Effects of prepubertal-onset exercise on body weight changes up to middle age in rats

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Shindo D, Matsuura T, Suzuki M. Effects of prepubertal-onset exercise on body weight changes up to middle age in rats. J Appl Physiol 116: 674–682, 2014. First published January 23, 2014; doi:10.1152/japplphysiol.00405.2013.—The present study was conducted to examine whether prepubertal-onset exercise might help adults maintain long-term body weight (BW) reduction and increased energy metabolism after the cessation of exercise. Furthermore, the effects of the exercise regimen were compared with those of food restriction. Twenty-three male obese-diabetic [Otsuka Long-Evans Tokushima Fatty (OLETF)] rats were randomly assigned to prepubertal-onset exercise (Childhood-Ex), food restriction (Childhood-Diet), and sedentary control (OLETF-Sed) groups. Childhood-Ex rats exercised voluntarily every day using a rotating wheel, while the food volume of the Childhood-Diet group was restricted to achieve a BW similar to that recorded in the Childhood-Ex group. Both treatments were conducted at 5–19 wk of age; after this period, the rats were kept sedentary and allowed ad libitum food intake until 45 wk of age. BW was significantly lower, and percent lean body mass was significantly higher, in the Childhood-Ex group compared with those in the Childhood-Diet and OLETF-Sed groups throughout maturation and middle age after cessation of the interventions. The Childhood-Ex group also demonstrated higher citrate synthase, succinate dehydrogenase, and phosphofructokinase activity levels, as well as uncoupling protein-3 mRNA expression in skeletal muscle. This study revealed that inhibited BW gain in an animal model of human obese diabetes by prepubertal-onset exercise lasted for a long period after the completion of the exercise intervention. This effect may be facilitated by increased energy metabolism. However, these benefits were not found by prepubertal food restriction treatment. Importantly, to allow translation of our work, these novel insights need to be assessed in obese human individuals.

body weight; body fat mass; lean body mass; energy metabolism-related enzymes; UCP-3 mRNA

BETWEEN 1978 AND 2007, THE prevalence of obesity increased by nearly threefold in young Japanese children (5–6 yr) and schoolchildren (7–17 yr) (44). Other developed countries have experienced similar epidemics (24, 37), although they have shown some signs of slowing (24, 44). The influence of heredity on obesity has been demonstrated by family studies (4, 39), especially Borjesson’s work on twins (4) and Stunkard and colleagues’ research on adopted children (39). However, reduced energy expenditure, particularly a lack of physical activity, is also an important factor in the rapid weight gain observed during the first year of life in infants born to overweight mothers (31). Research on an animal model genetically predisposed to become obese found that prepubertal-onset exercise treatment resulted in lowered body weight (BW) after cessation of exercise, whereas a food-restriction treatment resulted in rapid BW gain after the restriction period (27). Interestingly, sustained suppression of BW gain after exercise cessation was not observed in adult rats placed on a similar exercise regimen (22, 43).

Exercise-induced BW reductions are known to be regained quickly after cessation of exercise regimens in both obese humans and rodents (21, 42); such BW rebounds are at least partly driven by reductions in resting metabolic rate (RMR) and/or lean body mass (LBM) (21, 30). The latter is known as the major determinant of RMR. One reason for this recidivism might be a genetic predisposition to a particular BW in which lost BW is regained to the predetermined BW. Indeed, individuals of many species are known to regulate BW from a predetermined set point (11). However, studies have demonstrated that prepubertal-onset exercise may induce a sustained decrease in the higher-predisposed BW set points in obese individuals, even after the cessation of exercise regimens (15, 17, 18, 27).

To our knowledge, the mechanism of sustained suppression of BW gain by prepubertal-onset exercise remains unsolved, although this may be associated with increased “energy expenditure” in the skeletal muscles rather than decreased “energy intake” (15, 17, 18, 27). Indeed, animal studies have demonstrated that prepubertal-onset exercise did not affect anabolic or catabolic hypothalamic neuropeptide expression, both of which are associated with energy intake behavior (20). Thus we hypothesized that, regardless of an individual’s genetic makeup, daily physical activity early in life maintains higher levels of LBM and metabolic activity in the skeletal muscles, as well as reduces the future likelihood of becoming obese. As studies using an animal model of human obesity (13, 27) have investigated the effects of prepubertal exercise on BW during a relatively short term (~7 wk) in the developmental period, it is also of interest to discern whether the effect would be sustained in obese animals in later life.

