Progesterone receptor membrane component 1 reduces cardiac steatosis and lipotoxicity via activation of fatty acid oxidation and mitochondrial respiration

Sang R. Lee¹, Jun H. Heo¹, Seong Lae Jo¹, Globinna Kim², Su Jung Kim², Hyun Ju Yoo², Kyu-Pil Lee¹, Hye-Jung Kwun¹, Hyun-Jin Shin¹, In-Jeoung Baek²,² & Eui-Ju Hong¹

Obesity is implicated in cardiovascular disease and heart failure. When fatty acids are transported to and not adequately oxidized in cardiac cells, they accumulate, causing lipotoxicity in the heart. Since hepatic progesterone receptor membrane component 1 (Pgrmc1) suppressed de novo lipogenesis in a previous study, it was questioned whether cardiac Pgrmc1 protects against lipotoxicity. Hence, we focused on the role of cardiac Pgrmc1 in basal (Resting), glucose-dominant (Refed) and lipid-dominant high-fat diet (HFD) conditions. Pgrmc1 KO mice showed high FFA levels and low glucose levels compared to wild-type (WT) mice. Pgrmc1 KO mice presented low number of mitochondrial DNA copies in heart, and it was concomitantly observed with low expression of TCA cycle genes and oxidative phosphorylation genes. Pgrmc1 absence in heart presented low fatty acid oxidation activity in all conditions, but the production of acetyl-CoA and ATP was in pronounced suppression only in HFD condition. Furthermore, HFD Pgrmc1 KO mice resulted in high cardiac fatty acyl-CoA levels and TG level. Accordingly, HFD Pgrmc1 KO mice were prone to cardiac lipotoxicity, featuring high levels in markers of inflammation, endoplasmic reticulum stress, oxidative stress, fibrosis, and heart failure. In vitro study, it was also confirmed that Pgrmc1 enhances rates of mitochondrial respiration and fatty acid oxidation. This study is clinically important because mitochondrial defects in Pgrmc1 KO mice hearts represent the late phase of cardiac failure.

The incidence of heart disease is increasing in modern society. With dietary factors being strongly linked to life-threatening heart diseases, abnormal metabolism should be considered a trigger of cardiovascular conditions. Obesity is a fundamental disorder that is closely related to the pathogenesis of metabolic diseases, it promotes development of insulin resistance and an increase in free fatty acids (FFA) because of increased fat mass, decreased fatty acid disposal, and acquired insulin resistance. Some clinical studies have implied that characteristics of obesity, including increased fat mass, FFA, and insulin resistance, are associated with cardiac lipid accumulation and coronary heart disease.

Energy metabolism in the heart can be greatly affected by excess lipids under conditions of obesity, and it is important to note that the heart primarily relies on fatty acid oxidation for energy production. Fatty acids derived from nutrient sources or generated by lipolysis from adipose tissue enter the heart, and are either used for energy production processes or stored for later use. For energy production, fatty acyl-CoA enters the mitochondria to carry out fatty acid oxidation. Thus, it is converted into acetyl-CoA by oxidation, which is then metabolized in the course of the TCA cycle. NADH and FADH produced by a series of metabolic reactions in the TCA cycle serve to generate ATP via mitochondrial oxidative phosphorylation. However, fatty acyl-CoA can be stored by...
conversion into other forms of cardiac lipids, including ceramides, diacylglycerols (DAG), and triglycerides (TG). When storage outweighs oxidation, accumulation of ceramides, DAG, and TG can lead to cardiac pathogenesis. While TG is less toxic, DAG and ceramides are the major toxic compounds that affect the heart1,2.

Previous studies have shown that progesterone receptor membrane component 1 (Pgrmc1) suppresses fatty liver development and promotes pancreatic insulin secretion3,4. Although the role of Pgrmc1 has been studied in terms of various metabolic functions, including gluconeogenesis and cholesterol synthesis5,6, it has not yet been determined whether it is involved in cardiac metabolism. Based on the previous study involving the activation of P450 proteins of PGRMC17, the present study sought to investigate the relationship between Pgrmc1 expression and cardiac pathogenesis including dysfunction of mitochondrial metabolism. This study will firstly report the role of cardiac PGRMC1 protein. In vivo model using Pgrmc1 whole-body knockout (KO) mice, Pgrmc1 depletion presented a low expression of mitochondrial DNA (mtDNA) and suppressed fatty acid oxidation activity in the heart. This phenotype of Pgrmc1 KO mice resulted in low cardiac acetyl-CoA and ATP production when the diet was high in fat (HFD). Furthermore, lipid accumulation resulted in cardiac lipotoxicity upon Pgrmc1 loss. We compared three different feeding conditions to define the metabolic role of cardiac Pgrmc1 in animal models and confirmed the role of cardiac-specific Pgrmc1 in vitro conditions.

Results
Pgrmc1 absence resulted in a low number of cardiac mtDNA copies and suppressed cardiac mitochondrial metabolism, including TCA cycle, OXPHOS, and fatty acid oxidation. In resting condition when mice were fed ad libitum (Fig. 1A), levels of blood glucose were decreased (p < 0.05, 88.1% vs. WT) while levels of plasma FFA were increased (p < 0.05, 1.23-fold vs. WT) in Pgrmc1 KO mice (Fig. 1B). Interestingly, the expression of mtDNA was lower (p < 0.05, 51.2% vs. WT) in the Pgrmc1 KO hearts, suggesting the fundamental defect of cardiac mitochondria (Fig. 1C). Consistently, Pgrmc1 KO hearts significantly suppressed cardiac mRNA expression of TCA cycle genes, Idh3a, Ogld2, Sdhb, and Mdhd2 (p < 0.05, 74.6, 52.3, 49.5, 61.5, and 59.9%, respectively), and oxidative phosphorylation (OXPHOS) genes, Atp5b, Ckm12, and Ndufb5 (p < 0.05, 63, 61.7, and 56.4%, respectively), compared to WT hearts (Fig. 1C). These low energy mediated steps were reflected by suppressed (p < 0.05, 63.4, 61.4, and 19.4%, respectively, vs. WT) expression of contractile genes, Atp2a2, Hrc1, and Snc5a, in the hearts of Pgrmc1 KO mice (Fig. 1D). Using enzyme-dependent fatty acid oxidation measurement, fatty acid oxidation activity of heart samples, not in vivo fatty acid oxidation rate, was analyzed. As a primary cardiac mitochondrial metabolism, fatty acid oxidation activity was suppressed (p < 0.05, 70.3% vs. WT) in the hearts of Pgrmc1 KO mice (Fig. 1E). The mRNA expression of Cpt2, Mcad, and Vlcd was also suppressed (p < 0.05, 49.5, 61.4, 49.7%, respectively, vs. WT) in the hearts of Pgrmc1 KO mice (Fig. 1E).

