Fatty Acid Composition in 1,2-Diacylglycerol of Diabetic and Insulin-Treated Diabetic Rat Hearts

Kenji OKUMURA, M.D., Takuya NISHIURA, M.D.,
Kiyokazu SHIMIZU, M.D., Yoshio IWAMA, M.D.,
Junichiro KONDO, M.D., Hidekazu HASHIMOTO, M.D.,
and Takayuki ITO, M.D.

SUMMARY

Elevated levels of 1,2-diacylglycerol (DG) have been observed in streptozotocin-induced diabetic and insulin-treated diabetic rat hearts. The fatty acid moieties of 1,2-DG are considered to be related to its ability to activate protein kinase C. Therefore, we determined the fatty acids of 1,2-DG by gas chromatography and compared them with those of triglycerides in the myocardium. The triglyceride content returned to control levels after 4 weeks of untreated diabetes followed by 4 weeks of insulin treatment. There was a significant difference in the fatty acid composition of triglycerides between diabetic and control rats. Insulin treatment also returned the fatty acids of triglycerides in diabetes to the profile observed in control rats. On the other hand, insulin treatment of the diabetic rats did not normalize 1,2-DG content and its fatty acid composition. Fatty acid analysis of 1,2-DG showed that its profile in insulin-treated diabetic rats was different from that of either control or diabetic rats, suggesting that insulin-induced 1,2-DG differs from that seen in cases of diabetes.

Key Words:

1,2-Diacylglycerol Fatty acid compositions Diabetes mellitus
Insulin

1, 2-DIACYLGLYCEROL (DG) is recognized as an intracellular second messenger for signal transduction because of the activation of protein kinase C, which regulates muscle contraction¹⁾ and activates protein synthesis.²⁾ In recent years, we have demonstrated that 1,2-DG contents in the myocardium vary in genetic cardiomyopathy³⁾ and with the aging process.⁴⁾ We also showed that there are higher 1,2-DG levels in streptozotocin-induced diabetic and insulin-treated rat hearts than in control animals.⁵⁾ Therefore,

From the Second Department of Internal Medicine, Nagoya University School of Medicine, Nagoya, Japan.

Mailing address: Kenji Okumura, M.D., Second Department of Internal Medicine, Nagoya University School of Medicine, 65 Tsuruma-cho, Showa-ku, Nagoya 466, Japan.

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in this study we analyzed the fatty acid composition of 1,2-DG and triglycerides in diabetic and insulin-treated diabetic hearts and compared the results with a control.

MATERIALS AND METHODS

Male Wistar rats weighing 200–230 g were divided into 3 groups: control ($n=10$), diabetic ($n=8$; streptozotocin, 65 mg/kg, i.v.), and insulin-treated diabetic animals ($n=7$). All rats had unrestricted access to food and water until they were killed after 8 weeks. Animals in the insulin-treated diabetic group received 3 U of protamine zinc insulin subcutaneously daily for 4 weeks before being killed at 8 weeks. These models were examined for plasma glucose and insulin levels to determine whether the diabetic state was induced and whether the insulin effect was sufficient.

After the animals were anesthetized with ether, the hearts were rapidly excised and washed thoroughly with cold saline. Tissue samples of approximately 60–80 mg were obtained from the left ventricle near the apex and frozen immediately in liquid N_2 . Crude lipids were extracted according to Folch et al,⁶⁾ with some modification after the addition of 0.01% butylated hydroxytoluene as an antioxidant and 0.1 mg of cholesteryl acetate as an internal standard.⁷⁾ In order to eliminate most phospholipids, crude lipid was applied to a silicic acid column and neutral lipids were eluted with 7 ml of chloroform. Following the mass determinations of 1,2-DG and triglyceride by a TLC (thin layer chromatography)/FID (flame ionization detection) method as described previously,⁵⁾ the residual neutral lipids were separated by thin layer chromatography on silica gel plates (20 cm $\times$ 20 cm). The plates were subjected to development twice in a solvent system containing *n*-hexane/diethyl ether/acetic acid (80:35:1). The spots corresponding to 1,2-DG and triglyceride were scraped off and eluted with 1.5 ml of chloroform/methanol (9:1). After the extract was filtered through Whatman GF/CD glass fiber filters and evaporated to dryness with N_2 gas, fatty acids were transmethylated with boron trifluoride-methanol.⁸⁾ The fatty acid composition of 1,2-DG was analyzed by gas chromatography with a flame ionization detector and a silica capillary column of HR 20 M (Shinwakakoh, Kyoto, Japan).

Plasma samples were collected at death and analyzed for glucose by the glucose oxidase method. The results were expressed as the mean $\pm$ SEM, and comparisons between the 2 groups were assessed with a one-way analysis of variance and Duncan's test, except for the fatty acid compositions of 1,2-DG where the Mann-Whitney U-test was used. A *p* value of less than 0.05 was considered to be statistically significant.

