News Release

Title

LPL/AQP7/GPD2 promotes glycerol metabolism under hypoxia and prevents cardiac dysfunction during ischemia

Key Points

- **Cardiovascular diseases, such as myocardial infarction, remains the leading cause of mortality worldwide.**
- **The mechanism underlying the myocardial metabolic shift during ischemia remains unknown.**
- **Glycerol is a substrate for energy production in cardiomyocytes under hypoxic conditions.**
- **LPL/AQP7/GPD2-mediated glycerol metabolism plays an important role in preventing myocardial ischemia-related damage makes this glycerol pathway a promising target for therapeutic intervention in patients with acute MI.**

Summary

In the heart, fatty acid is a major energy substrate to fuel contraction under aerobic conditions. Ischemia downregulates fatty acid metabolism to adapt to the limited oxygen supply, making glucose the preferred substrate. However, the mechanism underlying the myocardial metabolic shift during ischemia remains unknown. Here, we show that lipoprotein lipase (LPL) expression in cardiomyocytes, a principal enzyme that converts triglycerides to free fatty acids and glycerol, increases during myocardial infarction (MI). Cardiomyocyte-specific LPL deficiency enhanced cardiac dysfunction and apoptosis following MI. Deficiency of aquaporin 7 (AQP7), a glycerol channel in cardiomyocytes, increased the myocardial infarct size and apoptosis in response to ischemia. Ischemic conditions activated glycerol-3-phosphate dehydrogenase 2 (GPD2), which converts glycerol-3-phosphate into dihydroxyacetone phosphate to facilitate adenosine triphosphate (ATP) synthesis from glycerol. Conversely, GPD2 deficiency exacerbated cardiac dysfunction after acute MI. Moreover, cardiomyocyte-specific LPL deficiency suppressed the effectiveness of peroxisome proliferator-activated receptor alpha (PPARα) agonist treatment for MI-induced cardiac dysfunction. These results suggest that LPL/AQP7/GPD2-mediated glycerol metabolism plays an important role in preventing myocardial ischemia-related damage

Research Background

The heart has a high adenosine triphosphate (ATP) demand to sustain contractile activity and maintain tissue perfusion. Approximately 60%–90% of cardiac ATP is produced via the oxidation of fatty acids, whereas the remaining 10%–40% is from the oxidation of glucose, lactate, ketone bodies and amino acids. Thus, although fatty acids constitute the predominant

substrate for energy in the heart, the cardiac metabolic network is highly flexible in terms of using other substrates depending on physiological and pathological stress, such as exercise, pregnancy, myocardial infarction (MI), and heart failure. The reciprocal relationship between fatty acid and glucose metabolism was first described in the 1960s. Specifically, the increased generation of acetyl CoA derived from fatty acid oxidation decreases glucose oxidation in the heart by inhibiting pyruvate dehydrogenase, the enzyme that catalyzes pyruvate decarboxylation, which is a key irreversible step in glucose oxidation. Conversely, increased acetyl CoA generation from glucose oxidation hinders fatty acid oxidation by suppressing carnitine palmitoyltransferase 1, which enhances fatty acid transport into the mitochondria. MI, which is defined as myocardial cell death owing to prolonged ischemia, remains the leading cause of mortality worldwide. The onset of myocardial ischemia results from an imbalance between oxygen supply and demand. It is generally accepted that under hypoxic conditions, cardiac metabolism shifts from fatty acids to glucose, which is more efficient with respect to ATP production per O2 consumed. Accumulating evidence suggests that modulating cardiac energy metabolism by increasing glucose oxidation and decreasing fatty acid oxidation can improve cardiac function in heart diseases ; however, various factors increase the concentration of plasma free fatty acids (FFA), such as the hormonal state in response to myocardial ischemia. This contradiction between the detrimental effects of fatty acid oxidation and increased plasma FFA levels in MI suggests that lipoprotein lipase (LPL), which is the principal enzyme that converts triglycerides in the circulation to FFA, mediates other metabolic pathways in MI. LPL is produced from cardiomyocytes, skeletal muscles and adipose tissues to control local fatty acid uptake, and a genetic study indicates that LPL activation reduces the risk of coronary artery disease. Glycerol is generated during the LPL-catalyzed breakdown of the triglyceride component of lipoproteins to provide fatty acids. Aquaporin 7 (AQP7) is an aquaglyceroporin that facilitates glycerol transport across cell membranes into the heart. AQP7 deficiency reduces glycerol uptake in the heart and exacerbates pressure overload-induced heart failure; however, the role of glycerol as a substrate for energy production in cardiomyocytes under hypoxic conditions remains unclear.

