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Perilipin 5 is protective in the ischemic heart

Christina Drevinge [a,b], Knut T. Dalen [c,d,e], Maria Nastase Mannila [h], Margareta Scharin Täng [a,b], Marcus Ståhlman [a,b], Martina Klevstig [a,b], Annika Lundqvist [a,b], Ismena Mardani [a,b], Fred Haugen [i], Per Fogelstrand [a,b], Martin Adiels [a,b], Jorge Asin-Cayuela [j], Charlotte Ekestam [h], Jesper R. Gådin [h], Yun K. Lee [e], Hilde Nebb [d], Sara Svedlund [a,b], Bengt R. Johansson [g], Lillemor Mattsson Hultén [a,b], Stefano Romeo [a,b], Björn Redfors [a,b], Elmir Omerovic [a,b], Max Levin [a,b], Li-Ming Gan [a,b,f], Per Eriksson [h], Linda Andersson [a,b], Ewa Ehrenborg [h], Alan R. Kimmel [e], Jan Borén [a,b,1], Malin C. Levin [a,b,*,1]

[a] Department of Molecular and Clinical Medicine, Institute of Medicine, University of Gothenburg and Sahlgrenska University Hospital, Gothenburg, Sweden
[b] Wallenberg Laboratory, Institute of Medicine, University of Gothenburg and Sahlgrenska University Hospital, Gothenburg, Sweden
[c] Norwegian Transgenic Center, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway
[d] Department of Nutrition, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway
[e] Laboratory of Cellular and Developmental Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, USA
[f] AstraZeneca R&D, Mölndal, Sweden
[g] Institute of Biomedicine, Department of Medical Biochemistry and Cell Biology, University of Gothenburg, Gothenburg, Sweden
[h] Atherosclerosis Research Unit, Center for Molecular Medicine, Karolinska University Hospital, Stockholm, Sweden
[i] Department of Physiology, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway
[j] Department of Clinical Chemistry, University of Gothenburg, Gothenburg, Sweden

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ABSTRACT

Background: Myocardial ischemia is associated with alterations in cardiac metabolism, resulting in decreased fatty acid oxidation and increased lipid accumulation. Here we investigate how myocardial lipid content and dynamics affect the function of the ischemic heart, and focus on the role of the lipid droplet protein perilipin 5 (Plin5) in the pathophysiology of myocardial ischemia.

Methods and results: We generated *Plin5*$^{-/-}$ mice and found that Plin5 deficiency dramatically reduced the triglyceride content in the heart. Under normal conditions, *Plin5*$^{-/-}$ mice maintained a close to normal heart function by decreasing fatty acid uptake and increasing glucose uptake, thus preserving the energy balance. However, during stress or myocardial ischemia, Plin5 deficiency resulted in myocardial reduced substrate availability, severely reduced heart function and increased mortality. Importantly, analysis of a human cohort with suspected coronary artery disease showed that a common noncoding polymorphism, rs884164, decreases the cardiac expression of *PLIN5* and is associated with reduced heart function following myocardial ischemia, indicating a role for Plin5 in cardiac dysfunction.

Conclusion: Our findings indicate that Plin5 deficiency alters cardiac lipid metabolism and associates with reduced survival following myocardial ischemia, suggesting that Plin5 plays a beneficial role in the heart following ischemia.

1. Introduction

Myocardial ischemia and ischemic heart disease are among the main causes of mortality worldwide. In the search for novel treatment strategies to improve cardiac function and outcome in patients with ischemic heart disease, researchers have extensively investigated metabolic abnormalities of the failing ischemic heart [1]. These abnormalities are characterized by reduced energy-efficient lipid oxidation [2,3], increased accumulation of myocardial lipids [4–6] and enhanced utilization of glucose for energy production [7–9]. It is unclear how myocardial lipid content and dynamics affect the function of the failing ischemic heart, but the balance between lipid utilization and storage appears to be crucial [10,11].

Myocardial lipids are stored in cytosolic droplets. These highly dynamic organelles consist of a core of neutral lipids surrounded by a complex surface containing numerous proteins of importance for the formation, stability, and trafficking of lipid droplets in the cell [12]. In

* Corresponding author at: Wallenberg Laboratory, Sahlgrenska University Hospital, 413 45 Gothenburg, Sweden.
E-mail address: malin.levin@wlab.gu.se (M.C. Levin).
[1] These authors are co-senior authors.

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addition, these proteins have been implicated in the regulation of cellular energy dynamics (*e.g.*, in mediating the recruitment of mitochondria to lipid droplets during conditions of high substrate availability) [13,14]. The lipid droplet protein perilipin 5 (Plin5), which is highly expressed in the heart and other oxidative tissues [15,16], has attracted interest because of its potential role in cardiac function. Plin5 stabilizes lipid droplets and may facilitate the association between lipid droplets and mitochondria [14]. However, *in vivo* studies of Plin5 deficiency have so far been elusive, revealing only minor effects on heart function [16–18]. It remains to be determined whether Plin5 plays a key role for pathophysiology of myocardial ischemia.

Here, we used *Plin5*$^{-/-}$ mice to investigate the role of Plin5 in myocardial lipid dynamics, cardiac function, and outcome after myocardial ischemia. We found that *Plin5* deficiency resulted in reduced cardiac lipid content, increased glucose uptake and was associated with a poorer outcome after myocardial ischemia. Furthermore, genetic variation of *PLIN5* in humans was associated with impaired cardiac function after myocardial ischemia.

2. Methods

2.1. Mice

Plin5$^{-/-}$ mice were generated in compliance with the guidelines of the Animal Care and Use Committee of the National Institutes of Health under a Division of Intramural Research, National Institute of Diabetes and Digestive and Kidney Diseases–approved animal study protocol. *Plin5*$^{-/-}$ mice were generated (Fig. S1) as described in the Supplemental information section. All animal studies were approved by the local animal ethics committee and conform to the guidelines from Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes. At the end of experiments, mice were sacrificed using 5% isoflurane and cervical dislocation.