In western blot, we observed the presence of cardiac PGRMC1 (Fig. 1F). While protein expression of hexokinase (HK1) was decreased (p < 0.05, 52.2% vs. WT), protein expression of pyruvate dehydrogenase (PDH) was increased (p < 0.05, 1.36-fold vs. WT) in the hearts of Pgrmc1 KO mice (Fig. 1F). However, hexokinase2 (HK2) and pyruvate kinase M2 (PKM2) protein expression did not show a significant difference (Fig. 1F). Despite the expression of mitochondrial metabolic genes and fatty acid oxidation activity in Pgrmc1 KO heart were suppressed, the amount of cardiac metabolites was not changed (Fig. 1G–K).

To investigate cardiac metabolism in Pgrmc1 KO mice according to defined nutritional conditions, we induced a refeed state, as illustrated in Fig. 2A. Both blood glucose levels (295 and 232.3 mg/dL; refeed WT and Pgrmc1 KO mice, respectively) and plasma FFA levels (957.6 and 1229.5 μM; refeed WT and Pgrmc1 KO mice, respectively) were enormously high under refeed conditions due to sudden feeding after prolonged fasting (Fig. 2B). Under refeed conditions, blood glucose levels were significantly decreased (p < 0.05, 78.8% vs. WT), while plasma FFA levels were increased (p < 0.05, 1.28-fold vs. refeed WT) in the hearts of refeed Pgrmc1 KO mice (Fig. 2B). Consistent with resting condition, the expression of mtDNA was lower (p < 0.05, 58.8% vs. refeed WT) in the hearts of refeed Pgrmc1 KO mice (Fig. 2C).

Suppression in mRNA expression of TCA cycle genes, Cs, Idh3a, Ogld2, Sucld2, Sdhb, and Mdhd2 (p < 0.05, 35.5, 42.2, 37.9, 36.4, 45.7, and 37.3%, respectively, vs. refeed WT) and OXPHOS genes, Atp5b, Ckm12, Ndufb5, and Slc25a4 (p < 0.05, 47.2, 31, 50.2, and 41.2%, respectively, vs. refeed WT) were still observed in the hearts of refeed Pgrmc1 KO mice (Fig. 2C). Contractility markers, Atp2a2, Hrc1, and Snc5a, also showed low (p < 0.05, 30.7, 34.7, and 29%, respectively, vs. refeed WT) mRNA expression in refeed Pgrmc1 KO hearts (Fig. 2D). Fatty acid oxidation activity was significantly lower (p < 0.05, 68.1% vs. refeed WT) in the hearts of refeed Pgrmc1 KO hearts (Fig. 2E). The mRNA expression of fatty acid oxidation genes, Cpt2 and Vlcad, was suppressed (p < 0.05, 42.3 and 36.1%, respectively, vs. refeed WT) in the hearts of refeed Pgrmc1 KO mice (Fig. 2E). Protein expression of glyceraldehyde genes, Hk1 and Pkm2, was increased (p < 0.05, 1.52- and 1.59-fold, respectively, vs. refeed WT) in the hearts of refeed Pgrmc1 KO mice (Fig. 2F).

Protein expression of the glucose oxidation gene Pdh1 was increased (p < 0.05, 1.33-fold vs. refeed WT) in the hearts of refeed Pgrmc1 KO mice (Fig. 2F).

Enrichment of glucose and fatty acid resulted in metabolite level changes according to metabolic gene alteration of Pgrmc1 KO hearts. Levels of FFA and palmitoyl-CoA (C16:0) in the hearts of refeed Pgrmc1 KO mice were higher (p < 0.05, 1.15- and 2.53-fold, respectively) than those of WT mice (Fig. 2G). Conversely, levels of fructose-1,6-bisphosphate (FBP), 3-phosphoglycerate (3-PG), pyruvate, and lactate were lower (p < 0.05, 1.15- and 2.53-fold, respectively, vs. refeed WT) in the hearts of refeed Pgrmc1 KO mice (Fig. 2H). While the acetyl-CoA level was not changed, fumarate and malate levels were lower (p < 0.05, 51.8 and 49.4%, respectively, vs. refeed WT) in the hearts of refeed Pgrmc1 KO mice (Fig. 2I–J). Nonetheless, ATP production was not suppressed in Pgrmc1 KO hearts (Fig. 2K).

Pgrmc1 absence possesses high cardiac fatty acyl-CoAs, but produce low cardiac TCA metabolites, acetyl-CoA, and ATP in HFD. To investigate how fat-dominant conditions could affect cardiac
metabolism in mitochondrial defective Pgrmc1 KO mice, we induced HFD conditions as shown in Fig. 3A. Under these conditions, blood glucose level was decreased (p < 0.05, 67.2% vs. HFD WT), while plasma FFA levels were increased (p < 0.05, 1.33-fold vs. HFD WT) in HFD Pgrmc1 KO mice (Fig. 3B). The expression of mtDNA was decreased (p < 0.05, 54.5% vs. HFD WT) in hearts of HFD Pgrmc1 KO mice (Fig. 3C). mRNA expression of TCA cycle genes, Cpt2, Mct2, and Vldld was suppressed (p < 0.05, 0.75, 0.37, and 0.37-fold, respectively, vs. HFD WT), and OXPHOS genes, Atp5b, Ckmt2, and Ndufb5, were increased (p < 0.05, 1.99, 2.26, and 1.65-fold, respectively, vs. HFD WT) in the hearts of HFD Pgrmc1 KO mice (Fig. 3E).

Levels of metabolites showed a more pronounced difference in HFD Pgrmc1 KO hearts. Levels of palmitoyl-CoA, stearoyl-CoA (C18:0), and oleoyl-CoA (C18:1) were increased (p < 0.05, 2.04, 1.28, and 1.6-fold, respectively, vs. HFD WT) in the hearts of HFD Pgrmc1 KO mice (Fig. 3E).
respectively, vs. HFD WT) in the hearts of HFD Pgrmc1 KO mice (Fig. 3G). Conversely, levels of glucose, FBP, 3-PG, pyruvate, and lactate were decreased (p < 0.05, 77.7, 8.22, 35.5, 45.1, and 52.4%, respectively, vs. HFD WT) in the hearts of HFD Pgrmc1 KO mice (Fig. 3H). Coenzyme A (CoA) was higher (1.19-fold), but the acetyl-CoA level was lower (86.2%), and the ratio of acetyl-CoA/CoA was also lower (75.9%) in the hearts of HFD Pgrmc1 KO mice compared to those of HFD WT and Pgrmc1 KO mice (p < 0.05, Fig. 3I). Furthermore, levels of citrate/isocitrate, fumarate, and malate were lower (p < 0.05, 10.4, 48.3, and 45.7%, respectively, vs. HFD WT) in the hearts of HFD Pgrmc1 KO mice (Fig. 3J). As a result, the ATP/ADP ratio was suppressed (p < 0.05, 85.3%, vs. HFD WT) in the hearts of HFD Pgrmc1 KO mice (Fig. 3K).

