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Potential clinical translation of juvenile rodent inactivity models to study the onset of childhood obesity

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Submitted 11 April 2012; accepted in final form 5 June 2012

Roberts MD, Company JM, Brown JD, Toedebusch RG, Padilla J, Jenkins NT, Laughlin MH, Booth FW. Potential clinical translation of juvenile rodent inactivity models to study the onset of childhood obesity. Am J Physiol Regul Integr Comp Physiol 303: R247–R258, 2012. First published June 13, 2012; doi:10.1152/ajpregu.00167.2012.— According to the latest data from the Center for Disease Control and Prevention 17%, or 12.5 million, of children and adolescents aged 2–19 years in the United States are obese. Physical inactivity is designated as one of the actual causes of US deaths and undoubtedly contributes to the obesity epidemic in children and adults. Examining the effects of inactivity on physiological homeostasis during youth is crucial given that 58% of children between the ages 6–11 yr old fail to obtain the recommended 60 min/day of physical activity and 92% of adolescents fail to achieve this goal [Troiano et al. Med Sci Sports Exerc. 40, 2008]. Nonetheless, invasive mechanistic studies in children linking diminished physical activity with metabolic maladies are lacking for obvious ethical reasons. The rodent wheel lock (WL) model was adopted by our laboratory and others to study how different organ systems of juvenile rats respond to a cessation of daily physical activity. Our WL model houses rats in cages equipped with voluntary running wheels starting at 28 days of age. After a certain period of voluntary running (3 to 6 wk), the wheels are locked, thus preventing the rats’ primary source of physical activity. The studies discussed herein suggest that obesity-associated maladies including skeletal muscle insulin resistance, hypothalamic leptin resistance, fatty acid oxidation impairments in skeletal muscle and adipose tissue, nonalcoholic fatty liver disease, and endothelial dysfunction are initiated in juvenile animals that are restrained from voluntary exercise via WL. The use of the juvenile rodent WL or other inactivity models will continue to provide a powerful clinical translational tool that can be used for primordial prevention of human childhood obesity.

juvenile obesity; rodent model; primordial prevention

THE TRIPLING OF CHILDHOOD OBESITY over the past three decades has garnered attention from the scientific community, clinicians, and policy makers. According to a Center for Disease Control and Prevention report (11) 17%, or 12.5 million, of children and adolescents aged 2–19 yr were obese as of 2009. In an American Heart Association (AHA) Scientific Statement, Balagopal et al. (4) contend, “Increased appreciation of the effects of long-term exposure to risk factors and concern about the epidemic of pediatric obesity have prompted a new sense of urgency for primordial and primary prevention strategies during childhood”; this being a position that we have been advocating for over a decade (9). Primordial prevention is defined as prevention of the development of risk factors in the first place, whereas primary prevention is defined as interventions designed to modify adverse levels of risk factors once present (79).

If actions against childhood obesity do not take place, the cruel reality may be what Olshansky predicted in 2005: that is, today’s children could be the first generation in over a century to experience a decline in life expectancy due to the epidemic of childhood obesity (53). He and others have extended their 2005 work by applying a three-dimensional technique that takes into account the delayed effects of health risks being
accumulated by today’s younger generations to forecast vital health and longevity to the US obesity epidemic. Their forecast for the US obesity epidemic suggests that future healthcare expenditures and death rates could be far worse when determined by the behaviors and characteristics of people alive now; not by the attributes of the recently deceased, who were born 60–90 years ago and lived under different circumstances (63).

Despite the fact that obesity rates have increased nearly threefold since 1960 (50) as time spent being physically active have fallen (13), a substantial amount of scientific effort has been placed in examining mechanisms that may explain a genetic predisposition toward the obesity phenotype. Outside of the laboratory setting, a compelling case can be made that sedentarism (not genetics) is the primary cause of the obesity epidemic. In this regard, Church et al. (13) have recently illustrated that the drop in occupational energy expenditure from 1960 to 2006 was able to predict body weight changes that have been observed from the US National Health and Nutrition Examination Surveys during this epoch. One could also logically argue that: 1) a majority of individuals possess a genetic predisposition toward obesity since nearly 70% of the US population is overweight or obese; and 2) environmental factors (i.e., sedentarism and poorer dietary habits) are the driving culprit of the obesity epidemic given that the human genome has likely changed very little since the appearance of Homo sapiens around ~40,000 years ago (i.e., spontaneous mutations in genetic alleles likely haven’t driven the epidemic) (16). Moreover, since the storage of fat was a survival mechanism during evolution, the mammalian genetic predisposition to obesity is not shocking. In fact, Reed et al. (62) report that 31% of knockout mouse strains weighed less than control mice, which reiterates the notion that numerous genes likely contribute to a predisposition toward obesity.

Nonetheless, the costly march toward finding associative relationships between genetic markers and the obesity phenotype continues. As an example, overwhelming research enthusiasm has surrounded elucidating variants of the “Fat mass and obesity-associated” (FTO) gene. The data seems to support the notion that variants in this gene are reliably associated with differing body mass indexes (60) and/or genetic predisposition to obesity (17, 23). However, recent evidence suggests that “obesogenic” variants of the FTO gene are not associated with an obesogenic phenotype in those that maintain “above average” physical activity levels (60). Furthermore, a large-scale meta-analysis of 218,166 adults and 19,268 children determined that the odds of obesity in those possessing the FTO risk allele (rs9939609) was attenuated by 27% in physically active adults (28). Thus, while a genetic predisposition to obesity may exist, recent evidence seems to suggest that physical activity levels are a stronger influence on obesity and/or other cardio-metabolic disease phenotypes compared with risk associated with FTO genotypes. In this review we present data from studies that have used juvenile rodent inactivity models which clearly demonstrate that insufficient physical activity is intimately linked to the mechanistic development of risk factors early in life that are associated with obesity and its associated comorbidities. Importantly, while dietary and genetic factors influence disease risk, we posit that results from studies presented herein support adopting physical activity during childhood as a means of primordial prevention against the initiation of obesity and its related comorbidities as emphasized in another recent AHA Policy Statement (79).

