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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Running head: High fat diet offsets the long-lasting effects of exercise

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Abstract

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 weeks of running wheel access beginning at 8 weeks 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 four weeks. We found that four weeks of running exercise significantly decreased food intake and body weight of OLETF rats. Consistent with prior results, four-week 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, high-fat diet access 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.
Introduction

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 II 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 post-weaning 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 diet (RC) (6).

The OLETF rat is established as an animal model of obesity and non-insulin-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 week of age) (15). OLETF rats congenitally lack the cholecystokinin (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 diet (HF)-induced obesity (3, 24). When maintained on HF, OLETF rats exhibited sustained over consumption 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 HF (3, 24).

In the present study, we aimed to determine whether diet modulates the lasting effects of physical exercise. By examining the effects of HF on food intake and body weight in OLETF rats that had prior access to running wheels for four weeks, we sought to explore the behavioral and neural mechanisms underlying the effects of exercise on weight maintenance or energy balance in relation to diet. Based on 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 HF 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 four weeks, that 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 the central nervous system differentially regulates visceral and subcutaneous WAT depots (1). In addition to determination of Npy gene expression in the DMH, we examined Npy, agouti-related peptide (Agrp) and Pomc mRNA in the ARC, and corticotrophin-releasing factor (Crf) in the paraventricular nucleus (PVN) as it is known that these hypothalamic peptide signals play major roles in the control of food intake and body weight, and that leptin, a hormone produced in the adipose tissue, regulates these ARC and PVN peptide signaling to influence food intake and energy balance (8, 9, 25, 29). The present results demonstrate that HF access overcomes the
Methods

Animals. Male OLETF and age-matched male lean LETO rats were provided by the Tokeshima Research Institute, Otsuka Pharmaceutical (Tokushima, Japan) as a generous gift. On arrival, rats were 4 wk old and were quarantined (for the clearance of any foreign pathogens) for 4 weeks in the University facilities. Rats were individually housed with a 12:12-h light-dark cycle in a temperature-controlled colony room (22 °C) with ad libitum access to standard regular chow and tap water. All procedures were approved by the Institutional Animal Care and Use Committee of Johns Hopkins University.

Running wheel access, food intake and body weight. At 8 wk of age, animals were transferred to individual running wheel stations which contained a running wheel and a nest box (15 x 25 x 15 cm) with wire-mesh floor as previous described (6). Access to food and water was in the running wheel enclosure. Food was delivered through automated pellet dispensers controlled by an infrared pellet-sensing photo beams (Med Associates Inc., Georgia, VT). A 45-mg chow pellet (Bio-Serv, Frenchtown, NJ) was delivered by the pellet dispenser in response to the removal of the previous pellet. The delivery of each pellet and the revolutions of the running wheels were both monitored, time stamped, and recorded 24 h/d in a computer (Med Associates). Initially, all running wheels were kept in a locked position for 7 days. After 7 days of habituation, OLETF rats were divided into two groups: sedentary OLETF rats (n=12, OLETF SED RC) and OLETF rats with access to running wheels (n=12, OLETF RW RC) such that the groups were weight matched. Six LETO rats were maintained on RC (LETO RC) in hanging wire cages comparable to wire-mesh nest box and served as the normal controls in this study. At 13 wk of age (after 4 wk of running wheel access), all the OLETF RW RC rats had their running wheels relocked. Half of them were randomly assigned to a high fat diet (n=6, OLETF
RW HF, 60% fat, 20% carbohydrate, and 20% protein in kcal%; 5.2 kcal/g; D12492; Research Diets), and the other half remained on regular chow diet (n=6 OLETF RW RC, 15.8% fat, 65.6% carbohydrate, 18.6% protein; ProLab RMH 1000). In addition, at 13 weeks of age half of the OLETF SED RC rats were assigned to a high fat diet (n=6, OLETF SED HF), and the other half remained on a regular chow diet (n=6). Both HF and RC diets were supplied as a pelleted chow in food hoppers. Hoppers were checked every morning. During the entire study, all rats had ad libitum access to food and water. Body weight was recorded every day and food intake was measured every week. At 21 wk of age, an intragastric glucose tolerance test was conducted, and one week later, all rats were sacrificed in the non-fasted state by decapitation under ether inhalation anesthesia between 0900 and 1100h. Sacrifice was conducted in two consecutive days (half of the rats from each group per day). Interscapular BAT, epididymal WAT, and subcutaneous inguinal WAT were collected and weighted. Trunk blood was taken for evaluation of leptin. Plasma leptin concentration was determined by using a rat leptin RIA kit (Linco Research Inc., St. Charles, MO). Brains were removed and rapidly frozen in isopentane on dry ice for subsequent analyses of hypothalamic gene expression.

