Original Article

MOLECULAR METABOLISM

Maternal exercise intervention in obese pregnancy improves the cardiovascular health of the adult male offspring

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ABSTRACT

Objective: Obesity during pregnancy is associated with an elevated risk of cardiovascular disease in the offspring. With increased numbers of women entering pregnancy overweight or obese, there is a requirement for targeted interventions to reduce disease risk in future generations. Using an established murine model of maternal obesity during pregnancy, we investigated if a treadmill exercise intervention in the mother could improve offspring cardiac health and explored potential underlying mechanisms.

Methods: A 20-minute treadmill exercise intervention protocol was performed 5 days a week in diet-induced obese female C57BL/6 mice 1 week prior to, and up to E17 of pregnancy. All male offspring were weaned onto a control diet and studied at 8 weeks of age when their cardiovascular physiology was assessed by *in vivo* echocardiography and non-invasive tail cuff plethysmography. Cardiomyocyte cell area, re-expression of fetal genes and the expression of calcium handling and sympathetic activation proteins were determined.

Results: At 8 weeks, there was no difference in bodyweight or fat mass between groups. Offspring of obese dams developed pathologic cardiac hypertrophy, hypertension and cardiac dysfunction characterized by reduced ejection fraction ($p < 0.001$). Maternal exercise prevented cardiac hypertrophy and dysfunction but failed to prevent hypertension. These offspring of exercised dams also had enhanced ($p < 0.001$) levels of calcium handling proteins and a sympathetic-activated inotropic response.

Conclusions: Exercise in obese pregnancy was beneficial to offspring cardiac function and structure but did not influence hypertension suggesting they are programmed by separate mechanistic pathways. These data suggest combination interventions in obese pregnancies will be required to improve all aspects of the cardiovascular health of the next generation.

Keywords Pregnancy; Obesity; Exercise; Hypertrophy; Echocardiography

1. INTRODUCTION

Cardiovascular disease (CVD) is the number one cause of death globally [1]. There is already considerable understanding in the cardiovascular research field that risk factors for disease development are both modifiable (e.g. poor diet, inactivity, smoking) and non-modifiable (e.g. genetic). Prevention strategies thus far have focused specifically on attenuating disease risk by reducing an individual's modifiable risk factors postnatally. However there is considerable evidence from human and animal studies that an adverse environment *in utero* increases the risk of cardio-metabolic disease in adult life independent of genetics or their postnatal adult environment [2–4]. Therefore,

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Abbreviations: BW, Body Weight; CVD, Cardiovascular Disease; ESV, End Systolic Volume; IVS, Interventricular Septum; LV, Left Ventricle; LVPW, Left Ventricular Posterior Wall; TD-NMR, Time Domain Nuclear Magnetic Resonance

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there is strong rationale that disease risk could be reduced prior to birth through targeted interventions to the mother before and during pregnancy [5].

The number of women entering pregnancy either overweight or obese has risen significantly over the last decade, and in 2014 these accounted for 50% of pregnancies in the US [6]. These statistics demonstrate the scale of the problem and highlight the importance of intervention strategies not only for the immediate [7] but also the long-term health of both the mother and her child. Studies in humans have consistently shown that long-term cardiovascular health is compromised in offspring exposed to an obese pregnancy [8–12]. Due to rising levels of obesity worldwide, it is critical to investigate a targeted intervention achievable during an obese pregnancy to prevent transmission of poor cardiometabolic health from mother to child. Without such intervention, the burden of CVD in future generations is likely to soar. Current guidelines for exercise in pregnant women from the American Congress of Obstetricians and Gynecologists advise women with uncomplicated pregnancies to participate in at least 20–30 min of moderate aerobic and strength conditioning physical activity on most days of the week [13]. However, most women do not achieve these recommendations [14]. A number of human trials worldwide have attempted to alter lifestyle through increased physical activity and/or dietary modification [15–18]. Although changing behavior is a challenge, a recent systematic review demonstrated that lifestyle (diet and physical activity) intervention strategies in pregnancy were enough to limit gestational weight gain irrespective of the mother's BMI, ethnicity and age [19]. Most human pregnancy intervention studies report only immediate effects during pregnancy and at the point of delivery. Recent findings from UPBEAT have shown that increased physical activity and a reduction in glycemic load during pregnancy, reduced offspring adiposity at 6 months of age [20]. However, due to the long-term nature of CVD and relative infancy of such intervention studies, data on such outcomes in humans will not be known for many years. There is, therefore, a need to model intervention strategies in animals to longitudinally determine the cardiometabolic effects on the offspring.

