Effect of maternal exercise and fetoplacental growth rate on serum erythropoietin concentrations

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OBJECTIVE: This study was undertaken to test the null hypotheses that neither weight-bearing exercise nor fetoplacental growth has a short- or long-term effect on the maternal serum erythropoietin level.

STUDY DESIGN: Serial blood samples were obtained before and after exercise from seven women who exercised regularly and seven physically active controls before pregnancy and at 8, 16, 24, 32, and 38 weeks' gestation. Fetoplacental growth was assessed both in midpregnancy (ultrasound) and at birth (morphometry).

RESULTS: Maternal serum erythropoietin levels rose with advancing gestation in both groups. Individual patterns, however, were quite variable and not related to differences in fetoplacental growth. There were no significant between-group differences at any time point, but levels rose after exercise in mid and late pregnancy.

CONCLUSION: The highly variable, pregnancy-associated changes in maternal serum erythropoietin were unrelated to variability in fetoplacental growth or maternal hematocrit. Absolute levels of erythropoietin are not influenced by regular exercise before or during pregnancy, but small acute elevations are seen after exercise in mid and late pregnancy. (Am J Obstet Gynecol 2003;188:1021-5.)

Key words: Pregnancy, exercise, erythropoietin

In humans, serum erythropoietin is a sensitive marker of acute and/or chronic, low-grade hypoxia before and after birth.1-4 After birth, the majority of erythropoietin is produced by renal peritubular interstitial cells with production rates being inversely related to tissue oxygen tension. During pregnancy, both maternal arterial oxygen tension and renal blood flow increase, which improves oxygen availability to the peritubular cells. Nonetheless, circulating maternal erythropoietin levels rise 2- to 4-fold in conjunction with an increase in human placental lactogen and total red blood cell mass.5,6 It is also notable that the timing, pattern, and magnitude of the pregnancy-associated increase in erythropoietin is extremely variable between women and that individual changes may correlate with concurrent changes in human placental lactogen, suggesting that nonhypoxic placental factors may initiate the changes in serum erythropoietin levels.5,6 The possible role of placenta factors is further supported by recent immunohistochemical data that demonstrate the presence of both erythropoietin and its receptor in placental syncytiotrophblast.7,8 This raises the possibility that the placenta is an extrarenal source of erythropoietin and that the pregnancy-associated rise in circulating levels may reflect a primary autocrine or paracrine role for erythropoietin as a regulator of placental development rather than a response to tissue hypoxia.

The impact of high-intensity endurance exercise on erythropoietin levels is equally intriguing. The physiologic adaptations to exercise training are similar to those of pregnancy in that training expands plasma volume with a resultant decrease in hemoglobin concentration.9,10 Endurance training also initiates prolonged intermittent decreases in renal perfusion.11 Both these changes suggest that endurance athletes should have increased erythropoietin levels especially after competition. This is not the case, however,9,10 suggesting that other adaptive mechanisms prevent renal tissue hypoxia during high-intensity, sustained exercise.

The combined effects of exercise and pregnancy on the serial changes in serum erythropoietin have not been examined. Given the observations that placental tissues have the potential to both produce and respond to erythropoietin7,8 and that exercise reduces both renal and placental perfusion,12,13 we believe it would be of interest to examine the interactions among exercise, pregnancy, fetoplacental growth, and erythropoietin to determine whether the stimulatory effects of exercise on placental development14 altered the magnitude of the serial changes in erythropoietin (chronic effect) or whether an
exercise session was associated with a change in circulating levels (acute effect), possibly reflecting a change in either placental production or peripheral clearance.

Thus, this study was designed to test two null hypotheses. First, that regular weight-bearing exercise during pregnancy has no short- or long-term effect on maternal serum erythropoietin levels. Second, that between-subject variability in the pregnancy-associated changes in erythropoietin is not explained by the concomitant between-subject variability in fetoplacental growth.

