Insulin receptor binding from mid-term and full-term placentas of patients with gestational diabetes mellitus and normal pregnant women

Omar S. Al-Attas
Associate Professor of Biochemistry, Department of Biochemistry, College of Science, King Saud University, PO Box 2455, Riyadh 11451, Saudi Arabia

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Abstract

Insulin receptor binding was examined in the microvillous membranes of mid-term (20–22 weeks of gestation, MT) and full-term (FT) placentas from patients with gestational diabetes mellitus (GDM) and in normal pregnant control (N). Mid-term placentas were obtained from patients who have had spontaneous abortion. The maximum per cent specific binding (%SB) in MT placenta for GDM was significantly lower (4.8%) compared with the FT placenta (22%, p < 0.001), while in the N group the maximum per cent specific binding for MT placenta was 14.1% compared with 26% for the FT placenta (p < 0.001). Binding data from FT placenta of well-controlled GDM patients were similar with the FT placenta from N group (22%SB for GDM VS 26% SB for N). Even as there were similarities in the binding characteristics of FT placentas from both groups the placental membrane protein content in the GDM group was lower by 50% compared with the N control (2.5 ± 0.11 VS 4.8 ± 0.15 mg protein/g placenta respectively, p < 0.001) suggesting that in the GDM group achieving a tight glycemic control could improve receptor affinities. Data from the competitive binding assay of GDM patients showed that the insulin necessary to achieve 50% inhibition (IDs0) was significantly lower in MT compared with the FT placenta (0.9 × 10⁻⁹ M VS 3.8 × 10⁻⁹ M, p < 0.001) but in the N placenta there was no alteration in the IDs0 of MT and FT placentas (3.1 × 10⁻⁹ M VS 4 × 10⁻⁹ M, p < 0.01, respectively). The present study demonstrated that in GDM the placental insulin receptor binding was significantly lower in spontaneously aborted placenta compared with placentas collected at full-term. Furthermore, these data suggest that the objective to achieve a tight glycemic control in GDM patients could optimize insulin receptor function similar to that of a normal pregnancy. Thus a full term placenta from GDM patients under a well managed glycemic control throughout the entire duration of pregnancy would result in an optimum insulin receptor function. (Mol Cell Biochem 151: 27–31, 1995)

Key words: insulin receptor, mid-term placenta, full-term placenta, gestational diabetes mellitus

Introduction

Gestational diabetes mellitus (GDM) is a well-defined condition that is characterized by carbohydrate intolerance of variable severity with onset or first recognition during the present pregnancy [1], which in some cases persists after the pregnancy or may have even predated this pregnancy. Available information suggests that GDM is associated with an increased perinatal mortality which can be averted by early recognition of the condition and by control of the hyperglycemia [2, 3].

Insulin receptor binding in GDM placentas has been the subject of various studies. A decrease in the percentage of ¹²⁵I-insulin receptor binding was found in placental membranes of GDM patients compared with normal control while the binding affinities were similar in both groups [4]. An improvement in insulin binding was demonstrated in placental membrane of well-controlled diabetic patients to an extent similar to a nondiabetic pregnant women [5]. On the other hand 50% reduction in insulin binding was observed in placentas of poorly controlled GDM patients [6]. Furthermore

Address for offprints: O.S. Al-Attas, Department of Biochemistry, College of Science, King Saud University, PO Box 2455, Riyadh 11451, Saudi Arabia
placental syncytiotrophoblasts from GDM patients under diet control alone expressed more insulin receptors in vitro [7]. These findings suggest a pathogenic role as one of the factors responsible for impaired insulin binding and insulin resistance in GDM. Insulin binding may however be optimized providing that adequate glycemic control is achieved. All previous studies were undertaken in GDM placentas collected at full term delivery. Hence the present study was conducted in order to assess insulin binding in placentas in their developmental stage and under hyperglycemia. To accomplish this insulin binding studies were done on placentas collected at mid-term pregnancy by spontaneous abortion, as well as in placentas of well controlled GDM collected at full term.

**Subjects and methods**

**Subjects**

Twenty seven pregnant women considered to be at high risk for developing GDM and 17 normal pregnant women (Table 1) with no previous history of GDM, attending the antenatal clinic at the Obstetric Unit of King Abdulaziz University Hospital and Otiqa Polyclinic, Riyadh, Saudi Arabia were included in this study. They were asked to undertake a 75 g oral glucose tolerance test (OGTT) and GDM was diagnosed according to World Health Organization (WHO) (8) criteria where either the fasting or 2 h blood glucose value of the OGTT exceeded 7.7 mmol/L and 11.1 mmol/L, respectively. Diagnosis for GDM was performed at the beginning of the second trimester (12–16 weeks gestation). Fourteen patients (14/27) were positively diagnosed with GDM and the rest were excluded from the study. These pregnant women with GDM were then placed on diet control consisting of 30-35 Kca/Kg ideal body weight [(body height in cm - 100) x 0.9]. All of the normal pregnant women were negative with the OGTT. None of the GDM patients or normal control pregnant were obese [Body Mass Index (BMI) >25] and had no endocrine disease and hypertension during the pregnancy. Informed consent from all patients and control subjects were obtained before they were allowed to participate in this study.

**Methods**

**Glycemic Control**: OGTT was performed in the morning after overnight fasting. Blood samples were collected in EDTA tubes and separated plasma were kept frozen at -20°C for use in glucose analysis in a glucose analyzer (Beckman Instruments Inc., Brea, California, USA); Glycosylated hemoglobin (HbAlc) was measured in whole blood by a microcolumn method (Helena Laboratories, Beaumont, Texas, USA) and insulin was measured by radioimmunoassay (CIS bio international, Cedex, France).

**Placental Investigations**: Only 8 placentas from the GDM patients and 8 placentas from normal control pregnant women were collected at full term delivery. Three of the GDM patients had spontaneous abortion at the 20–22 week of gestation and three pregnant women from the normal control group who had spontaneous abortion also on their 20–22 week of gestation were collected for use in this study. All these placentas collected during spontaneous abortion were labeled as mid-term placentas.

**Membrane Preparation**: Human mid-term and full-term placentas obtained after vaginal delivery were processed according to the method described by Posner [9] with modification as described elsewhere [4]. The final pellet containing microsomal membrane of syncytiotrophoblasts were stored at -70°C for further analysis.

**Marker enzyme analysis**: The marker enzyme 5'-nucleotidase (EC 3.1.3.5) activity was determined by measuring the liberation of adenosine from AMP [10]. Alkaline phosphatase (EC 3.1.3.1) was measured by the method of Bowers and McCombe [11]. Substrates were purchased from Sigma Chemicals (California, USA).

**Insulin receptor assay**: Insulin receptor analysis was performed by radioreceptor assay method as described elsewhere [12]. Briefly, placental plasma membranes (0.1 mg protein/tube) were incubated with 125I-insulin (approximately 20,000 cpm) in a final volume of 250 µl of binding buffer pH 7.8 containing 23 mMTris-HCL, 120 mMNaCl, 1.2 mM MgSO₄, 2.5 mM KCl 0.1% (w/v) Bovine Serum Albumin (BSA) and 1 mg Bacitracin. Non specific binding was determined as that radioactivity associated with the pellet obtained with a large excess of unlabelled insulin (8.5 x 10⁻⁵ M Actrapid Mono-