High-fat diet offsets the long-lasting effects of running-wheel access on food intake and body weight in OLETF rats

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Chao PT, Terrillion CE, Moran TH, Bi S. High-fat diet offsets the long-lasting effects of running-wheel access on food intake and body weight in OLETF rats. Am J Physiol Regul Integr Comp Physiol 300: R1459–R1467, 2011. First published March 2, 2011; doi:10.1152/ajpregu.00517.2010.—We have previously demonstrated that running-wheel access normalizes the food intake and body weight of Otsuka Long-Evens Tokushima Fatty (OLETF) rats. Following 6 wk of running-wheel access beginning at 8 wk of age, the body weight of OLETF rats remains reduced, demonstrating a lasting effect on their phenotype. In contrast, access to a high-fat diet exacerbates the hyperphagia and obesity of OLETF rats. To determine whether diet modulates the long-term effects of exercise, we examined the effects of high-fat diet on food intake and body weight in OLETF rats that had prior access to running wheels for 4 wk. We found that 4 wk of running exercise significantly decreased food intake and body weight of OLETF rats. Consistent with prior results, 4 wk of exercise also produced long-lasting effects on food intake and body weight in OLETF rats fed a regular chow. When running wheels were relocked, OLETF rats stabilized at lower levels of body weight than sedentary OLETF rats. However, access to a high-fat diet offset these effects. When OLETF rats were switched to a high-fat diet following wheel relocking, they significantly increased food intake and body weight, so that they reached levels similar to those of sedentary OLETF rats fed a high-fat diet. Gene expression determination of hypothalamic neuropeptides revealed changes that appeared to be appropriate responses to the effects of diet and running exercise. Together, these results demonstrate that high-fat diet modulates the long-lasting effects of exercise on food intake and body weight in OLETF rats.

neuropeptide Y; cholecystokinin 1 receptor; dorsomedial hypothalamic nucleus; proopiomelanocortin; Agouti-related peptide

OBESITY HAS BECOME A MAJOR public health problem. Rates of overweight and obesity have increased remarkably in the United States and throughout the developed countries (10, 31). Obesity has serious health consequences, such as type 2 diabetes, hypertension, and cardiovascular diseases (22). Changes in the availability of highly palatable, high-caloric density foods, and an overall decrease in physical activity have both been implicated in the current obesity epidemic.

How exercise and food intake interact in the overall control of energy balance is still not well understood. In a number of rodent obesity models, access to a running wheel and the subsequent increase in physical activity modulated both food intake and body weight, such that body weight was normalized during running-wheel access (12, 17, 26). In some models, a long-lasting effect of exercise on feeding and body weight has been identified. Thus, in both diet-induced obesity-prone Sprague-Dawley (DIO) rats and Otsuka Long-Evans Tokushima fatty (OLETF) rats, exercise during the postweaning period protected the rats from attaining the full-obesity phenotype, even though running-wheel access was restricted to this period (6, 21). In the OLETF rat, these results were attained when rats were maintained on a regular chow (RC) diet (6).

The OLETF rat is established as an animal model of obesity and noninsulin-dependent diabetes mellitus (NIDDM), characterized by hyperphagia (20), mild obesity (~40% heavier than the control Long-Evans Tokushima Otsuka rat, LETO) and late-onset hyperglycemia and NIDDM (after 18 wk of age) (15). OLETF rats congenitally lack the CCK-1 receptor (30) and show both deficits in the satiety actions of peripheral CCK and the central modulation of neuropeptide Y (NPY) signaling in the dorsomedial hypothalamus (DMH) (4, 20). These rats have also been characterized to be especially sensitive to high-fat (HF) diet-induced obesity (3, 24). When maintained on a HF diet, OLETF rats exhibited sustained overconsumption, resulting in a substantial increase in their obesity (3). Dietary fats are a primary secretagogue for peripheral CCK release (19). The absence of CCK signaling through the CCK-1 receptor both peripherally and centrally has been proposed to contribute to the OLETF rat’s overconsumption of a HF diet (3, 24).

