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Insulin-Receptor Interactions and Metabolic Effects of Essential Phospholipids in Pregnant Women with Diabetes Mellitus

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Insulin resistance in pregnant women with diabetes mellitus is shown to result from impaired sensitivity of their cells to threshold physiological and submaximal insulin doses. Maximal insulin doses lead to normalization of glucose utilization by the cells. However, high doses cause activation of lipid peroxidation, structural and functional alterations in cell membranes, and depressed function of insulin receptors. The use of essential phospholipids in women receiving insulin therapy promotes targeted modulation of insulin-binding receptor activity, reduced free-radical processes in cell membrane lipids, and improved metabolism in the cells.

Key Words: *insulin receptor; essential phospholipids; antioxidant activity; membrane modulator*

Published information on insulin-receptor interactions in patients with insulin-dependent diabetes mellitus (IDDM) is contradictory [7,11,12]. The rate of insulin binding by tissue cell receptors is variable and correlates inversely with levels of both endogenous and exogenous insulin [14]. Comparative studies of insulin-receptor interactions in erythrocytes, monocytes, and insulin target cells showed that although insulin binding to monocyte and erythrocyte receptors was normal, it was below normal in certain other types of living cells. This phenomenon of increased insulinization observed in peripheral tissues, notably fatty tissue, may be due to high levels of exogenous insulin in such tissues and its overwhelming influence on the insulin receptors. Blood cells can adapt to higher insulin concentrations so that administration of exogenous insulin does not appreciably alter the equilibrium between the levels of endogenous and exogenous insulin binding to receptors in these cells.

In pregnancy, glucose tolerance has been shown to be lowered despite a continual rise of the insulin concentration in the circulation [6]. The insulin dose administered to pregnant women with diabetes mellitus varies during the course of pregnancy. Patients with IDDM exhibit insulin resistance resulting from structural and functional abnormalities in cell membranes and altered insulin-receptor interactions in the cells [3-5].

In the present study, we focused our attention on insulin-receptor interactions in blood cells of pregnant women with IDDM and on the metabolic effects of essential phospholipids in such women.

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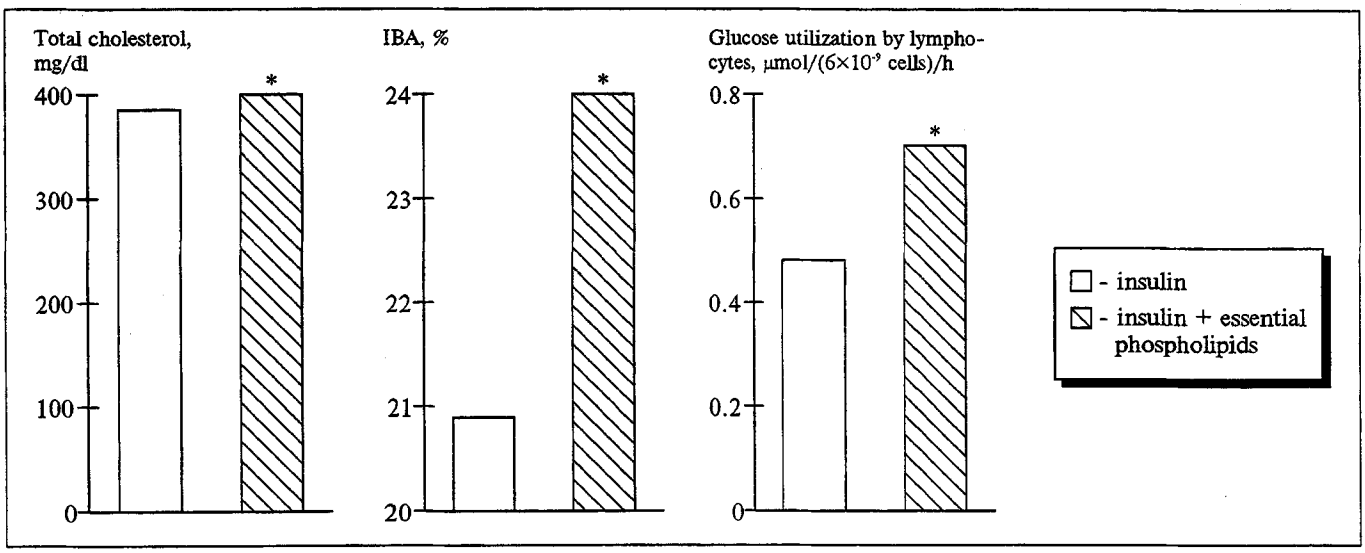

Fig. 1. Effect of essential phospholipids, administered to pregnant women with diabetes mellitus undergoing insulin therapy, on total serum insulin and IBA of lymphocytes. Here and in Fig. 2: the asterisk indicates a significant difference at $p<0.05$ from the group of diabetic women not treated with essential phospholipids.

