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Increased Fructose 2,6-bisphosphate in Peripheral Blood Mononuclear Cells of Patients with Diabetes

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Abstract. Fructose 2,6-bisphosphate (F2,6BP) is a powerful allosteric activator of 6-phosphofructo-1-kinase, which is the rate-limiting enzyme for glycolysis. Mitogenic stimulation of lymphocytes is related to an enhanced rate of glucose utilization and F2,6BP mediated activation of glycolysis. To determine the effect of hyperglycemia on intracellular glycolysis of lymphocytes, we measured intracellular F2,6BP content in peripheral blood mononuclear cells obtained from patients with diabetes and normal subjects. A total of 62 subjects participated in the present study. Venous blood samples were collected and peripheral blood mononuclear cells were separated by Ficoll gradients. Intracellular F2,6BP levels in peripheral blood mononuclear cells from normal control subjects were significantly lower than age-matched diabetic subjects. We observed a significant positive correlation between intracellular F2,6BP levels and long term glycemic control, as assessed by HbA1c. These data suggest that hyperglycemia increases intracellular F2,6BP in immune cells. These findings may help to clarify the impaired function in immune cells in patients with diabetes.

Key words: Glycolysis, Lymphocytes, Fructose 2,6-bisphosphate

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IMMUNE cell activation requires an increase in glucose uptake and anaerobic glycolysis, which are induced by antigenic or mitogenic challenge and serve to meet the acute metabolic demands of the cell [1–5]. Enhanced glycolysis provides ATP for synthetic functions and kinase reactions, and pentose sugars for the synthesis of the nucleotide precursors necessary for proliferative responses.

Fructose 2,6-bisphosphate (F2,6BP) is a powerful allosteric activator of 6-phosphofructo-1-kinase (PFK-1), which is the rate-limiting enzyme for glycolysis [6–9]. High F2,6BP levels mediate enhanced glycolysis, and F2,6BP in turn is produced by the bifunctional enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFK-2). We recently cloned an inducible isoform of PFK-2, termed iPFK-2 (encoded by PFKFB3 gene), that is responsible for increased intracellular F2,6BP levels in activated immune cells, thereby prompting our interest in the regulation and effector action of F2,6BP [10]. Infections occur with increased frequency and severity in diabetes [11]. However, potential mechanisms of impairment in immune cell function of patients with diabetes have not been clarified.

In this study, we measured intracellular F2,6BP content in peripheral blood mononuclear cells (PBMCs) obtained from 49 patients with diabetes and 13 normal subjects to investigate the level of F2,6BP in immune cells. We observed a significant relationship between intracellular F2,6BP levels and long term glycemic control, as assessed by HbA1c.

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Subjects and Methods

The diabetic patients were recruited from an outpatient endocrinology clinic at the Hokkaido University

Hospital. Venous blood samples were collected from subjects after an overnight fast, and PBMCs were separated by Ficoll gradients. PBMCs were homogenized in 50 mM NaOH and incubated at 80°C for 10 min. After centrifugation, the supernatants were used to assay for F2,6BP by Van Schaftingen's method [12]. Protein concentration was determined by Bio-Rad Reagent (Bio-Rad Laboratories Inc., Richmond, CA) according to the manufacture's protocol. HbA1c was measured by high performance liquid chromatography. The study protocol was approved by the institutional review boards of the ethics committee in Hokkaido University Graduate School of Medicine, Japan. All subjects gave written informed consent before entering the study. Statistical analysis was performed with Dr. SPSS II software. The level of significance was accepted at $P<0.05$ as assessed with Spearman's rank correlation.

Results

A total of 49 diabetic subjects with a mean ± SD age of 63.2 ± 12.4 years, 21 men and 28 women, participated in the present study. The clinical characteristics of the diabetic subjects are shown in Table 1. All participants had diabetes (six patients with type 1 diabetes and 43 patients with type 2 diabetes), but none had clinical symptoms for infection. All participants showed negative plasma CRP levels and were not taking any anti-inflammatory drugs. For the normal control subjects, non-diabetic healthy volunteers were recruited (mean ± SD age of 43.5 ± 6.5 years, n = 13).

