Effects of voluntary running on oxygen consumption, RQ, and energy expenditure during primary prevention of diet-induced obesity in C57BL/6N mice

Jacob D. Brown,1* Scott P. Naples,2* and Frank W. Booth1,2,3,4
1Department of Medical Pharmacology and Physiology; 2Department of Biomedical Sciences; 3Department of Nutrition and Exercise Physiology; and 4Dalton Cardiovascular Center, University of Missouri, Columbia, Missouri

Submitted 31 May 2011; accepted in final form 28 May 2012

Brown JD, Naples SP, Booth FW. Effects of voluntary running on oxygen consumption, RQ, and energy expenditure during primary prevention of diet-induced obesity in C57BL/6N mice. J Appl Physiol 113: 473–478, 2012. First published May 31, 2012; doi:10.1152/japplphysiol.00668.2011.—Diet-induced obesity (DIO) in C57BL/6 mice is the standard model for studying obesity in mice. The few reports of DIO utilizing voluntary running provide contradictory results with respect to prevention of obesity. However, total energy expenditures associated with voluntary running during DIO are unknown. We hypothesized that voluntary running would increase the amount of total energy expended during DIO. Female C57BL/6N mice were randomly assigned to one of three experimental groups [high-fat diet with voluntary running (HFRun); high-fat diet without running (HFSed); and low-fat diet without running (LFSed)] for a 10-wk period. We confirmed production of obesity in HFSed, and more importantly demonstrated primary prevention of obesity by voluntary running in a group of cohorts (HFRun). Indirect calorimetry was performed to determine oxygen consumption (VO2) and respiratory quotient (RQ). The following novel mechanisms were identified in female C57BL/6N mice: 1) HFRun showed ~2 times greater total energy expenditures during a day compared with HFSed and LFSed; 2) HFRun had increased VO2 compared with HFSed and LFSed, lower RQ in the light period than HFSed, and lower RQ in both light and dark periods compared with LFSed; and 3) in the HFRun group, the magnitude of change in VO2 and RQ differed in dark and light periods during voluntary running. Our data combined with existing literature point to a potential threshold of physical activity that would prevent DIO in this mouse model. These data give a mechanistic explanation to resolve contradictory reports on whether voluntary running can prevent obesity in the DIO mouse model. In conclusion, voluntary running rescues high-fat fed, female C57BL/6N mice from obesity in DIO by doubling energy expenditure during the dark period and significantly increasing energy expenditure during the light cycle.

C57BL/6 MICE become obese when fed a high-fat diet. This animal model is termed “diet-induced obesity” (DIO). DIO has been justified as an obesity model because it resembles and triggers the human metabolic syndrome (6, 8), and is possibly the best of available parallels to study human obesity (1). A controversy exists as to whether access to wheel running attenuates DIO. Multiple studies (12, 19, 20) found, in the words of one study, “Access to a running wheel had no apparent effect on body mass or percent body fat in mice that consumed a high-fat diet” (12). Other papers report access to wheels diminishes gain in body fat in C57BL/6 mice (11) and in rats (17, 24, 28). No previous study, to our knowledge, has measured oxygen consumption, respiratory quotient (RQ), and energy expenditure during voluntary running in a DIO mouse model over the course of a day. Therefore, we posed the question, “Does access to a wheel lead to a difference in energy expenditure when animals are switched to a high-fat diet?” as a means to provide insight to the controversy.

We selected the following approaches to address the question above: 1) mice as species since ~1,200 results were elicited in a PubMed search for the combined terms of “diet-induced obesity” and “mice”; 2) female C57BL/6 mice, since they voluntarily run longer distances than male mice to maximize energy expenditure from voluntary running (22, 26), and no previous reports using long-term DIO female rats or mice could be located; 3) 10-wk-old females since the growth of their body weights tend to plateau after 10 wk of age, which would minimize any increase in fat mass due to body growth; and 4) young, lean mice to study primary prevention of obesity [which can be defined as avoidance of obesity before it occurs (13, 27)], and since older, obese rodents demonstrate relatively lower running distances (25).

With all of the above considered, the major purpose of our present study was to determine if energy expenditures from voluntary running are increased in female, DIO C57BL/6N mice that are allowed wheel access for running at the start of a high-fat diet compared with a group in which voluntary running was “environmentally knocked-down” to sedentary levels due to the absence of running wheels. Consequently in this design, the single programmed variable was either the presence or absence of running wheels from the start of high-fat feeding. We also included a low-fat fed group without running wheels to allow comparison of how much obesity, if any, would be prevented by voluntary running with high-fat feeding in our model. Further, while it is already known that a high-fat diet will lower RQ (21), it is unknown how voluntary running plus a high-fat diet will impact RQ. Additionally, it is unknown if total energy expenditure will increase during the course of a day in the DIO model with running wheels. We hypothesized that voluntary running would increase the amount of total energy expended during DIO of female C57BL/6N mice.

