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Inactivity induces increases in abdominal fat

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Laye MJ, Thyfault JP, Stump CS, Booth FW. Inactivity induces increases in abdominal fat. J Appl Physiol 102: 1341–1347, 2007. First published November 22, 2006; doi:10.1152/japplphysiol.01018.2006.—Previously, inducing inactivity for 53 h after 21 days of voluntary running resulted in a 25 and 48% increase in epididymal and omental fat pad weights, respectively, while rats continued to eat more than a group that never had access to a running wheel (J Physiol 565: 911–925, 2005). We wanted to test the hypothesis that inactivity, independent of excessive caloric intake, could induce an increase in fat pad mass. Twenty-one-day-old rats were given access to voluntary running wheels for 42–43 days so that they were running ~9 km/day in the last week of running, after which wheels were locked for 5, 53, or 173 h (WL5, WL53, WL173) before the rats were killed. During the 53 and 173 h of inactivity, one group of animals was pair fed (PF) to match sedentary controls, whereas the other continued to eat ad libitum (AL). Epididymal and retroperitoneal fat masses were significantly increased in the WL173-PF vs. the WL5 group, whereas epididymal, perirenal, and retroperitoneal fat masses were all significantly increased in the WL173-AL group compared with the WL5 group. Additionally, hyperplasia, and not hypertrophy, of the epididymal fat mass was responsible for the increase at WL173-AL as demonstrated by a significant increase in cell number vs. WL5, with no change in cell diameter or volume. Thus two important findings have been elucidated: 1) increases in measured abdominal fat masses occur in both AL and PF groups at WL173, and 2) adipocyte expansion via hyperplasia occurred with an ad libitum diet following cessation of voluntary running.

exercise; obesity; food; hyperplasia

THE CENTERS FOR DISEASE CONTROL (CDC) has classified tobacco, poor diet, and physical inactivity as “actual causes” of premature death, distinguishing them from heart disease and malignant neoplasm as “leading causes” of death (20). The CDC defines physical inactivity as, “not engaging in any regular pattern of physical activity beyond daily functioning” (6); the model of inactivity employed here ceases voluntary running of rats with only regular cage activity remaining. Of the three major actual causes of death, physical inactivity has received the least attention. Having previously studied physical inactivity in rodents as a further reduction in activity from sedentary or caged conditions by physical restraint (4, 26), we have altered our approach by developing a model that would more closely approximate comparison between innate and voluntary physical activity with more sedentary but ambulatory conditions. It is known that the provision of running wheels to “caged” rats and mice results in their running 2–15 km per night, depending on the strain (9, 25); thus animals provided access to running wheels are naturally more physically active than caged animals. In conjunction with the CDC classification of physical inactivity as an actual cause of premature death, we speculated that allowing rats to have access to running wheels and then systematically locking the wheels would provide insight into processes important during the transition from the natural state of physical activity to a more sedentary existence in caged animals.

Upon locking the wheels of rats to induce physical inactivity, we unexpectedly observed a rapid (53 h) increase in epididymal and omental adipose tissue masses (15). This observation could be important because human visceral obesity is associated with higher incidence of premature death from the cardiometabolic syndrome (3, 10). However, in the “wheel-lock” (WL) model employed, rats ate ad libitum and consumed more food than sedentary rats during both the voluntary running and the 53 h of wheel lock (inactivity) period, compared with age-matched rats that never had access to running wheels (15). Therefore, it remained uncertain whether the increase in adipose tissue was solely due to excess food consumption rather than decreased physical activity. The present study was designed to specifically test the hypothesis that excess food intake was exclusively responsible for the increase in abdominal fat by feeding one group of rats ad libitum (AL) while limiting food intake of a second group (“pair fed” (PF)) to that of age-matched, always sedentary rats. The basis for selecting the three time points for AL and PF groups after locking wheels was determined from our laboratory’s previous findings and are as follows: 1) 5 h (WL5) because the acute exercise effects on basal glucose uptake into epitrochlearis muscle had disappeared (14); 2) 53 h (WL53) because enhanced insulin sensitivity had returned to sedentary levels (14) and epididymal and omental fat masses increased (15); and 3) 173 h (WL173) to ensure that the 53-h increase in fat mass was not a transient effect and that the period of inactivity was of sufficient length to test the hypothesis.

