±0.09	0.79±0.25
POMC	1.02±0.24[a]	1.12±0.17[a]	1.58±0.16[b]	2.29±0.36[c]
AgRP	0.17±0.00	0.88±0.18	1.07±0.16	1.02±0.34
NPY1R	1.19±0.17	1.38±0.12	1.43±0.17	1.99±0.31
NPY5R	0.82±0.07	1.05±0.12	0.96±0.09	1.17±0.17
MC3R	0.90±0.07	1.01±0.12	0.89±0.11	0.74±0.17
MC4R	2.01±0.32	1.88±0.24	1.73±0.25	1.14±0.21
lepr-b	1.11±0.17	1.06±0.10	0.97±0.14	1.31±0.31
BDNF	1.12±0.21	1.41±0.25	1.07±0.24	0.62±0.14
CRF1R	2.02±0.39	1.76±0.34	2.00±0.37	2.23±0.51
CRF2R	0.92±0.16	1.01±0.18	0.83±0.17	0.39±0.08
SOCS3	1.34±0.12	1.08±0.13	1.29±0.12	
		VMN		
CYC	0.72±0.15	0.82±0.14	0.97±0.10	0.49±0.12
NPY1R	0.81±0.12	1.02±0.09	1.07±0.06	0.73±0.13
NPY5R	0.93±0.09	1.03±0.07	1.06±0.04	1.32±0.10
MC3R	0.71±0.13[a]	0.70±0.15[a]	1.06±0.12[b]	0.38±0.10[c]
MC4R	1.00±0.13[a]	0.79±0.11[a]	0.72±0.09[a]	0.41±0.08[b]
lepr-b	0.84±0.14[a]	0.91±0.11[a]	0.93±0.07[a]	0.36±0.06[b]
BDNF	0.90±0.14	0.77±0.12	1.12±0.09	0.79±0.07
SOCS3	1.02±0.14	0.92±0.14	0.95±0.13	
		DMN		
CYC	0.90±0.27	0.83±0.22	0.90±0.23	0.34±0.10
NPY	0.97±0.17[b]	1.45±0.22[a]	0.83±0.12[b]	0.63±0.17[c]
NPY1R	1.29±0.18	1.10±0.15	1.03±0.09	1.10±0.19
NPY5R	1.12±0.16	0.77±0.13	0.81±0.12	1.06±0.20
MC3R	1.05±0.18	0.69±0.10	0.75±0.15	0.80±0.13
MC4R	0.96±0.08	0.57±0.14	0.71±0.13	0.55±0.05
lepr-b	0.88±0.10	0.73±0.09	0.77±0.08	0.94±0.23
BDNF	0.96±0.08	0.57±0.11	0.61±0.07	0.69±0.18
		PVN		
CYC	0.95±0.19	1.14±0.28	0.86±0.19	0.57±0.21
MC3R	1.27±0.12	0.87±0.19	1.03±0.10	0.65±0.24
MC4R	0.89±0.09	0.69±0.11	0.73±0.08	0.65±0.21
BDNF	0.69±0.13	0.63±0.13	0.65±0.09	0.42±0,15
CRF	0.80±0.25	0.80±0.24	0.97±0.27	0.11±0.06
		LH		
CYC	1.97±0.55	2.20±0.72	1.99±0.65	5.18±1.00
NPY1R	0.93±0.13	0.92±0.09	1.00±0.07	0.92±0.08
NPY5R	1.12±0.14	1.00±0,13	0.99±0.13	0.76±0.07
BDNF	1.29±0.16	1.46±0.25	1.64±0.29	0.79±0.08

Values are means ± SE ratios of the mRNA of interest relative to cyclophilin mRNA. mRNA expression data are derived from the brains of the rats in Table 2. Tissue from the arcuate nucleus (ARC), ventromedial nucleus (VMN), dorsomedial hypothalmic nucleus (DMN), paraventricular nucleus (PVN), and lateral hypothalmus (LH) were micropunched and assayed by quantitative PCR (QPCR). CYC, cyclophilin; NPY, neuropeptide Y; POMC, proopiomelanocortin; AgRP, agouti-related peptide; NPY1R, neuropeptide Y1 receptor; MC3R, melanocortin-3 receptor; lepr-b, leptin receptor-b; BDNF, brain-derived neurotropic factor; CRF1R, corticotrophin releasing factor-1 receptor; SOCS3, suppressor of cytokines signaling-3. [a,b,c]Parameters with differing superscripts differ from each other at the $P < 0.05$ level by post hoc Bonferroni adjustment after significant intergroup differences were found by 1-way ANOVA.

conflicting results represent differences in the way in which prepubertal rats respond to dietary restriction compared with adults.

