Effect of Maternal Exercise on Fetal and Placental Glycogen Storage in the Mature Rat

P.E. Houghton, M.F. Mottola, J.H. Plust, and C.L. Schachter

Catalog Data

Key words: age, exercise, pregnancy, fetal glycogen storage patterns
Mots-clés: âge, exercice, gestation, modalités de stockage hépatique du glycogène

Abstract/Résumé
The purpose was to determine the effects of exercise on fetal and placental glycogen storage patterns at 20 days gestation (term 21 days) in mature (approximately 12 months of age) Sprague-Dawley rats. The exercise protocol consisted of treadmill running at 30 m min⁻¹, on a 10 incline, for 60 min, 5 days per week, for 4 weeks prior to conception, which continued until day 19 of pregnancy. Exercise produced a significant reduction in fetal body weight, placental weight, and fetal organ weights (heart, kidney, brain, and liver) compared to sedentary control animals (p < .05). However, when fetal body size was taken into account, these differences disappeared, except for the fetal brain:body weight ratio, which was larger in the exercised animals compared to controls (p < .05). Fetal liver glycogen concentrations were significantly lower in exercised animals compared to nonrunning control animals (p < .05). These results demonstrate that exercise of mature rats may compromise fetal development and hepatic glycogen storage in the fetus.

Le but de cette étude est d'observer chez des rats adultes Sprague-Dawley âgées d'environ 12 mois les effets de l'exercice sur les modalités de stockage du glycogène dans le fœtus et

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le placenta après 20 jours de gestation (terme : 21 jours). La séance d'exercice consiste en une course de 60 min à une vitesse de 30 m·min⁻¹ sur tapis roulant dont la pente est de 10°; l'exercice est accompli 5 jours par semaine pendant les 4 semaines précédant la conception et les 19 jours suivants. Comparativement à des animaux témoins exemptés d'exercice (p < .05), l'exercice réduit la masse du placenta, celle du fœtus et de ses organes (cœur, rein, cerveau et foie). Toutefois, en prenant en compte le gabarit du fœtus, ces différences disparaissent sauf dans le cas du ratio du cerveau/masse chez le fœtus : ce ratio est plus important chez les animaux à l'effort (p < .05). Comparativement aux animaux du groupe témoin, la concentration hépatique de glycogène est significativement plus basse chez les animaux du groupe expérimental (p < .05). Ces observations indiquent que l'exercice chez des rates adultes peut compromettre le développement du fœtus et le stockage du glycogène dans le foie du fœtus.

Introduction

Glycogen storage has been considered an index of glucose availability to the fetus (Jones and Rolph, 1985), which in turn is a key factor controlling fetal growth. Placental metabolism is intertwined with that of the fetus and may maintain its own glucose supply at the expense of fetal glycogen stores (Jones and Rolph, 1985). This may occur when maternal placental blood flow decreases and may cause fetal hepatic glycogenolysis and a positive umbilical arteriovenous difference in glucose concentration (Jones and Rolph, 1985). Should this occur late in gestation, insufficient storage of glycogen in the fetal heart and liver prior to delivery may severely compromise early postnatal life when the placental supply of glucose is removed abruptly at birth (Garris et al., 1985; Koski and Hill, 1990; Shelley, 1961). Thus, alterations in fetal and placental glycogen storage patterns just prior to birth may not only alter fetal growth but may also affect neonatal survival.

Since it is well-established that chronic exercise can alter glycogen storage patterns, exercise during pregnancy has been of historic concern due to potential effects on fetal and neonatal morbidity. Previously, we have examined the effects of maternal exercise on glycogen storage patterns in the fetuses and placenta of young trained pregnant rats (Houghton et al., 1997). Our results suggested that in young animals, maternal exercise of moderate intensity did not compromise fetal or placental glycogen storage (Houghton et al., 1997) compared to control animals.

However, previous reports from our lab have also demonstrated that exercise performed by mature pregnant rats caused fetal growth restriction (Mottola et al., 1992). In these mature animals, fecundity was decreased, the number of living fetuses was diminished, and the number of resorption sites was increased compared to younger animals of the same species (Mottola et al., 1992).