Research Results

To investigate whether acute MI increases cardiac LPL expression, the left coronary artery in wild-type (WT) mice was ligated. LPL was assayed by immunostaining of sections using an antibody against LPL at 1 h after the ligation. MI increased the intensity of LPL in the infarct area, suggesting that the ischemic conditions enhanced LPL expression in vivo. To investigate whether hypoxic conditions affect LPL expression on isolated adult murine cardiomyocytes, LPL expression on these cells under 1-h of culture under hypoxic conditions was determined by immunostaining. Hypoxic conditions significantly increased the expression level of LPL on the cardiomyocyte surface, suggesting that hypoxia enhanced cardiac LPL expression. We also examined LPL activity in the culture medium, and our findings showed that hypoxic conditions significantly increased LPL activity. To examine the functional significance of cardiac LPL

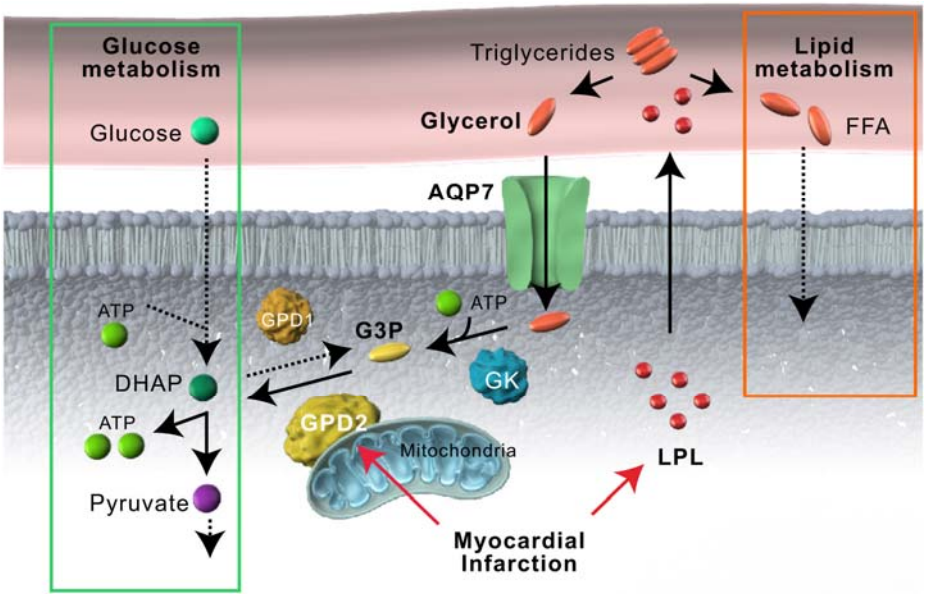
under ischemic conditions in vivo, we used mice with tamoxifen-inducible cardiomyocyte-specific deficiency for LPL (cmc-LPL KO). No differences were observed in basal cardiac function between cmc-LPL WT and cmc-LPL KO mice. Notably, cardiomyocyte-specific LPL deficiency suppressed MI induced LPL expression in the heart, suggesting that LPL is synthesized in cardiomyocytes under ischemic conditions. We performed echocardiography to assess cardiac functionality at 1 day after left coronary artery ligation. In control mice, 1-day coronary artery ligation resulted in significantly decreased cardiac function, with cardiac LPL deficiency exacerbating the MI-induced cardiac dysfunction. To examine whether cardiac LPL deficiency affects cardiac apoptosis induced by ligation, apoptotic cardiomyocytes were assessed by TUNEL staining of heart sections at 1 day after the ligation. LPL deficiency increased MI-induced apoptosis.

As glycerol is produced when LPL converts triglycerides to FFA, we next investigated whether the treatment with glycerol increased cardiomyocyte cell viability under hypoxic conditions. Isolated adult murine cardiomyocytes were incubated with glycerol and exposed to hypoxic conditions. After 4-h of exposure to normoxia or hypoxia, we identified damaged cardiomyocytes using trypan blue vital staining. Glycerol treatment increased cardiomyocyte cell viability under normoxic conditions in a dose-dependent manner; however, this effect was enhanced under hypoxic conditions. In addition, we confirmed that glycerol concentration decreased in the heart of cmc-LPL KO mice. AQP7 is expressed in adipose tissues, skeletal muscles, and the heart, and serves as a glycerol channel. To investigate whether the protective effect of glycerol under cardiac hypoxia requires AQP7, cardiomyocytes were isolated from AQP7+/+ and AQP7−/− mice. No differences were observed in cell viability between AQP7+/+ and AQP7−/− mice in the absence of glycerol; however, AQP7 deficiency decreased glycerol increased related cardiomyocyte cell viability. Moreover, AQP7 deficiency decreased glycerol concentration in the heart. To examine whether AQP7 deficiency increased MI-induced cardiac apoptosis, apoptotic cardiomyocytes were identified by TUNEL staining of heart sections at 1 day after left coronary artery ligation. No difference in apoptosis was observed between AQP7+/+ and AQP7−/− mice, but AQP7 deficiency significantly increased MI-induced apoptosis. To determine whether glycerol attenuates the progression of MI-induced cardiac dysfunction in mice, infarct changes were ascertained in PicroSirius red-stained sections at 7 days after the ligation. AQP7 deficiency increased infarct area in the heart. We also performed echocardiography to assess cardiac functionality. In control mice, 7-day MI resulted in a significantly decreased fractional shortening, with the response enhanced in AQP7−/− mice. In addition, atrial natriuretic peptide (ANP) in the heart, which is increased upon cardiac dysfunction, was elevated in AQP7−/− mice at 7 days after ligation.

