Sequential Studies of Glucose Tolerance and Red Blood Cell Insulin Receptors in Normal Human Pregnancy

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SUMMARY

Insulin binding to erythrocytes was sequentially studied in 12 healthy pregnant women during the anabolic (11–22 wk) and the catabolic (31–38 wk) gestational phases. For comparison, we studied 12 nonpregnant subjects at mid-luteal and mid-follicular menstrual phases. Oral glucose tolerance tests were also performed during these studies. There was a progressive worsening of the glucose tolerance from the anabolic to the catabolic phase associated with fasting hypoglycemia and hyperinsulinemia. The worsening of glucose tolerance was accompanied by a progressive increment of insulin secretion. Insulin binding to red blood cells increased progressively from the anabolic to the catabolic phase, due to an increased number of receptors per cell, associated with a reduction in the apparent affinity at the low occupancy levels. We concluded that the insulin resistance of pregnancy was not accompanied by an impaired binding of insulin to its receptors, at least in the RBC. The data suggest that the defect of insulin action lies at a site distal to the receptor.

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Pregnancy in normal women is accompanied by alterations in carbohydrate and lipid metabolism, persisting until parturition. In early gestation there is a tendency to fuel conservation by an increased maternal adipose tissue deposition (anabolic phase), whereas in late gestation, when fetal growth is predominant, there occurs an energetic depletion from maternal fat stores in order to maintain a continuous and adequate fuel flow throughout the placenta, necessary to fetal development (catabolic phase).[1,2] An increased insulin secretion, a tendency to develop fasting hypoglycemia, and a decreased tolerance to oral glucose are observed at this gestational catabolic period, suggesting an insulin-resistant state.[3–5] The factor(s) responsible for this decreased insulin action is (are) not perfectly understood, believed by some investigators to be related to hormones such as cortisol, progesterone, estrogens, and placental lactogen that are present in increased concentrations in plasma during late pregnancy.[6,7] The mechanism that mediates this altered sensitivity to insulin remains unknown, and studies on insulin receptors of circulating cells yielded conflicting results in insulin binding.

To evaluate the mechanism of the impaired insulin action observed during pregnancy, we prospectively studied a group of normal pregnant women in early and late gestation and compared the results to normal women in both phases of the menstrual cycle.

MATERIALS AND METHODS

Twelve healthy, pregnant women were studied, age 27.6 ± 5.1 yr (mean ± SD), height 158.0 ± 0.47 cm, during the anabolic and catabolic gestational phases and 12 normal women in luteal and follicular phases of the menstrual cycle. The composition of diet (30% fat, 50% carbohydrate, and 20% protein) was similar for all subjects with unrestricted total caloric intake. Body weights were in normal range for the controls according to the Metropolitan Insurance Company tables and pregnant weights were within the normal range according to the gestational weight gain slope described by Siqueira et al.[8] in the Brazilian, normal pregnant population. All subjects appeared to undertake equivalent physical activity, possibly reduced at the end of gestation.

Oral glucose tolerance tests (OGTT) were performed during the anabolic (wk 11–22) and catabolic (wk 31–37) gestational phases in the pregnant subjects and in the middle of the follicular (6 subjects) and luteal (6 subjects) menstrual phases of the nongravid controls. Blood samples were drawn, after an overnight fast, before starting the ingestion of 100 g of glucose as a 25% solution for glucose, for immunoreactive insulin (IRI), reticulocytes, prolactin (PRL), placental lactogen (HPL), estradiol (E_2), and progesterone (P) measurements (PRL and HPL only in the pregnant

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TABLE 1
Clinical data of pregnant women

