Original Article

Juvenile activity levels affect predisposition to metabolic syndrome induced by maternal hypoxia in male offspring rats

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Abstract: Background: An adverse intrauterine environment can predispose offspring to metabolic disorders. However, fetal programming maybe reversed by postnatal lifestyle especially physical activity levels. The goal of the present study was to determine whether predisposition induced by maternal hypoxia is aggravated or reversed by juvenile activity patterns, and to study the underlying molecular mechanisms. Methods: Sprague-Dawley rats experiencing maternal hypoxia were randomly assigned to juvenile activity patterns (sedentariness, cage activity and exercise). In male offspring, body weight, adiposity index, blood-borne factors, intramuscular oxidative/anti-oxidative status and the main proteins regulating glycolipid metabolism were evaluated by biochemical assays. Results: Maternal hypoxia offspring rats were born with reduced body weight and hyperlipemia. When full grown, lipid metabolism down-regulated and lipogenesis up-regulated. Juvenile sedentariness aggravated peripheral insulin resistance (Lg HOMA-IR, 1.02 ± 0.1 vs. 0.83 ± 0.0, \( P<0.05 \)), increased intramuscular triglyceride levels (94.63 ± 7.6 μmol/g vs. 59.08 ± 5.5 μmol/g, \( P<0.05 \)), and impaired glucose tolerance. The basal expression of fatty acid binding protein 3 and carnitine palmitoyl transferase 1 decreased, while the sterol regulatory element binding transcription factor 1c and fatty acid synthase increased. Following insulin stimulation, changes in membrane glucose transporter 4 and Akt expression regulating glucose metabolism occurred, and juvenile exercise normalized the observed aberrations. Conclusion: Juvenile sedentariness promoted the predisposition for metabolic syndrome in maternal hypoxia offspring, which was normalized by juvenile exercise. Changes in the intramuscular glucose uptake and lipid metabolism were responsible for the metabolic reprogramming.

Keywords: Maternal hypoxia, physical activity, sedentary lifestyle, fetal programming, metabolic syndrome

Introduction

Metabolic syndrome (MS) is a cluster of conditions, such as increased blood pressure, high blood sugar levels, excess body fat, and abnormal cholesterol levels, which increase the risk of heart disease, stroke and diabetes. MS has become more widespread over the last decade, probably influenced by the prevalence of factors, such as sedentary lifestyle and Western diet [1, 2]. The concept of fetal origins of adult disease [3] was introduced based on epidemiologic and experimental evidence, indicating that adverse intrauterine environment, including hypoxia, malnutrition, caffeine etc., can predispose offspring to susceptibility for metabolic disorders, such as MS, obesity, non-alcohol fatty liver disease, and atherosclerosis [4-6]. The fetal programming may also be modified by postnatal lifestyles especially different physical activity levels [7]. Therefore, our hypothesis is that reductions in daily ambulatory activity, a sedentary lifestyle, can aggravate metabolic disorders in offspring exposed to maternal hypoxia, and that exercise training reverses the metabolic aberrations.

Maternal hypoxia is quite a common insult during pregnancy, which independent of malnutrition, could promote molecular markers of insulin resistance in adult offspring born from a hypoxic pregnancy [8]. For instance, uteropl-
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cental insufficiency programmed glycolipid metabolism dysfunction, lowered pancreatic blood supply leading to pancreatic dysplasia and incompetence as a compensatory adaptation in the uterus, while generating maladaptation to a relative over-nutrition environment after birth [9]. Additionally, persistently advanced gluconeogenesis without suppression by insulin in intrauterine growth retardation (IUGR) offspring was demonstrated to contribute to the later impaired glucose tolerance and insulin resistance [10]. As previously reported, we have successfully established an intrauterine hypoxia model by sending pregnant rats to a hypoxia environment in middle and late pregnancy. We demonstrated that IUGR occurred in neonates with maternal hypoxia, and early pathologic atherosclerotic changes were detected in the adult thoracic aorta [11]. Moreover, demyelination, injured axons, and damaged microvasculars were observed in the periventricular white matter in adult offspring of the same model [12]. Finally, a synergistic effect of maternal hypoxia and postnatal environment (high-fat diet) was demonstrated in offspring rats in the intrauterine hypoxia model contributing to vulnerability for non-alcohol fatty liver disease [13].

