Diminished insulin clearance during late pregnancy in patients with Type I diabetes mellitus

Anders O. BJÖRKLUND, Ulf K. C. ADAMSON*, Per-Eric S. LINS* and L. Magnus R. WESTGREN†
Division of Obstetrics and Gynaecology, Karolinska Institute, Danderyd Hospital, SE-182 88 Danderyd, Sweden, *Division of Internal Medicine, Karolinska Institute, Danderyd Hospital, SE-182 88 Danderyd, Sweden, and †Department of Obstetrics and Gynaecology, Karolinska Institute, Huddinge University Hospital, SE-14186 Huddinge, Sweden

1. Intensive insulin treatment of patients with Type I diabetes mellitus during pregnancy is associated with a high frequency of serious hypoglycaemic events. A potential change in insulin metabolism during pregnancy may affect both the frequency and the severity of insulin-induced hypoglycaemia.

2. In 10 patients with Type I diabetes, during the third trimester of pregnancy and 5 to 13 months after delivery, hypoglycaemia was induced by the hyperinsulinaemic hypoglycaemic clamp technique. A constant high-dose intravenous insulin infusion was administered for 150 min and arterial blood glucose was clamped at 2.2 mmol/l by counterregulation with intravenous glucose. During the experiment venous samples were collected for later analysis of free plasma insulin, whereby the metabolic clearance rate of insulin could be calculated.

3. The desired blood glucose level was approached after approximately 60 min of insulin infusion. After just 30 min the insulin levels were significantly higher during pregnancy compared with after delivery. In addition, the steady-state insulin level from 90 to 150 min was significantly higher during pregnancy.

4. From the steady-state insulin levels at 90 to 150 min, the metabolic clearance rate of insulin was calculated, being 24% higher after delivery.

5. We conclude that there is a decreased metabolic clearance rate of insulin during pregnancy. This might be due to altered blood-flow distribution, decreased hepatic insulin extraction and relative increase in body fat during pregnancy. A decreased clearance of insulin will contribute to the risk for serious hypoglycaemic events in patients with Type I diabetes during pregnancy.

INTRODUCTION

Intensive insulin treatment is an established routine during pregnancy in women with Type I diabetes [1], a possible consequence of which being that severe hypoglycaemia is reported to occur in pregnancy at incidences as high as 44% [2–5]. Whether such a high frequency of hypoglycaemia is explicable by the intensive insulin treatment alone [6–8], or is also due to pregnancy-related factors, is not known. Our previous studies concerning...
the hormonal responses to hypoglycaemia did not reveal signs of apparent counterregulatory failure in pregnancy [9]. From a theoretical point of view, an altered metabolism of insulin during pregnancy, expressed as a change in clearance of insulin from the circulation, might influence the frequency of hypoglycaemia as well as the magnitude and duration of an insulin-induced hypoglycaemic event. This study therefore aimed to determine the metabolic clearance rates of intravenously (i.v.) infused insulin during hypoglycaemic clamps, performed in the third trimester as well as after delivery in patients with Type I diabetes.

**METHODS**

**Subjects**

The study group, consisting of 10 pregnant women with Type I diabetes, was recruited from the antenatal outpatient clinic for pregnant diabetic patients at the Department of Obstetrics and Gynaecology at Danderyd Hospital. Like all pregnant diabetic patients attending this unit, these women were intensively treated and counselled from the first trimester of pregnancy. Some relevant clinical data concerning the subjects are presented in Table 1. Details of the clinical management, as well as the uncomplicated fetal outcome, have been presented previously [9,10]. Endogenous insulin secretion was measured by plasma C-peptide determinations before breakfast and 2 h later, before hospital admittance.

The protocol was approved by the Local Ethics Committee of the Karolinska Hospital, and the study was carried out in accordance with the Declaration of Helsinki (1989). Before giving their consent all women had received full written and oral information.

**Experimental procedure and analysis**

The experimental procedure has been described in detail in our previous report [10]. This procedure, called hyperinsulinaemic hypoglycaemic clamp, was instituted in the third trimester (median 31 complete weeks of gestation, range 30–34 weeks), and was repeated as a control experiment 5 to 13 months after delivery. Twenty-four hours before induction of hypoglycaemia the women were admitted to hospital, whereupon no subcutaneous insulin injections were given in order to deplete the subcutaneous insulin depots. Insulin was delivered by an i.v. infusion with the aim of maintaining near-normoglycaemia 20–24 h before the investigation. After an overnight energy fast the women were transferred to the metabolic research laboratory.