We also examined changes in the mRNA expression of brain neuropeptides, receptors, and growth factors after 3 wk of exercise since this was the minimal amount of time required to provide sustained protection against the development of obesity. At this 3-wk time point, there were few differences among Sed, Ex, and Restr rats that might explain future differences in body weight gain and adiposity. Despite their lower levels of adiposity and leptin levels, NPY, AgRP, and POMC expression levels in Ex rats did not differ from those of Sed rats. This failure to alter the expression of these peptides or to increase their food intake in the face of decreasing adiposity and leptin levels is similar to what occurs in adult DIO rats after 6 wk of exercise (37). Thus, in some, as yet unidentified way, voluntary wheel running suppresses the expected compensatory responses to the negative energy balance and reduced leptin levels caused by such exercise (5, 36, 40, 48, 62). In fact, there were only minor reductions in ARC MC3R and NPY1 receptor and DMN SOCS3 mRNA levels in Ex compared with Sed rats at this time. Surprisingly, while food restriction would be expected to increase ARC NPY and AgRP and decrease

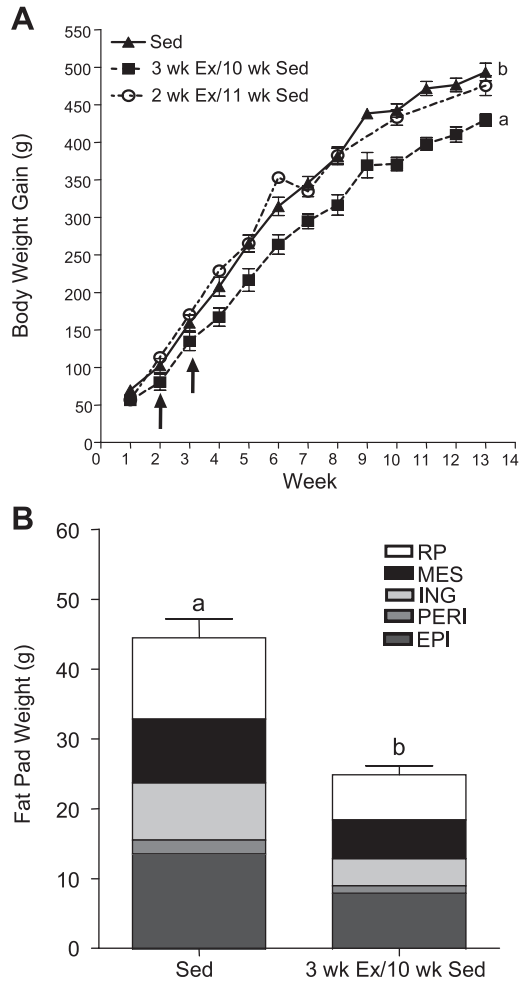

Fig. 4. At 4 wk of age, male DIO rats were weaned onto an HE diet and then remained sedentary for 13 wk ($n = 6$), or were given access to a running wheel for only 2 wk and then left sedentary for an additional 11 wk (2 wk Ex/11 wk Sed; $n = 6$), or were given access to a running wheel for only 3 wk and then left sedentary for an additional 10 wk (3 wk Ex/10 wk Sed; $n = 6$). *A*; body weight gain. *B*: terminal fat pad weights following 13 wk. Arrows denote removal of running wheel. Groups with differing superscripts differ from each other at the $P \leq 0.05$ level by post hoc Bonferroni adjustment after significant intergroup differences were found by repeated-measures or 1-way ANOVA.

Table 4. *Experiment 3: determination of the minimal amount of postweaning exercise required for sustained reduction in body weight gain, food intake, and adiposity*

	Sed	2 wk Ex/11 wk Sed	3 wk Ex/10 wk Sed
Initial body weight, g	72±5[a]	51±2[b]	67±8[a]
Body weight 2 wk, g	180±6[a]	164±3[b]	154±10[a]
Body weight 3 wk, g	240±7	221±4	211±9
Body weight 4 wk, g	287±7[a]	280±3[a]	246±12[b]
Body weight gain 2 wk, g	107±2	115±5	90±7
Body weight gain 3 wk, g	165±1	167±4	142±11
Body weight gain 4 wk, g	215±3[a]	231±5[a]	175±8[b]
Final body weight, g	570±5[a]	529±13[a]	496±10[b]
Final body weight gain, g	493±3[a]	478±13[a]	427±13[b]
Total intake 1–13 wk, kcal	7,894±597	7,236±147	7,212±832
Total feed efficiency, 1–13 wk, g/kcal × 1,000	68.1±2.2	66.1±2.1	65.3±4.6
Total fat pads, g	43.8±2.0[a]	ND	23.9±1.7[b]
Total fat pads/body weight, %	7.8±0.77[a]	ND	4.8±0.55[b]