It is widely accepted that the pathogenesis of MS is largely attributable to physical inactivity which has been recognized by the WHO as the fourth largest risk factor for mortality causing an estimated 3.2 million deaths globally [14]. While, Lees et al. [15] pointed out that most studies on chronic metabolic diseases neglected evaluating physical activity levels. Laufs et al. [16] confirmed that physical inactivity, independent of traditional risk factors, increased reactive oxygen species and inflammation, causing damages to artery endothelial function in animal experiments. Krogh-Madsen et al. [17] have shown that reduction of daily steps for 2 week significantly impaired peripheral insulin sensitivity and cardiovascular fitness in healthy, non-exercising young men. In young healthy males after bed-rest, the levels of glucose transporter 4 (GLUT4), hexokinase II, protein kinase B/serine-threonine kinase 1 (Akt 1), and Akt 2 were found to be reduced in vastus lateralis muscle, indicating that key proteins in glucose transport, storage, and phosphorylation might be responsible for sedentariness induced insulin resistance in muscle [18]. Additionally, a study demonstrated that juvenile inactivity could initiate changes in skeletal muscle architecture and mass, and caused metabolic abnormalities contributing to late life diseases in rats [19]. Normal cage activity and sedentariness, as compared to typical behavioral habits, such as climbing, chasing and rustling during the night cycle, in adolescence seemed to be especially important [20].

Exercise bears definitive benefits for MS prevention and treatment compared to physical inactivity and sedentariness. In a retrospective analysis, Kodama et al. [21] found that later mortality and incidence of cardiovascular diseases in diabetics were closely associated with physical inactivity, but that outcome improved by movement activity regardless of type, intensity, or lasting time. In offspring rat with inter-generational inheritance of perinatal high fat diet-induced metabolic disorders, early post-weaning exercise could restore fatty acid metabolic capacity and ameliorate adverse outcomes [22]. Gatford et al. [23], demonstrated that exercise training in early life partially or completely reversed predisposition for metabolic disorders in offspring rats with IUGR. In IUGR offspring, whose islet β-cell function was impaired and β-cell mass was reduced due to predisposition, aerobic exercise training improved metabolic function by suppressing glucose-stimulated insulin production and decreasing hepatic glucose production [24].

Although, the association between sedentary lifestyle and chronic metabolic diseases has been emphasized based on prospective observational study, animal models for the investigation of underlying molecular mechanism are scarce. Moreover, differences in the effects of juvenile activity patterns on metabolism in adult offspring after maternal hypoxia have not been examined yet. Thus, the aim of this study was to establish Sprague-Dawley rat models of sedentariness, cage activity, and exercise training and to determine whether the predisposition to MS induced by maternal hypoxia is aggravated or reversed by different juvenile activity patterns. Moreover, the underlying molecular mechanisms were investigated.

Materials and methods

Animals

Sprague-Dawley rats (Shanghai Slack Laboratory Animals LLC, Shanghai, China) (10 male,
20 female, weight 170-200 g) were bred in the Laboratory Animal Center under standard conditions with a 12/12 hours light/dark cycle and access to standard rat chow and water ad libitum. All experimental procedures were in accordance to the National Institutes of Health guidelines, and were approved by the Animal Experimentation Ethics Committee of Quanzhou Medical College, China.

**Maternal hypoxia model**

The maternal hypoxia model in rats was established according to a previously reported method [11, 25]. Briefly, after two-week acclimatization, rats were mated overnight, and pregnant rats were randomly divided into an intrauterine normoxia and hypoxia group. From day 7 to 21 of pregnancy, the hypoxia group was housed individually inside a low oxygen concentration (10 ± 1%) plexiglas eight hours per day where the oxygen concentration was monitored by an oxygen analyzer (S-450; IST-AIM). Normal control (NC) rats were housed in an identical chamber with an oxygen concentration around 21%. After natural delivery, litter size was randomly culled to six males for 28 days nursing then weaned to ad libitum laboratory chow. The surplus neonatal rats were sacrificed for birth weight and biomarkers detection.