Consequences of Physical Inactivity in Children

Physical inactivity was designated as one of the actual causes of US deaths in 1990 (46) and again as an actual cause of the leading causes of US deaths in 2004 (48). Convincing evidence suggests that in the absence of moderate or high level of cardiorespiratory fitness, an increased risk of all-cause and cardiovascular disease mortality exists in both adult men and women (37). With regard to studying physical activity and body composition in children, Schutz’s group has determined: 1) that time spent on sedentary activities was positively correlated to body fat percentage in 9-yr-old boys (40); and 2) 8- to 10-year-old obese children were more sedentary (i.e., 400 min/day vs. 295 min/day; P < 0.05) and spent less time performing nonsedentary activities (449 min/day vs. 563 min/day; P < 0.05) compared age-matched nonobese children (39). Interestingly, these authors reported that time spent performing moderate or vigorous activity (i.e., heart rates between 50% and 70% V˙O2 max for “moderate” and >70% V˙O2 max for “vigorous”) was not statistically different in obese versus nonobese children (moderate: 88 min/day vs. 52 min/day; vigorous: 20 min/day vs. 16 min/day, respectively). Nonetheless, little data exists regarding how a physically inactive childhood affects mortality decades later. A longitudinal study that examined physical activity patterns in Finnish boys and girls beginning at the ages of 3–18 yr determined that a high level of physical activity during this early period of life correlated to physical activity patterns 21 years later (75). A similar follow-up study (81) revealed an inverse relationship existed between higher levels of adult physical activity, which was maintained from high youthful physical activity, and low waist circumference.

Hence, these human experiments suggest that adopting a highly physically active childhood is important for sustaining a highly physically active adulthood which, in turn, combats the development of a marker of abdominal obesity.

Given the above findings, one may pose the question as to whether inactive, obese adults who were inactive and obese during childhood can become healthy by adopting a physically active lifestyle during adulthood. It is well known that, independent of caloric intake, overweight and obese adults who adopt a more physically active lifestyle improve biomarkers associated with obesity (71) insulin resistance (26) and markers of cardiovascular disease (72). Likewise, a moderate to high level of physical activity eliminates the higher risk of mortality associated with obesity (36). However, we posit that transitioning from a sedentary childhood to an active adulthood is extraordinarily challenging with regard to compliance limitations. In support of this hypothesis, in previously sedentary persons adherence to higher levels of physical activity over longer time periods (i.e., months to years) are very poor. A review by Laatakari et al. (33) eloquently summarizes this hypothesis: “While our understanding of health benefits of physical activity has rapidly increased in recent years, our ability to effect long-term changes in physical activity is lagging behind. As a matter of fact, for two decades it has been repeatedly documented that adherence to physical activity is poor even in supervised programs. One could legitimately ask
whether the long-term maintenance of physical activity is at all possible.”

Finally, longitudinal data from the Bogalusa heart study suggests that 77% of obese, and presumably sedentary, children remained obese during adulthood (18). Therefore, the lack of physical activity during childhood seems to exert two profound effects which include: 1) increasing the probability that physical activity during adulthood remains low, and 2) increasing the probability that the mechanistic initiation and development of obesity and its associated comorbidities that occur during childhood become difficult to manage during adulthood. Hence, current information establishes the importance and necessity to prevent the onset of obesity in children through childhood adequate and routine physical activity.

The Wheel Lock Model: Its Relevance to Human Findings and Its Validity in Studying the Initiation of Juvenile Obesity

The rodent wheel lock (WL) model was developed by Rhodes et al. (64) in 2003 and was subsequently employed in our laboratory in 2005 (30) and by Patterson et al. (57) in 2008 as a model to study how different organ systems of juvenile rats respond to a cessation of daily physical activity. Briefly, our WL model consists of 28-day-old rats housed in cages equipped with voluntary running wheels. After a period of voluntary running (3 to 6 wk), the wheels are locked, thus removing the rats’ primary source of physical activity. In such a manner, various physiological variables can be examined in animals that have: 1) continual running wheel access (i.e., are habitually active), 2) have their wheel access interrupted (i.e., become sedentary), and 3) animals that never have access to running wheels (i.e., are habitually sedentary). It is important to note that the rats examined in most of our studies were ∼40–60 days old at the time of data collection. Based upon classical literature studying sexual and social maturation patterns in rats (1), a 45-day-old rat is roughly equivalent to a 12.5-year-old in humans and is sexually immature. Therefore, this WL model parallels what is likely occurring in current-day adolescents with regard to the mechanistic initiation of risk factors associated with obesity, diabetes, and cardiovascular disease.

Our initial rationale for using the WL model was to gain a better understanding of mechanisms that regulated glucose uptake in a physical activity-dependent manner (30).

As proposed in our original WL study, allowing and subsequently denying access to running wheels provides a physiological model of intermittent physical activity that differs from forced exercise regimens (i.e., treadmill running or swimming) and/or drastic models of inactivity (i.e., hindlimb unloading, hindlimb immobilization). We propose that this approach to “knocking down” physical activity in juvenile animals provides a unique polygenic, human-relevant translational model to study the development of complex human diseases in the absence of physical activity. Conversely, while other studies knocking out or overexpressing single genes in sedentary mice to study resultant disease phenotypes are impressive and intuitive, some may argue that this is a “single bullet” approach to studying complex and polygenic human diseases. In this regard, the aforementioned article by Reed et al. (62) states, “Understanding how body weight is determined by this network of [2000–6000] genes presents an extraordinary challenge.” As will be discussed below, several physiological parameters (for instance insulin sensitivity and fat pad masses) “catch-up” to sedentary levels within 2–7 days of WL. Thus a critical assumption that we have made throughout is that sedentary juvenile animals (i.e., those that had not possessed running wheels) are 1) relatively unhealthier than animals with running wheels, and 2) in the process of mechanistically developing numerous obesity-related comorbidities that will be discussed herein. Martin et al. (43) agree with these assumptions, as they have also written, “Failure to recognize that many standard control rats and mice used in biomedical research are sedentary, obese, glucose intolerant, and are on a trajectory to premature death may confound data interpretation and outcomes of human studies.”