Intracellular F2,6BP levels in PBMCs from normal control subjects were significantly lower than age-matched diabetic subjects (n = 17, 4.62 ± 1.66 vs. 6.54 ± 2.87 pmol/mg protein, $P<0.04$) (Fig. 1). The HbA1c level in each group was 4.83 ± 0.26 and 8.28 ± 1.44, respectively. The concentration of F2,6BP was positively correlated with HbA1c in patients with diabetes and normal subjects ($r = 0.451$, $P<0.001$) (Fig. 2).

Table 1. Characteristics and laboratory findings in patients with diabetes and normal subjects

	Patients with diabetes	Normal subjects
Number (Male/Female)	49 (21/28)	13 (8/5)
Age (years)	63.2 ± 12.4	43.5 ± 6.5
Duration of diabetes (years)	15.9 ± 8.3	
BMI (kg/m^2)	23.2 ± 4.1	22.4 ± 4.3
HbA1c (%)	7.5 ± 1.2	4.8 ± 0.2

Values are the mean ± SD.

Discussion

In the present study, we have demonstrated for the first time a significant positive association between F2,6BP level in PBMCs and HbA1c level. In diabetes, enhanced glycolysis in the heart, kidney, and other organs [15, 16] can lead to an increase in the production

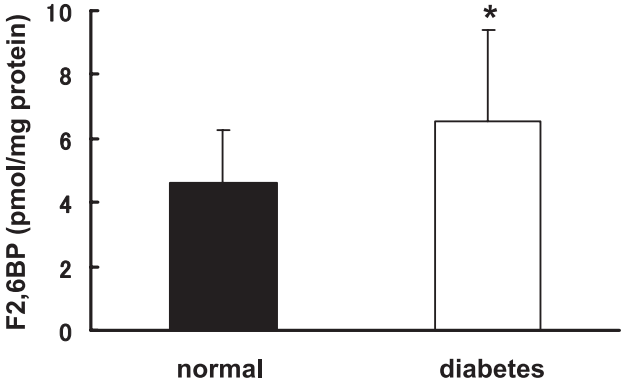

Fig. 1. Differences in intracellular F2,6BP content in PMBCs between patients with diabetes and control. (Data are shown as means ± SD, *$P<0.05$)

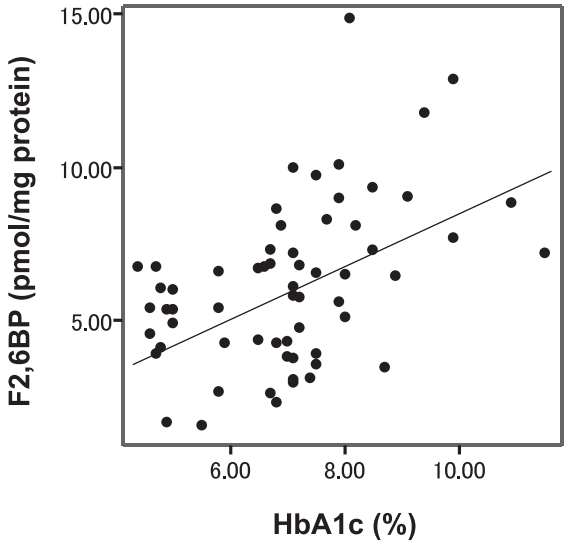

Fig. 2. Correlation between F2,6BP in PBMCs and HbA1c in patients with diabetes and normal subjects. ($r = 0.451$, $P<0.001$, n = 62)