Patient	Age (yr)	Height (m)	Weight (kg) Gest. phase 1	Weight (kg) Gest. phase 2	% IBW Gest. phase 1	% IBW Gest. phase 2	Weeks of pregnancy Gest. phase 1	Weeks of pregnancy Gest. phase 2	Reticulocytes (%) Gest. phase 1	Reticulocytes (%) Gest. phase 2
1	30	1.52	50.3	57.8	96	101	21	35	0.7	1.1
2	26	1.61	57.6	65.5	106	106	19	35	0.5	0.8
3	28	1.59	54.4	60.2	98	98	20	38	0.6	0.9
4	22	1.61	56.1	61.3	98	101	21	32	0.8	1.1
5	35	1.60	61.1	66.3	110	109	19	34	0.5	0.5
6	31	1.50	46.7	53.9	94	99	18	32	1.9	2.0
7	34	1.55	56.2	61.0	104	105	22	34	0.5	0.6
8	20	1.54	51.4	58.4	99	104	17	33	1.6	1.2
9	26	1.61	56.2	64.1	102	103	16	37	1.6	1.9
10	33	1.66	60.8	66.7	106	104	14	33	1.1	0.7
11	24	1.60	53.5	60.7	100	102	11	31	1.2	1.7
12	22	1.54	49.8	56.0	99	98	12	33	1.5	2.0
Mean	27.6	1.58	54.7	61.0	101.0	103.3	17.5	33.9	0.96	1.13
SD	5.1	0.47	4.6	4.1	4.7	3.3	3.6	2.1	0.49	0.52

women) and insulin binding to erythrocytes, and at 30, 60, 90, and 120 min afterward for glucose and IRI determinations.

Analytic procedures. Glucose concentrations in whole blood were determined by the Hoffman method.[9] Plasma IRI, PRL, and HPL were measured by standardized radioimmunoassays using ^{125}I-labeled hormones as tracers. Plasma progesterone and estradiol were quantified by radioimmunoassays using specific antibodies and H^3-steroids as tracers. Reticulocytes were counted in blood according to the technique described by Dacie et al.[10]

The total integrated areas circumscribed by the glucose and insulin response curves during OGTT, above or below fasting baseline level, were estimated on an Olivetti desk computer, employing a trapezoidal rule.[11] The data are expressed as mg · min/dl for glucose and μU · min/ml for insulin.

Cell preparation and insulin binding studies. Ten milliliters of venous blood was drawn into heparinized tubes and after centrifugation red cells were isolated by the technique of Boyum.[12] Isolated cells were incubated 90 min with 100 pg/ml of mono-^{125}I-(tyrosine A14)-porcine insulin, provided by Novo Research Institute, and unlabeled porcine insulin over a range of concentrations from 0.1 to 10^5 ng/ml according to the technique previously described.[13]

The data are presented in the following ways. (1) Competition-inhibition curves: in this presentation, the percentage of total radioactivity specifically bound to the cell pellet is graphically expressed as a function of the total insulin concentration. (2) Scatchard plot: the ratio of the bound-to-free ^{125}I-insulin (B/F) is plotted as a function of bound insulin (B) for each individual. The intercept of prolongation of the curves with the abscissa determines the maximal capacity for insulin binding (R_o), from which the total number of receptors per cell (N) can be calculated by the formula:

$$N = \frac{R_o \times 6.02 \times 10^{23}}{\text{Cell concentration/L}}$$

Although the explanation of the curvilinear shape of this plot remains debatable, we arbitrarily decided to describe it

TABLE 2
Plasma hormone concentrations of pregnant women

Patient	PRL (ng/ml) Gest. phase 1	PRL (ng/ml) Gest. phase 2	HPL (μg/ml) Gest. phase 1	HPL (μg/ml) Gest. phase 2	Progest. (ng/ml) Gest. phase 1	Progest. (ng/ml) Gest. phase 2	E_2 (ng/ml) Gest. phase 1	E_2 (ng/ml) Gest. phase 2
1	47	86	2.9	8.6	85	247	5.2	9.2
2	76	101	3.0	7.4	78	137	3.8	10.6
3	35	125	3.6	9.4	59	141	3.7	12.8
4	39	160	3.4	7.6	83	135	5.8	8.8
5	88	178	1.6	6.0	55	153	2.9	9.9
6	105	222	2.5	7.2	50	119	4.4	8.7
7	121	198	3.8	10.0	49	217	3.6	9.7
8	103	260	1.5	9.0	28	88	3.2	11.0
9	29	82	1.4	9.4	37	99	4.2	10.2
10	50	145	2.2	10.5	35	167	1.5	10.1
11	42	171	0.2	7.0	17	81	1.7	7.5
12	76	155	0.7	6.2	25	120	2.0	8.3
Mean	69.3	157.0	2.2	8.4	50.1	142.7	3.50	9.75
SD	30.2	54.0	1.1	1.5	23.0	57.0	1.33	1.38