Figure 2. Pgrmc1 KO mice show high levels of cardiac FFA and paltimoyl-CoA, but low levels of cardiac glucose metabolites and TCA cycle intermediates in refed state. (A) Schematic diagram which presents experimental schedule for refed state. (B) Levels of blood glucose (mg/dl) and plasma FFA (µM) in refed WT and Pgrmc1 KO mice. Nuclear DNA was used for an internal control. mRNA expression of TCA cycle and OXPHOS in hearts of refed WT and Pgrmc1 KO mice. Rplp0 was used for an internal control. (C) mRna expression of contractility markers in hearts of refed WT and Pgrmc1 KO mice. Rplp0 was used for an internal control. (E) Fatty acid oxidation activity in hearts of refed WT and Pgrmc1 KO mice. miRNA expression of fatty acid oxidation genes in hearts of refed WT and Pgrmc1 KO mice. Rplp0 was used for an internal control. (F) Western blot analysis and quantification of glycolysis and glucose oxidation genes in hearts of refed WT and Pgrmc1 KO mice. β-Actin was used for an internal control. (G) Cardiac FFA level (µM) and fatty acyl-CoA levels (pmol/mg) in refed WT and Pgrmc1 KO mice. (H) Levels of glucose metabolates (unit) in refed WT and Pgrmc1 KO mice. (I) Levels of coenzyme A (CoA) and acetyl-CoA in refed WT and Pgrmc1 KO mice. (J) Levels of TCA cycle intermediates in refed WT and Pgrmc1 KO mice. (K) Ratio of ATP per ADP in refed WT and Pgrmc1 KO mice. Values represent means ± SD. *p < 0.05. Student’s t test was performed. Total numbers of mice used for experiment were 8 (refed WT), and 6 (refed Pgrmc1 KO).

Pgrmc1 KO mice on a HFD are vulnerable to cardiac lipid accumulation. Excessive fatty acyl-CoA can be esterified to triacylglycerol (TG) or used for ceramide synthesis. The mRNA expression of Elovl6, which is responsible for fatty acid elongation, was increased (p < 0.05, 11.5-fold, vs. HFD WT) in the hearts of HFD Pgrmc1 KO mice (Fig. 4A). The mRNA expression of Scd1, which is responsible for fatty acid elongation, was also increased (p < 0.05, 1.6-fold, vs. HFD WT) in the hearts of HFD Pgrmc1 KO mice (Fig. 4A). Furthermore,
the mRNA expression of fatty acid esterification genes, Gpam, Agpat1, and Dgat1, was increased (p < 0.05, 1.78-, 2.06-, and 2.31-fold, respectively, vs. HFD WT) in the hearts of HFD Pgrmc1 KO mice (Fig. 4B). In addition, the protein expression of serine palmitoyltransferase 1 (SPT1), which is responsible for ceramide synthesis, was increased (p < 0.05, 1.66-fold vs. HFD WT) in the hearts of HFD Pgrmc1 KO mice (Fig. 4C). Accordingly, Oil-Red-O staining also showed profound lipid accumulation (p < 0.05, 1.45-fold vs. HFD WT) in the hearts of HFD Pgrmc1 KO mice (Fig. 4D). TG level was also higher (p < 0.05, 1.57-fold vs. HFD WT) in HFD Pgrmc1 KO hearts (Fig. 4E).

**Pgrmc1 KO mice on a HFD present various cardiac lipotoxic markers.** While the cardiac lipid accumulation and low ATP production were observed in HFD Pgrmc1 KO mice, the heart did not show hypertrophy, as shown in Fig. 5A. Conversely, the heart weight (HW) was decreased (p < 0.05, 86.5% vs. HFD WT) in HFD Pgrmc1 KO mice. However, the ratio of HW/body weight (BW) was not decreased in HFD Pgrmc1 KO mice. Consistently, HW/tibia length was similar between HFD WT and Pgrmc1 KO mice (Fig. 5A). In H&E staining, number of

**Figure 3.** Pgrmc1 KO mice increases cardiac fatty acyl-CoA levels, but suppresses production of acetyl-CoA and ATP. (A) Schematic diagram which presents experimental schedule for HFD state. (B) Levels of blood glucose (mg/dl) and plasma FFA (μM) in HFD WT and Pgrmc1 KO mice. (C) Expression of mitochondrial DNA (mtDNA) in hearts of HFD WT and Pgrmc1 KO mice. Nuclear DNA was used as an internal control. mRNA expression of TCA cycle and OXPHOS in hearts of HFD WT and Pgrmc1 KO mice. Rplp0 was used for an internal control. (D) mRNA expression of contractility markers in hearts of HFD WT and Pgrmc1 KO mice. Rplp0 was used for an internal control. (E) Fatty acid oxidation activity in hearts of HFD WT and Pgrmc1 KO mice. mRNA expression of fatty acid oxidation genes in hearts of HFD WT and Pgrmc1 KO mouse. Rplp0 was used for an internal control. (F) Western blot analysis and quantification of glycolysis and glucose oxidation genes in hearts of HFD WT and Pgrmc1 KO mouse. β-actin was used for an internal control. (G) Cardiac FFA level (μM) and fatty acyl-CoA levels (pmol/mg) in HFD WT and Pgrmc1 KO mouse. (H) Levels of glucose metabolites (unit) in HFD WT and Pgrmc1 KO mouse. (I) Levels of coenzyme A (CoA) and acetyl-CoA in HFD WT and Pgrmc1 KO mouse. (J) Levels of TCA cycle intermediates in HFD WT and Pgrmc1 KO mouse. (K) Ratio of ATP per ADP in HFD WT and Pgrmc1 KO mouse. Values represent means ± SD. “p<0.05. Student’s t test was performed. Total numbers of mice used for experiment were 10 (HFD WT) and 7 (HFD Pgrmc1 KO).
nuclei per area was increased (p < 0.05, 2.18-fold vs. HFD WT) in the hearts of HFD Pgrmc1 KO mice (Fig. 5B), suggesting the cardiac cell size is smaller.

