

Cellular insulin resistance in Epstein-Barr virus-transformed lymphoblasts from young insulin-resistant Japanese men^{☆,☆☆}

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Abstract

The metabolic syndrome is characterized by a blunted insulin-mediated glucose uptake in various cell types. We compared the glucose uptake characteristics of Epstein-Barr virus (EBV)-transformed lymphoblasts obtained from young men with vs without metabolic and cardiovascular evidence of metabolic syndrome. From a population of 218 men, 20- to 25-year-old, 10 men with a systolic blood pressure (BP) ≥ 130 mm Hg and family history of hypertension were assigned to a high BP (HBP) group, and 10 with a BP ≤ 110 mm Hg, and no family history of hypertension was assigned to a low BP (LBP) group. Multiple clinical and metabolic characteristics were examined in both groups and compared. Peripheral lymphocytes from HBP and LBP subjects were EBV-transformed, and the glucose transporter (Glut)-mediated glucose uptake from each group was compared in lymphoblasts. Body mass index, fasting glucose, immunoreactive insulin, insulin resistance index based on a homeostasis model assessment (HOMA-R), and total and low-density lipoprotein cholesterol were significantly higher in the HBP than the LBP subgroup (whole-body insulin resistance). Baseline Glut-mediated and Glut-mediated insulin-stimulated glucose uptake by lymphoblasts from the HBP group were significantly lower than by lymphoblasts from the LBP group (cellular insulin resistance). The net increment in Glut-mediated glucose uptake by insulin was inversely correlated with HOMA-R. In conclusion, cellular insulin resistance in EBV-transformed lymphoblasts is associated with young Japanese subjects with HBP. The net increment in Glut-mediated glucose uptake by insulin in lymphoblasts may be a useful intermediate phenotype to study genetic aspects of the metabolic syndrome.

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1. Introduction

Abnormal metabolism of glucose, insulin, and lipid is more prevalent in untreated hypertensive patients than in normotensive control subjects [1]. The cluster of these metabolic abnormalities associated with cardiovascular disease (CVD) has been named the metabolic syndrome.

Insulin resistance, a highly prevalent pathophysiological disorder implicated in the development of type 2 diabetes, obesity, hyperlipidemia, and hypertension, is expressed as a blunted biologic response to normal concentrations of circulating insulin. Compensatory hyperinsulinemia is one of its consequences.

The etiology of this syndrome, which promotes the development of premature atherosclerosis and increases significantly the risk of CVD early in life [2], remains controversial. Candidate mechanisms by which hypertension may develop include sodium retention, vascular hyperresponsiveness, arteriolar smooth muscle cell proliferation, abnormal cellular electrolyte transport and composition, stimulation of sympathoadrenergic activity, and

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growth-promoting effects [3]. Offspring studies have confirmed that insulin resistance associated with essential hypertension is inherited, though ambient environmental factors play an important role [4,5]. Measurements of insulin sensitivity by the glucose clamp technique have identified skeletal muscle and adipose tissue as major targets in the metabolic syndrome. However, these tissues are difficult to obtain and study in vitro. We have, therefore, created cell lines of Epstein-Barr virus (EBV)-transformed lymphoblasts. These lymphoblasts represent a useful cellular model with which to compare primary and secondary factors because (a) they reproduce the genetic characteristics of the donor, (b) the cells removed from the in vivo environment of the donor can be cultured under controlled conditions for an unlimited number of generations [6], (c) they enable repetitive measurements with minimally invasive sampling techniques.

Sesti et al [7] have studied the recycling of the insulin receptor and processing of insulin in EBV-transformed lymphoblasts from insulin-resistant patients, though they did not examine their glucose uptake. If in insulin-resistant patients, the blunted glucose uptake is due to an abnormality common to various cell types, this abnormality should be detectable in lymphoblasts. To clarify the mechanisms of cellular insulin resistance, we have focused our experiments on the glucose transport system. The glucose transporters are the main mediators of glucose entry into the cells. The glucose transporters, Glut1, abundant in various cells, and Glut4, mainly expressed in skeletal muscle and adipocytes, are implicated in insulin resistance [8].

