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Caffeine stimulates voluntary wheel running in mice without increasing aerobic capacity

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HIGHLIGHTS

- Caffeine, Red Bull, and Gatorade have not been tested in athletic rodent strains.
- Red Bull increased wheel running of both High Runner and control lines of mice.
- Males and females had similar responses for voluntary wheel-running behavior.
- Neither drink affected maximal oxygen consumption during forced exercise.
- Performance-enhancing effects of Red Bull may occur by delaying fatigue.

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ABSTRACT

The "energy drink" Red Bull and the "sports drink" Gatorade are often marketed to athletes, with claims that they cause performance gains. However, both are high in sugars, and also consumed by non-athletes. Few studies have addressed the effects of these drinks or their biologically active components in rodent exercise models. We used three experiments to test effects on both voluntary exercise behavior and maximal aerobic capacity in lines of mice known to differ in "athletic" traits. Mice from four replicate High Runner (HR) lines have been selectively bred for voluntary running on wheels, and run approximately three times as many revolutions per day as do mice from four non-selected Control (C) lines. HR mice also have higher endurance and maximal oxygen consumption (VO_2max) during forced treadmill exercise. In Experiment 1, we tested the hypothesis that Gatorade or Red Bull might cause or allow mice to increase their voluntary wheel running. On days 5 and 6 of 6 days of wheel access, as is used to select breeders, HR mice ran 3.3-fold more than C, and females ran 1.2-fold more than males, with no linetype by sex interaction. On day 7, mice were administered Gatorade, Red Bull or tap water. During the subsequent 19-hour period, Gatorade had no statistical effect on running, but Red Bull significantly increased distance run by both sexes and in both HR and C lines. The increase in distance run caused by Red Bull was attributable to time spent running, not an increase in mean (or maximum) speed. As previous studies have found that sucrose alone does not generally increase wheel running, we tested two other active ingredients in Red Bull, caffeine and taurine, in Experiment 2. With a similar testing protocol, caffeine alone and caffeine + taurine increased running by about half the magnitude of Red Bull. In Experiment 3, we tested the hypothesis that Red Bull or caffeine alone can increase physiological performance ability during aerobic exercise, measured as VO_2max. In a repeated-measures design spanning 6 days, females were housed with water bottles containing Red Bull, caffeine or water in a randomized order, and tested for VO_2max twice while receiving each fluid (6 total trials). Neither Red Bull nor caffeine significantly affected either VO_2max or a measure of trial cooperativity (rated on a scale of 1–5), but both treatments significantly reduced tiredness (rated on a scale of 1–3) scored at the end of trials for both HR and C lines. Taken together, our results suggest that caffeine increases voluntary exercise levels of mice by delaying fatigue, rather than increasing aerobic capacity.

1. Introduction

Red Bull and Gatorade are marketed as energy and performance enhancers. Their marketing schemes target active individuals, with advertisements that include images of extreme sports and sponsorships granted to popular athletes. Red Bull contains a stimulant, caffeine, as

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well as taurine, B-group vitamins, sodium, glucose, and sucrose. Gatorade contains sucrose, dextrose, sodium, and potassium (Table 1).

The purpose of the present study was to test the claims of improved athletic performance and/or voluntary exercise behavior for both of these sports drinks by use of a unique animal model, selectively bred High Runner lines of mice [1,2]. We used mice (*Mus domesticus*, original population from outbred Hsd:ICR strain) from an ongoing artificial selection experiment that breeds mice based on high voluntary wheel running. The mouse model consists of four replicate High Runner (HR) lines that voluntarily run up to 3-fold more revolutions per day than four non-selected Control (C) lines [3]. The difference in total wheel revolutions is caused primarily by an increase in average speed of running, rather than an increase in the amount of time spent running [4], although male HR mice do run for significantly longer per night than male controls [5]. The HR mice have been viewed as animal models of elite human athletes, exhibiting elevated endurance [6] and maximal aerobic metabolic rate during exercise [7–9], whereas the Control mice are seen as representing non-athletic humans [10]. Although HR mice voluntarily run faster in the wheels [5] they are not significantly better (or worse) sprinters (unpublished data), so no "trade-off" [11] in locomotor abilities is apparent.

Mice in the selected HR lines reached an evolutionary plateau and have remained at this plateau for about 40 generations [3]; however, previous studies show that they are physiologically capable of running more under some conditions, such as when given Western diet, high in fat and with added sucrose [10]. We hypothesized that the putative performance enhancers, Red Bull and Gatorade, would increase voluntary wheel running in both HR and C mice. Further, we predicted that the effects might differ in magnitude between HR and C mice, or between males and females, because of other physiological and neurobiological differences between the linetypes and sexes that are known to exist [2,12–17].

