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Treadmill Running of Mouse as a Model for Studying Influence of Maternal Exercise on Offspring

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[Abstract] Epidemiological studies robustly show the beneficial effects of maternal exercise in reducing maternal birth complications and improving neonatal outcomes, though underlying mechanisms remain poorly understood. To facilitate mechanistic exploration, a protocol for maternal exercise of mice is established, with the regimen following the exercise guidelines for pregnant women. Compared to volunteer wheel running, treadmill running allows precise control of exercise intensity and duration, dramatically reducing variations among individual mouse within treatments and facilitating translation into maternal exercise in humans. Based on the maximal oxygen consumption rate (VO_2max) before pregnancy, the treadmill exercise protocol is separated into three stages: early stage (E1.5 to E7.5 at 40% VO_2max), mid stage (E8.5 to E14.5 at 65% VO_2max), and late stage of pregnancy (E15.5 to birth at 50% VO_2max), which demonstrated persistent beneficial effects on maternal health and fetal development. This protocol can be useful for standardizing maternal treadmill exercise using mice as an experimental model.

Keywords: Maternal exercise, Pregnancy, Treadmill running, VO_2max, Mice, Protocol

[Background] Maternal exposure to adverse environmental stimuli leads to various physiological changes in the offspring (Hay *et al.,* 2016; Walejko *et al.,* 2019; Battarbee *et al.*, 2020), including predisposition of offspring to the development of obesity and type 2 diabetes (Godfrey *et al.*, 2017; Wesolowski *et al.*, 2018). Physical exercise is highly accessible and a prominent therapeutic tool to combat diet-induced obesity in adults (Peres Valgas da Silva *et al.*, 2019). Epidemiological studies robustly show the beneficial effects of maternal exercise in improving maternal health and neonatal outcomes (Hopkins and Cutfield, 2011; Nascimento *et al.*, 2012; Moyer *et al.*, 2016; da Silva *et al.*, 2017; Rogozinska *et al.*, 2017; Davenport *et al.*, 2018a and 2018b; Ming *et al.*, 2018; Beetham *et al.*, 2019; Hoover and Louis, 2019; Pastorino *et al.*, 2019; Wang *et al.*, 2019). But surprisingly, the biological effects of maternal exercise on offspring health have only been sparsely examined in the context of maternal obesity and high fat diet intake (Laker *et al.*, 2014; Stanford *et al.,* 2015; Stanford e*t al.,* 2017; Musial *et al.*, 2019; Uddin *et al.*, 2019), as well as normal chow diet (Raipuria *et al.*, 2015), the effects of maternal exercise of healthy mothers on fetal development remain largely unexplored. Mice is the most commonly used model for biomedical studies; to facilitate mechanistic exploration, an exercise protocol for pregnant mice which can be easily translated into exercise in pregnant women is needed.

Both treadmill exercise and voluntary wheel running are commonly used for exercise training in rodents. We recommend to use treadmill exercise because the exercise intensity and length can be precisely controlled, which is very similar to treadmill training in humans, increasing translational value. On the other hand, large variations in exercise intensity and duration among individual mouse exist in voluntary wheel running, making data interpretation difficult (Goh and Ladiges, 2015). Also, the exercise intensity and duration cannot be controlled in voluntary wheel running and thus difficult to be translated into human exercise. Nonetheless, treadmill exercise training induces low levels of acute stress to mice, but this concern can be alleviated by applying the same procedure to both control and exercised mice. Actually, regular treadmill exercise at moderate intensity has protective effects in stress-related symptoms (Patki *et al.*, 2014; Seo, 2018; Loprinzi and Frith, 2019). Based on exercise guidelines published by American College of Obstetricians and Gynecologists (Zavorsky and Longo, 2011), we propose the following protocol for treadmill exercise, which composes of three stages with exercise intensity calculated based on the maximal oxygen intake (VO_2max) before pregnancy. The initial stage is from embryonic day 1.5 (E1.5) to E7.5, mid-stage from E8.5 to E14.5, and late-stage from E15.5 to birth, with the intensity of exercise at 40%, 65%, and 50% of VO_2max, respectively. Using this protocol, we found that maternal exercise slightly reduced weight gain during pregnancy, but had no effect on birth weight; in addition, offspring metabolic health was improved due to maternal exercise and detailed changes were reported previously (Son *et al.*, 2019 and 2020). Thus, this protocol fits for studying maternal impacts on fetal development and long-term health of the next generation.