In the present study, we aimed to determine whether diet modulates the lasting effects of physical exercise. By examining the effects of a HF diet on food intake and body weight in OLETF rats that had prior access to running wheels for 4 wk, we sought to explore the behavioral and neural mechanisms underlying the effects of exercise on weight maintenance or energy balance in relation to diet. On the basis of previous results, indicating that OLETF rats have a primary deficit in modulating DMH NPY signaling but do not have a deficit in the regulation of peptide signaling, such as NPY and proopiomelanocortin (POMC), in the arcuate nucleus (ARC) (3, 4, 6), we hypothesized that a HF diet may alter the lasting effects of exercise on food intake and body weight in OLETF rats through the DMH NPY-signaling pathway.

We determined body weight, food intake, fat mass, running activity, plasma leptin level, and changes in mRNA levels of hypothalamic peptides involved in the control of food intake in OLETF and control LETO rats with or without prior access to running wheels for 4 wk, which were either assigned to HF or remained on RC. We specifically compared adipose depots in interscapular brown adipose tissue (BAT), epididymal white adipose tissue (WAT) (reflecting visceral WAT), and subcutaneous inguinal WAT (reflecting subcutaneous WAT) among the experimental groups of rats, since both exercise and diet affect BAT thermogenesis and since the central nervous system differentially regulates visceral and subcutaneous WAT depots (1). In addition to determining Npy gene expression in the DMH, we examined Npy, Agouti-related peptide (Agrp), and Pomc mRNA in the ARC, and corticotrophin-releasing factor...
Glucose Tolerance Test
After a 16-h overnight fast, rats were administered intragastric glucose at a dose of 2 g/kg by gavage. Tail blood was sampled by using a heparinized Natelson blood collection capillary tube (Fisher Scientific, Waltham, MA) before and 15, 30, 45, 60, and 120 min after giving glucose for the measurements of blood glucose and plasma insulin levels. Blood glucose levels were determined with a FreeStyle glucometer (TheraSense). Plasma insulin concentrations were determined by a rat insulin radioimmunoassay kit (Linco Research).

Riboprobes
As previously described (6), 35S-labeled antisense riboprobes of Npy, Pome, Agrp, and Crf were transcribed with in vitro transcription systems (Promega, Madison, WI) and then purified with Quick Spin RNA Columns (Roche, Indianapolis, IN).

In Situ Hybridization Determination
As previously described (6), frozen tissue slides were brought to room temperature and treated with TEA buffer and acetic anhydride. Then, the sections were incubated overnight in hybridization buffer containing 50% formamide, 0.3 M NaCl, 10 mM Tris-Cl in pH 7.5, 1 mM EDTA in pH 9.0, 1× Denhardt’s solution (Eppendorf), 10% dextran sulfate, 500 μg/ml yeast tRNA, 10 mM DTT, and 107 cpm/ml of [35S] UTP at 55°C. After hybridization, sections were washed three times with 2× SSC/1 mM DTT at 55°C, treated with 20 μg/ml RNase A (Sigma, St. Louis, MO) at 37°C for 30 min, washed twice in 2× SSC/1 mM DTT at 55°C for 5 min each, and wash twice in 0.1× SSC/1 mM DTT for 15 min at 55°C, and then dehydrated in gradient ethanol. The sections were air dried and exposed with BMR-2 film (Kodak) for 1–3 days.

Quantification analysis of the in situ hybridization images on developed films was done using National Institutes of Health (NIH) Scion Image Software (NIH, Bethesda, MD). Autoradiographic images were scanned with an Epson professional scanner (Epson, Long Beach, CA) and stored in a computer for subsequent analyses with Scion image program. Autoradiographic 14C microscales (Amersham, Piscataway, NJ) were used as a standard. The data obtained were calculated as the product of hybridization area × density on each section, and the background density was subtracted as the background noise. Data for each animal were an average of data generated from three anatomically matched sections. Data from each group were normalized to LETO RC rats as 100%, and all data are presented as means ± SE.