Both Gatorade and Red Bull contain glucose, sucrose, and fructose [18], and additionally Red Bull contains the psychologically and/or physiologically active compounds caffeine (0.32 mg/ml), taurine (4 mg/ml), and a mixture of B vitamins (Table 1). Of the advertised ingredients, caffeine has received the most study in the exercise literature, and has been shown to increase wheel running in gerbils [19] and mice [20–22] and to improve some measures of endurance exercise in humans (reviewed in [23,24]). The primary psychomotor effect of caffeine is apparently competitive inhibition of adenosine receptors in the striatum, a region that integrates signals crucial to the execution of voluntary movements [25]. Of relevance here, the striatum has been shown to respond differently between HR and C mice when wheel access is removed [26], and a preliminary study indicates differences in monoamine concentrations in this region [27]. Adenosine receptors are widespread in the body [28], so caffeine likely affects multiple systems contributing to performance. Taurine at doses of 100–500 mg/kg (two weeks of daily treatment by lavage) increased forced treadmill endurance-running capacity in rats [29] (the doses used for mice in the present study [see Methods] are somewhat higher than this, and were consumed freely over a period of ~19 h). Combinations of the active ingredients in Red Bull have been shown to increase aerobic endurance performance in non-athlete humans [30]. The psychomotor effect of taurine is also likely related to its actions on the striatum, as large doses of taurine increase extracellular dopamine in the striatum [31]. However, no previous study has examined the effect of Red Bull or Gatorade specifically on mammals that are genetically predisposed for high exercise abilities and high motivation to exercise, such as the HR mice.

2. Methods

2.1. Experimental animals

Mice were sampled from multiple generations of an ongoing artificial selection experiment that breeds mice for high levels of voluntary wheel running [1]. Briefly, mice from four replicate High Runner (HR) lines have been selected for the behavior of voluntary running on wheels (1.12 m circumference), and, at an apparent selection limit, run approximately three times as many revolutions per day as do mice from four non-selected Control (C) lines [3]. Mice are housed on a 12:12 photoperiod, with lights off 19:00–07:00. All procedures were approved by the University of California, Riverside, Institutional Animal Care and Use Committee, which follows the National Research Council Guide for the Care and Use of Laboratory Animals (revised 2011).

2.2. Experiments 1 and 2: effects of Red Bull, its components, and Gatorade on wheel running

We studied mice of both sexes from generation 70 for Experiment 1 (mean age at start of wheel testing = 58 days), and generation 71 for Experiment 2 (mean age = 54 days). In Experiment 1, after 6 days of wheel access as part of the routine testing to select breeders, 250 mice (both sexes) were randomly assigned to one of three treatments: tap water, Gatorade or Red Bull. In Experiment 2, after 6 days of wheel access, 587 mice (both sexes) were randomly assigned to one of four treatments: tap water, Red Bull, caffeine in water, or caffeine + taurine in water (caffeine and taurine each matching the concentration in water of Red Bull, Table 1). In both experiments, bottles were filled with 50 ml and provided to mice between 15:00 and 17:00, and left for the duration of the wheel test (19 h, 17:00–12:00; lights off 19:00–07:00).

Fluid consumption was measured by weighing water bottles as they were placed on cages on day 6 and when they were taken off cages on day 7, a period of 19 h. The difference in mass between days 6 and 7 cannot simply be interpreted as fluid consumption because of spilling and evaporation. Therefore, 10 bottles per treatment were placed on empty mouse cages at the same time we were recording wheel data. Any difference in these bottles could only be interpreted as spillage or evaporation. The average spillage for each drink type was subtracted from the apparent fluid consumption before statistical analyses. Any values of less than zero were set to zero for statistical analyses. During Experiment 1, we discovered that the carbonation of Red Bull caused some leakage, so for Experiments 2 and 3, we poured cans of Red Bull into a beaker with a stir bar at high speed for 15 min; as expected, this reduced the amount of leakage as compared with Experiment 1.

Wheel revolutions were measured automatically in one-minute bins using photocells attached to wheels from 17:00–12:00, i.e., over a 19-h period. From the revolutions recorded every minute, total revolutions, number of active minutes (minutes with revolutions >0), average speed (revolutions per active minute), and maximum speed (highest revolutions during a single minute) were calculated.

Table 1
Active ingredients in Red Bull and Gatorade. All units given are mg/ml. Caffeine, taurine, sodium, and potassium were taken from the nutrition label or product websites, and sugar information were measured values from Ventura et al. [18].

	Glucose	Sucrose	Fructose	Total sugar	Caffeine	Taurine	Sodium	Potassium
Red Bull	36	51	19	106	0.32	4	0.4	0
Gatorade	24	14	21	59	–	–	0.45	0.13

2.3. Experiment 3: effects of Red Bull and caffeine on VO_2max

Retired female breeders ($N = 63$) from generation 71 were chosen (mean age = 138 days). Females were used because they show less of a decline in voluntary wheel running with age as compared to males [32]. As mice are active on wheels almost entirely during the dark period [33,34], they were put on a reversed photoperiod to allow measurements during normal work hours. The light cycle was set to 12:12, with lights off at 11:00 and lights on at 23:00 for all mice 5 days prior to the first VO_2max test (see below). At 09:00 on the day of the VO_2max test (similar time-before-dark as in the wheel running experiments), bottles were provided with one of the three treatments: water, caffeine or Red Bull. The Red Bull was at room temperature and stirred to remove dissolved CO_2 from the drink for approximately 15 min using a magnetic stir bar. This was done to reduce bottle leakage caused by carbonation. Nonetheless, sufficient leakage sometimes occurred to prevent gathering of accurate data on fluid consumption. The treatment regimens were structured in different permutations of the treatments to maximize diversity in order of treatments given and minimize any possible carry-over effects (e.g., mice were given treatments in balanced, random orders). Each mouse was tested twice for each treatment for a total of six tests per mouse, across six, non-consecutive, testing days with 4 days between trials.