* J. D. Brown and S. P. Naples contributed equally to this study.
Address for reprint requests and other correspondence: F. W. Booth, Dept. of Biomedical Sciences, 1600 East Rollins St., Univ. of Missouri, Columbia, MO (e-mail: boothf@missouri.edu).

http://www.jappl.org
8750-7587/12 Copyright © 2012 the American Physiological Society
473
running had 24-h free access to a running wheel (diameter 11.5 cm, J.A. 862; Columbus, OH) for 22 h during the 8th and 9th week of this study. Daily weights for each mouse were averaged weekly and plotted on a graph. Daily weights for each mouse were averaged weekly and plotted on a graph. Daily weights for each mouse were averaged weekly and plotted on a graph."}

**MATERIALS AND METHODS**

**Animals.** The Institutional Animal Care and Use Committee approved all experimental protocols. Female, 10-wk-old, C57BL/6N mice (derived from a nullus colony obtained from the National Institutes of Health, Bethesda, MD; also designated as C57BL/6Nhsd; Harlan, Indianapolis, IN) were maintained in a 12:12-h light/dark cycle at 21–22°C. After acclimatization of the animals for 3 days on standard chow, the mice were randomly assigned to one of two diet groups for 10 wk: standard chow (Formulab 5008 from Lab Diets, Inc., Freeport, IL; 16.7% energy from fat), or a high-fat chow (Basal Purified Diet, TestDiet-Purina Mills, Richmond, IN; 45% energy from fat). High-fat-fed mice were further divided randomly into two subgroups for 10 wk, that either had access to voluntary running wheels (HFRun, high-fat diet with voluntary running; HFSed, high-fat diet without running; LFSed, low-fat diet without running; EE, energy expenditure; TEE, total EE; LME, lowest light-period metabolic rate; PAL, physical activity level. *Significantly different from HFRun (P < 0.05). †HFSed significantly different from LFSed (P < 0.05).

**Indirect calorimetry.** Indirect calorimetry was performed using the AccuScan Instruments, PhysioScan Metabolic System (Columbus Instruments, Columbus, OH) for ~22 h during the 8th and 9th week of the 10-wk study. This system uses zirconia and infrared sensors to monitor oxygen (O2) and carbon dioxide (CO2), respectively, inside respiratory chambers in which individual mice were tested. We modified the chamber height to allow for the same diameter running wheel as in their cages. All mice were monitored in the metabolic chambers for 22 h with ad libitum access to chow and water, and the HFRun group continued to have access to voluntary running wheels. Absence of automated food weighing led to a loss of indirect calorimetry in the final two h of light period for food collection and taking body weights prior to lights going off for the dark period, so data were extrapolated from the hour preceding the final 2 h of the light period. Gas samples were analyzed every 6 min per animal. Output parameters included VO2 and CO2 production (VCO2) (ml·kg⁻¹·min⁻¹). RQ was determined from RQ = VCO2/VO2.

**Calculation of total energy expenditure.** Energy expenditure (EE, kcal) was calculated separately for dark and light periods using average RQs and O2 consumed for these periods with the following equation from Longo et al. (18): total estimated energy expenditure (kcal) = [3.815 + (1.232 × RQ)] × VO2. Lowest light period energy expenditure was determined by using the lowest VO2 recorded during the light period and its corresponding RQ for the variables in the provided equation. Total energy expenditure (TEE) was calculated by the sum of the dark and light period EE. Dark period EE values were derived from the full 12 h of raw data from the metabolic chambers. Since light period metabolic chamber data for some of the animals was less than 12 h, RQ and accumulating O2 values were extrapolated to 12 h by extrapolating the average RQ and average accumulated O2 value for the 30 min of data points preceding the unmeasured time points. The extrapolated average RQ and accumulated O2 were then used to obtain the full 12 h values.

**Dual-energy X-ray absorptiometry.** On the day animals were euthanized, animals were anesthetized with ketamine (80 mg/kg), xylazine (10 mg/kg), and acepromazine (4 mg/kg), and whole body composition was measured using a Hologic QDR-1000/w dual-energy X-ray absorptiometry machine calibrated for mice.

