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Effects of early-onset voluntary exercise on adult physical activity and associated phenotypes in mice

Wendy Acosta^a, Thomas H. Meek^{a,b}, Heidi Schutz^{a,c}, Elizabeth M. Dlugosz^{a,d}, Kim T. Vu, Theodore Garland, Jr.^{a,*}

^a Department of Biology, University of California, Riverside, CA 92521, USA

^b Present address: Diabetes and Obesity Center of Excellence, University of Washington, Seattle, WA 98109, USA

^c Present address: Department of Biology, Pacific Lutheran University, Tacoma, WA 98447, USA

^d Present address: Department of Plant Sciences, University of Tennessee, Knoxville, TN 37996, USA

*Author for correspondence (e-mail: tgarland@ucr.edu)

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ABSTRACT

The purpose of this study was to evaluate the effects of early exercise on adult physical activity (wheel running, home-cage activity), body mass, food consumption, and circulating leptin levels in males from four replicate lines of mice selectively bred for high voluntary wheel running (High Runner or HR) and their four non-selected control (C) lines. Half of the mice were given wheel access shortly after weaning for three consecutive weeks. Wheel access was then removed for eight weeks, followed by two weeks of adult wheel access for all mice. A blood sample taken prior to adult wheel testing was analyzed for circulating leptin concentration. Early-life wheel access significantly increased adult voluntary exercise on wheels during the first week of the second period of wheel access, for both HR and C mice, and HR ran more than C. During this same time period, activity in the home cages was not affected by early-age wheel access, and did not differ statistically between HR and C mice. Throughout the study, all mice with early wheel access had lower body masses than their sedentary counterparts, and HR mice had lower body masses than C mice. With wheel access, HR mice also ate significantly more than C mice. Early-life wheel access increased plasma leptin levels (adjusted statistically for fat-pad mass as a covariate) in C mice, but decreased them in HR mice. At sacrifice, early-life exercise had no statistically significant effects on visceral fat pad, heart (ventricle), liver or spleen masses (all adjusted statistically for variation in body mass). Results support the hypothesis that early-age exercise in mice can have at least transitory positive effects on adult levels of voluntary exercise, in addition to reducing body mass, and may be relevant for the public policy debates concerning the importance of physical education for children.

Keywords: Early-life factors; Exercise; Food consumption; Genotype-by-environment interaction; Selection experiment; Spontaneous physical activity; Wheel running

1. Introduction

Human obesity and its negative health consequences are caused by interactions among diet, level of physical activity, environmental factors, sex, genetic predisposition, and socio-cultural factors [1-3]; [4]; [5]; [6]; [7]; [8]; [9]. Like obesity itself, levels of physical activity and diet/caloric intake are products of both genes and numerous environmental effects acting across ontogenetic development. Some human studies have identified early-life risk factors for a sedentary lifestyle (e.g., [10]; [7]) and parental characteristics that are somewhat predictive of adolescent physical activity [11]. Overall, however, early-life environmental determinants of adult physical activity levels are poorly understood [12]; [13]; [14, 15]; [16];[17]; [18] ; [19];[20].

Given that exercise is a fundamental tool in metabolic health and the control of body weight, an essential line of questioning pertains to understanding its regulation and programming ([21]; [22]). The dramatic increases of in the prevalence of obesity [23] heightens the need for new insights into mechanisms that govern energy balance and voluntary activity levels. This is especially critical because recovery from long-term obesity is particularly difficult due to an elevated defended body weight. Consistent with this concept, both juvenile obesity and diabetes tend to persist into adulthood, rendering such preventative measures as early exercise exposure a potentially useful option with long-term, beneficial effects [24]. Accumulating evidence suggests that early-life experiences can alter adult levels of voluntary exercise (VE) and/or spontaneous physical activity (SPA). For example, in a prospective birth-cohort study using accelerometers, parents' physical activity during pregnancy and early in the child's life showed a modest positive association with the child's physical activity at 11-12 years of age [11]. Less direct evidence comes from studies of individuals exposed to famine. Those exposed to the Dutch famine during gestation have increased adiposity and more atherogenic lipid profiles in later life that may be related to decreased physical activity [25]; [7]. Individuals exposed to the Chinese famine during

fetal life or infancy have an increased risk of metabolic syndrome in adulthood [20]. However, as these sorts of studies are not experimental (no interventions applied), it is difficult to identify causal relationships.