There are many risks to the fetus that have been associated with advancing maternal age. Growth restriction (Seidman et al., 1990; Sopelak and Butcher, 1982), shorter gestation (Seidman et al., 1990), and pathological glucose flux across the placenta (Thomas et al., 1990) are examples of problems associated with older pregnant individuals. Therefore, it is possible that compromised fetal growth and development occurring in fetuses of mature exercising rats may be due, at least in part, to aging and/or exercise-induced alterations in fetal glycogen storage patterns.
We have previously published maternal glycogen storage patterns, fetal body weight, number per litter, and resorption sites for the pregnant dams of fetuses examined in the present study (Mottola et al., 1992). The purposes of the present study were to (a) examine the effects of maternal age on glycogen storage patterns and organ mass in the placenta and fetal heart and liver, and (b) examine the combined effects of maternal age and chronic maternal exercise on glycogen storage patterns and organ mass in the placenta and fetal heart and liver. Thus, the present report provides new information about changes observed in organ mass and glycogen storage distribution, which occurred in the fetus and placenta of mature pregnant rats that engaged in a running exercise program.

Methods

ANIMALS

The treatment of the animals and the exercise protocol have been reported previously (Mottola et al., 1992) and were in compliance with the Canadian Council on Animal Care. Briefly, sedentary, virgin, female Sprague-Dawley rats (Zivic Miller, Zelienople, Pa) were received at 10 months of age (300 days). Since menopause in this species does not begin until 15 to 18 months of age (450-540 days; Baker et al., 1979), these mature rats had normal estrous cycles determined by vaginal smears (Mottola et al., 1992). Food and water were provided ad libitum, and the intake was similar (food pellet weight/day; water ml/day) between the groups (unpublished observations). Both food and water were removed from the cages of the sedentary animals while the other group exercised. In this way, both groups had similar exposure to food and water (Houghton et al., 1997).

EXERCISE PROTOCOL

The exercise protocol consisted of treadmill running at 30 m min⁻¹, on a 10 incline, for 60 min, 5 days per week, for 4 weeks. The animals were handled and exercised during the dark cycle, since rats are nocturnal and normally physically active at night (Mottola et al., 1992). The mature animals in the present study were given 4 weeks of exercise to ensure they were running well at this intensity before conception (Mottola et al., 1992).

GROUPS

After 4 weeks, the exercised animals (now approximately 11 months of age; 330 days) were mated using an LHRH injection technique (Mottola et al., 1992). Briefly, 29 exercising animals were mated, and only 5 became pregnant with live fetuses (PR; pregnant running group; N = 5). The other pregnancies contained resorption sites (Mottola et al., 1992). The animals in the PR group continued the exercise program until day 19 of gestation (term 21 days). Nonexercised virgin female Sprague-Dawley rats of the same age (11 months; 330 days) were also mated using the same technique (Mottola et al., 1992). A total of 27 sedentary control animals were injected with LHRH, and 7 became pregnant, 5 of which had live fetuses (PNR; pregnant non-running group; N = 5). Previously, we have shown that
in younger animals of the same species, this technique has produced pregnancies in >70% of the injected animals (Mottola and Christopher, 1991; Mottola et al., 1992). This fecundity rate for the younger animals was similar to that of other mating techniques used for younger animals (Mottola and Christopher, 1991).

TISSUE ANALYSIS

Pregnant animals in both PR and PNR groups were sacrificed on day 20 of gestation using methods reported previously (Mottola et al., 1992). Therefore, at the time of sacrifice, the mature pregnant rats would have achieved approximately 12 months of age. Sacrifice occurred 24 hr after the last exercise bout for the PR animals. This time frame is adequate for liver glycogen repletion in young (Mottola and Christopher, 1991; Terjung et al., 1974) and older (Mottola et al., 1992) trained rats. All animals were fed ad libitum with free access to water until sacrifice.

Fetal body mass, number of fetuses per litter and number of resorptions sites were recorded (PR = 1.6 ± 0.07 g; PNR = 2.0 ± 0.05 g; PR = 27; PNR = 40; PR = 4.6 ± 1.1, PNR = 5.7 ± 1.0, respectively, reported previously in Mottola et al., 1992). Fetal brain, heart, liver, and kidney were removed from each fetus, blotted, and weighed. These fetal organ weights as well as the placenta weight were recorded for each conceptus. As reported previously (Houghton et al., 1997), fetal heart, liver and placenta were frozen immediately in liquid nitrogen and stored at -80 °C for later glycogen analysis using the method of Lo and colleagues (1970). Sample absorbency was determined spectrophotometrically (Sequoia - Turner) against a reagent blank (500 μL of phenol; BDH Chemicals, Toronto, ON) and 2.5 mL of 96–98% sulphuric acid (BDH Chemicals) at 490 nm. Glycogen concentrations were calculated from the slope of the standard curve (Lo et al., 1970).

