Muscle ribosomal protein synthesis in normal pregnancy: implication for carrier detection in Duchenne muscular dystrophy*

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Summary. Muscle samples of rectus abdominis were obtained from 11 normal pregnant women 21–30 years old during elective Caesarean section and from 10 normal, non-pregnant controls of comparable age. The in-vitro amino-acid incorporation of polyribosomes extracted from the muscle homogenates of the pregnant women showed high activity for the synthesis of non-collagen protein in six cases, while control values were found in five cases. These results indicate that carrier detection in Duchenne muscular dystrophy based on increased muscle ribosomal protein synthesis cannot be done accurately during pregnancy, unless adequate values for known carriers at a comparable stage of gestation are available.

King, Spikesman, and Emery (1972) described a significant decrease in serum creatine phosphokinase (CPK) in early pregnancy of normal women. Usually increased levels of this enzyme are found in 54–80% of the known carriers of the X-linked recessive Duchenne muscular dystrophy (Gardner-Medwin, Pennington, and Walton, 1971; Moser, Mummenthaler, and Wiesmann, 1971), and these data are helpful in genetic counselling. However, a similar decrease of this enzyme was also found in four of seven pregnant carriers of the disease making its use in detecting the carrier state during pregnancy rather uncertain. The same carriers out of pregnancy showed the expected high levels of the enzyme (Blyth and Hughes, 1971).

The goal of the present study is to find out if the pregnancy of normal women also modifies the in-vitro synthesis of protein measured in the muscle extracts, since increased values recorded by this test have been used as well for carrier state detection of Duchenne muscular dystrophy (Ionasescu, Zellweger, and Conway, 1971 and 1973).

Materials and Methods

Samples of rectus abdominis were obtained from 11 normal pregnant women, 21–30 years, during elective Caesarean section and from 10 non-pregnant women of comparable age at operation for benign conditions such as prolapse, uterine myomas, or inguinal hernia. The parity of the pregnant women varied from one to eight and the duration of pregnancy was from 36 to 41 weeks. Nine of the 11 were scheduled repeat Caesareans and two were primary Caesareans. None of the 11 had been in labour. Epidural or spinal anaesthesia were used in both pregnant and non-pregnant women.

The procedure for the preparation of the muscle extracts and evaluation of ribosomal protein synthesis has been previously reported (Ionasescu et al, 1970 and 1971a and b). Determination of non-collagen protein in the muscle homogenate was done by the method of Lowry et al (1951) using bovine serum albumin as standard.

Results

Table I gives data on noncollagen protein and ribosome content of muscle from pregnant and non-pregnant women. The mean values obtained during
Muscle ribosomal protein synthesis in normal pregnancy

TABLE I

<table>
<thead>
<tr>
<th>Cases</th>
<th>Non-collagen Protein (mg/g Muscle ± SD)</th>
<th>Ribosomal Content (µg/mg protein ± SD)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Ribosomes</td>
<td>Major Ribosome Fraction</td>
</tr>
<tr>
<td>Pregnant women (11)</td>
<td>45 ± 18.7</td>
<td>9.5 ± 5.5</td>
</tr>
<tr>
<td>Controls (10)</td>
<td>48 ± 9.1</td>
<td>9.9 ± 1.7</td>
</tr>
<tr>
<td>t Value</td>
<td>&lt; 0.05</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The major ribosome fraction was obtained by extraction of an initial 122,000 g pellet with detergent. The free ribosomes were obtained by recentrifugation of the initial high speed supernatant fraction at 150,000 g for two hours. Further extractions of the 122,000 g pellet with a doubled concentration of detergent produced the re-extracted ribosome fraction.

TABLE II

<table>
<thead>
<tr>
<th>Cases</th>
<th>Monomeric Ribosomes</th>
<th>Total Polyribosomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnant women (11)</td>
<td>23.2 ± 10.0</td>
<td>134.0 ± 130.7</td>
</tr>
<tr>
<td>Controls (10)</td>
<td>15.1 ± 3.4</td>
<td>45.7 ± 12.6</td>
</tr>
<tr>
<td>t Value</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Measured as counts per minute per µg of ribosomes ± SD. Each sample contained 2 to 15 µg of ribosomes.