Values are means ± SE. At 4 wk of age, male DIO rats were weaned onto a high-energy diet and then remained sedentary for 13 wk (Sed; $n = 6$) or were given access to a running wheel for 2 wk ($n = 6$) or 3 wk ($n = 6$) and then left sedentary for an additional 11 wk (2 wk Ex/11 wk Sed) or 10 wk (3 wk Ex/10 wk Sed), respectively. ND, not determined. [a,b]Parameters with differing superscripts differ from each other at the $P < 0.05$ level by post hoc Bonferroni adjustment after significant intergroup differences were found by 1-way ANOVA.

POMC expression in adult rats (5, 12, 37, 40), this did not occur with the modest 3-wk 15% caloric restriction imposed on the postweanling Sed rats. The other differences in mRNA expression profiles between Restr and either Ex or Sed rats were also modest, at best. Probably the most notable difference was the reduction in VMN SOCS3 mRNA in Restr compared with Sed rats, a change that should lead to increased leptin receptor sensitivity (53) and a decreased drive to eat (16). On the other hand, Restr rats had reduced VMN BDNF mRNA expression compared with Sed rats. Such a reduction in this anorectic trophic factor would be expected to provide a drive to increase caloric intake in these food-deprived animals when they were allowed to eat ad libitum (69). However, VMN BDNF mRNA expression was similar between Rstr and Ex rats. Without further studies, it is not possible to know what these intergroup differences in mRNA expression might mean.

There are some important caveats to the interpretation of our findings. First, changes in mRNA expression are not necessarily representative of parallel changes in peptide release or receptor function. Second, in Ex rats, all neuropeptide and receptor mRNA levels were assessed at the onset of the dark cycle, 4 h after running wheels were removed during the end of the light cycle. Since the majority of the running takes place during the dark cycle, it is possible that we might have missed any temporal changes in neuropeptide expression that occur in Ex rats immediately following a bout of exercise. However, we expect that these changes if any, would only regulate energy homeostasis on a short-term basis, and the changes witnessed here would have a greater role in the long-term regulation of energy homeostasis in these rats.

Importantly, the long-term benefit of prior exercise on body weight and adiposity in rodents appears to be highly dependent on genetic background. For example, prior exercise leads to hyperphagia and increased obesity (28) or adipocyte cell numbers upon exercise cessation (4) in some rat strains, even when exercise is initiated during the juvenile period (32, 33). This is unlike our DIO rats which failed to show any compensatory increase in caloric intake either during or after exercise termination and the hyperphagic Otsuka Long-Evans Tokushima

Table 5. *Experiment 4: hypothalamic mRNA expression in DIO Sed, Ex, or Rstr rats for 3 wk postweaning*

	Sed	Ex	Rstr
		ARC	
CYC	1.35±0.27	0.92±0.19	1.20±0.16
NPY	0.93±0.15	0.92±0.07	0.90±0.14
AGRP	0.99±0.15	0.78±0.07	1.01±0.17
POMC	1.27±0.15	0.80±0.15	0.96±1.18
INSR	1.08±0.08	0.83±0.10	1.03±0.10
MC3R	0.94±0.04[b]	0.69±0.06[a]	1.02±0.04[b]
MC4R	1.16±0.09	0.91±0.07	0.97±0.10
NPY1R	1.13±0.09[b]	0.81±0.07[a]	0.92±0.10[ab]
NPY5R	1.22±0.07	0.88±0.11	0.98±0.11
lepr-b	1.17±0.10	0.88±0.08	1.17±0.12
SOCS3	1.52±0.22	1.09±0.21	0.83±0.21
CRF1R	1.12±0.13	1.14±0.11	1.16±0.17
CRF2R	0.78±0.13	0.62±0.09	1.03±0.21
		VMN	
CYC	0.45±0.07	0.61±0.06	0.40±0.06
INSR	1.06±0.08	0.86±0.06	0.83±0.14
MC3R	2.33±0.19[a]	2.10±0.26[a]	1.01±0.28[b]
MC4R	1.61±0.24	1.29±0.26	1.47±0.30
NPY1R	8.46±1.02	6.91±0.76	5.85±0.10
NPY5R	5.61±0.52	5.20±0.57	4.06±0.68
lepr-b	0.98±0.14	0.78±0.07	0.72±0.10
SOCS3	10.74±1.33[a]	8.37±1.42[ab]	4.73±0.95[b]
BDNF	4.46±0.58[a]	3.50±0.28[ab]	2.77±0.48[b]
		DMN	
CYC	0.16±0.05	0.11±0.04	0.12±0.05
INSR	0.15±0.03	0.15±0.04	0.15±0.03
MC3R	2.39±0.52	2.00±0.31	2.49±0.38
MC4R	2.14±0.25	1.91±0.27	1.77±0.38
NPY1R	2.70±0.29	2.39±0.32	2.67±0.41
NPY5R	0.63±0.07	0.62±0.11	0.68±0.11
SOCS3	9.72±0.89[b]	6.02±0.53[a]	6.19±0.52[a]
BDNF	2.36±0.23[a]	1.73±0.13[ab]	1.45±0.19[b]
CRF	0.29±0.04	0.29±0.02	0.35±0.03
		PVN	
CYC	1.15±0.17	1.16±0.18	0.82±0.24
MC3R	1.09±0.02	1.00±0.02	1.08±0.04
MC4R	1.04±0.07	1.08±0.07	1.36±0.16
BDNF	1.25±0.07	0.93±0.14	1.11±0.10
CRF	1.20±0.32	1.09±0.11	1.33±0.15
CRF1R	1.15±0.08	1.25±0.09	1.78±0.43
CRF2R	1.28±0.13	0.97±0.05	1.33±0.16
		LH	
CYC	0.96±0.29	1.03±0.18	1.27±0.19
NPY1R	2.57±0.96	2.12±0.62	1.71±0.13
NPY5R	1.82±0.29	1.78±0.36	1.73±0.10
BDNF	2.84±0.38	3.78±0.55	4.16±0.76
MCH	1.83±0.32	1.25±0.17	1.42±0.12