TABLE 3
Clinical and laboratory data in the normal control subjects

Patient	Age (yr)	Weight (kg)	Height (m)	% IBW*	E_2 (pg/ml) FP	E_2 (pg/ml) LP	Progest. (ng/ml) FP	Progest. (ng/ml) LP	Reticulocytes (%) FP	Reticulocytes (%) LP
1	33	57.0	1.60	108	50.1	116	0.8	8.3	0.6	0.6
2	34	47.5	1.52	98	50.1	155	0.3	6.3	1.3	1.5
3	26	50.0	1.54	101	50.9	215	0.8	6.0	0.5	0.5
4	22	49.0	1.59	94	47.8	184	0.7	3.4	1.0	0.6
5	31	58.5	1.55	115	42.3	168	0.7	3.0	0.7	0.5
6	22	64.0	1.72	105	57.2	166	0.8	4.0	0.9	0.9
7	34	49.8	1.61	93	41.5	204	0.4	3.0	0.4	0.5
8	29	58.5	1.70	78	38.4	140	0.8	10.4	0.5	0.6
9	32	47.5	1.50	101	71.5	150	0.9	4.7	0.7	0.9
10	29	52.0	1.59	100	87.0	150	0.8	12.5	0.5	0.7
11	22	50.5	1.60	96	53.0	121	0.5	4.3	1.4	2.0
12	39	57.8	1.60	110	60.3	142	0.3	4.3	0.7	0.8
Mean	29.4	53.5	1.59	101.6	54.1	159.3	0.65	5.86	0.77	0.84
SD	5.5	5.4	0.65	6.7	13.7	30.2	0.22	3.05	0.32	0.46

*Ideal body weight according to Metropolitan Life Insurance tables.
FP, follicular phase; and LP, luteal phase.

in terms of a negative cooperative model.[14] In this way, we constructed the average affinity profile as proposed by DeMeyts and Roth.[14,15] The high-affinity state at low levels of receptor occupancy is designated K_e. The low-affinity state at high levels of occupancy is designated K_f. The level of receptor occupancy at which the affinities start declining is called Y_e, whereas the levels of occupancy at which the affinity constants attain K_f is designated Y_f.

Student's *t*-test was used to evaluate differences between means. The comparison of the glucose tolerance tests between the groups of subjects was done by applying analysis of variance (ANOVA) with one-, two-, and three-way classification.[16]

RESULTS

Clinical and hormonal data. Tables 1 and 2 present the clinical and hormonal data from the pregnant women. As expected, a significant increase in the plasma placental hormone concentrations was observed in late gestation in comparison with early pregnancy.

The 12 normal subjects were of age 29.4 ± 5.5 yr (mean ± SD), of height 1.59 ± 0.65 cm, and of weight 53.5 ± 5.4 kg (body weights were within 10% of ideal body weight: 101.6 ± 6.7%). All women had normal menstrual cycles, confirmed by appropriate hormonal changes. The blood was collected for insulin receptors in both the first and second halves of the cycle, corresponding to the midfollicular and midluteal phases, with estradiol (E_2) values of 54.1 ± 13.7 and 159.3 ± 30.2 pg/ml and progesterone (P) levels of 0.65 ± 0.22 and 5.86 ± 3.05 ng/ml, respectively, in relation to the midcycle day. The reticulocyte count ranged from 0.4% to 2.0%, within the normal range (Table 3).

