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Determination of membrane lipid differences in insulin resistant diabetes mellitus type 2 in whites and blacks

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Abstract

Objectives: Insulin resistance in diabetes mellitus type 2 (DM2) can result from membrane lipid alterations. Blacks are at a higher risk of developing DM2; therefore, we investigated whether membrane lipid differences exist between blacks and whites and if differences contribute to impaired insulin binding in diabetes.

Methods: Subjects were recruited from four groups: white control ($n = 10$), black control ($n = 10$), white diabetic ($n = 5$), and black diabetic ($n = 10$). Diabetic subjects who had DM2 with insulin resistance on insulin monotherapy were matched by age and sex. The following determinations were made: fasting serum glucose, fasting serum insulin, plasma lipid profile, red blood cell (RBC) membrane lipids and cholesterol, and RBC insulin binding.

Results: The membrane lipid analysis showed racial differences in phosphatidyl ethanolamine (PE) and phosphatidyl choline (PC). The plasma membrane of whites showed higher PE and lower PC levels than that in blacks. The RBC rheologic (PE/phosphatidyl serine) properties (deformability) were lower in diabetics and black subjects. The saturated nature of RBC ([sphingomyelin + PC)/(PE + phosphatidyl serine]) was the lowest in white control subjects ($P < 0.056$).

Conclusion: The combination of increased saturated/polyunsaturated fatty acids, increased saturated nature, and increased cholesterol/phospholipid can contribute to decreased membrane fluidity, resulting in insulin resistance. Also, decreased RBC deformability can make oxygen delivery through the capillaries difficult, create tissue hypoxia, and contribute to some of the known complications of diabetes. Membrane lipid alteration may be one of the reasons for a higher incidence of diabetes among blacks.

Keywords: Diabetes; Membrane lipids; Insulin resistance; Red blood cell deformability; Fatty acids; Membrane fluidity

Introduction

Alterations in plasma membrane lipids have been demonstrated in various tissues in diabetic animal models [1,2]. Fatty acid (FA) desaturation has an important function in changing membrane fluidity in all cells. The main enzymes responsible for desaturation of ω-3 and ω-6 FAs and membrane fluidity are desaturase enzymes. The desaturase enzyme transcription is insulin dependent [3]. In diabetes decreased insulin action may be responsible for decreased enzyme transcription and activity [4]. Activity of desaturase enzymes are also sensitive to dietary changes [5], hormonal imbalance, developmental processes, temperature changes, metals, alcohol, peroxisomal proliferators, and phenolic compounds [6].

Erythrocytes (red blood cells [RBCs]) in diabetics are less flexible due to membrane lipid changes resulting in membrane rigidity, and this has been considered to be part of the pathology seen in diabetic microvascular complications [7]. Also, the metabolically labile pool of phosphatidate is decreased, which can lead to alteration in phosphatidyl inositol (PI) levels in RBC membranes from diabetic patients [8]. PI is an important phospholipid (PL) in insulin hormone signal transduction pathways.

The RBCs are a desirable sample for membrane lipid analysis because they lack the desaturase enzymes and the

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Table 1
Blood constituents in healthy (control) and diabetic subjects

Blood constituents	Control subjects		Diabetic subjects	
	CC	AC	CD	AD
Glucose (mg/dL)†	84.15 ± 15	81.65 ± 13	145.7 ± 41	244.625 ± 143
GHb (%)*	5.4 ± 0.8	6.0±1.3	6.7 ± 0.5	10 ± 3
Total cholesterol (mg/dL)	185 ± 17	185 ± 17	207 ± 41	222 ± 74
Triacylglycerol (mg/dL)†	91 ± 42	84 ± 34	154 ± 36	176 ± 95
VLDL (mg/dL)†	18 ± 8.4	17 ± 8	31 ± 7.5	35 ± 19
LDL (mg/dL)*	104 ± 17	108 ± 13	135 ± 36	143 ± 68
HDL (mg/dL)†	62 ± 14	62 ± 10.5	42 ± 11	45 ± 4
Insulin (μU/mL)	32 ± 25	59 ± 41	39 ± 23	43 ± 15

AC, black control; AD, black diabetic; CC, white control; CD, white diabetic; GHb, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein

* Control versus diabetic, $P < 0.05$.