Data Analyses
Data were analyzed using two-way repeated-measures ANOVA for changes in body weight and food intake among the three groups of LETO RC, OLETF SED RC, and OLETF RW RC, and three-factor repeated-measures ANOVA for changes in body weight and food intake among the four groups of OLETF rats. Student’s t-test for changes in feed efficiency between OLETF SED and OLETF RW during the exercise period, two-way ANOVA for changes in feed...
efficiency among the four groups of OLETF rats during the postexercise period, and one-way ANOVA for changes in blood glucose, plasma insulin, fat mass, plasma leptin, and hypothalamic gene expression among the five groups of rats. ANOVAs were followed by pairwise multiple Fisher least significant difference comparisons. \( P < 0.05 \) was interpreted as a significant difference.

**RESULTS**

Effects of Running-Wheel Access on Body Weight and Food Intake

As shown in Fig. 1, OLETF rats were given access to running wheels at 9 wk of age. They gradually increased running activity during the first 2 wk and eventually ran from 8,000 to 10,000 revolutions per day (8.0–10.0 km/day) (Fig. 1A). Running-wheel access resulted in significant effects on body weight [\( F(6,78) = 12.784, P < 0.0001 \)] and food intake [\( F(6,81) = 6.5707, P < 0.0001 \)]. During exercise, body weight was significantly decreased in OLETF RW rats compared with OLETF SED rats (Fig. 1B). Food intake of OLETF RW rats was initially decreased below that of LETO controls and then retained levels between those of OLETF SED rats and LETO controls (Fig. 1C). Moreover, 4 wk of exercise resulted in a 72% reduction in feed efficiency (from 48.7 ± 1.1 g/cal in OLETF SED rats to 13.7 ± 2.5 g/cal in OLETF RW rats, \( P < .001 \)). These results demonstrate that exercise not only increases energy expenditure but also affects food intake, and

Fig. 1. Effects of a high-fat diet on body weight and food intake in Otsuka Long-Evans Tokushima fatty (OLETF) rats that had prior access to running wheels for 4 wk. A: running wheel activity in OLETF rats. B: sedentary OLETF rats on a regular chow (OLETF SED RC) grew heavier than sedentary lean Long-Evans Tokushima (LETO) rats on a regular chow (LETO RC) (*\( P < 0.05 \) from 8 to 21 wk of age). Four weeks of running wheel (RW) access reduced body weight and produced a long-lasting body-weight effect in OLETF RW RC rats (#\( P < 0.05 \) compared with OLETF SED RC from 10 to 21 wk of age). High-fat diet access offset this effect. Body weight did not differ between the sedentary and prior exercised OLETF rats on a high-fat diet (OLETF SED HF and OLETF RW HF) by 17 wk of age (\( P > 0.05 \)). C: during running wheel access, daily food intake was significantly decreased in OLETF RW rats compared with OLETF SED rats. Food intake was initially increased in both OLETF RW RC and OLETF RW HF rats when wheels were relocked. After the initial increase, OLETF RW RC rats had a trend for decreases in food intake compared with OLETF SED RC rats, whereas daily food intake remained high in OLETF RW HF rats. D: OLETF SED RC rats consumed more cumulative energy than did LETO RC rats (*\( P < 0.05 \) from 9 to 21 wks of age). Exercise reduced this increase in OLETF RW RC rats (#\( P < 0.05 \) compared with OLETF SED RC from 10 to 13 wk of age) and produced a lasting effect (#\( P < 0.05 \) compared with OLETF SED RC from 14 to 21 wk of age). High-fat-diet access offset this effect. OLETF RW HF rats consumed a similar total amount of energy as that of OLETF SED HF rats (\( P > 0.05 \)). Values are expressed as means ± SE; \( n = 6 \)/group. *\( P < 0.05 \) vs. LETO RC; #\( P < 0.05 \) vs. OLETF SED RC; §\( P < 0.05 \) vs. OLETF RW RC and ‡\( P < 0.05 \) vs. OLETF SED HF.
both effects contribute to decreased body weight in OLETF RW rats.