A catheter was inserted into a vein of the left forearm for collection of blood samples every 15 min. An already indwelling venous catheter in the contralateral forearm was used for insulin and glucose infusions during the experiment. A third catheter which was introduced into the radial artery of the left forearm was connected to an automatic glucose analyser (Gambro Instrumenta AB, Lund, Sweden) for the monitoring of arterial blood glucose every 90 s, whereas venous blood glucose was determined every 15 min by a glucose analyser (Yellow Spring Instruments, Yellow Springs, OH, U.S.A.). To ensure that these analysers were operating accurately, the same calibration solution was used for both analysers throughout the test.

A 30-min period of normoglycaemia with blood

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (years)</th>
<th>Duration of Type I diabetes (years)</th>
<th>C-peptide, fasting (nmol/l)</th>
<th>C-peptide, 2 h after breakfast (nmol/l)</th>
<th>Late diabetic complications</th>
<th>HbA1c during pregnancy (%)</th>
<th>HbA1c after pregnancy (%)</th>
<th>Insulin dose, pregnant (i.u./24 h)</th>
<th>Insulin dose, non-pregnant (i.u./24 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>20</td>
<td>0.05</td>
<td>0.05</td>
<td>None</td>
<td>6.4</td>
<td>7.0</td>
<td>48</td>
<td>34</td>
</tr>
<tr>
<td>2</td>
<td>27</td>
<td>14</td>
<td>&lt; 0.05</td>
<td>0.34</td>
<td>Non-proliferative retinopathy</td>
<td>4.5</td>
<td>7.1</td>
<td>94</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>23</td>
<td>4</td>
<td>0.20</td>
<td>0.37</td>
<td>None</td>
<td>4.3</td>
<td>4.4</td>
<td>64</td>
<td>24</td>
</tr>
<tr>
<td>4</td>
<td>27</td>
<td>1</td>
<td>0.50</td>
<td>0.70</td>
<td>None</td>
<td>4.9</td>
<td>7.1</td>
<td>70</td>
<td>44</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>8</td>
<td>0.07</td>
<td>0.09</td>
<td>Non-proliferative retinopathy</td>
<td>4.7</td>
<td>7.8</td>
<td>126</td>
<td>46</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>5</td>
<td>&lt; 0.05</td>
<td>0.45</td>
<td>None</td>
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<td>6.9</td>
<td>89</td>
<td>26</td>
</tr>
<tr>
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<td>28</td>
<td>4</td>
<td>0.16</td>
<td>0.19</td>
<td>None</td>
<td>3.7</td>
<td>7.4</td>
<td>56</td>
<td>40</td>
</tr>
<tr>
<td>8</td>
<td>31</td>
<td>5</td>
<td>0.28</td>
<td>0.55</td>
<td>None</td>
<td>4.4</td>
<td>7.1</td>
<td>48</td>
<td>24</td>
</tr>
<tr>
<td>9</td>
<td>28</td>
<td>6</td>
<td>0.09</td>
<td>0.20</td>
<td>None</td>
<td>6.4</td>
<td>6.9</td>
<td>52</td>
<td>35</td>
</tr>
<tr>
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<td>32</td>
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<td>0.11</td>
<td>0.08</td>
<td>Proliferative retinopathy</td>
<td>5.9</td>
<td>10.7</td>
<td>54</td>
<td>24</td>
</tr>
</tbody>
</table>
glucose of about 5.0 mmol/l was instituted before the induction of hypoglycaemia. At the start of the hyper-insulinaemic hypoglycaemic clamp, at ‘time zero’, the i.v. insulin infusion was switched to a high-dose infusion (0.144 units h⁻¹·kg⁻¹) of insulin (Actrapid®, Novo-Nordisk), continuous during the entire 150-min clamp procedure. For calculation of the insulin infusion rate, body weight was reduced by the estimated weight of the feto-placental unit [11,12] since insulin does not pass the placenta [13]. An arterial blood glucose level of 2.2 mmol/l was achieved during the clamp by a variable i.v. infusion of 20% glucose (1.1 mol/l), guided by glucose determinations at 90-s intervals.

C-peptide was measured by a radioimmunoassay method using a commercial kit (RIA-gnost®, hC-Pep-tide, Hoechst Behringwerke, Germany). In the venous samples collected every 15 min for measurement of plasma free insulin, antibody-bound insulin was immediately precipitated by polyethylene glycol. The samples were then stored at −70 °C, pending concurrent analyses of specimens from both clamp occasions. A commercially available radioimmunoassay (Pharmacia Insulin RIA 100, Pharmacia Diagnostics AB, Uppsala, Sweden) was used for the determinations of free plasma insulin according to the method of Nakagawa et al. [14].

