Electron Microscopy Revealed Massive Lipid Droplets in Cardiomyocytes in a Patient with Cardiogenic Shock Following a Fulminant Type 1 Diabetes Mellitus

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Summary

A 52-year-old man with consciousness disorder following a 2-day history of general fatigue, diarrhea, vomiting and excessive thirst was admitted to our hospital. Severe hyperglycemia (1,739 mg/dL) with a slightly elevated HbA1c level (6.9%), ketonuria and low C-peptide level (0.07 ng/mL) confirmed the diagnosis of fulminant type 1 diabetes mellitus (FT1DM). Following sudden unexplained cardiogenic shock shortly after the initiation of insulin therapy with no evidence of myocardial ischemia assessed by coronary angiography, the patient was supported with percutaneous venoarterial extracorporeal membrane oxygenation. Electron microscopic analysis of the myocardium revealed massive lipid droplets without the infiltration of inflammatory cells. His left ventricular function began to recover during the following days and returned to a normal level on day 14. Currently, the impact of FT1DM on intramyocardial lipid deposition is poorly understood. However, this case suggests that even short-term exposure to high concentrations of glucose can be responsible for lipotoxicity followed by severe cardiac dysfunction.

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Key words: Lipotoxicity, Cardiac dysfunction, Diabetic cardiomyopathy

Fulminant type 1 diabetes mellitus (FT1DM) is a subtype of type 1 diabetes mellitus characterized by the abrupt onset of severe hyperglycemia leading to diabetic ketoacidosis without the presence of islet-related autoantibodies.¹⁾ Previous studies have demonstrated that in rare instances, FT1DM is accompanied by transient cardiac dysfunction diagnosed as myocarditis.²⁻⁵⁾ Viruses with overlapping infectivity in both the islet and myocardium were hypothesized to contribute to this simultaneous injury.⁶⁾ However, it is noteworthy that cardiac abnormalities did not precede the onset of FT1DM and were substantially displayed shortly after admission in these cases. There has been no mechanistic explanation for this stereotyped order of clinical onsets from the viewpoint of an infectious cause.

It has been recognized that one of the major mechanisms of diabetes-induced cardiac dysfunction is a maladaptive metabolic response in cardiomyocytes.⁷⁻⁹⁾ Long-term exposure to high glucose concentrations is responsible for myocardial lipid overload independent of the concentration of blood lipids that leads to non-ischemic cardiomyopathy, a phenomenon known as lipotoxicity.⁹⁻¹²⁾ How-

ever, the impact of FT1DM, which involves abrupt and short-term hyperglycemic crises, on intramyocardial lipid deposition with severe cardiac dysfunction remains to be fully elucidated.

Case Report

An otherwise healthy 52-year-old man was brought to our emergency department because of consciousness disorder. He had a 2-day history of general fatigue, diarrhea and vomiting and complained of excessive thirst. There was no family history of note. On admission, his heart rate was 68 beats/min with a blood pressure of 91/70 mmHg. Arterial blood gas analysis showed pH 7.123, pCO₂ 22.8 mmHg, pO₂ 78.1 mmHg, O₂ saturation 92.5%, and bicarbonate 7.2 mmol/L. Blood tests showed severe hyperglycemia (a blood glucose of 1,739 mg/dL). However, the HbA1c levels remained slightly elevated (6.9%), which indicated a short-term exposure to hyperglycemia. Autoantibodies against glutamic acid decarboxylase and islet antigen-2 were negative. Pancreatic amylase serum levels were slightly elevated (85 U/L), but abdominal

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Figure 1. Transthoracic echocardiographic images on days 0 and 14. **A:** Images on day 0 show left ventricular contractile dysfunction. No findings suggestive of myocardial edema or epicardial effusion were detected. **B:** Images on day 14 show improvement in left ventricular contractile function.

Figure 2. Cardiac biopsy findings. **A, B:** Hematoxylin and eosin staining. Small vacuoles are observed in the cytoplasm of cardiomyocytes. Bar = 50 μ m (**A**), 20 μ m (**B**). **C, D:** Immunohistochemical staining with a CD45RB antibody. Bar = 100 μ m (**C**), 50 μ m (**D**). **E:** Electron microscopic image. Massive lipid droplets are identified in the cytoplasm of cardiomyocytes. Bar = 5 μ m. **F:** Immunohistochemical staining with an adipose triglyceride lipase (ATGL) antibody. ATGL expression is confirmed in the cytoplasm of cardiomyocytes. Bar = 50 μ m.

computed tomography revealed no evidence of pancreatic diseases. Blood ketones were found to be elevated at 17.4 mmol/L, and urinalysis showed ketonuria. Serum peptide levels were also low (0.07 ng/mL). These findings confirmed the diagnosis of FT1D. An initial electrocardiogram (ECG) demonstrated no evidence of myocardial ischemia.