**Sedentary and exercise protocol**

Juvenile offspring rats (5 weeks old) in the maternal hypoxia model were assigned to 3 groups: sedentariness (HS), cage activity (HC), and exercise training (HE) according to methods reported by Suvorava et al. [26]. In the HS group, rats were housed individually in cages with a basal area of 300 cm² providing space for predominantly resting and normal posture adjustment. In the HC and HE groups, as well as the HC group, four rats were housed per litter in cages with a basal area of 1700 cm² providing space for climbing, chasing, fighting, and other spontaneous movements (Figure 1A). An incremental aerobic exercise protocol as described in Gomes et al. [27] was applied with minor modifications in the HE group. Briefly, treadmill (ZH-PT; Anhui Zheng Hua Biological Instrument Co., Ltd., Anhui, China) training was conducted during the dark cycle (8-10 p.m.), including an adaptive stage (2 weeks) and a formal training stage (11 weeks), for three times per week. Intolerant rats were excluded based on the maximum speed at the adaptive stage. Figure 1B illustrates the specific training protocol.

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**Figure 1.** Postnatal activity patterns imposed on hypoxic offspring rats. A. Sedentariness (left, 300 cm²) and cage activity (right, 1700 cm²). B. Treadmill for exercise training. C. Exercise protocol. Adaptive stage, exercise started at 10 m/min for 10 minutes on a 0° incline with the speed gradually increasing 2 m/min each time by the end of the 2nd week reaching 20 m/min. Formal stage, exercise started at 10 m/min for 10 minutes on a 0° incline with the speed and duration gradually increasing 1 m/min and 5 min per week, respectively, reaching 20 m/min and 60 min.
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Intraperitoneal glucose tolerance test (IPGTT)

Upon completion of the juvenile interventions in different groups (48 h later), rats were fasted overnight and injected intraperitoneally with a 50% glucose solution (2 g/kg). Blood glucose was detected by a glucose meter on the tail (Roche Diagnostics, Basel, Switzerland) in fasting condition and after glucose injection (at 0, 15, 30, 60, 90, and 120 min).

Tissue harvesting and blood sampling

After overnight fasting, rats were weighted and anesthetized by intraperitoneal injection (3 ml/100 g) with 2% sodium pentobarbital. Blood samples were collected by intracardiac puncture (decapitation for neonates) and after centrifuged at 12,000 g, 4°C for 15 min, serum specimens were stored at -20°C. Perirenal, epididymal, and retroperitoneal fat tissue was collected and weighed. Vastus lateralis muscle specimens were collected and snap frozen in liquid nitrogen or fixed in formaldehyde. 20 mins after either vehicle or insulin (8 U/kg) administration via bolus intraperitoneal injection, the same-level of vastus lateralis muscle specimens were collected and snap frozen. Specimens of neonates mixed three into one. Euthanasia was conducted by excess sodium pentobarbital intracardiac injection.

Serum biomarker detection

The concentrations of fasting glucose and blood lipids (triglycerides (TG), total cholesterol (TC), and free fatty acids (FFA)) were detected by standard enzymatic colorimetric methods with an auto-biochemical analyzer (Rapidlab-TM850, Beckman Coulter, Fullerton, CA, USA). Fasting serum insulin, insulin-like growth factor 1 (IGF-1) and interleukin-6 (IL-6) were quantified by commercially available RIA kits (Beijing North Institute of Biotechnology, Beijing, China). The homeostasis model assessment (HOMA) was adopted to evaluate the insulin resistance index (IR). HOMA-IR = serum glucose (mmol/L) x insulin (mIU/L)/22.5.

Intramuscular oxidative/antioxidative state, TG, and glycogen concentration assay

All experimental procedures were in accordance with operational guidelines. Briefly, homogenates of vastus lateralis muscle specimens were prepared by ball mill grinding (Qiagen, Hilden, Germany) in normal saline or extracting solution (1:9). Total citrate synthetase (CS) and superoxide dismutase (SOD) activity, concentration of malondialdehyde (MDA), intramuscular TG and glycogen levels were detected by colorimetric methods with commercial kits (Njjcbio, Nanjing, China) with a microplate reader (BIO-RAD, Hercules, CA, USA) at 412 nm, 450 nm, 532 nm, 546 nm, and 620 nm, respectively.