of diacylglycerol, which activates protein kinase C and alters downstream gene expression [17–20]. Hyperglycemia also leads to enhanced production of precursors of intracellular advanced glycation endproducts [21]. These altered metabolic products and oxidative stress play a role in the development of diabetic complications [21]. Diabetes is a risk factor for bacteremia in patients with pneumococcal pneumonia and is associated with increased mortality [22–24]. Iinfections occur with an increased severity in patients with diabetes [25]. Mitogenic stimulation of lymphocytes is related to an enhanced rate of glucose utilization and F2,6BP mediated activation of glycolysis [26]. In the previous report, elevated F2,6BP levels in thymus lymphocytes have been shown in streptozotocin (STZ) induced diabetic rats [27]. Altered functions of polymorphonuclear neutrophils in STZ-induced diabetic rats have been shown in a recent report [28]. These findings suggest the association between accelerated glycolysis due to hyperglycemia and alteration of the immune system during the diabetic state. HbA1c reflects the mean blood glucose levels during the past 1 to 2 months. We investigated the relationship between F2,6BP levels and plasma glucose levels. However, significant association was not observed (data not shown). Further studies are required to better understand the mechanism of regulation of F2,6BP levels in PBMCs.

Glycolysis is ultimately inhibited by several glycolytic metabolites such as glycerol 3-phosphate, ATP, phosphoenolpyruvate [10, 29] and citrate. Accelerated glycolysis may induce the accumulation of these metabolites, leading to a persistently unresponsive state for leukocytes in the diabetic milieu. It has been shown that neutorophils from diabetic rats are already activated at basal level and cannot show their normal response toward the stimulation [28]. Therefore, we hypothesize that activation of glycolysis by F2,6BP in PBMCs causes the accumulation of glycolytic metabolites, and inhibits the activation of immune cells.

In conclusion, this is the first report for a positive correlation between F2,6BP levels in PBMCs and HbA1c. Further investigation of the association between increased F2,6BP level in PBMCs and alteration of immune system in patients with diabetes may be a useful means to clarify the impaired function in immune cells in patients with diabetes.

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References

1. Sagone AL Jr, LoBuglio AF, Balcerzak SP (1974) Alterations in hexose monophosphate shunt during lymphoblastic transformation. *Cell Immunol* 14: 443–452.
2. Roos D, Loos JA (1970) Changes in the carbohydrate metabolism of mitogenically stimulated human peripheral lymphocytes. I. Stimulation by phytohaemagglutinin. *Biochim Biophys Acta* 222: 565–582.
3. Hedeskov CJ (1968) Early effects of phytohaemagglutinin on glucose metabolism of normal human lymphocytes. *Biochem J* 110: 373–380.
4. Cooper EH, Barkhan P, Hale AJ (1963) Observations on the proliferation of human leucocytes cultured with phytohaemagglutinin. *Br J Haematol* 9: 101–111.
5. Frauwirth KA, Riley JL, Harris MH, Parry RV, Rathmell JC, Plas DR, Elstrom RL, June CH, Thompson CB (2002) The CD28 signaling pathway regulates glucose metabolism. *Immunity* 16: 769–777.
6. Okar DA, Manzano A, Navarro-Sabate A, Riera L, Bartrons R, Lange AJ (2001) PFK-2/FBPase-2: maker and breaker of the essential biofactor fructose-2,6-bisphosphate. *Trends Biochem Sci* 26: 30–35.
7. Van Schaftingen E (1987) Fructose 2,6-bisphosphate. *Adv Enzymol Relat Areas Mol Biol* 59: 315–395.
8. Hue L, Rider MH (1987) Role of fructose 2,6-bisphosphate in the control of glycolysis in mammalian tissues. *Biochem J* 245: 313–324.
9. Pilkis SJ, Claus TH, Kurland IJ, Lange AJ (1995) 6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase: a metabolic signaling enzyme. *Annu Rev Biochem* 64: 799–835.
10. Chesney J, Mitchell R, Benigni F, Bacher M, Spiegel L, Al-Abed Y, Han JH, Metz C, Bucala R (1999) An inducible gene product for 6-phosphofructo-2-kinase with an AU-rich instability element: role in tumor cell glycolysis and the Warburg effect. *Proc Natl Acad Sci USA* 96: 3047–3052.
11. Guvener M, Pasaoglu I, Demircin M, Oc M (2002)