After 4 wk of running-wheel access, all of the running wheels were relocked, and half of the OLETF RW rats and OLETF SED rats were given access to a HF diet. During the 8-wk postexercise period, rates of body weight gain differed among the groups \([F(2,186) = 24.330, P < 0.0001]\). Both OLETF RW RC rats and OLETF RW HF rats gained body weight rapidly after the running wheels were relocked (Fig. 1B). However, the rate of weight gain in OLETF RW RC rats slowed so that they maintained a lower body weight compared with OLETF SED RC rats (Fig. 1B, \(P < 0.001\)). Similarly, food intake was initially increased in both OLETF RW RC rats and OLETF RW HF rats after running wheels were relocked (Fig. 1C), but the total cumulative food intake of OLETF RW RC rats remained significantly reduced relative to that of OLETF SED RC rats through 21 wk of age (\(P < 0.05\), Fig. 1D), suggesting that OLETF RW RC rats had incomplete compensation for the decreased food intake during running wheel access. These results demonstrate lasting effects of 4 wk of exercise (or postexercise effects) on body weight and food intake in OLETF RW RC rats.

In contrast to the patterns in OLETF RW RC rats, the body weight of OLETF RW HF rats continued to increase so that it was not different from that of OLETF SED RC rats by 15 wk of age (\(P > 0.05\)) and was not different from that of OLETF SED HF rats by 17 wk of age (\(P > .05\), Fig. 1B). OLETF RW HF rats also increased their food intake, retaining relatively high daily intake (Fig. 1C), resulting in no significant differences in their cumulative total caloric intake from that of OLETF SED RC rats by 16 wk of age (\(P > 0.05\)) and from that of the OLETF SED HF rats by 20 wk of age (\(P > 0.05\), Fig. 1D). Thus, access to a HF diet following 4 wk of exercise prevented the long-lasting effects of exercise on body weight and caloric intake. In addition, analysis of feed efficiency during the 8-wk postexercise period revealed significant main effects of prior exercise \([F(1,19) = 65.923, P < 0.001]\) and diet \([F(1,19) = 78.396, P < 0.001]\) but no significant interaction \([F(1,19) = 3.802, P = 0.066]\). Pairwise comparison further revealed that feed efficiency was significantly higher in OLETF RW RC rats (33.8 ± 1.1 g/cal) than that of OLETF SED RC rats (25.8 ± 1.3 g/cal, \(P < 0.001\)) during this period. Also, OLETF RW HF rats had significantly increased feed efficiency (47.8 ± 1.1 g/cal) compared with OLETF SED HF rats (34.8 ± 1.9 g/cal, \(P < 0.001\)) during this period.

**Effects of Running-Wheel Access on Glucose Tolerance**

Analyses of the data from the oral glucose tolerance test at week 21 of age showed a significant group effect on fasting basal glucose levels \([F(2,22) = 18.50, P < 0.0001]\). Post hoc analyses demonstrated that OLETF SED RC, OLETF SED HF, and OLETF RW HF had elevated basal values relative to the LETO RC group, whereas basal levels in the OLETF RW RC rats were not different from those in the LETO RC group. Fig. 2A shows the blood glucose values in response to the gastric glucose load across the various groups. Overall, all OLETF groups had sustained elevated values relative to those of the LETO rats. Within the OLETF groups, glucose values were lower in the OLETF RW RC rats compared with OLETF SED RC rats, indicating that prior exercise reduced hyperglycemia in OLETF RW RC rats. In contrast, OLETF SED HF rats and OLETF RW HF rats had significantly increased blood glucose levels compared with OLETF SED RC rats and OLETF RW RC rats, respectively. Analyses of the area under the curve (AUC) for glucose values (Fig. 2B) indicated significant group difference \([F(4,21) = 15.346, P < 0.0001]\). Again, all
OLETF groups had elevated values relative to those of LETO RC rats. The glucose AUCs of OLETF SED HF rats and OLETF RW HF rats were higher than those for the other groups \((P < 0.001)\) and did not differ from one another \((P = 0.156)\). The AUC glucose for the OLETF RW RC group was lower than that of the OLETF SED RC group, demonstrating a lasting effect of running-wheel access \((P < 0.001)\).