At 4 wk of age, DIO rats were weaned onto the high-energy diet and then left sedentary (Sed; $n = 8$), given access to a running wheel (Ex; $n = 8$), or calorically restricted (Rstr; $n = 8$) for 3 wk postweaning. Tissue from the ARC, VMN, DMN, PVN, and LH were micropunched and assayed by QPCR. INSR, insulin receptor. Values are means ± SE ratios of the mRNA of interest relative to cyclophilin mRNA. Parameters with differing superscripts differ from each other at the $P < 0.05$ level by post hoc Bonferroni adjustment after significant intergroup differences were found by 1-way ANOVA.

Fatty rats, which also fail to increase their intake during exercise (52). Thus, the way in which rodents regulate caloric intake and overall energy homeostasis in response to exercise and exercise termination may be a main determinant of the ability of exercise to produce long-term loss of weight and adiposity.

Voluntary exercise during the immediate postweaning period can override the genetic propensity of DIO rats to become obese, even when they are chronically exposed to a diet containing moderately elevated levels of fat and caloric density. The protective effect of continued exercise prevented the expected changes in anabolic and catabolic hypothalamic neuropeptide expression, despite the loss of adipose mass and reductions in plasma leptin and insulin levels. Importantly, only 3 wk of exercise was required to provide a long-lasting protective effect on the development of obesity. The association with increased ARC POMC expression 7 wk after cessation of exercise suggests that this finding may play an important role in their persistent obesity resistance. Perhaps of equal importance was the finding that 6 wk of postweaning caloric restriction led to increased obesity when these rats were allowed to eat ad libitum for another 7 wk. Thus, juvenile rats react very differently from adult rats to exercise and caloric restriction with regard to both long-term regulation of energy homeostasis and hypothalamic neuropeptide and receptor mRNA expression in response to these perturbations. We suggest that these differences may be due to an effect on the organization or function of the neural pathways involved in the regulation of energy homeostasis during this critical developmental period. While it known that the projections of critical NPY/AgRP and POMC neurons in the ARC reach their distal targets during the first 2–3 postnatal wk (9, 21), our data suggest that a potential window of opportunity for modifying these or other critical pathways may exist for several weeks beyond this time.