**Statistical procedure**

The mean free insulin concentrations between 90 and 150 min of the insulin infusion were calculated for each patient and represented the steady-state level of insulin at hypoglycaemia. The metabolic clearance rate (MCR) of insulin, defined as the volume of plasma completely and irreversibly cleared of the hormone in a given time (l·h⁻¹·kg⁻¹), was calculated for each patient by introducing the infusion rate and the steady-state insulin level in the following formula by Sønksen et al. [15]:

\[
\text{MCR (l·h}^{-1}\cdot\text{kg}^{-1}) = \frac{\text{Infusion rate (units h}^{-1}\cdot\text{kg}^{-1})}{\text{Steady-state plasma insulin concn. (units/L)}}
\]

Results are presented as mean values with S.E.M. The paired t-test was applied for statistical comparison of biochemical parameters in the pregnant and non-pregnant conditions. P-values < 0.05 were considered significant. Since the samples for free insulin were lost for one pregnant patient, the comparison regarding insulin levels and clearance was made on the samples of the remaining nine patients. The body weights of the non-pregnant subjects did not indicate a normal distribution, thus Wilcoxon signed rank test was applied for statistical comparison. The body weights are presented as medians with range.

**RESULTS**

All patients showed low or undetectable plasma C-peptide levels after fasting in the morning and 2 h after breakfast (Table 1).

At the start of the experiment the mean arterial blood glucose level was 5.0 mmol/l (SEM 0.2) in the pregnant state and 4.8 mmol/l (SEM 0.2) in the non-pregnant state, not differing significantly (P = 0.40). The blood glucose declined almost identically in both clamp studies, reaching a mean hypoglycaemic level between 90 and 150 min of 2.27 mmol/l (SEM 0.2) during pregnancy and 2.28 mmol/l (SEM 0.2) after delivery (not significant: P = 0.66; Figure 1). The median body weight was
Figure 2  Individual changes in the calculated MCR of insulin from pregnant to non-pregnant conditions (n = 9)

DISCUSSION

Our finding of significantly higher free insulin levels during pregnancy corresponds to a 24% lower MCR of insulin in the pregnant state, since equal amounts of insulin per kg of body weight were infused during and after pregnancy.

Our investigation was focused on insulin metabolism in pregnancy, as determined during hypoglycaemia. In previous investigations, also performed in patients with diabetes, we have documented that hypoglycaemia itself does not alter the metabolism of insulin [16], and therefore we believe that our observations are relevant for the third trimester of pregnancy as such. Notably, in the present investigation we observed significantly higher insulin levels after just 30 min, before established hypoglycaemia (mean blood glucose 3.7 mmol/l). All subjects in the present study had low or undetectable C-peptide levels, indicating deprivation of endogenous insulin secretion. It is well known that endogenous secretion of insulin is suppressed during hypoglycaemia in normal subjects [17,18], and that residual secretion of insulin is also inhibited during hypoglycaemia in Type I diabetes [19]. These findings indicate that, in the present study population, residual secretion of endogenous insulin was not a confounding factor for the calculation of the MCR of insulin.

As revealed by the HbA1c values in Table 1, the metabolic control, like in clinical practice, was better during pregnancy. However, there are no indications of long-term glycaemic control affecting insulin metabolism. In a previous study by our group we did not find altered free insulin levels when performing insulin-induced hypoglycaemias in patients with Type I diabetes before and after improved glycaemic control [20]. Thus there were no signs of altered insulin MCR despite a significant lowering of HbA1c after introduction of continuous subcutaneous insulin infusion.

In contrast to our present findings, four earlier studies on plasma clearance of insulin during pregnancy did not indicate altered clearance of insulin to be a phenomenon of pregnancy [21–24], suggesting that placental removal of insulin has a minimal impact on overall insulin clearance.
dynamics in pregnancy [25]. However, in a recently published study by Kautsky-Willer et al. [26], a 30% decrease of hepatic insulin extraction was found during pregnancy in lean subjects with gestational diabetes, an observation which is in line with our present calculations. Two of the earlier studies cited above [21,22] were based on analysis of insulin clearance after a single i.v. injection of insulin. Taking into account obvious changes in body composition during pregnancy, such an investigational procedure may be misleading. The increase in plasma volume related to pregnancy is likely to give rise to an altered initial distribution volume of insulin [27], which has not been taken into consideration in the MCR calculations presented. Due to the increased extracellular volume during pregnancy, one would expect lower insulin levels to appear if the MCR is unaltered. The method of calculating MCR used in our study is based on the assumption that the rate of removal of the hormone equals its rate of administration at stable plasma levels. Thus, this method obviates the necessity for analysing the multi-exponential plasma decay curves resulting from a single input of insulin [27]. With the normoglycaemic clamp technique, Gray et al. [24], in an investigation similar to ours, studied insulin pharmacokinetics during pregnancy and after delivery in seven patients with Type I diabetes. They detected significantly higher levels of plasma free insulin during pregnancy but failed to document a statistically significant decrease of MCR. However, this might only represent a β-error of statistics, since only seven subjects were included in their investigation.