At 12:00, mice were moved to the adjacent testing room and placed in the testing wheel one at a time over the period of 12:00 to 16:00 in the dark room, minimally lit with red light. The timing was chosen to match the peak wheel-running activity of mice [~1–3 h after the onset of darkness: 33, 34]. The O_2 analysis apparatus consisted of an incurrent H_2O scrubber (Drierite), mass flow controller, measurement wheel (effective volume 900 ml), H_2O and CO_2 scrubber (Drierite and indicating soda lime), followed by the O_2 sensor and O_2 analyzer (Fig. 1). The incurrent CO_2 was not scrubbed. Flow through the wheel metabolic chamber was set to 2000 ml/min, and instantaneous corrections were applied [35]. The O_2 consumption data were collected with Warthog Systems LabHelper X software (Mark A. Chappell and the Regents of the University of California, Riverside, CA, USA). This wheel apparatus for measuring VO_2max was chosen over the more traditional treadmill-based test because the former method obtains equivalent values [36] and more closely mimics the behavior for which the High Runner mice have been bred.

At the beginning of each run, a baseline oxygen concentration was recorded for approximately 1 min. Each mouse was then placed into the wheel metabolic chamber and forced to run until O_2 consumption plateaued and remained steady for ~75 s (trials averaged 6 min in length). At the end of each trial, each mouse was rated objectively for tiredness on a scale from 1 to 3. Tiredness was based on time spent motionless (typically prone) before the mouse resumed spontaneous locomotion, with the rating of 3 indicating >5 s before spontaneous locomotion and 1 indicating less than 1 sec before locomotion. Mice were also rated for trial quality, based on cooperativeness while being forced to run [37]. The scale was from 1 to 5, with 1 indicating no cooperation (the mouse would not attempt to run) and 5 being fully cooperative (mouse would continue to attempt to run even when pushed past the speed at which VO_2max was attained).

VO_2max values were obtained by processing and analyzing the $\%O_2$ data with Warthog LabHelper X software. The program recorded two channels: $\%O_2$ and flow. A typical graph for $\%O_2$ would contain the atmospheric baselines at the beginning and end of the tests, the resting O_2 consumption rate of the mouse placed on the wheel, followed by a steady increase in O_2 consumption as the mouse was forced to exercise until it reached VO_2max. The graphs were processed by creating a duplicate of the $\%O_2$ channel and creating a baseline collected from atmospheric air at the beginning and end of each trial. The $\%O_2$ samples were smoothed by the Warthog LabAnalyst X software over six consecutive measurements, with measurements recorded once per second. VO_2max was calculated from the $\%O_2$ (compared to baseline) and flow measurements, as the highest 60-second interval. Each mouse was tested twice per treatment, and the higher of the two measurements was used in the analysis.

2.4. Statistical analysis

For Experiments 1 and 2, we used the Mixed Procedure in SAS 9.1.3 (SAS Institute, Cary, NC, USA) to apply nested analysis of covariance (ANCOVA) to our data for wheel running. The main factors were drink type (treatment), sex, and linetype (HR vs. C), with replicate lines nested within linetype. The statistical interactions of sex by linetype, sex by drink, drink by linetype, and sex by drink by linetype were also tested, but were typically not significant. Degrees of freedom were 1 and 6 for testing the effects of linetype, drink type, sex, and the interaction terms (see tables in Results for full d.f.). Running data were analyzed as absolute responses, using only the data obtained on day 7. Outliers were removed if the standardized residual was > |3|.

The HR lines of mice have been bred for the amount of running over an entire daily cycle, on a 12:12 photoperiod as used in the present experiments, not the amount of wheel running restricted to the dark hours. As we wished to make the wheel measurements as relevant as possible to the conditions of this long-term selection experiment, we analyzed wheel running for as many hours as possible, which was 19 h, given the time required to administer the fluids. The number of revolutions run during the photophase is typically small for both HR and C lines of mice (e.g., see [33,34]).