Perspectives and Significance

Exercise has a selective but self-limited effect on lowering adiposity in obese adult rodents (4, 39, 51, 52, 64, 65, 75). However, we demonstrate here that rats selectively bred to develop DIO fail to become obese after only 3 wk of postweaning exercise, despite continued intake of a 31% fat HE diet for many weeks after exercise cessation. To our knowledge, this is the only example where such a short-term intervention produces such a long-lived prevention of obesity in genetically predisposed animals. Given the fact that the brain is still developing during this prepubertal period, we hypothesized that exercise might alter the formation of brain circuitry involved in the regulation of energy homeostasis. The failure of these animals to demonstrate differences in ARC NPY or AgRP mRNA expression is probably because these neurons have largely completed their intrahypothalamic target connections by the third week of life (11). On the other hand, it is possible that some factors (e.g., cytokines) released during exercise might either affect the early development of other neural pathways or in some way correct the inherited reductions of medial hypothalamic leptin (27, 41, 44) and insulin signaling (27) of DIO rats. Although exercise did not affect leptin or insulin receptor mRNA expression, it is still possible that binding to or downstream signaling of these receptors might be altered to explain why these rats had no compensatory hyperphagia to restore to their reduced adiposity once exercise was stopped. Unfortunately, it is difficult to extrapolate the present findings in the DIO rat to humans because of the marked differences in the temporal patterns of brain development between humans and rodents (10, 20, 21, 59, 60). Thus, even if early-onset exercise might prevent the development of obesity in children, it is unclear at what age they would have to begin exercising to obtain benefits similar to those seen in our rats. However, in keeping with our previous rat studies (19), the present ones do suggest that environmental perturbations during the prepubertal period might be able to modify the development of obesity in genetically predisposed humans.

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REFERENCES

1. **American Physiological Society.** Guiding principals for research involving animals and human beings. *Am J Physiol Regul Integr Comp Physiol* 283: R281–R283, 2002.
2. **Al-Almaie SM.** Prevalence of obesity and overweight among Saudi adolescents in Eastern Saudi Arabia. *Saudi Med J* 26: 607–611, 2005.
3. **Andersen LF, Lillegaard IT, Overby N, Lytle L, Klepp KI, Johansson L.** Overweight and obesity among Norwegian schoolchildren: changes from 1993 to 2000. *Scand J Public Health* 33: 99–106, 2005.
4. **Applegate EA, Upton DE, Stern JS.** Exercise and detraining: effect on food intake, adiposity and lipogenesis in Osborne-Mendel rats made obese by a high fat diet. *J Nutr* 114: 447–459, 1984.
5. **Bi S, Robinson BM, Moran TH.** Acute food deprivation and chronic food restriction differentially affect hypothalamic NPY mRNA expression. *Am J Physiol Regul Integr Comp Physiol* 285: R1030–R1036, 2003.
6. **Bi S, Scott KA, Hyun J, Ladenheim EE, Moran TH.** Running wheel activity prevents hyperphagia and obesity in OLETF rats: role of hypothalamic signaling. *Endocrinology* 146: 1676–1685, 2005.
7. **Billington CJ, Briggs JE, Grace M, Levine AS.** Effects of intracerebroventricular injection of neuropeptide Y on energy metabolism. *Am J Physiol Regul Integr Comp Physiol* 260: R321–R327, 1991.
8. **Blundell JE, Stubbs RJ, Hughes DA, Whybrow S, King NA.** Cross talk between physical activity and appetite control: does physical activity stimulate appetite? *Proc Nutr Soc* 62: 651–661, 2003.
9. **Bouret SG, Draper SJ, Simerly RB.** Formation of projection pathways from the arcuate nucleus of the hypothalamus to hypothalamic regions implicated in the neural control of feeding behavior in mice. *J Neurosci* 24: 2797–2805, 2004.
10. **Bouret SG, Draper SJ, Simerly RB.** Trophic action of leptin on hypothalamic neurons that regulate feeding. *Science* 304: 108–110, 2004.
11. **Bouret SG, Simerly RB.** Minireview: leptin and development of hypothalamic feeding circuits. *Endocrinology* 145: 2621–2626, 2004.
12. **Brady LS, Smith MA, Gold PW, Herkenham M.** Altered expression of hypothalamic neuropeptide mRNAs in food-restricted and food-deprived rats. *Neuroendocrinology* 52: 441–447, 1990.
13. **Butler AA, Marks DL, Fan W, Kuhn CM, Bartolome M, Cone RD.** Melanocortin-4 receptor is required for acute homeostatic responses to increased dietary fat. *Nat Neurosci* 4: 605–611, 2001.
14. **Chen AS, Metzger JM, Trumbauer ME, Guan XM, Yu H, Frazier EG, Marsh DJ, Forrest MJ, Gopal-Truter S, Fisher J, Camacho RE, Strack AM, Mellin TN, MacIntyre DE, Chen HY, Van der Ploeg LH.** Role of the melanocortin-4 receptor in metabolic rate and food intake in mice. *Transgenic Res* 9: 145–154, 2000.
15. **Corbett SW, Stern JS, Keesey RE.** Energy expenditure in rats with diet-induced obesity. *Am J Clin Nutr* 44: 173–180, 1986.

