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ORIGINAL ARTICLE

Comparison of lymphomononuclear cell energy metabolism between healthy, impaired glucose intolerance and type 2 diabetes mellitus patients

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Abstract Diabetes mellitus (DM) is a complex disease that affects many systems. The most important cells of the immune system are lymphomononuclear (LMN) cells. Here, we aimed to evaluate the energy metabolism of LMN cells in patients with diabetes and impaired glucose tolerance. We measured LMN cell energy metabolism in patients with type 2 diabetes mellitus, impaired glucose tolerance (IGT) and healthy subjects. Cells were freshly isolated from peripheral blood and the subgroups were determined by flow cytometric method. Lactate production and glycogen utilization were significantly increased in the LMN cells of patients with type 2 DM and IGT when compared with healthy volunteers. No statistical difference was observed between the patients with type 2 DM and IGT. There was a significant correlation between fasting plasma glucose and lactate production in LMN cells. LMN cells changed their energy pathway in a diabetic state and preferred anaerobic glycolysis. Prediabetic range also affected energy metabolism in LMN cells. This abnormal energy production might cause dysfunction in LMN cells and the immune system in diabetic and prediabetic patients. In conclusion, we concluded that impaired glucose metabolism could change energy metabolism.

Keywords Type 2 diabetes mellitus · Impaired glucose tolerance · Energy metabolism · Lymphomononuclear cell · Cell culture

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Introduction

Diabetes mellitus is one of the most serious metabolic diseases and affects approximately 2.5–3% of the world's population [1]. Infections occur with increased frequency and severity in diabetic patients [2]. Many studies have shown that it is hard to eliminate infectious diseases in a diabetic host [3]. However, the mechanism underlying immune cell dysfunction in diabetes is unclear. Interleukin (IL) 2, 4, 6, and 10 levels decrease and mononuclear cell proliferation is inhibited in diabetic rats [4]. Diabetic women with asymptomatic bacteriuria show low urinary IL-6 concentration [5]. Chemotaxis of polymorphonuclear leukocytes is significantly low in diabetic patients [6].

The mechanism underlying dysfunctional immune cells in diabetes is unclear. As in other body systems in diabetes, it is possible that the key is disruptions in immune cell metabolism. High glucose levels can also affect cell metabolism. Lymphocytes obtained from diabetic rats show decreased hexokinase and citrate synthase activity [7]. Glycolysis is necessary for DNA synthesis and lymphocyte proliferation [8]. High glucose levels suppress DNA synthesis. Impaired lymphocyte metabolism affects the immune system [9]. In diabetes, T-cell function is the most likely damaged function among the cellular immune

response [10, 11]. It has been demonstrated that impaired polymorphonuclear cell function can be reversed by insulin therapy [12–15].

It is important to estimate lymphomononuclear cell behavior in diabetic patients to determine the mechanisms underlying the high susceptibility of diabetic patients to infections. We have studied the production of lactate and breakdown of glycogen in cultures of peripheral-blood lymphomononuclear cells from type 2 diabetic and impaired glucose tolerance patients, and compared them with cell cultures from healthy volunteers.

Materials and methods

Seventeen type 2 diabetic patients and 17 impaired glucose tolerance (IGT) patients selected from the Endocrinology and Internal Medicine Clinics of the University Hospital of the Gulhane School of Medicine, and 16 healthy volunteers were included in our study (Table 1). Type 2 diabetic (glucose levels after 75 gr. OGTT higher than 200 mg/dl) and IGT patients (glucose levels after 75 gr. OGTT between 140 and 200 mg/dl) had no infectious diseases and were not using any drugs. The presence of macrovascular complications was determined by clinical and electrocardiographic evaluation. In addition to neurological examination, the patients were evaluated for the presence of microvascular complications using urine-protein measurement and ophthalmological examination. Type 2 diabetic and IGT patients had co-morbid problems such as hypertension and dislipidemia, and were using drugs for these problems. The Ethics Committee of our institution approved the study protocol according to the principles of the Helsinki Declaration, and patients gave their written informed consent to participate in the study.

Oral glucose tolerance test (OGTT) was performed after 10–12 h overnight fasting by ingesting 75 g oral glucose load over a 2 min period and obtaining blood samples at baseline and 2 h after glucose load for serum glucose measurements. Glucose tolerance status was defined as normal glucose tolerance (NGT), IGT or diabetes according to the WHO criteria [16, 17].