For Experiment 3, we used the Mixed Procedure in SAS 9.1.3 to apply repeated-measures (individual mouse as the unit of repeated measures) analysis of covariance (ANCOVA) models to the VO_2max values. The primary factors were linetype (HR or C) and drink type, as well as their interaction. The covariates of body mass, time spent running, and age were also used in the model (though time spent running and age were not predictive, and thus only body mass was included in the data presented here). Tiredness and run quality were considered as covariates for the analysis of VO_2max, but they had little predictive value and ultimately were not included in the reported model. An outlier was removed if the standardized residual was > |3|, and this procedure was repeated as necessary (in the case of VO_2max, four times). In four

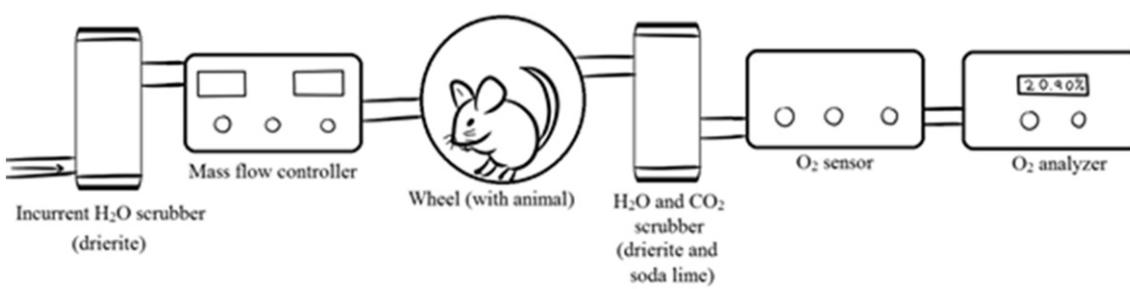

Fig. 1. Simplified schematic of apparatus to measure maximal oxygen consumption (VO_2max) during forced exercise in a wheel metabolic chamber (see [36,57]). CO_2 was not scrubbed from the incurrent air. The wheel metabolic chamber had an effective volume of 900 ml.

repetitions, a total of seven outliers from a total of 196 observations were removed (p-values were not viewed prior to the removal of outliers).

3. Results

3.1. Experiment 1: effects of Red Bull and Gatorade on wheel running

Averaging values for days 5 and 6 of wheel access, consistent with many previous studies of these lines of mice [5] HR mice ran 3.3-fold more revolutions than C ($p < 0.0001$), and females ran 1.2-fold more revolutions than males ($p < 0.05$), with no linetype by sex interaction ($p = 0.28$) (results not shown).

Based on analyses of absolute amounts of wheel running on day 7 (Table 2, Fig. 2), drink treatment affected both total revolutions run (+21% for Red Bull, −1% for Gatorade) and the number of active minutes (+23% for Red Bull, +2% for Gatorade), with no interactions.

The amount of fluid consumed (adjusted for spillage and evaporation) depended on drink type ($P < 0.05$), but was not significantly affected by any other factor or interaction term. On average, mice consumed 7.4 ± 0.88 g of water, 10.4 ± 0.84 g of Gatorade, and 8.4 ± 0.89 g of Red Bull. (Results were similar when body mass was used as a covariate.)

3.2. Experiment 2: effects of Red Bull, caffeine, and taurine on wheel running

Revolutions run was not significantly affected by drink type ($P = 0.15$), but HR mice ran more than C ($P < 0.0001$) and females ran more than males ($P < 0.05$), with no significant interactions (Table 2). We performed additional analyses to elucidate effects of caffeine. First, we computed an a priori contrast of water versus the three caffeine-containing fluids and found a marginally non-significant effect of caffeine ($F_{1,18} = 3.98$, $P = 0.06$). Second, we tested whether the amount of fluid containing caffeine, consumed during the 19-hour wheel trial, had an effect. This variable indicating caffeine dose was taken as the amount of Red Bull or caffeine or caffeine plus taurine solution consumed (the latter two were matched to Red Bull in terms of grams caffeine per ml of fluid), or given a value of zero for mice that had water. This covariate was a significant positive predictor of wheel revolutions ($F_{1,536} = 4.75$, $P = 0.03$) and the significance levels of the other factors were little changed (P for drink type = 0.16, P for linetype <0.0001, P for sex = 0.02, all other P = n.s.).

Drink treatment interacted with linetype in its effect on the number of minutes with any wheel revolutions ($P < 0.01$). Inspection of the values shown in Fig. 2 (and the online supplemental material) indicates that all three fluid treatments increased the number of active intervals, relative to water, regardless of sex or linetype, although this differential varied somewhat among the subgroups. In addition, HR mice of both sexes always had more active intervals than their C counterparts, regardless of the fluid being consumed.

The amount of fluid consumed (adjusted for spillage and evaporation) depended on drink type ($P < 0.0001$), was higher in males ($P = 0.001$), which are larger than females, but was not significantly affected by linetype or any interaction term. On average, mice consumed 8.0 ± 0.24 g of water, 5.1 ± 0.25 g of Red Bull, 8.0 ± 0.23 g of caffeine solution, and 8.4 ± 0.24 g of caffeine plus taurine solution. When body mass was included as a covariate ($P < 0.0001$), the effect of drink type was still highly significant ($P < 0.0001$), but the effect of sex was eliminated ($P = 0.89$), and again no other factor or interaciton term was significant. Adjusted for body mass (grand mean = 25.4 g), mice consumed 7.9 ± 0.26 g of water, 5.1 ± 0.27 g of Red Bull, 8.0 ± 0.25 g of caffeine solution, and 8.3 ± 0.26 g of caffeine plus taurine solution.