2.2. Immunoblotting

Briefly, frozen mouse heart tissue was homogenized and proteins were extracted with the Qproteosome Mammalian Protein Prep Kit (Qiagen, Hilden, Germany). Specific antibodies for Plin5 (GP31) and Na,K-ATPase (ab7671) (Abcam) were used. More detailed information can be found in the Supplemental information section.

2.3. Lipid analyses of heart muscle

Heart tissue was excised from fed wildtype (WT) and *Plin5*$^{-/-}$ mice, and 50–100 mg of tissue was used for lipid analyses, as described in the Supplemental information section.

2.4. Electron microscopy of mouse hearts

Hearts were harvested from fasted mice and prepared as described in the Supplemental information section.

2.5. GC–MS and MS/MS spectroscopy

Succinate and lactate from pulverized heart tissue (about 50 mg) were extracted using 1 ml of diethylether. 100 μl of the ether fraction was then mixed with 100 μl of N-methyl-N-tert-butyldimethylsilyltrifluoroacetamide (MTBSTFA, Sigma) and incubated at 70 °C for 1 h. The derivatized sample was then analyzed using GC–MS. The separation was done on a 60 m DB-5MS column and the detection was made in SIM mode. Extraction and analysis (HPLC-MS/MS) of acetylCoA were done as previously described [19].

2.6. mRNA expression in heart tissue from mice

Total RNA was extracted from snap-frozen mouse heart tissue with the RNeasy Fibrous Tissue Kit (Qiagen). cDNA was synthesized with the high-capacity cDNA Reverse Transcription Kit (Applied Biosystems) and random primers. mRNA expression of genes of interest was analyzed with TaqMan real-time PCR in an ABI Prism 7900 HT Detection System (Applied Biosystems). The TaqMan Gene Expression assays used were mm01135198_m1 (for CD36), mm00449511_m1 (fatty acid transfer protein), mm00434764_m1 (lipoprotein lipase), mm00440939_m1 (peroxosome proliferator-activated receptor α), mm01208835_m1 (peroxisome proliferator-activated receptor gamma coactivator 1α), mm004872206_m1 (carnitine palmitoyltransferase 1b), and mm00431611_m1 (median-chain acyl-coenzyme A dehydrogenase) (all from Applied Biosystems). HPRT (Applied Biosystems) was used as an internal control.

2.7. In vivo [^{3}H] palmitate uptake and incorporation into triglycerides, diglycerides and phospholipids

After a 5-h fast, mice were anesthetized with N-isoflurane and injected retroorbitally with [^{3}H]-palmitate (5 μCi; Amersham Biosciences) complexed with 6% bovine serum albumin in phosphate-buffered saline (PBS). Blood was taken from the tail 2 min after the injection. Mice were perfused with PBS 30 min after the injection. The heart was harvested, rinsed in PBS to remove all blood, and snap frozen in liquid nitrogen. Frozen tissue was pulverized into a fine powder and analyzed as described in the Supplemental information section.

2.8. In vivo glucose uptake

In vivo glucose uptake was measured as described [20], with some modifications. Briefly, after a 4-h fast, mice received an intraperitoneal injection of saline containing 50 Ci/kg 2-deoxy-D-[^{3}H]-glucose (specific activity, 9.0 Ci/mmol; Amersham Biosciences). Blood was collected after 5 min and 60 min. After 60 min, the heart was excised, snap-frozen in liquid nitrogen, and homogenized in ice-cold buffer 50 mM Tris–HCl (pH 7.4) and 250 mM sucrose. Radioactivity was counted in blood and homogenates.

2.9. Bioluminescence imaging of glycogen

Sections of snap-frozen infarcted and control hearts from WT and *Plin5*$^{-/-}$ mice were cut in 16-μm-thick sections, fixed on cover glasses, and heat-inactivated at 95 °C for 10 min. Bioluminescence imaging of glycogen was performed as described in the Supplemental information section.

2.10. Induction of myocardial infarction

Before induction of myocardial infarction, the mice were anesthetized with isoflurane, orally intubated, and connected to a small-animal ventilator (SAR-830, Geneq, Montreal, Canada) distributing a mixture of oxygen, air and 2–3% isoflurane. ECG electrodes were placed on the extremities, and cardiac rhythm was monitored during surgery. An incision was made between the 4th and 5th ribs to reveal the upper part of the anterior LV wall and the lower part of the left atrium. Myocardial infarction was induced by ligating the left anterior descending coronary artery immediately after the bifurcation of the left coronary artery. The efficacy of the procedure was immediately verified by characteristic ECG changes, and akinesis of the LV anterior wall. After verification of the infarction, the lungs were hyperinflated, positive end-expiratory pressure was applied, and the chest was closed. The mice received an intraperitoneal injection of 0.1 ml Temgesic to relieve post-operative pain and were allowed to recover spontaneously after isoflurane administration was stopped.

2.11. Echocardiography in mice

Mice were anesthetized with N-isofluorane (1.2%) and underwent a baseline echocardiographic examination with a VEVO 770 system (VisualSonics, Ontario, Canada), as further described in the Supplemental information section.

2.12. Dobutamine stress analysis

The mice were injected intraperitoneally with dobutamine (2 μg/g body weight) and echocardiography was performed. Mice were excluded from the study if their heart rate did not increase by >100 beats/min.

2.13. Human subjects

The Coronary Flow Reserve and Cardiovascular Events (CEVENT) study consists of 468 patients with clinically suspected coronary artery disease recruited at the Department of Clinical Physiology, Sahlgrenska University Hospital, Gothenburg, Sweden, from 2006 to 2008. Patient characteristics are presented in Table S1. The study was conducted in accordance with the Declaration of Helsinki. All participants gave written informed consent, and the study was approved by the local ethics committee in Gothenburg.