Table 1 summarizes the WL studies performed in our laboratory. Although we reference some of these studies throughout the review, we will briefly summarize these findings. The first WL study performed by Kump and Booth (30) revealed that insulin-stimulated glucose uptake was significantly greater in animals that underwent 5 and 29 h of WL compared with animals that underwent 53 h of WL and/or animals that never had running wheel access. The second study by Kump and Booth (31) revealed that food intakes were greater and feed efficiencies were lower in animals that had run for 21 days and were WL for 5, 10, 29, and 53 h compared with animals that had never had wheel access. Furthermore, WL generally increased relative omental and epididymal fat masses as well as epididymal triacylglycerol synthesis rates (higher than 3.5-fold) compared with animals that had continual running wheel access (Fig. 1).

The third study by Kump et al. (32) demonstrated that plasma insulin and triglyceride concentrations were higher by 53 h of WL compared with animals that had continuous running wheel access. Laye et al. (35) examined juvenile rats that were WL for 53 and 173 h and pair fed with animals that never had running wheel access to control for differences in caloric intakes. Interestingly, epididymal and perirenal fat masses in WL173-hour pair-fed animals were similar to non-pair-fed WL173-hour animals and greater than animals that had continual running wheel access, which suggests that increases in fat mass were strictly due to the cessation of habitual physical activity. Rector et al. (61) subsequently ran 28-day-old Otsuka Long Evans Tokushima Fatty (OLETF) rats for 16 wk before WL and determined that omental and retroperitoneal fat pad masses as well as hepatic triglycerides and protein markers of fatty acid synthesis were greater in sedentary versus WL5-, WL53-, and WL173-hour animals. Moreover, hepatic palmitate oxidation was greater in WL5- and WL53-hour animals compared with WL173-hour and sedentary animals. Laye et al. (34) studied 28-day-old Wistar rats that ran for 6 wk before WL for up to 173 h and reported that skeletal muscle, adipose tissue, and liver palmitate oxidation rates generally were higher in animals that had access to running wheels versus sedentary animals. Roberts et al. (66) recently examined the skeletal muscle translatome of WL5-, WL29-, and WL53-hour animals through polysome expression profiling and discovered that two mechanical stretch sensors (Ankrd2 and Csr3) generally decreased at the total mRNA, polyribosomal mRNA, and protein levels. Interestingly, these genes possess functions in skeletal muscle metabolism and hypertrophy, which may mechanistically link the onset of sedentarism with...
Table 1. Summary of WL studies performed by our group