Table 1 Comparison of age, laboratory parameters, lactate production and glycogen utilization between control, impaired glucose tolerance (IGT) and diabetic (DM) groups

	Control (*n*:16) (Mean ± SD)	IGT (*n*:17) (Mean ± SD)	Type 2 DM (*N*:17) (Mean ± SD)	*P*
Age (years)	59 ± 10.6	55 ± 7.1	60 ± 10.5	NS
Sex (M/F)	6/10	7/10	7/10	NS
Fasting plasma glucose (mg/dl)	90.2 ± 7.1	105.2 ± 16.7	168.9 ± 57.0	<0.0001*
Postprandial plasma glucose (mg/dl)	88 ± 3.3	160.6 ± 24.3	247.2 ± 75.3	<0.0001**
Triglyceride (mg/dl)	122 ± 58	139 ± 44	168 ± 33	0.022
HDL-cholesterol (mg/dl)	45 ± 7.2	44 ± 6.2	43 ± 5.9	NS
LDL-cholesterol (mg/dl)	110.9 ± 27.3	117.8 ± 34.9	128.8 ± 34.3	NS
Total cholesterol (mg/dl)	193 ± 28	206.8 ± 41	211.2 ± 42	NS
HbA1c (%)	5.5 ± 0.1	6 ± 0.3	8.2 ± 1.7	0.001
Diabetes duration (years)			4.5 ± 0.9	
Total live cell count	$3.32 \pm 0.91 \times 10^6$	$6.58 \pm 1.22 \times 10^6$	$4.97 \pm 1.42 \times 10^6$	0.206
Mean Protein Quantity (μgr./ml)	359.8 ± 148.3	229.7 ± 93.6	301.6 ± 110.68	0.011
Lactate production (M/F) (nmol/h μgr. protein)	317.3 ± 34.3/309.5 ± 69.2	375.3 ± 97.2/365.7 ± 31	429.6 ± 143.8/425.4 ± 135.4	0.008***
Glycogen utilization (M/F) (nmol/h μgr. protein)	792.2 ± 128.4/790.3 ± 110	1623.8 ± 689.6/1563.2 ± 669.4	1331.2 ± 615.9/1303.6 ± 609.4	<0.0001†

Fasting plasma glucose levels are expressed as Mean ± SD

* $P < 0.0001$ between control group and IGT group. $P < 0.0001$ between IGT group and DM group

Postprandial plasma glucose levels are expressed as Mean ± SD

** $P < 0.0001$ between control group and IGT group. $P < 0.0001$ between IGT group and DM group

Lactate production is expressed as Mean ± SD

*** $P < 0.008$ between control group and IGT group. $P < 0.008$ between IGT group and DM group

Glucogen utilization is expressed as Mean ± SD

† $P < 0.0001$ between control group and IGT group. $P < 0.0010$ between IGT group and DM group

Fasting plasma glucose (FPG), postprandial plasma glucose (PPG), total cholesterol (TC), triglyceride, and high density lipoprotein (HDL) cholesterol levels were measured by the enzymatic colorimetric method with Olympus AU 600 auto analyzer using reagents from Olympus Diagnostics, GmbH (Hamburg, Germany). Low density lipoprotein (LDL) cholesterol level was calculated by Friedwald's formula [18].

A whole blood (40 ml) sample was taken from each study participant after 12 h fasting and without medication, and placed in a sterile tube containing lithium heparin as anticoagulant for the cell culture and subgroup analysis. Cell cultures were based on a previously described protocol [19], using a Ficoll-Hypaque® density gradient for peripheral blood mononuclear cell separation. Lymphomononuclear cells, at a concentration of 1×10^6/ml, were exposed to RPMI 1640 (contains 11.1 mmol/l D-glucose) for 4 h at 37°C. The period between the collection of cells and incubation was very well controlled and almost the same for each participant. The supernatant was collected for lactate measurement and the cells were separated for glycogen measurement and subgroup analysis. Both measurements were initiated at the same time with no delays permitted.