In this study, we investigated the association of whole-body insulin resistance in individuals with high blood pressure (HBP) and a family history of hypertension, identified among a population participating in an annual health maintenance program. We then examined the cellular insulin resistance in their EBV-transformed lymphoblasts and the relationship between whole-body and cellular insulin resistance.

2. Materials and methods

2.1. Study population, physical findings, and laboratory measurements

A population of 218 men between the ages of 20 and 25 years was recruited from an annual health maintenance program at Keio University, Tokyo, Japan. This study was approved by the Institutional Ethic Committee of Keio University, and written informed consent was obtained from all participants who underwent (a) a physical examination, including measurements of body mass index (BMI), systolic and diastolic BP, and heart rate; (b) fasting blood chemistry, including fasting blood glucose (FBG), immunoreactive insulin (IRI), insulin resistance index based on a homeostasis model assessment (HOMA-R, calculated as $\text{FBG [mg/dL]} \times \text{IRI [\mu U/mL]/405}$), total cholesterol, low-density

lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides, free fatty acids, and uric acid; and (c) a medical questionnaire probing into the personal and family history.

High BP and low BP (LBP) groups were selected from the study population. A study participant was included in the HBP group if he had (1) a systolic BP ≥ 130 mm Hg and (2) a family history of hypertension or CVD among second-degree relatives. He was included in the LBP group if he had (1) a systolic BP ≤ 110 mm Hg and (2) no family history of hypertension or CVD among second-degree relatives.

2.2. immortalization of lymphocytes and culture of lymphoblasts

A 7-mL blood sample was drawn from a peripheral vein, and the circulating lymphocytes were isolated on a Ficoll conray (IBL, Gunma, Japan). B lymphocytes were immortalized by incubation with EBV-containing supernatant. Lymphocytes from peripheral blood of HBP and LBP subjects were transformed with EBV by a modification of the method of Sesti et al [9]. Cells were grown and routinely cultured in Rapid Prototyping and Manufacturing Institute 1640 (RPMI) medium (Manufacturing Research Institute, Georgia Institute of Technology, Atlanta, Ga) supplemented with 100 $\mu\text{g/mL}$ kanamycin and 10% heat-inactivated fetal calf serum (FCS; Gibco, Grand Island, NY). When the lymphoblasts had become confluent, cyclosporine A, 200 ng/mL, was added to the RPMI medium. Passages were performed at least twice a week. The same lot of FCS was used throughout the experiments. After immortalization, the lymphoblasts were grown for 4 to 8 weeks and stocked frozen. Fresh cultures were thawed from the frozen stocks for each experiment. Cells were grown in RPMI medium supplemented with 10% FCS and subcultured for 3 days before the experiments.

2.3. Uptake of 2-deoxyglucose into lymphoblasts

The uptake of 2-deoxyglucose (DG) into lymphoblasts was measured by a modified method of Daneman et al [10]. At first, to determine the optimal insulin concentration, preliminary experiments were performed using lymphoblasts derived from randomly selected subjects ($n = 5$ in each group). We resuspended 500 μL of lymphoblasts in concentration of 2×10^6 cells/mL in *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES)-buffered RPMI medium (glucose concentration, 100 mg/dL), and preincubated for 1 hour with insulin in concentrations of 0, 0.3, 1.0, 3.0, and 10 nmol/L, and with 100- $\mu\text{mol/L}$ concentration of cytochalasin B, a fungal metabolite inhibitor of the insulin-regulated transport of glucose [11–13]. The lymphoblasts were incubated in presence of 100-mg/dL concentration of [^3H]-2-DG (40 μL) at 37°C and gently agitated for 20 minutes. The cells were thoroughly rinsed 3 times with glucose-free HEPES-buffered saline solution and centrifuged. The cell pellets were lysed by

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