Materials and Reagents

1. Adult C57BL/6J pregnant mice
2. High-fat diet (HFD), 60% energy from fat (Research Diets，D12492)

Equipment

1. Oxymax Fast 4 lane modular treadmill system (Columbus Instruments, Columbus, OH, USA)
2. Indirect open circuit calorimetry system (Comprehensive Lab Animal Monitoring System, CLAMS; Columbus Instruments, Columbus, OH, USA)

Software

1. Oxymax for Windows v4.93 (Columbus Instruments, Columbus, OH, USA) with listing parameters: VO_2, O_2 in&out, DO_2, $ACCO_2$, VCO_2, CO_2 in&out, DCO_2, $ACCCO_2$, RER, HEAT, Flow (Figure 1)

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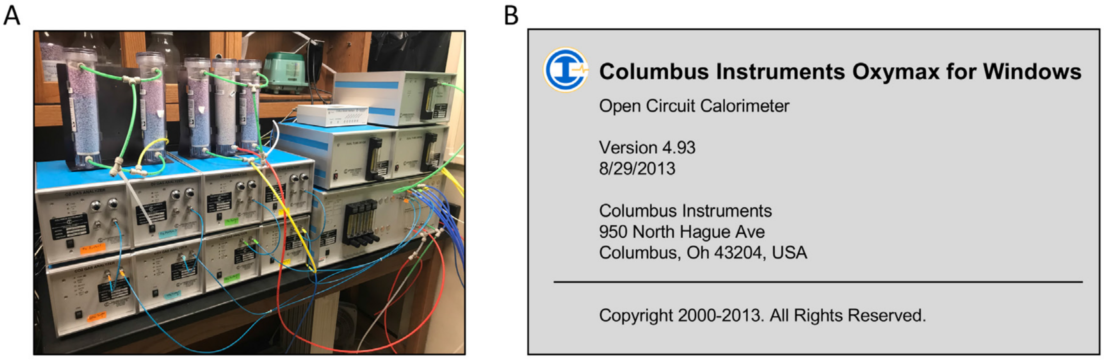


Figure 1. Calorimeter and software for measuring oxygen consumption. A. Open circuit calorimeter. B. Software for open circuit calorimeter.

Procedure

A. Setting target intensities

1. Before mating and initiating exercise training, female mice need to be accustomed with treadmill running by either training daily or every alternative days for one week (10 m/min for 10 min once)
2. Measuring the maximal oxygen consumption rates (VO_2max)
   a. Before mating, female mice are subjected to VO_2max measurement. The body weight (g) and age of mice need to be recorded, which are needed for normalization of oxygen consumption rate by the software.
   b. Before treadmill running, mice are put on the treadmill with indirect open circuit calorimetry system for 5 min to calm down.
   c. Treadmill running (without incline) is initiated following the progressive loading in intensity shown in Table 1.
   d. Running is stopped when mice exhibit exhaustion: When mice touch the electric shock bar three times within five seconds, those mice are regarded as exhaustion.
   e. The target intensities (40%, 65%, and 50% of VO_2max) will be calculated (Zavorsky and Longo, 2011).