The enzymatic method (Sigma kit 826-UV, Sigma Chemical, St. Louis, MO) was used for lactate measurement. Lactate was converted to pyruvate and hydrogen peroxide by lactate oxidase. Horseradish peroxidase (HRP) catalyzes the oxidative condensation of chromogen precursors to produce a colored dye with an absorption maximum at 540 nm in the presence of H_2O_2. Measurement was initiated by adding 10 ml of sample solution to 1 ml of testing reagent, with an incubation time of 5–10 min. The increase in absorbance at 540 nm (monitored spectrophotometrically; Shimadzu, Japan) was directly proportional to the lactate concentration in the sample.

The flow cytometry method with antihuman CD45, CD14, CD19, and CD3 antibody was used (Becton Dickson Sanjose, California, USA) for subgroup analysis of the cells. Cell viability was determined by trypan blue. Cells were destructed by 5 M KOH in pellet and protein was measured by using the modified Lowry method [20]. Glycogen production per protein was estimated by Hassid and Abraham's enzymatic method [21, 22].

All analyses were performed using SPSS/PC statistical program (Version 15.0 for Windows; SPSS, Chicago, IL, USA). Results have been reported as mean ± SD. The one sample Kolmogorov–Smirnov test was used to evaluate the distribution characteristics of variables. The differences between the groups were tested for significance by one-way ANOVA, chi-square and Kruskall–Wallis tests. The relationship between variables was analyzed by Pearson's correlation or Spearman's rho correlation test as appropriate. Differences and correlations were considered significant at $P < 0.05$.

Results

Age, sex, total cholesterol, HDL-cholesterol, and LDL-cholesterol levels were similar among the three groups. FPG, PPG, HbA1c, and triglyceride levels were significantly higher in the diabetes group than the IGT and control groups, and in the IGT than the control group (Table 1). None of the diabetic patients had chronic diabetic macro- and microvascular or neurologic complications.

Mean lactate production was 313.4 ± 82.3 nmol/h μg protein in the control group, 370.8 ± 61.2 nmol/h μg protein in the IGT group and 427.9 ± 139.7 nmol/h μg protein in the DM group. Lactate production was higher than the control group in both IGT ($P = 0.004$) and DM ($P = 0.008$) groups. There was no statistically significant difference for lactate production between the IGT and DM groups ($P = 0.88$; Table 1). No difference was observed between men and women regarding lactate production in LMN cells (Table 1). There was a significant correlation between FPG and lactate production in LMN cells ($r = 0.313$, $P = 0.03$; Fig. 1). There were no correlations between FPG and lactate production in each group separately. No correlation was found between other parameters and lactate production in LMN cells.

Mean glycogen utilization was 791.6 ± 119.9 nmol/h μg protein in the control group, 1593.5 ± 679.2 nmol/h μg protein in the IGT group and 1317.8 ± 612.8 nmol/h μg protein in the DM group. Glycogen utilization was higher than the control group in both the IGT ($P < 0.0001$) and DM ($P < 0.0001$) groups. There was no statistically significant difference between the IGT and DM groups ($P = 0.28$; Table 1).

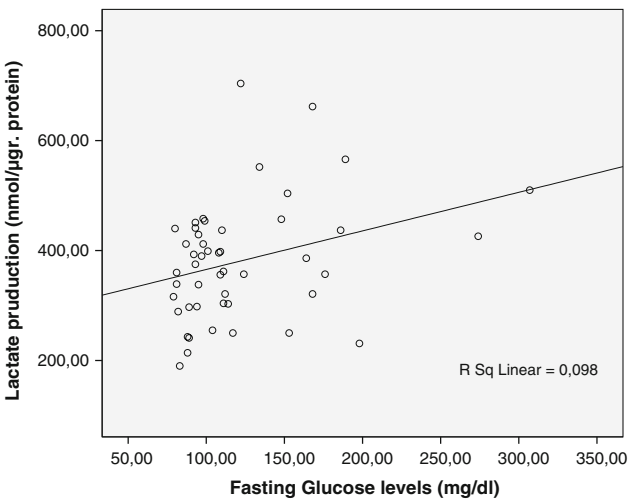

Fig. 1 Correlation between lactate production and fasting plasma glucose (FPG) levels ($r = 0.313$, $P = 0.03$). There was a significant correlation between FPG and lactate production in LMN cells ($r = 0.313$, $P = 0.03$)

Discussion

The purpose of this study was to examine the effects of hyperglycemic state on LMN cells. We collected LMN cells from patients with type 2 DM, those with IGT and normal subjects. Hyperglycemic state was observed in patients with type 2 DM and IGT. LMN cells changed their energy metabolism in the hyperglycemic state and tended to use anaerobic glycolysis. An altered glycolytic pathway could affect their capacity to respond to immune stimuli [3–6].

