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CHANGES IN PLASMA GROWTH HORMONE
AND INSULIN OF THE HUMAN FOETUS FOLLOWING
HYSTEROTOMY

By
R. C. Turner, B. Schneeloch and P. Paterson

ABSTRACT

Plasma immunoreactive insulin and growth hormone of 20–24 weeks' gestation human foetuses were assayed in serial samples following delivery by hysterotomy. The mean umbilical cord plasma growth hormone concentration was 71 ng/ml (range 13–120 ng/ml) and the mean plasma insulin was 5 μU/ml (range 2–8 μU/ml). Following delivery the growth hormone levels increased, but there was no significant change in plasma insulin concentration. The hypothalamic-hypophyseal axis controlling growth hormone secretion appears to be developed by 20 weeks' gestation, and "stress" appears to be a provocative stimulus.

Only limited information is available regarding the plasma concentrations of growth hormone and insulin in the human foetus. The exact role of these hormones, and the mechanisms which control their secretion during intrauterine life are largely unknown.

Growth hormone has been demonstrated in the human pituitary by bioassay at the 18th gestational week (Levina 1968) and by immunoassay at the 8th week of intrauterine life (Matsuzaki & Shizume 1968). Plasma concentrations in older foetuses (Kaplan & Grumbach 1967) and in umbilical cord plasma at term are higher than those found in adults (Kaplan & Grumbach 1965; Cornblath et al. 1965).

The human foetal pancreas contains insulin from the 12th gestational week (Steinke & Driscoll 1965). Plasma insulin may be detected by the 14th to 16th week of gestation, but at this stage plasma insulin levels do not rise in

The present study was designed to investigate the levels of these hormones in the immature human foetus and to examine the degree to which the plasma hormone levels change in response to the altered environment following delivery by hysterotomy. Growth hormone levels were found to rise significantly. The role of hypoglycaemia in provoking this elevation has been investigated by infusing glucose to the mother and foetus.

**MATERIALS AND METHODS**

Ten foetuses of 20–24 weeks' gestational age were studied. Termination of the pregnancy by hysterotomy was necessary in every case on sociopsychiatric grounds and no maternal physical disease was present. A lumbar epidural anaesthetic was employed followed by 20–30 mg intravenous Diazepam® and inhalation of a mixture of 75 % nitrous oxide and 25 % oxygen.

Within 2 min of incising the uterus, the foetus was delivered and an umbilical cord segment isolated at the earliest opportunity between clamps; the right carotid artery was cannulated. Deep body temperature was monitored with an Ellab TE3 rectal probe (Sierex Ltd.) and heart rate with a monitor provided by SE Laboratories (Slough). The foetus was placed on a heating pad during the study. The rectal temperature was found to remain constant and the foetal heart rate was between 70 and 100 beats per minute during the investigation.

A blood sample was taken from the isolated cord segment and from the carotid artery, 5, 10, 15 and 20 min after cord clamping. Maternal venous blood was also obtained at delivery. A portion of each sample was immediately centrifuged and a plasma fraction frozen at -20°C for subsequent glucose, insulin and growth hormone determinations. The remainder of the heparinised whole blood was placed into anaerobic containers and immersed in ice-cold water, and the pH was measured within 60 min using a Radiometer E5021 microelectrode assembly. The period of gestation was confirmed after delivery by reference to formulae relating crown-heel length and weight to gestation (Boyd 1941).

One foetus of 19 weeks' gestation was infused with 0.5 g/kg glucose via an umbilical catheter during the ten minutes immediately following hysterotomy, and samples collected as above. In a further study, 15 mothers of 19–22 weeks' gestation were infused with 3 litres of intravenous fluid during the 24 hours to hysterotomy; seven received 10 % dextrose (300 g) whilst eight »control« mothers received 0.45 % sodium chloride. A carotid artery blood sample was collected from each foetus five min after delivery, and glucose and hormone concentrations were estimated.