**Statistical analyses.** One-way and two-way ANOVA with a post hoc Tukey or Dunn test were employed using SigmaPlot (SPSS) and SAS (SAS Institute) software. Significance was set at P < 0.05. Longo et al. (18) stated, “In practical terms, ensuring that all of the

---

**Table 1. Body composition, food intake, energy expenditure, and running distance**

<table>
<thead>
<tr>
<th></th>
<th>HFRun</th>
<th>HFSed</th>
<th>LFSed</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-wk study period</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial body weight, g</td>
<td>20.78 ± 0.59</td>
<td>19.58 ± 0.48</td>
<td>18.89 ± 0.63</td>
</tr>
<tr>
<td>Final body weight, g</td>
<td>24.87 ± 0.77</td>
<td>30.68 ± 1.88†</td>
<td>23.84 ± 0.97</td>
</tr>
<tr>
<td>Weight gain, g</td>
<td>4.09 ± 0.50</td>
<td>11.10 ± 1.59†</td>
<td>4.95 ± 0.50</td>
</tr>
<tr>
<td>%Change in body weight</td>
<td>19.76 ± 2.49</td>
<td>56.19 ± 2.44*</td>
<td>26.15 ± 2.44</td>
</tr>
<tr>
<td>Fat mass, g</td>
<td>4.34 ± 0.39</td>
<td>13.58 ± 1.91†</td>
<td>3.07 ± 0.57</td>
</tr>
<tr>
<td>Lean mass, g</td>
<td>20.11 ± 0.48</td>
<td>16.75 ± 0.39†</td>
<td>19.97 ± 0.69</td>
</tr>
<tr>
<td>Average daily running distance, km/day</td>
<td>16.98 ± 0.61</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total 10-wk running distance, km</td>
<td>1,188.54 ± 42.7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Citrate synthase activity (HFSed, n = 6)</td>
<td>709.4 ± 47.8</td>
<td>564.0 ± 64.9*</td>
<td>494.6 ± 39.9*</td>
</tr>
<tr>
<td>One-day in metabolic chamber</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TEE, kcal/day</td>
<td>14.98 ± 0.62</td>
<td>9.60 ± 0.32*</td>
<td>8.30 ± 0.27*</td>
</tr>
<tr>
<td>Normalized EE, kcal/g body wt</td>
<td>0.66 ± 0.03</td>
<td>0.35 ± 0.02*</td>
<td>0.36 ± 0.01*</td>
</tr>
<tr>
<td>LME, kcal/day</td>
<td>6.85 ± 0.32</td>
<td>6.02 ± 0.25†</td>
<td>5.05 ± 0.17*</td>
</tr>
<tr>
<td>PAL (TEE/LME)</td>
<td>2.20 ± 0.06</td>
<td>1.61 ± 0.05*</td>
<td>1.64 ± 0.02*</td>
</tr>
<tr>
<td>Running distance within metabolic chamber, km</td>
<td>11.51 ± 0.23</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE; n = 7, 10, and 6 (n = 4, 9, and 6) for HFRun, HFSed, and LFSed groups, respectively. HFRun, high-fat diet with voluntary wheel running; HFSed, high-fat diet without running; LFSed, low-fat diet without running; EE, energy expenditure; TEE, total EE; LME, lowest light-period metabolic rate; PAL, physical activity level. *Significantly different from HFRun (P < 0.05). †HFSed significantly different from LFSed (P < 0.05).
animals in a particular experiment remained in energy balance would be unfeasible; however, this could be achieved statistically by adjusting the data for changes in body mass during calorimetry—potentially improving the power of any comparison.” Consistent with Longo et al. (18), we performed analysis of covariance (ANCOVA) on energy expenditure values with the change in body weight during the time in the metabolic chamber as the covariate. Since no significant change for metabolic rate was found for adjustment in body mass, we do not show these data.

RESULTS

Voluntary running rescues mice from DIO. Average running distance for HFRun was 16.36 km/day (Table 1). No differences in body weights existed during the first 5 wk of treatments among the three experimental groups, or between HFRun and LFSed groups during the 10-wk treatments (Fig. 1). However, beginning at the 6th treatment week, HFSed body weights were greater than either LFSed or HFRun. At the end of the 10-wk treatment period, the gain in body mass of HFRun (4.09 g) and LFSed (4.95 g) were half as much in HFSed (11.10 g) (Table 1 and Fig. 1), implying that all groups were in positive energy balance at the end of the experiment (food intakes are not presented). HFSed (13.78 g) had a 3 times greater mass of total body fat than HFRun (4.36 g) and LFSed (3.07 g) at the end of the 10-wk treatments (Table 1). Thus HFRun was rescued from obesity. HFRun and LFSed groups did not differ after 10-wk treatments in any the aforementioned determinations [final body weight and % body fat; or in percentage gains in body weight and fat mass (Table 1)]. Mice averaged 11.5 km of voluntary running distance while in the metabolic chamber for 1 day (Table 1). A 26% higher citrate synthase activity in gastrocnemius muscle of HFRun than in HFSed provides evidence for an aerobic training effect after 10 wk of voluntary running (Table 1).