3.3. Experiment 3: effects of Red Bull and caffeine on VO_2max

The effect of treatment on VO_2max was not statistically significant (Table 3, Fig. 3). Linetype had a significant effect on VO_2max ($P < 0.05$: Table 3, Fig. 3), consistent with previous findings that HR mice have an increased maximal aerobic capacity [8,37–39]. Trial duration was not a significant predictor of VO_2max (weak positive effect, results not shown). Tiredness and run quality scores did not affect VO_2max, nor did their inclusion in the model have an appreciable effect on the effect sizes or significance level of other factors (results not shown). Thus, these scores were not included in the reported model. Cooperativeness was not significantly affected by treatment (Table 3, Fig. 4). HR mice tended to be more cooperative than C mice, but not significantly so ($P = 0.05$). Body mass was used as a covariate for VO_2max and was highly predictive ($P < 0.0001$, Table 3).

Although tiredness did not affect VO_2max, tiredness was significantly affected by treatment ($P < 0.01$; Table 3, Fig. 4), with caffeine-treated mice being rated as less tired than water-treated mice, and Red Bull-treated mice even less tired than those receiving caffeine. Test number (each mouse was tested six times for VO_2max) was also considered as a covariate, but was not a significant predictor ($P = 0.3$) and so was not included in the final model. Linetype, body mass, trial length, and age did not affect tiredness ($P > 0.05$; results not shown).

Table 2
ANOVA results for experiments 1 and 2: absolute responses. Drink type (Treatment) had a significant effect on revolutions run per day and number of active minutes in Experiment 1, as well as minutes run in Experiment 2 (Fig. 2). As compared with mice drinking tap water, Red Bull increased voluntary wheel running (total revolutions/day) in both Experiment 1 (differences of least squares means, $P < 0.05$) and Experiment 2 ($P < 0.05$). This increase in wheel running was caused by an increase in the time spent running, rather than the average or max speed of running. Least squares means and associated standard errors are presented in online supplemental material.

		Revolutions		Max speed		Mean speed		Active minutes	
Experiment 1									
Factor	df	F	P	F	P	F	P	F	P
Sex	(1, 6)	4.12	0.09	0.11	0.750	0.61	0.47	10.98	0.02
Linetype	(1, 6)	185.79	<0.0001	150.42	<0.0001	98.38	<0.0001	12.36	0.01
Treatment	(2, 12)	4.87	0.03	0.23	0.80	0.96	0.41	15.07	<0.001
Sex * treatment	(2, 214)	0.10	0.91	0.19	0.83	0.01	0.99	0.25	0.78
Linetype * treatment	(2, 12)	0.07	0.93	0.56	0.59	0.78	0.48	1.05	0.38
Sex * linetype	(1, 6)	0.60	0.47	0.28	0.61	0.73	0.43	0.65	0.45
Sex * linetype * treatment	(2, 214)	0.29	0.75	0.74	0.48	0.34	0.72	0.18	0.84
Experiment 2									
Factor	df	F	P	F	P	F	P	F	P
Sex	(1, 6)	7.96	0.03	4.06	0.09	3.35	0.12	13.98	<0.01
Linetype	(1, 6)	166.48	<0.0001	141.81	<0.0001	107.68	<0.0001	4.71	0.07
Treatment	(3, 18)	2.01	0.15	1.39	0.28	0.83	0.50	13.22	<0.0001
Sex * treatment	(3, 540)	0.22	0.88	0.55	0.65	0.47	0.70	0.71	0.55
Linetype * treatment	(3, 18)	1.05	0.40	0.49	0.69	0.26	0.85	5.27	<0.01
Sex * linetype	(1, 6)	0.77	0.41	1.93	0.21	1.30	0.30	1.99	0.21
Sex * linetype * treatment	(3, 540)	0.87	0.45	0.13	0.94	0.65	0.58	1.56	0.20

