Effects of fibroblast growth factor 21 on the heart

Pongpan Tanajak[1,2], Siriporn C Chattipakorn[1,3,4] and Nipon Chattipakorn[1,2,3]

[1]Cardiac Electrophysiology Research and Training Center, Faculty of Medicine, [2]Cardiac Electrophysiology Unit, Department of Physiology, Faculty of Medicine, [3]Center of Excellence in Cardiac Electrophysiology Research, and [4]Department of Oral Biology and Diagnostic Sciences, Faculty of Dentistry, Chiang Mai University, Chiang Mai 50200, Thailand

Correspondence should be addressed to N Chattipakorn
Email
ncchattip@gmail.com

Abstract

Fibroblast growth factor 21 (FGF21) is a novel polypeptide ligand that has been shown to be involved in several physiological and pathological processes including regulation of glucose and lipids as well as reduction of arteriosclerotic plaque formation in the great vessels. It has also been shown to exert cardioprotective effects in myocardial infarction, cardiac ischemia-reperfusion injury, cardiac hypertrophy and diabetic cardiomyopathy. Moreover, FGF21 protects the myocardium and great arteries by attenuating remodeling, inflammation, oxidative stress and also promoting the energy supply to the heart through fatty acid β-oxidation. This growing evidence emphasizes the important roles of FGF21 in cardioprotection. This review comprehensively summarizes and discusses the consistent and inconsistent findings regarding the beneficial effects of FGF21 on the heart available from both basic research and clinical reports. The details of the signaling, biological and pharmacological effects of FGF21 with regard to its protection of the heart are also presented and discussed in this review.

Key Words
- fibroblast growth factor 21
- myocardial injury
- cardiac metabolism
- oxidative stress

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Introduction

Fibroblast growth factors (FGFs) are polypeptide chains that have paracrine, autocrine or endocrine functions. The paracrine FGFs are further divided into five subfamilies, whereas the autocrine and endocrine FGFs are composed of one subfamily each (Itoh & Ornitz 2011, Itoh & Ohta 2013) (Fig. 1). FGFs act through cell surface FGF receptors (FGFRs), which are regulated by four types of genes including FGFR1, FGFR2, FGFR3 and FGFR4 (Mohammadi *et al*. 2005, Beenken & Mohammadi 2009, Goetz & Mohammadi 2013). Although FGFRs are essential for FGF action on the target cells, they cannot activate intracellular signaling without co-receptors (Kharitonenkov 2008). Previous studies show that heparan sulphate proteoglycans are essential co-receptors for paracrine and autocrine FGFs (Beenken & Mohammadi 2009, Goetz & Mohammadi 2013), whereas Klothos are essential co-receptors for endocrine FGFs to mediate their attachment to and activation of target FGFRs (Suzuki *et al*. 2008, Beenken & Mohammadi 2009, Goetz & Mohammadi 2013).

FGF21 is an endocrine FGF that consists of 209 amino acids. The FGF21 ligand is produced from several organs such as the liver and adipose tissue (Ito *et al*. 2000), skeletal muscle (Joki *et al*. 2015), and the heart (Nishimura *et al*. 2000, Kharitonenkov 2009, Planavila *et al*. 2013, Patel *et al*. 2014). To activate FGF21 signaling, FGF21 binds to FGFR1c with its C-terminus, and also with β-Klotho as its co-receptor with its N-terminus, to form the FGFR/β-Klotho complex (Kharitonenkov 2008, Suzuki *et al*. 2008, Yie *et al*. 2009, Ding *et al*. 2012, Hale *et al*. 2012).

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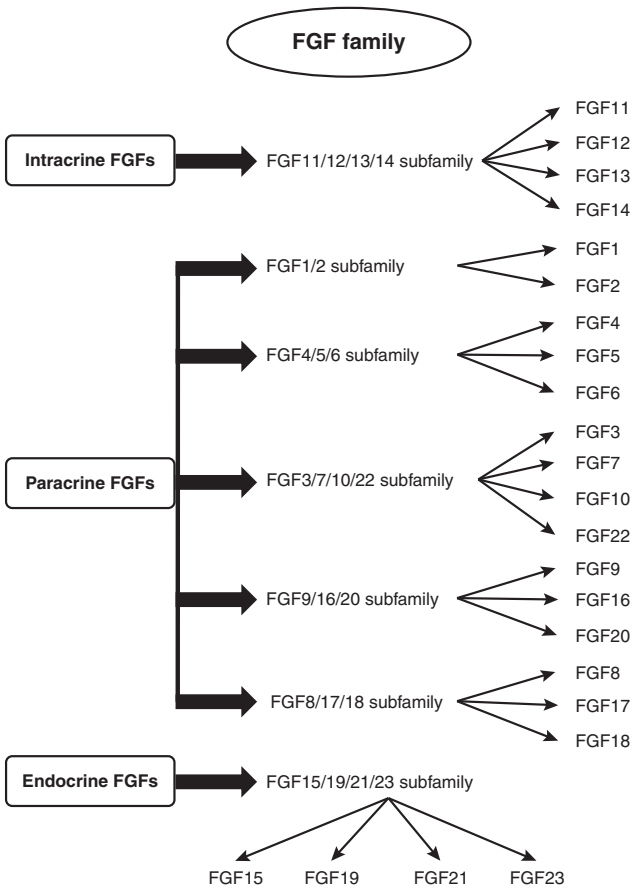

Figure 1
Fibroblast growth factors. FGFs have 22 members which can be divided into three classes and subdivided into seven subfamilies. Intracrine FGFs (11/12/13/14 subfamily); Paracrine FGFs (1/2 subfamily, 4/5/6 subfamily, 3/7/10/22 subfamily, 9/16/20 subfamily, and 8/17/18 subfamily); Endocrine FGFs (15/19/21/23 subfamily). Data from Itoh & Ohta (2013) and Itoh & Ornitz (2011).

The FGFR/β-Klotho complex then stimulates the autophosphorylation of the fibroblast receptor substrate 2 alpha (FRS2α), which is the first step in the downstream signaling of FGF21 (Kharitonenkov 2008, Suzuki *et al.* 2008). However, FGF21 is believed to have no action in physiological conditions since FGF21 knockout (FGF21-KO) mice were found to have normal development (Badman *et al.* 2009), and did not develop any pathological conditions such as insulin resistance (Hotta *et al.* 2009, Potthoff *et al.* 2009). Nevertheless, future studies are needed to evaluate this hypothesis.

FGF21 has been shown to play an important role in pathological processes, such as the regulation of plasma glucose level (Nishimura *et al.* 2000) and fatty acid β oxidation (FAO) which is the primary energy source for the myocardium (Vega *et al.* 2000, Planavila *et al.* 2013). Under stress conditions, FGF21 has been shown to reduce the apoptosis of Islet β cells (Wente *et al.* 2006), hepatocytes (Yu *et al.* 2015), vascular cells (Wu *et al.* 2014), cardiac endothelial cells (Lu *et al.* 2010) and cardiomyocytes (Cong *et al.* 2013, Liu *et al.* 2013). Interestingly, FGF21 also protects the heart from apoptosis and remodeling through the activation of adiponectin release to activate the adiponectin signaling pathways (Joki *et al.* 2015). Currently, the biological and pharmacological mechanism of FGF21 in cardioprotection is still to be elucidated. This review will focus on the effects of FGF21 and its roles in the heart. The consistent and inconsistent findings regarding the beneficial effects of FGF21 in the heart available from both basic research and clinical reports are comprehensively summarized and discussed. The details of the signaling, biological and pharmacological effects of FGF21 with regards to its protection of the heart are also presented and discussed in this review.