16. **Dhillon H, Zigman JM, Ye C, Lee CE, McGovern RA, Tang V, Kenny CD, Christiansen LM, White RD, Edelstein EA, Coppari R, Balthasar N, Cowley MA, Chua S Jr, Elmquist JK, Lowell BB.** Leptin directly activates SF1 neurons in the VMH, and this action by leptin is required for normal body-weight homeostasis. *Neuron* 49: 191–203, 2006.
17. **Donnelly JE, Hill JO, Jacobsen DJ, Potteiger J, Sullivan DK, Johnson SL, Heelan K, Hise M, Fennessey PV, Sonko B, Sharp T, Jakicic JM, Blair SN, Tran ZV, Mayo M, Gibson C, Washburn RA.** Effects of a 16-month randomized controlled exercise trial on body weight and composition in young, overweight men and women: the Midwest Exercise Trial. *Arch Intern Med* 163: 1343–1350, 2003.
18. **Ford ES, Giles WH, Mokdad AH.** Increasing prevalence of the metabolic syndrome among U.S. adults. *Diabetes Care* 27: 2444–2449, 2004.
19. **Gorski JN, Dunn-Meynell AA, Levin BE.** Maternal obesity increases hypothalamic leptin receptor expression and sensitivity in juvenile obesity-prone rats. *Am J Physiol Regul Integr Comp Physiol* 292: R1782–R1791, 2007.
20. **Grove KL, Allen S, Grayson BE, Smith MS.** Postnatal development of the hypothalamic neuropeptide Y system. *Neuroscience* 116: 393–406, 2003.
21. **Grove KL, Smith MS.** Ontogeny of the hypothalamic neuropeptide Y system. *Physiol Behav* 79: 47–63, 2003.
22. **Harrold JA, Widdowson PS, Williams G.** Altered energy balance causes selective changes in melanocortin-4 (MC4-R), but not melanocortin-3 (MC3-R), receptors in specific hypothalamic regions: further evidence that activation of MC4-R is a physiological inhibitor of feeding. *Diabetes* 48: 267–271, 1999.
23. **Haynes WG, Morgan DA, Djalali A, Sivitz WI, Mark AL.** Interactions between the melanocortin system and leptin in control of sympathetic nerve traffic. *Hypertension* 33: 542–547, 1999.
24. **Hill JO, Davis JR, Tagliaferro AR.** Effects of diet and exercise training on thermogenesis in adult female rats. *Physiol Behav* 31: 133–135, 1983.
25. **Hill JO, Davis JR, Tagliaferro AR, Stewart J.** Dietary obesity and exercise in young rats. *Physiol Behav* 33: 321–328, 1984.
26. **Hill JO, Thacker S, Newby D, Sykes MN, Digirolamo M.** Influence of food restriction coupled with weight cycling on carcass energy restoration during ad-libitum refeeding. *Int J Obes* 11: 251–262, 1987.
27. **Irani BG, Dunn-Meynell AA, Levin BE.** Altered hypothalamic leptin, insulin and melanocortin binding associated with moderate fat diet and predisposition to obesity. *Endocrinology* 148: 310–316, 2007.
28. **Jen KL, Almario R, Ilagan J, Zhong S, Archer P, Lin PK.** Long-term exercise training and retirement in genetically obese rats: effects on food intake, feeding efficiency and carcass composition. *Int J Obes Relat Metab Disord* 16: 519–527, 1992.
29. **Keinan-Boker L, Noyman N, Chinich A, Green MS, Nitzan-Kaluski D.** Overweight and obesity prevalence in Israel: findings of the first national health and nutrition survey (MABAT). *Isr Med Assoc J* 7: 219–223, 2005.
30. **Kim DM, Ahn CW, Nam SY.** Prevalence of obesity in Korea. *Obes Res* 6: 117–121, 2005.
31. **Kramer FM, Jeffery RW, Forster JL, Snell MK.** Long-term follow-up of behavioral treatment for obesity: patterns of weight regain among men and women. *Int J Obes* 13: 123–136, 1989.
32. **Kump D, Booth F.** Sustained rise in triacylglycerol synthesis and increased epididymal fat mass when rats cease voluntary wheel running. *J Physiol* 565: 911–925, 2005.
33. **Laye M, Thyfault J, Stump C, Booth F.** Inactivity induces increases in abdominal fat. *J Appl Physiol* 102: 1341–1347, 2007.
34. **Leibel RL, Hirsch J.** Diminished energy requirements in reduced-obese patients. *Metabolism* 33: 164–170, 1984.
35. **Leibel RL, Rosenbaum M, Hirsch J.** Changes in energy expenditure resulting from altered body weight. *N Engl J Med* 332: 621–628, 1995.
36. **Levin BE.** Arcuate NPY neurons and energy homeostasis in diet-induced obese and resistant rats. *Am J Physiol Regul Integr Comp Physiol* 276: R382–R387, 1999.
37. **Levin BE, Dunn-Meynell AA.** 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.
38. **Levin BE, Dunn-Meynell AA.** Defense of body weight against chronic caloric restriction in obesity-prone and -resistant rats. *Am J Physiol Regul Integr Comp Physiol* 278: R231–R237, 2000.
39. **Levin BE, Dunn-Meynell AA.** Differential effects of exercise on body weight gain and adiposity in obesity-prone and -resistant rats. *Int J Obes* 30: 722–727, 2006.