**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) 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).

**Cryosections.** Coronal sections (14 μm) through the hypothalamus were cut via a cryostat, mounted on superfrost/plus slides (Fisher Scientific) as series of six slides (section 1: slide 1, section 2: slide 2, etc; section 7: slide 1, etc). Sections were fixed with 4% paraformaldehyde
and stored at -80 °C for in situ hybridization determinations. One slide from each series was stained with cresyl violet acetate and used for the selection of sections for the following in situ hybridization determinations. Sections for mRNA determinations of DHM Npy, ARC Npy, ARC Pomc, and ARC Agrp (3.1-3.3 mm caudal to bregma) and PVN Crf (1.8-2.0 mm caudal to bregma) were selected (6).

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

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-HCl in pH 7.5, 1 mM EDTA in pH 9.0, 1xDenhardt's solution (Eppendorf), 10% dextran sulfate, 500 μg/ml Yeast tRNA, 10 mM DTT, and 10^7 cpm/ml of [35S] UTP at 55°C. After hybridization, sections were washed three times with 2 x SSC/1mM DTT at 55°C, treated with 20 μg/ml RNase A (sigma) at 37°C for 30 minutes, washed twice in 2 x SSC/1mM DTT at 55°C for 5 minutes each, and wash twice in 0.1 x SSC/1mM DTT for 15 minutes 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 NIH Scion Image Software (National Institutes, of Health, 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) were used as a standard. The data obtained were calculated as the product of hybridization area x 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 ± SEM.

**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-factors 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 post-exercise 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. ANOVA’s were followed by pairwise multiple Fisher least significant difference (LSD) 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 Figure 1, OLETF rats were given access to running wheels at 9 weeks of age. They gradually increased running activity during the first two weeks and eventually ran from 8,000 to 10,000 revolutions per day (8.0-10.0 km/d) (Fig. 1A). Running wheel access resulted in significant effects on body weight \( [F(6,78) = 12.784, p < .0001] \) and food intake \( [F(6,81) = 6.5707, p < .0001] \). 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, four weeks 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 both effects contribute to decreased body weight in OLETF RW rats.

After four weeks of running wheel access, all the running wheels were relocked, and half of the OLETF RW rats and OLETF SED rats were given access to HF. During the 8-week post exercise period, rates of body weight gain differed among the groups \( F(28,168) = 24.330, p < .0001 \). 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 in comparison with OLETF SED RC (Fig 1B, \( p < .001 \)). 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 weeks of age (\( p < .05 \), 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 four weeks of exercise (or post-exercise 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 weeks of age (\( p > .05 \)) and was not different from that of OLETF SED HF rats by 17 weeks 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 weeks of age (\( p > .05 \)) and from that of the OLETF SED HF rats by 20 weeks of age (\( p > .05 \), Fig 1D). Thus, access to HF following 4 weeks of exercise prevented the long lasting effects of exercise on body weight and caloric intake. In addition, analysis of feed efficiency during the 8 week post exercise period revealed significant main effects of prior exercise \([F(1,19) = 65.923, p < .001]\) and diet \([F(1,19) = 78.396, p < .001]\), but no significant interaction \([F(1,19) = 3.8022, p =0.066]\). 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 < .001) during this period. As well, OLETF RW HF rats had significantly increased feed efficiency (47.8 ± 1.1 g/cal) compared to OLETF SED HF rats (34.8 ± 1.9 g/cal, p < .001) during this period.

Effects of running wheel access on glucose tolerance. Analyses of the data from the oral glucose tolerance test (OGTT) at week 21 of age showed a significant group effect on fasting basal glucose levels [F(2,22) = 18.50, p < .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. Figure 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 < .00001]. Again, all OLETF groups had elevated values relative to those of LETO RC rats. The glucose AUC’s of OLETF SED HF rats and OLETF RW HF rats were higher than those for the other groups (p < .001) and did not differ from one another (p = .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 < .001).