In the present study, we examined whether maintenance of prepubertal exercise-induced BW loss in genetically predisposed obese rats can be sustained until the period corresponding to middle age in humans. Furthermore, we investigated whether inhibition of BW gain is facilitated by increased energy metabolism. Our results further confirmed the benefits of prepubertal exercise for inhibiting BW gain in obese individuals in later life.

METHODS

Animals. The Tokushima Research Institute (Otsuka Pharmaceutical, Tokushima, Japan) provided all 31 rats: 23 male Otsuka Long-Evans Tokushima Fatty (OLETF) rats, which are characterized by insulin resistance, accumulated intra-abdominal fat, dyslipidemia, and type 2 diabetes (22, 23, 40), and 8 male Long-Evans Tokushima Otsuka (LETO) rats, which were used as control rats. As it has been reported that running wheel activity efficiently prevented obesity

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in OLETF rats, but not in Zucker fatty rats (32), we used this animal model of human obesity in this study.

All rats were 4 wk old at the time of purchase. The rats were placed in conventional plastic cages (36 × 26 × 20 cm) for 1 wk to become acclimatized to the experimental environment. They were fed standard solid rodent chow (CE-II, CLEA, Tokyo, Japan) and tap water ad libitum and were housed in dedicated facilities under specific pathogen-free conditions. Temperature (20.5 ± 1.2°C), humidity (43.0 ± 7.9%), lighting (0600–1800), and air conditioning were controlled throughout the experimental period. This study was conducted in accordance with the guiding principles of The Physiological Society of Japan. The experimental protocol was approved by the Committee for the Care and Use of Animals of the Jikei University School of Medicine.

Experimental design. It has been reported that a higher incidence of obesity-related diabetes in humans appears from the maturation stage to the middle-age period (35–50 yr of age). OLETF rats develop diabetes around 20 wk of age, and their levels of serum glucose, triglyceride (TG), and urine protein are known to dramatically increase around 30 wk of age (16, 35). Therefore, in the present study, childhood (including adolescence) was defined as the period of 5–19 wk; adulthood (maturation stage) as the period of 20–34 wk; and middle age as the period after 34 wk. The OLETF rats were randomly assigned to one of three groups: a childhood exercise group (Childhood-Ex, n = 7), in which the rats exercised at 5–19 wk of age; a childhood food-restriction group (Childhood-Diet, n = 8), in which food intake (FI) was limited while the rats were 5–19 wk of age; and a sedentary control group (OLETF-Sed, n = 8), in which the rats were kept sedentary and allowed food and tap water ad libitum throughout the experiment. The Childhood-Ex group exercised voluntarily using a rotating wheel made of wire mesh (20 × 20 × 25 cm, Shinano, Tokyo, Japan) attached to the cage. To mimic a BW similar to that recorded in the Childhood-Ex group, we restricted the FI of the Childhood-Diet group to roughly 70–85% of the food volume consumed by the OLETF-Sed group (40).

Rats in the Childhood-Ex and Childhood-Diet groups were allowed to have resting periods and take food freely when they were 20–45 wk of age. Eight male LETO rats were assigned to the sedentary normal control group (LETO-Sed).

BW, FI, and running distance measurements. BW (g), FI (g), volume of water consumed (ml), and running distance (m) were measured weekly throughout the experiment. Running distance was recorded by a cyclometer attached to the wheel’s axis, which recorded the number of wheel revolutions per week.

Measurements of changes in fasting blood glucose, insulin, and leptin levels. When the rats were 7, 12, 20, 27, 35, and 45 wk of age, we placed them under diethyl ether anesthesia and collected blood (10 ml) from the retroorbital sinus; before sample collection, all rats were subjected to an overnight fast at 1800–0900 (24, 41). Serum specimens were stored at −20°C until fasting serum glucose (FSG; Glucose C II-test, Wako, Wako Pure Chemical Industries, Osaka, Japan), insulin (FSI; ELISA kit, Shibayagi, Gunma, Japan), and leptin (FSI; Rat Leptin ELISA kit, Yanaihara Lab, Shizuoka, Japan) levels could be examined. The homeostasis model assessment of insulin resistance (HOMA-IR), which was developed to assess hepatic insulin resistance in humans, was used to calculate insulin resistance according to the following formula: HOMA-IR = FSI (µU/ml) × FSG (mg/dl)/405. The resulting value was then used to assess hepatic insulin resistance in rats according to the methods of Anwer et al. (1).