DATA ANALYSES

The proportion of each fetal organ to total fetal body mass was calculated for the fetal liver, kidney, heart, and brain and expressed as a percentage of total fetal body mass (Mottola et al., 1989). Placental mass was compared to fetal mass by calculating the ratio of the placental mass to fetal mass. Values for organ and placental mass and ratio to fetal body mass are expressed as mean ± SEM, where N is the number of fetuses per group. The mean ± SEM per group for fetal and placental glycogen concentrations were determined from 5 samples that were randomly analyzed per litter. These values were then averaged to represent each pregnant animal. Thus, total observations would be 25 per group.

STATISTICAL ANALYSES

Comparisons were made between the pregnant running group (PR) and the sedentary controls (PNR) using a Student's t-statistic for independent samples. Statistical significance was accepted at the p ≤ 0.05 level. Fetal organ mass (mg and percent of fetal body mass), placenta glycogen concentration, and placental:fetal mass ratio were also compared using a Mann-Whitney Rank Sum Test for non-parametric data. Statistical results were the same regardless of whether the data were compared between groups using parametric or nonparametric tests.
Results

All fetal organ masses (Table 1) from the exercised animals (PR) were significantly less than those of the sedentary pregnant controls (PNR; $p \leq .05$). The mean placental mass of the pregnant running rats was also significantly smaller than the mean placental mass measured in the nonrunning pregnant rats ($p \leq .05$). Therefore, chronic exercise in mature animals was associated with lower fetal organ mass and placental mass values compared to sedentary animals of the same age.

Because of the differential growth patterns of the placenta and fetus, placenta to fetal body mass ratios were determined for each conceptus (Table 2). In addition, the mass of the heart, liver, kidney, and brain were calculated as a percentage of fetal body mass (Table 2). When fetal brain mass was expressed as a

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Fetal mass (g)</th>
<th>Placental mass (g)</th>
<th>Heart mass (mg)</th>
<th>Liver mass (mg)</th>
<th>Kidney mass (mg)</th>
<th>Brain mass (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNR</td>
<td>40</td>
<td>2.00</td>
<td>0.60</td>
<td>10.55</td>
<td>17.92</td>
<td>12.97</td>
<td>120.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.05</td>
<td>±0.02</td>
<td>±0.02</td>
<td>±0.56</td>
<td>±0.60</td>
<td>±1.1</td>
</tr>
<tr>
<td>PR</td>
<td>27</td>
<td>1.60*</td>
<td>0.48*</td>
<td>8.78*</td>
<td>14.20*</td>
<td>10.52*</td>
<td>105.2*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.07</td>
<td>±0.02</td>
<td>±0.04</td>
<td>±0.60</td>
<td>±0.60</td>
<td>±2.0</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM where N is the number of fetuses per group. PNR = pregnant nonrunning group; PR = pregnant running group.

*PR significantly smaller than PNR group, $p \leq 0.05$.

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Placenta /fetal mass ratio</th>
<th>Heart % fetal body mass</th>
<th>Liver % fetal body mass</th>
<th>Kidney % fetal body mass</th>
<th>Brain % fetal body mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNR</td>
<td>40</td>
<td>0.31</td>
<td>0.54</td>
<td>8.96</td>
<td>0.64</td>
<td>5.96*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.01</td>
<td>±0.01</td>
<td>±0.24</td>
<td>±0.03</td>
<td>±0.12</td>
</tr>
<tr>
<td>PR</td>
<td>27</td>
<td>0.32</td>
<td>0.56</td>
<td>9.15</td>
<td>0.67</td>
<td>6.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.02</td>
<td>±0.01</td>
<td>±0.13</td>
<td>±0.02</td>
<td>±0.19</td>
</tr>
</tbody>
</table>

Values are means ± SEM where N is the number of fetuses per group. PNR = pregnant nonrunning group; PR = pregnant running group.

*PNR is significantly smaller than PR group, $p \leq 0.05$. 