TABLE III

<table>
<thead>
<tr>
<th>Cases</th>
<th>Non-collagen Protein</th>
<th>Collagen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnant women (11)</td>
<td>354 ± 70</td>
<td>46.7 ± 67</td>
</tr>
<tr>
<td>Controls (10)</td>
<td>172 ± 64</td>
<td>36.0 ± 23</td>
</tr>
<tr>
<td>t Value</td>
<td>&lt; 0.05</td>
<td>0.48</td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

* Sedimented polyribosomes are the heaviest muscle polyribosomes pelleted to the bottom of the 15 to 50% linear sucrose gradients. Protein synthesis is measured as counts per minute/µg of ribosomes ± SD. Each sample contained 1 to 4 µg of ribosomes.

pregnancy do not differ from those in non-pregnant women; however, in pregnancy the variation in both protein and ribosome contents are considerably wider than that of the controls. The distribution of ribosomes on linear sucrose density gradients is identical for the two compared lots. Table II shows the results for amino-acid incorporation in monomeric ribosomes and total polyribosomes extracted from rectus abdominis. The mean values show a significant increase (P < 0.05) in protein synthesis of monomeric ribosomes and of total polyribosomes in the preparations from pregnant versus non-pregnant women. Analysis of the data from individual cases shows an increase in specific activity of in-vitro amino-acid incorporation of polyribosomes only in six cases, while control values were found in five cases. This distribution into two groups might account for the high standard deviations (SD) and the low t values. The mean value for collagen synthesis by the sedimented muscle polyribosomes from the pregnant women is within normal limits as shown in Table III. However, three of our cases show slight increase in their collagen synthesis. The average value of 46.7 cpm/µg of ribosomes represents 13.3% of the total specific activity of this class of polyribosomes. The mean value for non-collagen protein synthesis by the sedimented muscle polyribosomes is significantly increased (P < 0.05) and accounts for the increase in specific activity of total polyribosomes.

Discussion

The evaluation of in-vitro ribosomal protein synthesis in fractionated extracts of biopsied muscle from normal pregnant women shows variable results. About half of our cases give control values while the other half show increased formation of non-collagen protein. The latter increase in activity of the muscle polyribosomes is suggestive of high muscle regeneration.

The possibility of an increase in muscle ribosomal protein synthesis in normal women during pregnancy has, however, important implications in genetic counselling of carriers of Duchenne muscular dystrophy. Our previous studies (Ionasescu et al, 1973) have shown that muscle extracts from carriers of this disease have an increased ability to form protein in vitro. In this connection more than 70% of the carriers under 30 years of age had polyribosomes which synthesized higher amounts of collagen than matched controls. These values were intermediate between the controls and the patients with Duchenne muscular dystrophy. Thus the increased collagen synthesis seen in the carriers differs from the increase in the synthesis of non-collagen protein noted in the normal pregnant women. Nevertheless, in some carriers of Duchenne muscular dystrophy, the increase in ribosomal protein synthesis affects primarily non-collagen
protein, and collagen formation is not significantly increased. Furthermore, a slight increase in collagen formation was seen in three of the eleven normal pregnant women. Also it is possible that pregnancy might induce unexpected changes in ribosomal protein synthesis of pregnant carriers as it does in reducing the level of serum CPK (Blyth and Hughes, 1971). For all these reasons, at the present time it would be unwise to do a muscle biopsy for evaluation of ribosomal protein synthesis in a potential carrier with normal serum CPK who seeks genetic advice during pregnancy.

In the future, it may well become possible to evaluate accurately the carrier state during pregnancy using either ribosomal protein synthesis or serum CPK provided we have adequate values for known carriers (as well as normals) at a comparable stage of gestation. And if these methods are to be used for genetic counselling and antenatal diagnosis, values for controls and known carriers would have to be determined in early pregnancy. The acquisition of such information for known carriers would probably take some time because of the small numbers available and most likely would require collaboration between different centres.

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REFERENCES
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