Material and methods

After approval of the study by our Human Subjects Committee, 14 healthy, nonsmoking women (7 exercisers and 7 physically active controls) were recruited and evaluated before pregnancy. Preconceptional fitness was assessed by measuring both maximal oxygen consumption and percentage of body fat, and actual exercise performance by measuring usual exercise intensity, expressed as a percentage of maximal effort (\%VO_{2max}), and whole blood lactate.\(^{12,15}\) Serial changes in erythropoietin and exercise response were assessed by obtaining blood samples immediately (within 1 minute) before and after 20 minutes of treadmill exercise before pregnancy and at 8, 16, 24, 32, and 38 weeks’ gestation. Exercise intensity was assessed by measuring oxygen consumption.\(^{12,15}\) Hematocrit, which was used as an index of changes in plasma volume,\(^{16}\) and whole blood lactate were measured on the blood samples within 2 to 3 minutes of collection with the use of standard techniques,\(^{15,16}\) and then the remainder of the blood sample was spun, the serum removed and stored at -70°C until analysis. All samples were available in 13 of the 14 subjects. The remaining subject delivered 2 days before her 38-week study, so the final set of samples was not obtained.

At the time of analysis, the samples were batched by subject and analyzed in duplicate by using a specific double-antibody radioimmunoassay with a sensitivity of 1.2 mU/mL and a intra-assay coefficient of variation of 4%.\(^{17}\) Placental volumes and the rate of midtrimester placental growth were estimated with a standardized B-mode ultrasound technique (accuracy ±5%).\(^{14}\) and placental and neonatal morphometrics were obtained in a standard fashion at the time of birth.\(^{18}\)

The erythropoietin levels were rounded up to the next whole number before statistical analysis and the average of the individual pre-exercise and postexercise values were used for assessing both between-group differences at each time point and the longitudinal changes during pregnancy. Data are presented as ranges and means ± SD.

To clearly separate the short- and long-term effects of exercise from the effect of pregnancy, we used a stepwise approach to data analysis. First, we sought to determine whether regular exercise altered the magnitude or pattern of the response to pregnancy. Thus, significant between-group differences in the average erythropoietin level at each experimental time point were initially sought by using one-way analysis of variance (ANOVA) and Fisher exact test. As none were found, the data from the two groups were combined and significant changes with advancing pregnancy were sought with the use of repeated measures ANOVA and the significant changes identified were validated with MANOVA. Then, significant acute changes in erythropoietin with exercise at each experimental time point were sought with a paired \(t\) test. Finally, least squares regression was used to assess relationships between erythropoietin levels and indices of fetoplacental growth.

Power analysis was performed before subject enrollment by using estimates obtained from nonpregnant data to determine the sample size necessary to detect a significant between-group difference at a single time point at the .05 level with a power of .80. We chose mean values of 20 and 16 (a 20% difference) with an SD of 2.5 in each group and found that a sample size of seven subjects in each group would be required. This also had adequate power to detect a 5% to 10% acute or chronic change in the sample as a whole over time.

Results

Study population characteristics. Maternal age at study ranged between 26 and 35 years (mean = 31 ± 2 years); 10 were primigravid and all had healthy uncomplicated singleton pregnancies. All exercised regularly before preg-

<table>
<thead>
<tr>
<th>Time point</th>
<th>Exercise group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before pregnancy</td>
<td>20 ± 6</td>
<td>18 ± 4</td>
</tr>
<tr>
<td>8th wk</td>
<td>17 ± 3</td>
<td>18 ± 4</td>
</tr>
<tr>
<td>16th wk</td>
<td>25 ± 9</td>
<td>24 ± 13</td>
</tr>
<tr>
<td>24th wk</td>
<td>32 ± 11</td>
<td>30 ± 12</td>
</tr>
<tr>
<td>32nd wk</td>
<td>33 ± 12</td>
<td>34 ± 8</td>
</tr>
<tr>
<td>38th wk</td>
<td>34 ± 10</td>
<td>32 ± 13</td>
</tr>
</tbody>
</table>

Data presented as mean ± SD in mU/mL. No significant between-group differences were detected.

