Running Throughout Pregnancy: Effect on Placental Villous Vascular Volume and Cell Proliferation

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Many studies have documented that placental development is altered by a variety of environmental factors which alter placental bed blood flow and/or oxygen delivery. One of these is sustained weight-bearing exercise. The purpose of this investigation was to examine the effects of running throughout pregnancy on villous vascular development and cell proliferation by testing the null hypothesis that continuing a regular running regimen throughout pregnancy has no effect on villous vascular volume or cell proliferation at term. Accordingly, placentae of 11 healthy runners with uncomplicated pregnancies were matched by placental weight, maternal diet and birth weight with those of 11 healthy controls and examined using systematic random sampling and point counting of placental tissues stained immunohistochemically with either an endothelial (CD 31, PECAM-1, endoCam) or proliferative (Ki-67, MIB-1) marker. The placentae of the runners had greater villous vascular volumes in both absolute weight, maternal diet and birth weight with those of 11 healthy controls and examined using systematic random sampling and point counting of placental tissues stained immunohistochemically with either an endothelial (CD 31, PECAM-1, endoCam) or proliferative (Ki-67, MIB-1) marker. The placentae of the runners had greater villous vascular volumes in both absolute (77 ± 20 cm³ versus 47 ± 18 cm³, p < 0.02) and relative (% of total villous volume: 29 ± 5% versus 20 ± 6%, p < 0.003) terms. Likewise, they had a greater proliferation index (45 ± 14 mitoses/1000 nuclei versus 29 ± 10 mitoses/1000 nuclei, p < 0.008). We conclude that continuing to run regularly throughout pregnancy increases both absolute and relative villous vascular volume and cell proliferation at term. We also speculate that this exercise effect may have clinical value in cases at risk for anomalous feto-placental growth as increased villous vascular volume should improve feto-placental growth by enhancing placental transfer of oxygen and diffusible substrate.

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INTRODUCTION

Normal placental development is linked to its functional capacity which is an important determinant of fetal growth potential. Indeed, many of its morphometric characteristics correlate directly with size at birth [1–4]. Placental development is influenced by a variety of environmental factors which alter oxygen tension, nutrient availability and/or placental perfusion such as altitude, chronic pulmonary disease, diabetes, smoking, diet and sustained exercise [4–16]. These effects appear to be gestational-age specific and are evidenced primarily by changes in the pattern and degree of villous growth and vascularization. Recent studies also suggest that the site of the stimulus (pre-placental, placental or fetal) is a primary determinant of the pattern and timing of the response [7,13].

Regular, sustained forms of weight-bearing exercise (running, aerobics, stair-stepping, etc.) during human pregnancy stimulate mid-trimester placental growth and placental size at birth [3,4,9,10]. The latter reflects an increase in placental volume, placental functional volume (villous volume + intervillous space volume) and villous vascular volume. The level of the response in villous vascular volume within the villous vascular tree varies in a time-specific fashion during pregnancy (early versus late pregnancy) and appears to be dependent on the ratio between exercise intensity prior to pregnancy and exercise intensity at various time-points during pregnancy [4,9,10]. For example, a decrease in the exercise intensity ratio in early pregnancy suppresses villous vascular growth, whereas a decrease in late pregnancy stimulates it [10].

Interpretation of these exercise-associated placental effects, however, has been somewhat confounded by both between-exercise-group differences in placental and fetal size at birth and the effects of maternal diet [4,11,17]. Since both birth weight and placental weight correlate directly with villous vascular volume, the effects of maternal exercise and diet on the latter could be entirely secondary to their effects on the former. Therefore, this study was undertaken in an attempt to determine if weight-bearing exercise had a primary or only a secondary effect on both absolute (total villous vascular volume) and relative (total villous vascular volume/total villous volume) villous vascular volume by controlling these three potentially confounding factors. Thus, the placentae of 11 runners were matched with those of 11 physically active controls.
for placentae and neonatal size at birth and maternal diet. As there is no direct information currently on the effects of maternal exercise on the rate of cell proliferation, the matched placentae were also used to provide additional data in that area.