40. **Levin BE, Dunn-Meynell AA.** Dysregulation of arcuate nucleus preproneuropeptide Y mRNA in diet-induced obese rats. *Am J Physiol Regul Integr Comp Physiol* 272: R1365–R1370, 1997.
41. **Levin BE, Dunn-Meynell AA.** Reduced central leptin sensitivity in rats with diet-induced obesity. *Am J Physiol Regul Integr Comp Physiol* 283: R941–R948, 2002.
42. **Levin BE, Dunn-Meynell AA.** Sibutramine alters the central mechanisms regulating the defended body weight in diet-induced obese rats. *Am J Physiol Regul Integr Comp Physiol* 279: R2222–R2228, 2000.
43. **Levin BE, Dunn-Meynell AA, Balkan B, Keesey RE.** Selective breeding for diet-induced obesity and resistance in Sprague-Dawley rats. *Am J Physiol Regul Integr Comp Physiol* 273: R725–R730, 1997.
44. **Levin BE, Dunn-Meynell AA, Banks WA.** Obesity-prone rats have normal blood-brain barrier transport but defective central leptin signaling prior to obesity onset. *Am J Physiol Regul Integr Comp Physiol* 286: R143–R150, 2004.
45. **Levin BE, Hogan S, Sullivan AC.** Initiation and perpetuation of obesity and obesity resistance in rats. *Am J Physiol Regul Integr Comp Physiol* 256: R766–R771, 1989.
46. **Levin BE, Keesey RE.** Defense of differing body weight set-points in diet-induced obese and resistant rats. *Am J Physiol Regul Integr Comp Physiol* 274: R412–R419, 1998.
47. **Lewis DE, Shellard L, Koeslag DG, Boer DE, McCarthy HD, McKibbin PE, Russell JC, Williams G.** Intense exercise and food restriction cause similar hypothalamic neuropeptide Y increases in rats. *Am J Physiol Endocrinol Metab* 264: E279–E284, 1993.
48. **Li C, Chen P, Smith MS.** Neuropeptide Y (NPY) neurons in the arcuate nucleus (ARH) and dorsomedial nucleus (DMH), areas activated during lactation, project to the paraventricular nucleus of the hypothalamus (PVH). *Regu Pept* 98–100, 1988.
49. **MacLean PS, Higgins JA, Johnson GC, Fleming-Elder BK, Peters JC, Hill JO.** Metabolic adjustments with the development, treatment, and recurrence of obesity in obesity-prone rats. *Am J Physiol Regul Integr Comp Physiol* 287: R288–R297, 2004.
50. **Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, Fei H, Kim S, Lallone R, Ranganathan S, Kern PA, Friedman JM.** Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nat Med* 1: 1155–1161, 1995.
51. **Mayer J, Marshall NB, Vitale JJ, Christensen JH, Mashayekhi MB, Stare FJ.** Exercise, food intake and body weight in normal rats and genetically obese adult mice. *Am J Physiol* 177: 544–548, 1954.
52. **Moran T, Bi S.** Hyperphagia and obesity of OLETF rats lacking CCK1 receptors: Developmental aspects. *Dev Psychobiol* 48: 360–367, 2006.
53. **Mori H, Hanada R, Hanada T, Aki D, Mashima R, Nishinakamura H, Torisu T, Chien KR, Yasukawa H, Yoshimura A.** Socs3 deficiency in the brain elevates leptin sensitivity and confers resistance to diet-induced obesity. *Nat Med* 10: 739–743, 2004.
54. **Murphy B, Nunes CN, Ronan JJ, Harper CM, Beall MJ, Hanaway M, Fairhurst AM, Van der Ploeg LH, MacIntyre DE, Mellin TN.** Melanocortin mediated inhibition of feeding behavior in rats. *Neuropeptides* 32: 491–497, 1998.
55. **Ogden CL, Flegal KM, Carroll MD, Johnson CL.** Prevalence and trends in overweight among US children and adolescents, 1999–2000. *JAMA* 288: 1728–1732, 2002.
56. **Palkovits M.** Isolated removal of hypothalamic or other brain nuclei of the rat. *Brain Res* 59: 449–450, 1973.
57. **Popkin BM.** The nutrition transition and obesity in the developing world. *J Nutr* 131: 871S–873S, 2001.
58. **Popkin BM, Doak CM.** The obesity epidemic is a worldwide phenomenon. *Nutr Rev* 56: 106–114, 1999.
59. **Rinaman L.** Postnatal development of catecholamine inputs to the paraventricular nucleus of the hypothalamus in rats. *J Comp Neurol* 438: 411–422, 2001.
60. **Rinaman L.** Postnatal development of hypothalamic inputs to the dorsal vagal complex in rats. *Physiol Behav* 79: 65–70, 2003.
61. **Rosenbloom AL, Joe JR, Young RS, Winter WE.** Emerging epidemic of type 2 diabetes in youth. *Diabetes Care* 22: 345–354, 1999.
62. **Schwartz MW, Seeley RJ, Campfield LA, Burn P, Baskin DG.** Identification of targets of leptin action in rat hypothalamus. *J Clin Invest* 98: 1101–1106, 1996.
63. **Schwartz MW, Woods SC, Porte D Jr, Seeley RJ, Baskin DG.** Central nervous system control of food intake. *Nature* 404: 661–671, 2000.
64. **Shima K, Shi K, Mizuno A, Sano T, Ishida K, Noma Y.** Exercise training has a long-lasting effect on prevention of non-insulin-dependent