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<tr>
<th>Study (Year)</th>
<th>Methods Summary</th>
<th>Major Findings</th>
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<tr>
<td>Kump and Booth (2005), (30)</td>
<td>28- to 30-day-old F-BN rats wheel ran for 3 wk and were WL for 5 h (WL5), 29 h (WL29) and 53 h (WL53); aged-matched sedentary animals were also studied (SED)</td>
<td>Submaximal insulin-stimulated 2-DOG uptake in epicthroclearis muscle: WL5/WL29 &gt; WL53/SED (P &lt; 0.05)</td>
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<td>Muscle insulin receptor ligation and signaling: WL5/WL29 &gt; WL53/SED (P &lt; 0.05)</td>
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<td>Muscle GLUT4 protein: WL5/WL29 &gt; WL53/SED (P &lt; 0.05)</td>
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<td>28- to 30-day-old F-BN rats wheel ran for 3 wk and were WL for 5 h (WL5), 10 h (WL10), 29 h (WL29) and 53 h (WL53); aged-matched sedentary animals were also euthanized the same time as WL5 (SED5) and WL10 (SED10)</td>
<td>Body mass: WL5/WL10/WL29/WL53 &gt; SED5/SED10 (P = 0.001–0.05)</td>
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<td>Relative gastroc/plantaris mass: SED5 &gt; WL5 (P &lt; 0.05)</td>
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<td>Food intake over 21-day intervention: WL5/WL10/WL29/WL53 &gt; SED5/SED10 (P = 0.001–0.05)</td>
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<td>Relative epididymal fat mass: WL29/WL53/SED5 &gt; WL5 (P = 0.005–0.05)</td>
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<td>Relative omental fat mass: WL53/SED5 &gt; WL5/WL29 (P = 0.005–0.05)</td>
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<td>Mean cell volume of epididymal fat: WL53 &gt; WL5/WL29/SED (P &lt; 0.05)</td>
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<td>Triacylglycerol synthesis in epididymal fat: WL10/WL29/WL53/SED5 &gt; WL5/SED10 (P = 0.001–0.005)</td>
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<td>21-day food efficiency (g body mass/food intake): all SED groups &gt; all WL groups (P &lt; 0.05)</td>
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<td>Kump et al. (2006), (32)</td>
<td>28- to 30-day-old F-BN rats wheel ran for 3 wk and were WL for 5 h (WL5), 10 h (WL10), 29 h (WL29) and 53 h (WL53); aged-matched sedentary animals were also studied (SED)</td>
<td>Plasma insulin: WL53 &gt; WL5 (P = 0.001–0.05)</td>
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<td>Plasma triacylglycerol: WL53 &gt; WL5/WL29/SED (P = 0.001–0.05)</td>
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<td>Epididymal triglycerol synthesis: SED5 &gt; WL5 (P &lt; 0.01)</td>
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<td>Laye et al. (2007), (35)</td>
<td>21-day-old F-BN rats wheel ran for 6 wk and were WL for 5 h (WL5), WL53 (WL53) and 173 h (WL173); aged-matched sedentary animals were also studied (SED)</td>
<td>Epididymal/perirenal fat mass: WL173/WL173-PF &gt; WL5 (P &lt; 0.05)</td>
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<td>*during WL53 and WL173, a subgroup of these groups were pair-fed (PF) to match SED food intakes</td>
<td>Epididymal fat cell number: WL173 &gt; WL5 (P &lt; 0.05)</td>
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<td>Rector et al. (2008), (61)</td>
<td>28-day-old OLETF rats wheel ran for 16 wk and were WL</td>
<td>Body mass: WL5/WL53/WL173 &gt; SED (P &lt; 0.05)</td>
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<td>Food intake over intervention (g wk⁻¹ g body mass⁻¹): WL5/WL53/WL173 &gt; SED (P &lt; 0.05)</td>
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<td>Omental and retroperitoneal fat mass: SED &gt; WL5/WL53/WL173 (P &lt; 0.05)</td>
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<td>Serum glucose/insulin/triglycerides: SED &gt; WL5/WL53/WL173 (P &lt; 0.05)</td>
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<td>Hepatic triglycerides: SED &gt; WL5/WL53/WL173 (P &lt; 0.05)</td>
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<td>Hepatic triglyceride synthesis: WL5/WL53/SED &gt; SED (P &lt; 0.05)</td>
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<td>Hepatic protein markers for fatty acid synthesis (FAS, SCD-1, PPARγ): SED &gt; WL5/WL53/WL173 (P &lt; 0.05)</td>
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<td>Laye et al. (2009), (34)</td>
<td>21-day-old F-BN rats wheel ran for 6 wk and were WL for 5 h (WL5) and 173 h (WL173); aged-matched sedentary animals were also studied (SED)</td>
<td>ΔBody mass during last week of intervention: only increased within WL173 group (P &lt; 0.05)</td>
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<td>*during the last week of the intervention, WL173 animals had their wheels locked whereas WL5 animals did not</td>
<td>ΔLean mass during last week of intervention: increased in WL5 and SED groups (P &lt; 0.05); did not increase in WL173 group</td>
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<td>Food intake during last week of intervention: WL5 &gt; WL53/SED (P &lt; 0.05)</td>
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<td>Relative epididymal fat mass: WL173/SED &gt; WL5 (P &lt; 0.05)</td>
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<td>Palmitate oxidation (in vitro)</td>
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<td>Red gastrocnemius: WL5 &gt; SED (P &lt; 0.05)</td>
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<td>Epididymal fat adipocytes (normalized to volume of cells): WL5/WL173 &gt; SED (P &lt; 0.05)</td>
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<td>Liver: WL173 &gt; WL5/SED (P &lt; 0.05)</td>
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<td>PGC-1α mRNA expression</td>
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<td>Red gastrocnemius: WL5 &gt; SED (P &lt; 0.05)</td>
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<td>Liver: WL5 &gt; SED &gt; WL173 (P &lt; 0.05)</td>
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<td>Roberts et al. (2012), (66)</td>
<td>28-day-old Wistar rats wheel ran for 3 wk and were WL for 5 h (WL5), 29 h (WL29), 53 h (WL53), and 173 h; aged-matched sedentary animals were also studied (SED)</td>
<td>Expression profiling of polyribosomal RNA in plantaris muscle: 8 mRNAs were altered in WL5 vs. WL53 rats (FDR &lt;0.15)</td>
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<td>mRNAs downregulated encoded mechanical stretch sensors (Csrp3 &amp; Ankrd2)</td>
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<td>Ankrd2 protein tended to decrease in WL53 vs. WL5 rats (P = 0.054)</td>
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<td>Csrp3 protein was lower in WL173/SED vs. WL5 rats (P &lt; 0.05)</td>
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<td>Padilla et al. (2012), (55)</td>
<td>28-day-old Wistar rats wheel ran for 30 days (RUN30) or ran for 23 days and WL 173 h (WL7d)</td>
<td>Total mRNA alterations in iliac artery with WL</td>
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<td>TNFR1: WL7d &gt; RUN30 (P &lt; 0.05)</td>
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<td>Endothelin-1: WL7d &gt; RUN30 (P &lt; 0.05)</td>
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<td>Lipoxygenase-1: WL7d &gt; RUN30 (P &lt; 0.05)</td>
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See text and referenced articles for definitions of abbreviations.
metabolic maladies that ensue later in life. Finally, Padilla et al. (55) discovered that WL173-hour rats exhibited mRNA alterations in the hindlimb iliac artery associated with inflammation [endothelin-1 (ET-1) and tumor necrosis factor receptor 1 (TNFR1)] as well as oxidative stress (lectin-like oxidized low-density lipoprotein receptor-1, LOX-1).

Using Rodent Inactivity Models to Study the Initiation of Juvenile Obesity

Levin’s group assessed how access to voluntary running wheels for different periods of time affected fat mass accrual in juvenile rats selectively bred to become obese (DIO rats). DIO rats aged 28 days old that were subsequently provided access to running wheels for 13 wk gained 22% less body weight and had 39% lighter fat pads compared with their sedentary counterparts (57). Rats that had their running wheels removed after 6 wk: 1) gained less body weight over the last 7 wk compared with rats that never had wheel access; and 2) exhibited similar adipose pad weights relative to rats that had exercised over the entire 13-wk period. Intriguingly, these investigators discovered that 3 wk of wheel access was enough to prevent the development of abdominal obesity for up to 10 wk after wheel removal. The authors attributed the reduced adiposity to an increased expression of arcuate nucleus proopiomelanocortin mRNA during wheel running access and concluded that exercise during juvenile states positively enhances the development of the hypothalamic pathways controlling energy homeostasis. A follow-up study (56) by the same researchers examined leptin sensitivity in 4-wk-old DIO rats that were fed a 31% fat high-energy diet (HE) and were either: 1) given no access to running wheels (Sed), 2) given access to running wheels for 3 wk followed by 10 additional weeks of WL (Ex/Sed), or 3) given access to running wheels for 13 wk (Ex). At 3 and 7 wk of treatments, leptin injections (5 mg/kg ip) decreased 24-h food intake in the Ex and Ex/Sed rats, whereas Sed rats exhibited no alterations in food intake. Ex and Ex/Sed rats also presented more leptin-responsive phospho-STAT3 (pSTAT3) neurons in the arcuate nucleus versus Sed rats. However, by week 10 of running cessation, the anorectic effect of leptin was lost. Finally, after the 13-wk intervention, the Ex and Ex/Sed rats had 58% and 38% less fat, respectively, compared with Sed rats. These data led the authors to conclude that providing juvenile DIO rats (which are inherently leptin resistant) access to voluntary running wheels for merely 3 wk makes them more resistant to obesity for up to 10 wk of no running due to increased central leptin sensitivity. Importantly, data from Levin’s group continues to support the notion that juvenile physical activity combats biomarkers associated with obesity in obesity-prone animals.