The main placental outcome variables were absolute and relative villous vascular volume (synonym villous vascular density) and cell proliferation. These were determined using immunohistochemical identification of the outcome variables and conventional stereological techniques (systematic random sampling and point counting) developed by Weibel [18]. The null hypothesis to be tested was that continuing a regular running regimen throughout pregnancy has no effect on either absolute or relative villous vascular volume or cell proliferation at term.

MATERIALS AND METHODS

Study materials

The 22 placentae studied were obtained from women who were enrolled in a larger study designed to assess the effects of maternal exercise on the course and outcome of pregnancy [19]. Eleven were obtained from women who continued to run regularly throughout pregnancy and were matched for placental weight, birth weight and maternal diet with 11 from controls. All women were healthy with uncomplicated singleton pregnancies. Exercise performance was monitored prospectively in both groups using a daily exercise/activity log and, in the exercise group, additional direct monitoring was carried out in the laboratory (one or two sessions each week) to assess exercise intensity [10]. Diet was assessed weekly using 24 hour dietary recall which was routinely obtained by a dietician using randomly timed phone interviews.

Tissue preparation and sampling

At the time of vaginal delivery, the time of cord clamping and neonatal position prior to cord clamping were not standardized. Rather all placentae were allowed to deliver spontaneously and within the hour, membranes, umbilical cord and surface clots were removed, blood in the major fetal vessels on the chorionic plate was completely expressed to the point of tissue pallor and the surfaces blotted dry. It should be recognized that, although this preparative technique produces more reproducible results, it may disproportionately decrease the size of the intervillous space, thereby making between-group differences in intervillous space volume of same-sized placentas more difficult to detect [4].

The placentae were then weighed and the volume estimated by fluid displacement [4]. The latter value was used for all later volume calculations. Then all placentae were fixed by immersion fixation in 10% formalin for a minimum of 6 weeks prior to random sampling.

Random sampling was accomplished using a modification of the technique initially described by Jackson and colleagues [5]. Briefly, a 3 cm squared grid was randomly oriented over the fetal surface of the placenta and a full-thickness cube of tissue obtained at each intersection. Each tissue sample was then randomly oriented and a 5 mm thick section was removed and embedded in paraffin. Several 5 µm sections from each paraffin block were mounted on electrostatically coated slides and stained with either CD 31 (Synonym PECAM-1, endoCam) or Ki-67 (Synonym MIB-1) monoclonal antibody and then counterstained with hematoxylin using the techniques described by Wienhard and her colleagues [20].

Then, 3 randomly selected microscopic fields (200×) from each slide were analyzed stereologically for component volumes or cell proliferation using the conventional point counting technique [3,16,18]. One hundred fifty points were counted in each field. No attempt was made to divide total villous volume or villous vascular volume into their component parts (stem, intermediate and terminal) because the method of processing fresh placental tissues at birth in these studies was different from that employed in the studies which used size to define the difference between intermediate and terminal villi. Figure 1 illustrates positive staining with the Ki-67 proliferative marker.

Data analysis

Absolute component volumes were calculated as the quotient of the number of points overlying the specific structure and the total number of points, multiplied by the fresh placental volume. Relative volumes (Synonym volume densities) [21] were calculated as a percentage of either total villous volume or total placental volume (i.e. relative villous vascular volume = villous vascular volume/villous volume). As far as the vessel volume is concerned, points over CD 31 positive structures as well as points over vessel lumens were counted, i.e. vessel volume included endothelial cells and lumen. Absolute cell proliferation was expressed as the total number of dividing nuclei counted in 30 fields using point counting and a proliferation index (dividing nuclei/1000 nuclei) was calculated to assess it in relative terms.

