

Preference for Western diet coadapts in High Runner mice and affects voluntary exercise and spontaneous physical activity in a genotype-dependent manner

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ABSTRACT

Do animals evolve (coadapt) to choose diets that positively affect their performance abilities? We addressed this question from a microevolutionary perspective by examining preference for Western diet (WD: high in fat and sugar, but lower in protein) versus standard rodent chow in adults of both sexes from 4 lines of mice selectively bred for high levels of voluntary wheel running (High Runner or HR lines) and 4 non-selected control (C) lines. We also assessed whether food preference or substitution affects physical activity (wheel running and/or spontaneous physical activity [SPA] in the attached home cages). In experiment 1 (generation 56), mice were given 6 days of wheel acclimation (as is used routinely to pick breeders in the selection experiment) prior to a 2-day food choice trial. In experiment 2 (generation 56), 17 days of wheel acclimation allowed mice to reach a stable level of daily running, followed by a 7-day food-choice trial. In experiment 3 (generation 58), mice had 6 days of wheel acclimation with standard chow, after which half were switched to WD for two days. In experiment 1, WD was highly preferred by all mice, with somewhat greater preference in male C mice. In experiment 2, wheel running increased and SPA decreased continuously for the first 14 days of adult wheel testing, followed by 3-day plateaus in both. During the subsequent 7-day food choice trial, HR mice of both sexes preferred WD significantly more than did C mice; moreover, wheel running increased in all groups except males from C lines, with the increase being significantly greater in HR than C, while SPA declined further in all groups. In experiment 3, the effect of being switched to WD depended on both linetype and sex. On standard chow, only HR females showed a significant change in wheel running during nights 7 + 8, increasing by 10%. In contrast, when switched to WD, C females (+28%), HR females (+33%), and HR males (+10%) all significantly increased their daily wheel-running distances. Our results show for the first time that dietary preferences can coadapt in response to selection on activity levels.

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1. Introduction

Evolution occurs via more-or-less coordinated genetic and phenotypic changes at multiple levels of biological organization. Even within a level, such as behaviour, coadaptation is expected (e.g.,

see Brashares and Arcese, 2000; Jarman, 1974). Two aspects of behaviour that seem likely to exhibit coadaptation are diet and physical activity. For example, the classic (but oversimplified) dichotomy between active and sit-and-wait foragers entails variation in both activity patterns and diet (as well as life-history traits, locomotor abilities, the predation regime experienced, social behaviour, and mating systems (Huey et al., 2001; Perry et al., 1990; Verwajen and Van Damme, 2008).

In addition to an expectation of correlated evolutionary changes, dietary choices and physical activity can influence each other during an individual's ontogeny, both acutely and chronically. Effects of physical activity on appetite have been studied extensively (Blundell and King, 1998; Hopkins et al., 2014; Westerterp, 2010). Generally, food or energy intake increases as physical activity

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increases (Koteja et al., 1999; Melzer et al., 2005; Stevenson, 1967; Westerterp, 2010). In a few cases, physical activity has been shown to affect dietary choice. For example, Tour de France cyclists voluntarily consumed more carbohydrates as their primary fuel source during their training than when not training (Saris et al., 1989). The underpinnings of these bidirectional, within-individual effects may themselves have evolved in response to past correlational selection, as part of the homeostatic mechanisms underlying both physiology and behaviour. From the perspective of coadaptation, we would expect that animals may generally evolve to choose diets that enhance their ability to perform various functions, such as particular types of locomotion or reproductive activities.

Studies of laboratory rodents show that diet choice and composition can affect both physical activity and whole-animal performance capacities. For example, 3 months of high-fat diet increased maximal aerobic capacity ($VO_2\max$) by 15 percent in sedentary rats, whereas endurance training for 3 months increased $VO_2\max$ by 20 percent (Simi et al., 1991), and the effects were additive. A different study of rats compared effects of a high-fat versus a high-carbohydrate diet fed for 2 months on exercise endurance and body composition. Half of the rats had their diets switched for 3 days prior to endurance testing. Rats that received the high-fat diet first, followed by the high-carbohydrate diet, fatigued later than the rats fed a high-carbohydrate or high-fat diet only (Lapachet et al., 1996). In another study of rats fed one of three diets (high in saturated fat, high in n-3 fatty acids, or high in n-6 fatty acids), those fed the high n-6 diet increased treadmill endurance-running performance compared to the other groups (Ayre and Hulbert, 1997). Increased consumption of polyunsaturated fatty acids (PUFAs) has also increased treadmill endurance-running performance in rats (Ayre and Hulbert, 1997). A large and often conflicting literature also concerns effects of dietary supplements and carbohydrate loading on human athletic performance (e.g., Bucci, 2000).

Diet choice and composition can also affect physical activity and performance ability in wild animals. A field study demonstrated that just prior to their long-distance migration from Canada to South America, sandpipers consumed a diet rich in amphipod crustaceans, which have greater polyunsaturated fatty acids (PUFA) content than other crustaceans (Weber, 2009). Unsaturated fatty acids have increased fluidity, accelerating peroxidation rates and influx into cells more than saturated fatty acids (Maillet and Weber, 2006). Enhanced fuel accessibility may be the reason sandpipers consume certain crustaceans prior to migration. Similarly, migratory red-eyed vireos prefer long-chain unsaturated fatty acids over long-chain saturated fatty acids (Pierce et al., 2004). In a different experiment, vireos had improved aerobic performance (mass-specific peak metabolic rate during forced exercise) with a diet containing lower unsaturated fatty acids as compared with a diet containing more unsaturated fatty acid (Pierce et al., 2005). These and other studies (e.g., Frank et al., 1998; Weber, 2009) support the general hypothesis that dietary preferences should coadapt with other aspects of behavioural and physiological ecology in ways that facilitate organismal performance abilities (Angilletta et al., 2006; Bauwens et al., 1995).