Energy expenditures. Energy expenditure in the dark period for HFRun was approximately twice that of either LFSed or HFSed, both not differing from one another (Table 2). In the light period, energy expenditure for HFRun was 34% higher than LFSed, but not greater than HFSed. HFSed energy expenditure in the light period exceeded LFSed by 18%.

Postexercise effects of voluntary running independently lowered RQ during the light period in high-fat-fed C57BL/6N mice (Fig. 2C; Table 2). A linear increase in accumulated V\(_{\text{O}_2}\) existed with running distance (Fig. 3A), as mice performed intermittent voluntary running throughout the dark period (Fig. 2A). Values are expressed as means ± SE; \(n = 7, 10, \) and 6 for HFRun, HFSed, and LFSed, respectively. *Significantly different from HFRun within period (\(P < 0.05\)). †HFSed is significantly different from LFSed (\(P < 0.05\)). ‡Respiratory quotient (RQ) is significantly different between dark and light periods in HFRun (\(P < 0.05\)).
Increased Energy Expenditure Prevents Obesity in DIO Mice • Brown JD et al.

2A). Total VO₂ and energy expenditures for voluntary running in HFRun were twice as great as HFSed during the dark (voluntary running) period (P < 0.05) (Fig. 2B; Table 2). HFRun total VO₂ and energy expenditures remained higher (~20%) in the first 6 h of the light (sleep) period (P < 0.05) (Fig. 2B; Table 2) compared with HFSed group. The increase in total energy expenditures during the first 6 h of the light period in HFRun was not due to running during that period (Fig. 2A).

TEE was 56% and 80% greater in HFRun than in HFSed and LFSed, respectively (Table 1). Using the calculation for physiological activity level (PAL = TEE/lowest light-period metabolic rate), PAL was significantly higher in HFRun than both HFSed and LFSed (Table 1).

High-fat diet lowered average RQ. Addition of high fat to the diet of sedentary mice without voluntary running lowered RQ from 0.90 to 0.85 and from 0.90 to 0.82 during dark and light periods, respectively (comparison of LFSed to HFSed in Table 2).

Post-voluntary running effect in light period lowers RQ. Since our automated counter detected no wheel running in the light period, the lowering of RQ from 0.82 to 0.77 in the light period (comparison of HFSed to HFRun in Table 2) could be considered a post-voluntary running effect.

DISCUSSION

We show for the first time, to our knowledge, that prevention of DIO in the C57BL/6 mouse by voluntary running is associated with a doubling of energy expenditure during the dark (active) period and a significant increase in energy expenditure during the light (sleep) period. Further, our energy expenditure and running distance data provide insight leading to a novel interpretation and potential resolution of a current literature discrepancy in DIO voluntary running publications; specifically, why do not all papers using DIO voluntary running show that voluntary running prevents obesity in DIO rodent models (11, 12, 17, 19, 20, 24, 28). During our data interpretation, we posited that varying running distances, as reported in the literature, might provide a potential explanation for contradictory outcomes in publications differing on whether access to wheels for voluntary running prevents DIO. We propose that voluntary running distances below an undefined threshold increase energy expenditure to a level sufficient to prevent obesity in the DIO mouse model.

The few reports of DIO utilizing voluntary running provide contradictory results with respect to prevention of obesity. For example, in a recent study Jung and Luthin (12) concluded, “Access to a running wheel had no protective effect on body mass or %body fat in C57BL/6 mice that consumed either a high-fat or a high-carbohydrate diet during a 12-wk period.” In contrast, other reports show attenuation of obesity when high-fat-fed, C57BL/6, DIO mice while running distances above this undefined threshold increase energy expenditure to a level sufficient to prevent obesity in the DIO mouse model.
model. We suggest that this new knowledge needs to be considered in future DIO papers as “knocking down” instinctive voluntary running of sufficient distance alters the outcome of obesity.