Measurements of changes in whole body subcutaneous fat mass, visceral fat mass, and LBM. Whole body subcutaneous fat mass (SFM), visceral fat mass (VFM), and LBM were measured when the rats were 4, 7, 12, 20, 27, 35, and 45 wk of age using a radiographic computed tomography scan apparatus (25). The rats were anesthetized via isoflurane inhalation 5–6 h after the absorptive period. The anesthetized rats were placed in the computed tomography scanner, and the entire body of each was scanned along the body axis at 2-mm intervals. Latheta software version 1.10 with a Latheta LCT-200 (Aloka, Tokyo, Japan), was used to analyze contiguous 2-mm slice images to quantify SFM, VFM, and LBM volumes (25). Values are reported as %fat {[SFM + VFM]/BW} × 100, and %LBM {[LBM/BW] × 100}.

Analysis of biochemical components in the blood. When the rats were 46 wk of age (±1 wk after completing the final blood sampling and body composition analysis), they were placed on an overnight fast and then anesthetized with pentobarbital (50 mg/kg ip) via a catheter placed in the external jugular vein. We rapidly drew blood samples (~10 ml), which were used to measure levels of serum free fatty acid, TG, total cholesterol (Tcho), and HDL-cholesterol (HDL-C) using a Hitachi 7600–210 automatic analyzer (Hitachi, Tokyo, Japan). HDL-cholesterol (HDL-C) levels were calculated using the Friedewald equation (LDL-C, mg/dl = Tcho – HDL-C – TG/5).

Measurements of energy metabolism-related enzyme activity and uncoupling protein-3 mRNA expression in the skeletal muscle. All rats were euthanized by bloodletting, after which one quadriceps femoris muscle per animal was removed and weighed. A portion of the muscle was stored at −80°C for later measurement of citrate synthase (CS), succinate dehydrogenase (SDH), and phosphofructokinase (PFK). Uncoupling protein-3 (UCP-3) mRNA expression was quantified by real-time PCR using the ABI StepOnePlus system and software (8, 9, 14). CS, SDH, and PFK are the key regulatory enzymes in the energy-generating metabolic pathway, while UCP-3 may serve as a mitochondrial mediator of fatty acid metabolism in the muscle (5, 9).

Statistical analysis. All data are expressed as means ± SE. Differences among the treatment groups were examined using one-way ANOVA, followed by post hoc tests (Fisher’s protected least significant difference). Student’s paired r-tests were used to investigate within-group changes in variables over the course of the study. Pearson’s tests were used to examine correlations between parameters. A 95% confidence level was accepted as significant for all of the statistical tests.

RESULTS

Changes in BW and food consumption. The mean BW of the OLETF rats at 5 wk of age (135.3 ± 6.5 g, n = 23) was significantly higher than that of the LETO rats (118.3 ± 1.8 g, P < 0.05, n = 8); however, there were no differences in BW among the three OLETF groups. As the rats aged (from 7 to 45 wk), gradual increases in BW were observed in the OLETF-Sed and LETO-Sed groups; however, rats in the OLETF-Sed group had significantly higher BW compared with that of those in the LETO-Sed group (P < 0.001, Fig. 1A).

The Childhood-Ex group and Childhood-Diet group experienced comparable changes in BW between 5 and 19 wk of age. From 15 wk of age, the mean BW in both the Childhood-Ex and Childhood-Diet groups was significantly lower compared with that in the OLETF-Sed group. At the end of the study period, the mean BW in the Childhood-Ex, Childhood-Diet, and OLETF-Sed groups were 460.8 ± 15.3, 460.9 ± 16.0, and 579.6 ± 13.3 g, respectively. The mean BW was significantly lower in the Childhood-Ex group than in the OLETF-Sed group until the rats were 40 wk of age, after which this difference in BW was only a trend. BW in the Childhood-Diet group increased rapidly after completion of the food restriction treatment and was significantly different from that in the Childhood-Ex group when the rats were 24 wk of age. Surprisingly, however, it was not significantly different from that in the OLETF-Sed group at 28 wk of age.