Selection experiments and experimental evolution can provide powerful tools for elucidating the coadaptation of behavioural and other traits (Garland et al., 2016; Garland and Rose, 2009; Rhodes and Kawecki, 2009). The purpose of the present study was to test the coadaptation hypothesis by examination of preference for “Western diet” (relatively high in fat and sugar, but low in protein) and the effects of Western diet on physical activity in a unique rodent model: four replicate lines of High Runner (HR) mice that have experienced long-term selective breeding for voluntary exercise on wheels, as compared with four non-selected control (C) lines (Acosta et al., 2015; Careau et al., 2013; Copes et al., 2015; Swallow et al., 2009, 1998). In addition to running voluntarily 2.5-

3-fold more revolutions per day, HR mice have increased endurance capacity (Meek et al., 2009) and $VO_2\max$ (Dlugosz et al., 2013; Kolb et al., 2010; and references therein), reduced body fat (Girard et al., 2007; Swallow et al., 2001), lower circulating leptin levels that are not explained solely by their lower body fat (Acosta et al., 2015; Girard et al., 2007), alterations in the brain reward system (Belke and Garland, 2007; Caetano-Anollés et al., 2016; Claghorn et al., 2016; Kolb et al., 2013; Rhodes et al., 2005; Saul et al., 2016), and may experience withdrawal symptoms when wheel access is removed (Kolb et al., 2013) (review in Wallace and Garland, 2016).

Previous studies found that Western diet can positively affect wheel running in male HR mice but not in C mice (Meek et al., 2014, 2010). This unusual response of HR mice could be related to constraints that their hyper-lean phenotype places on sustained endurance exercise (given that they have inherently high motivation for voluntary exercise) and/or an effect on the reward they receive from running, alternatives that have yet to be discriminated. Here, we report on three separate experiments, all that used both sexes, designed to address several hypotheses and predictions about the HR mice. Most generally, we hypothesized that HR mice would have elevated preference for Western diet compared to C mice (Experiments 1 and 2), and that this difference might depend on context, e.g., whether they were fully acclimated to wheels when tested (6 vs. 17 days in Experiment 1 vs. 2). In addition, we expected that HR mice might respond differently from C mice, in terms of wheel running and/or home-cage activity, when Western diet was substituted (Experiment 3) for standard chow (Meek et al., 2014, 2012, 2010). Finally, we expected males and females might differ in food preferences (Experiments 1 and 2) and responses (Experiment 3) to Western diet (Asarian and Geary, 2013; Bender, 1976; Fukushima et al., 2015) in a manner that depends on genetic background (Svenson et al., 2007; C vs. HR lines in the present study) (C vs. HR lines: all three experiments).

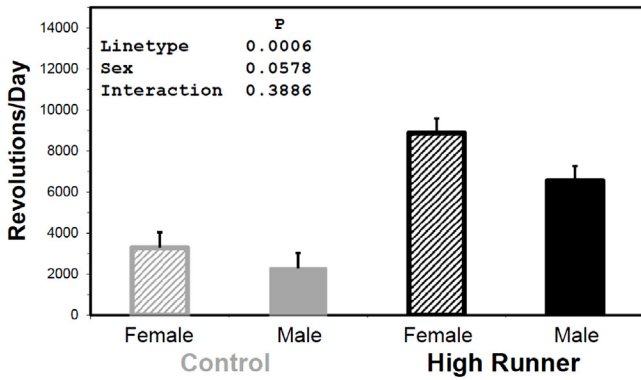
2. Materials and procedures

2.1. Experimental animals

Mice were from generations 56 and 58 of an ongoing artificial selection experiment that breeds for high voluntary wheel running activity (for more details on the selection process, see (Careau et al., 2013; Swallow et al., 1998)). The base population consisted of 224 outbred, genetically variable Hsd:ICR house mice (*Mus musculus*). Ten pairs of mice were used to create eight closed lines, four of which were randomly designated and bred for high running (HR) on wheels and the other four were non-selected control (C) lines, bred without regard to wheel running. In each generation, mice are weaned at 21 days of age and housed in same-sex groups of four until approximately 6–8 weeks of age, when they 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 that records revolutions in 1-min intervals continuously for 6 days of wheel testing. In the HR lines, breeders for the next generation are chosen based on their wheel running for days 5 and 6. For the HR lines, the highest-running males and females within each family are chosen as breeders, whereas random males and females are chosen from within families in the C lines (sibling mating is disallowed in all lines).

Room temperature was maintained at approximately 22 °C. Lights were on at 0700 with a photoperiod of 12:12 h. Water and food (Harlan Teklad Laboratory Rodent Diet [W]-8604 or Harlan Teklad Custom Research Western Diet TD.88137) were available *ad libitum*. Many different “Western” and “high-fat” diets are used in research with rodents, including several that are commercially

A) Wheel Running on days 5 + 6 of short-term Mice



B) Wheel Running on days 14-17 of long-term Mice

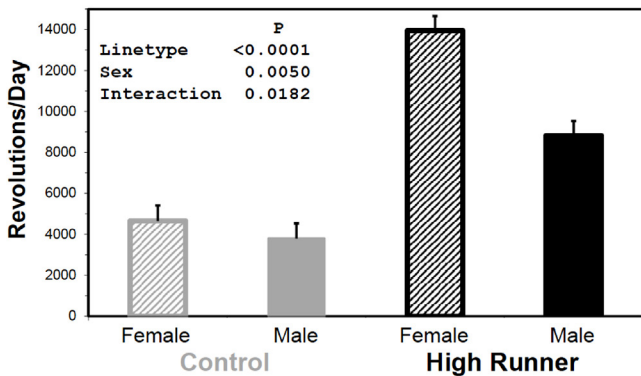


Fig. 1. Mice from the four replicate High Runner lines ran on wheels significantly more than those from the four non-selected Control lines. For each linetype and sex, values are least squares means (LSM) + associated SEs from SAS Procedure Mixed. A) Values from days 5 and 6, prior to mice having a choice between standard chow and Western diet (Experiment 1, short-term mice, generation 56). B) Values from days 14–17, prior to mice having a choice between standard chow and Western diet (Experiment 2, long-term mice, generation 56). Comparison of panel A and B demonstrates that mice continue to increase their average daily running levels for almost two weeks when first granted wheel access (see also Fig. 4A).

available. The specific composition of these diets can have large effects on diet composition (Guyenet, 2009). We used the particular diets listed above because (1) they are widely used, (2) they have been used for several previous studies in our laboratory with these lines of mice, and (3) we have previously shown linetype-specific responses to this Western diet (Meek et al., 2014, 2010). Table 1 in Meek et al. (Meek et al., 2010) provides for a listing of the composition of the two diets used.