Interestingly, increased oxygen consumption and lower RQ occurred in the excessive postexercise oxygen consumption (EPOC) period during the early hours of the light period for mice in our study, to our knowledge, for the first time. This observation is relevant to human postexercise responses, as described next. Børsheim and Bahr (5) and LaForgia et al. (15) in two EPOC reviews both concluded that sufficient exercise quantity in humans is required to pass a threshold effect to observe EPOC. LaForgia et al. (15) point out that an intensity threshold of ~50–60% of maximal VO₂ (VO₂ max) must be reached before EPOC manifests. The basis of the increased energy expenditure in EPOC from the current experiment is unknown. However, Kiens and Richter (14) suggest that muscle glycogen resynthesis has such a high metabolic priority postexercise that lipids are used to cover the energy expenditures instead of blood glucose, and this could be the cause of the increased VO₂ and decreased RQ observed in HFRun. Percentage contributions from foraging activity and feeding thermogenesis as well as muscle glycogen resynthesis measurements during EPOC were not determined in our experiment.

Biases exist in our experimental selection criteria. We selected C57BL/6N mice as the strain and species due to their popularity in DIO publications, as well as being the only C57BL/6 mouse strain supplied by our vendor during the development of this study. Serendipitously, C57BL/6N mice seem to voluntarily run longer distances than other C57BL/6 strains, such as C57BL/6J (19, 20). This coincidence contributes to interpretations in this paper. We restricted our experimental design to primary prevention of obesity in young, lean mice because most older, obese rodents voluntarily run short distances that may not have been sufficient to test our hypothesis (25). Female mice were selected as the sex because they voluntarily run longer distances than do male mice (22, 26), and thus should increase their energy expenditure from voluntary running more than male mice. On the whole then, the bias of our experimental conditions was in the direction to exaggerate differences for obesity between HFSed and HFRun C57BL/6N mice.

Multiple limitations exist for experimental design and in available equipment. Approximately 99% of the 70-day access to the voluntary running wheel was performed in large, conventional mouse cages. Thus energy expenditure data are limited to day of residence in the indirect calorimetry chamber with the assumption that chamber data may underrepresent the other 69 days when mice ran an average of 48% longer distances daily (Table 1) in conventional mouse cages with the same type and diameter of running wheel as in the chamber. Importantly, however, the underestimation of the increased energy expenditure in HFRun did not underestimate the doubling of total energy expenditure in the dark period. The indirect calorimetry chamber likely also underestimated energy expenditure as the chamber ceiling had a solid ceiling as opposed to a wire cage top that mice normally use for hanging. The metabolic chamber had neither apparatus for determining cage locomotor activity, collecting urine, or automatically weighing food so our data are restricted to energy expenditures, running distances, and body fat. Thus exact fat oxidation values could not be made without determinations of energy balance status, protein turnover, and de novo lipogenesis. While this study did not study the effects of secondary prevention, or reversal, of obesity through exercise, it did demonstrate that primary prevention of obesity in DIO through voluntary running of sufficient distance prevents fat gain.

Primary prevention of a risk factor, such as obesity, is gaining clinical significance (3, 4, 7, 10, 27). For example, improvements in aerobic fitness from childhood to adolescence were associated with a lower risk of becoming overweight/obese in adolescence (23). Doubling the frequency of physical education sessions of 12- to 14-yr-old adolescents was a sufficient stimulus to improve aerobic fitness, which has been shown to primarily prevent many chronic diseases and decrease mortality (2). Our current interpretations provide additional insight as for the need of sufficient energy expenditure to prevent obesity in the first place.

In conclusion, we reveal here for the first time that a major mechanism of total energy expenditures in high-fat-fed, female, C57BL/6N mice is the presence or absence of voluntary wheel running. The current study demonstrates primary prevention of DIO C57BL/6 mouse model is efficacious if total energy expenditures are increased by addition of sufficient voluntary running distance. Alternatively, knocking down the dark and light energy expenditures by the lack of voluntary running is shown here for the first time to be a contributory mechanism to obesity in DIO mice.

ACKNOWLEDGMENTS

We thank Richard Madsen, Greg Petroski, and Bin Ge for analysis of indirect calorimetry data and Joe Company and Nathan Jenkins for offering comments on the manuscript. Mission Enhancement Funds from the University of Missouri purchased calorimetry equipment. The experimental study was funded by anonymous gifts.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: J.D.B., S.P.N., and F.W.B. analyzed data; J.D.B., S.P.N., and F.W.B. interpreted results of experiments; J.D.B. and F.W.B. prepared figures; J.D.B. and F.W.B. drafted manuscript; J.D.B. and F.W.B. edited and revised manuscript; J.D.B., S.P.N., and F.W.B. approved final version of manuscript; S.P.N. and F.W.B. performed experiments; F.W.B. conception and design of research.

REFERENCES


J Appl Physiol • doi:10.1152/japplphysiol.00668.2011 • www.jappl.org

477

Increased Energy Expenditure Prevents Obesity in DIO Mice • Brown JD et al.
Increased Energy Expenditure Prevents Obesity in DIO Mice • Brown JD et al.