Table II. Effect of sustained exercise on serum erythropoietin before and during pregnancy

<table>
<thead>
<tr>
<th>Time point</th>
<th>Before exercise</th>
<th>After exercise*</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before pregnancy</td>
<td>19 ± 5</td>
<td>19 ± 5</td>
<td>.63</td>
</tr>
<tr>
<td>8th wk</td>
<td>17 ± 3</td>
<td>17 ± 3</td>
<td>.34</td>
</tr>
<tr>
<td>16th wk</td>
<td>24 ± 10</td>
<td>25 ± 11</td>
<td>.08</td>
</tr>
<tr>
<td>24th wk</td>
<td>30 ± 12</td>
<td>31 ± 11</td>
<td>.03</td>
</tr>
<tr>
<td>32nd wk</td>
<td>33 ± 9</td>
<td>35 ± 9</td>
<td>.01</td>
</tr>
<tr>
<td>38th wk</td>
<td>31 ± 11</td>
<td>34 ± 11</td>
<td>.0001</td>
</tr>
</tbody>
</table>

Data presented as the mean ± SD in mU/mL. Samples obtained immediately before and after 20 minutes of exercise (see text).
nancy and all were well above the 50th percentile for age-corrected fitness as assessed by maximal oxygen consumption and percentage of body fat (VO\textsubscript{max}\textsubscript{2max} > 40 mL/kg/min, % body fat < 22%). There were no preconceptional differences in either weekly exercise performance or these fitness parameters between the groups and the seven women who chose to stop exercising during pregnancy and the seven who continued (VO\textsubscript{max}\textsubscript{2max} = 47 ± 4 mL/kg/min and 49 ± 5 mL/kg/min, % body fat 18 ± 5 vs 18 ± 6). During pregnancy the seven women who continued regular exercise ran or performed aerobics five or more times each week (range 5-12 exercise sessions), whereas those who stopped did no regular sustained exercise after their pregnancy was confirmed by a positive urine test. During the testing sessions, the seven women who continued exercised at intensities between 63% and 77% of their preconceptional VO\textsubscript{max} (mean = 68% ± 4%) and the seven physically active controls exercised at 55% to 60% of their preconceptional VO\textsubscript{max}. All exercised below their lactate threshold (postexercise whole blood lactate levels < 2.5 mmol/L).

Before pregnancy, pre-exercise hematocrit values ranged between 37% and 44% (mean = 40% ± 2%). During pregnancy, hematocrit decreased in all subjects, reaching a nadir at 32 weeks’ gestation (range 33% to 38%, mean = 36% ± 3%). Twenty minutes of exercise produced a variable increase in hematocrit at all time points, which averaged between 1% and 2% (range -1% to +3.5%) and was consistently greater in the women who were exercising at higher intensities.

All morphometric outcome parameters varied widely within the study population. The midtrimester placental volume growth rate, which correlates with placental and fetal weight at birth,\textsuperscript{19} ranged from 16 to 39 mL per week (mean = 26 ± 6 mL/wk) and trimmed, blotted placental weight at term ranged from 330 to 823 g (mean = 461 ± 122 g). Birth weights of the seven male and seven female infants ranged between 2.63 and 4.18 kg (mean = 3.28 ± 0.45 kg) with estimated fat mass\textsuperscript{18} ranging between 80 and 510 g (mean = 286 ± 123 g).

**Effect of regular exercise during pregnancy on serum erythropoietin.** There were no significant (P all > 0.60) differences in pre-exercise or postexercise serum erythropoietin levels between the control and continued exercise group either before pregnancy or at any of the five time points during pregnancy. The average of the two (pre- and postexercise levels) are presented in Table I. Note that the maximum between-group difference in the means was only 2 mU/mL.

**Effect of acute exercise on serum erythropoietin.** The 20-minute exercise session did not change serum erythropoietin levels before pregnancy (Table II). During pregnancy, however, acute exercise was associated with a small increase in maternal serum erythropoietin level (Δ = 1.2 mU/mL) that became consistent and statistically significant (P < 0.05) in the 24th week of gestation with the level of significance rising to P < 0.0001 near term.

**Effect of pregnancy on serum erythropoietin.** Pregnancy-associated changes in serum erythropoietin were extremely variable within the study population. The maximum increase varied between 1- and 3-fold above prepregnancy levels (mean = 2-fold ± 1-fold) with peak values (range 24-54 mU/mL) occurring with near equal frequency at 24, 32, and 36 weeks’ gestation. The individual patterns in the control and continued exercise groups are detailed in Figs 1 and 2. Note that they ranged from no real change until the 38th week (one subject) to a steady progressive increase throughout pregnancy (one subject) with the majority (10 subjects) decreasing in the 8th week and then rising to peak at 24, 32, or 38 weeks. There was no detectable inverse relationship between these changes and those observed in hematocrit.