Absolute FI nearly plateaued in each group by the time the rats were 8 wk of age (Fig. 1B). However, FI in the Childhood-Diet group gradually increased during the latter half of the
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Fig. 1. Changes in body weight (BW; g; A), absolute quantity of food intake (g/day; B), and relative quantity of food intake per unit BW (g·kg⁻¹·day⁻¹; C) throughout the experimental period (5–45 wk of age). Values are means ± SE. A: note that, in BW, significant differences (P < 0.05) were found between Otsuka Long-Evans Tokushima Fatty (OLETF) obese-diabetic sedentary (OLETF-Sed) and prepubertal-onset exercise (Childhood-Ex) at 7–40 wk of age, between OLETF-Sed and Childhood-Diet at 7–28 wk of age, and between OLETF-Sed and Long-Evans Tokushima Otsuka (LETO)-Sed at 5–45 wk of age. B: in terms of food intake, significant differences (P < 0.05) were found between the OLETF-Sed and Childhood-Diet groups at 34–38 wk of age, between the OLETF-Sed and Childhood-Diet groups at 5–28 wk of age, between the Childhood-Ex and Childhood-Diet groups at 5–20 and 24–26 wk of age, and between the OLETF-Sed and LETO-Sed groups at 5–45 wk of age. C: in relative quantity of food intake, significant differences (P < 0.05) were found between the OLETF-Sed and Childhood-Ex groups at 7–33 wk of age, between the OLETF-Sed and Childhood-Diet groups at 6–8, 11, and 12 wk of age, between the Childhood-Ex and Childhood-Diet groups at 5–33 wk of age, and between the Childhood-Ex and LETO-Sed groups at 5–22 wk of age.

treatment until it was comparable to the FI in the OLETF-Sed group. Directly after completion of the food-restrictive treatment, the FI in the Childhood-Diet group temporarily increased but then remained constant. The Childhood-Ex group experienced a gradual reduction of FI from 27 wk of age onward; rats in this group had significantly lower consumption from 35 wk of age onward than individuals in the OLETF-Sed group. FI relative to BW was significantly higher in the Childhood-Ex group than in the other three groups from 7 to 33 wk of age (Fig. 1C); however, this value did not vary significantly among the OLETF-Sed, Childhood-Diet, and LETO-Sed groups. The mean total running distance during the exercise intervention period in the Childhood-Ex group was 350 ± 20.0 km (Σ350 ± 20.0 km). The amount of daily activity in the OLETF-Sed and Childhood-Diet groups was not measured throughout the experiment. Each animal in each experimental group was allowed to move freely in a normal cage. However, the Childhood-Ex group was given voluntary exercise opportunities using a rotator wheel attached to the normal cage. The amount of activity in the normal cage was estimated to be almost the same among the OLETF-Sed, Childhood-Diet, and Childhood-Ex groups. The total amount of physical activity during the exercise intervention period of the Childhood-Ex group was the sum of the daily activity in the normal cage and voluntary exercise (Σ350 ± 20.0 km). Therefore, it is evident that the total amount of activity during the childhood intervention period was greater in the Childhood-Ex group than in the OLETF-Sed and Childhood-Diet groups.

Changes in HOMA-IR and FSG, insulin, and leptin levels. Changes in HOMA-IR as well as FSG, FSI, and FSL levels are shown in Table 1. The mean HOMA-IR value in the Childhood-Ex group was consistent throughout the 15-wk treatment, after which it was significantly lower than that in the OLETF-Sed and Childhood-Diet groups. FSG levels were similar in all four groups when the rats were 7–12 wk of age. At 20 wk of age, however, FSG levels were significantly lower in the Childhood-Ex and Childhood-Diet groups than in the OLETF-Sed group. Thereafter, the mean FSG level was significantly lower in the Childhood-Ex group until the rats were 45 wk of age. In contrast, the mean FSG level in the Childhood-Diet group gradually increased over this period, approaching the value recorded in the OLETF-Sed group. Throughout the experiment, changes in FSI levels were similar to those of FSG in the Childhood-Ex, Childhood-Diet, and OLETF-Sed groups.
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Table 1. Changes in fasting serum glucose, insulin, and leptin concentrations, as well as HOMA-IR values