Animal models are widely used for studies of early-life effects because they allow manipulations that would be neither feasible nor ethical in humans [26]. A few rodent studies demonstrate that juvenile exercise can affect adult activity levels. In male rats, 3 weeks of wheel access begun at 36 days of age reduced weight gain over the next 10 weeks [27]. Three weeks of post-weaning exercise in leptin-resistant rats bred to develop diet-induced obesity caused a sustained resistance to obesity on high-fat diet, in part due to increased central leptin sensitivity [28]. In rats genetically prone to early-onset, hyperphagia-induced obesity, post-weaning voluntary exercise for ~3 weeks caused long-term moderation of adiposity in males but not females [29].

In the present study we examined the effects of early-life wheel access on adult physical activity in a unique, genetically defined animal model, four selectively bred High Runner (HR) mouse lines and their four non-selected Control (C) lines [30-32]. The HR and C lines differ markedly in both voluntary wheel-running behavior [32] and baseline activity in the home cage when wheels are absent [33] [34], which also allows tests for genotype-by-environment interactions. Previous studies of a subset of these lines have identified quantitative trait loci (QTL) that influence voluntary wheel running and body composition (e.g. [35]; [36]; [37]; [38]; [39], but the importance of early-life environmental factors is unknown. Furthermore, using lines of mice with a wide range of running distances allows for assessment of possible threshold effects, or plateaus in benefit. Additionally, use of multiple genetic strains to some extent better mimics human ethnic and racial diversity in proneness to physical activity, obesity, metabolic syndrome, and related diseases (e.g., see [40]; [41]; [42].

Based on associations observed in humans (e.g., [40] several differences between HR and C mice suggest they are likely to respond differently to early-life factors. For example, as compared with C, HR mice have higher VO₂max and endurance during forced exercise, an altered brain reward system, elevated baseline circulating corticosterone levels (for possible relevance, see [43], and increased depressive-like behaviors when wheel-deprived [44]; [45]; [46]; [47]; [33]; [48]; [49]. Moreover, previous studies document significant genotype-by-environment interactions in adult HR vs. C mice challenged with Western diet and housed with versus without wheels ([50] [51] [52]; [48]).

2. Materials & procedures

2.1. Experimental animals

Mice were from an artificial selection experiment that breeds for high voluntary wheel running activity [32]. Briefly, the base population was 224 outbred, genetically variable Hsd:ICR house mice. After two generations of random mating in our animal facility, 10 pairs of mice were used to create each of eight closed lines, four of which were randomly designated and bred for high running (HR) on wheels and the other four were the control (C) lines bred without regard to wheel running. During the normal selection experiment process, mice approximately 6-8 week old are individually housed in standard cages attached to a Wahman-type activity wheel (1.12 m circumference, 35.7 cm diameter, 10 cm wide running surface). Wheels are interfaced to a computer, which records revolutions in 1-minute intervals continuously for 6 days of wheel testing. Breeders for the next generation are chosen based on their wheel running on days 5 and 6. Within-family selection is applied. For the HR lines, the highest-running male and female within each family are chosen as breeders, whereas a random male and female are chosen from each family within the C lines (disallowing sib mating in all lines). Room temperatures are maintained

at approximately 22°C. Lights were on at 0700 with a 12:12 photoperiod. Water and food (Harlan Teklad Laboratory Rodent Diet [W]-8604) are available *ad libitum*. Pregnant dams are given a breeder diet (Harlan Teklad Mouse Breeder Diet [S-2335] 7004) through weaning.

2.2. Early-life wheel access

Males from generation 59 were weaned at 21 days of age and housed individually in standard cages (Total N = 98). Half of the experimental mice were allowed wheel access when they were approximately 24 ± 1 days old for a total of 21 days. The other subset of mice remained with their wheel access blocked and were designated as the young sedentary group. All mice had their home-cage activity (HCA, also referred to as spontaneous physical activity) monitored (see below). In addition, all mice had their food consumption and body mass monitored weekly throughout the experimental period.

2.3. Adult wheel testing

Wheels were removed after 3 weeks and all individuals remained in standard home cages for an additional 7⁺ weeks (52 days). Following this sedentary phase, all mice were then granted wheel access for 2⁺ weeks (16 days), with continued monitoring of home-cage activity, food consumption, and body mass.