OGTTs. Figure 1 shows the glucose tolerance curves of the two gestational phases and the mean curve of the nongravid controls, obtained by pooling the results from both menstrual phases (six in each phase), since no significant differences were noticed at any times of sampling. To compare the means of the blood glucose values at the different times of sampling between the control and pregnant subjects' data, we used

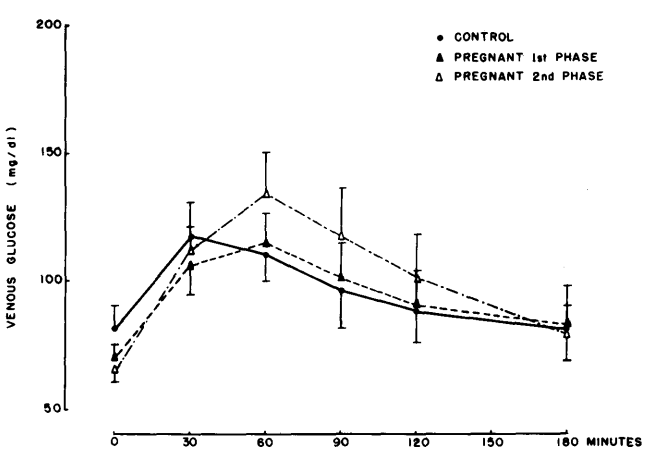

FIGURE 1. Blood glucose levels during a 100-g OGTT in 12 normal women and 12 healthy pregnant women during the first and second phases of gestation. Bars denote mean ± SD.

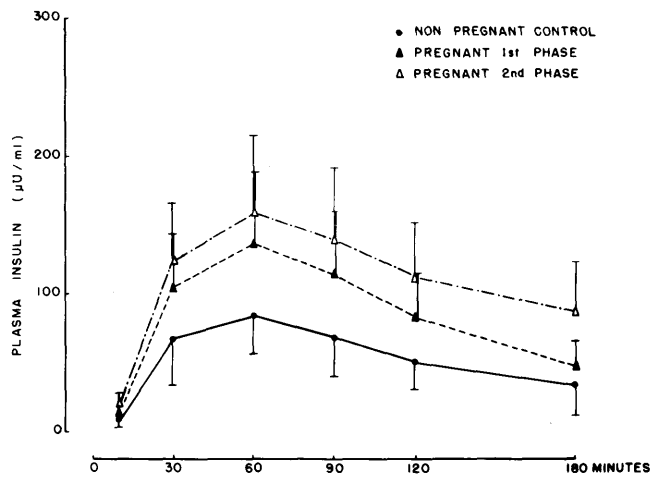

FIGURE 2. Plasma insulin levels during a 100-g OGTT in 12 normal women and 12 healthy pregnant women during the first and second phases of gestation. Points and bars denote mean ± SD.

TABLE 4
One-way ANOVA for comparison of the blood glucose and plasma insulin levels between control and pregnant subjects

Time (min)	Blood glucose				Plasma insulin			
	Controls vs. 1st phase preg.		Controls vs. 2nd phase preg.		Controls vs. 1st phase preg.		Controls vs. 2nd phase preg.	
	F*	P†	F	P	F	P	F	P
0	20.18	0.00018	48.27	0.00001	6.49	0.02	16.56	0.0005
30	6.18	0.022	1.56	NS	5.16	0.03	14.52	0.001
60	0.92	NS	16.80	0.0005	5.26	0.03	12.41	0.002
90	0.69	NS	9.25	0.006	8.20	0.009	17.05	0.0004
120	0.54	NS	6.25	0.02	10.19	0.004	24.00	0.00007
180	0.10	NS	0.05	NS	2.76	NS	18.47	0.0003
Areas 0–180 (min)	9.98	0.005	42.87	0.00005	7.85	0.011	16.09	0.0006

*F, variance ratio; and †P, probability.

one-way analysis of variance (ANOVA), showing that there were only significant differences between controls and first phase of pregnancy at fasting and 30 min, while between controls and the catabolic pregnant period there were significant differences in the blood glucose levels at fasting, 60, and 90 min after the glucose load. In relation to the insulin concentrations, there were significant differences between controls and first phase of pregnancy at all times of sampling except at 180 min (Figure 2). When the second phase of pregnancy and control subjects were compared, the mean insulin levels were significantly higher at all times of sampling during the glucose tolerance test (Table 4). To compare the mean blood glucose levels between the first and second phases of pregnancy, we used the two-way (no replicates) ANOVA, since the two groups were related, evaluating subjects and time of sampling effects. Regarding glucose values, they were significantly higher at all times of sampling in the catabolic phase except at 30 and 180 min. In the same way, the insulin levels were higher in the second phase of pregnancy at all times of sampling except at 60 min (Table 5). The three-way ANOVA used to compare the pregnant subjects, considering patients, times of sampling, and phases of pregnancy, showed that there was a significant difference in blood glucose ($F = 15.67$, $P = 0.00013$) and plasma insulin levels ($F = 29.50$, $P = 0.000001$) between the phases of pregnancy.