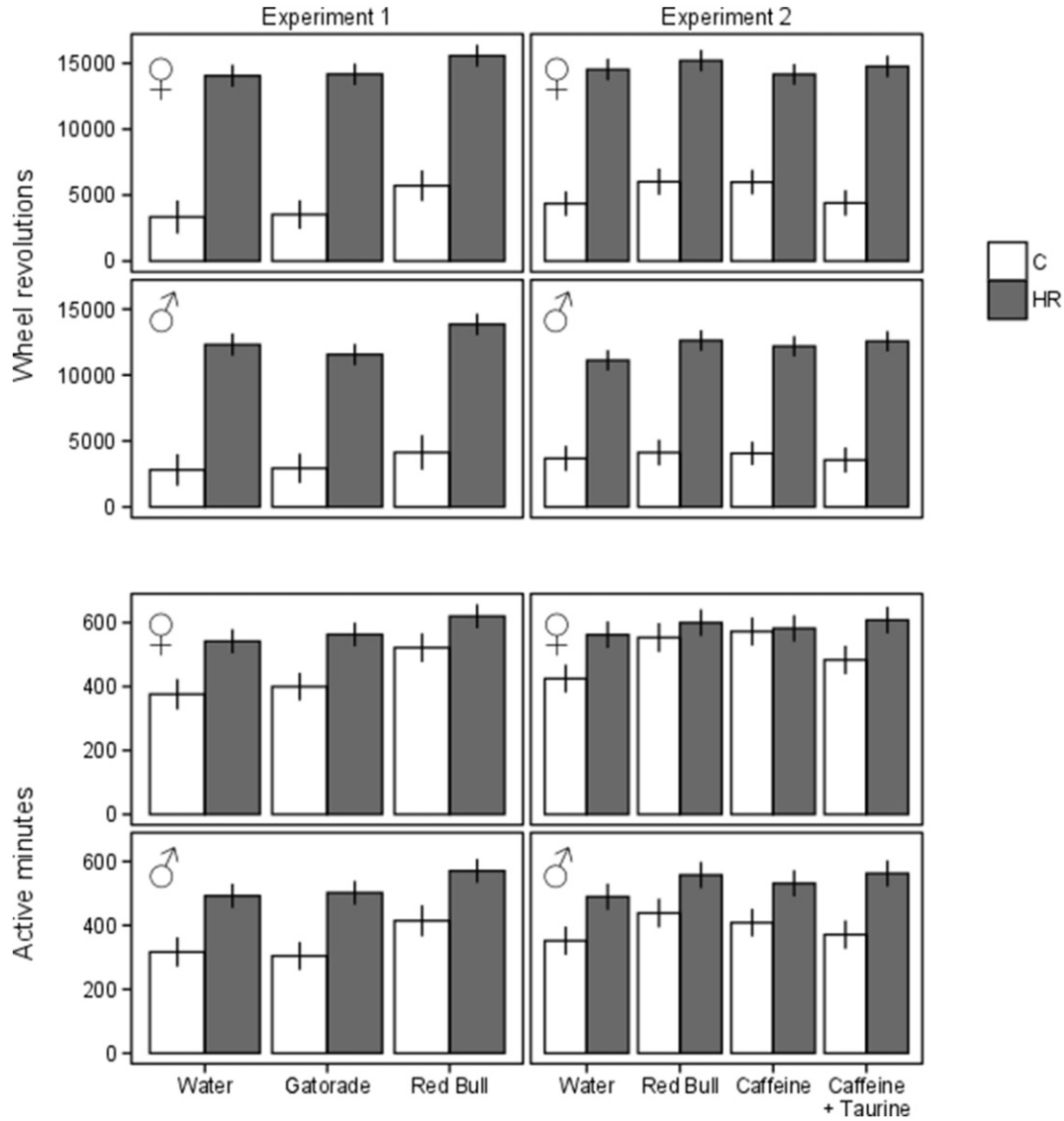

Fig. 2. Absolute responses for wheel revolutions and the number of active minutes. In experiment 1, both total revolutions run and the number of active minutes were significantly increased by Red Bull (see Table 2). In experiment 2, revolutions were increased by Red Bull. Wheel circumference = 1.12 m. Values are least squares means and associated standard errors from an ANOVA conducted in SAS Procedure Mixed.

We also analyzed the amount of fluid consumed immediately prior to initiation of VO_2max trials, adjusted for spillage and evaporation as described above. The amount of time between placing bottles on the cages and the start of the VO_2max trial averaged 293 min, but varied from 178 to 456 min among individual trials, so we used this time interval as a covariate. Adjusting for elapsed time ($P < 0.0001$), fluid consumption depended on drink type ($P < 0.001$), but was not affected by linetype ($P = 0.64$) nor the drink type by linetype interaction ($P = 0.72$). On average, mice consumed 4.7 ± 0.51 g of water, 5.3 ± 0.51 g of Red Bull, 8.2 ± 0.51 g of caffeine solution. (Results were similar when body mass was used as a covariate.)

Finally, we tested whether the amount of fluid containing caffeine, consumed prior to VO_2max trials, had an effect. This variable indicating caffeine dose was taken as the amount of Red Bull or caffeine solution consumed (the latter was matched to Red Bull in terms of grams caffeine per ml of fluid), or given a value of zero for mice that had water. This covariate was not a significant predictor of VO_2max ($P = 0.67$) and the significance levels of the other factors and body mass were scarcely changed. Results for analyses of cooperativeness and tiredness as dependent variables were also unaffected by inclusion of caffeine dose as a covariate.

Table 3
Repeated measures ANOVA results for experiment 3, which measured maximal aerobic capacity during forced treadmill exercise. The higher of the two measurements for an individual mouse and treatment combination were used in the analysis. Nested, repeated-measures ANCOVA indicated that body mass was a significant predictor of VO_2max ($P < 0.0001$), and HR mice had greater VO_2max than control mice ($P < 0.05$), but treatment did not significantly affect VO_2max and there was no linetype by treatment interaction. HR mice tended to be more cooperative, but not significantly so ($P = 0.05$).