Table 1. The intensity of single bout of exhaustive treadmill exercise for maximal oxygen consumption rates

Progressive loading in intensity (speed)										
Intensity (m/min)	0.0	5.0	8.0	12.0	15.0	17.2	21.8	24.0	26.0	28.0
Time (s)	300	300	300	300	300	300	300	300	300	300

B. Exercise during pregnancy

1. After mating which is confirmed in the morning by the presence of vaginal smear and designated

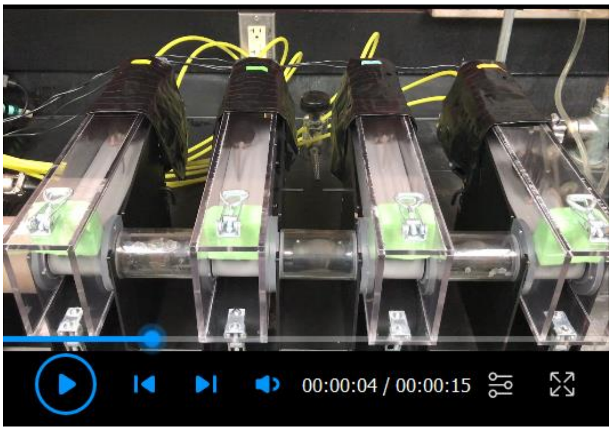
as embryonic day 0.5 (E0.5), pregnant mice are subjected to treadmill exercise training without incline every morning starting at E1.5.

a. Based on the guideline for pregnancy (Zavorsky and Longo, 2011), a three stage exercise is used: the early stage (E1.5 to E7.5), mid-stage (E8.5 to E14.5), and late stage (E15.5 to birth) with target intensity of 40%, 65%, and 50% VO_2max, respectively.
b. As an example, the target intensities of healthy or obese adult C57BL/6J female mice (16-week-old) are shown in Table 2 (Son *et al.*, 2019 and 2020).
c. During exercise training, electric shocks are used to motivate individual mouse which may be reluctant to run, but the usage for pregnant mice should be avoided in order to minimize stress. Instead, tail touching is recommended to encourage run.
d. In a single training session, individual mice will only be allowed to be tail-touched twice without being stopped. If mice refuse to run upon 2nd touch, mice will be allowed to rest for 5 min before resuming the training/testing protocol. Mice will be removed from the trial if a 4th touch occurs.
e. To encourage mice to rum, a black cover in the front end of treadmill can be used, which creates a dark area (see Video 1).

Table 2. The target intensities for adult C57BL/6J pregnant mice

Target % of VO_2max (intensity) for healthy pregnant mice			
Trimester stages	Early stage	Mid-term	Late stage
Warming up (10 min)	5 m/min		
Main exercise (40 min)	40% (11 m/min)	65% (14 m/min)	50% (12.5 m/min)
Cooling down (10 min)	5 m/min		
Target % of VO_2max (intensity) for obese pregnant mice*			
Warming up (10 min)	5 m/min		
Main exercise (40 min)	40% (10 m/min)	65% (13 m/min)	50% (12 m/min)
Cooling down (10 min)	5 m/min		

*Obese adult mice are fed a high-fat diet (HFD, 60% energy from fat; D12492, Research Diets) for 8 weeks. The mice had gained over 25% of initial body weight due to HFD.



Video 1. Mice during treadmill exercise training

Data analysis

Indirect open circuit calorimetry system is utilized to measure oxygen consumption (VO_2), carbon dioxide production (VCO_2), and respiratory exchange ratio (RER). For setting target intensity, VO_2max is determined as the highest value with RER over 1.0. Then, the target percentages (40, 65, and 50%) of intensities (speeds) are calculated to be proportional to the speed at VO_2max. For example, if the VO_2max is 10,000 ml/kg/hr when the speed is 20 m/min, the 50% of exercise intensity is the speed when the oxygen consumption rate is:
(10,000 – resting value) x 0.5 + resting value.

Notes

To set the target intensities, heart rate and total calorie use are alternative endpoints. We recommend to use the target intensities (speeds) which proportionally match with target percentages of VO_2max and can be easily performed.

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Competing interests

The authors declare that they have no competing interests.

Ethics

All animal experiments were approved by the Institute of Animal Care and Use Committees (IACUC) at the Washington State University (Protocol number: 6704; valid between Sept. 25, 2020-Sept. 24, 2023).

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