All methods were approved by and are in agreement with the regulations of the Institutional Animal Use and Care Committee of the University of California, Riverside, which follows the U.S. National Institutes of Health guide for the care and use of laboratory animals.

2.2. Experiment 1 (N = 326) and experiment 2 (N = 96): preference for western diet

Two sets of mice sampled from generation 56 were studied. Both groups were tested at approximately 6–8 weeks of age, similar to the standard selection procedures. In both experiments, sample sizes were equally distributed between the sexes and linytypes. In experiment 1, mice were given short-term wheel access prior to food choice trials (6 days, as is used routinely to pick breeders). In experiment 2, mice had 17 days of wheel access (long-term) prior to food choice trials. This extended period of wheel access allowed

mice to reach a plateau in wheel running, as has been reported previously (e.g., Meek et al., 2010; Swallow et al., 2001). Diet preference was measured by dividing the standard food hoppers into two subsections with a stainless steel plate. Each half was filled with a weighed amount of either standard chow or Western diet. Food hoppers were reweighed after seven additional days of wheel access, and the bedding (wood shavings) was checked for pieces of uneaten food that were weighed and accounted for in determination of food intake (Koteja et al., 2003). Previously, with the standard chow used here, we have reported that the amount of spillage does not differ between the High Runner and Control lines of mice (Koteja et al., 2003). We have not previously quantified food wasting or spillage on the Western diet that we used, but it certainly crumbles more easily than the standard chow and almost certainly the amount of spillage would be greater. Some of this “spillage” becomes powder, and so accurate quantification requires housing animals on plastic grids, rather than with the standard bedding, and that potentially creates novelty/stress for the animals. In the present study, we did not attempt to compare the amounts of spillage among groups. Food consumption is shown in graphs as grams, but for statistical analyses food choice (preference) was calculated based on caloric equivalents using the conversion factors shown in Table 1 of (Meek et al., 2010) as: Western kJ/(Western kJ + Standard kJ). All mice were monitored for wheel running, food consumption, and body mass; home-cage activity was also monitored in experiment 2 (long-term) mice.

2.3. Experiment 3 (N = 313): substitution of western diet

Mice from generation 58 were subjected to food substitution. Sample sizes were equally distributed between the sexes, linytypes, and diet manipulation. After having wheel access and standard chow for 6 days (the usual for the selection experiment protocol) half of the mice were switched to Western diet for two days. We used these last two days because the selection criterion in the ongoing experiment uses two days of wheel-running data. Each food hopper was weighed as described above, before and after the two days of additional wheel running.

2.4. Measurement of wheel running and home-cage activity

Mice were individually housed in standard cages attached to a Wahman-type activity wheel, as used in the ongoing selection experiment described above. Wheel running was recorded for 23 h each day (1300–1200), with the final hour used for data collection and checks of wheel function and mouse health.

Spontaneous physical activity (SPA) in the attached home cages was monitored as previously described (Copes et al., 2015), using infrared motion-detector sensors (similar to Gebczyński and Konarzewski, 2009) that detect movement within the standard housing cages. Briefly, sensors were interfaced to a Macintosh personal computer running custom recording software. The software measured activity summed over every 1-min interval for 23 h. Sensor status was recorded 3 times per second as either movement (1) or no movement (0). A mean value (0–1) with arbitrary units was computed for each minute. Total home-cage activity (referred to as spontaneous physical activity or SPA) was taken as the sum of all activity over 23 h. The number of 1-min intervals showing any SPA were also computed and tallied to indicate the duration (minutes) of SPA during the entire daily period. Dividing daily activity by the number of 1-min 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 SPA. This system for measuring SPA detects all types of physical activity in the cage, but cannot discriminate among activities (e.g., grooming versus rearing versus feeding). The SPA measures were recorded