		VO_2max		Tiredness		Cooperativity	
Factor	df	F	P	F	P	F	P
Linetype	(1, 6)	8.96	0.02	0.78	0.41	5.74	0.05
Treatment	(2, 12)	1.01	0.39	9.11	<0.005	0.89	0.44
Linetype * treatment	(2, 12)	0.44	0.65	0.31	0.74	0.41	0.67
Body mass	(1, 164)	28.14	<0.0001				

4. Discussion

As compared with mice drinking tap water, Red Bull increased voluntary wheel running (total revolutions/day) in both Experiment 1 (differences of least squares means, $P < 0.05$) and Experiment 2 ($P < 0.05$).

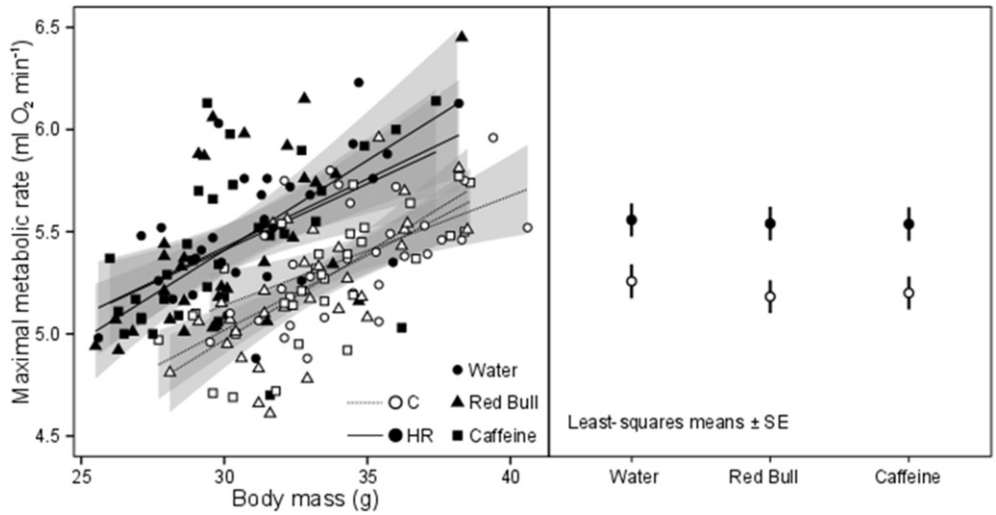

Fig. 3. VO_2max is greater in HR mice, but not affected by treatment. Left: VO_2max vs. body mass, separated by treatment and linetype. Symbols are individual treatments (the higher of two trials per mouse per treatment). Lines indicate least squares regressions within treatment and linetype, and the shaded area indicates 95% confidence intervals about the lines. Right: Average VO_2max values over the 3 treatments for HR and C mice. Values are least squares means and associated standard errors from a repeated-measures ANCOVA conducted in SAS Procedure Mixed.

The increase in total distance run was caused by a greater amount of time spent running (active minutes), not an increase in average (or maximum) running speed. When caffeine or caffeine + taurine was given in water, in the same concentrations as found in Red Bull (Experiment 2), the increase in wheel running relative to water was only about half that of Red Bull (Fig. 2) and not statistically significant ($P = 0.13$ and $P = 0.38$, respectively). If caffeine is the only ingredient affecting wheel running duration, then the differential effects versus Red Bull observed in Experiment 2 potentially could be explained if mice drank circa twice as much Red Bull as compared with the caffeine-containing solutions. However, the difference was opposite to this, with mice consuming, on average, about 8 ml of caffeine-containing solutions versus only 5 ml of Red Bull. (Previous studies have also showed that mice will consume more fluid when given caffeine solutions vs water [20,21,22]). Sugars alone can increase exercise performance in some contexts. In human studies, carbohydrates increase exercise performance, even if the carbohydrate is not consumed, suggesting powerful central in addition to peripheral effects of carbohydrate consumption or carbohydrate sensing in the mouth [40–42]. However, in the present study, a treatment of sugar, sodium, and potassium, but not caffeine (Gatorade) did not increase wheel running, and in similar experiments performed on females at generation 42, sucrose solutions increased wheel running revolutions per day in C but not HR mice [43]. Thus, the differential increase in wheel running (in both HR and C mice) between Red Bull and caffeine or caffeine + taurine must be attributed to additive or possibly interactive effects of one or more of the other ingredients of Red Bull (i.e., one of the sugars or B vitamins).

Maximal oxygen consumption (VO_2max) was not significantly affected by the fluid treatments (Table 3, Fig. 3). However, both Red Bull and caffeine reduced tiredness ratings of both HR and C mice, so the finding that Red Bull and caffeine caused an increase in wheel-running behavior may be attributable to decreased or delayed fatigue by affecting central nervous system fatigue (reviewed in [23]). Such an effect could be attributable to caffeine's known effects as an adenosine receptor antagonist [44], and/or it's complex effects on other neurotransmitters [45].