RESULTS

The presence of diabetes in streptozotocin-injected rats was confirmed by the increased levels of plasma glucose compared with the control group (183 ± 8 vs. 639 ± 48 mg/dl, $p < 0.01$). Insulin treatment significantly decreased the hyperglycemia seen in the untreated diabetic animals (187 ± 22 mg/dl, $p < 0.01$). The diabetic animals exhibited significantly depressed body weights and ventricular weights compared to the controls after the onset of diabetes (data not shown). Significantly elevated ratios of ventricular weight to body weight were observed in the diabetic animals (2.86 ± 0.03 vs. the control value of 2.36 ± 0.05 mg/g, $p < 0.01$). These changes observed in the diabetic group were reversed by insulin treatment (2.66 ± 0.07 mg/g as ventricular/body weight ratio).

Hearts from the 8-week diabetic animals contained 2.5-fold more triglyceride than did the control animals (control, 1.18 ± 0.08 vs. diabetic, 2.98 ± 0.42 μ g/mg wet wt, Fig. 1). Insulin treatment completely returned the triglyceride content to the control level (1.12 ± 0.14 μ g/mg wet wt). A significant difference in the percentages of fatty acid composition of triglycerides was observed in 14:0, 16:1 and 18:1 between diabetic and control rats (Fig. 2). Arachidonate, expressed as 20:4, also increased in diabetic hearts although the value was not significant. These changes in the fatty acid compositions of triglycerides were almost completely similar to the control fatty acid profile after insulin treatment, including the amount of total triglycerides.

Fig. 1. Triglyceride and 1,2-diacylglycerol contents in control, diabetic, and insulin-treated diabetic rat hearts. Results represent mean $\pm$ SEM obtained from 7–10 rats 8 weeks after streptozotocin injection.

** and ## indicate $p < 0.05$ compared to control and diabetic rats, respectively.

On the other hand, the 1,2-DG content determined in the myocardium of diabetic rats was 68.2 ± 2.1 ng/mg wet wt, a value that was significantly higher than that in the control rats (57.0 ± 1.7 ng/mg wet wt, Fig. 1). However, it should be noted that insulin treatment in the diabetic rats did not normalize

Fig. 2. Fatty acid compositions of triglycerides in control, diabetic, and insulin-treated diabetic rat hearts. Results represent mean $\pm$ SEM obtained from 6–8 rats 8 weeks after streptozotocin injection. The fatty acids are expressed as % area of chromatograms.

* and ** indicate $p < 0.05$ and $p < 0.01$, respectively, compared to control rats by the Mann-Whitney U-test.

and ## indicate $p < 0.05$ and $p < 0.01$, respectively, compared to diabetic rats by the Mann-Whitney U-test.

Fig. 3. Fatty acid compositions of 1,2-diacylglycerol in control, diabetic, and insulin-treated diabetic rat hearts. Results represent mean $\pm$ SEM obtained from 6–8 rats 8 weeks after streptozotocin injection. The fatty acids are expressed as % area of chromatograms.

* and ** indicate $p < 0.05$ and $p < 0.01$, respectively, compared to control rats by the Mann-Whitney U-test.

and ## indicate $p < 0.05$ and $p < 0.01$, respectively, compared to diabetic rats by the Mann-Whitney U-test.

the 1,2-DG content in the myocardium (68.2 ± 3.1 ng/mg wet wt). Analysis of the fatty acid composition of 1,2-DG revealed differences from those of triglycerides. 1,2-DG contained greater percentages of 18:2 and 22:6, and less 16:1 than triglycerides (Figs. 2 and 3). The 1,2-DG of diabetic animals showed significantly lower percentages of 14:0 and 20:4 than the controls, but the differences were small. Although the total content of myocardial 1,2-DG in insulin-treated diabetic rats was as high as in diabetic rats, the profile of fatty acid composition seemed to be different from that of diabetic rats as well as that of the control animals (Fig. 3).

DISCUSSION

Diabetic animals show increased plasma triglyceride and cholesterol concentrations, and insulin treatment significantly lowers levels of both triglyceride and cholesterol.⁹⁾ Cardiomyopathy associated with diabetes appears to be related to elevated circulating lipid concentrations and myocardial cholesterol and triglyceride content.¹⁰⁾ Moreover, it is known that a drug which has been proven to prevent the development of diabetic cardiomyopathy also reduces the accumulation of circulating lipids, but does not affect blood glucose levels in experimental diabetes.¹¹⁾ 1,2-DG is a distinct lipid in tissues where it plays the role of an activator of protein kinase C. Furthermore, this lipid is a central intermediary metabolite in phospholipid and triglyceride syntheses and a potential source of arachidonate, the precursor of prostaglandins.¹²⁾ Therefore, changes in 1,2-DG content in the tissue may imply disorders in intracellular biochemical activity under pathological conditions. We observed that 1,2-DG contents are at higher levels in both diabetic and insulin-treated diabetic rat hearts than in controls. Insulin is known to be a stimulant of 1,2-DG production. In response to many hormones and neurotransmitters the 1,2-DG signal can be generated by the hydrolysis of phosphoinositides.¹³⁾ However, 1,2-DG accumulation is likely to result from the degradation of other phospholipids as well.^{14),15)} Farese et al suggested that insulin increases 1,2-DG in myocytes both by increases in *de novo* phosphatidic acid synthesis and by hydrolysis of non-inositol-containing phospholipids such as phosphatidylcholine and phosphatidylethanolamine.¹⁶⁾ Our analysis of 1,2-DG fatty acid composition suggests different sources for the generation of this lipid between insulin-treated diabetic hearts and diabetic hearts or controls, although the implication of the fatty acid species involved in 1,2-DG has not been well explored. The elevated levels of 1,2-DG might be due mostly to the insulin treatment. Therefore, 1,2-DG content was not normalized by daily insulin injections in spite of evidence