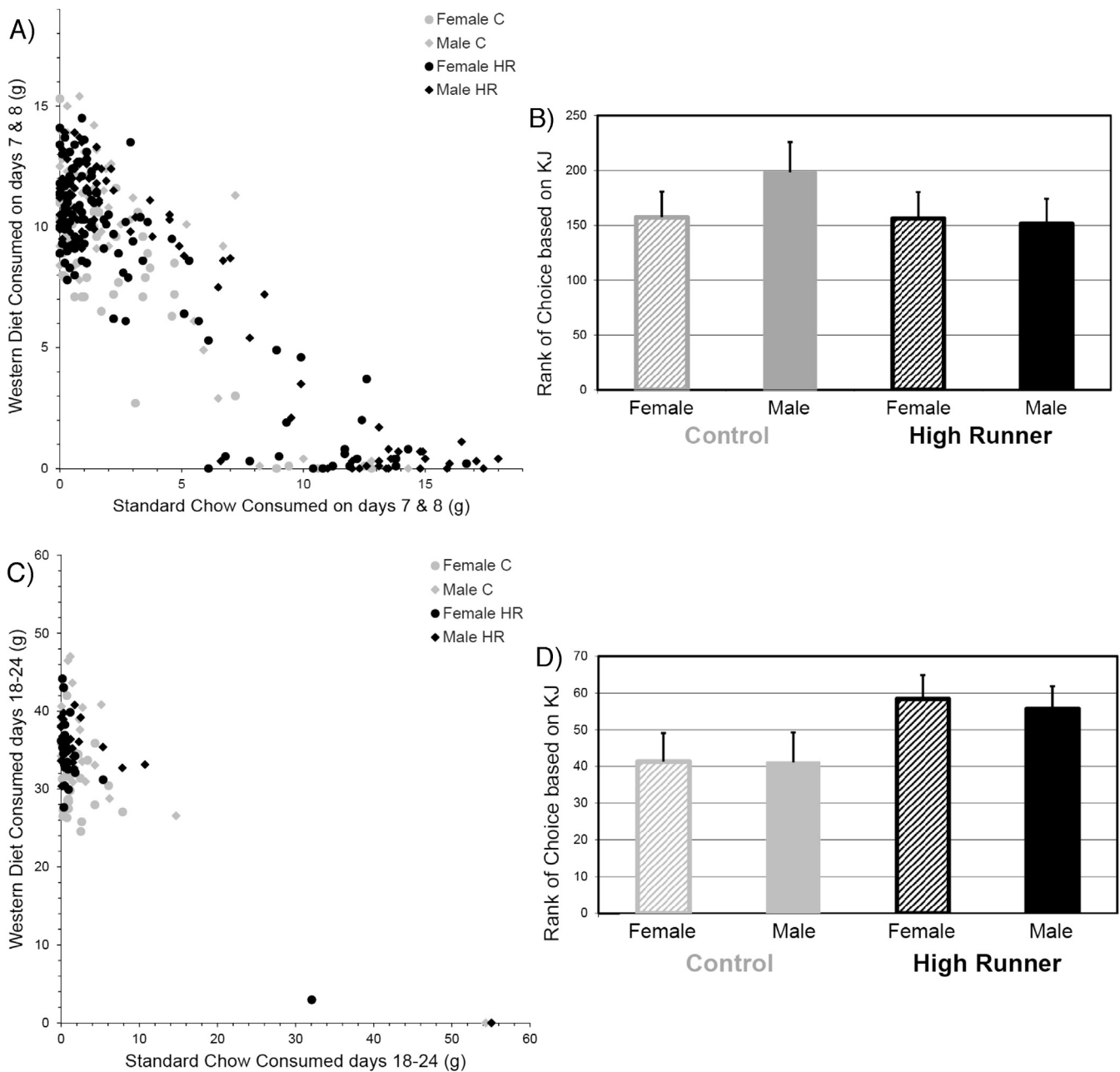


Fig. 2. Food consumption and preference when mice were offered a choice between standard chow and Western diet (same mice as in Fig. 1). A) After six days of wheel access (as is used routinely in the selection experiment for choosing breeders), on days 7 & 8 most mice preferred Western diet, although some individuals ate little of it. B) On days 7 & 8, on average, all four groups of mice consumed more Western Diet than standard chow (in terms of caloric intake), with a marginally significant interaction between sex and linetype ($P=0.0612$), indicating that males from the Control lines showed somewhat stronger preferences than did the other three groups (sex $P=0.3610$, linetype $P=0.2413$). Values are least squares means (LSM) + associated SEs from SAS Procedure Mixed, based on analyses of the rank-transformed values for kJ of Western diet/(Western diet + standard chow). C) Preference for Western diet was even stronger after mice had 17 days of wheel access prior to the choice trial, when only three of 97 individuals ate little WD across days 18–24. D) During days 18–24, all groups of mice again preferred WD (in caloric terms), but, as predicted, the preference was stronger for mice from High Runner lines of both sexes (1-tailed $P=0.0424$), with no main effect of sex ($P=0.8185$) nor a sex-by-linetype interaction ($P=0.8277$).

simultaneously with wheel running. Data for both SPA and wheel running were downloaded daily between 1200 and 1300 h.

2.5. Statistical analyses

As in numerous previous studies of these eight lines of mice (e.g., Acosta et al., 2015; Copes et al., 2015; Girard et al., 2007; Kolb et al., 2013; Meek et al., 2010), 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. Linetype (HR or C), diet type (if applicable), and sex were treated as fixed

effects; line was nested within line type as a random effect. Effects of linetype, sex, and diet and their respective interactions 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 (Copes et al., 2015), SPA sensor calibration (measure of sensitivity to motion (Copes et al., 2015)), and total amount of wheel running. The dependent variable and/or covariates were transformed as necessary to improve the homogeneity of the spread of the covariates, linearity of relations, and the normality of the residuals. The distribution of food choice

values (based on caloric equivalents) was U-shaped, with most individuals consuming far more kJ of Western diet than standard chow, but some (Experiment 1) or a few (Experiment 2) individuals instead consuming more kJ of standard chow than Western diet; therefore, the choice variable was rank-transformed prior to statistical analyses (Conover and Iman, 1981) (for an example with these lines of mice, see Klomberg et al., 2002).

3. Results

3.1. Experiment 1 and experiment 2: preference for western diet

3.1.1. Wheel running before food choice

Mice from the High Runner lines, as expected from numerous previous studies (e.g., Acosta et al., 2015; Copes et al., 2015; Girard et al., 2007; Kolb et al., 2013; Meek et al., 2014), ran significantly more than those from Control lines in both short-term (experiment 1, Fig. 1A) and long-term (experiment 2, Fig. 1B) wheel-access groups. HR mice ran significantly more than C mice before the food choice on days 5+6 of wheel access ($P=0.0006$), showing a nearly-significant sex effect, with females running more than males on days 5 and 6 ($P=0.0578$), and a non-significant sex-by-linetype interaction ($P=0.3886$) (Fig. 1A). For the long-term mice (experiment 2), HR ran significantly more than C mice before the food choice on days 14–17 of wheel access ($P<0.0001$), females ran more than males ($P=0.0050$), and a significant sex-by-linetype interaction ($P=0.0182$) was found, indicating a larger differential in females than males on both an absolute basis (9298 versus 5029 revolutions/day, respectively) and proportional basis (increase of 199.8% in females versus increase of 132.7% in males) (Fig. 1b).

3.1.2. Food choice

Most mice exhibited a preference for Western diet after 6 days of wheel access (Fig. 2A), and this pattern was accentuated after 17 days of wheel access, when only three of 97 individuals ate more standard chow than Western diet (Fig. 2C). Beyond the overall tendency of mice to prefer Western diet, we measured differences among groups in the strength of this preference, after converting grams eaten to an energetic equivalent based on values in Table 1 of (Meek et al., 2010). After 6 days of wheel access, male mice from the Control lines tended to show stronger preferences than male HR mice (Fig. 2B: interaction $P=0.0612$). The pattern was different after 17 days of wheel acclimation, with High Runner mice of both sexes showing stronger preference for Western diet based on caloric intake (Fig. 2D: linetype 1-tailed $P=0.0424$).