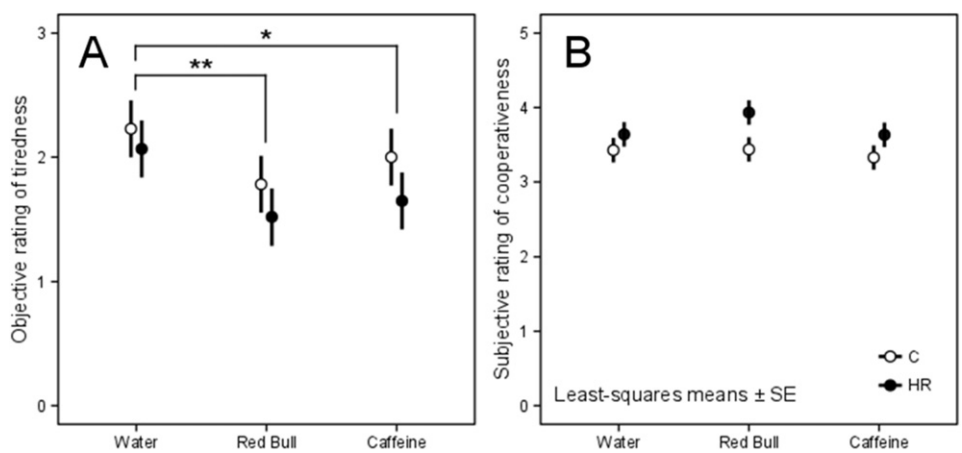

Fig. 4. Tiredness and cooperativity during VO_2max trials. Values are least squares means and associated standard errors from a repeated-measures ANOVA conducted in SAS Procedure Mixed. A single asterisk indicates a significant difference of least squares means between treatments at $P < 0.05$, and two asterisks indicate a significant difference of least squares means between treatments at $P < 0.001$. A) Left: average tiredness ratings at the end of VO_2max trials for the linetypes across treatment. Treatment significantly affected tiredness scores ($P < 0.01$), with Red Bull-treated mice being the least tired, and water-treated mice being the most tired. HR mice scored lower on average, but not significantly, and there was no linetype by treatment interaction ($P = 0.74$, Table 3). B) Right: average cooperativeness ratings during tests of VO_2max for the linetypes across treatment. HR mice tended to be more cooperative, but not significantly so ($P = 0.05$). Treatment did not affect these scores and the interaction was not significant ($P = 0.67$, Table 3).

Post-trial tiredness ratings did not significantly differ between HR and C lines, but tiredness was significantly reduced by caffeine and Red Bull (Table 3, Fig. 4). This effect might also be caused by the action of caffeine on the perception of fatigue, as caffeine acts as a stimulant on the central nervous system and can delay fatigue by blocking adenosine receptors [44]. Cooperativeness scored during VO_2max trials tended to be higher in HR than C lines of mice (but not significantly so, $P = 0.05$), supporting previous evidence of a motivational difference between the linetypes [12,46,47]. Studies of these lines at generation 10 [37] and 35 [48] did not find a significant difference in cooperativity between the HR and C lines, so if there is indeed a difference in cooperativity, it may have evolved in subsequent generations.

The mechanism by which caffeine increased wheel running and decreased tiredness in mice is unclear, but one or more of many proposed mechanisms may be involved. Caffeine has been shown to delay fatigue in a variety of studies on humans [30,49–51], but fewer studies have been conducted on rodents (but notably, see [19,20,21,22]). In mice, ad-lib access to bottles containing caffeine solution (similar to our method) or caffeinated tea increased wheel running and fluid consumption [20–22]. In gerbils, caffeine increased wheel revolutions when animals were placed inside a wheel and measured for 10 min, 30 min after receiving an intraperitoneal injection of caffeine [19]. If and how this sort of wheel-running test relates to the prolonged (19 h) test used in the present study is unknown. Ryu et al. [52] showed that rats which received caffeine ran significantly longer in a treadmill endurance trial and had significantly lower blood glucose, higher blood lactate, and higher liver glycogen at exhaustion than those receiving placebo, and attributed this to the effect of caffeine on lipolysis and "glycogen sparing". However, the evidence for changes in substrate utilization as a result of caffeine is mixed [53,54]. Lim et al. [55] also showed that rats injected subcutaneously with caffeine ran significantly longer in a treadmill endurance trial, and that caffeine-treated animals had lower tryptophan hydroxylase in the raphe nuclei, suggesting that caffeine suppressed serotonin-related central fatigue. Serotonin-induced central fatigue has been suggested to have been altered by selective breeding in HR mice, but in this case, caffeine seems to have affected both HR and C mice in the same way [16]. Davis et al. [44] showed that caffeine injected intracerebroventricularly increased spontaneous activity and time to exhaustion in a forced treadmill exercise trial, whereas intraperitoneal caffeine injection had no such effect, and concluded that a substantial part of the effect of caffeine on endurance was due to central nervous system effects. Central effects of caffeine in humans have been well reviewed [23,49,56].

In conclusion, Red Bull (and to a lesser extent, caffeine in water) increased voluntary wheel running by mice, and decreased tiredness following maximal metabolic rate trials, while not increasing aerobic capacity itself. The mechanism of action is unknown, but could be a combination of central and peripheral effects. In any case, positive effects on exercise behavior, if they can be shown to occur in humans, could have important implications for promoting voluntary exercise.

Conflict of interest statement

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.physbeh.2016.12.031.

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