2.14. Myocardial perfusion scintigraphy

All patients underwent myocardial perfusion scintigraphy to detect coronary artery disease. A 2-day nongated stress/gated rest test was performed with the 99 mTc-sestamibi protocol. Patients underwent a symptom-limited ergometry test, maximal exercise, or pharmacological testing with adenosine or dobutamine. Images were acquired with a dual-head SPECT camera (Infinia or Millenium VG, General Electric). Automatically generated information on myocardial ischemia and infarction [21] was interpreted by one experienced physician. Wall motion scores were calculated as the sum of wall motion abnormalities in a 17-segment model. The patients were grouped according to the absence or presence of left ventricle perfusion defects (clinical score) or wall motion abnormalities.

2.15. Selection of SNPs, genotyping, and expression analysis

The *PLIN5* SNPs we investigated were selected based on an expected minor allele frequency >5%, no or weak linkage disequilibrium, and presumed potential effects on protein function (mediated by amino acid change) and protein concentration (mediated by mRNA stability and splicing pattern). Genomic DNA was isolated from whole blood by standard protocols. Four SNPs in *PLIN5* (rs11085080G, rs11610090, rs1062223, rs884164) were successfully genotyped in 466 of 468 patients (DNA was unavailable from 2 patients) with TaqMan technology (Applied Biosystems, Foster City, CA) (Table S2). Human heart biopsies were obtained from patients undergoing valve surgery, and mRNA expression according to genotype was assessed as described [22].

2.16. Statistical analysis

Statistical analyses for genetic studies were performed in R [23] and SAS 9.2 (SAS Institute, Cary, NC). Hardy–Weinberg equilibrium and pairwise linkage disequilibrium coefficients expressed as r^2 were calculated with HaploView 3.32 [24]. All skewed variables were log transformed to obtain a normal distribution before statistical computations and significance testing. The effect size of genotypes was estimated from β coefficients per minor allele tested under an additive genetic model, using linear regression models for continuous and logistic regression models for dichotomized dependent variables, adjusting for age, gender, myocardial infarction, and ejection fraction. Groups were compared by two-tailed *t* test. An additive linear model was used to estimate the fold-change in gene expression per minor allele. Values <1 indicate decreased expression with the minor allele of the SNP rs884164. For mouse studies, data are shown as mean ± SEM. Measurements were compared with the two-tailed *t* test or Mann–Whitney rank-sum test.

3. Results

3.1. Reduced myocardial triglycerides in *Plin5*$^{-/-}$ hearts

To investigate the role of Plin5 in myocardial lipid metabolism and heart function, we generated *Plin5*$^{-/-}$ mice (Fig. S1) and assessed their cardiac physiology and biochemistry. *Plin5*$^{-/-}$ mice did not express Plin5 in the heart (Fig. 1A). Body weight and plasma cholesterol and triglyceride levels did not differ between 3-month-old WT and *Plin5*$^{-/-}$ mice on a chow diet (data not shown).

To determine whether Plin5 deficiency affects cardiac lipid accumulation, we used lipidomics to analyze heart tissue from WT and *Plin5*$^{-/-}$ mice. Triglyceride levels in the heart were markedly lower in *Plin5*$^{-/-}$ mice than in WT mice (Fig. 1B). Diacylglycerol levels were also lower in *Plin5*$^{-/-}$ hearts, but surprisingly there were no differences in other analyzed glycerolipid species or membrane lipids (Fig. 1B). In addition, *Plin5*$^{-/-}$ hearts contained a higher percentage of triglycerides with medium-chain fatty acids and a lower percentage of triglycerides with long-chain and very-long-chain fatty acids (Fig. S2).

As shown by electron microscopy of left ventricular sections, lipid droplets were large and located close to mitochondria in WT hearts, but were smaller, tended to be less numerous, and were not closely associated with mitochondria in *Plin5*$^{-/-}$ hearts (Fig. 1C–F). These observations indicate that Plin5 stabilizes lipid droplets, thus allowing increased storage of triglycerides, and facilitates their association with mitochondria *in vivo*.

3.2. Reduced fatty acid uptake in *Plin5*$^{-/-}$ hearts

Plin5 has previously been suggested to play a role as a lipolytic barrier on the lipid droplet surface, [25] protecting lipids from hydrolysis. We next investigated whether Plin5 deficiency also resulted in altered fatty acid uptake. Importantly, we found that fatty acid uptake was markedly decreased in *Plin5*$^{-/-}$ hearts (Fig. 2A). After adjusting for the reduced uptake of fatty acids in *Plin5*$^{-/-}$ hearts, the incorporation of fatty acids into triglycerides was reduced (Fig. 2B), there was no difference in the incorporation of fatty acids into phosphatidic acid and diglycerides (Fig. 2C), and the incorporation of fatty acids into phospholipids was markedly increased (Fig. 2D) in *Plin5*$^{-/-}$ compared with WT hearts. The altered glycerolipid pathway in the *Plin5*$^{-/-}$ hearts is shown schematically in Fig. 2E.

These data indicate the *Plin5*$^{-/-}$ heart compensates for its reduced capacity to store triglycerides by decreasing fatty acid uptake and suggest that the incorporation of fatty acids in the glycerolipid pathway is shifted from triglycerides to phospholipids, thus maintaining membrane homeostasis.

3.3. Increased glucose uptake in *Plin5*$^{-/-}$ hearts

Given the marked reductions in triglyceride levels and fatty acid uptake in *Plin5*$^{-/-}$ hearts, we tested whether these hearts use glucose as an energy source instead of lipids. As shown by ^{3}H-2-deoxyglucose injections, glucose uptake was 2-fold higher in *Plin5*$^{-/-}$ than in WT hearts (Fig. 3). Collectively, our results suggest that *Plin5*$^{-/-}$ mice undergo a metabolic shift leading to reduced fatty acid uptake and increased glucose uptake to maintain their energy metabolism under normal conditions.