Histopathological procedures

For light microscopic analysis, following formaldehyde-fixation overnight, vastus lateralis muscle tissues were processed by standard procedures in gradients of alcohol and xylene, and paraffin embedded. Tissue slices of 5 μm thickness were hematoxylin-eosin stained, and photographed with an Olympus AH-2 light microscope (Olympus, Japan).
Western blot analysis

Western blot analysis was performed according to standard protocols. Briefly, vastus lateralis muscle total or membrane protein was extracted using protein extraction reagent (Beyotime Institute of Biotechnology, Haimen, China) containing protease inhibitors and phosphatase inhibitors, and separated by electrophoresis on SDS-polyacrylamide gels and transferred to nitrocellulose membranes. Then muscle homogenates were incubated with a rabbit polyclonal antibody against target proteins (AMP-activated protein kinase (AMPK), phosphorylated AMPK (p-AMPK Thr172), fatty acid binding protein 3 (FABP3), carnitine palmitoyl transferase 1 (CPT1), up-regulated sterol regulatory element binding transcription factor 1c (SREBP-1c), fatty acid synthase (FAS), Akt, phosphorylated Akt (p-Akt Ser473), membrane GLUT4 (m-GLUT4) and total GLUT4 (Santa Cruz Biotechnology, Santa Cruz, CA, USA. or Cell Signaling, Beverly, MA, USA) 1:500 in skim milk at 4°C overnight. The second antibody, goat anti-rabbit IgG antibody (1:3000 in skim milk) (EarthOx, USA), was incubated for 2 h at room-temperature, and the blots were developed by the ECL method. The relative protein levels were normalized to the β-actin expression levels and quantified using the Image-Pro Plus (6.0) software (Media Cybernetics, Bethesda, MA, USA).

Statistical analysis

Statistical analysis was conducted using the SPSS statistical software version 17.0 (SPSS Inc., Chicago, IL, USA). Serum insulin and HOMA-IR were logarithmically transformed to normally distribution. All values were expressed as means ± SD. The analysis of variance (ANOVA) models were used to compare various treatment groups and Fisher’s paired least significant difference test was performed to determine intergroup differences. Statistically significance at $P<0.05$ was accepted.

Results

Effect of maternal hypoxia and juvenile activity patterns on general physiological index in offspring rats

Whereas maternal hypoxia in rats did not alter litter size (11.75 ± 2.1 vs. 10.34 ± 1.7 in the NC and HC group, respectively, $P>0.05$), the neonates from maternal hypoxia rats were born...
with lower birth weight compared to the normoxia control group (5.28 ± 0.48 g vs. 6.68 ± 0.41 g, P<0.05). However, the difference in body weight vanished after breastfeeding at 28 days of age (Figure 2A).

No significant change was found in fat content of adult rats after maternal hypoxia. Juvenile exercise training (HE group) resulted in a leaner phenotype in adulthood compared to the other groups (Figure 2B). No differences were found in body weight between NC, HC, and HS groups (P>0.05). Moreover fat mass was reduced in the HE group, but was increased in the HS group compared to the NC and HC groups (P<0.05). Maternal hypoxia increased the adiposity index in the HC and HS group when compared to the NC group. The adiposity index was significantly elevated after sedentariness (Figure 2B, P<0.05). In contrast, the adiposity index was significantly reduced in the HE group (Figure 2B, P<0.05). It is worth mentioning that explorative willingness and behavior of rats after juvenile sedentariness were reduced with obvious anxiety in the open-field assay.

Effect of maternal hypoxia and juvenile activity patterns on blood-borne biomarkers for inflammation, glucose and glycolipid metabolism

In neonates, maternal hypoxia resulted in a significant increase in serum lipids TG and FFA (TG, 0.4 ± 0.2 mmol/L vs. 0.2 ± 0.1 mmol/L; FFA, 314 ± 102.9 μmol/L vs. 176 ± 89.3 μmol/L; P<0.05), and IGF-1 levels while serum levels for glucose and insulin remained normal (Figure 3A).

In adult offspring rats, the above effects of maternal hypoxia on serum lipids were still present (Figure 3B) with a tendency of increased IR (Lg HOMA-IR, 0.83 ± 0.0 vs. 0.79 ± 0.0, P>0.05). Although most blood-borne metabolic parameters affected by maternal hypoxia were not additionally altered by postnatal sedentariness, impaired insulin function was aggravated (Lg HOMA-IR, 1.02 ± 0.1 vs. 0.83 ± 0.0, P<0.05). Notably, exercise training significantly normalized metabolic parameters and insulin function disturbance (Lg HOMA-IR, 0.74 ± 0.0 vs. 0.83 ± 0.0, P<0.05).