Two of our original WL studies (31, 32) examined how the cessation of daily physical activity after 3 wk of voluntary wheel access affected adipose tissue physiology as well as body mass, muscle mass, region-specific adipose tissue mass, daily feeding patterns, and food efficiency (or the change in body mass divided by food intake) in 28- to 49-day-old Fischer 344 × Brown Norway F1 hybrid rats. Kump et al. (31) reported that relative omental fat mass was 30% greater in rats after 53 h of WL compared with rats that had wheel access until euthanized, suggesting that the cessation of physical activity can quickly lead to an accrual of visceral adiposity. In the second study, Kump et al. (32) reported that the circadian rhythm of epididymal adipocyte triacylglycerol synthesis was lost after 53 h of WL (Fig. 1).

Laye et al. (35) subsequently reported that forced inactivity via WL for 7 days after 6 wk of running resulted in significant fat cell hyperplasia (i.e., and increase in fat cell number) in the epididymal fat pad of 63-day-old rats; a finding that was independent of food intake. In a follow-up commentary, Roberts (65) stated that the data from Laye et al. provided invaluable insight into adipogenic mechanisms during childhood given the difficulties in obtaining fat biopsies from youth. Extrapolating these findings to humans suggests that physical inactivity during early childhood may lead to adipose tissue expansion via hyperplasia; an effect that has been posited to impair weight loss capabilities during adulthood (38). Laye et al. (34) subsequently published a second WL study demonstrating that epididymal adipocyte palmitate oxidation was significantly greater in 63-day-old rats that voluntarily ran for 6 wk versus those that never ran. Again, applying these data to humans suggests the hypothesis that fatty acid oxidation in...
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adipose tissue may be impaired in sedentary children. We speculate that this impaired fatty acid oxidation in adipose tissue leads to a rapid expansion in adipose tissue mass in sedentary children which could then continue throughout adolescence and adulthood. Hence, the two WL studies of Laye et al. (34, 35) provided invaluable information regarding how physical inactivity in juvenile rats negatively affects adipose tissue metabolism. In this regard, more research is needed using the WL model to determine whether the reintroduction of voluntary exercise in juvenile rats can revert adipose tissue back to a “healthy” state.

OLETF rats have a spontaneous mutation of the cholecystokinin-1 receptor that renders them hyperphagic and causes obesity and type II diabetes to develop at a young age (49). A recent study (68) used juvenile OLETF rats to determine how access to running wheels affected adipose tissue and feeding characteristics. Relative to sedentary male OLETF rats, running wheel access reduced food intake, adiposity, circulating leptin, and adipocyte size despite an increase in fat cell number. Likewise, OLETF females that were given running wheel access exhibited a reduction in feeding efficiency (i.e., less body mass gained per unit of food intake) and liver fat as well as a significant increase in brown fat. Both genders of OLETF rats that were given running wheel access also presented greater serum adiponectin levels (68).

It is well known that visceral adiposity is a predominant risk factor for the development of the metabolic syndrome in humans and is associated with an increased pro-inflammatory cytokine blood profile as well as lower adiponectin levels (45). Stimulated by the investigations by Kump et al. (30, 31), Bente Pederson’s group reported that a 2-wk reduction in daily steps in 24-yr-old healthy males produced a significant 6.7% increase in intra-abdominal fat mass (52). Slentz et al. (70) also reported that overweight men and women jogging 20 miles per week for 6 mo significantly reduced visceral fat mass by 6.9% while those that did not exercise experienced an 8.6% increase in visceral fat mass without a reduction in caloric intake. A subsequent meta-analysis by Ohkawara et al. (51) reported that subjects (group means ranged from 22 to 67 yr old) performing at least 10 MET-h per week exhibited a dose-dependent decrease in visceral fat mass with aerobic exercise training. These human data demonstrate that regular physical activity is associated with lower visceral adiposity. Importantly, our juvenile rat WL model parallels human findings suggesting that regular physical activity suppresses gains in visceral adiposity; perhaps through decreases in fat cell mass through diminished triacylglycerol synthesis rates. Our findings also suggest that 1) visceral fat mass accrual is greater in either sedentary or WL juvenile rats compared with more physically active rats; and 2) that the persistent lack of regular physical activity in young animals may be an early initiator in the development of obesity-related comorbidities including diabetes, systemic low-grade inflammation, and atherosclerosis earlier in life.

Transgenerational Inheritance of Obesity with Maternal Inactivity

A recent study reported that allowing juvenile rats access to running wheels could attenuate the transgenerational inheritance of metabolic diseases from obese/sedentary high-fat diet (HFD)-fed Sprague-Dawley dams (59). In the aforementioned study, the offspring were either fed standard chow or a HFD. Relative to chow-fed mothers, maternal obesity induced by HFD-feeding during pregnancy increased the body weight of juvenile offspring by 12%, increased plasma lipids, and reduced glucose tolerance. These effects were significantly augmented by postweaning HFD feeding in juvenile offspring, albeit allowing running wheel access to the juvenile offspring of obese HFD-fed dams significantly reduced fat mass, plasma lipids, homeostatic model assessment (HOMA), and fasting glucose. Human data suggests that women who continue regular physical activity in the last two trimesters of pregnancy have less risk for high offspring birth weights (24). However, well-controlled human studies examining how structured exercise regimens in concert with nutritional status during pregnancy effect offspring birth weight and subsequent health markers are apparently lacking and future studies are needed (24). Nonetheless, the aforementioned animal data provide important preliminary evidence suggesting that the transgenerational inheritance of metabolic maladies can be offset in offspring that exhibit increased physical activity patterns.