Plasma insulin secretion stimulated by the gastric glucose load was different among the groups [F(94,21) = 15.436, p < .0001]. AUC plasma insulin was significantly greater in the OLETF SED RC and the OLETF HF groups compared to both the LETO RC and the OLETF
RW RC groups (p < .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 HF. Although values were lower in the OLETF RW HF group compared to the OLETF SED HF group, this did not quite reach statistical significance (p = .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<.0001], epididymal WAT [F(4,22) = 54.098, p<.0001] and subcutaneous WAT [F(4,22) = 131.46, p<.0001]. At sacrifice, all OLETF rats had significant increases in BAT relative to LETO controls (p <.001) and both OLETF SED HF rats and OLETF RW HF rats had significantly increased BAT relative to OLETF rats on RC (p<.001). Prior exercise did reduce BAT in OLETF rats on HF (p<.05). OLETF rats in all groups had increased epididymal WAT and subcutaneous WAT relative to LETO control rats (p<.001) and OLETF rats on HF had increased fat pad weights as compared to OLETF SED RC rats and OLETF RW RC rats (p<.001). Prior exercise resulted in reduced epididymal and subcutaneous fat in the OLETF RW RC rats relative to the OLETF SED RC rats (p<.05). However, there were no post-exercise effects on the weight of white fat pads in the rats on HF.

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<.0001). Prior exercise normalized plasma leptin levels in OLETF RW RC rats such that they were not different from those of LETO controls (p>.05). OLETF SED HF rats and OLETF RW HF rats had significantly increased plasma leptin levels compared to OLETF SED RC rats (p<.01) and OLETF RW RC rats (p<.05) respectively. Prior exercise also resulted in decreased leptin levels in rats on HF (p<.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<.001]. *Npy* mRNA levels in the ARC were significantly decreased in OLETF SED RC rats, OLETF SED HF rats, and OLETF RW HF rats compared to those of LETO RC rats (p<.05, Fig. 4A). Prior exercise resulted in significant increases in *Npy* mRNA levels in the ARC of OLETF RW RC rats compared to OLETF SED RC rats (p<.05), reaching levels above those of LETO RC rats (p<.05, Fig. 4A). This increase was prevented in the RW rats on HF. *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 to OLETF RW RC rats, becoming similar to those of OLETF SED HF rats (p<.05, 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 to all other groups (p<.05, 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<.001]. *Agrp* expression levels were significantly decreased in OLETF SED RC rats, OLETF SED HF rats, and OLETF RW HF rats as compared to LETO RC rats (p<.01, Fig. 4C). Prior exercise resulted in significant increases in *Agrp* mRNA levels in the ARC of OLETF RW RC rats compared to OLETF SED RC rats (p<.05, Fig. 4C). HF access prevented this increase. *Agrp* mRNA levels in the ARC did not differ between the two groups of OLETF rats on HF (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*. As compared to LETO RC rats, *Pomc* mRNA levels were significantly
increased in the ARC in all OLETF rats (p<.05, Fig. 4D). Although levels of Pomc mRNA expression in the ARC were higher in OLETF SED HF rats compared to OLETF SED RC rats, the difference did not reach statistically significance (p>.05, Fig. 4D). Prior exercise resulted in a trend for decreases in Pomc mRNA levels in the ARC of OLETF RW RC compared to OLETF SED RC rats (p=0.146, Fig. 4D). HF access increased ARC Pomc mRNA expression in RW rats (p<.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 to OLETF SED RC rats and in OLETF RW HF rats compared to OLETF RW RC rats in response to HF (p<.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 access following a period of voluntary running wheel exercise altered the lasting effects of exercise on the eventual phenotype in OLETF 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, four weeks 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 HF access on these post-exercise effects. When switched to HF 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 HF. Similarly, HF access 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 weeks following running wheel access, HF access offset these lasting effects.