METHODS

Materials. Nylon mesh was from Sefar America (Kansas City, MO). All other reagents were either from Sigma or Fisher.

Animal protocol. The animal protocol was approved by the Institutional Animal Care and Use Committee at the University of Missouri-Columbia. Thirty-six, male Fischer 344 × Brown Norway F1 hybrid rats (Harlan) were obtained in the fourth week of life. Animals were randomly separated into those with access to running wheels (WL) and those without access to the wheels (Sed). Rats assigned to running groups were immediately housed (at the age of 21 days) in cages equipped with a voluntary running wheels outfitted with a Sigma Sport BC 800 bicycle computer (Cherry Creek Cyclery, Missouri-Columbia. Thirty-six, male Fischer 344 × Brown Norway F1 hybrid rats (Harlan) were obtained in the fourth week of life. Animals were randomly separated into those with access to running wheels (WL) and those without access to the wheels (Sed). Rats assigned to running groups were immediately housed (at the age of 21 days) in cages equipped with a voluntary running wheels outfitted with a Sigma Sport BC 800 bicycle computer (Cherry Creek Cyclery, Columbia, Missouri. The animal protocol was approved by the Institutional Animal Care and Use Committee at the University of Missouri-Columbia. Thirty-six, male Fischer 344 × Brown Norway F1 hybrid rats (Harlan) were obtained in the fourth week of life. Animals were randomly separated into those with access to running wheels (WL) and those without access to the wheels (Sed). Rats assigned to running groups were immediately housed (at the age of 21 days) in cages equipped with a voluntary running wheels outfitted with a Sigma Sport BC 800 bicycle computer (Cherry Creek Cyclery, Columbia, Missouri.

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Foster Falls, VA) for measuring daily running activity. Voluntary running was selected to approximate the more natural activity state of the animal. The selection of the age of the rats was based on our laboratory’s previous observation that the first night’s running distance in voluntary wheels by rats in the fifth week of life was threefold longer than rats in the eighth week of life (6 vs. 1.5 km) (15, 16). Therefore, we hypothesized rats in their fourth week of life would run further total distances than those in their fifth week. Cages were in temperature-controlled animal quarters (21°C) with a 0600–1800 light:1800–0600 dark cycle that was maintained throughout the experimental period. Two cohorts of equal numbers were obtained that had initial body weights of 38.8 ± 1.0 and 33.0 ± 0.9 g and who ran an average of 9.49 ± 1.00 and 8.16 ± 0.74 km daily, respectively, during the last week of running.

All animals were provided 200 g of standard rodent chow (Formula-5008, Purina Mills, St. Louis, MO) in new cages at the beginning of each week when cages were changed and body weights obtained between 0800 and 1000. Animal cages were changed 7 days before the rats were killed for each group based on a dip in food intake that was noted the day after changing cages (see Fig. 2A). Running activity (for groups with wheel access) was obtained every day of running between 0800 and 1000. Body mass and food intake were measured daily during the fifth and sixth week of running and following locking of the wheels.

Rats in running groups had access to wheels and food and water ad libitum for 42 or 43 days, at which time (0600) wheels were locked for all groups (Fig. 1). One group of rats was anesthetized [ketamine (80 mg/kg), xylazine (10 mg/kg), and acepromazine (4 mg/kg)] and killed by exsanguination by removal of the heart 5 h (1100; WL5) after locking of wheels; a group of sedentary rats were killed at the same time. The remaining four groups of rats were divided into two main groups (either fed AL or PF to the predicted amount of food being eaten per gram of body weight by an age-matched group that never had access to running wheels). These two main groups of rats were further assigned so that they underwent 53 h (WL53-AL and WL53-PF) or 173 h (WL173-AL and WL173-PF) of wheel lock (designated “inactivity”); these groups were killed after being anesthetized (as above) at 1100 after either 53 or 173 h of inactivity. All animals had access to food until the day of death, when food was removed at 0600.