Table 1 Placental, Fetal Body, and Organ Masses

Table 2 Fetal Organ Mass as a Percentage of Fetal Body Mass and the Placenta/Fetal and Placenta/Total Mass Ratio
Table 3  Glycogen Concentrations in the Placenta, Fetal Heart, and Fetal Liver

<table>
<thead>
<tr>
<th>Group</th>
<th>Placenta mg/g tissue</th>
<th>Fetal heart mg/g tissue</th>
<th>Fetal liver mg/g tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNR (N = 5)</td>
<td>8.29 ± 2.61</td>
<td>45.96 ± 10.68</td>
<td>17.44 ± 1.51</td>
</tr>
<tr>
<td>PR (N = 5)</td>
<td>9.01 ± 0.66</td>
<td>51.41 ± 3.37</td>
<td>10.96 ± 2.42*</td>
</tr>
</tbody>
</table>

Values are the means ± SEM per group (refer to text for explanation of total observations). PNR = pregnant nonrunning group; PR = pregnant running group.

*PNR is greater than PR, p ≤ 0.05.

percentage of total fetal body mass, a significantly higher value in the PR group compared to the PNR group was noted (p ≤ .05). No other differences were found between the PR and PNR groups for any of the other organ masses when calculated as a percentage of total fetal body mass. This would indicate that, except for the fetal brain, the size of each organ was proportional to fetal body mass in both exercised and sedentary mature pregnant animals. Glycogen concentrations measured in the placenta and fetal heart were not different between the mature PR and PNR groups; however, fetal liver glycogen concentration was significantly less in the chronically exercised mature animals (PR) compared to the sedentary mature (PNR) group (Table 3).

Discussion

The results showed that in mature exercised animals, fetal organ masses were markedly reduced compared to sedentary control animals. When the differences in fetal body mass were eliminated through calculating the fetal organ mass:fetal body mass ratio, it was found that except for the fetal brain, the reduction in each organ size was proportional to the smaller size of the fetus. Fetal brain mass expressed as a percentage of total fetal body mass was significantly higher in exercised pregnant animals than sedentary controls. In addition, glycogen concentrations in the fetal liver, but not placenta or fetal heart, were lower in exercised animals compared to nonrunning controls. These changes in fetal organ masses and fetal liver glycogen concentrations suggested that this intensity of exercise in mature pregnant rats may significantly affect fetal carbohydrate metabolism and alter the development of the fetus.

Previous studies have reported that fetuses under hypoxic stress maintain brain, heart, and liver mass at the expense of other nonvital organs (Gilbert et al., 1979), such as kidneys, due to the redistribution of fetal blood flow to vital organs. This adaptive mechanism results in smaller fetal body size and the disproportionately larger vital organs, which is manifested by relatively larger ratios of vital fetal organ masses:fetal body mass. The fetal brain:fetal body mass ratio was larger in the exercised group of the present study compared to controls, which suggests
that the change in fetal development observed in the present study following exercise in the mature animals may be due in part to fetal hypoxia, perhaps due to a redistribution of maternal blood flow from the gut (uterus) to favour the exercising muscles of the mother. Other mechanisms that may alter fetal development may include diminished fetal glucose availability, problems in thermoregulation, or a combination of these factors, all of which may occur with maternal exercise (Wolfe et al., 1994).

It is well-known that maternal blood glucose is the primary energy substrate for fetal growth and development (Hay et al. 1983). Glucose availability and transplacental glucose transport are the key factors involved in this process (Battaglia et al., 1978). Previous reports from this laboratory have demonstrated that glucose uptake in the fetus and placenta are not altered by exercise in trained pregnant rats (Mottola et al., 1993). However, these studies were performed in younger pregnant rats in which placental mass, fetal mass, and fetal organ masses as well as fetal glycogen storage patterns were not altered by the maternal exercise (Houghton et al., 1997).

In the present study, liver glycogen concentrations were significantly lower in the fetuses of the mature pregnant rats that exercised compared to those of the sedentary controls. Altered fetal glycogen storage pattern in mature animals may reflect the “pathological glucose flux across the placenta” that has been associated with advancing maternal age (Thomas et al., 1990). Furthermore, we have shown that liver glycogen concentrations in the mature dams of the fetuses under current investigation were significantly increased following chronic exercise exposure (Mottola et al., 1992). Exercise in the mature pregnant rat may enhance the storage of maternal glucose in the form of liver glycogen in the mother at the expense of making glucose available to the fetus. Thus, either a reduction in maternal glucose availability to the fetus or a problem in transplacental glucose transfer, or both, may be the underlying mechanism for lower fetal liver glycogen stored in mature exercising pregnant animals.