The mean levels of serum erythropoietin for the entire study population are shown in Fig 3. The increase over time was highly significant (P < 0.0001) with the first significant increase (P < 0.05) above nonpregnant levels occurring in the 16th week.

**Correlations between the pregnancy-associated changes in erythropoietin and the morphometric parameters.** We were unable to detect any significant association between either the absolute levels of serum erythropoietin or changes in maternal serum erythropoietin levels and midtrimester placental growth, placental weight at term, birth weight, or neonatal fat mass (r², all < 0.1).

**Comment**

This study reports two new findings and confirms several previous observations dealing with the effects of ex-
exercise and pregnancy on serum erythropoietin levels. The first new finding is that, even though the human trophoblast has the capacity to produce erythropoietin, the changing levels in the maternal compartment during pregnancy do not reflect changes in either placental or fetal growth rate. This indicates that it is unlikely that trophoblast is a significant extrarenal source of erythropoietin during pregnancy and that the variability in and magnitude of the maternal erythropoietin response is probably not related to placental functional development or substrate availability at the placental site. These conclusions are supported by 2 additional observations. First, the fact that maternal erythropoietin levels remain elevated in the immediate postpartum period suggests that the placental tissues do not play a large role in the production of circulating erythropoietin. Second, renal failure during pregnancy is associated with anemia and low serum levels of erythropoietin. This also suggests that the presence of a significant extrarenal source of circulating erythropoietin during pregnancy is unlikely.

The second new finding is that short bouts of moderate-to-high-intensity, weight-bearing exercise induce a small but significant increase in erythropoietin levels during mid and late pregnancy. The reason for this, however, is unclear. If it was related to exercise-induced hemocoagulation then its magnitude should be related to the exercise-induced change in hematocrit and it is not. Likewise, the same change should have been present before pregnancy and it was not.

As it was observed only during pregnancy, it is possible that it is related to exercise-induced, acute decreases in splanchnic and/or placental bed blood flow that influence tissue PO2. It is unlikely, however, that this is the case as experimental data indicates that it takes approximately 4 hours for a sudden change in tissue PO2 to produce an increase in circulating erythropoietin. It is more likely that same decreases in visceral blood flow transiently decrease erythropoietin clearance resulting in a small but consistent increase in its circulating level. This interpretation is also supported by the observation that the subjects with the greatest decrease in visceral flow (those who worked at the higher intensities) had the greatest increase in circulating erythropoietin level. Currently, however, we have no additional data to support this conclusion.

These results also confirm the earlier finding that regular exercise in the nonpregnant woman has no short- or long-term effect on serum erythropoietin levels. Likewise, the levels of erythropoietin obtained in this study are no different than those previously reported in pregnant sedentary women. The reasons for this remain unclear. As noted earlier, it is unlikely that the variability is due to differences in placental erythropoietin production. Similarly, it is unlikely that the variability was due to individual differences in exercise habit. Nor does it appear to be due to differences in renal oxygenation because the longitudinal changes in hematocrit were typical of normal pregnancy and did not correlate with changes in erythropoietin levels. However, we cannot be sure that the latter inference is correct because hematocrit is only an indirect measure of acute and
chronic changes in plasma volume and red blood cell mass.\textsuperscript{16,21} The latter were not measured directly for practical reasons (use of labels during pregnancy, indwelling lines, and the time required for dilution techniques makes them impractical for assessing acute change).

Although the current sample size proved adequate to detect acute and chronic longitudinal changes in erythropoietin level, the magnitude of the between-subject variability in erythropoietin level at each experimental time point markedly reduced our ability to detect significant differences between the control and continued exercise groups (post hoc power calculation = 0.20). Thus, we are unable to state with reasonable certainty that exercise during pregnancy has a clinically significant (20\%) effect on circulating erythropoietin levels. However, because the actual mean differences during pregnancy were consistently 6\% or less, it is unlikely that we would have identified a 20\% difference by enlarging the sample size to that calculated post hoc (60 in each group).

In conclusion, the highly variable, pregnancy-associated changes in maternal serum erythropoietin are unrelated to variability in fetoplacental growth, exercise habit, or maternal hematocrit. Absolute levels of erythropoietin are not greatly or consistently influenced by regular exercise before or during pregnancy. However, acute, small elevations are seen after exercise in mid and late pregnancy.

REFERENCES