Effects of FGF21 on the heart

FGF21 is synthesized and expressed in the heart by cardiomyocytes (Planavila *et al.* 2013) and cardiac microvascular endothelial cells (CMECs) (Lu *et al.* 2010). A previous study demonstrated that cardiomyocytes secrete FGF21 into the media culture in basal conditions at a rate of ~0.05 ng/ml per 24 h (Planavila *et al.* 2013). In the heart, FGF21 ligands act via the FGFR1c (Suzuki *et al.* 2008, Liu *et al.* 2013, Planavila *et al.* 2013, Wu *et al.* 2014), and FGFR3 (Suzuki *et al.* 2008, Liu *et al.* 2013), utilizing β-Klotho as a co-receptor (Suzuki *et al.* 2008, Liu *et al.* 2013, Planavila *et al.* 2013). Endogenous and exogenous FGF21 plays an anti-apoptotic role in both *in vitro* and *in vivo* models, partially through the adiponectin signaling cascade (Joki *et al.* 2015). Recent studies found that FGF21 protects against isoproterenol (ISO) induced cardiac hypertrophy by activating anti-oxidative pathways (Planavila *et al.* 2013, 2014) and promoting FAO (Planavila *et al.* 2013). FGF21 also protects the heart from ischemic reperfusion (I/R) injury and myocardial infarction (MI) by activating several survival pathways (Cong *et al.* 2013, Liu *et al.* 2013, Patel *et al.* 2014). Moreover, FGF21 deficiency accelerated the development of diabetic cardiomyopathy (DCM) (Yan *et al.* 2015). In contrast, FGF21 administration also prevents lipotoxicity and diabetes induced cardiac apoptosis in DCM (Zhang *et al.* 2015a).

Interestingly, Liu and colleague demonstrated that the endogenous FGF21 which acted as endocrine protection in the ischemic myocardium was not from the heart but from the liver and adipose tissue (Liu *et al.* 2013),

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indicating that the major endogenous FGF21 proteins which preserve cardiac function are from the liver and adipose tissues. Although FGF21 from cardiomyocytes is not a major source, previous studies demonstrated that the autocrine action of FGF21 from cardiomyocytes is essential and could protect the heart from pathological conditions such as cardiomyocyte hypertrophy and I/R injury (Planavila *et al*. 2013, 2014).

Effects of FGF21 on myocyte apoptosis and myocardial infarction

Myocardial ischemia and I/R injury induce cell apoptosis and MI, leading to an impairment in cardiac function. Growing evidence from both *in vitro* and *in vivo* studies demonstrate that exogenous FGF21 protected the cardiomyocytes from apoptosis and MI, and improved cardiac function through activating the PI3K-Akt1-BAD pathway in FGF21-KO mice (Liu *et al*. 2013), and Akt-GSK3β-caspase 3 dependent pathways in H9c2 cell lines (Cong *et al*. 2013), resulting in the suppression of caspase 3 induced apoptosis. It was proposed that the activation of these pathways would lead to a decrease in the myocardial infarct area and increase cardiac function (Liu *et al*. 2013, Patel *et al*. 2014).

Evidence regarding the effects of FGF21 on inhibiting cardiovascular cell apoptosis in *in vitro* models is summarized in Table 1. FGF21 protects H9c2 cells from I/R injury in a dose dependent manner by promoting the energy supply, and reducing inflammation and apoptosis through the Akt-GSK3β pathway (Cong *et al*. 2013). On other hand, a previous study found peroxisome proliferator activated receptor alpha (PPARα) activation led to the synthesis and release of FGF21. FGF21 was released into the culture media, and protected the CMECs from lipotoxicity induced by Ox-LDL by decreasing DNA fragmentation in an autocrine manner (Lu *et al*. 2010). In an *ex vivo* model of global cardiac ischemia, it has been shown that recombinant rat FGF21 infusion 10 min prior to ischemia can protect the heart from I/R injury by decreasing MI and increasing the cardiac function through activation of the MAPK-PI3k-Akt signaling pathway (Patel *et al*. 2014). Moreover, FGF21 prevented oxidative stress (Cong *et al*. 2013, Planavila *et al*. 2014), and also increased the energy supply for cardiomyocytes in H9c2 cell lines under I/R injury conditions (Cong *et al*. 2013).

In addition to *in vitro* reports, evidence regarding the effects of FGF21 on cell apoptosis and myocardial infarction in *in vivo* models is summarized in Table 2. In FGF21-KO mice, FGF21 given intravenously at 50 ng/g per day for 3 days with the first dose being given immediately after I/R injury ($I=30$ min, $R=1$–30 days), had been shown to protect the heart from apoptosis, MI, and also increase cardiac function through activation of the FGFR1/β-Klotho-PI3K-Akt1-BAD signaling cascade (Liu *et al*. 2013). The acute MI in C57BL/6 mice showed that an i.v. injection of Recombinant mouse FGF21 10 ng/g in a single dose immediately post MI, which was caused by a left anterior descending coronary artery ligation, decreased the infarction area. It was also shown that these protective effects could be reversed by SiRNA-FGF21 intravenously injected 1 day prior to MI (Liu *et al*. 2012). Moreover, in chronic MI (2 weeks) C57BL6 and adiponectin-KO mice models it was demonstrated that FGF21 protein derived from skeletal muscles protected the heart from apoptosis through adiponectin signaling (Joki *et al*. 2015). In addition, FGF21 100 μg/kg per day s.c. injections for 4 weeks could protect the abdominal aorta from arteriosclerotic lesions through lipid regulation and ER stress induced vascular cell apoptosis in the ApoE-KO model (Wu *et al*. 2014).

All of these findings indicate that exogenous and endogenous FGF21 play an important role in protecting the heart from apoptosis via several pathways including PI3K-Akt1-BAD and Akt-GSK3β-caspase 3 dependent mechanisms, leading to decreased infarction and increased left ventricular function under I/R injury, lipotoxic and MI conditions.

Molecular basis of anti-apoptosis signaling cascades of FGF21

The anti-apoptotic signaling cascade of FGF21 from in *in vitro* and in *in vivo* models previously mentioned are summarized in Fig. 2. After FGF21 binding to FGFR1 and β-klotho via its N-terminus and C-terminus, respectively, the FGF21 ligand induces dimerization of receptors, and the autophosphorylation of tyrosine kinase recruits and phosphorylates FRS2α. In later steps, the anti-apoptotic signaling pathways in cardiomyocytes could be activated through 4 major survival pathways, including Erk1/2, RORα, PI3k-Akt and AMPK signaling pathways. Currently, the downstream signaling proteins involved in these processes are still unclear (Patel *et al*. 2014).

Previous studies demonstrated that the downstream signaling cascades of FGF21 begin with the autophosphorylation of the receptor after the binding of FGF21. This leads to the phosphorylation of FRS2α, and subsequent activation of PI3K (Liu *et al*. 2013, Patel *et al*. 2014, Yu *et al*. 2015) following its phosphorylation at Serin458

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Table 1 Effects of FGF21 on apoptosis in cardiomyocytes and endothelial cells