MATERIALS AND METHODS

Insulin-receptor interactions were studied in 90 women with IDDM in the third trimester of pregnancy. The patients ranged in age from 18 to 40 years and had been suffering from diabetes for 5 to 16 years. The daily dose of insulin they received was 72 units on average. By the time of the study, most of the patients had attained compensation, as was confirmed by normoglycemia (both in the fasting state and postprandially throughout the day) and by the absence of ketoacidosis and glucosuria. The control group comprised 20 healthy women in the third trimester of pregnancy.

Insulin concentration was measured using radioimmunoassay kits. The number of insulin receptors on erythrocyte plasma membranes was determined by the method we described earlier [4]. ^{125}I-insulin binding to erythrocyte plasma membrane receptors was assessed by the method of Meyts and Roth [9] using a technique in which ^{125}I-insulin is displaced from its complex with receptors by increasing amounts of unlabeled insulin under equilibrium conditions [8]. The total number of insulin-binding sites and receptor affinity for the hormone were also estimated by previously described methods [9,12]. The sensitivity of cells to insulin was evaluated from the degree of glucose utilization by the cells. The rate of lipid peroxidation (LPO) in isolated erythrocyte plasma membranes was estimated from the levels of hydroperoxides [1] and malonic dialdehyde (MDA) using spectrophotometry with thiobarbituric acid [10]; the antioxidant activity of blood samples was determined as described previously [2]. Structural and functional properties of erythrocyte membranes were evaluated by changes in their levels of MDA, hydroperoxides, and antioxidant activity and also by measuring the concentration of thiol groups in these membranes and their microviscosity and hy-

TABLE 1. Parameters of Insulin–Receptor Interaction in Mononuclear Cells from Pregnant Women with Diabetes Mellitus

Group	% of bound ^{125}I-insulin relative to total ^{125}I-insulin		Receptor concentration		
	maximal specific binding	nonspecific binding	insulin receptors, nmol/ml	bound receptors, 10^7 M	free receptors, 10^7 M
Control group	29.3±2.9	3.2±0.9	0.55±0.07	18.0±2.3	5.1±0.9
Test group 1:	30.4±3.4	7.4±0.9	0.53±0.1	31.0±4.9	8.7±1.2
p_1	>0.05	<0.01	>0.1	<0.01	<0.05
p_2	<0.05	<0.01	<0.05	<0.01	<0.05
Test group 2:	13.9±1.8	3.3±1.09	0.16±0.03	16.3±1.1	5.9±0.4
p_1	<0.01	>0.1	<0.001	>0.1	>0.1
p_2	<0.01	<0.05	<0.05	<0.01	<0.05

Note. p_1: difference from the control group; p_2: difference between test groups 1 (patients with increased ^{125}I–insulin binding) and 2 (those with decreased binding).

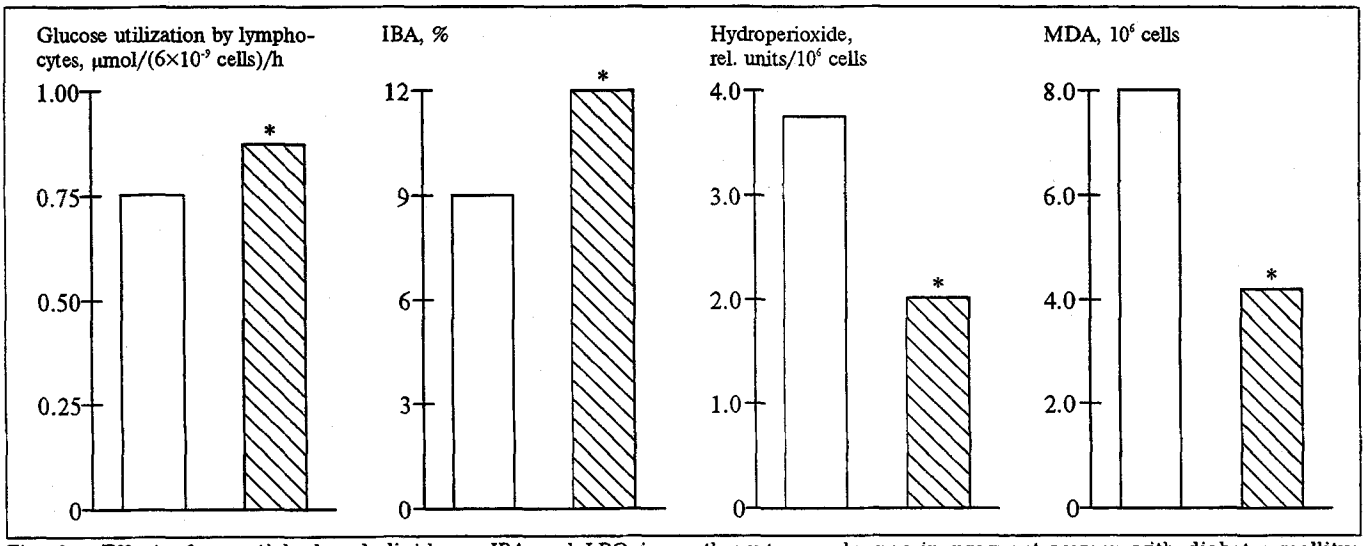

Fig. 2. Effect of essential phospholipids on IBA and LPO in erythrocyte membranes in pregnant women with diabetes mellitus undergoing insulin therapy.