| Table 1. Changes in fasting serum glucose, insulin, and leptin concentrations, as well as HOMA-IR values |
|--------------------------------------------------|------------------|------------------|------------------|------------------|------------------ |
| Serum glucose, mg/dl                             | Treatment Period | Posttreatment Period |
|                                                  | 7 wk             | 12 wk             | 20 wk            | 27 wk            | 35 wk            | 45 wk            |
| OLETF-Sed                                        | 8                | 89.1 ± 4.3        | 88.5 ± 5.1       | 123.1 ± 10.2     | 141.8 ± 7.0      | 185.2 ± 14.4     | 229.4 ± 22.1     |
| Childhood-Ex                                    | 7                | 88.8 ± 2.8        | 84.1 ± 5.9       | 93.3 ± 7.2*      | 117.0 ± 3.9*     | 148.3 ± 9.0*     | 182.2 ± 16.8*    |
| Childhood-Diet                                  | 8                | 82.9 ± 1.5        | 85.7 ± 4.4       | 102.8 ± 5.4†     | 130.9 ± 4.8      | 167.3 ± 8.1      | 204.7 ± 11.9     |
| LETO-Sed                                        | 8                | 82.9 ± 1.2        | 83.6 ± 2.1       | 84.1 ± 2.8       | 80.0 ± 2.1       | 112.7 ± 3.6      | 145.4 ± 7.3      |
| Serum insulin, pg/ml                            | OLETF-Sed        | 8                | 475.2 ± 57.4     | 767.6 ± 163.9    | 1,036.3 ± 101.7  | 1,327.1 ± 88.2   | 1,327.2 ± 45.5   | 1,327.2 ± 51.9   |
|                                                | Childhood-Ex     | 7                | 469.9 ± 37.5     | 617.3 ± 97.6     | 536.5 ± 61.9*    | 745.6 ± 169.8*   | 868.3 ± 182.0*   | 955.5 ± 163.7*   |
|                                                | Childhood-Diet   | 8                | 391.5 ± 20.7     | 652.7 ± 109.4    | 844.1 ± 154.3    | 955.3 ± 145.7*   | 1,091.5 ± 138.0  | 1,234.5 ± 133.8  |
|                                                | LETO-Sed         | 8                | 437.9 ± 48.3     | 544.1 ± 145.4    | 586.7 ± 99.0     | 491.8 ± 88.2     | 430.3 ± 30.1     | 560.4 ± 101.9    |
| Serum leptin, pg/ml                             | OLETF-Sed        | 8                | 452.9 ± 7.6      | 745.5 ± 31.8     | 1,299.0 ± 62.8   | 1,828.8 ± 148.1  | 1,973.0 ± 130.6  | 2,475.4 ± 135.2  |
|                                                | Childhood-Ex     | 7                | 447.0 ± 19.1     | 427.6 ± 17.7*    | 592.1 ± 57.9*    | 875.8 ± 105.7*   | 1,179.8 ± 91.8*  | 1,734.8 ± 131.4* |
|                                                | Childhood-Diet   | 8                | 465.3 ± 19.3     | 529.7 ± 19.8*†   | 622.8 ± 23.6*†   | 1,601.4 ± 119.8† | 1,788.5 ± 80.7†  | 2,465.3 ± 77.1†  |
|                                                | LETO-Sed         | 8                | 426.7 ± 19.8     | 504.6 ± 15.4     | 677.2 ± 37.4     | 837.7 ± 51.1     | 911.9 ± 92.3     | 966.4 ± 93.1     |
| HOMA-IR                                         | OLETF-Sed        | 8                | 2.8 ± 0.5        | 4.4 ± 0.9        | 7.7 ± 0.6        | 12.3 ± 1.3       | 15.4 ± 1.6       | 18.7 ± 2.4       |
|                                                | Childhood-Ex     | 7                | 2.7 ± 0.3        | 3.2 ± 0.4        | 3.1 ± 0.5*       | 5.7 ± 1.5*       | 7.4 ± 1.8*       | 10.3 ± 1.3*      |
|                                                | Childhood-Diet   | 8                | 2.1 ± 0.2        | 3.6 ± 0.6        | 5.6 ± 1.1†       | 8.0 ± 1.3*       | 11.6 ± 1.7†      | 15.8 ± 2.4       |
|                                                | LETO-Sed         | 8                | 2.1 ± 0.1        | 2.2 ± 0.2        | 3.2 ± 0.6        | 2.6 ± 0.5        | 3.1 ± 0.2        | 5.2 ± 0.9        |

Values are means ± SE; n, no. of rats; HOMA-IR, homeostasis model assessment of insulin resistance; OLETF-Sed, Otsuka Long-Evans Tokushima Fatty-sedentary; Childhood-Ex, childhood-exercise; Childhood-Diet, childhood-dietary; LETO-Sed, Long-Evans Tokushima Otsuka-sedentary. *P < 0.05 vs. OLETF-Sed; †P < 0.05 vs. Childhood-Ex.