Interestingly, circulating IL-6, an inflammatory factor, was found unchanged after maternal hypoxia (98.3 ± 23.0 ng/L vs. 90.1 ± 20.5 ng/L, P>0.05), while increased in the HE group when compared with the HS group (152.4 ± 20.4 ng/L vs. 123.2 ± 26.9 ng/L, P<0.05).

Postprandial blood glucose in IPGTT

Maternal hypoxia interacted with the juvenile sedentary lifestyle contributing to the increase of postprandial blood glucose at 15 min, 30 min, and 60 min following injection in adult offspring rats (Figure 4). Even after 120 min, blood glucose levels were still significantly higher in the HS group compared to the NC group. In contrast, juvenile exercise down-regulated postprandial blood glucose levels in the adult offspring but without reaching significance comparing with HC levels.

Intramuscular oxidative/antioxidative state, TG, and glycerogen concentration

Maternal hypoxia had no effect on the intramuscular oxidative/antioxidative status in adult rats as measured by the MDA content and SOD activity (Table 1). Citrate synthetase (CS) and glycerogen concentrations were found to be slightly but not significantly down-regulated after sedentariness, but were both significantly up-regulated after training (P>0.05). Thus, the changes in glycerogen concentration mimicked CS activity. Juvenile sedentariness (HS) after maternal hypoxia led to the increase of the intramuscular TG concentration compared with cage activity, while exercise normalized TG significantly (P<0.05, Table 1).

### Table 1. Intramuscular oxidative/antioxidative status, glycogen and triglyceride concentration in adult offspring rats (n=10)

<table>
<thead>
<tr>
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<th>NC</th>
<th>HC</th>
<th>HS</th>
<th>HE</th>
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<tbody>
<tr>
<td>CS (U/mg)</td>
<td>162.3 ± 19.7</td>
<td>151.0 ± 42.1</td>
<td>126.1 ± 33.6</td>
<td>171.1 ± 37.5*</td>
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<tr>
<td>MDA (nmol/mg)</td>
<td>1.3 ± 0.3</td>
<td>1.5 ± 0.6</td>
<td>1.7 ± 0.4</td>
<td>1.5 ± 0.6</td>
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<tr>
<td>SOD (U/mg)</td>
<td>33.8 ± 8.3</td>
<td>31.6 ± 7.5</td>
<td>30.1 ± 6.7</td>
<td>38.0 ± 9.9</td>
</tr>
<tr>
<td>Glycogen (mg/g)</td>
<td>0.9 ± 0.2</td>
<td>3.0 ± 0.3</td>
<td>0.8 ± 0.2</td>
<td>1.2 ± 0.3*</td>
</tr>
<tr>
<td>TG (μmol/g)</td>
<td>43.3 ± 5.1</td>
<td>59.1 ± 5.5*</td>
<td>94.6 ± 7.6*</td>
<td>37.2 ± 4.7*</td>
</tr>
</tbody>
</table>

NC, normal controls; HC, hypoxia cage activity; HS, hypoxia sedentariness; HE, hypoxia exercise; CS, citrate synthase; MDA, malondialdehyde; SOD, super oxide dismutase; TG, triglyceride. P<0.05, compared with NC; *P<0.05, compared with HC; **P<0.05, compared with HS.
Figure 5. Western blot analysis of signal molecules regulating glycolipid metabolism in skeletal muscle of adult rats. n=10; NC, normal controls; HC, hypoxia cage activity; HS, hypoxia sedentariness; HE, hypoxia exercise; AMPK, AMP-activated protein kinase; p-AMPK, phosphorylated AMPK; FABP3, fatty acid binding protein 3; CPT1, carnitine palmitoyl transferase 1; SREBP-1c, sterol regulatory element binding transcription factor 1c; FAS, fatty acid synthase; Akt, serine-threonine kinase; p-Akt, phosphorylated Akt; m-GLUT4, membrane glucose transporter 4; INS-, baseline; INS+, insulin stimulation; *P<0.05, compared with NC; **P<0.05, compared with HC; ***P<0.05, compared with HE; ++P<0.05, INS+ vs. INS- of the same group.
Morphology observations after HE staining and western blot analysis of the vastus lateralis muscle to detect specific markers for glycolipid metabolism

Maternal hypoxia and juvenile activity patterns did not alter structure and morphology of the vastus lateralis muscle in adult offspring rats as observed under light microscopy (data not shown).