2.4. Home-cage activity

Home-cage activity (HCA) was monitored using passive infrared motion-detector sensors [34] similar to [53]) that detect movement within the standard housing cages attached to the wheels. Sensors were interfaced to a Macintosh personal computer that had custom Activity Recording Software developed by Dr. Mark A. Chappell. The software measured activity summed over every 1-minute interval for 23 hours. The computer records 3 times per second and reads if there is movement (1) or no movement (0). Recordings are then averaged over 1-minute

intervals and given values with arbitrary units. Total activity values in each 1-minute interval were summed to get total HCA for the entire daily period. The number of 1-minute intervals that show *any* HCA were also computed and tallied to indicate the duration (minutes) of HCA during the entire daily period. Dividing daily activity by the number of 1-minute intervals with any activity then gives an indication of the average intensity of activity when active. We also determined the single minute with the highest amount of HCA. All of these HCA measures had direct parallels from the wheel-data analyses [34]. Data for both HCA and wheel running were downloaded daily between 1200-1300 hours.

2.5. Blood sampling, leptin assay, dissections

Prior to the second wheel-testing period, mice were anesthetized with isoflurane and bled through the retroorbital sinus [54]. Blood samples were spun at 13,000 RPM for 12 minutes and collected plasma was stored at -20°C. Plasma leptin was measured using a Millipore Enzyme-linked Immunosorbent Assay (ELISA) kit (Mouse Leptin Assay Catalog # EZML-82K). Plasma samples were diluted and measured in duplicate in 96-well plates. Absorbances were read at 450 nm in a SpectraMax Plus microplate reader (Molecular Devices, Sunnyvale, CA, USA) and compared with a standard curve generated individually for each plate.

After the 2-week period of adult wheel testing, mice were euthanized via CO₂ asphyxiation and dissected. Body mass and body length measurements from the tip of the nose to the base of the tail were taken. Visceral fat pad (combined visceral perirenal, periovaric, parametrial, and perivescical fat masses [55] along with heart ventricles, liver, and spleen were dissected and weighed. All methods were approved by the Institutional Animal Use and Care Committee of the University of California, Riverside.

2.6. Statistical analyses

Sample sizes were chosen based on previous experimental studies of these lines of mice. Analyses were performed using the Mixed Procedure in SAS 9.1.3 (SAS Institute, Cary, NC, USA) to apply analysis of covariance models with Type III tests of fixed effects and REML estimation. Linetype (HR or C) and wheel access (if applicable) were fixed effects; line was nested within line type as a random effect. Effects of linetype, wheel access, and their interaction were tested relative to the variance among replicate lines, and degrees of freedom were always 1 and 6. Covariates depended on the trait being analyzed and included age, body mass, wheel freeness (an inverse measure of rotational resistance), home cage sensor calibration (measure of sensor motion sensitivity), and total wheel running. Dependent variable and/or covariates were transformed as necessary to improve the homogeneity of the spread of the covariates, linearity of relations with covariates, and normality of residuals. All P values are 2-tailed unless otherwise indicated.

3. Results

3.1. Body mass

Early-life wheel access tended to decrease body mass in both Control and High Runner lines of mice (**Fig. 1**), but the effect was never statistically significant (Online Supplemental Material Tables S1-S3). Mice from HR lines were significantly lighter than C from week 3 through the end of the experiment. No interaction between early-life wheel access and linetype was ever statistically significant. A repeated-measures analysis supported the foregoing results, with a highly significant effect of week ($F = 135.07$; d.f. = 12,72; $P < 0.0001$), a trend for wheel access to reduce body mass ($F = 3.99$; d.f. = 1,6; $P = 0.0926$), a trend for HR mice to be smaller

than C mice ($F = 4.93$; d.f. = 1,6; $P = 0.0682$), a strong week-by-linetype interaction ($F = 4.11$; d.f. = 12,72; $P < 0.0001$), no week-by-early-life exercise interaction ($F = 0.77$; d.f. = 12,72; $P = 0.6745$), and no week-by-early-life exercise-by-linetype interaction ($F = 0.45$; d.f. = 13,72; $P = 0.9450$).