As indicators of ER stress, the ratio of pEIF2α/EIF2α protein and the expression of CHOP protein were increased (p < 0.05, 1.86- and 1.78-fold, respectively, vs. HFD WT) in the hearts of HFD Pgrmc1 KO mice (Fig. 5C). Furthermore, oxidative stress was increased in the hearts of HFD Pgrmc1 KO mice; the level of reduced glutathione (GSH) was decreased (p < 0.05, 85.9% vs. HFD WT), while the levels of oxidized glutathione (GSSG) and the GSSG/GSH ratio had increased (p < 0.05, 1.2- and 1.4-fold, respectively, vs. HFD WT), as shown in Fig. 5D. The mRNA expression of pro-inflammatory cytokine genes, including Tnf, Il-1β, and Il-6, was increased (p < 0.05, 1.37-, 25-, and 6.02-fold, respectively, vs. HFD WT) in HFD Pgrmc1 KO mice (Fig. 5E). The level of plasma creatine phosphokinase (CPK) was increased (p < 0.05, 1.33-fold vs. HFD WT) in HFD Pgrmc1 KO mice (Fig. 5F). Abnormal stress led to the induction of cardiac fibrosis, as observed by Masson's trichrome staining. A significant increase (p < 0.05, 1.44-fold vs. HFD WT) in fibroblasts (blue staining) was observed in the hearts of HFD Pgrmc1 KO mice (Fig. 5G). Furthermore, as a cardiac failure marker, the expression of ANP was increased (p < 0.05, 2.44-fold vs. HFD WT) in the hearts of HFD Pgrmc1 KO mice (Fig. 5H). Based on these results, we concluded that the metabolic phenotype of Pgrmc1 KO mice leads to cardiac lipotoxicity under HFD conditions, but does not trigger cardiac hypertrophy.

Pgrmc1 increases mitochondrial respiration and fatty acid oxidation in H9c2 cells. H9c2 cells were transfected with Pgrmc1 siRNA, and the protein expression of PGRMC1 was suppressed (p < 0.05, 67.8% vs. control siRNA (CON)) in the Pgrmc1 siRNA group (Fig. 6A). The number of mtDNA copies was decreased (p < 0.05, 54.1% vs. CON) in the Pgrmc1 siRNA group (Fig. 6B). Using the seahorse flux analyzer in H9c2 cells, we monitored the oxygen consumption rate (OCR), wherein other endocrine factors were not considered.

The level of maximal respiration was suppressed (p < 0.05, 72.2% vs. CON) in the Pgrmc1 siRNA group (Fig. 6C). To measure the rate of fatty acid oxidation, palmitate was conjugated with CD-BSA (charcoal dextran-bovine serum albumin) and used for treatment. The level of maximal respiration was decreased (p < 0.05, 76.8% vs. CON) in the Pgrmc1 siRNA group (Fig. 6D). Conversely, the levels of glycolysis and glycolytic capacity, which were measured by the extracellular acidification rate (ECAR), were increased (p < 0.05, 1.97- and 1.76-fold, respectively, vs. CON) in the Pgrmc1 siRNA group (Fig. 6E). In the palmitate-BSA-treated condition, the positive area for Oil-Red-O staining was increased (p < 0.05, 1.86-fold vs. CON) in the Pgrmc1 siRNA group (Fig. 6F). According to the in vitro results, we confirmed that Pgrmc1 is involved in mitochondrial metabolism, especially for fatty acid oxidation in cardiac cells.
Discussion

Obesity is characterized by the gain of body weight and fat mass. When fat mass accumulates around abdominal organs, visceral obesity occurs, leading to susceptibility to cardiovascular conditions. Regarding physiological aspects of the heart, cardiac TG accumulation is a common characteristic of most animal models of obesity, as the heart promotes fatty acid uptake even in a lipotoxic state. Therefore, fatty acid disposal in the heart is crucial to maintain intracardiac lipid balance, but fatty acid oxidation is limited under condition of failing heart. Compensatory glucose utilization is activated, but ATP production cannot be fully replenished. Consequently, the lack of fatty acid oxidation leads to impairment of cardiac contractility. In a HFD mouse model, Pgrmc1 KO hearts presented lipid accumulation and suppressed ATP production. This was due to impaired fatty acid oxidation and mitochondrial dysfunction of Pgrmc1 KO hearts.

Cardiac mitochondrial function is crucial for energy metabolism because the heart relies on oxidative phosphorylation for energy production. Cardiac cells possess large numbers of mitochondria, and ATP produced by mitochondrial oxidative phosphorylation is used for cardiac contractile function, as it is hydrolyzed to ADP.

Figure 5. Pgrmc1 KO mice shows lipotoxicity in HFD condition. (A) Gross image of hearts of HFD WT and Pgrmc1 KO mice. Heart weight (HW), HW per body weight (BW) and HW per tibia length were measured. (B) H&E staining of hearts of HFD WT and Pgrmc1 KO mice (scale bar 50 µm). Nucleus per area was measured by Image J program. (C) Western blot analysis and quantification of ER stress genes in hearts of HFD WT and Pgrmc1 KO mice. β-Actin was used for an internal control. (D) Cardiac levels of reduced (GSH) and oxidized (GSSG) glutathione (µM), and ratio of GSSG:GSH in HFD WT and Pgrmc1 KO mice. (E) mRNA expression of pro-inflammatory genes in hearts of HFD WT and Pgrmc1 KO mice. Rplp0 was used for an internal control. (F) Plasma CPK (U/I) level of HFD WT and Pgrmc1 KO mice. (G) Masson trichrome staining of hearts of HFD WT and Pgrmc1 KO mice (scale bar 30 µm). Positive area was measured by Image J program. Blue fibroblasts were set as positive. (H) Western blot analysis and quantification of ANP in hearts of HFD WT and Pgrmc1 KO mice. β-Actin was used for an internal control. Values represent means ± SD. *p < 0.05. Student’s t test was performed. Total numbers of mice used for experiment were 10 (HFD WT) and 7 (HFD Pgrmc1 KO).
Pgrmc1 KO hearts in resting, refed, and HFD conditions all presented low expression of mtDNA and mRNA expression of contractile markers. Furthermore, mRNA expression of TCA cycle genes and OXPHOS genes was all decreased in Pgrmc1 KO hearts. Our results show that Pgrmc1 KO hearts are phenotypically lacking mitochondrial function.