3.1.3. Wheel running during short-term food choice

As shown in Fig. 3, all mice in experiment 1 increased wheel running during the food-choice trial on days 7 & 8. The difference in wheel running was positive for all groups, and was significant for Control Females ($P=0.0428$), HR Females ($P<0.0001$), and HR Males ($P=0.0011$), but not for Control Males ($P=0.3716$). Females and HR mice showed significantly larger increases than males and C mice (sex $P=0.0115$, linetype $P=0.0033$), respectively, with no significant interaction ($P=0.1992$).

3.1.4. Wheel running and home-cage activity during long-term food choice

As expected, HR mice ran also more than C mice during long-term wheel access (Fig. 4A). Food choice was tested after mice appeared to have reached a running plateau (days 14–17 in Fig. 4A). During the diet-choice trial (days 18–24), when HR mice of both sexes showed elevated preferences for Western diet (see above and Fig. 2D), wheel running increased in all mice as compared with days 14–17 (Fig. 4B: Control Female $P=0.0106$, Control Male $P=0.1032$,

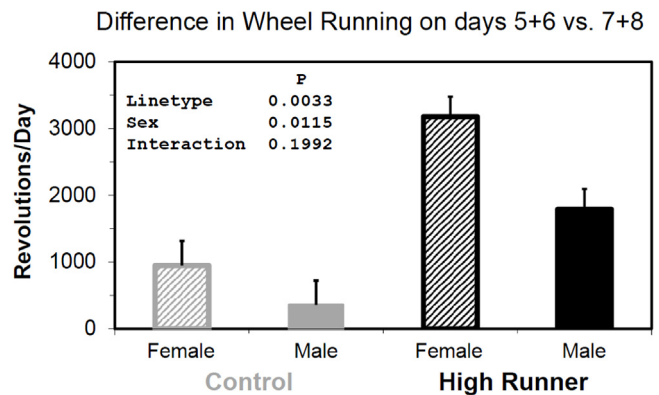


Fig. 3. In Experiment 1 (short-term mice), all groups except Control males ran significantly more on days 7 & 8 of wheel access, when they had a choice between Western diet and standard chow, as compared with days 5 & 6. Values are least squares means (LSM) + associated SEs from SAS Procedure Mixed. The increase in running was significantly greater for HR mice and for females rather than C mice or males (linetype $P=0.0033$, sex $P=0.0115$), with no interaction ($P=0.1992$).

HR Female $P=0.0008$, HR Male $P=0.0012$). Analysis of these differences in wheel running indicated the increases were significantly greater in the HR mice (linetype $P=0.0200$), with no effect of sex ($P=0.2699$) and no interaction ($P=0.8006$).

Reciprocal to increased wheel running, SPA decreased throughout the experiment across all groups (Fig. 4C). After food choice was introduced, SPA tended to decrease further in all groups (Control Female $P=0.0686$, Control Male $P=0.0407$, HR Female $P=0.0246$, HR Male $P=0.1258$), with no statistical difference between line-types or sexes, nor an interaction (Fig. 4D).

3.2. Experiment 3: substitution of western diet

3.2.1. Effects of western diet on wheel running

On days 5 & 6 of the 6-day wheel exposure (Fig. 5A) with standard chow ($N=313$), HR mice ran much more than C ($P<0.0001$), as expected, but with no sex effect ($P=0.1469$) or interaction ($P=0.6629$). The effects of administering Western diet to half of the mice on days 7 & 8 depended on sex and linetype in a complex manner. Statistically significant interactions occurred for sex*diet ($P=0.0174$) and sex*linetype ($P=0.0061$), and marginally for diet*linetype ($P=0.0868$) and sex*diet*linetype ($P=0.1102$). For females (Fig. 5B), mice from Control lines remaining on standard chow showed no statistical change in wheel running from days 5 & 6–7 & 8. In contrast, HR females on standard diet (+9.9%, $P=0.0070$), Control females on WD (+27.8%, $P=0.0176$), and especially HR females on WD (+32.9%, $P<0.0001$) all showed significant increases (based on comparisons of least squares means with the null hypothesis of no change; % changes calculated by comparison with least squares means from combined-sex analysis). Note that the figures depict absolute changes in revolutions run per day, and not the percentage changes, which are presented parenthetically. For males (Fig. 5C), the only group showing a statistically significant change was HR switched to Western diet, which increased approximately 10% in average revolutions/day ($P=0.0138$).

3.2.2. Effects of western diet on home-cage activity

On days 5 & 6 of the 6-day wheel exposure, males were less active in their home cages than females ($P=0.0295$), but HR and C mice did not differ statistically ($P=0.8146$), nor was there an interaction ($P=0.5487$). On days 7 & 8, with Western diet, males were still less active than females ($P=0.0454$), HR and C did not differ ($P=0.9281$), and Western diet reduced activity ($P=0.0430$). None of the interaction terms were statistically significant (all $P>0.29$). When the changes in home-cage activity were analyzed, all mice