The plasma glucose was measured by a glucose oxidase method (Technicon Autoanalyser). Plasma insulin and growth hormone were measured simultaneously by immunoassay using albumen-coated charcoal to separate antibody bound and free hormone. Ten µl aliquots were placed in incubation tubes with 40 µl »insulin and growth hormone free« charcoal-treated human plasma for comparison with 50 µl of the same charcoal-treated plasma in the tubes containing standard hormone. Plasma containing high growth hormone concentrations were re-assayed using 10 µl aliquots.
of a 1:5 dilution of the sample in charcoal-treated plasma. The results shown are reported as the mean ± 2 SEM of the quadruplicate estimations. The overall precision of the quadruplicate insulin estimations is 1.8 ± µU/ml (± 2 SEM). Thirty of the plasma samples were re-assayed with a single 25 µl aliquot of plasma plus 25 µl of charcoal-treated plasma per incubation tube; there was no significant difference between the results assayed at the two plasma dilutions, the latter method giving a mean insulin value 0.5 µU/ml lower than the results of the quadruplicate estimations. A human insulin standard (MRC 63/304 supplied by Dr. M. Cotes) and a human growth hormone standard (NIH-GS-HS 722A, Wilhelmi, supplied by the National Pituitary Agency) were used. We are indebted to Dr. J. Ellis for the antiinsulin antiserum, and Dr. A. D. Wright for the antigrowth hormone serum. The degree to which the proinsulin cross-react with the insulin antiserum is not known.

RESULTS

The cord plasma growth hormone levels of the ten foetuses range from 13 to 120 ng/ml (mean ± 1 sd, 71 ± 37 ng/ml) (Table 1). No significant correlation was found between the umbilical cord growth hormone levels and the foetal crown-heel length. The cord levels were significantly inversely correlated with the plasma glucose concentrations (r = -0.91, P < 0.001), and to a lesser extent with the cord blood pH (r = -0.61, P = 0.05). Following delivery of the foetus the growth hormone levels rose significantly (P < 0.001), the 20 minute value ranging from 92 to 462 ng/ml (mean ± 1 sd, 205 ± 117 ng/ml growth hormone) (Table 1, Fig. 1). During the same period the glucose concentration fell from 81 ± 10 mg/100 ml (mean ± 1 sd) to 49 ± 16 mg/100 ml (P < 0.001) and the pH from 7.33 ± 0.05 to 6.90 ± 0.05 (P < 0.001). If pooled values from all sampling times are considered there is an inverse correlation between growth hormone and glucose (r = -0.81, P < 0.001) and growth hormone and pH (r = -0.64, P < 0.001). In several foetuses the plasma growth hormone fell in the latter part of the test period.

Following maternal glucose infusion, the glucose concentration in the five minute foetal carotid artery sample was raised (mean ± 1 sd, 131 ± 54 mg/100 ml) compared with the control group (53 ± 11 mg/100 ml). There was no difference between the foetal growth hormone levels in the two groups (Table 2). Direct glucose infusion to the delivered foetus also raised the plasma glucose level but nevertheless the plasma growth hormone levels rose to a degree comparable to the other ten non-infused foetuses which were studied (Table 3).

The cord plasma insulin of the ten foetuses ranged from 2 to 8 µU/ml (mean ± 1 sd, 5.3 ± 2.4 µU/ml). There was no significant change in the plasma insulin level following delivery, the 20 minute level being 5.0 ± 2.5 µU/ml (Table 4). There was no change in foetal plasma insulin concentrations following either maternal or foetal glucose infusions (Tables 2 and 3).
Table 1.
Plasma glucose and growth hormone levels following hysterotomy.