drophobicity by the method of electron paramagnetic resonance (EPR) on an E-4 radiospectrometer (Varian) using spin probes.

RESULTS

The specific insulin binding by erythrocytes from pregnant diabetic women was significantly less than in healthy individuals because of decreased concentrations of receptors on these cells. Evaluation of insulin-receptor interactions showed that insulin binding to erythrocyte receptors in women with compensated IDDM in the third trimester of pregnancy was significantly reduced as compared to their healthy counterparts. The reduced binding appears to have been mainly due to lower numbers of receptors on erythrocytes rather than to reduced receptor affinity for insulin.

In vitro examination of dose-response relationships revealed that when insulin was used in physiological doses, erythrocytes from women with IDDM utilized less glucose than did those from healthy controls. In hyperinsulinemia, insulin concentrations in the samples increased fourfold while the rate of glucose uptake by erythrocytes approached normal levels. This indicates that pregnant women with long-standing IDDM develop insulin resistance, manifested in a lowered sensitivity to threshold physiological insulin doses and in a normal response to maximal insulin concentrations. This defect was expressed only at the level of insulin binding by erythrocyte plasma membrane receptors. Further evidence for insulin resistance in most IDDM patients was provided by the observation that the therapeutic dose of insulin for them far exceeded the level of its physiological secretion (30-40 units per day). Insulin resistance in pregnant IDDM women is therefore secondary and depends on the state of the insulin receptors and the insulin dose.

In vitro tests of various insulin doses for their impact on the rate of free-radical oxidation and the activity of endogenous antioxidants revealed intensified LPO and depressed antioxidant activity in blood serum samples from all IDDM patients as compared to those from normal subjects. For erythrocyte membranes, in contrast, no significant differences in antioxidant activity were found between IDDM patients and controls. After a 2-hour incubation with insulin in a concentration of 20 or 80 pg/ml, erythrocytes from IDDM women contained MDA and hydroperoxides at levels that differed only slightly from their levels in similarly incubated erythrocytes of healthy controls. When erythrocytes from IDDM patients were exposed to a much higher insulin concentration (1000 pg/ml), LPO increased markedly (as indicated by sharp rises in MDA and hydroperoxide concentrations; $p<0.05$) while the level of antioxidant activity remained unchanged, i.e., insulin in high concentrations exerted a prooxidant effect. On the other hand, in high concentrations (80 and 1000 pg/ml) insulin increased glucose utilization by erythrocytes, and a close correlation was found to exist between antioxidant activity and glucose uptake rate at these insulin levels ($r=0.65$ and $r=0.76$, respectively). This suggests that the activity of antioxidant enzymes was insufficiently high when LPO was enhanced under the influence of a high insulin concentration.

Following LPO activation, erythrocyte membranes failed to function adequately despite a com-

pensatory rise in the concentration of lipid phosphorus in IDDM (3.82±0.19 mmol/10^6 cells *vs.* 2.67±0.11 mmol/10^6 cells in control samples; $p<0.001$), as was evidenced by the reduced sensitivity of erythrocytes to insulin and the low concentration of their insulin receptors.

Similar *in vitro* tests staged with washed mononuclear cells from IDDM patients showed significantly increased glucose uptake and specific insulin binding by these cells in response to high insulin concentrations ($p<0.05$), indicating that the degree of glucose utilization by mononuclears and their receptor activity were directly related to the insulin dosage ($r=0.78$, $p<0.05$). Succinate dehydrogenase activity in lymphocytes from IDDM women was only half that in the control samples ($p<0.01$) - an indication of reduced efficiency of the glycerophosphate shunt and of severe disturbances in the energy-producing system of the cell. When insulin was added to the *in vitro* system in a large concentration, succinate dehydrogenase activity in lymphocytes rose and their oxidative metabolism was restored.

These *in vitro* tests show that the defect in IDDM is not confined to the level of insulin binding by cell receptors but extends to the postreceptor level, i.e., to that of mitochondrial enzymes, since lowered succinate dehydrogenase activity reflects an impaired energy-producing function of the mitochondria.