Increases in upper body fat in humans are reported to worsen metabolic risk factors, but whether this incremental effect is due to abdominal subcutaneous or intraperitoneal fat is disputed (see Ref. 27 for references). Selection of fat depots was based on body cavity: epididymal (intraperitoneal), perirenal (intraperitoneal), and retroperitoneal (extraperitoneal) adipose tissues were removed from exsanguinated animals and weighed. Epididymal fat was divided and placed into osmium tetraoxide or blocked in paraffin following an overnight fix in formalin for microscopic examination. Epididymal fat was selected because of its well-defined anatomic boundaries in the intraperitoneal cavity.

Adipocyte isolation. Adipocytes from epididymal fat pads were isolated by a modification of the Rodbell method (24) as modified by our laboratory (15).

Adipocyte size and number. Preparation of epididymal adipose tissue for determination of cell size and number was performed essentially as described by Cartwright (5), as previously delineated by our laboratory (15). Cell volumes were directly measured with a Coulter counter, and the total number of cells was then calculated from the average cell volume and the weight of the entire epididymal fat pad. For morphometric determinations, sections were stained with hematoxylin and eosin. Thirty random adipocytes from two different areas of the microscopic section for each sample to verify Coulter counter data were measured at a ×20 magnification using Image Pro (Silver Spring, MD) imaging software.

Statistics. For each outcome measure, a one-way analysis of variance was done using the MIXED procedure (SAS, Cary, NC). Pairwise comparisons were done using least squares means (SAS) but only for the five pairs of interest (WL5 vs. WL53-AL; WL5 vs. WL53-PF; WL5 vs. WL173-AL; WL5 vs. WL173-PF; and WL173-AL vs. WL173-PF). The 25 P values obtained from these comparisons were then used in the PROC MULTTEST (the procedure approaches the multiple testing problem by adjusting the p-values from a family of hypothesis tests, SAS) to obtain the set of comparisons that met an approximate 0.05 false discovery rate adjustment (2). Significance for all tests was set at P < 0.05. All data are presented as means ± SE.

RESULTS

In the final week of running, rats ran ~9 km/day (Fig. 1B). On the final day of 42 or 43 days of running, the absolute food intakes of WL rats (23.5 ± 0.5 g/day) was 23% more (P < 0.001) than Sed rats (19.1 ± 0.7 g/day) (Fig. 2A). In the first 24 h, when wheels were locked, there was an immediate drop in food intake of 11 and 24% for the WL-AL and WL-PF groups, respectively (Fig. 2B); WL-PF rats consumed what Sed rats were eating per gram of body weight (Fig. 2, C and D). The WL-AL group consumed significantly more food during days 1–3 of wheel lock (P < 0.005) (Fig. 2B). However, by day 4 of wheel lock, this statistical difference no longer existed as determined by repeated-measures ANOVA (P = 0.06–0.08 for days 4–6, P = 0.57 for day 7) (Fig. 2B).
Body weights (g) at death were not statistically different between groups (Table 1). During the 7 days of inactivity, the WL53-PF and WL53-AL groups gained 9.14 g and 4.2 g of body weight, respectively. In the first cohort (n = 5 for Sed, n = 2–3 for other groups) of animals, muscle weights for soleus and plantaris were taken and determined to not differ among groups (data not shown).