One hour after he was started on a continuous insulin infusion, ECG monitoring showed bradycardia accompanied by frequent pauses, and ventricular stimulation by external pacemakers was introduced. Transthoracic echocardiography (TTE) showed that left ventricle contraction remained within normal limits in this instance. The blood glucose level decreased to 639 mg/dL during the first 18 hours. However, it was becoming more difficult to maintain normal blood pressure despite fluid resuscitation and combined inotropic/vasopressor therapy. TTE at that time

revealed severe cardiac dysfunction [left ventricular end-diastolic diameter (LVDd)/left ventricular end-systolic diameter (LVDs), 48/44 mm and left ventricular ejection fraction (LVEF), 16%], which indicated cardiogenic shock (Figure 1A). Mechanical circulatory support with percutaneous venoarterial extracorporeal membrane oxygenation and intra-aortic balloon pumping were initiated. Although laboratory findings showed enhanced levels of creatine kinase (CK) (2,833 IU/L), CK-MB isoenzyme (83 IU/L) and troponin-I (11,383 pg/mL), coronary angiography did not show any evidence of coronary artery disease.

Hematoxylin and eosin staining (Figure 2A and B) and immunohistochemical staining with a CD45RB antibody (Figure 2C and D) of endomyocardial biopsy samples (a total of 4 specimens) revealed small vacuoles in the cytoplasm of cardiomyocytes and only a minority of inflammatory cell infiltration without edema or fibrosis in

the myocardium. Electron microscopy analyses of the myocardium showed massive lipid droplets in the cytoplasm of cardiomyocytes (Figure 2E). Immunohistochemical studies confirmed the presence of adipose triglyceride lipase (ATGL) (Figure 2F). The peak levels of CK, CK-MB, troponin-I and B-type natriuretic peptide during the course of illness were 3,221 IU/L on day 3, 89 IU/L on day 1, 24,020 pg/mL on day 4, and 371.9 pg/mL on day 2, respectively.

Left ventricular systolic function began to recover during the following days, and the patient was successfully weaned off mechanical circulatory support on day 9. TTE revealed that left ventricular function returned to a normal level on day 14 (LVDd/LVDs, 43/25 mm and LVEF, 73%; Figure 1B).

Discussion

The main significant finding of this case report is that massive lipid droplets in cardiomyocytes are present in the acute phase of FT1D complicated by the abrupt and serious but completely reversible cardiogenic shock. A general model for the development of non-ischemic diabetic cardiomyopathy includes the excessive reliance on fatty acids (FAs) for energy production leading to the accumulation of lipids in the heart (so-called lipotoxicity).¹³⁻¹⁵⁾ At present, it remains unknown whether cardiac steatosis leading to massive lipid droplets emerged as an acute phase response to FT1DM. Another point to note is that cardiac function deteriorated shortly after the initiation of insulin therapy and was not present on admission. Developments leading up to the fulminant heart failure occurred during the first several days after admission, showing that our case is consistent with previous reports of FT1D complicated by myocarditis.²⁻⁵⁾ However, pathological findings in our case ruled out the possibility of inflammatory tissue injury. Based on these observations, metabolic abnormalities may be implicated as a leading cause of the myocardiopathy.

The abundant neutral lipid droplets in cardiomyocytes is a hallmark of triglyceride deposit cardiomyovasculopathy (TGCV), a chronic systemic disease that presents as severe cardiac dysfunction often requiring subsequent heart transplantation.^{16,17)} A prominent feature of its pathophysiology is the disruption of long-chain fatty acid (LCFA) metabolism, which is caused by abnormalities in ATGL or other functional components of metabolic pathways.^{18,19)} A histopathological examination of this patient excluded the possibility of primary TGCV caused by a genetic deficiency in ATGL. Conversely, idiopathic TGCV, which shows abnormal LCFA metabolism without ATGL gene-deficiency, is thought to be a possible cause of the combination of severe cardiac dysfunction and abnormal accumulation of intramyocardial lipid droplets.²⁰⁾ However, the specific clinical characteristics of TGCV, including slow-progressive and irreversible cardiac dysfunction and the development of diffuse coronary arteriosclerosis, differ from our case in many ways²¹⁾.