TABLE 5
Two-way ANOVA for comparison of the blood glucose and plasma insulin levels between first and second phases of pregnancy

Time (min)	Blood glucose		Plasma insulin	
	F*	P†	F	P
0	23.22	0.0005	20.12	0.0009
30	3.98	NS	5.44	0.04
60	52.83	0.00002	3.83	NS
90	13.00	0.004	6.57	0.026
120	6.58	0.03	9.99	0.009
180	0.50	NS	19.24	0.001
Areas 0–180 (min)	34.03	0.0001	10.03	0.009

*F, variance ratio; and †P, probability.

Figure 3 shows the blood glucose and plasma IRI incremental areas from baseline fasting levels up to 180 min of the testing period. One-way ANOVA indicated statistical differences of glucose areas between controls and first and second phases of pregnancy. The same was found when insulin areas were compared. The two-way ANOVA for comparison of the two phases of pregnancy showed significantly greater glucose and insulin areas in the second when compared with the first phase of pregnancy.

There were significant positive correlations between E_2 ($r = 0.62$, $P < 0.01$), P ($r = 0.73$, $P < 0.01$), HPL ($r = 0.57$, $P < 0.01$), and PRL ($r = 0.50$, $P < 0.05$) plasma concentrations and the glucose areas. However, no significant correlation between these hormone concentrations and the insulin areas was observed.

Insulin receptor studies. The mean insulin specific binding curves at progressive insulin concentrations in pregnant and control subjects are shown in Figure 4. Insulin binding at tracer concentrations was significantly elevated during the

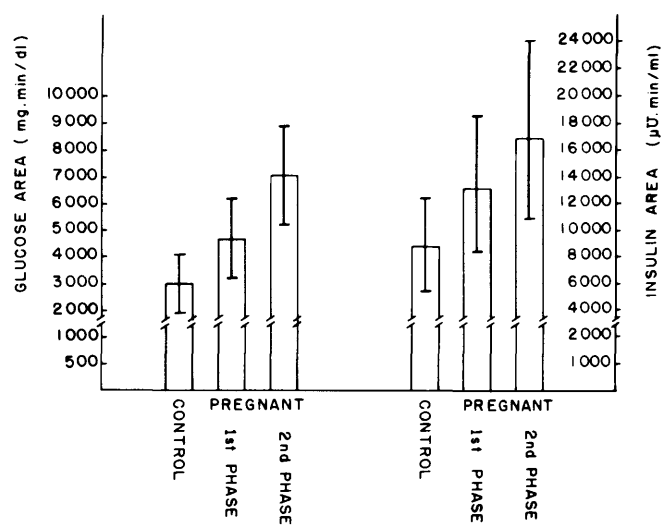

FIGURE 3. Incremental total glucose and insulin areas during the OGTT in 12 normal women and 12 healthy pregnant women during the first and second phases of gestation. Columns and bars indicate the mean values ± SD.

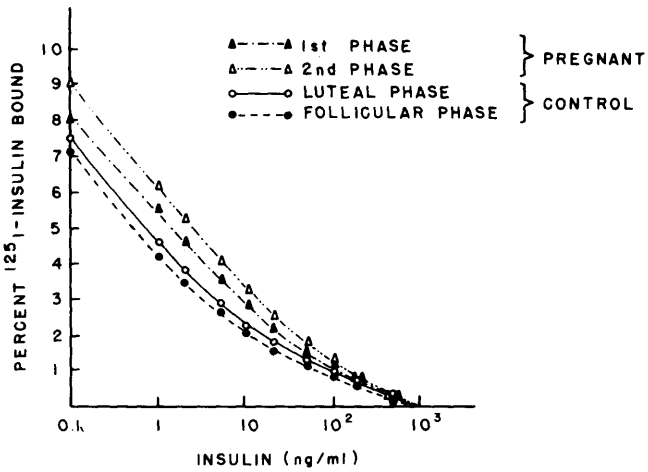

FIGURE 4. Specific binding of ^{125}I-insulin to RBC plotted as a function of insulin concentration. Mean values for 12 normal women at midfollicular and midluteal phases and for 12 normal healthy pregnant women at the first and second phases of gestation are indicated.