FSL levels gradually increased over time in the OLETF-Sed group, exhibiting a fivefold increase between 7 and 45 wk (Table 1). Indeed, FSL levels were much higher in the OLETF-Sed group compared with those in the Childhood-Ex and Childhood-Diet groups throughout the 15-wk treatment. However, levels increased markedly in the Childhood-Diet group after the treatment was completed; when the rats were 27–45 wk of age, FSL levels did not differ between the Childhood-Diet and OLETF-Sed groups (Table 1). Levels in the Childhood-Ex group, on the other hand, remained significantly lower throughout the remainder of the study, although they did increase slightly as the experiment progressed.

Serum lipid levels. Serum levels of Tcho, TG, HDL-C, and LDL-C were significantly lower in the Childhood-Ex group than in the OLETF-Sed and Childhood-Diet groups (Fig. 2). Rats in the Childhood-Ex group had a significantly higher HDL-C-to-Tcho ratio compared with those for rats in the OLETF-Sed group; however, LDL-C-to-Tcho ratios did not differ significantly among the other three groups.

Changes in whole body SFM, VFM, and LBM. SFM, VFM, %fat, and %LBM were comparable across groups (n = 23

Fig. 2. Effects of the Childhood-Ex and Childhood-Diet regimens on serum lipid levels (A) and serum lipid ratios (B) during childhood. Values are means ± SE. Tcho, total cholesterol; TG, triglyceride; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; HDL-C/Tcho, ratio of HDL-C to Tcho; LDL-C/Tcho, ratio of LDL-C to Tcho. P < 0.05 vs. §Childhood-Ex, Childhood-Diet, and LETO-Sed; *OLETF-Sed; †Childhood-Ex; &Childhood-Diet; and #Childhood-Ex and Childhood-Diet.
OLETF and n = 8 LETO rats) before the study (i.e., at 4 wk of age). At 7 wk of age, we observed significantly lower SFM, VFM, and %fat as well as significantly higher %LBM in the Childhood-Ex compared with values measured in both the OLETF-Sed and Childhood-Diet groups (Fig. 3). These trends lasted until the termination of the study, when the rats were 45 wk of age.

Energy metabolism-related enzyme activities and UCP-3 mRNA expression in skeletal muscle. At 46 wk of age, rats in the Childhood-Ex group demonstrated higher levels of CS, SDH, and PFK enzyme activity as well as greater UCP-3 mRNA expression compared with those in rats in the other three groups (Fig. 4). No such differences were observed among the OLETF-Sed, Childhood-Diet, and LETO-Sed groups. UCP-3 mRNA expression levels were significantly correlated with the activity of both PFK (r = 0.560, P < 0.01) and SDH (r = 0.580, P < 0.01) in rats from the OLETF-Sed, Childhood-Ex, and Childhood-Diet groups. However, no significant correlations were observed between enzyme activity and mRNA expression in the skeletal muscles of rats from the LETO group.

Correlations between body composition, serum leptin, and metabolism-related parameters. Changes in VFM were significantly correlated with changes in FSG throughout the experiment (OLETF groups: r = 0.830, P < 0.001; LETO group: r = 0.660, P < 0.001; OLETF and LETO groups combined: r = 0.830, P < 0.001). We also found a similar correlation between the changes in SFM and FSG (OLETF groups: r = 0.770, P < 0.001; LETO group: r = 0.690, P < 0.001; OLETF and LETO groups combined: r = 0.80, P < 0.001). Changes in FSL levels strongly correlated with the changes in %fat (Fig. 5) and %LBM. The relationship between FSL level and %fat in the OLETF group was described by the equation y = 94.8x − 1,306 (r = 0.90, P < 0.001); for the LETO group, this relationship was described as follows: y = 47.9x − 419.8 (r = 0.83, P < 0.001).

FSL level was strongly correlated with serum TG level measured at the completion of the experiment in both the OLETF (r = 0.830, P < 0.001) and LETO (r = 0.925, P < 0.001) groups. Rats in the OLETF group, however, displayed a broader range of both FSL and TG levels relative to those found in the LETO group. Serum TG levels were also closely related to %fat (OLETF groups: r = 0.662, P < 0.001; LETO group: r = 0.681, P < 0.001; OLETF and LETO groups: r = 0.728, P < 0.001) and BW. Changes in HOMA-IR positively correlated with BW (r = 0.742, P < 0.001) and VFM (r = 0.817, P < 0.001) in the OLETF group. Although the same