3.1. Food consumption

With body mass as a covariate, food consumption during week 3 of early-life wheel access (**Fig. 2A**) was significantly increased by wheel access in HR mice, but not in C (wheel access by linetype interaction $P = 0.0183$). Results were similar during week 2 (Supplemental Material Table S4). During the first week of adult wheel access (**Fig. 2B**), food consumption was higher in HR mice (linetype $P = 0.0310$), but was not affected by early-life wheel access ($P = 0.8793$; interaction $P = 0.2301$). Similarly, during the second week of adult wheel access, food consumption was higher in HR mice (linetype $P = 0.0470$), with neither an effect of early-life wheel access ($P = 0.7845$) nor an interaction ($P = 0.9992$) (Supplemental Material Table S6).

3.1. Wheel running

During week 3 of early-life wheel access ($N = 47$), HR mice ran approximately 6-fold more revolutions per day than C ($P = 0.0018$) (**Fig. 3A**). When tested as adults ($N = 89$), mice that experienced early-life exercise ran more than sedentary individuals ($P = 0.0168$), HR mice ran more than C ($P = 0.0021$), with no statistical interaction ($P = 0.2245$) (**Fig. 3B**). During the second week of adult wheel access (Table S7), HR mice continued to run more than C mice ($P < 0.0001$), but the effect of early-life wheel access had disappeared ($P = 0.9261$), with no linetype-by-early exercise interaction ($P = 0.5929$).

3.1. Home-cage activity

During the third week of early-life wheel access (**Fig. 4A**), all mice with wheels had reduced spontaneous physical activity in their home cages ($P < 0.0001$), but the reduction was greater in HR mice than in C (interaction $P = 0.0388$). During the first week of adult wheel access, HCA was not statistically affected by any factor (**Fig. 4B**: early exercise $P=0.8773$, linetype $P=0.5155$, interaction $P=0.9728$). Results were similar during the second week of adult wheel access (early exercise $P=0.3991$, linetype $P=0.5069$, interaction $P=0.5779$: Supplemental Material Table S7).

3.1. Plasma leptin concentrations

Plasma leptin levels (measured one week prior to adult wheel testing) were strongly positively correlated with fat pad mass in both C (**Fig. 5A**) and HR (**Fig. 5B**) mice. Adjusted for fat-pad mass as a covariate, leptin levels were increased in C mice that had early-life wheel access, but decreased in HR mice that had early exercise (**Fig. 5C**: linetype x wheel access interaction $P = 0.0206$).

3.1. Organ masses

As shown in the Supplemental Material (Table S8), early-life exercise did not have a statistically significant effect on fat pad mass, heart ventricle, liver or spleen masses (with either body mass or body length as a covariate), nor did we find any interaction effects. However, early-life exercise did tend to reduce fat pad mass in both C and HR mice ($P = 0.1228$, adjusted for body mass as a covariate). In addition, HR mice tended to have had larger hearts (adjusted for body mass) than C mice ($P = 0.0616$).

4. Discussion

We tested the hypothesis that early-life exposure to exercise (voluntary wheel running) would affect the propensity to exercise in adult mice. Consistent with our hypothesis, three weeks of wheel access beginning at weaning significantly increased adult wheel running in both genetically selected High Runner lines and in non-selected control lines (Fig. 3B), whereas simultaneously measured home-cage activity was unaffected (Fig. 4B). However, this positive effect on adult wheel running was transitory, occurring during the first week of adult testing but not the second.

Wheel access caused a statistically significant increase in mass-adjusted food consumption by juvenile HR but not C mice (Fig. 2A, Table 4). The explanation for a lack of increase in C mice is unclear, but a few previous studies of other strains of mice have also reported no increase in food consumption with wheel access ([56, 57]). In general, the effect of wheel access on food consumption will be fairly directly related to the amount of running in rodents, and so should be greater in strains that run more (e.g., see [58]). Our observation for C mice is of interest, as it suggests modest levels of exercise can perhaps lower body mass without necessarily triggering homeostatic compensatory responses in food intake. If true, and if this relationship exists in humans, then it could prove of value for ultimately determining recommended daily exercise criteria.