Model	Methods	Dose	Results	Interpretation	References
H9c2 cells (rat cardiomyocytes)	I/R injury ($I = 3$ h, $R = 1$, 3, 6 h)	FGF21 0.25, 0.5, 1, 1.5, 2, 4, 5, 6, 7, 8 μg/ml applied immediately at reperfusion	↑ Cell viability (dose-dependent) ↑ pAkt, GSK3β ↑ ATP synthase-α, Energy supply ↓ Apoptosis ↓ Cleaved caspase-3/pro-caspase 3 ratio ↓ TNFα, PAI1 ↓ H_2O_2 damaged cells	FGF21 protects H9c2 cells from I/R injury by promoting the energy supply, and reducing inflammation and apoptosis in cardiomyocytes through Akt-GSK3β pathway in a dose-dependent manner.	Cong *et al.* (2013)
CMECs	Lipotoxicity induced by Ox-LDL50 or 100 μg/ml 37 °C for 12 h	Bezafibrate (PPAR-α ligand) 50, 100, 200 μmol/l for 12 h, Transfected with shRNA-FGF21 vector	PPAR-α ligand 200 μmol/l ↑ Culture media FGF21 concentration ↓ DNA fragmentation shRNA-FGF21 transfection ↑ DNA fragmentation ↓ Culture media FGF21 concentration	PPAR-α activation increases FGF21 synthesis and release into culture media and protects the CMECs from lipotoxicity.	Lu *et al.* (2010)
Male wistar rats	I/R injury; *ex vivo* (*I*: 30 min, *R*: 120 min)	Rr FGF21 100 nM (in normal tyrodes solution) infusion 10 min prior to ischemia	↑ Rate pressure production ↑ ±dp/dt ↑ MAPK-PI3k-Akt signaling ↓ Total infarct size, LVDP	FGF21 increases cardiac function and decreases infarct size due to I/R injury through the activation of MAPK-PI3k-Akt signaling.	Patel *et al.* (2014)

I/R, ischemic reperfusion; GSK3β, glycogen synthase kinase 3β; TNF α, tumor necrosis factors alpha; PAI 1, plasminogen activator inhibitor 1; CMECs, Cultured cardiac micro vascular endothelial cells; OX-LDL, oxidized low density lipoprotein; PPARα, Peroxisome proliferator-activated receptor alpha; PGC1α, PPARγ coactivated 1 alpha; Rr, recombinant rats; LVDP, left ventricular diastolic pressure; MAPK, AMP-activated protein kinase; PI3K, phosphatidylinositol 3 kinase.

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Table 2 Effects of FGF21 on myocardial infarction and apoptosis in *in vivo* models

Model	Methods	Dose	Results	Interpretation	References
FGF21-KO mice	I/R injury ($I=30$ min, $R=1$, 3, 5, 10, 20, 30 days)	FGF21 50 ng/g per day, i.v. immediately after myocardial injury, and for the next 3 days	↑ ± dp/dt, %FS ↑ FGF21 sensitivity ↓ Apoptosis, MI ↑ FGFR1/β-Klotho–PI3K–Akt1–BAD signaling	FGF21 decreases cellular apoptosis and increases cardiac function due to I/R injury through an activation of FGFR1/β-Klotho–PI3K–Akt1–BAD signaling.	Liu *et al.* (2013)
ApoE$^{-/-}$ mice	Abdominal aortic (AA) arteriosclerosis for 4 weeks	FGF21 100 μg/kg per day, s.c. injection for 4 weeks	↑ AA luminar diameter ↓ Arteriosclerotic lesion area ↓ TC, TG ↓ Cleaved caspase 12, CHOP, GRP94 ↓ Apoptotic rate	FGF21 protects the AA from arteriosclerotic lesion through lipid regulation and ER stress induced by vascular cell apoptosis.	Wu *et al.* (2014)
C57BL/6 mice	MI for 24 h	At MI, RmFGF21 10 ng/g, i.v. injection	↑ Infarct size by siRNA-FGF21 ↓ Infarct size by FGF211	FGF21 protects heart from acute MI by decreasing myocardial infarct area	Liu *et al.* (2012)
C57BL6, adiponectin-KO mice	Chronic MI (2 weeks)	Ad-FGF21 1×10^{-9} pfu/mouse, i.m. injection 3 days prior to MI	↑ Capillary density (CD31) ↑ Cardiac function ↓ TNFα, IL6 mRNA Reversed by adiponectin-KO	FGF21 protein was derived from skeletal muscle and protects the heart from apoptosis through adiponectin signaling.	Joki *et al.* (2015)

FGF21-KO, FGF21 knockout; ApoE$^{-/-}$, apolioprotein E knockout; I/R, ischemic reperfusion; MI, myocardial ischemia; Rm, Recombinant mouse; i.v., intravenous; PI3K, phosphatidylinositol 3 kinase; BAD, BCL2 antagonist of cell death; AA, Abdominal aortic; TC, total cholesterol; TG, triglyceride; CHOP, C/EBP homologous protein; GRP94, glucose-regulated proteins 94; ER, endoplasmic reticulum; LVDP, left ventricular diastolic pressure; MAPK, AMP-activated protein kinase.