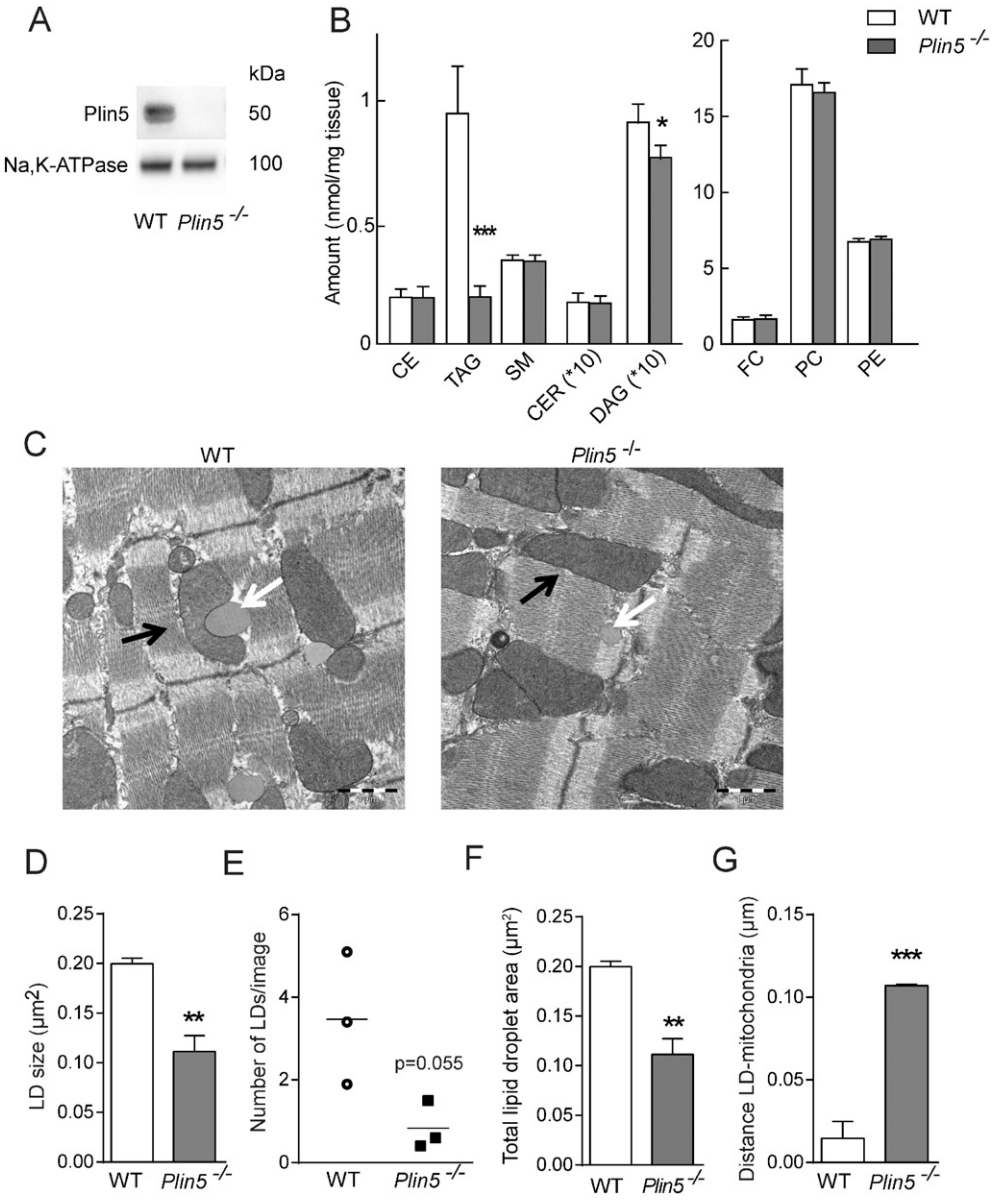

Fig. 1. Reduced myocardial triglycerides in *Plin5*$^{-/-}$ mice. (A) Immunoblot of Plin5 protein in hearts from WT and *Plin5*$^{-/-}$ mice. (B) Lipid content in heart of 12-week-old WT and *Plin5*$^{-/-}$ mice on a chow diet. Glycero- and sphingolipids were measured with high-performance liquid chromatography and mass spectrometry (n = 5–8). (C) Representative electron microscopy images of WT and *Plin5*$^{-/-}$ hearts. Lipid droplets are marked with a white arrow and mitochondria are marked with a black arrow. (D) Lipid droplet size measured as the cross-sectional area of lipid droplets in WT and *Plin5*$^{-/-}$ hearts. 40 lipid droplets per mouse were averaged. (E) Number of lipid droplets per image in WT and *Plin5*$^{-/-}$ hearts, quantified in 10 images per mouse. (F) Distance between the lipid droplet and the closest mitochondrion (D–F; n = 3 per genotype) Values are mean ± SEM. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ *vs* WT. CE, cholesterol ester; TAG, triglyceride; SM, sphingomyelin, CER, ceramide; DAG, diacylglycerol; FC, free cholesterol; PC, phosphatidylcholine; PE phosphatidylethanolamine; LD, lipid droplet.

3.4. Dysregulated metabolism in Plin5$^{-/-}$ *hearts following ischemia*

Next, we examined the effect of Plin5 deficiency following myocardial ischemia in mice. We observed significantly lower levels of C2 and C4 acylcarnitines after a myocardial infarction (induced by ligation of the left anterior descending artery) in *Plin5*$^{-/-}$ hearts compared with WT hearts (Fig. 4A), indicating that fatty acid oxidation is downregulated in *Plin5*$^{-/-}$ hearts. In addition, we found that accumulation of glycogen was markedly increased in the septum of WT but not *Plin5*$^{-/-}$ hearts after a myocardial infarction compared with baseline (Fig. 4B and C), suggesting that *Plin5*$^{-/-}$ hearts utilize all available glucose because of their reduced triglyceride content. Thus, our results indicate that *Plin5*$^{-/-}$ hearts display dysregulated metabolism following a myocardial infarction.

3.5. Impaired cardiac function and reduced survival in Plin5$^{-/-}$ *mice after myocardial stress*

We next investigated whether the dysregulated metabolism in *Plin5*$^{-/-}$ hearts was paralleled by impaired heart function and survival after myocardial stress in mice. Under baseline conditions, *Plin5*$^{-/-}$ mice displayed no differences in heart dimension or stroke volume and a modest reduction in ejection fraction (Fig. 5A and B), indicating that *Plin5*$^{-/-}$ mice and WT mice have similar heart function. However, when we used the β-adrenergic agonist dobutamine to maximize the heart's capacity, we observed dramatic differences in diastolic dimensions and stroke volume between WT and *Plin5*$^{-/-}$ mice (Fig. 5C and Table S3). Importantly, we also showed that *Plin5*$^{-/-}$ mice had reduced survival after an induced myocardial infarction (Fig. 5D).