Plasma insulin secretion stimulated by the gastric glucose load was different among the groups \((F(94,21) = 131.46, P < 0.0001)\). AUC plasma insulin was significantly greater in the OLETF RW RC group compared with both the LETO RC and the OLETF RW RC groups \((P < 0.001)\). Thus, although AUC blood glucose was significantly elevated in the OLETF RW RC group, handling this glucose load required less insulin release than in the other OLETF groups. The effect of running-wheel access on insulin secretion was essentially overwhelmed by subsequent exposure to a HF diet. Although values were lower in the OLETF RW HF group compared with the OLETF SED HF group, this did not quite reach statistical significance \((P = 0.06)\).

**Effects of Running-Wheel Access on Fat Mass and Plasma Leptin Levels**

Analyses of the weights of different fat pads revealed significant effects on intrascapular BAT \(F(4,22) = 30.413, P < 0.0001\), epididymal WAT \(F(4,22) = 54.098, P < 0.0001\), and subcutaneous WAT \(F(4,22) = 131.46, P < 0.0001\) (Fig. 3A). At death, all OLETF rats had significant increases in BAT relative to LETO controls \((P < 0.001)\), and both OLETF SED HF rats and OLETF RW HF rats had significantly increased BAT relative to OLETF rats on RC \((P < 0.001)\). Prior exercise did reduce BAT in OLETF rats on a HF diet \((P < 0.05)\). OLETF rats in all groups had increased epididymal WAT and subcutaneous WAT relative to LETO control rats \((P < 0.001)\) and OLETF rats on a HF diet had increased fat pad weights compared with OLETF SED RC rats and OLETF RW RC rats \((P < 0.001)\). Prior exercise resulted in reduced epididymal and subcutaneous fat in the OLETF RW RC rats relative to the OLETF SED RC rats \((P < 0.05)\). However, there were no postexercise effects on the weight of white fat pads in the rats on a HF diet.

Consistent with the results on fat pad weight, analyses of plasma leptin levels showed significant effects of strain and treatment \(F(4,23) = 9.948, P < 0.0001\) (Fig. 3B). Prior exercise normalized plasma leptin levels in OLETF RW RC rats, such that they were not different from those of LETO controls \((P > 0.05)\). OLETF SED HF rats and OLETF RW HF rats had significantly increased plasma leptin levels compared with OLETF SED RC rats \((P < 0.01)\) and OLETF RW RC rats \((P < 0.05)\), respectively. Prior exercise also resulted in decreased leptin levels in rats on a HF diet \((P < 0.05)\). Thus, these results demonstrate opposing effects of HF diet and exercise on plasma leptin levels.

**Effects of Running-Wheel Access on Hypothalamic Npy, Agrp, Pomc, and Crf mRNA Levels**

*Npy* mRNA levels. *Npy* mRNA levels in the ARC differed by strain and treatment \(F(4,22) = 19.941, P < 0.0001\). *Npy* mRNA levels in the ARC were significantly decreased in OLETF SED RC rats, OLETF SED HF rats, and OLETF RW HF rats compared with those of LETO RC rats \((P < 0.05, \text{Fig. 4A})\). Prior exercise resulted in significant increases in *Npy* mRNA levels in the ARC of OLETF RW RC rats compared with OLETF SED RC rats \((P < 0.05)\), reaching levels above those of LETO RC rats \((P < 0.05, \text{Fig. 4A})\). This increase was prevented in the RW rats on a HF diet. *Npy* mRNA levels in the ARC did not differ between the sedentary OLETF rats on RC and HF, whereas *Npy* mRNA levels in the ARC were significantly decreased in OLETF RW HF rats compared with OLETF RW RC rats, becoming similar to those of OLETF SED HF rats \((P < 0.05, \text{Fig. 4A})\). ANOVA also identified a significant effect of strain and treatment on *Npy* gene expression in the DMH \(F(4,23) = 4.534, P = 0.008\). *Npy* mRNA levels were significantly increased in the DMH of OLETF RW HF rats compared with all other groups \((P < 0.05, \text{Fig. 4B})\).