<table>
<thead>
<tr>
<th>Gestational period (weeks)</th>
<th>Plasma growth hormone ng/ml (± 2 SEM)</th>
<th>Plasma glucose mg/100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maternal Umbilical Times after hysterotomy (min)</td>
<td>Maternal Umbilical Times after hysterotomy (min)</td>
</tr>
<tr>
<td></td>
<td>Artery Vein 5' 10' 15' 20'</td>
<td>Artery Vein 5' 10' 15' 20'</td>
</tr>
<tr>
<td>20</td>
<td>7 ± 2 88 ± 14 102 ± 8 191 ± 26 219 ± 26 200 ± 13</td>
<td>95 82 67 60 55 55</td>
</tr>
<tr>
<td>20</td>
<td>8 ± 2 49 ± 6 115 ± 11 214 ± 13 144 ± 12 129 ± 12</td>
<td>107 89 67 61 49 47</td>
</tr>
<tr>
<td>23</td>
<td>14 ± 1 72 ± 5 185 ± 25 148 ± 17 138 ± 16 137 ± 5</td>
<td>88</td>
</tr>
<tr>
<td>20</td>
<td>10 ± 2 63 ± 2 107 ± 7 162 ± 5 123 ± 15 122 ± 11</td>
<td>115 85 55 50 50 58</td>
</tr>
<tr>
<td>24</td>
<td>12 ± 6 13 ± 1 20 ± 1 79 ± 22 80 ± 8 92 ± 11</td>
<td>82 95 68 62 61 50</td>
</tr>
<tr>
<td>24</td>
<td>7 ± 1 103 ± 4 176 ± 18 181 ± 14 135 ± 16 188 ± 17</td>
<td>81 67 55 53 49 44</td>
</tr>
<tr>
<td>21</td>
<td>22 ± 1 57 ± 3 114 ± 17 230 ± 12 248 ± 10 316 ± 26</td>
<td>84 83 49 35 29 30</td>
</tr>
<tr>
<td>20</td>
<td>6 ± 1 120 ± 6 163 ± 21 140 ± 12 156 ± 14</td>
<td>78 70 59 59 51</td>
</tr>
<tr>
<td>24</td>
<td>7 ± 1 27 ± 1 106 ± 4 148 ± 15 167 ± 24 201 ± 13</td>
<td>100 85 60 67 62 65</td>
</tr>
<tr>
<td>21</td>
<td>6 ± 1 120 ± 22 201 ± 7 297 ± 11 423 ± 24 462 ± 33</td>
<td>73 69 48 25 20 22</td>
</tr>
</tbody>
</table>

Mean
± 1 sd 22 10 ± 5 71 ± 37 129 ± 54 179 ± 59 183 ± 97 205 ± 117 90 ± 14 81 ± 10 60 ± 8 55 ± 15 50 ± 15 49 ± 16
DISCUSSION

The foetal plasma growth hormone is almost certainly of foetal origin as the levels are higher than the maternal levels, and the foetal levels rise after delivery. Placental lactogen only appears in the foetal plasma in very small amounts (Kaplan & Grumbach 1965; Greenwood et al. 1964). Human foetal growth hormone appears to be antigenically similar to the purified adult hormone (Greenwood et al. 1964; Makler 1968).

The growth hormone levels found in the umbilical cord plasma at 20–24 weeks are slightly higher than those found in scalp plasma obtained before the onset of labour at term (Turner, Beard & Oakley, pers. observ. 1970), range 10–48, mean ± 1 so, 22 ± 10 ng/ml. The levels are similar to those reported in cord blood at term by Cornblath et al. (1965), range 9–320 (mean 66 ng/ml), and by Kaplan & Grumbach (1965), range 6–93, mean 34 ng/ml. One cannot be certain that any of these observations represent basal foetal plasma growth hormone levels as the delivery of the foetus and the sampling techniques may in each case produce sufficient stress to elevate the growth hormone levels.
Table 2.
Foetal glucose, growth hormone and insulin levels.