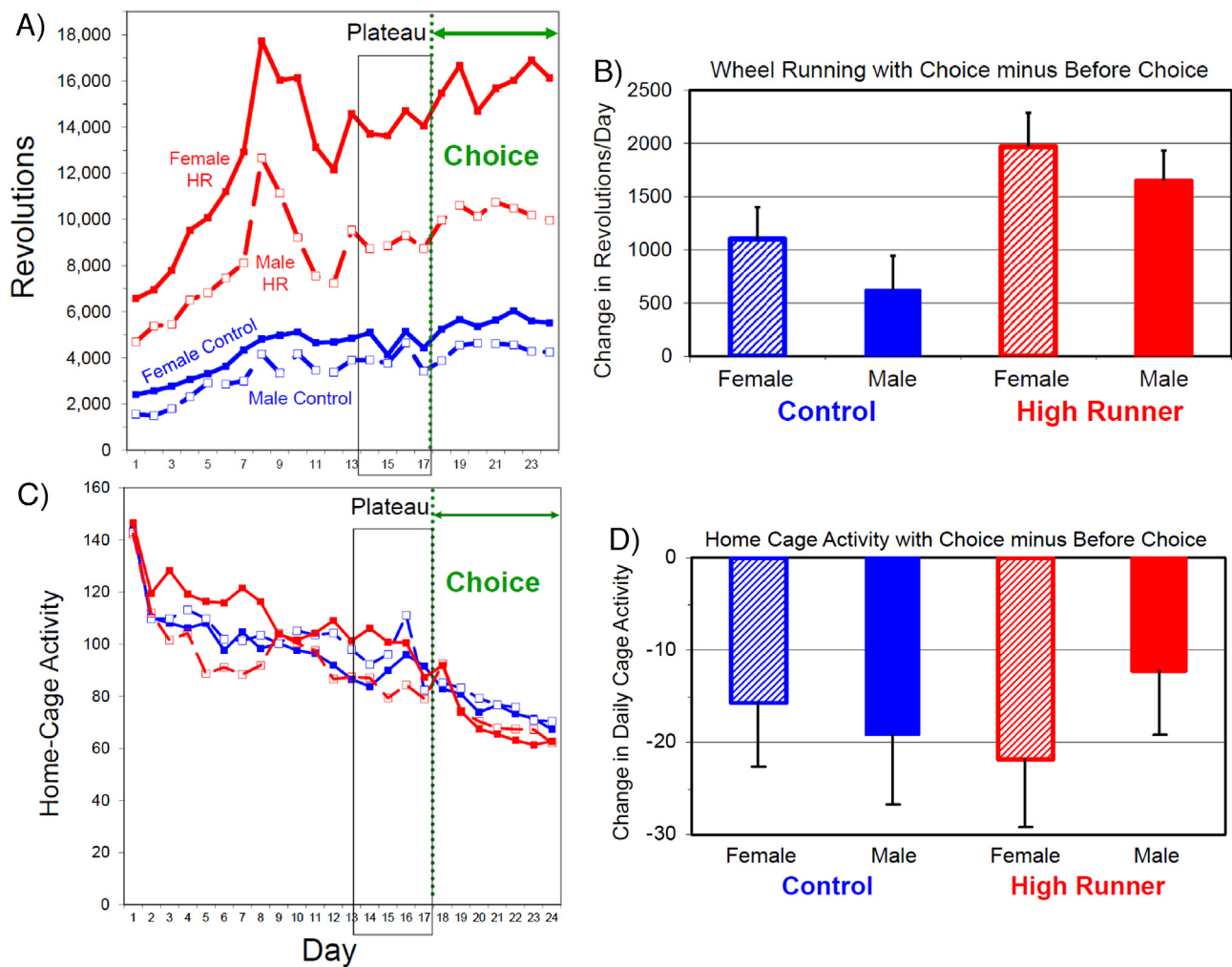


Fig. 4. A) In experiment 2 (long-term mice), both sexes of High Runner mice ran significantly more than C lines at all times. B) During the diet-choice trial (days 18–24), when HR mice of both sexes showed elevated preferences for Western diet (see Fig. 2D), wheel running increased in all mice as compared with days 14–17. Analysis of these differences in wheel running indicated the increases were significantly greater in the HR mice (linetype $P = 0.0200$), with no effect of sex ($P = 0.2699$) and no interaction ($P = 0.8006$). C) As wheel running increased across the experiment, home-cage activity decreased for all four groups. After food choice was introduced, SPA tended to decrease further in all groups. D) During the food-choice trial, home-cage activity of all groups decreased (while wheel running increased: Fig. 4B), with no statistical difference between linetypes ($P = 0.9613$) or sexes ($P = 0.6384$), nor an interaction ($P = 0.2788$).

tended to become less active with Western diet ($P = 0.0074$). None of the other main effects or interaction terms was statistically significant (all $P > 0.24$).

3.2.3. Effects of western diet on body mass

In principle, the complicated patterns of wheel-running response to Western diet (Fig. 5b and 5c) may have affected, or been affected by, changes in body mass over the two-day trial. Over such short time periods, change in body mass might reflect changes in energy balance and real alterations in fat or even lean mass, but they could also reflect variation in the degree of gut fill, bladder fill or hydration state. We observed that some individuals lost or gained several grams during the trial, and analysis of mass change revealed a positive effect of Western diet ($P < 0.05$), but no robust statistical effects of sex, linetype or any of the interaction terms (results not shown).

4. Discussion

We tested the hypotheses that mice from lines selectively bred for high levels of voluntary wheel running would have altered preferences for Western diet, as compared with mice from non-selected

control lines, and that their response to Western diet in terms of physical activity might also differ from C mice. Elevated preferences for WD in HR mice could arise from a general alteration of the reward system (Belke and Garland, 2007; Caetano-Anollés et al., 2016; Claghorn et al., 2016; Kolb et al., 2013; Rhodes et al., 2005; Saul et al., 2016) and/or a greater need for lipids associated with their higher levels of physical activity and energy expenditure (even when housed without wheels: (Copes et al., 2015 and references therein; Swallow et al., 2001)). As expected from many previous studies of rodents (e.g., Laugerette et al., 2005; Levine et al., 2003; Reed et al., 1997), Western diet was highly preferred by most mice, regardless of sex or linetype (Fig. 2A,C). Consistent with our hypothesis, HR mice of both sexes showed enhanced preferences after 17 days of wheel acclimation (Fig. 2D), although not after 6 days (Fig. 2B). Moreover, administration of WD after 6 days of wheel access (no choice given) had a complex interactive effect on wheel running that depended on both sex and linetype (Fig. 5).