*Agrp* mRNA levels. Consistent with the patterns of change in *Npy* mRNA levels in the ARC, *Agrp* mRNA levels in the ARC varied by strain and treatment \(F(4,23) = 7.546, P < 0.001\). *Agrp* expression levels were significantly decreased in OLETF SED RC rats, OLETF SED HF rats, and OLETF RW HF rats compared with LETO RC rats \((P < 0.01, \text{Fig. 4C})\). Prior exercise resulted in significant increases in *Agrp* mRNA levels in the ARC of OLETF RW RC rats compared with...
OLETF SED RC rats ($P < 0.05$, Fig. 4C). Access to a HF diet prevented this increase. *Agrp* mRNA levels in the ARC did not differ between the two groups of OLETF rats on a HF diet (Fig. 4C).

*Pomc* mRNA levels. *Pomc* mRNA expression in the ARC was significantly affected by strain and treatment [$F(4,19) = 10.251, P < .001$], but the patterns of change were opposite to those of ARC *Npy* and *Agrp*. Compared with LETO RC rats, *Pomc* mRNA levels were significantly increased in the ARC in all OLETF rats ($P < 0.05$, Fig. 4D). Although levels of *Pomc* mRNA expression in the ARC were higher in OLETF SED HF rats compared with OLETF SED RC rats, the difference did not reach statistical significance ($P > 0.05$, Fig. 4D). Prior exercise resulted in a trend for decreases in *Pomc* mRNA levels in the ARC of OLETF RW RC compared with OLETF SED RC rats ($P = 0.146$, Fig. 4D). Access to a HF diet increased ARC *Pomc* mRNA expression in RW rats ($P < 0.01$), reaching levels similar to those of OLETF SED HF rats (Fig. 4D).

*Crf* mRNA levels. *Crf* gene expression in the PVN also differed by strain and treatment [$F(4,21) = 5.264, P < .01$]. *Crf* mRNA levels were significantly increased in the PVN in both OLETF SED HF rats compared with OLETF SED RC rats and in OLETF RW HF rats compared with OLETF RW RC rats in response to a HF diet ($P < 0.05$, Fig. 4E). Prior exercise did not produce a significant effect on *Crf* gene expression in the PVN of OLETF rats (Fig. 4E).

**DISCUSSION**

The present study demonstrated that HF diet access following a period of voluntary running wheel exercise altered the lasting effects of exercise on the eventual phenotype in OLETF rats.
rats. OLETF rats on RC are hyperphagic and become obese, eventually developing insulin resistance and a variety of characteristics of the metabolic syndrome (14). Prior work has demonstrated that access to a running wheel and the resulting voluntary activity prevented these alterations (6, 26, 27) and that a period of exercise also produced a lasting effect on their food intake, body weight, and glucose homeostasis (6, 26). The present results from OLETF rats maintained on RC replicate these previous findings, that is, 4 wk of running-wheel access beginning at 9 wk of age significantly decreased food intake and body weight, and when running wheels were locked, food intake increased and some body weight was regained, but during the 8-wk observation period, body weight stabilized at a level significantly below that of OLETF rats that had no exercise experience. This early period of exercise improved glucose tolerance and enhanced insulin sensitivity in OLETF rats. In addition, the present study demonstrated the impact of access to a HF diet on these postexercise effects. When switched to a HF diet following exercise, OLETF rats continued to eat a large amount of food and gain body weight rapidly, so that their body weight exceeded those of unexercised OLETF rats on RC and eventually reached those of unexercised OLETF rats on a HF diet. Similarly, access to a HF diet resulted in poor glucose tolerance and insulin sensitivity in both sedentary and prior exercised OLETF rats. Together, our present results demonstrated that while prior exercise produced effects on food intake, body weight and glucose homeostasis in OLETF rats for at least 8 wk following running-wheel access, access to a HF diet offset these lasting effects.