catabolic when compared with the anabolic phase of gestation and in controls in both menstrual phases (Table 6). However, insulin binding to erythrocytes was not statistically different when the anabolic phase was compared with the follicular and luteal phases of the controls, or when the two menstrual phases of the normal controls were compared. Mean Scatchard plots of the binding data are shown in Figure 5. The calculated receptor number per cell was significantly higher during the catabolic phase in comparison to the anabolic phase and controls.

The mean affinity profiles are shown in Figure 6. Affinity constants at the low levels of occupancy (K_e) were significantly lower in the catabolic phase than in the anabolic phase, and were also lower when luteal phase values were compared with the follicular values. The values in the luteal phase were also significantly lower than in the anabolic phase of pregnancy, but similar to those observed in the catabolic phase. Affinity constants at the high levels of occupancy and the K_f/K_e ratios were not statistically different in all studied groups.

A positive and significant correlation was observed between the insulin binding to erythrocytes at tracer concentrations (B_o) and the plasma concentrations of E_2 ($r = 0.56$, $P < 0.01$), P ($r = 0.46$, $P < 0.05$), and HPL ($r = 0.57$, $P < 0.01$) during the progression of pregnancy. Similarly, a positive significant correlation was seen between the number of receptors per cell (N) and these hormone levels (E_2: $r = 0.63$, $P < 0.01$; P: $r = 0.050$, $P < 0.05$; and HPL: $r = 0.66$, $P < 0.01$). A significant correlation was also observed between N and PRL concentrations, except when these hormone concentrations were correlated to B_o. No significant correlation was observed between B_o and basal plasma insulin concentrations.

DISCUSSION

Alterations in maternal carbohydrate metabolism during pregnancy, as mentioned in the literature, were also observed in our pregnant subjects: a reduction of plasma glucose and increased insulin concentration during fasting, elevated glucose levels, and a marked increased insulin secretion after the oral glucose load.

While the blood glucose curves were similar in the control and the pregnant subjects at the anabolic phase during OGTT, except at some times of sampling (particularly at fasting) but with significantly higher mean blood glucose levels during the second phase of pregnancy from 60 to 120 min, the incremental areas, which are strongly influenced by the lower fasting glucose levels in pregnancy, were significantly higher during pregnancy, and still greater in the catabolic than in the anabolic gestational phase. However, from a physiologic standpoint, it can be stated that glucose tolerances were similar. The glucose areas correlated significantly with the measured plasma hormones (E_2, P, HPL, and PRL); however, this correlation was not found with corresponding insulin areas. This discrepancy could be related to the wider range of values of the insulin, as opposed to glucose, areas and is therefore more a statistical anomaly than a biologically meaningful observation.

The increased insulin secretion associated with normal or

TABLE 6
Parameters of ^{125}I-insulin binding to erythrocytes in pregnant women and controls (mean ± SD)

Group	%B_o	N	R_o*	K_e (10^8 M^{-1})	K_f (10^8 M^{-1})	Y_e (%)	Y_f (%)	K_f/K_e	Reticulocytes (%)
Pregnant 1st phase	8.13 ± 0.66	63.2 ± 15.6	2.83 ± 0.70	1.92 ± 0.18	0.37 ± 0.13	0.29 ± 0.03	51.2 ± 12.0	0.19 ± 0.07	0.96 ± 0.49
Pregnant 2nd phase	8.80 ± 0.76	73.3 ± 4.0	3.29 ± 0.18	1.80 ± 0.18	0.35 ± 0.06	0.27 ± 0.03	50.8 ± 7.8	0.19 ± 0.03	1.13 ± 0.52
Control F. phase	7.22 ± 1.74	56.0 ± 11.3	2.51 ± 0.51	1.97 ± 0.41	0.30 ± 0.06	0.29 ± 0.05	44.7 ± 7.0	0.16 ± 0.04	0.77 ± 0.32
Control L. phase	7.48 ± 1.71	64.0 ± 12.6	2.87 ± 0.56	1.62 ± 0.27	0.31 ± 0.09	0.26 ± 0.04	43.2 ± 7.8	0.19 ± 0.07	0.84 ± 0.46
Statist. signif.	§‖ ¶	§‖ ¶#	§‖ ¶#	‡§ #	—	§#	—	—	—