In a pathological murine model, a high-fat, high-sucrose (HFHS) diet causes cardiac mitochondrial dysfunction and decreased cardiac ATP production. From a biochemical perspective, ATP is generated in the electron transport system by NADH and FADH2, which are produced in the TCA cycle. Fatty acid and glucose oxidation both produce acetyl-CoA, which drives the TCA cycle, and the acetyl-CoA/CoA ratio indicates acetyl-CoA production. Fatty acid oxidation is the primary pathway for ATP production in the heart, while glucose oxidation is the more efficient pathway. Fatty acid oxidation inhibits glucose oxidation, thereby balancing cardiac metabolism. While Pgrmc1 KO hearts lack fatty acid oxidation activity, the acetyl-CoA production was maintained in a resting and refed state. This result can be interpreted in two ways: (1) decrease of fatty acid oxidation was not enough to trigger a physiological response. (2) acetyl-CoA from glucose source compensated the level. Especially in the refed state, where glucose is enriched, the increased expression of glycolysis and glucose oxidation and low level of glucose metabolites in Pgrmc1 KO hearts were observed, suggesting compensatory recovery of acetyl-CoA from glucose. Conversely, in fat-rich condition (HFD), Pgrmc1 KO hearts presented compensatory response from glucose according to the induction of glucose metabolic pathway and low glucose metabolite levels, but acetyl-CoA production was suppressed. Furthermore, TCA cycle intermediates levels were low, especially for citrate/isocitrate, fumarate, and malate. According to the low capacity of ATP production in HFD Pgrmc1 KO hearts, these comparative results suggest that lack of fatty acid oxidation activity in fat-enriched conditions leads Pgrmc1 KO hearts to pronounced impairment of ATP production. While Pgrmc1 KO hearts
could spend the cellular fatty acid in resting and refed conditions, they failed to fully use the cellular fatty acid in long-term HFD conditions.

Low fatty acid usage can result in excessive residual fatty acyl-CoA and induce lipid accumulation in the heart. Palmitoyl-CoA is the first form of fatty acyl-CoA synthesized from acetyl-CoA, which is converted to stearyl-CoA by elongation. Scd1 desaturates both palmitoyl-CoA and stearyl-CoA to form oleoyl-CoA. Afterward, desaturated fatty acyl-CoA is esterified through catalysis by a series of enzymes, including Gpam, Agpat1, Mogat1, and Dgat1, to form triglycerides. Meanwhile, ceramide also would be synthesized in the condition of lipid overload by conjugation of serine and palmitoyl CoA. The first and rate-limiting step of ceramide synthesis is controlled by SPT1. When the heart of HFD Pgrmc1 KO mice has high levels of fatty acyl-CoA due to limited fatty acid oxidation activity, they could be successfully esterified to triglycerides or used for ceramide synthesis considering the expression of genes. Accordingly, lipid accumulation was observed in the heart of HFD Pgrmc1 KO mice.

Lipotoxicity triggered by lipid is controversial as it differs by lipid species, including TG, DAG, and ceramide. Nonetheless, as the heart is not a conventional lipid-storing organ, lipid overload in the heart is still unusual and can cause cardiac abnormalities. Lipid-induced heart failure accompanies hypertrophy, fibrosis, inflammation, and mitochondrial dysfunction, which lead to contractile dysfunction. HFD Pgrmc1 KO hearts was not linked with cardiac hypertrophy according to similar HW/BW and HW/tibial length to those of HFD WT mice. Instead, HW and BW of HFD Pgrmc1 KO mice were both low. As weight gain during the HFD period was similar between WT and Pgrmc1 KO mice (Fig. S1), Pgrmc1 KO mice are phenotypically lighter than WT mice. Conversely, HFD Pgrmc1 KO hearts induced various heart failure markers. Increased pEIF2α and CHOP expression, high GSSG and low GSH levels, and increased inflammatory gene expression in Pgrmc1 KO heart are indicative of heart failure. Plasma CPK was also increased, which serves as a cardiac damage marker.

Finally, fibrosis and cardiac failure markers suggest the cardiac failure of Pgrmc1 KO mice.

Collectively, this study highlights the role of Pgrmc1 as a metabolic modulator of cardiac health. Since suppression of fatty acid oxidation without decreasing mitochondrial biogenesis is observed in the early stages of heart failure, individuals with low cardiac Pgrmc1 expression might be prone to mitochondrial impairment and progression to heart failure. According to our study, induction of Pgrmc1 expression improves the fundamental cardiac energy capacity and reduces cardiac lipid accumulation by increasing fatty acid oxidation and mitochondrial respiration. This is important because a fundamental increase in ATP production capacity is suggested for the targeted treatment of cardiomyopathy.

Methods
Animals. C57BL/6J mice were housed in a pathogen-free facility at Chungnam National University under a standard 12 h light:12 h dark cycle and fed standard chow or high-fat diet, with water provided ad libitum. All animal experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Republic of Korea), and in compliance with ARRIVE guidelines. Animal experiment was approved by the Institutional Animal Care and Use Committee of Chungnam National University (IACUC; approval no. CNU-01145). As reported previously, Pgrmc1 KO mice and wild-type littermates were used in this study. Fed conditions were induced by 6 h of feeding after 18 h of fasting. HFD conditions were induced by injecting streptozotocin (30 mg/kg) and feeding a high-fat diet for 8 weeks according to previous studies. The high-fat diet (#D12492, Research Diets, Inc., New Brunswick, NJ) was composed of carbohydrate (20% kcal), protein (20% kcal), and fat (60% kcal).

Fatty acid oxidation activity. Fatty acid oxidation kit (E-141) was obtained from Biomedical Research (BMR, Buffalo, USA). All tissue was processed according to the manufacturer’s protocol. Values were normalized by protein concentration.

Measurements of biochemical values. All plasma samples were diluted 1/5-fold with PBS. Plasma FFA levels were measured employing a commercial kit (BM-FFA100) obtained from Biomax (Seoul). Blood glucose levels were measured by tail snipping using an Accu-Chek Active (Roche, 07124112) kit.