In general, the regulation of lipid intake is a complex behaviour controlled by both instantaneous orosensory stimuli (i.e., texture, odor, and taste) and delayed post-ingestive signals (Drewnowski, 2007; Gilbertson, 1998). For rodents, various studies attribute the palatability of high-fat foods to such factors as texture, flavor,

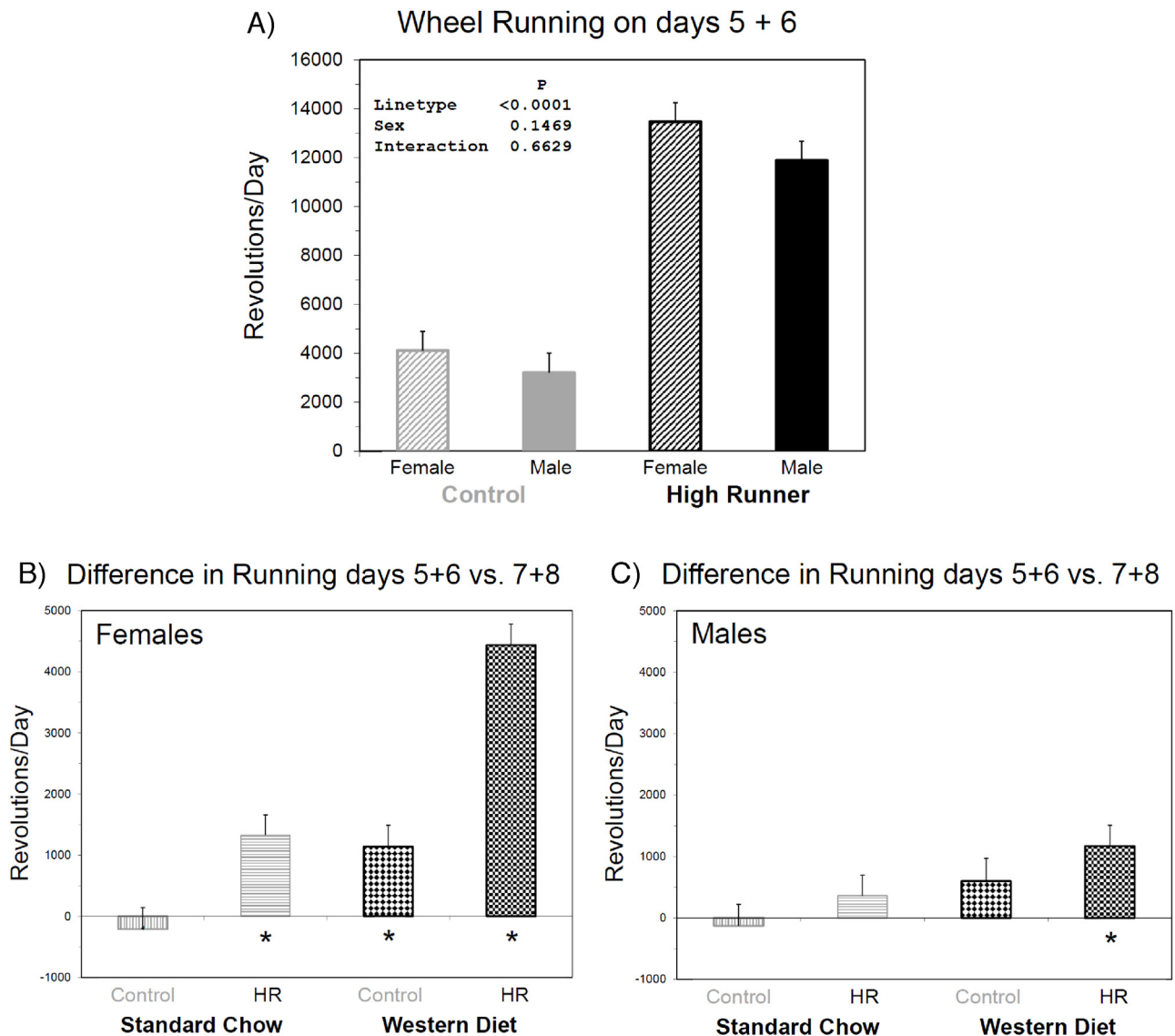


Fig. 5. A) During days 5 & 6 prior to food-substitution in Experiment 3 (generation 58), HR mice ran significantly more than C, and females showed no statistically significant difference from males, with no interaction (N = 313). Age and a measure of wheel freeness were also included in the statistical model (results not shown). When half of the mice were switched to Western diet on days 7 & 8, the change in wheel running depended on linetype in both B) females and C) males. See text for further statistical results. Asterisks indicate that the change in wheel running within a given group differed significantly ($P < 0.05$) from zero. All values are least squares means and standard errors from SAS Procedure Mixed.

taste, and post-ingestive effects (Laugerette et al., 2007; Levine et al., 2003; Manabe et al., 2010). The Western and standard diets used here do differ in color and texture, in addition to nutrient characteristics, which may well affect preferences by mice, but all experiments used the same two diets and so all individuals had equal opportunities to exhibit preferences. Traits that might account for an elevated preference for high-fat foods (as observed in the HR mice) include altered numbers of fatty acid receptors on the tongue, which could increase palatability of fatty foods, possibly affect appetite for dietary fat, and potentially enhance the hedonic response.

Alternatively, the promotion of wheel running by WD may serve as an indirect mechanism leading to the reinforcement of the WD preference. In other words, the dietary change allowed the HR mice to engage in additional activity, which itself is known to be rewarding (Belke and Garland, 2007; Greenwood et al., 2011; Lett et al., 2000; Novak et al., 2012; Sherwin, 1998), and through this process they might have developed a stronger preference for WD. Although this possibility has not been specifically tested, it is consistent with

our data indicating that the elevated preference for Western diet in HR mice does not arise within the first week (Fig. 2B), but appears to require more time (Fig. 2D), which is what would be expected for the gradual elevation in running to generate a learned (reinforced) response.

The Western diet used here contains not only more fat but also more sucrose than standard chow. Numerous studies show that rodents generally prefer sweet tastes, especially as compared with bitter-tasting foods (Lemon, 2015; Reed et al., 1997). Consuming sugar-sweetened foods or beverages in humans, rodents, and other mammals leads to rewarding sensations, as indicated by the upregulation of opioid and dopamine receptors in the nucleus accumbens (Gosnell and Levine, 2009). Hence, both sugar and fat rewards may explain why both HR and C mice preferred Western diet. Future studies will be required to determine the proximate physiological and neurobiological bases of elevated fat and/or sucrose preferences in the HR lines of mice.