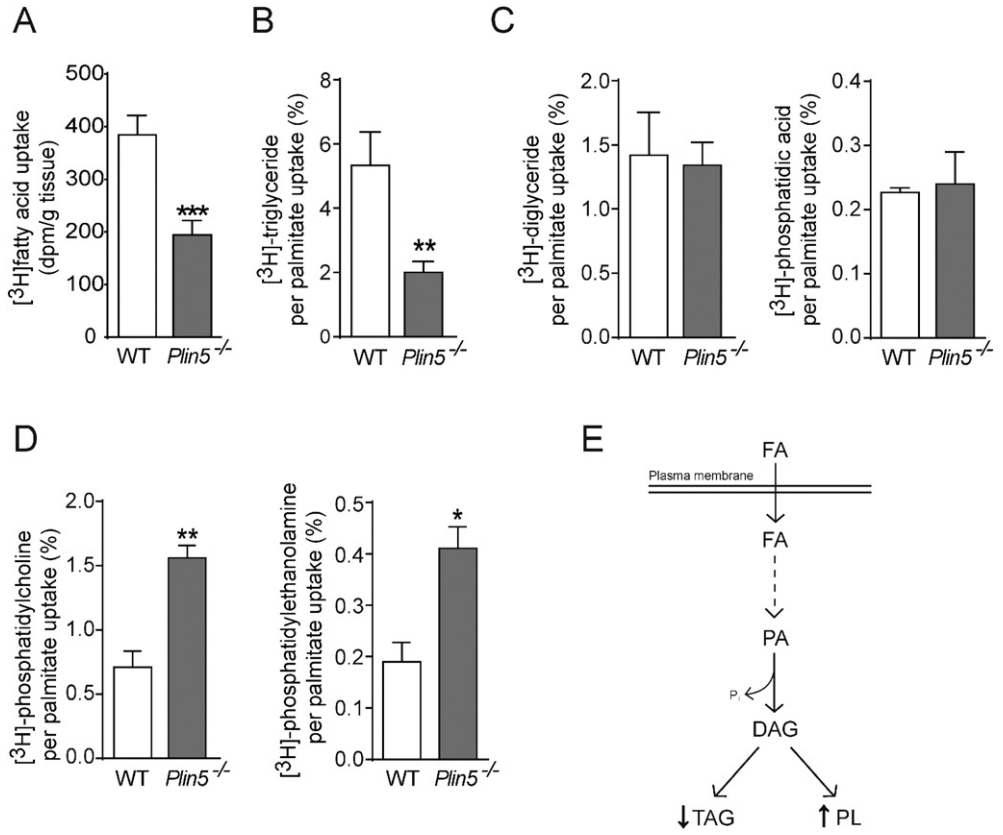

Fig. 2. Reduced fatty acid uptake and shifted incorporation of fatty acids into phospholipids in *Plin5*$^{-/-}$ hearts. (A) [^{3}H]-palmitate uptake in WT and *Plin5*$^{-/-}$ hearts (n = 4–7). (B) [^{3}H]-palmitate incorporation into triglycerides adjusted for fatty acid uptake (n = 4–7). (C) [^{3}H]-palmitate incorporation into diglycerides and phosphatidic acid adjusted for fatty acid uptake (n = 4–7). (D) [^{3}H]-palmitate incorporation into phosphatidylcholine and phosphatidylethanolamine adjusted for fatty acid uptake (n = 4–7). (E) Schematic overview of the effect of Plin5 deficiency on the cardiac glycerolipid synthesis after corrected for fatty acid uptake. The flux of fatty acid was shifted from incorporation into triglycerides towards incorporation into phospholipids. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ *vs* WT. PA, phosphatidic acid; PL phospholipids.

Plin5$^{-/-}$ mice started to die at a much higher rate than the WT mice from three days after induced ischemia (Fig. 5D).

Together, these data indicate that, although *Plin5*$^{-/-}$ mice maintain relatively normal heart function under baseline conditions, cardiac function is reduced during stress and mortality is increased following myocardial ischemia.

3.6. Genetic variation in PLIN5 is associated with impaired cardiac function after myocardial ischemia in humans

To elucidate if Plin5 is relevant to myocardial physiology in humans, especially in the setting of myocardial ischemia, we investigated whether genetic variation in *PLIN5* is associated with cardiac dysfunction after myocardial ischemia. 466 patients with suspected coronary artery disease underwent myocardial perfusion scintigraphy examinations (Table S1) and were genotyped for four single nucleotide polymorphisms (SNPs) across *PLIN5* (Fig. 1). All SNPs had a minor allele frequency >5% and were in Hardy–Weinberg equilibrium without linkage disequilibrium (Table S2). Importantly, *PLIN5* rs884164 was consistently and significantly associated with several variables (Table 1). The minor rs884164 allele was significantly associated with a higher wall motion score, reflecting reduced heart muscle motion due to myocardial ischemia. Furthermore, a larger reversibility mass (a significant predictor of major adverse cardiovascular events [26]), was also significantly associated with the minor rs884164 allele.

Next, we assessed the summed stress score (SSS), which indicates perfusion abnormalities during stress, reflecting both myocardial ischemia and infarction. Since an SSS >8 is associated with higher mortality from cardiac events [27], we used this cut-off to dichotomize the participants. The minor rs884164 allele was associated with an SSS >8 ($\beta \pm$ se: 0.862 ± 0.374, $P = 0.02$), which suggests that patients carrying this allele are at higher risk of cardiovascular morbidity and mortality after myocardial ischemia.