According to the degree of ^{125}I-insulin binding by mononuclears, the IDDM patients tested for this binding could be divided into two groups - those with increased (near-normal) binding (group 1, $n=19$) and those with decreased binding (group 2, $n=17$). Parameters of insulin-receptor interactions, including maximal specific binding of ^{125}I-insulin, calculated for these groups are given in Table 1. It follows from this table that the reduced binding in group 2 is determined by the number of insulin receptors and the higher binding in group 1 is determined by the insulin affinity of free and bound receptors. Worthy of note is the significantly higher nonspecific binding of labeled hormone in group 2; this may be attributed to altered properties of plasma membranes, which can affect the affinity of insulin receptors.

The near-normal maximal specific binding in group 1 suggests that insulin therapy may normalize the activity of insulin receptors in patients whose receptors have a relatively high affinity for insulin unless their numbers are too low initially.

Impaired insulin receptor activity in patients with low receptor numbers appears to play a greater role in the pathogenesis of IDDM than in patients whose receptors have an increased affinity for insulin, which may be a consequence of metabolic disturbances due to insulin deficiency. The results of this study point to a heterogeneity of IDDM in pregnant women.

As the population of mononuclears is heterogeneous and different types of these cells show unequal capacities for insulin binding, we also tested the activity of insulin receptors in T lymphocytes in some of the patients. The tests showed that these patients could be divided into two contrasting groups in terms of the ^{125}I-insulin-binding activity (IBA) of their T cells - high (36.82±1.31% [range 32.5-43.4%]; $p<0.01$) or low (9.86±1.44% [range 3.3-13.0%]; $p<0.01$). Pregnant women with a low IBA of their T cells were receiving insulin in high doses, and this probably accounts for insulin resistance in decompensated patients and for insulin excess in compensated ones.

The detected differences in IBA between mononuclears and T lymphocytes (Table 2) appear to be associated both with the heterogeneity of the studied population and with the multifactorial influences of hormonal and metabolic alterations caused by pregnancy on the one hand and diabetes mellitus on the other.

Essential phospholipids were administered in combination with insulin to 46 women with IDDM and severe hyperlipidemia; 25 of them were treated

TABLE 2. Insulin-Binding Activity of Mononuclear Cells and T Lymphocytes from Pregnant Women with Compensated and Decompensated Insulin-Dependent Diabetes Mellitus (IDDM)

Group	Insulin-binding activity, %	
	mononuclears	T lymphocytes
Healthy subjects ($n=11$)	34.02±3.93	24.50±2.53
IDDM ($n=17$):	23.50±2.61*	22.44±3.21
decompensated	26.64±2.90	24.85±4.13
compensated[1]	20.28±4.09*	20.33±5.1
gestation diabetes	10.1±5.02*	16.23±3.1

Note. An asterisk denotes $p<0.05$ in comparison with healthy subjects. [1]Clinical or clinical and metabolic compensation.

with essential phospholipids intravenously (5 ml on alternate days) for 4 weeks and then only orally (6 capsules/day) over the next 4 weeks, while the rest were treated only intravenously for about 8 weeks. After this treatment, total lipids were decreased by 14.5%, total cholesterol by 22% ($p<0.05$), and triglycerides by 53% ($p<0.01$); IBA of lymphocytes was 15% higher ($p<0.05$) and glucose utilization by lymphocytes was 22% higher ($p<0.05$) (Fig. 1). Hydroperoxide and MDA concentrations in erythrocyte plasma membranes decreased approximately twofold ($p<0.01$). The antioxidant activity of erythrocytes rose by 13%, their IBA by 41% ($p<0.01$), and their glucose utilization by 42% ($p<0.01$) (Fig. 2), i.e., the phospholipid therapy improved not only structural properties of the erythrocyte plasma membranes but also their functional characteristics. These results indicate that essential phospholipids used by pregnant women with diabetes mellitus exert a membrane-modulating effect, manifested in enhanced insulin binding and antioxidant activities of erythrocytes, reduced LPO, and improved cell metabolism.

To summarize, the present study suggests that insulin resistance in pregnant women with IDDM is due to impaired sensitivity of their cells to threshold physiological and submaximal insulin doses, and that insulin administered in maximal doses returns glucose consumption by the cells to normal. High insulin doses intensify LPO and reduce antioxidant activity in the cell membranes and, consequently, their ability to bind reactive peroxide radicals. The structural and functional changes in the membranes are then accompanied by reductions in the affinity of insulin receptors and in the number of insulin-binding sites in the membranes, with a resultant disruption of the intracellular effects of insulin.

This study indicates that essential phospholipids used in the context of insulin therapy can promote targeted modulation of insulin-binding receptor activity, reduce LPO processes in the cell membranes, elevate the activity of some components of the antioxidant defense of erythrocytes, and improve cell metabolism. Essential phospholipids may, therefore, prove useful in the treatment not only of IDDM but also of other diseases in which the state of insulin receptors is altered.

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