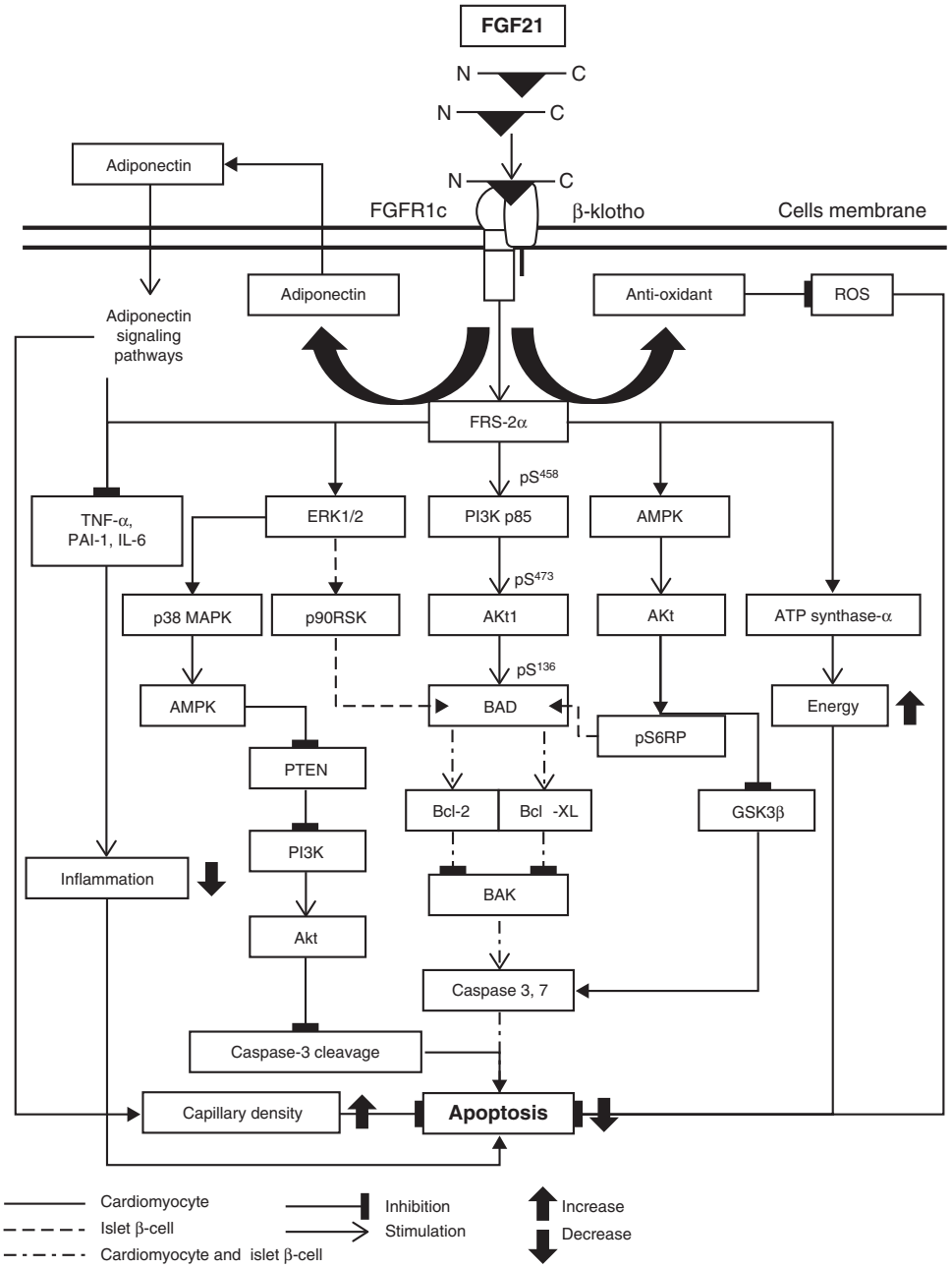

Figure 2

FGF21 signaling cascade in anti-apoptotic effects. FGF21 exerts anti-apoptotic effects in cardiomyocytes through decreased inflammation, improved FAO metabolism, increased capillary density and anti-oxidative stress. FGF21, Fibroblast growth factors 21; FGFR1c, Fibroblast growth factors receptors 1c; N, N-terminus residue of FGF21 or Amino acid terminal; C, C-terminus residue of FGF21 or Carboxylic terminal; FRS2α, Fibroblast growth factors substrate 2α; ROR, Retinoic acid receptor-related receptor; Erk1/2, Extracellular signal-regulated kinases 1/2; p90RSK, p90 ribosomal s6 kinase; pS6RP, pS6 ribosomal protein; AMPK, AMP dependent protein kinase; PI3K P85, Phosphatidylinositide-3 kinase P85; GSK3β, Glycogen synthase kinase-3b; BAD, BCL2 antagonist of cell death; BCL2, B cell lymphoma 2; BCL-XL, B cell lymphoma-extra-large; BAX, Bcl2 associated X protein; BAK, Bcl2 homologous antagonist killer; TNFα, tumor necrosis factors α; IL6, interleukin 6; PAI1, plasminogen activator inhibitor 1.

(pS^{458}). This leads to the recruitment and phosphorylation of a secondary messenger Akt1 by phosphorylation at Serine473 (pS^{473}). Akt1 in turn activates the BCL2 antagonist of cell death (BAD) by inducing the phosphorylation of BAD at Serine136 (pS^{136}). his causes BCL2 and BCL-XL to inhibit BAX and BAK induced caspase3/7 activity, which leads to decreased apoptosis in cardiomyocytes (Liu *et al*. 2013). In addition, FGF21 has

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been shown to inhibit apoptosis through another alternative pathway by activating Akt, thereby inhibiting GSK3β, thus leading to decreased caspase 3 activity (Akt-GSK3β-caspase 3 dependent pathways) (Cong *et al*. 2013) (Fig. 2). Moreover, FGF21 can protect the heart from apoptosis by activation of the Erk1/2-p38 MAPK-AMPK survival pathway (Zhang *et al*. 2015a). Evidence from these reports confirmed that FGF21 plays a critical role in myocardial protection and anti-apoptosis following myocardial injury (Patel *et al*. 2014, Joki *et al*. 2015, Zhang *et al*. 2015*a*).

Due to the potential cardioprotective benefits of FGF21, it is possible that FGF21 could be used to prevent and/or treat the myocardial apoptosis due to I/R injury or MI. However, evidence related to the roles of the time course of FGF21 administration and its beneficial effects to the pathological heart are still lacking.

Effects of FGF21 on cardiac hypertrophy and adverse cardiac remodeling

Myocardial ischemia resulting from coronary artery disease (CAD) is the primary cause of MI which could impair cardiac function by reducing the ejection fraction (EF), leading to insufficient oxygen supply to body tissues (Gheorghiade & Bonow 1998, Joki *et al*. 2015). This contributes to progression to cardiac hypertrophy and heart failure due to the compensatory mechanisms of the circulatory system to maintain the EF and carry oxygen to peripheral metabolic tissues, known as cardiac remodeling. This long-term maladaptive remodeling can cause increased ventricular hypertrophy, ventricular dilatation, interstitial growth and cardiac fibrosis (Neely *et al*. 1972).

Evidence regarding the effects of FGF21 on protection against adverse cardiac remodeling and hypertrophy in *in vitro* and *in vivo* models is summarized in Table 3. In a single *in vitro* study, pre-treatment with FGF21 protects neonatal cardiomyocytes (NCMs) from phenylephrine induced hypertrophy by promoting FAO gene expression, attenuating inflammation and oxidative stress through the activation of Sirt1 and Erk1/2-CREB signaling pathways (Planavila *et al*. 2013). This study also demonstrated that the Sirt1-PPARα pathway plays an important role in the control of FGF21 expression in the heart.

Evidence from *in vivo* studies demonstrate that continuous administration of ISO via s.c. infusion for 7 days in FGF21-KO mice induced cardiomyopathy and led to MI, impaired cardiac metabolism and loss of cardiac function in the rat heart (Heather *et al*. 2009, Planavila *et al*. 2013). Interestingly, the endocrine function of FGF21 derived from skeletal muscles attenuated cardiac hypertrophy, and reversed the adverse cardiac remodeling process, leading to improved left ventricular function in this chronic MI mice model (Joki *et al*. 2015). In FGF21-KO mice, it has been shown that FGF21 attenuated cardiac hypertrophy by decreasing hypertrophic markers including atrial natriuretic factor (ANF) and α skeletal actin (αSKA) (Planavila *et al*. 2013). Moreover, FGF21 decreased the heart weight/body weight ratio and cardiomyocytes area, and also improved cardiac function (Planavila *et al*. 2013, 2014).

In summary, the protective effects of FGF21 against cardiac hypertrophic damage have been evidenced. Conversely, FGF21 deficiency was found to enhance the induction of cardiac hypertrophy by promoting pro-inflammatory pathways, oxidative stress, cardiac fibrosis and impairing cardiac metabolism (Planavila *et al*. 2013, 2014). Results confirmed that cardiac FGF21 has an impact on activation of the autocrine loop and plays a protective role against cardiac hypertrophy and remodeling. However, further investigation and clinical studies are needed to warrant the usefulness of FGF21 against cardiac hypertrophy.

Molecular basis of anti-hypertrophic signaling cascades of FGF21

The FGF21 activates cells to autocrine function by binding to FGFR1 on the cell membrane, using β-Klotho as a co receptor. This event activates the dimerization of the receptor and causes autophosphorylation of tyrosine kinase. Tyrosine kinase then recruits and phosphorylates FRS2α. The FRS2α in turn affects four primary pathways, which in turn leads to the attenuation of cardiac hypertrophy. An illustrated diagram of the anti-hypertrophic effects of FGF21 in both the autocrine and endocrine manner by the loop autocrine function of FGF21 through the Sirt1/PPARα pathway is shown in Fig. 3.