Altered organ masses and glycogen storage patterns in fetuses of mature pregnant rats reported herein are in contrast to an earlier study from this laboratory, which employed an identical exercise protocol to examine the effects of exercise in younger pregnant rats (90–100 days) of the same strain (Mottola and Christopher, 1991). Therefore, the effect of this exercise protocol on fetal outcome may depend on maternal age. Alternatively, differences between young and mature rats may also be due to the higher relative work intensity in these older, heavier mature pregnant rats. In this regard, the exercise protocol of the present study is of moderate intensity for younger animals of this species and produces enzymatic changes in skeletal muscle within 4 weeks that are indicative of a training effect in these younger animals (Mottola et al., 1993). Either way, it is clear that fetal outcome and glycogen deposition represent a unique complex interplay between maternal age and exercise.

No studies to date have examined whether fetal glucose uptake is affected by exercise in mature pregnant rats. Further investigations are required to determine underlying mechanisms involved in altered placental glucose metabolism in mature animals. These studies may include ultrastructural analysis of the placenta, examination of the nutrient transfer across the placenta using stable isotope methodology, or examination of placental glucose transporters.
It is also noteworthy that mature sedentary control animals appear to have higher placental mass values than younger sedentary pregnant animals of the same species (Houghton et al., 1997). It has been suggested that increases in placental size occur to enhance nutrient delivery to the fetus (McCrab et al., 1991). This apparent increase in placental mass in the mature sedentary group was associated with fetuses that were within normal body mass values for this species (Mottola and Christopher, 1991). Therefore, reduced placental transfer of glucose early in pregnancy may stimulate placental enlargement as an adaptation to maintain nutrient delivery to the fetus (McCrab et al., 1991).

Although placental enlargement may not result in immediate health concerns to the fetus or neonate, it has been suggested that redistribution of resources to favour the placenta instead of the fetus is associated with cardiovascular problems in later adult life (Barker, 1997) and future programming of adult health (Barker et al., 1990).

Although both fetal body masses and fetal organ masses of the mature exercised animal appeared to be compromised, the placental mass and glycogen concentrations were similar to younger animals of the same species that underwent an identical exercise protocol (Houghton et al., 1997). It is interesting to note that when exercise was added into the equation in the older pregnant animals of the present study, the placenta did not enlarge in size, and yet there was no change in placental glycogen deposition. However, fetal liver glycogen was significantly reduced in these older exercised animals.

Glucose is stored as glycogen in the fetal liver during late gestation and is used as an energy source during the early neonatal period, prior to the establishment of suckling. Thus, a reduction of fetal liver glycogen storage may have serious consequences on the survival of the fetus during labour and delivery and through early neonatal life (Garris et al., 1985; Gutierrez-Corra et al. 1991; Koski and Hill, 1990; Shelley 1961). Low fetal liver glycogen just prior to parturition may reflect a major perturbation over most of gestation in these mature pregnant rats. Placental metabolism affects that of the fetus, and the placenta may maintain its own glucose supply at the expense of fetal stores (Jones and Rolph, 1985). This may occur when maternal placental blood flow decreases, which may cause fetal hepatic glycogenolysis and a positive umbilical arteriovenous glucose concentration difference (Jones and Rolph, 1985). Should this occur late in gestation, insufficient storage of glycogen in the fetal heart and liver prior to delivery may severely compromise early postnatal life when the placental supply of glucose is removed abruptly at birth (Garris et al. 1985; Koski and Hill, 1990; Shelley 1961). Thus, alterations in fetal glycogen storage patterns just prior to birth may not only alter fetal growth but may also affect neonatal survival.

Although we did not measure maternal circulating stress hormones in the present study, catecholamines are known to cross the placenta (Artal, 1986) and exercise has been shown to induce catecholamine release (Calles-Escandon and Felig, 1984). Once in the fetal circulation, stress hormones may act to alter hepatic glycogenolytic enzymes (Stratford and Hooper, 1997). Similar stress-induced changes in fetal liver enzymes could be responsible for the observed depletion in hepatic glycogen stores in the fetuses of the older pregnant rats that exercised. Thus, differences observed between the younger and older animals may also be
due to the higher relative work rate endured by the older heavier animals, which may have produced more stress hormones.

It is possible that the changes observed in the present study to the placenta and fetus could affect fetal and neonatal survival and may have serious health consequences for later adult life. Although alternative fuel sources may be available to the fetus, the primary source is maternal glucose. We cannot predict what may happen to the offspring in early postnatal life and beyond, but the potential remains for future health concerns. Thus, human observation is necessary for future exercise studies to examine metabolic relationships in the older pregnant population.

References


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