SNP rs884164 is located in an intergenic region at the 3′ end of *PLIN5* and 5′ of *PLIN4*. These noncoding parts of the genome are believed to be vital for correct spatial and temporal gene expression [28]. We analyzed the mRNA expression of *PLIN5* and *PLIN4* in human hearts (n = 127) and found that *PLIN5* expression was reduced if the subject had the minor rs884164 allele. The per-allele effect on expression was 0.78 (95% confidence interval (CI) 0.61–1.00; $P = 0.04$; values below 1 indicate decreased expression with the minor rs884164 allele), revealing that this SNP affects *PLIN5* expression. rs884164 did not

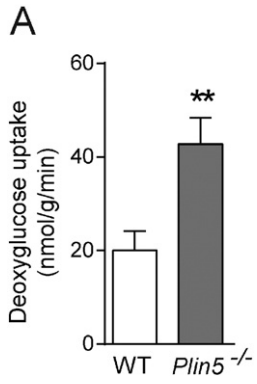

Fig. 3. Increased glucose uptake Plin5$^{-/-}$ hearts. (A) Basal glucose uptake in heart of WT and *Plin5*$^{-/-}$ mice on a chow diet was assessed after a single injection of 2-deoxyglucose (n = 10–12). *$P < 0.05$; **$P < 0.01$ *vs* WT.

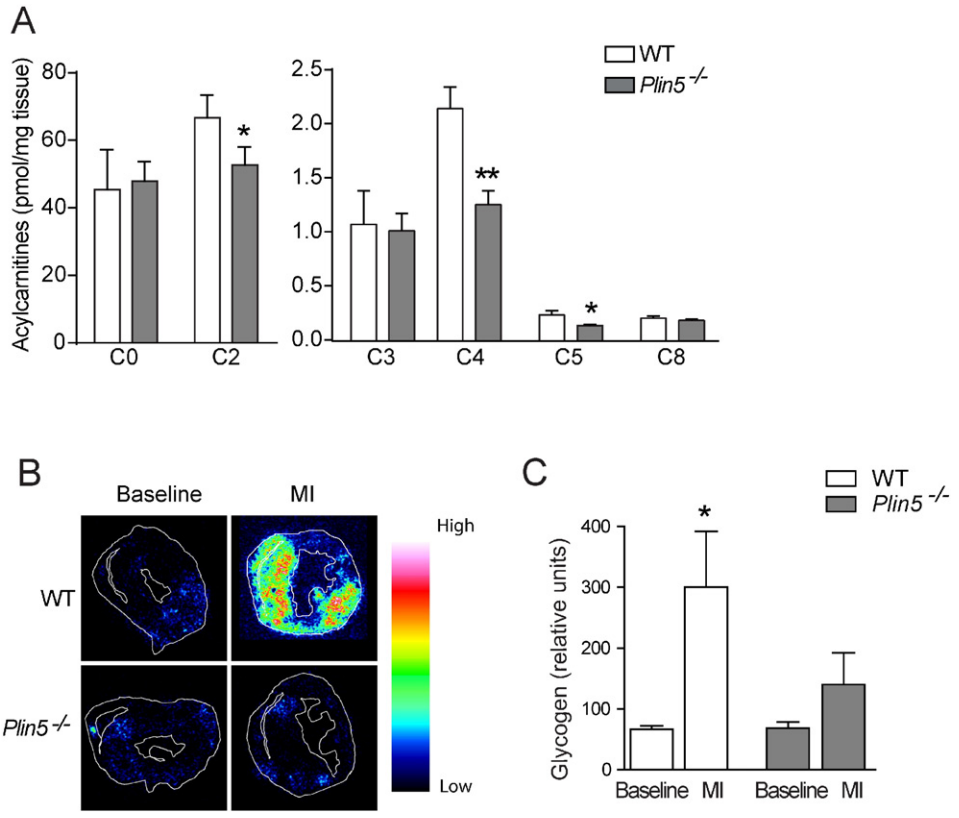

Fig. 4. Reduced substrate availability in *Plin5*$^{-/-}$ hearts. (A) Acyl carnitine species, markers of substrate oxidation, in hearts of WT and *Plin5*$^{-/-}$ mice measured with mass spectrometry (n = 5–8). (B) Representative images showing glycogen accumulation in WT and *Plin5*$^{-/-}$ hearts at baseline and after an induced myocardial infarction (MI). (C) Quantification of glycogen in the interventricular septal wall (n = 4–6). Values are mean ± SEM. $^{*}P < 0.05$; $^{**}P < 0.01$ *vs* WT.

influence *PLIN4* expression (per-allele effect, 0.93; 95% CI 0.76–1.13; $P = 0.44$).

Thus, genetic variation in *PLIN5* associates with impaired cardiac function after myocardial ischemia in humans, indicating that PLIN5 function is relevant to human cardiac physiology.

4. Discussion

In this study, we investigated Plin5 and its role in cardiac dysfunction and outcome after myocardial ischemia. In mice, Plin5 deficiency resulted in a dramatic reduction in myocardial lipid accumulation. Under normal conditions, the energy balance in *Plin5*$^{-/-}$ hearts was maintained by increasing glucose uptake and utilization, allowing a close to normal heart function. However, Plin5 deficiency in the ischemic heart resulted in dysregulated metabolism, which was accompanied by markedly reduced heart function and decreased survival. In humans, we found that the common SNP rs884164 alters cardiac expression of *PLIN5* and associates with several variables linked to reduced heart function and adverse outcome after myocardial ischemia. Thus, our results suggest that Plin5 plays a role in cardioprotection during myocardial ischemia.