Measurements of cellular mitochondrial respiration, glycolysis and fatty acid oxidation. H9C2 cells were transfected and then transferred to a seahorse cell culture plate. The cells were then cultured in DMEM serum-starved medium [i.e., without the addition of fetal bovine serum (FBS)] for 5–6 h to remove endogenous hormones. For only glycolysis stress test, cells were further incubated in DMEM low-glucose medium [glucose 50 mg/dl without FBS]. Before the experiment, the cells were decarboxylated for 40 min to 1 h in XfP medium (103575-100, Agilent Technologies) containing the same amount of glutamine, sodium pyruvate, and glucose as that of the medium in which cells were grown. For the mitochondrial stress test (mitochondrial respiration and fatty acid oxidation), oligomycin (2 μM), FCCP (5 μM), and rotenone/antimycin (0.5 μM) were used, and oxygen consumption rate (OCR) was measured. For fatty acid oxidation measurement, BSA (34 μM)-conjugated palmitate (200 μM) was added to seahorse cell culture plate. For the glycolysis stress test, glucose (25 mM), oligomycin (2 μM), and 2-deoxy-D-glucose (2-DG, 100 μM) were used, and extracellular acidification rate (ECAR)
was measured. Seahorse XFp analyzer (Agilent Technologies), Seahorse XFp, XFp FluxPak (103022-100, Agilent Technologies), and XFp Cell Mito Stress test kit (103010-100, Agilent Technologies) were used for analysis.

**Measurements of metabolites (HPLC–MS/MS).** Standard metabolites and internal standards were purchased from Sigma-Aldrich and CDN isotopes. All solvents including water were purchased from J. T. Baker. Tissue was homogenized using TissueLyzer (Qiagen) after adding 400 μL chloroform/methanol (2/1, v/v) and 100 μL internal standard solution containing 10 μM 13C5-glutamine for metabolites related to energy metabolism and 5 μM malonyl-13C3 CoA for fatty acyl CoA. Metabolites were extracted from aqueous phase by liquid–liquid extraction. The aqueous phase was dried using vacuum centrifuge, and the sample was reconstituted with 50 μL of H2O/acetonitrile (50/50 v/v) prior to LC–MS/MS analysis. Metabolites were analyzed with LC–ware (GraphPad Inc., San Diego, CA, USA).

Lipid extraction from heart was done with Folch method. Briefly, tissue was homogenized with beads and 0.9% NaCl solution. Homogenates were mixed with chloroform and methanol and incubated. Chloroform and distilled water were added and homogenates were centrifuged, and lower phase was collected. Steps after homogenization were 3 times repeated. Samples were dried and dissolved in chloroform and 2-propanol. TG level was analyzed by TG measurement solution (AM1575-S, Asan-Set).

**Western blotting.** Protein samples were quantified by Bradford assay using PRO-Measure solution (Intron, #21011) and subjected to protein electrophoresis (SDS-PAGE). Gels were blotted by wet transfer using a Bio-Rad Power Pac at 350 V for 1 or 2 h. Membranes were blocked and incubated with primary antibodies for overnight at 4 °C. After washing, the membranes were incubated with secondary antibodies overnight at 4 °C. Next, after a washing step, the protein bands were detected using an ECL kit (XLS025-0000, Cyanagen) on the ChemiDoc system (Fusion Solo, Vilber Lourmat).

The primary antibodies used were: β-actin [Rabbit polyclonal antibody (Rab Poly Ab), Santa Cruz, sc-130656], HK1, HK2, PKM2, PDH1 [Rab Poly Ab, Cell Signaling Technology (CST), #8337], eIF2α [Rab Poly Ab, CST, #9722], p-eIF2α [Rabbit monoclonal antibody (Rab mono Ab), CST, #3597], CHOP [Mouse Monoclonal antibody, Invitrogen, #MA1-250], ANP [Rabbit polyclonal antibody (Rab Poly Ab, Abcam, ab14348), SPT1 [Rab Poly Ab, Abclonal, A6750], and PGRMC1 [Rab mono Ab, CST, #13856]. The secondary antibodies used were: Mouse anti-rabbit IgG (211-032-171, Jackson ImmunoResearch, 1:5000) and Goat anti-mouse IgG antibody (BS-0296G-HRP, Bioss, 1:5000).

**Statistical analysis.** Data are reported as mean ± SD. Student’s t test was performed using Graph Pad Software (GraphPad Inc., San Diego, CA, USA).
### Table 1. Primers used for real-time PCR.

| Gene name | Upper primer (5’-3’) | Lower primer (5’-3’) | Species |
|-----------|----------------------|----------------------|---------|
| Cpt2      | CAG CAC AGC ATC GTA CCC A | TCC CAA TGC GCT TCT CAA AAT | Mouse |
| Mcad      | ACG TTT CAA GAT CGC AAT GG | CTC CTT GGT GCT CCA CTA GC | Mouse |
| Vldl      | TAT CTC TGC CCA GGC ACT TT | TGG GTA TGG GAA CAC ATG TT | Mouse |
| Cs        | CCT GAG TGC CAG AAA ATG CTG | CCA CAT GAG AAG GCA GAG GT | Mouse |
| Acox2     | ACA AGT AGG AGC GCA AAG AC | AGC ATT GCG TAG AAG ATG GGC | Mouse |
| Idh3a     | TGC TTC GCC ACA TGG GAC TT | CGT TGC CTC CCA GAT CTT TT | Mouse |
| Udh4      | AAT GAT GGT CCT GCC TGG TG | TCA GGT GTG TTT TCT TGT TGC C | Mouse |
| Sru52     | GTC TGC CAT CAT TGC CAA CG | ATG GGG AGT CCG CTG CTC TT | Mouse |
| Sidh      | TCA GAC CCG CTT ATG TGT C | CAG CCC CAA CAG CAG AAC AC | Mouse |
| Fh1       | GTG GAA GTT CAC AAG GTC CTG | GGA CTT GCT CAA GGT AAC CAC | Mouse |
| Mdh2      | ATG CTC GAT CCC GCC TGG TC | GAG GGA TAG CCG CAG CAA TC | Mouse |
| Atp5b     | GTA CTG GAT TCA GGG GCA CC | CTA TGA ACT CAG GAG CCT G | Mouse |
| Ckmt2     | GTG CGG ACT ACC CTG ACC TTC | CCG TAT GGT CTA TCC CCC | Mouse |
| Ndufb5    | CGA GCT TGC AGA AAT CCC AGA AGG C | GTC CAT CAC CTC GGG CAC GCA TCA G | Mouse |
| Slc25a    | CGG CTC CTT GCA GGC TGT G | CAA TGC TGG CTT TGT ACT GC | Mouse |
| Atp2a2    | GAG AAC GCT CAC ACA AAG ACC | CAA TTC GTT GGA GCC CCA T | Mouse |
| Hcrt1     | CAA CGG ATG TAC CCA ATG TGA | GAT AGC AAG ATT GAC AGT GCT G | Mouse |
| Scn5a     | TGC TGA ATA AGG GCA AAA CCA | GCT GAA GAG CAG ATG TAC CAA AA | Mouse |
| Elovl6    | AGC AAA GCA CCC GAA CAA GTA G | CCA GGA GTA CAG CAG A | Mouse |
| Scd1      | GTC TCT GTT AGC ACC TTC TTT | CAG AGT ATG CGA AGG GGA AG | Mouse |
| Gpam      | AGA GGC TTC TAC CTG CCA GG | TTC ACG AGA CAG TAT GTG GC | Mouse |
| Agpat1    | TAA GAT GGC CTT CTA CAA CGG C | CCA TAC AGG TAT TTG ACG TGG AG | Mouse |
| Mogat1    | TGG TGC CAT TTT GTC CGG TG | TGC TAT GGT GTC GGG TGG TA | Mouse |
| Dque1     | TCC GTC CAT GGT GGT AGT G | TGA CAA AAG AAT CTT GCC GAC GA | Mouse |
| Tnf       | CCT GTC GAC CAC GTC GTA G | GGG AGT AGA CAA GTC AGA ACC C | Mouse |
| Il-1β     | GAA ATG CCA CCT TTT GAC AGT G | CTG GAT GCT CAG ATG AGG ACA | Mouse |
| Il-6      | CTG CAA GAG ACT TCC ATC AGC | AGT GGA GTA AAG AGG TCT GTT GG | Mouse |
| mtDNA     | CCT ATC ACC CTT GCC ATC AT | GAG GCT GTG CTT GTG AGC | Mouse |
| nDNA      | ATG GAA AGC CTG CCA TCA TG | TCC TTT TTG TTT AGC ATC AC | Mouse |
| mdDNA     | AAG TGG CTT CTG AGA CAT TC | TCT GTG TTT GAT TCC TGG CT | Rat |
| tDNA      | TCT CCT ACT TGG ATC ACT GTG G | GGC GAC TAC CAT CGA AAG TTG | Rat |