At the molecular level in the basal state, maternal hypoxia resulted in down-regulation of p-AMPK/AMPK, FABP3, and CPT1, as well as up-regulation of SREBP1c and FAS in skeletal muscle of adult rats compared to those in NC group ($P<0.05$, Figure 5A, 5B). The aberrations were exacerbated in the HS group, while dramatically alleviated after exercise. Moreover, at the base level there were no significant differences in m-GLUT4/total GLUT4 and p-Akt/Akt levels among all groups compared to the NC group (Figure 5C, 5D).

In response to insulin, p-Akt/Akt levels increased significantly in all groups ($P<0.05$). In the HC and HS groups, insulin stimulation of p-Akt/Akt was lower compared with controls, and juvenile training normalized the ratio. In contrast to NC, with insulin stimulation, m-GLUT4/total GLUT4 did not correspondingly increase with p-Akt/Akt in HC and HS (Figure 5C, 5D). Nevertheless, juvenile exercise strongly enhanced insulin stimulated m-GLUT4/total GLUT4 ($P<0.05$, Figure 5D).

Discussion

This study determined whether predisposition to MS induced by maternal hypoxia was aggravated or reversed by juvenile activity patterns, such as sedentariness, cage activity and exercise, and evaluated underlying molecular mechanisms. The main findings of our study were as follows: 1. Maternal hypoxia independently resulted in IUGR and hyperlipemia in neonates. 2. Juvenile sedentariness exacerbated the susceptibility to impaired glucose tolerance, ectopic lipid deposited and peripheral IR. 3. Metabolic aberrations were partly reversed by exercise. 4. Changes in intramuscular glucose uptake and lipid metabolism were responsible for the reprogramming effect of different juvenile activity patterns in maternal hypoxia offspring.

Importantly, we established the sedentary lifestyle rat model by singularizing and reducing room for locomotor activity, which differed from other studies. In most experimental models, sedentary lifestyle or physical inactivity are defined by the paradigms of bed-rest, hind limb unloading, transgenic inactive mice, and detraining [17, 19]. However, bed rest is more typical for patients after surgery or paralysis, and hindlimb unloading is the preferable local braking model for patients with bone fractures. These models, together with transgenic animals, are not appropriately reflecting the typical changes of a sedentary lifestyle [14]. Although, detraining reflects a realistic condition of low energy consumption, the persistence of a high basal metabolic rate would affect resting metabolism [17, 28]. The Sedentary Behavior Research Network defines “sedentary behavior” as any waking behavior, such as sitting/reclining posture, etc., which is characterized by an energy expenditure ≤1.5 metabolic equivalents [29]. We assumed that a modern sedentary lifestyle is characterized by reduced ambulatory activity, somewhat solitary but non mobility-restricted living conditions. Accordingly, our sedentariness model reflecting these conditions appears to be more suitable for investigating the effects of a sedentary lifestyle on average persons. It needs to be pointed out that psychological factors induced by a solitary lifestyle are reported to affect the immune system [30]. However, no consensus on how to assess solitary and its relationship with metabolic factors in animals has been reported so far.

Our findings are supported by previous studies [8, 11, 13, 31, 32] showing that maternal hypoxia resulted in IUGR and hyperlipemia in neonates followed by a “catch-up growth” in early adulthood. Maternal hypoxia did not alter body weight, but the adipose index increased overtly in adulthood. These results were confirmed by finding reported by Rueda-Clausen et al. [4] in a late pregnancy hypoxia model. Biron-Shental et al. [33] showed that lipid accumulation in trophoblast markedly increased and lipid availability was regulated after fetal hypoxia. Given that stressors, including hypoxia or low energy conditions, can trigger glycolysis and, thus, reduced glucose level can stimulate compensatory increases of lipolytic hormones, e.g. cortisol, both in pregnant and fetal sheep [34].
Hence, a high level catabolic switch could create a concentration gradient driving lipid flux to the fetus. Additionally, in our model of maternal hypoxia lasted from mid to late trimester of pregnancy, which might result in an early coming of lipid flux to the fetus [35]. This would give rise to mass lipid metathesis deposition in fetal organs and accordingly predispose the fetus to lipotoxicity. With this in mind, we reasoned it may explain the increased levels of fetal serum FFA and TG.