*Maximal insulin binding capacity (ng/ml).
‡Difference statistically significant at 5% level between anabolic and control luteal phase.
§Difference statistically significant at 5% level between anabolic and catabolic phase.
‖Difference statistically significant at 5% level between catabolic and control follicular phase.
¶Difference statistically significant at 5% level between catabolic and control luteal phase.
#Difference statistically significant at 5% level between control follicular and luteal phase.

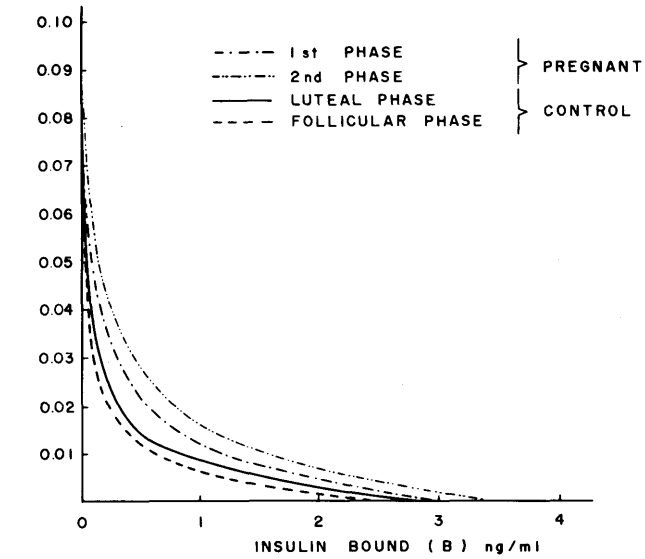

FIGURE 5. Scatchard plot of the insulin binding data shown in Figure 4. Values shown are means.

greater-than-normal glucose levels after the glucose load in pregnancy, when compared with the normal controls, is suggestive of a maternal insulin-resistant state. Rushakoff and Kalkhoff[17] observed, in rat hind limb perfusion studies, a decreased glucose uptake in response to increased insulin concentrations in late gestation, demonstrating a reduced skeletal muscle sensitivity to insulin action. This could explain the greater plasma glucose excursions after feeding and would assure availability of this substrate, rather than the continuous drainage of this nutrient, to the rapidly growing conceptus throughout the placenta. The reduced sensitivity to insulin was not apparently related to the increased plasma progesterone and estradiol levels, since metabolic alterations similar to those found in pregnancy were not observed after P and E_2 benzoate injections in normal female rats.[17] Leturque et al.'s in vivo experiments on insulin effect on glucose kinetics in pregnant rats confirmed this decreased sensitivity to insulin in late gestation, showing that this resistance involves both glucose-producing (liver) and glucose-utilizing tissues.[18]

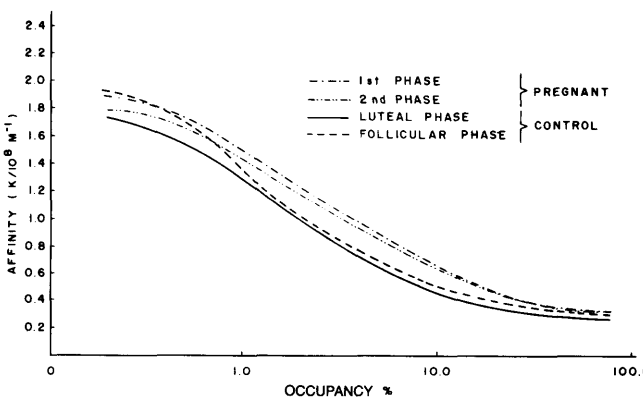

FIGURE 6. Affinity profiles of the insulin binding data shown in Figure 4. The average affinities are plotted against the receptor occupancy. Values shown are means.