Perioperative hyperglycemia is a strong correlate of postoperative infection in type II diabetic patients after coronary artery bypass grafting. *Endocr J* 49: 531–537.
12. Van Schaftingen E, Lederer B, Bartrons R, Hers HG (1982) A kinetic study of pyrophosphate: fructose-6-phosphate phosphotransferase from potato tubers. Application to a microassay of fructose 2,6-bisphosphate. *Eur J Biochem* 129: 191–195.
13. Atsumi T, Nishio T, Niwa H, Takeuchi J, Bando H, Shimizu C, Yoshioka N, Bucala R, Koike T (2005) Expression of inducible 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase/PFKFB3 isoforms in adipocytes and their potential role in glycolytic regulation. *Diabetes* 54: 3349–3357.
14. Haneda M, Koya D, Isono M, Kikkawa R (2003) Overview of glucose signaling in mesangial cells in diabetic nephropathy. *J Am Soc Nephrol* 14: 1374–1382.
15. Crepin KM, Darville MI, Hue L, Rousseau GG (1988) Starvation or diabetes decreases the content but not the mRNA of 6-phosphofructo-2-kinase in rat liver. *FEBS Lett* 227: 136–140.
16. Khandelwal RL, Zinman SM, Knull HR (1979) The effect of streptozotocin-induced diabetes on glycogen metabolism in rat kidney and its relationship to the liver system. *Arch Biochem Biophys* 197: 310–316.
17. Koya D, King GL (1998) Protein kinase C activation and the development of diabetic complications. *Diabetes* 47: 859–866.
18. Derubertis FR, Craven PA (1994) Activation of protein kinase C in glomerular cells in diabetes. Mechanisms and potential links to the pathogenesis of diabetic glomerulopathy. *Diabetes* 43: 1–8.
19. Xia P, Inoguchi T, Kern TS, Engerman RL, Oates PJ, King GL (1994) Characterization of the mechanism for the chronic activation of diacylglycerol-protein kinase C pathway in diabetes and hypergalactosemia. *Diabetes* 43: 1122–1129.
20. Koya D, Jirousek MR, Lin YW, Ishii H, Kuboki K, King GL (1997) Characterization of protein kinase C beta isoform activation on the gene expression of transforming growth factor-beta, extracellular matrix components, and prostanoids in the glomeruli of diabetic rats. *J Clin Invest* 100: 115–126.
21. Brownlee M (2005) The pathobiology of diabetic complications: a unifying mechanism. *Diabetes* 54: 1615–1625.
22. Bouter KP, Diepersloot RJ, van Romunde LK, Uitslager R, Masurel N, Hoekstra JB, Erkelens DW (1991) Effect of epidemic influenza on ketoacidosis, pneumonia and death in diabetes mellitus: a hospital register survey of 1976–1979 in The Netherlands. *Diabetes Res Clin Pract* 12: 61–68.
23. Marrie TJ (1992) Bacteraemic pneumococcal pneumonia: a continuously evolving disease. *J Infect* 24: 247–255.
24. Koziel H, Koziel MJ (1995) Pulmonary complications of diabetes mellitus. Pneumonia. *Infect Dis Clin North Am* 9: 65–96.
25. Shah BR, Hux JE (2003) Quantifying the risk of infectious diseases for people with diabetes. *Diabetes Care* 26: 510–513.
26. Bosca L, Mojena M, Diaz-Guerra JM, Marquez C (1988) Phorbol 12,13-dibutyrate and mitogens increase fructose 2,6-bisphosphate in lymphocytes. Comparison of lymphocyte and rat-liver 6-phosphofructo-2-kinase. *Eur J Biochem* 175: 317–323.
27. Moreno-Aurioles VR, Montano R, Conde M, Bustos R, Sobrino F (1996) Streptozotocin-induced diabetes increases fructose 2,6-biphosphate levels and glucose metabolism in thymus lymphocytes. *Life Sci* 58: 477–484.
28. Nabi AH, Islam LN, Rahman MM, Biswas KB (2005) Polymorphonuclear neutrophil dysfunctions in streptozotocin-induced type 1 diabetic rats. *J Biochem Mol Biol* 38: 661–667.
29. Manes NP, El-Maghrabi MR (2005) The kinase activity of human brain 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase is regulated via inhibition by phosphoenolpyruvate. *Arch Biochem Biophys* 438: 125–136.