Using the Wheel Lock Model to Study the Initiation of Juvenile Insulin Resistance

In our original WL study (30), it was determined that skeletal muscle glucose uptake and insulin signaling became significantly reduced after 2 days of WL in 49-day-old lean Fischer 344 × Brown Norway F1 hybrid rats that had run 3 wk prior. Kump’s subsequent data (32) revealed that plasma insulin significantly increased after 53 h of WL. These findings are similar to previous human research whereby insulin sensitivity was significantly reduced in as little as 38 h to 7 days following cessation of habitual exercise in endurance-trained athletes (3, 10, 21, 25, 54), albeit these investigations examined adults that discontinued structured exercise regimens. As a follow-up to Kump’s animal findings (30), Pederson’s group determined that a 2-wk reduction in step count (from 10,501 to 1,344) in 24-year-old humans significantly reduced insulin sensitivity and Akt-mediated insulin signaling as assessed during a hyperinsulinemic-euglycemic clamp test (29). Thyfauld and coworkers (47) subsequently reported that a 3-day reduction in physical activity (from 12,956 to 4,319 steps per day) significantly impaired glycemic control in 29-year-old healthy individuals as assessed by continuous glucose monitors. Taken together, these data suggest that substantially reducing daily physical activity levels in juvenile rats and adult humans causes a dysregulation in insulin signaling and glucose uptake; these being biomarkers that precede the development of insulin resistance, obesity, and diabetes. Importantly, the adult human data continue to validate the use of the rodent WL model to reveal mechanisms concerning how juvenile animals develop diabetes.

Using the Wheel Lock Model to Study the Initiation of Nonalcoholic Fatty Liver Disease in OLETF Rats

Nonalcoholic fatty liver disease (NAFLD) is a condition whereby a hepatic resistance to insulin-stimulated glucose uptake exists and symptoms can range from simple steatosis (i.e., retention of fat) to hepatocellular damage coupled with inflammation (41). Interestingly, the prevalence of NAFLD parallels increases in obesity trends as 17% of a cohort of 15-
to 19-yr-old US adolescents have been reported to present symptoms of NAFLD (69) and 17% of adolescents are obese (2). Furthermore, NAFLD is thought to initiate during childhood as it has been reported to occur in children as young as 3 yr old (42). Rector et al. (61) used the WL model to determine whether 4-wk-old OLETF rats that were given access to voluntary running wheels for 16 wk were able to prevent the development of hepatic steatosis. WL was then applied to these animals from 5 to 173 h, which transitioned them to a sedentary condition. Complete hepatic fatty acid oxidation and animals from 5 to 173 h, which transitioned them to a sedentary condition. Complete hepatic fatty acid oxidation and synthesis as well as an increase in hepatic malonyl-CoA concentrations. Together, these changes in the liver likely increase susceptibility to NAFLD. Thus the animal WL model provides valuable data relative to the mechanisms whereby physical activity blunts and/or prevents the initiation of NAFLD.

**Using the Wheel Lock Model to Study the Potential Initiation of Sarcopenic Obesity**

Sarcopenic obesity is the convergence aging and obesity whereby skeletal muscle loss with aging is accelerated with an increase in adiposity (8). Nonetheless, while maintaining a physically active lifestyle throughout an individual’s lifetime has been posited to combat age-related losses in muscle mass and increases in adiposity (8), we suggest that sarcopenic obesity observed during late adulthood may be initiated in children that remain sedentary. In this regard, Kump et al. (31) reported that soleus, gastrocnemius/plantarlis, quadriceps, triceps, trapezius, and latissimus dorsi muscle weights were significantly less in sedentary rats versus rats that ran for 3 wk between the ages of 28–49 days of age before WL. In contrast, Laye et al. (34) determined that juvenile rats that had access to running wheels for 6 wk and were wheel locked for 173 h presented impairments in total body lean muscle mass growth over the 1-wk wheel lock period compared with rats that continued to run during this period, which was associated with a 4% increase in lean mass. Hence, these two reports demonstrate that skeletal muscle growth in juvenile rats is enhanced with regular physical activity.

We recently employed the juvenile WL model to unveil intramuscular mechanisms that occur within hours of reduced voluntary activity (66). Specifically, we employed mRNA expression profiling of intramuscular polyribosomal fractions to identify targets that were altered at the mRNA and protein levels up to 7 days of WL in 49-day-old rats that ran 3 wk prior. We hypothesized that: 1) polyribosomal mRNA profiling would act as a filter to identify mRNAs that were altered at the translational level, and 2) identified targets could be used to examine upstream intramuscular signaling pathways initiated by inactivity. Interestingly, polyribosomal mRNA expression profiling identified two muscle-specific mechanosensors (Ankrd2 and Csrp3/muscle LIM protein) that were downregulated by 53 h of WL. RT-PCR confirmed these targets were downregulated at the polyribosomal and total mRNA levels, and Western blotting analyses indicated both proteins either tended to be (Ankrd2) or were downregulated (Csrp3) in rats that underwent 53 h of WL. Interestingly, a recent study (7) has used small interfering RNA (siRNA)-mediated Ankrd2 knockdown in myotubes to illustrate that this protein affects mRNA expression patterns of genes related to insulin signaling, focal adhesion, mitogen-activated protein kinase (MAPK) signaling, cytokine receptor signaling, Wnt signaling, and p53 signaling among other identified pathways. Likewise, Lieber’s group has examined the skeletal muscle architecture of Ankrd2- (5) and Csrp3- (6) knockout mice housed without running wheels and reported that these two genes regulated skeletal muscle architecture. Hence, our recent WL study highlights early mechanistic intramuscular changes that occur when active juvenile animals become less active which, in turn, may initiate metabolic abnormalities in skeletal muscle architecture and mass that ensue in late life.