Several pathologic, resistant states have been demonstrated to be associated with a decreased insulin binding to its specific receptors. However, the evaluation of the effect of pregnancy on insulin receptors has not shown uniform results, as indicated in Table 7, which summarizes the published data on the insulin receptor in circulating cells. In our study, we observed a progressive increase in insulin binding to RBC from the anabolic to the catabolic state due to an increased number of receptor sites/cell with a proportionate decrease in apparent receptor affinity at low occupancy levels (K_e). As affinity constant ratios (K_f/K_e) were not statistically different when all groups were compared, the proportional alterations in K_e and K_f, without any change in their ratios, suggested that no primary changes in receptor affinity occurred.[19]

Since a trend toward higher reticulocyte counts was observed from early to late pregnancy (Table 6), while not statistically significant, it could be a factor for the significantly higher insulin binding found in the catabolic phase. It has been shown that the younger the RBC population, with greater reticulocyte count being one index of RBC age, the more insulin-binding sites are available.[20]

A positive relation between insulin binding to receptors or receptor number per cell and PRL, P, and E_2 concentrations was observed during pregnancy. This observation does not allow us, however, to conclude that a cause-and-effect relationship exists. As suggested by the rat hind limb perfusion studies, as pregnancy[17] progresses, the increase in placental hormones is accompanied by the progressive increase in the factor(s) responsible for the development of the maternal insulin-resistant state that allows adequate substrate flow to the conceptus.

Furthermore, we were unable, as were Moore et al.,[21] to find any correlation between the circulating plasma insulin level and the degree of insulin binding. This is markedly different from a variety of other situations of insulin resistance in which a strong inverse correlation has been observed between plasma insulin levels and insulin binding.[22] Since pregnant women have higher circulating insulin levels than do controls, this suggests that some factor(s) exists during pregnancy that modulates insulin receptors in a positive manner, preventing a fall in insulin binding.[21]

We cannot associate the insulin-resistant state of pregnancy with a reduction in insulin binding to the cells, at least when circulating cells reflecting target tissues are used, since a reduction in insulin binding during pregnancy was not consistently demonstrated. (In fact, *increased* insulin binding to the receptor is frequently observed.) Rather, this insulin-re-

TABLE 7
Summary of the insulin receptor studies in circulating cells during normal human pregnancy

Authors	Cell system	Gestation phase	B_o
Neufeld et al., 1979[25]	Monocytes	Late gestation	↑
Beck-Nielsen et al., 1979[26]	Monocytes	3rd trimester	↓
Soman et al., 1979[27]	Monocytes	3rd trimester	↑
Moore et al., 1981[21]	Erythrocytes	3rd trimester	N/↑
Tsibris et al., 1980[28]	Monocytes	Late gestation	N
	Erythrocytes	Late gestation	N
Puavilai et al., 1982[29]	Monocytes	3rd trimester	↑

sistant state is probably related to a postreceptor abnormality.

The mechanism whereby the postreceptor defect is associated with enhanced insulin binding is not known, probably being related to the slightly increased levels of FFA and β-hydroxybutyrate in the fasting state during the catabolic phase[2,5] with their well-known blocking effect on insulin action,[23] probably at a postreceptor locus. Increased levels of FFA would induce a state of insulin resistance, inferred from the findings that increments in plasma glucose after oral glucose load in late pregnancy are correlated with basal level of plasma FFA[5] at the time of glucose administration. This postreceptor defect could lead to a compensatory increase in receptor concentration independent of the RBC age, since such independence was described in monocytes. Furthermore, it has been demonstrated that, in the presence of β-hydroxybutyrate, there is an increase in insulin binding to cultured human lymphocytes.[24] Such a situation is not observed in the anabolic phase, in which the insulin resistance is of a mild degree, eventually related to the development of the increase in fat stores.[5] In effect, the insulin receptor binding characteristics in this phase were no different than those in the nonpregnant state either at the follicular or luteal phases.

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