diabetes mellitus in Otsuka-Long-Evans-Tokushima Fatty rats. *Metabolism* 45: 475–480, 1996.
65. **Shima K, Shi K, Mizuno A, Sano T, Ishida K, Yoshimoto S.** Effects of difference in amount of exercise training on prevention of diabetes mellitus in the Otsuka-Long-Evans-Tokushima fatty rats, a model of spontaneous non-insulin-dependent diabetes mellitus. *Diabetes Res Clin Pract* 23: 147–154, 1994.
66. **Stubbs RJ, Sepp A, Hughes DA, Johnstone AM, King N, Horgan G, Blundell JE.** The effect of graded levels of exercise on energy intake and balance in free-living women. *Int J Obes Relat Metab Disord* 26: 866–869, 2002.
67. **Wadden TA, Phelan S.** Behavioral assessment of the obese patient. In: *Handbook of Obesity Treatment*, edited by Wadden TA and Stunkard AJ. New York: Guilford 2002, p. 186–226.
68. **Walker HC, Romsos DR.** Similar effects of NPY on energy metabolism and on plasma insulin in adrenalectomized ob/ob and lean mice. *Am J Physiol Endocrinol Metab* 264: E226–E230, 1993.
69. **Wang C, Bomberg E, Levine A, Billington C, Kotz CM.** Brain-derived neurotrophic factor in the ventromedial nucleus of the hypothalamus reduces energy intake. *Am J Physiol Regul Integr Comp Physiol* 293: R1037–R1045, 2007.
70. **Wang Y.** Cross-national comparison of childhood obesity: the epidemic and the relationship between obesity and socioeconomic status. *Int J Epidemiol* 30: 1129–1136, 2001.
71. **Weigle DS, Duell PB, Connor WE, Steiner RA, Soules MR, Kuijper JL.** Effect of fasting, refeeding, and dietary fat restriction on plasma leptin levels. *J Clin Endocrinol Metab* 82: 561–565, 1997.
72. **Weinsier RL, Nelson KM, Hensrud KM, Darnell BE, Hunter GR, Schutz Y.** Metabolic predictors of obesity: contribution of resting energy expenditure, thermic effect of food, and fuel utilization to four-year weight gain of post-obese and never-obese women. *J Clin Invest* 95: 980–985, 1995.
73. **Wing RR.** Physical activity in the treatment of the adulthood overweight and obesity: current evidence and research issues. *Med Sci Sports Exerc* 31: S547–S552, 1999.
74. **Wyatt HR, Grunwald GK, Seagle HM, Klem ML, McGuire MT, Wing RR, Hill JO.** Resting energy expenditure in reduced-obese subjects in the National Weight Control Registry. *Am J Clin Nutr* 69: 1189–1193, 1999.
75. **Yasari S, Paquette A, Charbonneau A, Gauthier MS, Savard R, Lavoie JM.** Effects of ingesting a high-fat diet upon exercise-training cessation on fat accretion in the liver and adipose tissue of rats. *Appl Physiol Nutr Metab* 31: 367–375, 2006.
76. **Zachwieja JJ, Hendry SL, Smith SR, Harris RB.** Voluntary wheel running decreases adipose tissue mass and expression of leptin mRNA in Osborne-Mendel rats. *Diabetes* 46: 1159–1166, 1997.