Our observation that hearts of *Plin5*$^{-/-}$ mice had markedly lower triglyceride levels and fewer lipid droplets is in agreement with results from a previous mouse model of Plin5 deficiency [16]. We also showed that the distance between lipid droplets and mitochondria was greater in *Plin5*$^{-/-}$ hearts than in WT hearts, suggesting dysfunctional lipid utilization. Previous studies in cell lines showed that Plin5 protects lipid droplets from lipolysis but paradoxically also increases fatty acid oxidation [31,32], which could potentially be explained by Plin5 regulating the transfer of fatty acids from lipid droplets to mitochondria. Importantly, our data suggest that *Plin5*$^{-/-}$ hearts compensate for reduced triglyceride storage capacity by markedly decreasing fatty acid uptake and incorporation into triglycerides. Furthermore, we found that the incorporation of fatty acids into phospholipids was increased in *Plin5*$^{-/-}$ hearts after accounting for the reduced fatty acid uptake. Maintaining the homeostasis of cellular membranes (*e.g.* the endoplasmic reticulum) is crucial for many cellular functions including protein folding [33]. Similar changes in fatty acid flux into phospholipids rather than storage of triglycerides have previously been shown in Scd1-deficient mice [34]. Taken together, our data suggest that the decreased triglyceride levels in *Plin5*$^{-/-}$ hearts are not explained by increased fatty acid oxidation but instead by decreased fatty acid uptake and a shift of fatty acid incorporation from triglycerides to phospholipids.

Because glucose uptake and utilization were higher in *Plin5*$^{-/-}$ hearts compared with WT hearts, we propose that the *Plin5*$^{-/-}$ mice maintain nearly normal heart function under baseline conditions by increasing glucose utilization. During stress and conditions of reduced oxygen supply, it is well known that glucose uptake is increased in the heart [35,36]. The increased glucose uptake is seen even in regions remote from the ischemic area, and glucose that is not consumed can be stored as glycogen for later use [35,36]. We observed a marked accumulation of glycogen in the interventricular wall in WT mice following an induced myocardial infarction, suggesting that energy production through lipid oxidation is maintained in this region under ischemic conditions, and thus the heart does not need to use large amounts of glucose. By contrast, despite the higher level of glucose uptake in *Plin5*$^{-/-}$ hearts under baseline conditions, *Plin5*$^{-/-}$ hearts were not capable of accumulating glycogen in the interventricular wall following a myocardial infarction, indicating that they instead utilized all the glucose in an attempt to sustain energy production. However, this increased glucose utilization in *Plin5*$^{-/-}$ hearts did not appear to be sufficient to maintain function as survival was reduced in *Plin5*$^{-/-}$ mice after a myocardial infarction.

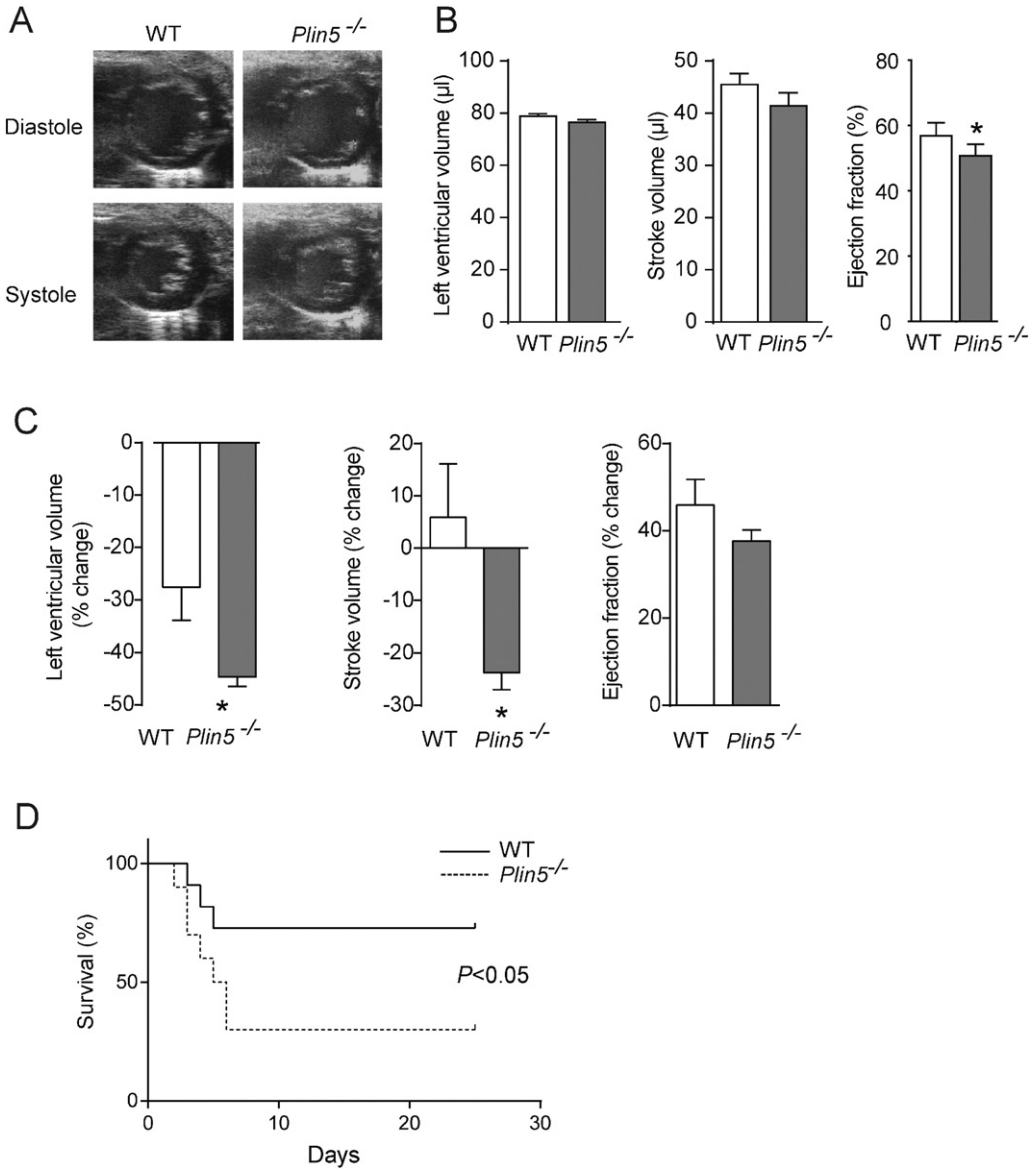

Fig. 5. Impaired cardiac function and reduced survival in *Plin5*$^{-/-}$ mice after myocardial stress. (A) Representative images of the end-diastolic and end-systolic phase of WT and *Plin5*$^{-/-}$ hearts under baseline conditions. (B) Baseline left ventricular diastolic volume, stroke volume and ejection fraction in WT and *Plin5*$^{-/-}$ mice assessed with echocardiography (n = 13–14). (C) Change relative to baseline in left ventricular diastolic volume, stroke volume and ejection fraction in WT and *Plin5*$^{-/-}$ mice after stimulation with dobutamine (n = 5). (D) Survival of WT and *Plin5*$^{-/-}$ mice after an induced myocardial infarction (n = 11). Values are mean ± SEM. **P* < 0.05 *vs* WT.