that diabetic cardiomyopathy induced by streptozotocin was improved with insulin treatment. A study using lipids labeled with [H^3] fatty acids showed that 1,2-DG with 14:0 was rapidly produced and that 1,2-DG with 20:4 was released in response to insulin stimulation,¹⁵⁾ suggesting that insulin increases 1,2-DG by two or more mechanisms.^{15),17),18)} The fact that there was no difference in the fatty acid profiles of triglycerides between control and insulin-treated diabetic hearts appears to favor our hypothesis. Although we also conducted an analysis of the fatty acid composition of phospholipid species, these results did not help us to clarify the sources of 1,2-DG production. At present, the implications of elevated 1,2-DG with different fatty acid profiles in insulin-treated diabetic rats remain unexplored.

REFERENCES

1. Vivaudou MB, Clapp LH, Walsh JV Jr, Singer JJ: Regulation of one type of Ca^{2+} current in smooth muscle cells by diacylglycerol and acetylcholine. *FASEB J* **2**: 2497, 1988
2. Henrich CJ, Simpson PC: Differential acute and chronic response of protein kinase C in cultured neonatal rat heart myocytes to α_1 -adrenergic and phorbol ester stimulation. *J Mol Cell Cardiol* **20**: 1081, 1988
3. Okumura K, Yamada Y, Kondo J, Ishida A, Hashimoto H, Ito T, Ogawa K, Kitoh J: Increased 1,2-diacylglycerol content in myopathic hamster hearts at a preneurotic stage. *Life Sci* **43**: 1371, 1988
4. Okumura K, Yamada Y, Kondo J, Hashimoto H, Ito T, Ogawa K: Alteration in 1,2-diacylglycerol level and its fatty acid composition in hearts during the growth of hamsters. *Bas Res Cardiol* **85**: 164, 1990
5. Okumura K, Akiyama N, Hashimoto H, Ogawa K, Satake T: Alteration of 1,2-diacylglycerol content in myocardium from diabetic rats. *Diabetes* **37**: 1168, 1988
6. Folch J, Lees M, Sloane-Stanley GH: A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* **226**: 497, 1957
7. Okumura K, Hashimoto H, Ito T, Ogawa K, Satake T: Quantitation of 1,2-diacylglycerol in rat heart by Iatroscan TLC/FID. *Lipids* **23**: 253, 1988
8. Morrison WR, Smith LH: Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. *J Lipid Res* **5**: 600, 1964
9. Bhimji S, Godin DV, McNeill JH: Insulin reversal of biochemical changes in hearts from diabetic rats. *Am J Physiol* **251**: H670, 1986
10. Fields LE, Daugherty A, Bergmann SR: Effects of fatty acid on performance and lipid content of hearts from diabetic rabbits. *Am J Physiol* **250**: H1079, 1986
11. Tahiliani GA, McNeill JH: Diabetes-induced abnormalities in the myocardium. *Life Sci* **38**: 959, 1986
12. Natarajan R, Stern N, Nadler J: Diacylglycerol provides arachidonic acid for lipoxygenase products that mediate angiotensin II-induced aldosterone synthesis. *Biochem Biophys Res Commun* **156**: 717, 1988
13. Nishizuka Y: Turnover of inositol phospholipids and signal transduction. *Science* **225**: 1365, 1984
14. Besterman JM, Duronio V, Cuatrecasas P: Rapid formation of diacylglycerol from phosphatidylcholine: a pathway for generation of a second messenger. *Proc Natl Acad Sci USA* **83**: 6785, 1986
15. Saltiel AR, Sherline P, Judith AF: Insulin-stimulated diacylglycerol production results from

- the hydrolysis of a novel phosphatidylinositol glycan. *J Biol Chem* **262**: 1116, 1987
16. Farese RV, Cooper DR, Konda TS, Nair G, Standaert ML, Davis JS, Pollet RJ: Mechanisms whereby insulin increases diacylglycerol in BC3H-1 myocytes. *Biochem J* **256**: 586, 1987
 17. Farese RV, Konda TS, Davis JS, Standaert ML, Pollet RJ, Cooper DR: Insulin rapidly increases diacylglycerol by activating de novo phosphatidic acid synthesis. *Science* **236**: 586, 1987
 18. Farese RV, Barnes DE, Davis JS, Standaert ML, Pollet RJ: Effects of insulin and protein synthesis inhibitors on phospholipid metabolism, diacylglycerol levels, and pyruvate dehydrogenase activity in BC3H-1 cultured myocytes. *J Biol Chem* **259**: 7094, 1984