In addition to most mice consuming more of the Western diet, most individuals also increased wheel running when they had

access to Western diet (Figs. 3–5). As expected from many previous studies (e.g., Acosta et al., 2015; Dlugosz et al., 2013; Kolb et al., 2010; Meek et al., 2010; Swallow et al., 1998), HR mice continued to run more than C mice in all experiments when they had access to WD (Figs. 1,3–5). Based on two previous studies that examined only males (Meek et al., 2012, 2010), we expected that HR mice would run even more with the WD, whereas this effect might not occur in C mice. Our results were only partially consistent with these expectations. After 6 days of wheel acclimation, access to WD for two days in a choice paradigm (Experiment 1) resulted in significantly increased wheel running for all mice except Control males. After 17 days of wheel acclimation (Experiment 2), by which time mice had reached stable (plateaued) levels of running, access to WD for seven days increased wheel running (revolutions/day) in both HR and C mice of both sexes, but with a statistically greater effect in HR mice of both sexes (Fig. 4A,B). In experiment 3, mice had 6 days of wheel access (as used to choose breeders in the ongoing selection experiment) followed by replacement of standard chow with Western diet for half of the mice. This treatment demonstrated a complex interactive effect on wheel running, modulated by both sex and linetype (Fig. 5).

Meek and colleagues (Meek et al., 2010), who studied only males of the HR and C lines, suggested that ingestion of certain macronutrients is important for muscular activity and delaying fatigue during exercise, although not via polyunsaturated fatty acids (PUFAs), as they are not the major component of the WD used in our experiments. As Meek et al. further proposed, HR mice might run more with a WD because of the neuro-hormonal system that maintains energetic balance and regulates total body weight. In particular, the increased wheel running by HR mice on WD may be a compensatory mechanism to deal with the excess caloric intake making them run more as a consequence of eating more.

Alternatively, Western Diet may increase running because diets high in fat have many physiological effects that act to promote endurance performance. Consumption of high-fat diet may increase fatty acid metabolism, which would allow fat to fuel muscular activity (Templeman et al., 2012; Turner et al., 2007). More specifically, chronic consumption of high-fat diet (HFD) increases fatty acid oxidative capacity of skeletal muscle in rats and mice, as indicated by increased activity of β -hydroxyacyl CoA dehydrogenase (β HAD), medium-chain acyl-CoA dehydrogenase (MCAD), carnitine palmitoyl-transferase (CPT-1), and citrate synthase (CS), as well as an elevated protein expression of PGC-1 α , UCP3, and mitochondrial respiratory chain subunits (Turner et al., 2007). A subset of these and other factors (CCO, CS, CPT, and HK enzyme activities) are indeed higher in the HR mice, as compared with C, when given chronic (8 weeks) wheel access on standard chow (Houle-Leroy et al., 2000). The effects of WD on wheel running observed in Experiment 3 (Fig. 5) occurred during days 7 and 8 of wheel access, and it is unknown if any of the biochemical and molecular changes described above could have occurred by that time (see also Gomes et al., 2009).

Whatever the mechanism that underlies a stimulatory effect of WD on wheel running, our finding that the effects of WD on physical activity depend on genetic background is consistent with studies of a different selection experiment, in which mice were bred to be lean or fat (Simončić et al., 2008). On a regular diet, mice from the lean line had higher wheel running and greater home-cage activity (measured via vertical posture allocation) than those from the fat line. When mice were switched to a calorie-matched HFD, mice from the fat line significantly increased their wheel running, up to the level of lean-line mice, while mice from the lean line were unaffected (Simončić et al., 2008).

Several previous studies have shown that home-cage activity of rodents tends to decline when wheel access is provided (Copes et al., 2015 and references therein; de Carvalho et al., 2016). Here,

we present novel evidence that home-cage activity decreases with access to Western diet, possibly as compensation for increases in wheel running, and that this effect is general for both sexes and both linetypes that we studied (Fig. 4). The decreased home-cage activity may be directly related to the increase in wheel running through homeostatic compensatory mechanisms targeting energy balance or potentially the overall level of physical activity (i.e., the “activity-stat” hypothesis: Eisenmann and Wickel, 2009; Garland et al., 2011; Rowland, 1998). Alternatively, some sort of reward substitution could be occurring (Belke et al., 2006; Darlington et al., 2014). However, we cannot rule out the possibility that WD has some sort of direct effect on SPA (e.g., see Vaanholt et al., 2008).

The present study is not without limitations. In particular, the diets used differ in several ways beyond fat and sugar content (see Table 1 in Meek et al., 2010). First, the fat composition of the two diets differs. Second, the protein content of the WD is less than half that of the standard diet (as%kJ). Protein intake can modulate reward-driven feeding behaviours and, more generally, plays a role in the neural regulation of appetite and feeding (Cuenca-Sanchez et al., 2015; Journal et al., 2012; Leidy et al., 2013). Moreover, many studies report a negative relationship between dietary protein concentration and total food intake. Third, the standard chow contains natural ingredients with phytonutrients not present in semi-purified diets (e.g., phytoestrogens, beta-carotenes) that potentially have a variety of physiological and behavioural effects. Further, the natural compounds (and micronutrients) are likely to vary seasonally or in other ways that cause batch-to-batch variation in diet composition (Barnard et al., 2009). Therefore, our interpretation of coadaptive evolution of dietary preferences because they facilitate running performance in the HR lines of mice should be viewed as a working hypothesis.

In conclusion, the present results demonstrate for the first time that dietary preferences can evolve as a byproduct of selective breeding for activity levels, and that the effects of a Western diet can vary depending on sex and genetic background. Moreover, the dietary preference of HR mice has evolved in a way that facilitates the trait for which they have been bred, i.e., high levels of voluntary wheel running, and hence this qualifies as an example of coadaptation (Angilletta et al., 2006). Further studies will be required to elucidate the mechanistic basis of these differences in preference and behavioural response to Western diet, as well as the basis of sex differences (e.g., Fig. 5). Nonetheless, the present findings are important for understanding behavioural evolution, and have practical implications for studies of diet, locomotor behaviour, and energy balance.

Conflict of interest statement

The authors declare no conflict of interest.

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