Epididymal fat masses (g) of WL53-PF and WL53-AL were both significantly (P < 0.05) greater than WL5 (Fig. 3A, Table 1). However, WL5 was not different from WL53-PF, WL53-AL, and Sed. Perirenal fat mass (g) of WL53-AL was significantly greater (P < 0.05) than WL5 (Fig. 3B, Table 1). However, WL5 was not different from WL53-PF, WL53-AL, WL53-AL, or Sed. Retropertioneal fat masses (g) of WL53-PF and WL53-AL were both significantly (P < 0.05) greater than WL5 (Fig. 3C, Table 1). However, WL5 was not different than WL53-PF, WL53-AL, or Sed. WL53-PF tended to have slightly, nonsignificantly, less fat gains than WL53-AL for all three fat masses, which could be related to WL53-AL consuming more food on the first 3 days of inactivity (Fig. 2). Altogether then, the hypothesis that excess food intake was exclusively responsible for the increase in abdominal fat from WL5 to WL173 is deemed not to be held for two of the three determinations; i.e., epididymal and retropertioneal fat increased at WL173 in the PF condition.

Epididymal adipose cell volume did not significantly differ between WL5 and any group as determined with a Coulter counter (see methods for details) (Fig. 4, Table 1). No differences in adipocyte diameters were observed between WL5 and WL53-AL (Fig. 5). Only diameters (μm)
from WL5 and WL173-AL were calculated via microscope method to verify Coulter counter data. The values were 71.1 ± 1.99, and 74.4 ± 3.45 for WL5 and WL173-AL, respectively. The diameters (µm) from the Coulter counter were not different between WL5 and other groups (Table 1). The Coulter counter and microscopic (Fig. 5) methods were in agreement that no difference existed for adipocyte cell size. Both methods gave a 4.6%, nonsignificant, higher value for cell diameter in WL173-AL than in WL5.

Adipocyte number ($\times 10^6$) in the epididymal fat pad was significantly greater ($P < 0.05$) in WL173-AL (10.76 ± 1.06) than WL5 (7.63 ± 0.64) (Fig. 6, Table 1). Adipocyte numbers per epididymal fat pads for remaining groups were not different from WL5.

**DISCUSSION**

The sole purpose of the present study was to test whether pair-feeding (food intake levels provided at levels of age-matched rats never having access to running wheels) would entirely prevent the increase abdominal fat that occurs in ad libitum eating upon locking running wheels. A major finding of the present study is that pair feeding during inactivity did not prevent epididymal and retroperitoneal, but it did prevent the perirenal, fat masses from increasing in size after rats ceased 42–43 days of daily voluntary running. Therefore, the hypothesis that excess food intake was exclusively responsible for the increase in abdominal fat is rejected; the alternative hypothesis that excess food intake was exclusively responsible for the increase in abdominal fat in the model employed in the present experiments. Another major finding is that adipocyte hyperplasia was present at the seventh day after ceasing voluntary running in those rats ad libitum fed.

Pair feeding during inactivity did not totally prevent the gain in mass of epididymal and retroperitoneal fat masses at the seventh day of inactivity. When comparing the 173rd with 5th h of inactivity after ending 42 nights of voluntary running, PF and AL groups had increases in epididymal fat mass of 34 and 50% and in retroperitoneal fat mass of 93 and 101% (Fig. 3, A and C, black bars). Importantly, body weights only increased by 0.5 and 3.5% during the 7 days of inactivity, in the WL173-PF and WL173-AL, respectively. Thus body growth alone cannot fully account for the increases in fat mass. For perirenal fat mass, an increase of 85% occurred from the 5th to the 173rd h of inactivity for the AL-ed group. However, the difference from the 5th to the 173rd h of inactivity was 52% in the pair-fed group ($P = 0.19$). No significant differences existed at the 173rd h of inactivity between PF and AL groups for any of the three fat masses. Similar results were obtained when specific fat masses were normalized to body weight (Fig. 3, A–C, gray bars), suggesting that increases in fat masses during inactivity were in large part independent of body mass. These observations suggest that inactivity after voluntary running, independent of excess caloric intake, leads to the enlargement of abdominal fat. The major limitation to the interpretation is the absence of indirect calorimetry to more precisely balance caloric intake with expenditure in the inactivity period; therefore, our interpretations are based on pair feeding. An alternative, but not contradictory, interpretation is that physical activity performed voluntarily slowed the growth of abdominal adipose tissue, and the ensuing physical inactivity permitted catch-up growth. Nonetheless, whatever the interpretation, the rapid growth of abdominal adipose tissue opens a future opportunity to dissect mechanisms by which reductions in the caloric expenditure of physical activity increases the partition of calories to fat masses.