**Using Inactivity Models to Study the Initiation of Vascular Dysfunction in Juvenile Rats**

The rapid increase in the prevalence of childhood obesity is likely to lower the age of onset for cardiovascular disease (4). AHA Statements published in 2011 include: “The rapid increase in the prevalence and severity of obesity in children is likely to lower the age of onset and increase the incidence of cardiovascular disease worldwide.” Concern about the epidemic of pediatric obesity have prompted a new sense of urgency for primordial and primary prevention strategies during childhood (4); and “Preclinical substrates for clinical cardiovascular disease (e.g., fatty streaks and atherosclerosis) begin early in life and are influenced over time by potentially modifiable risk factors, behaviors, and environmental exposures” (79). Nonetheless, while “preclinical substrates” related to atherosclerosis are evident early in life in those that are predisposed to develop overt cardiovascular disease later in life, little is known regarding how atherosclerosis is mechanistically initiated in children/adolescents. We recently conducted a WL study in juvenile rats to evaluate whether short-term inactivity alters proatherogenic vascular mRNA levels in conduit arteries perfusing the hindlimb skeletal muscle (e.g., iliac artery) and arteries feeding noncontracting organs (e.g., renal artery). Twenty-eight-day-old rats were given access to running wheels for 23 days. Rats in one group were transitioned to a sedentary state by locking the wheels for 7 days, whereas rats in the second group were allowed access to the running wheel and remained active for an additional 7 days. Seven days of inactivity significantly increased mRNA levels of proatherogenic genes such as TNFR1, LOX-1, ET-1, in the iliac, but not renal, artery; thus suggesting that cessation of voluntary wheel running produces differential changes in mRNA levels between the iliac and renal arteries of juvenile rats (55). We hypothesize that this heterogeneous influence of short-term physical inactivity is attributed to the distinct alteration in hemodynamic forces between arteries resulting from the decreased physical activity. The finding that vascular ET-1 gene expression is upregulated following 1 wk of inactivity may be of particular relevance in view of evidence from human studies indicating that increased leg vascular tone of extremely inactive legs of spinal cord injury patients is largely ET-1 mediated (77).
Findings in human and other animal models indicate that physical inactivity confers negative vascular consequences. For example, improvements in rat aortic endothelium-dependent dilation associated with exercise training (1 h/day treadmill running) are partially lost within 2 days and completely lost within 1 wk of detraining (19). Physical inactivity was also shown to induce endothelial dysfunction in an interesting study in which young mice were either housed in groups of five per cage or individually (73). The mice in grouped housing were documented to engage in “running, climbing, and fighting during their active cycle,” whereas the single-housed mice exhibited mostly inactive behavior. The inactive single-housed mice displayed a ~45% reduction in endothelium-dependent dilation compared with the active controls and an impairment in endothelial nitric oxide synthase activity. It is important to note, however, that the only reported index of improved physical activity in the aforementioned study was improvements in skeletal and cardiac muscle citrate synthase activity. Finally, a recent study indicated that as little as 1 day of physical inactivity can adversely and profoundly affect endothelial phenotype in humans (27). Circulating levels of microparticles shed from activated (CD62E+) and apoptotic (CD31+/CD42b−) vascular endothelial cells were measured in plasma of regularly active healthy young men under two experimental conditions. In one condition, subjects performed a laboratory exercise protocol designed to be similar to one of their regular training bouts (i.e., 60% maximal oxygen uptake for ~50 min on the prior day). In the other condition they were instructed to perform as little physical activity as possible on the day before testing. The results indicated that 1 day of physical inactivity caused a ~2.5-fold increase in plasma concentrations of activated endothelial cell markers (CD62E+ endothelial microparticles) and a 75% increase in the markers of apoptotic endothelium (CD31+/CD42b−). Importantly, CD62E (or E-selectin) is expressed exclusively on endothelial cells in response to activation by an inflammatory or pro-oxidant insult, whereas CD31+/CD42b− microparticles derive from endothelial cells that have undergone cell death and subsequent shedding from the vascular wall (12). These results suggest that in humans, physical inactivity induces a rapid and profound increase in endothelial damage and death through mechanisms that require substantial further investigation.

Taken with these previous results from human and animal studies, we propose that more WL studies in juvenile animals are needed to: 1) determine whether vascular function is concomitantly affected during WL as in previous studies of exercise cessation (19); 2) continue examining whether longer WL periods affect gene targets implicated in the development of atherosclerosis in humans; 3) examine whether the reintroduction of running wheels and/or other exercise modalities reverts the mRNA expression patterns of pro-atherogenic/pro-oxidant genes; and 4) examine the molecular initiating events underlying acute endothelial cell activation and death induced by physical inactivity that was recently documented in healthy humans.

Conclusions: Strengths From Multiple Interpretations of WL Models

The studies summarized herein suggest that obesity-associated maladies including skeletal muscle insulin resistance, hypothalamic leptin resistance, fatty acid oxidation impairments in skeletal muscle and adipose tissue, nonalcoholic fatty liver disease, and endothelial dysfunction are initiated in juvenile animals that are restrained from voluntary exercise (summarized in Fig. 2).