**Fig. 3.** Changes in body composition throughout the experiment. A: subcutaneous fat mass (SFM). B: visceral fat mass (VFM). C: %fat (a quantity obtained by dividing the sum of SFM and VFM by BW, then multiplying by 100). D: %lean body mass (LBM; a quantity obtained by dividing LBM by BW and then multiplying by 100). Values are means ± SE. *P < 0.05 vs. §Childhood-Ex, Childhood-Diet, and LETO-Sed; †OLETF-Sed; ‡Childhood-Ex; &Childhood-Diet; and #Childhood-Ex and Childhood-Diet.
general pattern was true for the LETO group, the relationships between these variables were much weaker (BW: $r = 0.389$, $P < 0.01$; VFM: $r = 0.437$, $P < 0.01$). Changes in serum free fatty acid level were not significantly correlated with changes in body composition in either the OLETF or the LETO group.

**DISCUSSION**

OLETF rats that engaged in childhood exercise maintained significantly lower BWs and higher %LBM throughout the maturation stage and middle age; this was not observed in rats subjected to childhood food restriction. Rats placed on an exercise regimen also displayed higher activity levels of energy metabolism enzymes CS, SDH, and PFK, as well as greater expression of UCP-3 mRNA in the skeletal muscle 25 wk after the cessation of childhood exercise. Cumulatively, our results suggest that the characteristics of energy metabolism obtained through a childhood exercise regimen in an animal model of human obese diabetes may be sustained more or less throughout maturation and middle age, which may contribute to the sustained suppression of BW gain. Importantly, to allow translation of our work, these novel insights need to be assessed in obese humans.

We found that absolute FI in the Childhood-Ex group gradually decreased 9 wk after cessation of exercise. At present, we do not know the reason for this observation. However, this is unlikely the main reason for the reduced BW and fat

Fig. 4. Activity of phosphofructokinase (PFK), citrate synthase (CS), and succinate dehydrogenase (SDH), as well as uncoupling protein-3 (UCP-3) mRNA expression level in the quadriceps femoris muscle at the completion of the experiment. Values are means ± SE. §$P < 0.05$ vs. OLETF-Sed, Childhood-Diet, and LETO-Sed.

Fig. 5. Relationship between fasting serum leptin (FSL) and %fat in the OLETF (A) and LETO (B) rat groups. The box indicates the range of measurements observed in the LETO rat group.
mass parameters in the Childhood-Ex group. Indeed, BW and all fat mass parameters after cessation of exercise in the Childhood-Ex group demonstrated significantly lower values than those in the Childhood-Diet and OLETF-Sed groups even before showing significant reductions in FI in the Childhood-Ex group. Moreover, we found that absolute FI in the Childhood-Diet group was lower than that in the Childhood-Ex group up to ~10 wk after cessation of the interventions, whereas BW in this group during this period demonstrated higher levels than that in the Childhood-Ex group. These findings indicate that absolute FI levels are not only the factor for determining BW levels in OLETF rats.

In an earlier study in rats that are genetically predisposed to become obese, Patterson et al. (27) found that early-onset exercise in the postweaning period prevented the development of obesity for up to 7 wk after exercise cessation, whereas rapid increases in BW and intake were observed after the cessation of a food-restriction treatment. However, exercise and food restriction treatments have not been found (by these or any other researchers, Ref. 20) to cause significantly different changes in anabolic and catabolic hypothalamic neuropeptide expression, suggesting that the hypothalamic roles in regulating energy intake might not be involved in the mechanisms underlying the sustained suppression of BW gain after exercise cessation.

In the case of exercise treatment in adult humans and obese rats, BW gain is typically observed once exercise treatments have been discontinued; no studies to date (21, 22, 42, 43) have observed continuous suppression of BW after exercise cessation. This recidivism is explained by the set point theory for BW (19), which states that individuals are predisposed to return to a genetically determined BW. This process has been associated with reductions of LBM and/or RMR in individuals who have lost weight through exercise (21, 30). RMR is the largest component (50–70%) of daily energy expenditure (29), and the major determinant of RMR is fat-free mass (LBM) quantities. In the present study, we found that rats that engaged in “childhood” exercise, but not food restriction, maintained ~10% lower BW and significantly higher %LBM compared with individuals who performed no exercise (OLETF-Sed). These results suggest that sustained higher %LBM may be an important factor for contributing the long-term suppression of BW gain in the Childhood-Ex group.