ARC neuropeptides, such as the orexigenic peptides, NPY, and AgRP, and the anorexigenic precursor peptide POMC (precursor of α-melanocyte-stimulating hormone) play an important role in the control of food intake and body weight, and their peptide-signaling pathways have been demonstrated to be under the control of leptin (8, 9, 25, 29). Previous reports have shown appropriate regulation of gene expression for these ARC peptides in OLETF rats. In response to increased body weight and circulating leptin levels, OLETF rats have decreased Npy and increased Pome mRNA expression in the ARC compared with LETO controls (4). Access to a HF diet resulted in a trend for even greater decreases in Npy expression and increases in Pome mRNA expression in the ARC of OLETF rats (3). Consistent with these reports, the present study found that overall effects of exercise and a HF diet on Npy, Agpr, and Pomc mRNA expression in the ARC corresponded to their effects on body weight and/or leptin levels. Levels of Npy and Agpr mRNA expression were elevated in the ARC of prior exercised OLETF rats on RC compared with their unexercised counterparts. Access to a HF diet offset these elevations, while prior exercised rats had the same body weight as unexercised OLETF rats. Similarly, prior exercise did not produce a significant impact on Pome mRNA expression in the ARC in OLETF rats on a HF diet. The PVN is the target of ARC neuropeptides and PVN CRF is also partially under the control of leptin (9, 25). Results for CRF mRNA expression in the PVN were similar to those for Arc POMC and reflect a secondary response to the weight gain in HF-fed OLETF rats, with no modulating effect of prior exercise experience.

A role for DMH NPY in the control of food intake and body weight has been suggested (2). Npy mRNA expression was increased in the DMH in response to situations of increased energy demand, such as lactation (28), chronic food restriction (5), and exercise (13). We have documented that Npy gene expression is differentially regulated in the ARC and the DMH and that while ARC NPY is under the control of leptin as discussed above, the regulation of DMH NPY is leptin independent (5). In addition, elevation or induction of Npy mRNA expression in the DMH has been shown in several rodent models of obesity (11, 16, 32). We further demonstrated an etiological role for DMH NPY in OLETF rats. Premature OLETF rats or OLETF rats pair fed to amounts consumed by LETO controls have greatly elevated levels of Npy mRNA expression in the DMH (4, 23) where CCK-1 receptors are normally colocalized within NPY-containing neurons and mediate the inhibitory effects of CCK on DMH NPY signal and food intake (7). Knockdown of NPY expression in the DMH prevents hyperphagia and obesity of OLETF rats (33). Thus, we have suggested that the congenital lack of CCK-1 receptors in OLETF rats resulted in two deficits in controlling energy balance. A deficit in satiety actions of peripheral CCK leads to impaired short-term control of food intake (20), and a deficit in central control of DMH NPY signaling results in alterations in overall control of energy balance, eventually leading to hyperphagia and obesity of OLETF rats.

Data from the present study provide additional support for such a role for DMH NPY in OLETF rats. Our previous report showed that exercise prevented hyperphagia and obesity of OLETF rats, and an early period of exercise produced a long-lasting effect on their eventual phenotypes (6). Exercise also limited the DMH NPY overexpression seen in pair-fed OLETF rats, i.e., while both exercise and pair-feeding normalized body weight of OLETF rats, DMH NPY overexpression was not observed in exercised OLETF rats (6). This suggests that exercise influences DMH NPY signaling to affect food intake and energy balance, and this action is likely independent of DMH CCK signaling. On the other hand, consistent with the evidence that dietary fats serve as a primary secretagogue for CCK release (19), presumably producing a strong feeding inhibition as satiety feedback signaling, OLETF rats lacking CCK-1 receptors became more vulnerable to diet-induced obesity, exhibiting sustained overconsumption and exacerbation of obesity (3). While Npy gene expression was appropriately decreased in the DMH of lean LETO controls, defending against increased energy intake, its expression was dysregulated in OLETF rats, showing no significant difference between the two groups of OLETF rats on chow and HF diets (3). This suggests that the dysregulation of DMH NPY may contribute to the effects of a HF diet on obese phenotypes of OLETF rats. The present study further found that access to a HF diet offset the lasting effects of exercise on food intake and body weight, as well as resulted in increased DMH NPY expression in OLETF rats that formerly exercised. Thus, these results imply that a HF diet may not only influence CCK’s actions, but also may particularly limit the actions of factors that normally mediate the effects of exercise on DMH NPY signaling to affect food intake and body weight. How such factors act to modulate DMH NPY signaling or the features of factors contributing to the long-lasting effects of exercise on food intake and body weight in OLETF rats remains to be determined.