<table>
<thead>
<tr>
<th></th>
<th>Glucose mg/100 ml</th>
<th>266</th>
<th>253</th>
<th>161</th>
<th>182</th>
<th>382</th>
<th>147</th>
<th>151</th>
<th>Mean ± 1 sd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carotid artery</td>
<td>Glucose mg/100 ml</td>
<td>239</td>
<td>128</td>
<td>88</td>
<td>154</td>
<td>133</td>
<td>100</td>
<td>78</td>
<td>131 ± 54</td>
</tr>
<tr>
<td>sample 5 min</td>
<td>Growth hormone ng/ml</td>
<td>30 ± 6</td>
<td>76 ± 9</td>
<td>78 ± 4</td>
<td>127 ± 3</td>
<td>81 ± 13</td>
<td>116 ± 15</td>
<td>135 ± 16</td>
<td>91 ± 37</td>
</tr>
<tr>
<td>after hysterotomy</td>
<td>Insulin μU/ml</td>
<td>10 ± 2</td>
<td>4 ± 2</td>
<td>6 ± 2</td>
<td>4 ± 2</td>
<td>5 ± 1</td>
<td>9 ± 2</td>
<td>8 ± 1</td>
<td>7 ± 2</td>
</tr>
</tbody>
</table>

|                   | Glucose mg/100 ml | 71  | 71  | 76  | 74  | 74  | 77  | 86  | 91  | 78 ± 7    |
| Carotid artery    | Glucose mg/100 ml | 45  | 54  | 58  | 53  | 37  | 48  | 72  | 60  | 53 ± 11   |
| sample 5 min      | Growth hormone ng/ml | 59 ± 22 | 42 ± 13 | 35 ± 1 | 55 ± 6 | 147 ± 4 | 83 ± 3 | 140 ± 13 | >160 | 90 ± 51   |
| after hysterotomy | Insulin μU/ml     | 6 ± 1 | 4 ± 2 | 4 ± 1 | 9 ± 2 | 3 ± 2 | 10 ± 3 | 7 ± 2 | 6 ± 1 | 6 ± 2     |
Table 3.
Glucose infusion to delivered foetus.

<table>
<thead>
<tr>
<th></th>
<th>Umbilical vein</th>
<th>Times after hysterotomy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Glucose mg/100 ml</td>
<td>59</td>
<td>107</td>
</tr>
<tr>
<td>Growth hormone ng/ml</td>
<td>95 ± 12</td>
<td>94 ± 24</td>
</tr>
<tr>
<td>Insulin µU/ml</td>
<td>5 ± 1</td>
<td>3 ± 2</td>
</tr>
</tbody>
</table>

Table 4.
Plasma insulin levels following hysterotomy.

<table>
<thead>
<tr>
<th>Umbilical cord</th>
<th>Plasma insulin µU/ml (± 2 SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Times after hysterotomy (min)</td>
</tr>
<tr>
<td></td>
<td>5'</td>
</tr>
<tr>
<td>4.0 ± 2.0</td>
<td>3.8 ± 1.7</td>
</tr>
<tr>
<td>7.7 ± 2.9</td>
<td>7.1 ± 0.8</td>
</tr>
<tr>
<td>3.6 ± 3.3</td>
<td>4.6 ± 3.1</td>
</tr>
<tr>
<td>2.4 ± 0.8</td>
<td>5.3 ± 0.8</td>
</tr>
<tr>
<td>8.0 ± 1.6</td>
<td>6.9 ± 1.0</td>
</tr>
<tr>
<td>6.0 ± 3.3</td>
<td>6.0 ± 0.8</td>
</tr>
<tr>
<td>7.1 ± 1.4</td>
<td>6.4 ± 1.0</td>
</tr>
<tr>
<td>1.5 ± 1.3</td>
<td>2.5 ± 1.8</td>
</tr>
<tr>
<td>8.1 ± 1.5</td>
<td>6.3 ± 0.7</td>
</tr>
<tr>
<td>4.4 ± 1.9</td>
<td>3.3 ± 1.8</td>
</tr>
</tbody>
</table>

Mean
± 1 sd 5.3 ± 2.4 | 5.2 ± 1.6 | 4.7 ± 1.8 | 5.0 ± 2.0 | 5.0 ± 2.5

In the present study the cord blood pH values were all within normal limits, which suggests the foetuses were not markedly asphyxiated before the cord samples were taken. Nevertheless there was a correlation between growth hormone concentration and pH, and one cannot exclude the possibility that the cord values represent a stressed state. Growth hormone levels have been found to be raised during the first 48 hours after delivery, and to fall to adult levels during the next eight weeks (Cornblath et al. 1965). Premature neonates tend to have high plasma growth hormone levels for a longer period after delivery than do full-term neonates.