The first of these four pathways is the activation of the Erk1/2-CREB-Sirt1-PGC1α signaling pathway as an autocrine function and autocrine loop regulation in FGF21-KO cardiomyocytes. This pathway leads to increased mitochondrial FAO enzyme genes expression including MCAD and mcpt1α, indicating increased cardiac mitochondrial FAO (Planavila *et al*. 2013). The second pathway involves the inhibition of the translocation of pro-inflammatory cytokines NFkβ into the nucleus to activate inflammatory cytokine expression including TNFα, IL6, and MCP1, resulting in a decrease in the

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Table 3 Effects of FGF21 on cardiac hypertrophy and remodeling

Model	Methods	Dose	Results	Interpretation	References
FGF21-KO mice	Cardiac hypertrophy induced by ISO 15 mg/kg per day for 7 days via s.c. Osmotic pump	FGF21 2.5 μg/kg per day, s.c. for 7 days	↓ HW/BW, cardiomyocyte area ↓ ANF, IL6 mRNA and protein ↓ αSKA mRNA ↑ PGC1α, MCAD, mcpt1α	FGF21 protects heart from ISO induced cardiac hypertrophy via promoting FAO gene expression, and reducing inflammation.	Planavila *et al.* (2013)
FGF21-KO mice	Cardiac hypertrophy induced by ISO	ISO 15 mg/kg per day, s.c. for 7 days	In FGF21-KO mice ↓ Antioxidant genes ↑ ROS production ↓ Aconitase activity ↑ Protein carbonyl	FGF21 protects heart from ISO induced cardiac hypertrophy through an anti-oxidative stress mechanism.	Planavila *et al.* (2014)
C57BL6 and adiponectin-KO mice	Chronic MI (2 weeks)	Ad-FGF21 1×10^{-9} pfu/mouse i.m. injection 3 days prior MI	↑ Capillary density (CD31) ↑ Cardiac function ↓ Cardiac hypertrophy ↓ Apoptosis ↓ TNFα, IL6 mRNA – Reversed by adiponectin-KO	FGF21 protein was derived from skeletal muscle, it protects the heart by attenuating cardiac remodeling in chronic MI.	Joki *et al.* (2015)
NCMs	Cardiomyocyte hypertrophy induced by PE	FGF21 pre-treatment (5 nM) for 24 h	↑ PGC1α, mcpt1α genes ↑ pErk1/2, pCREB ↑ FGF21 mRNA and protein levels by Sirt1 overexpression ↓ ROS production ↓ NFKB activity, IL6 ↓ ANF, αSKA ↓ Cardiomyocyte area ↓ FGF21mRNA expression in PPARα-KO	FGF21 protects NCMs from PE induced hypertrophy by promoting FAO gene expression, attenuating inflammation and oxidative stress through the activation of Sirt1 and Erk1/2-CREP signaling pathways.	Planavila *et al.* (2013)

FGF21-KO, FGF21 knockout; ANF, atrial natriuretic factor; αSKA, alpha skeletal actin; IL6, interleukin 6; PGC1α, PPARγ coactivated 1 alpha; MCAD, medium chain acetyl CoA dehydrogenase; mcpt1α, mitochondrial carnitine palmitoyltransferase 1; ISO, isoproterenol; LPS, lipopolysaccharide; ROS, reactive oxygen species; pfu, plaque-forming units; i.m., intramuscular; TNFα, TNFα tumor necrosis factor alpha; NCMs, neonatal cardiomyocytes; ROS, reactive oxygen species; NFkB, nuclear factor kappa beta; IL6, interleukin 6; PE, phenylephrine; PPARα, Peroxisome proliferator-activated receptor alpha; FAO, fatty acid oxidation; Sirt1, Sirtuin 1; Erk1/2, Extracellular signal-regulated kinases 1/2; CREB, cAMP response element-binding protein.

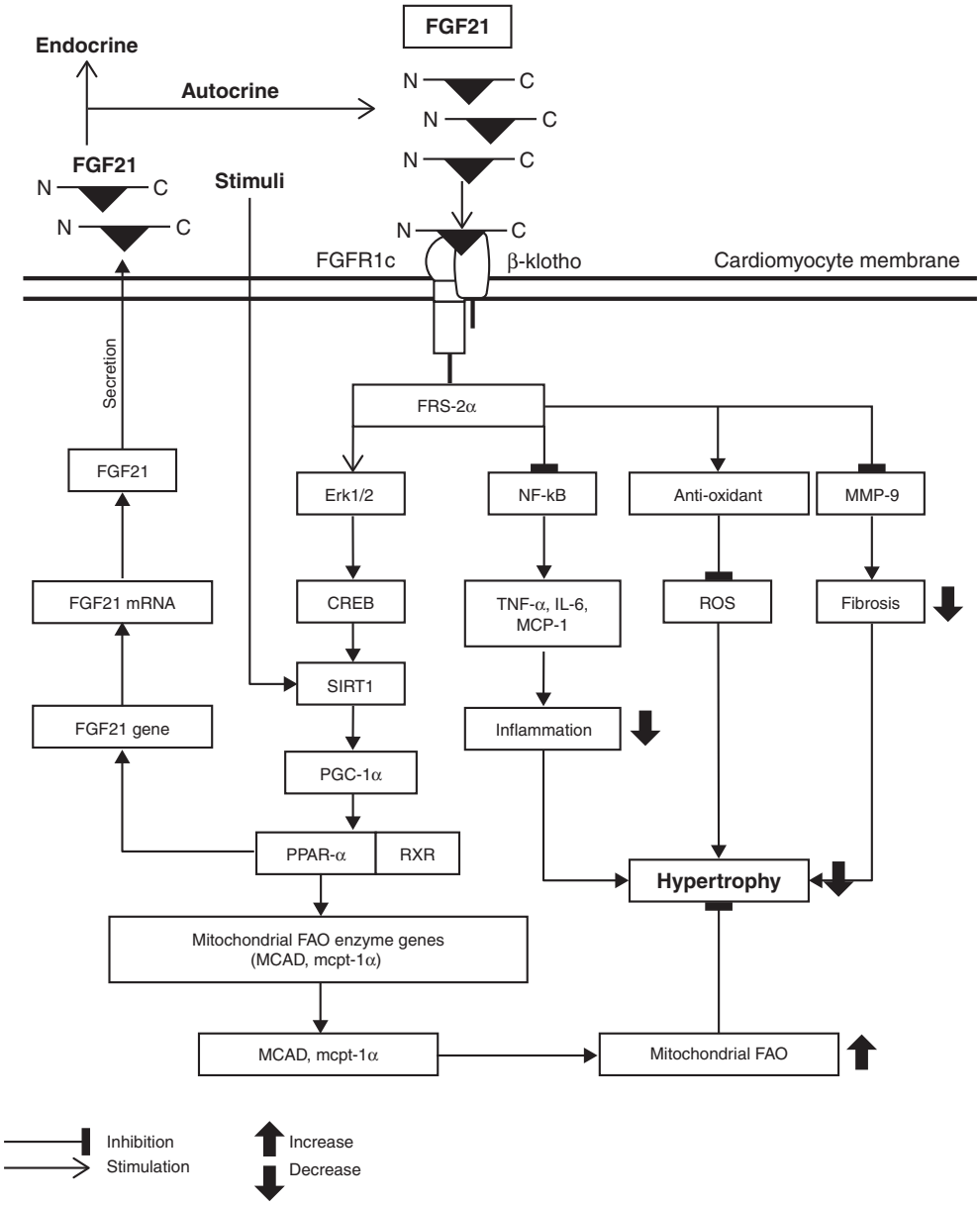

Figure 3
FGF21 signaling cascade in anti-hypertrophic effects of FGF21. FGF21 exerts anti-hypertrophic effects in autocrine and endocrine manners by loop autocrine function through the Sirt1/PPARα pathway. FAO, fatty acid β oxidation; PGC1α, peroxisome proliferator-activated receptor 1γ coactivator 1α; NFkB, nuclear factor Keppa B; SIRT1, sirtuin1; FGF21, fibroblast growth factors 21; FGFR1, fibroblast growth factors receptors 1; PPARα, peroxisome proliferator activated receptor α; MCAD, medium-chain acyl-CoA dehydrogenase; mcpt 1α, carnitine palmitoyltransferase Iα; Erk1/2, extracellular-signal-regulated kinases 1/2; CREB, cAMP response element-binding protein; RXR, retinoid X receptor; TNFα, tumour necrosis factors α; IL6, interleukin 6; MCP1, monocyte chemoattractant protein 1; MMP9, matrix metallopeptidase 9.

inflammatory processes (Planavila *et al*. 2013). The third pathway involves the inhibition of cardiac MMP9, which indicates a decrease in cardiac fibrotic formation following cardiac remodeling (Planavila *et al*. 2013). Finally, FGF21 activates the anti-oxidative pathway, resulting in the reduction of oxidative stress in the cells (Planavila *et al*. 2013, 2014).