In adult mice, no effect of early-life wheel access on mass-adjusted food consumption was detected (Fig. 2B, Supplemental Material Tables S5 and S6). Overall, mice with wheel access as juveniles tended to be smaller than no-wheel-access mice, but the effect was not statistically significant (Fig. 1 and Online Supplemental Material Tables S1-S3). Reductions in growth rate and body mass caused by chronic wheel access have been reported many times previously in rodents, including in the HR and C lines of mice ([59]; references therein).

Leptin is a hormone secreted by adipocytes, generally in proportion to fat mass. In the present study, as would be expected, visceral fat pad mass was a strong positive predictor of circulating leptin concentrations (Fig. 5). Leptin affects neuroendocrine regulation of body weight by inhibiting food intake and/or increasing energy expenditure. We found that early-life wheel access increased adult plasma leptin levels (adjusted statistically for fat-pad mass as a covariate) in C mice, but decreased them in HR, thus demonstrating a genotype-by-environment interaction (Fig. 5). Previously, we found that long-term wheel exposure results in low body fat and leptin levels in HR mice, and that either Western diet (high in fat and with added sucrose) or exogenous leptin treatment increased wheel running in adult male HR mice ([50-52]. This work, together with the current observations, suggests leptin availability is one of the contributing factors regulating wheel running in HR mice. Moreover, leptin appears to play a role in the bidirectional control of activity. This assertion is supported by the fact that restoration of low leptin levels promotes even greater running distances in HR mice, indicating that insufficient leptin concentrations may limit wheel running by serving as a “stop” signal to reduce motivation for activity. Furthermore, elevated levels of leptin, whether through chronic inactivity (Fig. 5A) or experimental high fat diet feeding [51], appear to initiate marked homeostatic compensatory increases in volitional physical activity in the form of wheel running, presumably to prevent further weight gain. Collectively, these findings implicate leptin as a critical mediator of physical activity, and in coordinating energetic outputs, such as exercise, with energy intake.

The compensatory hyperphagia observed in HR mice when given access to wheels during the present study suggests that exercise modifies the responses of the central nervous system to adjust energetic needs. Consistent with this hypothesis, the adiposity signal leptin, which acts primarily in the CNS to exert its behavioral effects, remains suppressed through at least 7 week after juvenile wheel exposure in HR mice (Fig. 5), indicating a long-lasting improved sensitivity

to the hormone. Interestingly, the C mice did not exhibit this pattern, but instead developed elevated leptin levels 7 weeks after wheel exposure (Fig. 5). This change in leptin occurred despite no statistically significant effect on body mass or visceral fat pad mass (Fig. 1, Supplemental Material), indicating some disassociation between circulating leptin concentrations and body fat per se. Further quantitative measures of adiposity, including whole-body levels, will be needed to test this possibility under conditions of altered exercise and/or diet. Another consideration when interpreting changes in circulating leptin concentrations is that although leptin treatment does not alter wheel running in wild type mice [60, 61] (but does in our HR mice: [52], exercise alone does improve leptin sensitivity in rodents, even on a high fat diet. This improvement in leptin sensitivity was demonstrated by greater weight loss and reductions in food intake after an ICV leptin injection, as compared to sedentary mice, and this effect occurred independently of baseline adiposity [61] . Thus, although we observed an increase in leptin 7 weeks after juvenile wheel exposure in our C mice, this response may be a harbinger of leptin resistance and future weight gain (none occurred during the present study) in response to the cessation of exercise, or possibly a long lasting physiological response to reverse the exercise induced hyperphagia after wheel access was revoked.

We did not observe a statistically significant effect of early-life wheel access on the trajectory of body-mass gain with age, but mice given early-exercise did tend to be lighter (Fig. 1). The diet used here, as in our routine selective breeding protocols (Teklad Laboratory Rodent Diet [W]-8604), is not considered obesogenic for mice, so it would be of interest to repeat these studies with a high-fat or Western diet, which is obesogenic for these lines of mice ([51, 52, 62]). Another point worth mentioning is that "room temperature," as used in the present study and in most studies of laboratory house mice, generally imposes a mild cold stress ([63]: [at least when mice are housed individually and/or without nesting materials]). As this sort of cold exposure can

protect against obesity in some mice [64], and housing under different thermal conditions results in alterations in energy expenditure and food intake, it would also be of interest to repeat the present study in mice housed within their thermal-neutral zone [65].