The part played by growth hormone in the development and metabolism of
the foetus in incompletely understood. Growth hormone deficient foetuses tend
to be short although they usually have a normal body weight (Laron & Pert-
zelan 1969). We have shown that in immature foetuses there is a marked rise
in foetal growth hormone levels following delivery. This is presumably due to
increased secretion and it suggests that the hypothalamic-hypophyseal axis is
well developed by 20 weeks gestation. The exact stimulus to the secretion
cannot be determined. It could be due to the stress of delivery and the catheter-
isation of the carotid artery, or to asphyxia or hypoglycaemia. In adults
stress and hypoglycaemia stimulate growth hormone release. The inverse cor-
relation between plasma growth hormone and glucose concentrations both in
the cord and subsequent values of the initial ten foetuses raised the possibility
that blood glucose concentration may be a factor regulating growth hormone
secretion in the human foetus. However, direct infusion of glucose to the foetus
after hysterotomy did not inhibit the rise in plasma growth hormone at this
time, and prolonged elevation of the foetal plasma glucose levels as a result
of maternal glucose infusions did not affect the foetal plasma growth levels.
It is thus improbable that plasma glucose concentrations is a major factor con-
trolling foetal growth hormone secretion. In foetal monkeys the plasma growth
hormone levels do not change in response to infusions of glucose or arginine,
although the levels appear to be labile (Mintz et al. 1969). It seems likely that
stress of delivery is the provocative stimulus causing the rise in growth
hormone levels following delivery of the foetus. The inverse correlation be-
tween the umbilical cord glucose and growth hormone levels may be due to
the stress of the hysterotomy inducing both slight hypoglycaemia and growth
hormone release. Stress has been shown to stimulate the release of hypo-
thalamic growth hormone releasing factor and of pituitary growth hormone in
neonatal rats (Pecile et al. 1969). Human neonatal growth hormone levels
increase in response both to insulin-induced hypoglycaemia and to intravenous
glucose (Cornblath et al. 1965; Westphal 1967) and stress may be the
stimulus in both situations.

The role of growth hormone in the foetus is uncertain, and the apparent
presence of stress stimulation of growth hormone release in the foetus at an
early stage of development is surprising. A possible teleological explanation
is that growth hormone secreted in response to the stress of labour and de-
livery may assist in the prevention of neonatal hypoglycaemia, and may play
a part in the changing metabolic requirements of the neonate compared with
the foetus.

The basal insulin levels are comparable to those reported by Adams et al.
(1968), 5.9 ± 2.3 μU/ml in midtrimester foetuses, but are lower than those
reported by Thorell (1970) (mean 29 μU/ml). The levels are slightly lower
than those found using the same assay in the mature human foetus before the
onset of labour (Turner, Beard & Oakley, unpubl. observ., range 4–14 μU/ml,
mean 11 μU/ml); adult fasting values measured with this assay range from 2 to 14 μU/ml, mean 5.3 μU/ml. The failure of plasma insulin levels to respond to hyperglycaemia at this stage of gestation is in accord with the observation that glucose does not stimulate insulin secretion in mid-term foetuses (Adam et al. 1968; Thorell 1970). In adults there is marked suppression of insulin levels in response to alcohol-induced hypoglycaemia (Roddam et al. 1968; Bagdade et al. 1969; Turner, Beard & Oakley, unpubl. observ.). In the present study there does not appear to be such marked suppression in response to hypoglycaemia in the foetus, but this is not certain as the plasma disappearance rate of insulin in these foetuses is not known. There is no rise in foetal monkey insulin levels in response to infused glucose or arginine, although tolbutamide does increase the plasma levels (Mintz et al. 1969). In the human foetus a variable insulin response to glucose has developed at term (Paterson et al. 1968; Coltart et al. 1969) and similar findings have been demonstrated in the neonate (Tierman et al. 1967; Isles et al. 1968; Gentz et al. 1969).

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