FGF21 protects the heart from diabetes induced cardiomyopathy

Evidence regarding the protection of the heart from diabetes induced cardiomyopathy by FGF21 is summarized in Table 4. In FGF21 deficient mice, it has been shown that FGF21 is essential in the prevention of the

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Table 4 Protective effects of FGF21 on the heart from diabetes induced cardiomyopathy

Model	Methods	Dose	Results	Interpretation	References
FGF21-KO mice	T1DM was induced by STZ 60 mg/kg once a day via i.p. injection for 6 days	–	↑ Blood glucose, TG ↑ Cardiac Nrf2 and CD36 ↑ Cardiac 3NT and 4HNE ↑ Cardiac TG ↑ Cardiac lipid accumulation ↑ Cardiac collagen accumulation ↑ Cardiac CTGF ↓ Cardiac PGC1α ↓ %EF, %FS	FGF21 deficiency up-regulation of Nrf2 driven CD36 expression exacerbates cardiac lipid uptake and accumulation, oxidative stress, impairs cardiac lipid and glucose utilization, leading to acceleration of the development of DCM.	Yan *et al*. (2015)
FGF21-KO and WT mice	NEFA 0.1 g/10 g, i.p. injection T1DM was induced by STZ	FGF21 100 μg/kg per day for 10 days, i.p. injection	↑ Cardiac function ↓ Blood glucose, plasma TAG ↓ Cleaved caspase 3 ↓ Apoptosis ↓ Collagen contents	FGF21 protects the heart from lipotoxicity and diabetes induced apoptosis through activation of the Erk1/2-p38MAPK-AMPK signaling pathway.	Zhang *et al*. (2015*a*,*b*)
H9C2 cells and cardiomyocytes	Pre-treated with pharmaceutical inhibitors or specific small interfering (si) RNAs against Erk1/2, p38MAPK, AMPK, PTEN and pPTEN	FGF21 50 ng/ml, followed palmitate treatment for 15 min	↑ Erk1/2-p38MAPK-AMPK signaling pathway ↓ tPTEN, pPTEN ↓ Apoptosis		

FGF21-KO, FGF21 knockout; T1DM, type 1 diabetes mellitus; STZ, streptozotocin; i.p., intraperitoneal; TG, triglyceride; Nrf2, nuclear factor (erythroid-derived 2)-like 2; 3NT, 3 nitrotyrosine; 4HNE, 4 hydroxynonenal; CTGF, connective tissue growth factor; PGC1α, peroxisome proliferator-activated receptor gamma co-activator 1α; %EF, percent ejection fraction; % FS, percent fractional shortening; DCM, diabetic cardiomyopathy; NEFA, non esterified fatty acid.

progression of T1DM induced cardiomyopathy (Yan *et al.* 2015). It has been proposed that four potential mechanisms are responsible for this adverse effect due to FGF21 deficiency. First, increased cardiac oxidative stress was observed, as shown by increased 3 nitrotyrosine (3NT) and 4 hydroxynonenal (4HNE). Second, increased nuclear factor (erythroid derived 2) like 2 (Nrf2) activated CD36 expression was seen, which led to increased plasma lipid uptake and accumulation into the cells. Third, decreased PGC1α protein expression was observed, which led to decreased FAO thus promoting lipid uptake and accumulation in cardiomyocytes via the CD36 receptor on the cell membranes. Fourth, increased myocardial collagen accumulation was observed as shown by increased connective tissue growth factor (CTGF). In contrast, FGF21 administration can protect the heart from lipotoxicity and diabetes induced cardiac apoptosis by the activating of the Erk1/2-p38 MAPK-AMPK survival pathway leading to decreased cardiac apoptosis and improved cardiac function (Zhang *et al.* 2015a). An illustrated diagram of these mechanisms is shown in Fig. 4. It has been proposed that these four potential mechanisms cause left ventricular dysfunction and accelerate the development and progression of DCM (Yan *et al.* 2015) and the effects can be reversed by FGF21 treatment (Zhang *et al.* 2015a).

FGF21 regulates energy supply in the heart

FAO is the major source of energy for cardiomyocytes, generating 50–70% of ATP in a normal adult heart, while only 20–30% of energy is released by glycolysis, and <5% from other sources (Neely *et al.* 1972, Neely & Morgan 1974). It has been shown that the transition from fetal glycolysis (fetal pattern) to FAO in the neonatal stage (Lockwood & Bailey 1970, Kelly *et al.* 1989) is brought about by increased PGC1α, PPARα, and FGF21 mRNA expression (Planavila *et al.* 2013). In contrast, the chicken ovalbumin upstream promoter transcription factor (COUP-TF) that regulates glycolysis is down regulated (Sack *et al.* 1997). PPARα is expressed at a high rate in mitochondrial FAO tissue, and is situated on the nuclear membrane with the retinoid X receptor (RXR). PGC1α binding to the PPARα/RXR on the nuclear membrane leads to the increased expression of FAO genes including MCAD and mcpt1α, hence promoting increased FAO and synthesis of the ATP supply for the heart in physiological conditions (Vega *et al.* 2000) (Fig. 5A).

Under pathological conditions such as cardiac hypertrophy, myocardium FAO enzyme genes are down regulated (Sack *et al.* 1997, Razeghi *et al.* 2001), while the COUP-TF is up regulated (Sack *et al.* 1997). This caused the switch of the energy source back to the fetal glycolysis pattern again (Fig. 5B). A recent study demonstrated that myocardium PGC1α, MCAD, and mcpt1α mRNA expression is regulated by FGF21 to promote FAO for the energy supply to the heart (Planavila *et al.* 2013). The deletion of FGF21 has been shown to increase CD36 and decrease PGC1α, leading to acceleration of DCM through aggravating cardiac lipid accumulation (Yan *et al.* 2015). Therefore, FGF21 might be beneficial as a pharmacological intervention under these conditions. Further studies are needed to give more evidence for the substantiation to this hypothesis.

Molecular basis of the antioxidant signaling cascade of FGF21 in cardiomyocytes

Previous studies demonstrated that following ISO induced cardiac hypertrophy by causing cardiac oxidative stress and inflammation, and that FGF21 was secreted from cardiomyocytes via Sirt1 activation. Sirt1 was found to stimulate FGF21mRNA and protein expression and secretion into the circulation, where FGF21 proceeded to act in a paracrine, autocrine and endocrine manner. n the autocrine loop function, FGF21 induces anti-oxidant gene expression through the Erk/Sirt1 pathway, including uncoupling protein 3 (UCP3), superoxide dismutase 2 (Sod 2), peroxiredoxin 5 (Prdx5), glutathione peroxidase 1 (GPX1), Catalase (CAT) and Sequestosome 1 (Sqstm1), resulting in a reduction in cardiac tissue injury (Planavila *et al.* 2014). Furthermore, FGF21 has been shown to activate the Nrf2 pathway in hepatocytes, which was found to lead to increased anti-oxidant gene expression, resulting in the reduction of liver tissue injury (Yu *et al.* 2015) (Fig. 6).

The stimulation of antioxidative pathways by FGF21 led to an increase in antioxidative gene and enzyme expression, and prevented oxidative stress by decreasing ROS production in cardiomocytes. Therefore, this protected the myocardium (Planavila *et al.* 2014) from oxidative stress and subsequent injury. Interestingly, a clinical study demonstrated that FGF21, UCP3, and Sod2 levels were increased in dilated cardiomyopathy patients in the final stages of heart failure (Planavila *et al.* 2014). This indicated that FGF21 could protect the cardiomyocytes or slow down the degree of damage following oxidative stress in a failing human heart.