† Control versus diabetic, $P < 0.001$.

membrane lipids are taken up from the plasma [9]; therefore, RBC membrane serves as a useful tissue sample to evaluate alterations in a disease state [2]. Because plasma lipid composition in a fasting state mostly signifies hepatic lipid synthesis, RBC membrane lipid composition can be used as a surrogate for lipid metabolism in diabetes and other diseases.

Materials and methods

The research project was approved by the institutional review board of North Carolina State University at Raleigh. The subjects were recruited from four groups: white control (CC; $n = 10$), black control (AC; $n = 10$), white diabetics (CD; $n = 5$), and black diabetics (AD; $n = 10$). The diabetic subjects had type 2 diabetes with insulin resistance and used daily insulin injections. The diabetic and control subjects were matched by age and sex in both racial groups. Subjects' ages ranged from 23 to 60 y and equal numbers of men and women were in each group, except in CD group (three men and two women). The control subjects were recruited based on the following eligibility requirements: (1) must have no health problems, (2) must not be taking any medications, and (3) female subjects must not be taking estrogen. Blood was collected during the last week of the menstrual cycle from female subjects taking birth control pills or on estrogen therapy.

Blood samples were collected by venipuncture after an overnight fast. The serum samples were analyzed for total serum glucose (GLU), triacylglycerol, total cholesterol (CH), very low-density lipoprotein, low-density lipoprotein, high-density lipoprotein (Cholestech Instrument, Haywood, CA), and insulin level (RIA Kit, Diagnostic Products Corporation, Los Angeles, CA, USA). The RBCs were used for determining glycated hemoglobin (GHb; Sigma, St. Louis, MO, USA) and lipid extraction [9]. The RBC membrane FA and CH contents were determined by using gas chromatography and the phospholipid content was determined by using high-performance liquid chromatography [10]. The remaining RBC samples were prepared for insulin-binding studies [11].

Statistical analysis was performed with SAS computer software (SAS Institute, Cary, NC, USA). Data were analyzed by two-way analysis of variance using a general linear model and Pearson's correlation procedure. The data were split by health status (diabetic versus non-diabetic) and race (white versus black). In addition, age was used as a covariant in analysis of all dependent variables. Analysis means were compared by least significant differences at $P < 0.05$.

Results

Blood constituents

Serum evaluation of GLU levels (Table 1) showed that the diabetic subjects had higher fasting GLU levels ($P < 0.01$), as expected. There were no racial differences seen in the GLU levels (Fig. 1) in the diabetic and control groups. The GHb levels were significantly different between the AD

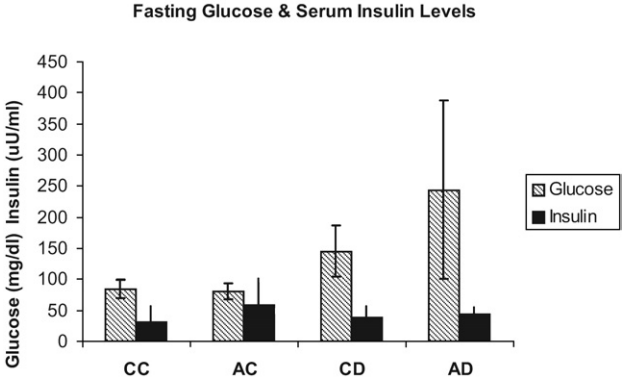

Fig. 1. Fasting glucose and serum insulin levels. AC, black control group; AD, black diabetic group; CC, white control group; CD, white diabetic group.