Between-group differences were sought using the unpaired t-test. Structure–function correlations were sought between absolute volumes and corrected birth weight using linear regression analysis. Sample size was calculated using half the variance encountered in earlier studies [9,10]. This was done because we thought that matching the placentae for size, birth weight and maternal diet would decrease the variance. This calculation predicted that an n of 10 per group was more than adequate to detect a 10% difference at the 0.05 level with a power of 0.80. Significance was set at the 0.05 level and all the tabulated data are presented as the mean and standard deviation or range and coefficient of variation.

RESULTS

Subjects

All subjects were healthy, non-substance abusing, physically active women between the ages of 29 and 35, who experienced uncomplicated singleton pregnancies. The women in both groups all ate a relatively high carbohydrate diet (55–65% of calories from carbohydrate) with an average glycemic index less than 80 [11], all were from middle or upper socioeconomic
class and all worked outside the home. All but 2 (one in each group) were primigravid.

Each of the 11 runners continued to run 4 or more times each week throughout pregnancy for 40–60 min a session at an intensity between 55 and 65% of their preconceptional maximal aerobic capacity. The 11 controls were physically active (gardening, golf, occasional tennis or hikes) but did no moderate to high intensity sustained exercise on a regular basis during pregnancy. Pregnancy outcome is detailed in Table 1 and the ranges and coefficients of variation for primary outcome variables are shown in Table 3. Note that there were no significant between-group differences in either size at birth or placental weight.

Placental composition and cellular proliferation

The data on placental composition and cellular proliferation are shown in Table 2. Although there appeared to be an increase in absolute villous volume in the runner group (\(p = 0.07\)), the only significant between-group difference in absolute volumes was that villous vascular volume was significantly greater in the runner group. However, as shown in Table 3, the within-group ranges were wide and the coefficients of variation larger than originally anticipated which reduced the power of these negative findings. Thus, \(\beta\) error (not identifying a significant between-group difference when one exists) cannot be ruled out.

Relative villous vascular volumes or densities were also calculated as a means of normalizing the data [21] and again they were significantly greater in the runner group (\(p < 0.001\)). In the runners, villous vascular volume comprised 18 ± 3% of total placental volume and 29 ± 5% of villous volume versus 12 ± 4% and 20 ± 6%, respectively, in the controls.

All absolute indices of mitotic activity and the overall proliferation index were significantly greater in the runner group. The percentage of dividing cells that were cytotrophoblast (90%, SD = 4), endothelial or stromal (10%, SD = 4), however, were similar in both groups indicating that the

![Figure 1. A projection of a microscopic field to illustrate the use of Ki-67 immunostaining for examining mitotic activity in placental tissue at a magnification of 200 X.](image_url)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls ((n=11))</th>
<th>Runners ((n=11))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (days)</td>
<td>278 (5)</td>
<td>280 (4)</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>3.46 (0.45)</td>
<td>3.48 (0.39)</td>
</tr>
<tr>
<td>Corrected birth weight (kg)</td>
<td>3.53 (0.42)</td>
<td>3.44 (0.30)</td>
</tr>
<tr>
<td>Neonatal fat mass (kg)</td>
<td>0.42 (0.22)</td>
<td>0.38 (0.14)</td>
</tr>
<tr>
<td>Placental weight (kg)</td>
<td>0.40 (0.08)</td>
<td>0.44 (0.08)</td>
</tr>
</tbody>
</table>

Data are presented as the mean and (SD). Note that no significant between-group differences were present. Corrected birth weight = crude birth weight corrected for gestational age, sex and race.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls ((n=11))</th>
<th>Runners ((n=11))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total placental volume (cm³)</td>
<td>391 (80)</td>
<td>431 (76)</td>
</tr>
<tr>
<td>Intervillous space (cm³)</td>
<td>136 (31)</td>
<td>136 (26)</td>
</tr>
<tr>
<td>Villous volume (cm³)</td>
<td>237 (56)</td>
<td>284 (65)</td>
</tr>
<tr>
<td>Villous vascular volume (cm³)</td>
<td>47 (18)</td>
<td>77 (20)*</td>
</tr>
</tbody>
</table>