Our present study does not elucidate the mechanism behind the finding of an altered MCR of insulin during pregnancy. A number of factors may be relevant; firstly, the radical haemodynamic changes that occur during pregnancy represent a possible physiological background to the concurrent decrease in insulin clearance. Parallel to a large increase in cardiac output during pregnancy, the distribution of this blood flow is changed. The proportion destined for the uterine circulation increases the most, from 2% to 17% of the total cardiac output [28]. Since insulin does not cross the placenta [13], the insulin degradation in the uterine circulation is mainly dependent on the binding and degradation of insulin in the placenta and in the uterine smooth muscle wall. Since the fractional extraction of insulin here can be assumed to be lower than in the liver, which is the predominant organ clearing insulin [27], the effect of the relatively smaller fraction of cardiac output entering the hepatic circulation would be a decrease in the plasma clearance rate of insulin during pregnancy.

Secondly, by analysing the ratio between C-peptide and insulin in the fasting state: Kühl et al. [23] studied hepatic insulin extraction during pregnancy, and did not find any signs of a decreased MCR. However, in a recent study using a similar method to estimate fractional hepatic insulin extraction during oral and i.v. glucose tolerance tests, a diminished hepatic insulin extraction during pregnancy was reported [26]. Such a decrease in the extraction of insulin by the liver could at least partly explain our findings. Levels of circulating non-esterified fatty acids are increased during normal pregnancy [29], and since other studies indicate that elevated non-esterified fatty acid levels may in the liver reduce insulin-mediated glucose uptake and enhance glucose production [30,31], the altered non-esterified fatty acid levels during pregnancy might influence the hepatic extraction of insulin.

Thirdly, we reduced the pregnant woman’s body weight by the estimated weight of the feto-placental unit when calculating the insulin dose to be administered. However, the body weight changes during pregnancy are disproportional in different body compartments. The volume of plasma and subcutaneous fat increases by a greater proportion during pregnancy compared with other tissues [32], thus influencing the distribution of insulin. Adipose tissue may degrade insulin, but much less than organs such as liver, kidney and muscle [27]. The increase in adipose tissue represents 44% of the maternal tissue and fluid weight gain during pregnancy (mean of 7650 g at term) [32]. In analogy with this discussion, two groups have shown an inverse relationship between the MCR of insulin and body mass index [33,34]. Castillo et al. [34] studied a female population, ranging from anorexia nervosa to severe obesity, and Davidson et al. [33] examined humans whose body mass indices ranged from 18.0 to 34.1 kg/m². Furthermore, Yki-Järvinen et al. [35] showed a strong inverse correlation between insulin clearance and the relative size of body adipose tissue, and suggested that this was due to a decreased adsorption surface of the capillaries, which have a density that is considerably lower in fat tissue than in, for example, muscle. In addition, a decrease in the binding of insulin to adipocytes from pregnant compared with non-pregnant women, reported in several papers cited by Buchanan [36], will be additive to this effect. However, the general insulin resistance observed during pregnancy is considered to be mainly a post-insulin receptor effect. The binding of insulin to receptors in the skeletal muscle, quantitatively the most important target tissue for total-body insulin-mediated glucose uptake, has been reported to be similar in non-pregnant and pregnant women [37]. Taken together, the increase of adipose tissue and the decreased insulin binding to adipocytes in pregnancy might be important in explaining the observed alterations of insulin metabolism in pregnancy.

The amounts of glucose needed to maintain the desired blood glucose level were significantly lower during pregnancy, an observation which reflects the marked and well-described insulin resistance in the third trimester of pregnancy [38]. Hence, the hyperinsulinaemia observed during pregnancy in non-diabetic women, mainly be-
lieved to be due to increased insulin release [36], may be seen as a physiological adaptation to the state of insulin resistance. The decreased insulin clearance which we observed in the present study, could represent an additional compensatory mechanism contributing to the physiological augmentation of peripheral insulin availability. In healthy pregnant women this will have an insulin-sparing effect, but in pregnant individuals with Type I diabetes it will tend to increase the risk for serious hypoglycaemic events.

In conclusion, we found a decreased MCR of insulin during late pregnancy. Altered blood-flow distribution, decreased hepatic insulin extraction and increased adipose tissue may all be factors contributing to this phenomenon. Whatever the underlying physiological mechanism involved, it is clear that an altered metabolism of insulin during late pregnancy is likely to increase the frequency and severity of hypoglycaemia in patients with Type I diabetes.

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