Three observations from our laboratory’s earlier study (15) provide notions as to how maladaptations to physical inactivity may have played some role in the increased epididymal fat mass, which are 1) inactivity would omit the nightly 80% suppression of palmitate incorporation into triacylglycerol and increase in lipolysis; 2) palmitate incorporation into triacylglycerol of epididymal fat overshoot sedentary values by fourfold at the 10th h of the light cycle (i.e., the 10th h after the wheels were locked to prevent further running); and 3) a three- to fourfold overshoot in palmitate incorporation into triacylglycerol was maintained at the 29th and 53rd h of inactivity after the last night (in contrast to the 80% suppression seen 24 and 48 h earlier at the 5th h after the last night of running). The overshoot of palmitate incorporation into triacylglycerol at the 29th and 53rd h after running did not occur after only a single night of running (16), suggesting a potential enzymatic adaptation, such as mitochondrial glycerol-3-phosphate acyltransferase enzyme activity, an enzyme that catalyzes the first committed step in triacylglycerol and phospholipid biosynthesis, was 48, 45, and 58% higher than sedentary values at 10, 29, and 53 h of wheel lock, respectively (16). Because the percent increase in palmitate incorporation into triacylglycerol was manifold greater than the increase in glycerol-3-phosphate.
acyltransferase activity, increases triacylglycerol hydrolysis (lipoprotein lipase), free fatty acid transport (fatty acid translocase/CD36), or other potential adaptations could also enhance triacylglycerol stores.

These findings may apply to the gains in fat mass found in human subjects. For example, Yanovski et al. (28) observed almost 10% of subjects gained 2.3 kg in an 6-wk period; the greatest gain being 4.07 kg. The laboratory of Bouchard et al. (23) reported that human fat mass increased an average of 14.3% over a 22-day period; the largest gain was 3.2 kg. The two major differences between the above-mentioned human studies and the present rodent study are the percent increases and rate of increase in fat mass gained in humans are less than rats. Potential explanations are the larger relative metabolic rate seen in rats and/or shorter life span of the rat compared with humans. Additionally, in human studies total fat mass rather than depot specific masses were measured, which may mask gains in a few specific fat depots.

Some (8, 11, 15, 17), but not all (1), studies have reported increases in abdominal fat following the cessation of physical activity in rats. For example, there is a 41% increase in epididymal fat mass (11), a 53% increase in parametrial adipocyte volume (8), a 23% increase in epididymal adipocyte diameter (17), and 25 and 18% increases in epididymal mass and adipocyte volume (15), respectively, 14, 4, 7, and 2 days, respectively, following the cessation of exercise training. In the latter study, our laboratory used a model of inactivity where wheels are locked after voluntary running; the wheel locked (former runners) continued to consume 20% more food for the first 2 days of wheel lock than age-matched sedentary (15), raising the concern that the 25% gain in epididymal fat mass was because of higher food consumption and because of not physical inactivity.

The enlargement of fat masses raises the question of whether the increase is associated with adipocyte hypertrophy or hyperplasia, or both. In our laboratory’s previous study (15), no
adipocyte hyperplasia was observed at the 53rd h of inactivity. In the present study, an unanticipated 41% increase in epididymal fat pad cell number was observed at the 173rd h of inactivity in WL173-AL with no change in cell size. However, in WL173-PF with a 33% increase in epididymal mass, neither the 26% increase in cell number (P = 0.16) nor the 6% increase in cell volume (P = 0.48) was statistically significant. The mechanism for the increase in epididymal fat mass in WL173-PF is likely to be hyperplasia, but a larger number of observations are necessary to reach statistical significance. A power calculation based on the standard deviations and means of the cell number data for the PF group, as well as the assumption that a difference of 50% would be clinically meaningful, found that nine per group would give a power of ≥80% in the WL173-PF group.