Data are presented as the mean and (SD). *\(p < 0.02\).
Table 3. Ranges and coefficients of variation for select outcome variables

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls (CV)</th>
<th>Runners (CV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight (kg)</td>
<td>2.75–4.13 (13)</td>
<td>2.87–4.20 (12)</td>
</tr>
<tr>
<td>Placental volume (cm³)</td>
<td>252–460 (21)</td>
<td>340–560 (18)</td>
</tr>
<tr>
<td>Intervillous space volume (cm³)</td>
<td>100–216 (23)</td>
<td>105–194 (23)</td>
</tr>
<tr>
<td>Villous volume (cm³)</td>
<td>109–302 (24)</td>
<td>214–358 (23)</td>
</tr>
<tr>
<td>Villous vascular volume (cm³)</td>
<td>16–72 (39)</td>
<td>52–113 (27)</td>
</tr>
</tbody>
</table>

CV = coefficient of variation as a percent.

The fact that regular weight-bearing exercise is associated with evidence of increased cell proliferation is new information which supports the idea that the exercise stimulates placental growth. Furthermore, the proliferative activity appears to remain balanced between cytotrophoblast, endothelial and stromal cells. While the increase in absolute villous vascular volume is confirmatory, the matching of gross morphometric outcomes and maternal diet provides further assurance that this exercise effect is primary rather than just secondary to larger placentae per se. This conclusion is also supported by the finding of increased relative villous vascular volumes in the runners. The balanced increase in proliferative activity amongst the three tissues of interest however conflicts with this finding and, at the present time, we have no explanation for the absence of an increase in endothelial proliferation in the runner group.

The increase in absolute villous vascular volume in the current study is virtually identical to that found in an earlier study that examined the placentae of similar subjects using the same stereological techniques [4]. However, others who use early cord clamping with immersion fixation and/or perfusion fixation report absolute values that indicate greater placental volumes and greater absolute component volumes (especially intervillous space volumes and villous vascular volumes) than obtained with the technique used in the current study [5,12,14,16,23–26]. Nonetheless, relative villous vascular volumes are quite similar in all the studies that used systematic random sampling which suggests that the differences in absolute volumes are simply the result of differences in the protocol used for tissue preparation.

The assumptions made about variance in calculating the sample size for this study appear to have been incorrect because we did not simultaneously limit the range of birth weight and placental weight in our matched sample. Unfortunately, this increases the chances of β interpretive error which suggests that between-group differences with p values between 0.1 and 0.05 are probably significant. The only volume parameter which fell in this range was absolute villous volume (p = 0.07) and, in an earlier study with similar subjects, a significant between-group difference was detected with a sample size of 20 [4]. Thus, it is probable that the difference observed in villous vascular volume in the current study would have reached statistical significance if we had restricted the range of placental weight examined prior to matching or increased our sample size. If this assumption is correct, then the significant exercise-induced changes in villous vascular volume are partially due to differences in the villous volume. Nonetheless, relative villous vascular volume or density was also significantly greater in the runner group indicating that exercise stimulated vessel growth to a greater degree than overall villous growth.

The mechanism underlying the stimulatory effect of maternal weight-bearing exercise on villous vascular growth and cell proliferation was not elucidated in this study. However, the results of others and current thought [27–29] regarding both angiogenesis and exercise effects on placental perfusion [30] lead us to speculate that the underlying
mechanism involves an intermittent reduction in placental perfusion which initiates a response in the VEGF–IGF II growth regulating cascades.

The resultant structural effects could be of significant preventive or therapeutic value in pregnancies at risk for anomalous fetoplacental growth, specifically those with a history of growth restriction in a previous pregnancy or those with clinical evidence of poor placentation. However, the effect of beginning a weight-bearing exercise regimen in mid to late pregnancy on fetoplacental growth and anatomical indices of placentation function has not been studied. This would appear to be the next logical step explore.

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REFERENCES