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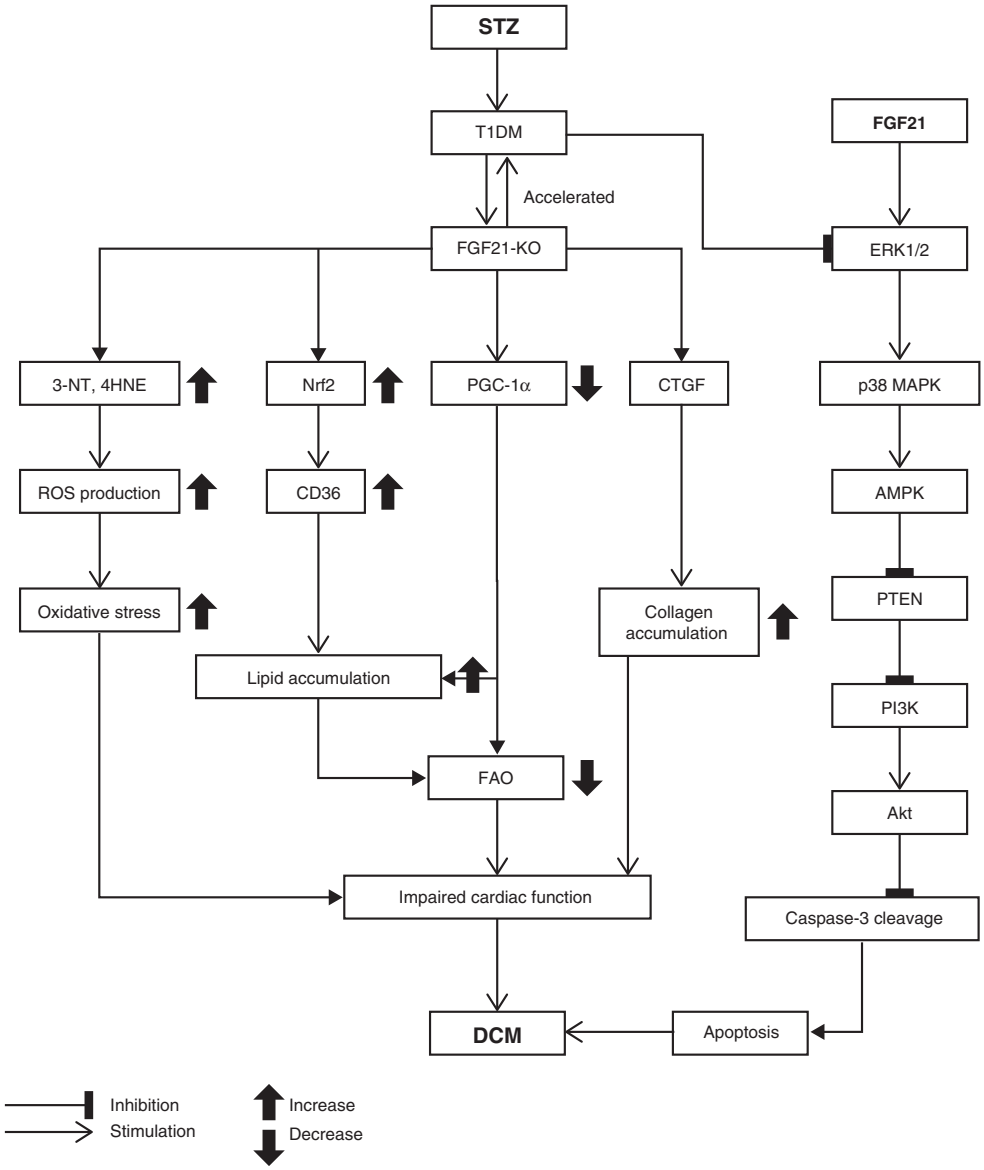

Figure 4
FGF21 protects the heart from diabetes induced cardiomyopathy. STZ, streptozotocin; T1DM, type 1 diabetes mellitus; FGF21-KO, FGF21 knock-out; Nrf2, nuclear factor (erythroid-derived 2)-like 2; 3NT, 3 nitrotyrosine; 4HNE, 4 hydroxynonenal; CTGF, connective tissue growth factor; PGC1α, peroxisome proliferator activated receptor gamma co-activator 1α; TG, triglyceride; FAO, fatty β acid oxidation; DCM, diabetic cardiomyopathy; Erk, extracellular signal-regulated kinase; p38 MAPK, mitogen-activated protein kinase 14; PTEN, phosphatase and tensin homologue; PI3K, phosphatidylinositol 3-kinase. Data from Yan *et al*. 2015, Zhang *et al*. 2015*a*.

It has been shown that these signaling pathways of FGF21 have the crosstalk at the secondary messenger levels such as ERK1/2 for anti-apoptosis (Zhang *et al*. 2015*a*), anti-hypertrophic (Planavila *et al*. 2013, 2014), and anti-oxidative stress (Yu *et al*. 2015) in the heart. Activation of FGFR/β-Klotho complex therefore could activate these effects of FGF21 simultaneously at this crosstalk. However, further studies are needed to investigate this issue.

Clinical evidence of the association between FGF21 and cardiovascular alteration

Evidence regarding the correlation of plasma FGF21 with cardiovascular alteration in clinical reports are summarized in Table 5. Serum FGF21 levels have been shown to have a strong correlation with waist circumference, systolic blood pressure, lower extremity arteriosclerotic disease (Zhang *et al*. 2015*b*) and carotid intima

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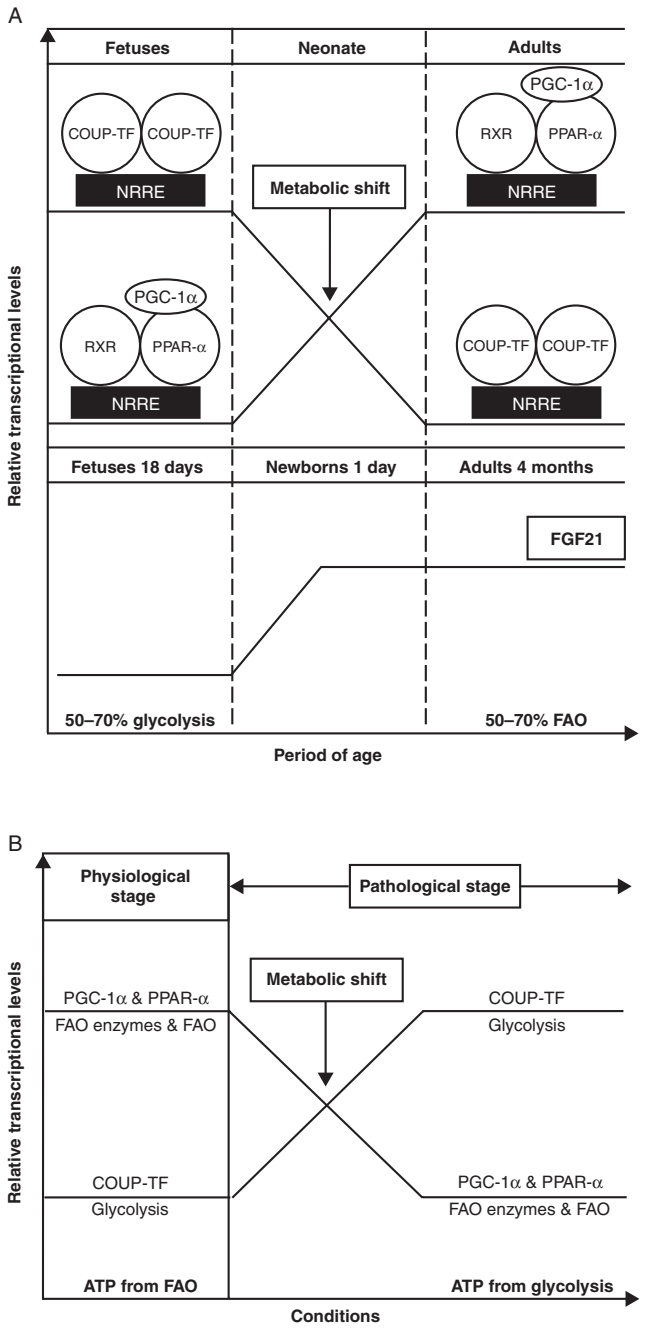