**Received:** 13 October 2020; **Accepted:** 5 April 2021

**Published online:** 22 April 2021

**References**

1. Rabinovich-Nikitin, I., Dhingra, R. & Kirshenbaum, L. A. Activation of mitophagy in high-fat diet-induced diabetic cardiomyopathy. *Circ. Res.* **124**, 1288–1290. [https://doi.org/10.1161/CIRCRESAHA.119.314967](https://doi.org/10.1161/CIRCRESAHA.119.314967) (2019).

2. Siri-Tarino, P. W., Sun, Q., Hu, F. B. & Krauss, R. M. Saturated fatty acids and risk of coronary heart disease: Modulation by replacement nutrients. *Curr. Atheroscler. Rep.* **12**, 384–390. [https://doi.org/10.1007/s11883-010-0131-6](https://doi.org/10.1007/s11883-010-0131-6) (2010).

3. Boden, G. Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. *Diabetes* **46**, 3–10 (1997).

4. Boden, G. Obesity and free fatty acids. *Endocrinol. Metab. Clin. N. Am.* **37**, 635–646. [https://doi.org/10.1016/j.ecl.2008.06.007](https://doi.org/10.1016/j.ecl.2008.06.007) (2008).

5. Abbasi, F., Brown, B. W. Jr., Lamendola, C., McLaughlin, T. & Reaven, G. M. Relationship between obesity, insulin resistance, and coronary heart disease risk. *J. Am. Coll. Cardiol.* **40**, 937–943. [https://doi.org/10.1016/s0735-1097(02)02051-x](https://doi.org/10.1016/s0735-1097(02)02051-x) (2002).

6. Kankaanpaa, M. et al. Myocardial triglyceride content and epicardial fat mass in human obesity: Relationship to left ventricular function and serum free fatty acid levels. *J. Clin. Endocrinol. Metab.* **91**, 4689–4695. [https://doi.org/10.1210/jc.2006-0584](https://doi.org/10.1210/jc.2006-0584) (2006).

7. Nagoshi, T., Yoshimura, M., Rosano, G. M., Lopaschuk, G. D. & Mochizuki, S. Optimization of cardiac metabolism in heart failure. *Circ. Res.* **117**, 2646–2653. [https://doi.org/10.1161/CIRCRESAHA.117.310520](https://doi.org/10.1161/CIRCRESAHA.117.310520) (2015).

8. Goldberg, I. J., Trent, C. M. & Schulze, P. C. Lipid metabolism and toxicity in the heart. *Cell Metab.* **15**, 805–812. [https://doi.org/10.1016/j.cmet.2012.04.006](https://doi.org/10.1016/j.cmet.2012.04.006) (2012).

9. Lee, S. R. et al. Loss of progesterone receptor membrane component 1 promotes hepatic steatosis via the induced de novo lipo genesis. *Sci. Rep.* **8**, 15711. [https://doi.org/10.1038/s41598-018-34148-6](https://doi.org/10.1038/s41598-018-34148-6) (2018).

10. Zhang, M. et al. Progesterone receptor membrane component 1 is a functional part of the glucagon-like peptide-1 (GLP-1) receptor complex in pancreatic beta cells. *Mol. Cell Proteom.* **13**, 3049–3062. [https://doi.org/10.1074/mcp.M114.041966](https://doi.org/10.1074/mcp.M114.041966) (2014).

11. Lee, S. R. et al. Progesterone increases blood glucose via hepatic progesterone receptor membrane component 1 under limited or impaired action of insulin. *Sci. Rep.* **10**, 16316. [https://doi.org/10.1038/s41598-020-73330-7](https://doi.org/10.1038/s41598-020-73330-7) (2020).

12. Rohé, H. J., Ahmed, I. S., Twist, K. E. & Craven, R. J. PGRMC1 (progesterone receptor membrane component 1): A targetable protein with multiple functions in steroid signaling, P4S5 activation and drug binding. *Pharmacol. Ther.* **121**, 14–19. [https://doi.org/10.1016/j.pharmthera.2008.09.006](https://doi.org/10.1016/j.pharmthera.2008.09.006) (2009).