We also examined the effects of access to a HF diet on the lasting effects of exercise on glucose homeostasis in OLETF
rats. Adult OLETF rats are hyperglycemic and hyperinsulinemic and eventually develop NIDDM (15). Exercise prevents these alterations, and such preventive effects against the development of NIDDM last for at least 3 mo after the cessation of exercise in this model (26). The present study found that while an early period of exercise produced a lasting effect on glucose homeostasis, improving glucose tolerance and enhancing insulin sensitivity, in OLETF rats fed RC, HF access overwhelmed these postexercise effects. Exercised OLETF rats on a HF diet became glucose intolerant and insulin insensitive to the same degree as sedentary OLETF rats on a HF diet. Because body weight and fat mass were significantly decreased in OLETF RW rats on RC compared with sedentary OLETF rats on RC and greatly increased in exercised OLETF rats on a HF diet as high as those of sedentary OLETF rats on a HF diet, the changes in glucose homeostasis in OLETF rats likely resulted from their alterations in body weight and fat mass.

Results of the present study are in contrast with the effects of early exercise in obesity-prone DIO Sprague-Dawley rats that were established on the basis of responses to a high-fat/energy diet (18). Patterson et al. (21) have reported that the obesity of DIO rats was completely prevented when the rats were given postweaning access to a running wheel, even when they were maintained on a high-fat/energy diet. Thus, 6 wk of running-wheel access prevented obesity for a subsequent 7 wk in DIO rats on a high-fat/energy diet (21). Even 3 wk of postweaning access to a running wheel was sufficient to significantly attenuate obesity development for subsequent 10 wk in DIO rats on a high-fat/energy diet (21). There are multiple differences between the studies with obesity-prone DIO rats and the present experimental design that may account for the divergent long-term effects of exercise in the two models. The high-fat/energy diet used with the obesity-prone DIO rats was 31% rather than 60% fat. It may be the case that the higher level of fat content simply overwhelmed any effects of the exercise. It is also the case that the obesity-prone DIO rats had the high-energy diet both during and following the period of running-wheel access. It could be the case that the contrast between the regular chow and HF diets in OLETF rats stimulated greater intake during the postexercise period. The timing of the running-wheel access also differs. Obesity-prone DIO rats had access to a running wheel at an earlier time point (postweaning) and that could contribute to the different findings. Finally, the obesity-prone trait is undoubtedly polygenic (18), while the genetic deficit in the OLETF rat derives from a specific deletion (30). Nevertheless, the mechanisms underlying the differences in the postexercise effects on the obese phenotypes between the two models merit further investigation.

In summary, the present results indicate that while running-wheel access reduces food intake, normalizes body weight, and improves glucose homeostasis in the OLETF rat, the lasting effects of that access on food intake, body weight, and glucose homeostasis depend upon the diet in the post-running-wheel access period. Access to a HF diet following 4 wk of running wheel access overwhelms the documented 8-wk effects of running wheel access. The dysregulation of DMH NPY in OLETF rats may contribute to these alterations.

**Perspectives and Significance**

Diet and physical activity are two major factors impacting energy homeostasis that is under the control of hypothalamic peptide systems. Disordered energy balance due to either increased energy intake or decreased physical activity can eventually cause obesity and diabetes. Preventive effects of exercise on obesity have been shown in various rodent obesity models, and in some cases, the effects of exercise have been long-lasting, altering the eventual phenotype (6, 12, 17, 21, 26). Although leptin plays an important role in maintaining energy homeostasis through affecting hypothalamic peptide signaling (25), a role for leptin in mediating effects of exercise on food intake and body weight is undetermined. Our previous results have implied a potential role for DMH NPY in this exercise-induced effect. Exercise-limited DMH NPY overexpression in OLETF rats, likely leading to normalization of body weight of OLETF rats (6). The present study further found that access to a HF diet offset the long-lasting effects of exercise on the obese phenotype of OLETF rats, as well as resulted in increased DMH NPY expression, suggesting that DMH NPY is an important contributing factor to both the effects of exercise and diet on energy balance. Thus, a complete determination of how DMH NPY mediates the effects of the diet and exercise on energy balance would not only advance our understanding of overall energy balance control but also provide a potential target for combating obesity and/or diabetes.