Environmental oxygen concentration determines the metabolic pathway of a cell [21, 23]. When the environmental oxygen level is lower, anaerobic glycolysis increases to compensate for ATP production by utilizing glucose faster [24]. Glycogen utilization is the best way to measure anaerobic glycolysis [24]. We therefore measured glycogen utilization instead of glucose to show the shift from slow aerobic to rapid anaerobic consumption of glucose. It has previously been reported that LMN cells cannot oxidize glucose effectively in a diabetic state [7]. We found glycogen utilization greater than lactate production (Table 1). Glucose can serve as a primary substrate for the generation of ATP; it can supply a carbon source for the synthesis of other macronutrients such as nucleic acids and phospholipids; and it can be metabolized by the pentose phosphate pathway to generate NADPH [25]. LMN cells prefer anaerobic glycolysis and the Pasteur effect is evident in the hyperglycemic state.

In our study, glycogen utilization and lactate production were both elevated significantly in the DM and IGT groups. There was no statistically significant difference between the DM and IGT groups for LMN cell energy metabolism. Prediabetic period (IGT patients) could change cell metabolism. Despite the high glucose levels, diabetic lymphocytes were not able to oxidize this metabolite efficiently. They used anaerobic glycolysis to get ATP. Similar observations have been previously reported in thymus lymphocytes from streptozotocin-induced diabetic rats [26].

In diabetic rat studies, lymphocytes, and neutrophils changed their metabolic pathway to anaerobic glycolysis. After insulin treatment, aerobic glycolysis occurred [27, 28]. Insulin treatment improved the impaired neutrophil function in diabetic rats [28]. We showed that a diabetic state changed human LMN cell metabolism. Metabolic control could also influence immunological parameters such as the number of peripheral CD-4 and CD-8 positive cells [29]. However, one study reported that there is no relationship between the number of mononuclear cells in peripheral blood and blood glucose level [30]. It is still unclear whether a hyperglycemic state affects mononuclear cell count.

LMN cell proliferation and ATP, DNA, RNA, and phospholipid synthesis were decreased in diabetic state [31]. Changes in LMN cell energy metabolism might contribute to immune dysfunction and cause enhanced susceptibility to infections in DM patients. Until now, LMN cell metabolism has received little attention in hyperglycemic state. Energy metabolism was very important for LMN cell function. This study showed that changes in energy metabolism could be one of the mechanisms underlying impaired immune function in diabetic patients.

In this study, we emphasize the role of high glucose on immune cell function. However, our IGT subjects displayed normal HbA1c and fasting glucose, suggesting that the differences seen between IGT and type 2 DM are more likely to be due to insulin resistance and not to hyperglycemia. Recently, it was demonstrated that monocytes have insulin receptors and insulin has limited action on monocyte function [32]. Lymphocytes require glucose uptake and metabolism for normal survival and function. Insulin resistance may play a direct role in impaired LMN cell leucocyte function [25]. However, reverse lactate also induces insulin resistance and diabetes [25]. LMN cell energy metabolism may also play role in the etiology of insulin resistance.

It appears that we should not only control diabetes in the early stages but also control blood glucose levels in a very narrow range. Hyperglycemia is a major cause of diabetic complications [33]. As LMN cell energy metabolism is affected early in the progression of diabetes, we think that early diagnosis is important. Appropriate measures should be taken before diabetes is established and normal glucose levels should be restored in prediabetics and diabetics. The Diabetes Control and Complications Trial (DCCT) [33] and the UK Prospective Diabetes Study (UKPDS) [34, 35] both concluded that DM and IGT patients required the same efforts for glycemic control.

In conclusion, this was the first human study to demonstrate that a hyperglycemic state affects LMN cell energy metabolism. LMN cells from DM and IGT patients prefer an anaerobic glycolytic pathway for energy yield. This adaptation starts before diabetes occurs. DM and IGT patients would therefore benefit from early diagnosis. New studies should be undertaken to evaluate the aerobic glycolytic pathway in human diabetic LMN cells.

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