After cells capture glycerol, glycerol kinase catalyzes the phosphorylation of glycerol to yield glycerol-3-phosphate (G3P). Glycerol kinase expression is considered to be restricted to the liver, kidney, and skeletal muscle. To examine Gk gene expression in the heart, we performed qPCR analysis of various murine tissues, which revealed Gk was also expressed in the heart. In turn, glycerol-3-phosphate dehydrogenase 2 (GPD2), which is anchored to the mitochondrial

membrane, oxidizes G3P to dihydroxyacetone phosphate (DHAP) in the cytoplasm. To examine GPD2 enzymatic activity on cardiac mitochondria, mitochondria were isolated from the heart; they exhibited GPD2 activity in vitro. Next, as Ca2+ increases GPD2 activity on the mitochondria in the liver, but not in the brain of rats, cardiac mitochondria were incubated with a high concentration of Ca2+. This increased GPD2 activity, whereas treatment with EDTA suppressed Ca2+-induced GPD2 activation, suggesting that the high Ca2+ concentration enhances GPD2 activity in the heart. To examine whether MI affects GPD2 enzymatic activity in the heart, mitochondria were isolated from the heart at 1 h after sham surgery or coronary artery ligation. Ischemic conditions significantly increased cardiac GPD2 enzymatic activity. Next, we examined whether glycerol is involved in energy metabolism in cardiomyocytes. As 4-h hypoxia was shown to decrease cardiomyocyte cell viability, we examined intracellular ATP concentrations in isolated adult murine cardiomyocytes after 1-h of culture under hypoxic condition to avoid the effects of cell viability on ATP production. Notably, 1-h hypoxia did not affect cell viability, and no difference between control and glycerol treatment was observed. Conversely, 1-h hypoxia significantly decreased ATP production in cardiomyocytes. Although glycerol treatment did not increase ATP production under normoxic conditions, glycerol-mediated ATP production was significantly increased under hypoxic conditions. In comparison, glycerol-dependent ATP production under early hypoxic conditions was suppressed by the GPD2 inhibitor in a dose-dependent manner, whereas significant effects on ATP production under normoxic conditions were not detected. The GPD2 inhibitor did not significantly decrease ATP production in the absence of glycerol. To further evaluate the role of GPD2 in vivo, we performed coronary artery ligation in GPD2+/+ and GPD2−/− mice. In control mice, cardiac dysfunction was observed at 2 and 4 weeks after ligation. GPD2 deficiency significantly exacerbated MI-induced cardiac dysfunction and increased infarct area in the heart.

We next investigated whether the activation of LPL/AQP7/GPD2-mediated glycerol metabolism would attenuate the progression of MI-induced cardiac dysfunction. For this, GPD2+/+ and GPD2−/− mice were orally administered glycerol at 0, 8, and 16 h after coronary artery ligation. In control mice, glycerol treatment significantly suppressed MI-induced cardiac dysfunction. GPD2 deficiency resulted in reduced therapeutic response. Finally, to examine whether peroxisome proliferator activated receptor alpha (PPARα), which increases the hydrolysis of plasma triglycerides due to induction of LPL expression in the liver and adipose tissues, mediated cardiac glycerol metabolism under ischemic conditions, oral treatment with fenofibrate, a PPARα agonist, was initiated 5 days before coronary artery ligation in cmc-LPL WT and cmc-LPL KO mice. Treatment with the PPARα agonist protected mice from MI-induced cardiac dysfunction, and cardiomyocyte-specific LPL deficiency suppressed the therapeutic effect of the PPARα agonist. These findings suggest that LPL/AQP7/GPD2-mediated glycerol metabolism in the heart attenuates cardiac dysfunction during acute MI.



Research Summary and Future Perspective

Our findings revealed that MI-induced an increase in LPL expression in the heart, preventing myocardial ischemia. AQP7 acts as a glycerol channel in cardiomyocytes during MI. GPD2 increases ATP synthesis from glycerol under hypoxic conditions. The discovery that LPL/AQP7/GPD2-mediated glycerol metabolism plays an important role in preventing myocardial ischemia-related damage makes this glycerol pathway a promising target for therapeutic intervention in patients with acute MI.

Publication

FASEB J. 2021 Dec;35(12): e22048.
https://doi.org/10.1096/fj.202100882R

Japanese ver.
https://www.med.nagoya-u.ac.jp/medical_J/research/pdf/Fas_Jou_20211122.pdf