Our laboratory uses a well-established murine model of maternal diet-induced obesity in which we mimic human obesity by feeding murine dams a Western-style diet that is high in sugar and fat. Offspring develop pathologic cardiac hypertrophy [21] and importantly, the hearts of these offspring demonstrate impaired function *in vivo* and *ex vivo*, with evidence of sympathetic dominance [22,23] as well as hypertension [24]. The aim of the current study was to use this model to test the effectiveness of exercise intervention during obese pregnancy at preventing programmed cardiovascular dysfunction in the adult offspring and to identify underlying molecular mechanisms.

2. METHODS

2.1. Animals

Studies were conducted according to the UK Home Office Animals (Scientific Procedures) Act 1986 and following ethical review and approval by the University of Cambridge Animal Welfare and Ethical Review Board. This study capitalized on an already established maternal diet-induced obesity model using C57BL/6J mice [25]. At 3 weeks of age, female mice were randomly assigned to receive either a standard laboratory RM1 diet (approx. 7% simple sugars, 3% fat, 50% polysaccharide, 15% protein [w/w], 10.74 kJ/g)] or a semi-synthetic, energy-rich, highly palatable obesogenic diet (approx. 10% simple sugars, 20% animal lard, 28% polysaccharide, 23% protein [w/w], 28.43 kJ/g) supplemented with sweetened condensed milk (Nestle, UK) (approx. 16% fat, 33% simple sugars, 15% protein, 13.7 kJ/g), which was fortified with mineral and vitamin mix AIN93G. Both diets were purchased from Special Dietary Services (UK). Detailed energy content is supplied in Supplementary Table 1. A first pregnancy is undertaken to prove fertility and establish good maternal care. The interval between the weaning of the first pregnancy and mating for the second pregnancy was at least one week and animals were maintained on the same diet throughout both pregnancies. From this point, body composition was assessed using Time Domain-Nuclear Magnetic Resonance (TD-NMR); this method was chosen to allow longitudinal non-invasive measurement of body composition in the dams both before and during pregnancy in the same animal. Once females on the obesogenic diet reached an absolute fat mass greater than 10 g, they were considered obese and were mated for the second pregnancy. Control females were only entered into the study if they had an absolute fat mass under 5 g. These 2 groups of females were rested for one week before mating (to match for the training week undertaken by the obese-exercised group), and weekly TD-NMR measurements were taken before mating and throughout pregnancy. Females were mated with previously LAD1 fed males (diets were matched to the incoming female during the period of mating) and maintained on their respective diets throughout pregnancy and lactation. At the time females were mated for their second pregnancy, they had been on the diet for approximately 15 weeks and were approximately 18 weeks old.

2.2. Maternal exercise intervention

For the third group, obese females ($>=$ 10 g fat mass) were trained to run on a treadmill. Treadmill speed was gradually increased over 5 days of training. By day 5, mice were running 20 min per day at a top speed of 12.5 m/min. Mice were time mated after 1 week of training. A 5-day exercise protocol (weekdays) followed by 2 days rest (weekend) was continued up to and including gestational day 17. These mice are referred to as Obese-Exercise (Ob-Ex). The experimental protocol is shown schematically as part of the graphical abstract.

Litter size (all groups) was standardized to 6 pups on postnatal day 2, and only male offspring were studied to avoid the influence of sex. At weaning, all offspring were weaned onto RM1. The N for all experiments corresponds to the number of independent litters (number of dams, Control n = 16, Obese n = 16 and Ob-Ex n = 7). To eliminate confounding within litter effects, only one male per litter was used for each outcome measure. TD-NMR measurements were undertaken weekly in male offspring from 4 to 8 weeks of age. At eight weeks of age, mice were fasted overnight (16 h). Tail blood glucose was measured (Alphatrak 2, Zoetis, USA) before the mice were killed by rising CO_2. Immediately *postmortem*, blood was collected by cardiac puncture and tissue was isolated. Our study aimed to address if exercise during an obese pregnancy was effective at reducing the long-term detrimental cardiovascular effects on the offspring. As we were not seeking to investigate the impact of exercise during a lean pregnancy, we did not include an exercised control group of dams. This was to ensure that we were in keeping with the ARRIVE guidelines (NC3Rs) and only utilized animals required to fulfil our specific research aims.

2.3. Serum analysis

Total serum cholesterol, free fatty acids and triglycerides were measured by the MRC MDU Mouse Biochemistry Laboratory (Addenbrooke's Hospital, UK).

2.4. Histology

Hearts were immersion-fixed in 10% neutral buffered formalin, processed and sectioned at 10 μm. Using a previously published method [23], three mid-cardiac sections from each heart were stained; six