Diabetes mellitus is associated with cardiac dysfunction, even in the absence of coronary atherosclerosis. The incorporation of excess free FAs into the myocardium

promotes oxidative stress, insulin resistance and mitochondrial dysfunction through an increase in lipotoxic intermediates, such as ceramide and diacylglycerol.²²⁾ Previous studies have demonstrated that the upregulated gene expression of peroxisome proliferator-activated receptor (PPAR) alpha, which is responsible for myocardial fatty acid use, leads to enhanced lipotoxicity in diabetic cardiomyopathy.²³⁾ However, for nondiabetic patients, there were inconsistent associations between PPAR alpha expression levels and cardiac dysfunction; therefore, it is unclear how the regulation of FA metabolism contributes to the pathogenesis of heart failure.²⁴⁾

Current evidence does not provide an answer to the fundamental question of why cardiac dysfunction appeared at a rapid rate immediately after the start of treatment. In our case, a distinctive feature of the metabolic aspect is the drastic switching of energy substrates between FAs and glucose during the clinical course. Accordingly, it is assumed that the unsuccessful modulation of energy use is a potential mechanism of FT1DM-associated abrupt deterioration in myocardial function. FT1DM is characterized by rapid and severe depletion of insulin, indicating glucose starvation followed by significant inhibition of glucose metabolic pathways in cardiomyocytes via the Randle cycle.^{1,25)} A key step in the alteration of intracellular metabolic regulation triggered by insulin reintroduction is the rapid stimulation of glucose transporter 4 translocation and subsequent extensive glucose influx into cardiomyocytes within 30 minutes of insulin administration.²⁶⁾ Under the condition of impaired glucose oxidation, a high rate of glucose uptake may overwhelm the rate of glucose oxidation and lead to the accumulation of glycolytic intermediates and advanced glycation end-product-induced reactive oxygen species involved in the initiation of apoptosis.²⁷⁾ Furthermore, it is noteworthy that subsequent high concentrations of intracellular glucose also provoke the inhibition of FA oxidation in the heart through the repression of PPAR alpha and related genes, suggesting the complete deprivation of principal energy sources.²⁸⁾ This hypothesis for metabolic adaptation is supported by a previous study demonstrating fatal cardiac injury and accumulated neutral lipids in cardiomyocytes in conditional PPAR alpha-knockout mice during fasting.²⁹⁾

The main limitations of this case report include the lack of histological follow-up, not feasible for ethical reasons, and certification for improvement of cardiac lipid metabolism. Further studies are needed to elucidate whether the myocardial lipid accumulation is reduced in accordance with recovery of cardiac function. Previous experiments had suggested substantial influence of systemic or local inflammatory response on myocardial lipid accumulation.¹³⁾ Severe cardiac dysfunction itself is also positively associated with lipid droplets in the electron microscopy of the cardiomyocytes. A wide variety of diseased conditions would be taken into consideration in the pathogenesis of this case.

The elevation of serum enzymes including CK, CK-MB and troponin-I seems inconsistent with a lack of histopathological features of myocyte necrosis. However, CK-MB is a very low percentage of total CK in our case, which indicate high probabilities of skeletal muscle dam-

age evoked by diabetic ketoacidosis. Furthermore, the cellular pathophysiology of cardiac troponin release includes enhanced cell permeability followed by the extrusion from reversibly injured myocytes in the absence of cell death.³⁰⁾

Conclusion

In this report, we presented a case of FT1DM followed by the abrupt onset of severe transient cardiac dysfunction and massive lipid droplets in cardiomyocytes, a characteristic pathological feature common to TGCv, detected in a heart biopsy sample. Without follow-up histopathological examinations of the myocardium in the convalescent phase, we can only make inferences about whether lipid droplet formation is coincident with the onset of severe contractile dysfunction in our case. Moreover, the causal relationship between lipid deposition and cardiac dysfunction also remains uncertain. However, our observation provides the concept of metabolic maladaptation associated with insulin-mediated regulation of energy substrate use among patients with FT1DM.

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Disclosure

Conflicts of interest: The authors declare that there is no conflict of interest.

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