13. Piel, R. B. 3rd. et al. A Novel role for progesterone receptor membrane component 1 (PGRMC1): A partner and regulator of ferrochelatase. *Biochemistry* **55**, 5204–5217. [https://doi.org/10.1021/acs.biochem.6b00756](https://doi.org/10.1021/acs.biochem.6b00756) (2016).
14. Shuster, A., Patlas, M., Pintus, J. H. & Mourtzakis, M. The clinical importance of visceral adiposity: A critical review of methods for visceral adipose tissue analysis. Br. J. Radiol. 85, 1–10. https://doi.org/10.1259/bjr/38447338 (2012).
15. Christoffersen, C. et al. Cardiac lipid accumulation associated with diastolic dysfunction in obese mice. Endocrinology 144, 3483–3490. https://doi.org/10.1210/en.2003-0242 (2003).
16. Ingwall, J. S. Energy metabolism in heart failure and remodelling. Cardiovasc. Res. 81, 412–419. https://doi.org/10.1093/cvr/cvn301 (2009).
17. Ashrafian, H., Frenneaux, M. P. & Opie, L. H. Metabolic mechanisms in heart failure. Circulation 116, 434–448. https://doi.org/10.1161/CIRCULATIONAHA.107.702795 (2007).
18. Hom, J. & Sheu, S. S. Morphological dynamics of mitochondria—a special emphasis on cardiac muscle cells. J. Mol. Cell Cardiol. 46, 811–820. https://doi.org/10.1016/j.yjmcc.2009.02.023 (2009).
19. Stanley, W. C., Recchia, F. A. & Lopaschuk, G. D. Myocardial substrate metabolism in the normal and failing heart. Physiol. Rev. 85, 1093–1129. https://doi.org/10.1152/physrev.00006.2004 (2005).
20. Sverdlov, A. L. et al. High fat, high sucrose diet causes cardiac mitochondrial dysfunction due in part to oxidative post-translational modification of mitochondrial complex II. J. Mol. Cell Cardiol. 78, 165–173. https://doi.org/10.1016/j.yjmcc.2014.07.018 (2015).
21. Abo Alroh, O. & Lopaschuk, G. D. Role of CoA and acetyl-CoA in regulating cardiac fatty acid and glucose oxidation. Biochem. Soc. Trans. 42, 1043–1051. https://doi.org/10.1042/BST20140094 (2014).
22. Huang, B., Wu, P., Bowker-Kinley, M. M. & Harris, R. A. Regulation of pyruvate dehydrogenase kinase expression by peroxisome proliferator-activated receptor-alpha ligands, glucocorticoids, and insulin. Diabetes 51, 276–283. https://doi.org/10.2337/diabetes.51.2.276 (2002).
23. Larsen, P. J. & Tennagels, N. On ceramides, other sphingolipids and impaired glucose homeostasis. Mol. Metab. 3, 252–260. https://doi.org/10.1016/j.molmet.2014.01.011 (2014).
24. Zlobine, I., Gopal, K. & Usber, J. R. Lipotoxicity in obesity and diabetes-related cardiac dysfunction. Biochem. Biophys. Acta 1555–1568, 2016. https://doi.org/10.1016/j.bbapal.2016.02.011 (1861).
25. Schulze, P. C., Drosatos, K. & Goldberg, I. J. Lipid use and misuse by the heart. Circ. Res. 118, 1736–1751. https://doi.org/10.1161/CIRCRESAHA.116.366842 (2016).
26. Stanley, W. C. & Recchia, F. A. Lipotoxicity and the development of heart failure: Moving from mouse to man. Cell Metab. 12, 555–556. https://doi.org/10.1016/j.cmet.2010.11.016 (2010).
27. Segura, A. M., Frazier, O. H. & Buja, L. M. Fibrosis and heart failure. Heart Fail. Rev. 19, 173–185. https://doi.org/10.1007/s11799-012-9365-4 (2014).
28. Shirazi, L. F., Bissett, J., Romeo, F. & Mehta, J. L. Role of inflammation in heart failure. Curr. Atheroscler. Rep. 19, 27. https://doi.org/10.1007/s11883-017-0660-3 (2017).
29. Ghosh, S., Silkysioningrum, D. C., Giler, M. B., Verchere, C. B. & Devlin, A. M. Altered glutathione homeostasis in heart augments cardiac lipotoxicity associated with diet-induced obesity in mice. J. Biol. Chem. 286, 42483–42493. https://doi.org/10.1074/jbc.M111.304592 (2011).
30. Minamino, T. & Kitakaze, M. ER stress in cardiovascular disease. J. Mol. Cell Cardiol. 48, 1105–1110. https://doi.org/10.1016/j.yjmcc.2009.10.026 (2010).
31. Myhill, S. & Malahni, N. Diagnostic markers of acute myocardial infarction. Biomed. Rep. 3, 743–748. https://doi.org/10.3892/br.2015.300 (2015).
32. Saito, Y. et al. Augmented expression of atrial natriuretic polypeptide gene in ventricle of human failing heart. J. Clin. Invest. 83, 298–305. https://doi.org/10.1172/JCI133872 (1989).
33. Lai, L. et al. Energy metabolic reprogramming in the hypertrophied and early stage failing heart: A multisystems approach. Circ. Heart Fail. 7, 1022–1031. https://doi.org/10.1161/CIRCHEARTFAILURE.114.001469 (2014).
34. Rosca, M. G. & Hoppel, C. L. Mitochondria in heart failure. Cardiovasc. Res. 88, 40–50. https://doi.org/10.1093/cvr/cvr071 (2011).
35. Skovso, S. Modeling type 2 diabetes in rats using high fat diet and streptozocin. J. Diabetes Investig. 5, 349–358. https://doi.org/10.1111/jdi.12235 (2014).
36. Folch, J., Lees, M. & Sloane Stanley, G. H. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. 226, 497–509 (1957).

Acknowledgements
We thank the GEAR core, HTS core, and Metabolomics core at the Convergence Medicine Research Center, Asan Medical Center for support and instrumentation.

Author contributions
S.R.L., and E.-J.H. designed research; S.R.L., J.H.H., S.L.J., G.K., and S.J.K. performed research; S.R.L., H.J.Y, K.-P.L., H.-J.K., H.-J.S., I.-J.B. and E.-J.H. analyzed data; S.R.L. and E.-J.H. wrote the initial draft; S.R.L., I.-J.B. and E.-J.H. revised the paper.

Funding
This research was supported by National Research Foundation of Korea (NRF) Grants (2018R1D1A1A02043102 to EJH). This research was supported by a Grant from the Korea Health Technology R&D Project through the Korea Health Industry Development Institute, funded by the Ministry of Health & Welfare, Republic of Korea (HI17C1408). This work was also supported by grants (2018-695, 2019-849) from the Asan Institute for Life Sciences, Asan Medical Center, Seoul, Korea. This work was supported by NRF (National Research Foundation of Korea) Grant funded by the Korean Government (NRF-2019-Global Ph.D. Fellowship Program).

Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-021-88251-2.

Correspondence and requests for materials should be addressed to I.-J.B. or E.-J.H.

Reprints and permissions information is available at www.nature.com/reprints.