Various interpretations are available for gains in adiposity that arise upon the cessation of physical activity in juvenile rodent WL models. A first interpretation is that the WL model is a tool to study mechanisms of how reductions in physical activity cause increases in visceral adipose tissue in childhood obesity. Data from the model, in our opinion, could be translated to modern-day living conditions in which young children decrease physical activity when summer ends. Upon starting school in the fall, many children ride in motor vehicles to school, sit in classrooms, and often are denied either sufficient recess or physical education classes, or both. Given that sedentary behavior in children (39, 40) and animals (76) is linked with an increased adiposity, we favor this interpretation of the model. Likewise, the model is valuable because visceral adipose tissue biopsies in children and adults participating in intervention studies are unethical. A second interpretation could be that the WL model is a tool to studying “rebound mechanisms” and/or “catch-up fat” since neither locomotor x-y-z activity nor energy expenditure has been previously determined; better stated, WL animals may experience swift initial increases in adiposity with the cessation of physical activity followed by a normal growth curve thereafter. If the WL model is indeed a template for this phenomenon, then we are in agreement with Dulloo (15) who provides evidence to suggest that people who exhibit “catch-up growth” have higher susceptibility to abdominal obesity, glucose intolerance, type 2 diabetes, and/or cardiovascular diseases later in life. Finally, a third interpretation has been made with the aforementioned WL model in which a 30% high-fat diet (that causes diet-induced obesity in the animals of the study) was fed to rats that were selectively bred to be genetically prone to obesity. With this model, Patterson et al. (57) showed that 3 wk of early-onset voluntary wheel running prolongs increases in visceral adiposity for 10 wk in rats selectively bred to develop diet-induced obesity. As stated previously, their interpretations of results include: 1) early-onset exercise may favorably alter the development of the hypothalamic pathways controlling energy homeostasis during brain development; 2) rats had a 55% increase in arcuate nucleus proopiomelanocortin mRNA expression versus rats never voluntary run in wheels, suggesting that this may have contributed to their sustained obesity resistance; and 3) a potential postnatal window of opportunity exists for physical activity to modify critical pathways to adjust body fat may exist. Taken together, multiple interpretations and usages of this model are, in our opinion, a strength of the WL model that can be used as a tool to uncover mechanisms related to energy balance.

Perspectives and Significance

Inactivity models of rodents in early life stages have just begun to be exploited for purposes of identifying candidate biomarkers and risk factors that initiate a sequential chain of major chronic diseases associated with obesity (i.e., cardiovascular disease, NAFLD, insulin resistance, proinflammatory phenotype during obesity, sarcopenic obesity). Interestingly,
resultant juvenile rodent data have predicted results from recent work performed in younger human adults (29) who similarly demonstrate that reduced ambulatory activity (independent of food intake) initiates a reduction in insulin sensitivity, decreases leg muscle mass, and increases in omental fat mass. The following bullet points are only a few recommendations as to how the scientific community can continue using juvenile rodent inactivity models:

- Use longer-term juvenile rodent WL studies (i.e., months) to examine how the initial tissue-specific events presented herein progress toward risk factor phenotypes during adulthood.
- Use longer-term juvenile WL studies to examine whether inactivity-related disease phenotypes affect serum metabolomic profiles. In this regard, new screening methodologies can allow for over 2,000 serum metabolites to be assayed, which can be indicative of perturbations in various tissues (74). Thus examining how WL affects an array of serum metabolites may 1) continue to shed insight into which tissues are most affected with inactivity (for instance, examine whether WL affects metabolites associated with liver, kidney, or muscle perturbations); and 2) allow for the identification of novel biomarkers that are altered with sedentarism and can be assayed via minimal invasiveness (i.e., blood draws from children).
- Follow longer-term juvenile WL studies with the reintroduction of exercise paradigms (i.e., give wheel access 1–3 days/wk or perform treadmill training) to establish how much “minimal” activity is needed to maintain healthy tissue and/or serum metabolomic phenotypes throughout adulthood. Classical literature has established how different treadmill training intensities and frequencies affect skeletal muscle phenotypes (20, 22). Likewise, it is well known that exercising previously sedentary adults improves various metabolic parameters. However, it is not known how much physical activity is needed to optimize tissue-specific metabolic processes throughout childhood and adulthood. Hence, allowing juvenile rats limited wheel access and examining the dose-dependent effects that voluntary physical activity exhibits on tissue phenotypes is warranted.
- Examine how short-term and longer-term juvenile WL studies affect exercise motivation during adulthood. In this regard, Rhodes et al. (64) demonstrated that mice undergoing WL had high Fos immunoreactivity (Fos+/− cells, biomarker for neuronal activation within various brain regions) in 16 of 25 brain regions compared with mice that continued to run; these regions included: caudate putamen, prefrontal cortex, medial frontal cortex, nucleus accumbens, amygdala, piriform cortex, paraventricular hypothalamic nucleus, ventral anterior thalamic nucleus, sensory cortex (trunk region), lateral hypothalamus, substantia nigra, lateral periaqueductal gray, dorsal raphe nucleus, cuneiform nucleus, pedunculo tegmental nucleus, and pontine nucleus. Conversely, high voluntary runners exhibited a greater percentage of Fos−/+ cells in the dendrite gyrus, medial entoral cortex, hippocampal subregions CA2/3, and bed nucleus of the stria terminalis. §2 and §3: exercise has been shown to increase regional cerebral blood flow to the brain (reviewed in Ref. 58), and chronic endurance exercise has been shown to increase brain mass (14) and markers of cognition in elder persons (2). TG, triacylglycerol.

Fig. 2. Summary of how acute and chronic sedentarism affects various tissues. ‡1: Rhodes et al. (64) demonstrated that mice undergoing WL had high Fos immunoreactivity (Fos+/− cells, biomarker for neuronal activation within various brain regions) in 16 of 25 brain regions compared with mice that continued to run; these regions included: caudate putamen, prefrontal cortex, medial frontal cortex, nucleus accumbens, amygdala, piriform cortex, paraventricular hypothalamic nucleus, ventral anterior thalamic nucleus, sensory cortex (trunk region), lateral hypothalamus, substantia nigra, lateral periaqueductal gray, dorsal raphe nucleus, cuneiform nucleus, pedunculo tegmental nucleus, and pontine nucleus. Conversely, high voluntary runners exhibited a greater percentage of Fos−/+ cells in the dendrite gyrus, medial entoral cortex, hippocampal subregions CA2/3, and bed nucleus of the stria terminalis. §2 and §3: exercise has been shown to increase regional cerebral blood flow to the brain (reviewed in Ref. 58), and chronic endurance exercise has been shown to increase brain mass (14) and markers of cognition in elder persons (2). TG, triacylglycerol.
Continuing to examine the effects of inactivity on physiological homeostasis during youth is crucial given that 58% of children between the ages 6–11 yr fail to obtain the recommended 60 min/day of physical activity and 92% of adolescents fail to achieve this goal (78). Importantly, we contend that using juvenile rodent inactivity models will continue to provide a valuable clinical translational tool that can be used for primordial prevention of human childhood obesity.

REFERENCES