Although mice with early-life wheel access ran more on wheels when retested as adults, the effect disappeared after approximately one week because both C (+33.5%) and HR (+58.6%) mice that did not have early-life wheel access increased their wheel running more than those that did have early-life exercise (C -4.0%, HR +27.9%)(see Supplemental Material Table S8).

Although the positive effect of early-life exercise lasted for only one week, it is important to note that one week in the life of a mouse is equivalent to approximately nine months for humans ([66];[67]). On the other hand, some early-life human intervention studies indicate that beneficial effects can last for at least one year (e.g., [68]). In any case, our results potentially suggest that any positive effects of early-life exercise on adult exercise propensity will require reinforcement and maintenance if they are to be long-lasting.

In summary, we found that early-life access to voluntary exercise had lasting effects on the behavior and physiology of adult mice in ways that depended on genetic background. Throughout the study, all mice with early exercise were lighter than their non-exercised counterparts, but the effect on plasma leptin concentrations and food consumption depended on genetic background. In all mice, early-life wheel access specifically increased adult wheel running and not cage activity. Use of the polygenic High Runner mouse lines and their controls, the former produced by selective breeding rather than manipulation of a single gene, allows exploration of wide variation in genetically based levels of voluntary exercise, aerobic capacity, body fat, food consumption [34], and leptin levels.

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Figure Legends

Figure 1. Early-life wheel access tended to decrease body mass in both Control and High Runner (HR) lines of mice, but the effect was never statistically significant for any measure (Online Supplemental Material Tables S1-S3). Mice from HR lines were lighter than C, and the difference reached statistical significance (2-tailed $P < 0.05$ at the time points indicated by *). No interaction between early-life wheel access and linetype was ever statistically significant. Values are least square means (LSM) + associated SE from SAS Procedure Mixed. Each of the four groups included 24-25 males.

Figure 2. Wheel access significantly increased food consumption during week 3 of early-life wheel access (A) in HR mice, but not in C (wheel access by linetype interaction $P = 0.0143$). During the first week of adult wheel access (B), food consumption was higher in HR mice (linetype $P = 0.0384$), but was not affected by early-life wheel access ($P = 0.7819$; interaction $P = 0.4779$). Mass-adjusted food consumption $LSM \pm SE$.

Figure 3. Wheel running during the third week of early-life wheel access (beginning just after weaning) and following two months of no wheel access for any group. During week 3, HR mice ran more than non-selected control lines, as reported in numerous previous studies. During week 12, HR mice again ran more than C, and for both linetypes those individual who had early-life wheel access ran significantly more than those that did not. Wheel revolutions per day $LSM \pm SE$.

Figure 4. During the third week of early-life wheel access, mice housed with wheels showed reduced activity in the home cages attached to the wheels. Relative to mice housed without wheels, mice from the HR lines showed a greater reduction in SPA than did C mice. During week

12, the amount of home-cage activity was somewhat reduced in all groups, and no differences among groups were apparent. Values are LSM $\pm$ SE.

Figure 5. Early-life wheel access increased adult plasma leptin levels (adjusted statistically for fat-pad mass) in C mice (+29% on the backtransformed raw scale), but decreased them in HR (-20%) with juvenile exercise. A) leptin levels (square-root transformed) plotted versus visceral fat pad mass ($\log_{10}$ -transformed) for mice from non-selected Control lines (without wheel access [open symbols], with early life wheel access [solid symbols]). B) leptin levels versus visceral fat pad mass for mice from selectively bred HR lines. C) Least squares means and standard errors from nested analysis of covariance in SAS Procedure Mixed, with $\log_{10}$ fat pad mass as a covariate. These transformations of leptin and fat pad mass were chosen because they best achieved homogeneity of the spread of the covariate, linearity of the relation with the covariate, and normality of residuals from the statistical model.

B) End of Week 3

Effect	P
Early Exercise	0.2399
Linetype	0.0835
Interaction	0.7197

C) End of Week 12

Effect	P
Early Exercise	0.1045
Linetype	0.0473*
Interaction	0.5489

Figure 1

A) Week 3

Effect	P
Early Exercise	0.0042*
Linetype	0.1007*
Interaction	0.0183*
Body Mass	<.0001*

B) Week 12

Effect	P
Early Exercise	0.8793
Linetype	0.0310*
Interaction	0.2301
Body Mass	0.5215

Figure 2

Figure 3

Figure 4

Figure 5

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