The presence of adipocyte hyperplasia in 7 days of physical inactivity in ad libitum-fed rats as employed here is somewhat faster than that occurring in a diet-induced obesity model, described next. At 6 wk of age, rats given a 60% fat diet for 3 wk quadrupled epididymal fat mass (19). However, increases in adipocyte size reached an essential plateau after the first week, which Li et al. (19) interpreted to imply that any subsequent increase in epididymal wet weight from 1 to 3 wk of the high-fat diet was largely the result of hyperplasia. Similarly, human subjects overfed for 8 wk gain 3–4 kg in fat mass, which according to Levine et al. (18) is likely due to hyperplasia as mean adipocyte size in subcutaneous adipose tissue did not increase. Therefore, there is evidence that humans can undergo adipocyte hyperplasia in the relatively short duration of weeks similar to rats when in a positive-calorie state.

Previous studies have shown adipocyte hyperplasia, but these studies compared separate groups of exercising and sedentary animals and also were not designed to measure durations of inactivity as short as in the present study. Sedentary rats, either pair weighted to the exercising group or ad libitum fed, had 28 or 54%, respectively, more adipocytes per epididymal fat pad than a group of rats participating in a 14- to 16-wk swim training program (22). Craig et al. (7) showed that sedentary rats had 108% more epididymal adipocytes than rats that had voluntarily ran in wheel from 6 to 12 mo of age (and subjected to an ~8% food restriction for approximately the last 2 mo). In addition, we have observed that 87-wk-old sedentary female rats had 123% greater number of adipocytes in the ovarian fat pad than age-matched rats that had access to running wheels beginning at 4 wk of age (D. S. Kump and F. W. Booth, unpublished observation; n = 3 per group). Taken together, our results extend these observations to show...
that it takes only 7 days of inactivity for adipocyte hyperplasia to occur in ad libitum-fed rats. Future studies need to determine whether known inducers of adipocyte hyperplasia (13) occur between inactivity days 2–7 because our laboratory’s earlier paper (15) found no increases in peroxisome proliferator-activated receptor-γ and CCAAT/enhancer binding protein-α protein levels in epididymal fat at 53 h of inactivity after 21 days of voluntary running.

Disproportional greater rates of fat deposition relative to lean tissue are well documented in infants born small for gestational age and/or whose growth faltered during infancy and childhood, but who show subsequent catch-up growth (21), and in adults (12) recovering body weight after weight loss due to a variety of conditions (war-related famine, poverty-related undernutrition, experimental starvation, anorexia nervosa, and other clinical hypermetabolic conditions such as cancer, septic shock, and acquired immunodeficiency syndrome). The rapid increase in abdominal fat encountered after running ceases in young rats may be have similar biochemical drives as the aforementioned examples.

Differences in outcomes for adipocyte hypertrophy have been noted between the present and our laboratory’s previous study (15); that may be, in part, due to the two experimental designs used. Rats in the present and previous reports (14–16) were given access to running wheels in the fourth and fifth week of life, respectively, ran 2 and 6 km the first night, respectively, and had access to running wheels for 6 and 3 wk, respectively. The two designs produced remarkably different outcomes. Rats in the present and our laboratory’s previous reports (14–16) ran distances of 9 and 5 km/day during the last week of running, respectively, and they had body masses, skeletal muscles, and epididymal adipocyte volumes that were similar in size and larger, respectively.

Attention to the specificity of the model needs to be made. Inactivity (and the lagging attenuation in caloric expenditure) follows a period of caloric intake and expenditure that are greater than in rats never having access to voluntary running. Thus it is inactivity going after a chronic period of daily running that produces the enlargement of fat pads. A potential speculation is that daily activity inhibits adipocyte proliferation in young animals and that the induction of physical inactivity allows for adipocyte hyperplasia. Because very young rats were used in the study, a future study needs to examine more mature rats.

In summary, physical inactivity, independent of excess caloric intake, is associated with rapid increases abdominal adipose tissue masses. Furthermore, adipocyte hyperplasia in epididymal fat was present by the seventh day of inactivity in the ad libitum-eating group.

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