Although some studies found that physical inactivity led to a stronger inflammatory reaction and oxidative stress in the skeletal muscle [36]. In the present study we found that serum IL-6 levels and the intramuscular oxidative balance were kept unchanged after sedentariness. Similarly, others have reported that there was no change in pro-inflammatory factors, such as TNF-α and IL-6, between detrained and control groups [17]. Additionally, a recent study suggested that exercise induced IL-6 release which resulted in favorable regulatory effects on local fatty acid oxidation [37]. This effect may explain to a certain extent the relationship between exercise-induced serum IL-6 increasing and partly reversed metabolic disorders in our study.

In contrast to the observed complete reversal effect of juvenile exercise on β-cell mass in rats with IUGR [38], our data suggested that moderate prolonged exercise during adolescence enhanced mitochondrial function without changing muscular structure and morphology. In support of our findings, Huber et al. [39] demonstrated that long term moderate to intense exercise enhanced substrate metabolism in skeletal muscle without retailing the size and distribution. Interestingly, normal lactational feeding was shown to restore cardiomyocytes numbers after uteroplacental insufficiency [40]. According to this study, normal lactational feeding would have already normalized muscular structure and morphology. In addition, in accordance with the “thrifty phenotype” hypothesis [41], we discovered that the ratio of p-AMPK to total AMPK, as an energy sensor, was down-regulated in response to the postnatal over-nutrition status relative to prenatal intrauterine hypoxia. The thrifty phenotype hypothesis proposes that the epidemiological associations between poor fetal and infant growth and the subsequent development of type 2 diabetes and the metabolic syndrome result from the effects of poor nutrition in early life, which produces permanent changes in glucose-insulin metabolism. One of the expected metabolic consequences of sedentariness would be ectopic lipid deposits and blunt insulin sensitivity, as revealed in our study, and which was normalized again by juvenile exercise. Similarly, Laye et al. [42] found that inactivity contributed to increased fat mass independent of excessive caloric intake. Mass lipid metathesis deposits drive IRS-1 serine phosphorylation, while insulin-stimulated activation of Akt and downstream signal signaling was diminished [43]. Increased intramuscular TG, especially accompanied by a reduction of oxidation ability, contributed to impaired glucose utilization and IR [44]. Additionally, exercise was proven to increase insulin sensitivity through reducing TG levels and increasing CS and β-HAD activity in patients with type 2 diabetes [45].

Biensø et al. [18], reported that GLUT4 and glycogen synthase were key players responsible for 7 days bed-rest induced IR in healthy subjects. Also, Caponi et al. [46] showed that aerobic exercise increased GLUT4-related glucose intake. In line with these results, we found that insulin stimulated the increase of glucose uptake (m-GLUT4/t-GLUT4 ratio), which was diminished by juvenile sedentariness in adult offspring with maternal hypoxia without corresponding alterations in insulin action (p-Akt/Akt ratio). Furthermore, the observed increase of p-Akt and m-GLUT4 levels were in accordance with an exercise improved metabolic phenotype. These findings are in accordance with these data demonstrating that sedentariness facilitated the occurrence of exogenous IR via perturbing insulin responsive GLUT4 translocation independent of Akt suppression in adult offspring with maternal hypoxia [47].

Meanwhile, our data showed that glycogen levels increased in accordance with high CS activity, a marker of mitochondrial respiratory chain activity, and an increased ratio of m-GLUT4 to total GLUT4 in male rats of HE group, which indicated the increased switch of fuel utilization to glucose. Thus, increasing glucose utilization contributed to prevent and improve metabolic aberrations induced by maternal hypoxia.

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However, Garg et al. [24] found that exercise did not alter insulin responsive GLUT4 in skeletal muscle in malnutrition-induced IUGR female rats. Since estradiol was demonstrated to favor GLUT4 translocation in cardiac muscles [48], we are not sure whether the exercise related improvement in insulin action was related to gender differences. It was speculated that, the pre-existing increase of basal m-GLUT4 levels before exercise as a compensatory adaptation in IUGR females might partially cover exercise-dependent GLUT4 translocation.