ARC neuropeptides such as the orexigenic peptides, NPY and AgRP, and the anorexic precursor peptide POMC (precursor of α-melanocyte-stimulating hormone, α-MSH) 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 \( Pomc \) gene expression in the ARC compared to LETO controls (4). HF access resulted in a trend for even greater decreases in \( Npy \) expression and increases in \( Pomc \) expression in the ARC of OLETF rats (3). Consistent with these reports, the present study found that overall effects of exercise and HF 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 to their unexercised counterparts. HF access 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 \( Pomc \) mRNA expression in the ARC in OLETF rats on HF. 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. Pre-obese 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 feed-back 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, it expression was dysregulated in OLETF rats, showing no significant difference between the two groups of OLETF rats on chow and HF (3). This suggests that the dysregulation of DMH NPY may contribute to the effects of HF on obese phenotypes of OLETF rats. The present study further found that HF access 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 HF may not only influence CCK’s actions, but also may particularly limit the actions of factor(s) that normally mediate the effects of exercise on DMH NPY signaling to affect food intake and body weight. How such factor(s) act to modulate DMH NPY signaling or the features of factor(s) 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 HF access 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 months 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 on RC, HF access overwhelmed these post-exercise effects. Exercised OLETF rats on HF became glucose intolerant and insulin insensitive to the same degree as sedentary OLETF rats on HF. Since body weight and fat mass were significantly decreased in OLETF RW rats on RC compared to sedentary OLETF rats on RC
and greatly increased in exercised OLETF rats on HF as high as those of sedentary OLETF rats on HF, 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 based on responses to a high fat/energy diet (18). Patterson and colleagues (21) have reported that the obesity of DIO rats was completely prevented when the rats were given post weaning access to a running wheel even when they maintained on a high fat/energy diet. Thus, six weeks of running wheel access prevented obesity for subsequent 7 weeks in DIO rats on a high fat/energy diet (21). Even 3 weeks of post weaning access to a running wheel was sufficient to significantly attenuate obesity development for subsequent 10 weeks 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 chow and HF diet in OLETF rats stimulated greater intake during the post exercise 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 (post weaning) 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 post-exercise 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 HF following 4 weeks of running wheel
access overwhelms the documented 8 week 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 HF access 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 understating of overall
energy balance control, but also provide a potential target for combating obesity and/or
diabetes.
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References


20. **Moran TH, Katz LF, Plata-Salaman CR, and Schwartz GJ.** Disordered food intake and


Figure 1. Effects of 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 four weeks.  (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 wks of age).  Four wks of running wheel access reduced body weight and produced a long-lasting body-weight effect in OLETF RW RC rats (#P <0.05 compared to OLETF SED RC from 10 to 21 wks 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 wks of age (P >0.05).  (C) During running wheel access, daily food intake was significantly decreased in OLETF RW rats compared to 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 to 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 to OLETF SED RC from 10 to 13 wks of age) and produced a lasting effect (#P <0.05 compared to OLETF SED RC from 14 to 21 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 means + SEM.  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.

Figure 2. Effects of high fat diet on glucose and insulin levels in prior exercised OLETF rats.  (A) Blood glucose response to oral glucose administration; (B) Area under the curve (AUC) of blood glucose; and (C) Plasma insulin AUC.  OLETF SED RC rats became glucose intolerant and
insulin insensitive, and high fat diet access exacerbated this impairment in OLETF SED HF rats. 

Prior exercise ameliorated this impairment in OLETF RW RC rats, but high fat diet access offset these effects in OLETF RW HF rats. Values are means ± SEM. \( n = 6 \) /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.

Figure 3. Effects of high fat diet on fat mass and plasma leptin levels in prior exercised rats.

OLETF SED RC rats had increased body fat accumulation (A) and became hyperleptinemic (B). Prior exercise lowered these increases, but high fat diet access offset these effects. Values are means ± SEM. \( n = 6 \) /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.

Figure 4. Effects of high fat diet on hypothalamic \( Npy, Agrp, Pomc \) and \( Crf \) gene expression in prior exercised OLETF rats. (A) \( Npy \) gene expression in the arcuate nucleus (ARC); (B) \( Npy \) gene expression in the dorsomedial hypothalamus (DMH); (C) \( Agrp \) gene expression in the ARC; (D) \( Pomc \) gene expression in the ARC and (E) \( Crf \) gene expression in the paraventricular nucleus (PVN). Values are means ± SEM. \( n = 6 \) /group. *P <0.05 vs. LETO RC, \(^{#}P <0.05 \) vs. OLETF SED RC, \(^{§}P <0.05 \) vs. OLETF SED HF.
A

Blood glucose levels (mg/dl)

LETO RC
OLETF SED RC
OLETF SED HF
OLETF RW RC
OLETF RW HF

***
*
*
*
*
*
*
*

Time (minute)

B

Glucose AUC

LETO RC
OLETF SED RC
OLETF SED HF
OLETF RW RC
OLETF RW HF

*
*
*
*

C

Plasma insulin AUC

LETO RC
OLETF SED RC
OLETF SED HF
OLETF RW RC
OLETF RW HF

*
Interscapular brown fat

Fat weight (g)

LETO RC
OLETF SED RC
OLETF SED HF
OLETF RW RC
OLETF RW HF

Plasma leptin levels (ng/ml)

LETO RC
OLETF SED RC
OLETF SED HF
OLETF RW RC
OLETF RW HF

A

B