The question arises as to which mechanisms are involved in the long-term maintenance of high levels of %LBM in the Childhood-Ex group, even after the termination of their exercise treatment. This may be, in part, explained by the “muscle memory” theory, which explains a phenomenon that individuals who have previously undergone athletic training quickly acquire force when retraining (for example, Refs. 38, 41). Bruusgaard et al. (6) previously reported that the major cause of hypertrophy is the long-term (>3 mo after subsequent denervation) maintenance of myonuclei that have been newly recruited after overload. Thus “muscle memory” may be facilitated by a lasting change in the number of myonuclei in muscle cells (6, 38, 41); furthermore, because the ability to create myonuclei is impaired at older ages, individuals may particularly benefit from strength training at an early age (10). These mechanisms may be involved in the long-term maintenance of high levels of %LBM in the Childhood-Ex group after the termination of their exercise treatment, although confirmation of this hypothesis requires further investigation.

Our study also demonstrated that, even at 46 wk of age (e.g., 25 wk after the cessation of childhood exercise), rats in the Childhood-Ex group had 1.7- to 2.1-fold higher activity levels of CS, SDH, and PFK in the quadriceps femoris muscle relative to rats in the OLETF-Sed group; in contrast, the activity of these enzymes did not differ among the OLETF-Sed, Childhood-Diet, and LETO-Sed groups. Extensive research on metabolic enzymes in the skeletal muscles of both rodents and humans (9, 14, 36) identified CS as an important regulatory enzyme in the energy-generating metabolic pathway; this has since been used as a metabolic marker for assessing oxidative and respiratory capacity (36). With regard to UCP-3 mRNA, the expression levels measured in the quadriceps femoris muscle upon the completion of the experiment were 2.6- to 4.0-fold higher in the Childhood-Ex group than in the other three groups. UCP-3, which is found only in muscles, is thought to be involved in the regulation of energy expenditure (5). In both humans and rats, UCP-3 protein and/or UCP-3 mRNA expression in the skeletal muscle increases markedly within 3 h after a single exercise session (12, 28). Furthermore, among Pima Indians, UCP-3 mRNA expression in the skeletal muscle is positively correlated with sleeping metabolic rate (33). Taken together, these findings appear to suggest that childhood exercise training produces a sustained adaptive physiological response in skeletal muscle (5, 12, 28, 34), such as increased energy metabolism and increased RMR. Further studies are required to understand the mechanisms underlying the sustained high levels of metabolism-related enzymes and UCP-3 mRNA expression in skeletal muscle. Moreover, changes in RMR need to be directly measured.

Rats in the OLETF groups displayed a broader range of FSL values than those in the LETO group (Fig. 5). Circulating leptin level closely relates to body fat stores (7), since increased adiposity induces greater leptin production. However, the high leptin levels generally observed in obese individuals do not induce loss of fat mass (7, 26). This apparent paradox is described as “leptin resistance.”

Hypertriglyceridemia markedly impairs the rate of leptin transport into the central nervous system (2). The broad range of FSL levels observed in the OLETF group, as well as the markedly higher correlation between FSL and TG levels (compared with the LETO group), may reflect reduced leptin activity in these rats; in other words, the OLETF rats may be more leptin resistant than the LETO rats. Individuals in the Childhood-Ex group may have demonstrated long-term reductions in leptin level because of their equally long-term reductions in serum TG level, as well as their lower levels of body fat mass induced by prepubertal-onset exercise. In the present study, the strong relationship between FSL and TG levels may have been driven by the over-accumulation of body fat, leading to hypertriglyceridemia and leptin resistance.

In summary, we speculate that prepubertal-onset exercise affected the regulation of skeletal muscle mass and energy metabolism in the skeletal muscle rather than the feeding center in the hypothalamus. Although the exact mechanisms by which prepubertal-onset exercise alters skeletal muscle properties remain to be determined, our findings further support the concept that prepubertal-onset exercise induces a sustained decrease in the higher-predisposed BW set points in obese
individuals even after cessation of exercise regimens. Additional future work might compare the effects of childhood exercise and food-restriction treatments to those during adulthood (i.e., 20–34 wk of age in OLETF rats). Such work would be vital for confirming that the present results stem from prepubertal onset of the exercise regimen.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Authors: D.S. conducted experiments; D.S. interpreted results of D.S. prepared figures; T.M. drafted manuscript; T.M. edited and revised manuscript; M.S. conception and design of research; M.S. interpreted results of experiments; M.S. approved final version of manuscript.

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