In the present study, TG increases were associated with the expression of enzymes (SREBP1c, FAS involved in de novo lipogenesis), and negatively correlated with enzymes (FABP3) for lipid transportation and oxidative lipid metabolism (CPT1) in sedentary adults. The experimental results implied that sedentary lifestyle or exercise aggravated or alleviated TG deposits via regulation of lipogenesis, lipid transportation, and β-oxidation. Similarly, the decline of FABP3 expression, a membrane related fatty acid binding protein, was an extremely important cause in hind limb unloading induced IR in humans as revealed by gene expression microarrays [49]. In in vitro muscle cells cultures, FABP3 was reported as a stimulator that activates AS160 phosphorylation thereby enhancing glucose uptake by facilitating GLUT4 translocation [50]. Additionally, FABP3 was increased by endurance training as an adaptation to the improvement of lipid oxidation and insulin sensitivity [51]. On the contrary, in rats with low birth weight, Laker et al. [44] speculated exercise did not reprogram skeletal muscle mitochondrial biogenesis by detecting PGC1α, a mitochondrial biogenesis markers in gastrocnemius muscle. However, since substrate metabolism likely differs in different types of muscular fibers, detecting PGC1α in fast-twitch fibers (glycolysis oriented) might not fully reflect the metabolic changes induced by exercise. In addition, Huber et al. [39] introduced malnutrition-induced IUGR models and documented that glycogen storage in fast-twitch gastrocnemius muscle fibers increased in association with plasma lactate accumulation after exercise. They speculated that exercise could increase anaerobic glucose metabolism rather than fat utilization in IUGR offspring [39]. However, the diet-induced susceptibility in their study somewhat differed from the predisposition for metabolic disorders induced by maternal hypoxia in our study [8]. Moreover, the disparity in exercise-induced glucose metabolism depended on the predisposition for metabolic disorders, as well as exercise speed, intensity, and lasting time [24]. Therefore, more researches were needed to confirm and improve our findings.

In the present study, preliminary trials were made to introduce a new model of juvenile sedentariness in maternal hypoxia offspring and study the effect of juvenile activity on MS predisposition. Our findings have important implications for study design and interpretation in regard to social habits activity patterns for the investigation of metabolic aberrations in rats. Furthermore, we confirmed the juvenile period as a critical window for interventions that could prevent or aggravate maternal hypoxia related MS diseases by different activity patterns. The predisposition for MS in maternal hypoxia offspring was exacerbated by sedentary, while regular exercise launched in early adolescence effectively reversed MS. These results confirm the findings by Laker et al. [37], suggesting that long-lasting effects of exercise are less effective and lasting in adult compared to that in the adolescent stage. Given that intrauterine hypoxia of varying degrees is a very common complication in obstetrics, we recommend that regular exercise should be initiated in early life, regardless of clear experience of intrauterine hypoxia.

Nevertheless, there were several limitations of the present study. Although, IUGR induced by maternal hypoxia was widely applied as an animal model in previous studies [11-13, 26], the possibility that repetitive ischemia-reperfusion may impact fetal development in a variety of different ways should be factored in. Moreover, reductions in litter size might independently impose mammary dysfunction on offspring growth as a postnatal restriction [52]. Last but not least, additional evaluation of the relationship between psychological stressors induced by a singularized sedentary lifestyle and glycolipid metabolism needs to be considered. Consequently, future studies are needed to address two major questions: 1. How to evaluate the effect of psychological factors during singularized residence on metabolism. 2. What is the most effective juvenile locomotor therapy.
for preventing adult metabolic diseases induced by maternal hypoxia?

Conclusions

In conclusion, the sedentariness model established in our study is well suitable for investigations of the effect of social habit deficiency or different lifestyles on physiology and psychology of subjects. The predisposition for MS induced by maternal hypoxia was successfully reprogrammed by different juvenile activity patterns. Changes in intramuscular glucose uptake, and lipid metabolism were underlying molecular mechanism of reprogramming. Future studies are required to confirm our finding and to determine the most effective juvenile locomotor therapy for prevention of “fetal origins of adult diseases”, especially induced by maternal hypoxia.

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Disclosure of conflict of interest

None.

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References


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