Our findings suggest that the presence of Plin5 affects metabolic flexibility of the heart. In agreement with a role for Plin5 in metabolic flexibility, a recent study in mice with Plin5 overexpression in cardiac muscle showed that Plin5 regulation of lipolysis is abolished by protein kinase A (PKA) [37], suggesting that epinephrine could activate PKA to increase the release of fatty acids from lipid droplets under conditions of stress and high workload. Since the total energy pool can turn over rapidly at high workload, even modest alterations in myocardial lipid metabolism may have a substantial effect on contractile function. We therefore speculate that Plin5 is important for metabolic flexibility during conditions of myocardial stress by regulating the release of fatty acids from the triglyceride storage pool for transfer to the mitochondria and fatty acid oxidation.

Earlier reports have shown that genetic variations in perilipins 1 and 4 are associated with human disease [29], and here we found that patients carrying the SNP rs884164, a minor allele of *PLIN5*, are at higher risk of cardiovascular morbidity after myocardial ischemic events. rs884164 is located 3′ of the coding region of *PLIN5* and 5′ of *PLIN4* on chromosome 19, a part of the genome believed to be vital for correct spatial and temporal gene expression.[28] We found that the minor allele of the rs884164 was associated with reduced expression of *PLIN5* in human hearts. A meta-analysis has shown that rs884164 is associated with high plasma levels of polyunsaturated fatty acid and triglycerides [30], and we observed that patients with rs884164 responded worse to ischemia as indicated by a larger reversibility mass and wall motion abnormalities. Thus, a genetic variation that reduces cardiac *PLIN5* expression is associated with reduced cardiac function after myocardial ischemia in humans.

There are limitations to this study. The experiments were performed in a mouse model with whole body Plin5 deficiency. Therefore, contributing effects of other cell types and organs on cardiac metabolism cannot be excluded. However, Plin5 is not ubiquitously expressed, but rather expressed in oxidative tissues such as heart, liver and skeletal muscle. Furthermore, cell types in the heart other than cardiomyocytes, *e.g.* endothelial cells and fibroblasts, do not normally express high levels of Plin5. Thus, knockout of Plin5 would not substantially affect the function of these cells. In accordance with this, we observed large lipid droplets in endothelial cells in EM images of *Plin5*$^{-/-}$ hearts in contrast

Table 1
Association of *PLIN5* polymorphisms with myocardial perfusion imaging.

Variable	rs884164		rs1062223		rs1610090		rs11085080	
	β ± se	*P*	β ± se	*P*	β ± se	*P*	β ± se	*P*
Infarct score	0.944 ± 0.463	**0.042**	0.081 ± 0.348	0.815	−0.073 ± 0.485	0.880	0.192 ± 0.658	0.771
Infarct area	1.021 ± 0.436	**0.019**	−0.146 ± 0.340	0.668	−0.230 ± 0.479	0.631	0.315 ± 0.614	0.608
Ischemia score	0.414 ± 0.296	0.161	0.004 ± 0.207	0.985	0.094 ± 0.265	0.723	0.390 ± 0.341	0.254
Ischemia area	0.478 ± 0.295	0.105	0.039 ± 0.207	0.849	0.053 ± 0.267	0.842	0.317 ± 0.345	0.358
Ischemia severity	0.499 ± 0.295	0.090	−0.035 ± 0.208	0.866	0.130 ± 0.264	0.622	0.375 ± 0.343	0.273
SSS[a]	0.862 ± 0.374	**0.021**	−0.538 ± 0.279	0.054	−0.190 ± 0.356	0.593	0.119 ± 0.424	0.779
Wall motion score[b]	1.066 ± 0.441	**0.016**	−0.437 ± 0.375	0.244	−0.668 ± 0.541	0.217	0.356 ± 0.606	0.557
Reversible ischemia[c]	1.163 ± 0.502	**0.020**	0.013 ± 0.427	0.976	−0.245 ± 0.613	0.690	1.125 ± 0.574	0.050

The beta refers to the effect of each additional copy of the minor allele with corresponding standard errors and *P* values adjusted for age, gender, prior myocardial infarction, and ejection fraction.
Bold numbers indicate significance at $P < 0.05$.
[a] SSS, summed stress score dichotomized using a score >8 as cut-off.
[b] Wall motion score dichotomized using a score ≥2 as cut-off.
[c] Reversible ischemia, the reversibility mass percent of myocardium dichotomized using a score ≥10% as cut-off.

to the few, small lipid droplets seen in the cardiomyocytes. Another potentially confounding effect is that insulin sensitivity and/or levels of circulating lipids may be altered due to the absence of Plin5 in non-cardiac tissue [38]. However, we used young *Plin5*$^{-/-}$ mice fed a chow diet for our experiments. These mice did not differ in weight compared with WT mice and plasma levels of triglycerides, cholesterol, glucose and insulin were similar between the genotypes. In addition, no differences in circulating triglycerides, fatty acids or insulin were found between WT and *Plin5*$^{-/-}$ mice following myocardial ischemia. Thus, our results describing the effect of Plin5 on heart function and cardiac lipid metabolism are not likely due to systemic effects of Plin5 knockout.

In conclusion, our findings elucidate the role of Plin5 in cardiac lipid metabolism and identify Plin5 as a potential cardioprotector following myocardial ischemia.

Conflict of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ijcard.2016.06.037.

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