Figure 5
Relationship of FGF21 mRNA expression and FAO control in cardiomyocytes in different periods of age, and its alteration under physiological and pathological stages. (A) Relative transcriptional level of FGF21 mRNA and cardiac FAO enzyme gene regulator expression in different age brackets. (B) Relative transcriptional level of FGF21 mRNA and cardiac FAO enzyme gene regulator expression in physiological and pathological stages. PGC1α, peroxisome proliferator-activated receptor 1γ coactivator 1α; RXR, retinoid X receptor; PPARα, peroxisome proliferator activated receptor-α; COUP-TF, chicken ovalbumin upstream promoter transcription factor; NRRE, nuclear receptor response element; FAO, fatty acid β oxidation; ATP, Adenosine triphosphate.

media thickness (Chow *et al.* 2013) in T2DM (type 2 diabetes mellitus) patients. FGF21 level was also increased in atrial fibrillation (AF) patients and was shown to be an independent risk factor for AF (Han *et al.* 2015). In the cases of non-alcoholic fatty liver disease (NAFLD) and CAD, the serum FGF21 was associated with an adverse lipid profile and also showed a positive correlation with total cholesterol (TC) and triglycerides (TG) (Shen *et al.* 2013). Moreover, the CAD patient's serum FGF21 levels were also positively correlated with TG, fasting blood glucose, ApoB100, insulin, and HOMA-IR, and also have a negative correlation with HDL, and ApoA1 (Lin *et al.* 2010). Recent studies demonstrated that serum FGF21 levels correlate with metabolic status in patients. High serum FGF21 levels in several pathological conditions of the heart under metabolic dysregulation may be explained by FGF21 resistance conditions, which have been observed in *ex vivo* experiments with obese rat hearts (Patel *et al.* 2014) and *in vivo* experiments with DIO mice liver and white adipose tissue (Fisher *et al.* 2010). Therefore, serum FGF21 levels may be indicators of adverse metabolic dysregulation and prognosis for CVD.

The term 'FGF21 resistance' in the heart was first mentioned in chronic DIO rats by Patel and colleagues (Patel *et al.* 2014). They found that obese rat hearts had increased FGF21 mRNA, and FGF21 protein expression and secretion levels. Despite the high level of FGF21, disrupted FGF21-FGFR1-β-Klotho signaling and decreased ERK1/2, Akt and AMPK phosphorylation were observed under this condition (Patel *et al.* 2014). These findings indicate that obese condition caused the impairment of the FGF21 signaling cascades, and that the feedback mechanism allowed the increased production of FGF21 to overcome the FGF21 receptor signaling dysfunction. Unfortunately, the increased endogenous FGF21 level was not sufficient when the exogenous FGF21 administration comes into play a role for therapeutic strategy. The FGF21 resistance was also observed in clinical reports where serum FGF21 level was significantly increased in non-NAFLD (Shen *et al.* 2013), coronary heart disease (Lin *et al.* 2010, Shen *et al.* 2013), metabolic syndrome (Lee *et al.* 2014), and T2DM (Lenart-Lipinska *et al.* 2013). This condition is similar to what has been observed in subjects under 'insulin resistance' condition in which the impairment of insulin receptor and signaling cascades was found with increased plasma insulin level (Pratchayasakul *et al.* 2011, Pipatpiboon *et al.* 2012).

The cross sectional study in 15 male patients who underwent aorto-coronary bypass surgery showed that

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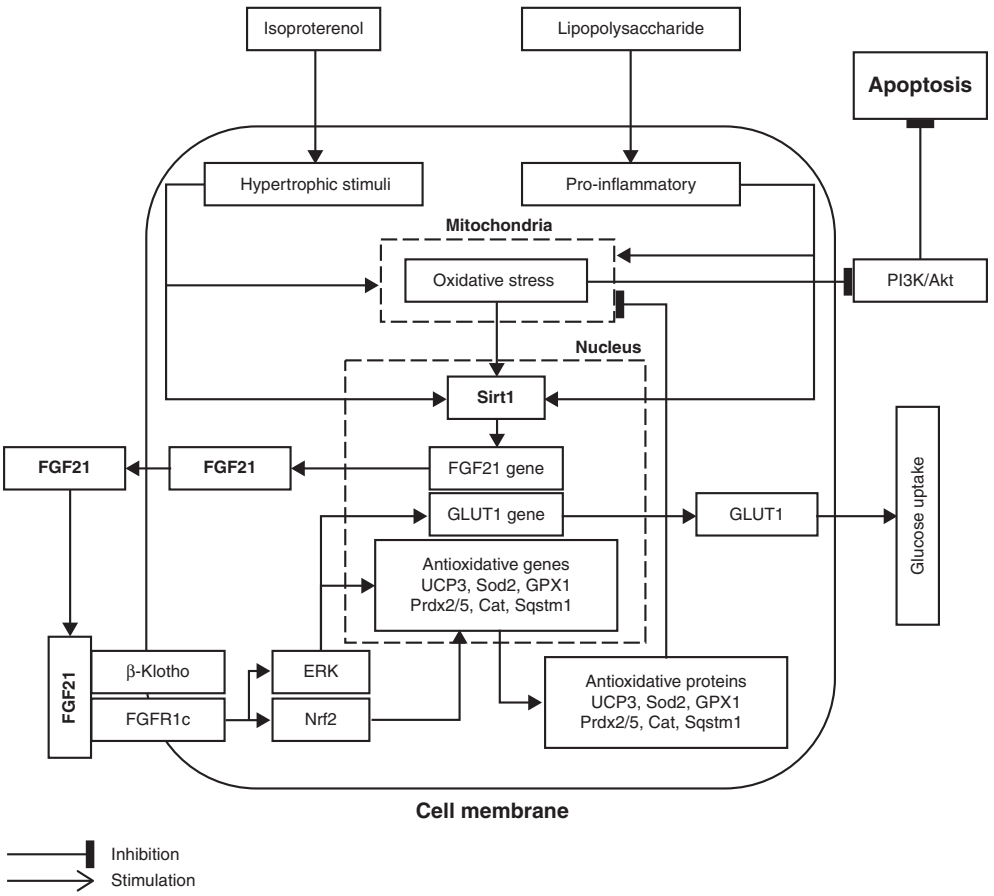

Figure 6
FGF21 mediates and plays a role in protecting against oxidative stress in cardiomyocytes. Sirt1, sirtuin 1; UCP3, uncoupling protein-3; Sod2, superoxide dismutase-2; Prdx2/5, peroxiredoxin-2/5; Cat, catalase; GPX1, glutathione peroxidase 1; Sqstm1, sequestosome 1; ROS, reactive oxygen species; Nrf2, Nuclear factor erythroid 2-related factor 2; PI3K, phosphatidylinositol 3-kinase. Data from Planavila *et al.* (2014) and Yu *et al.* (2015).

serum FGF21 levels increased to a peak at 6 h into surgery, and were associated with increased serum glucose, insulin, pro-inflammatory cytokines (TNFα, MCP1) and inflammatory cytokines (IL6, 8), but returned to baseline at 96 h after surgery (Kotulak *et al.* 2011). Moreover, epicardial fat and muscular FGF21 mRNA expression increased after surgery has reacted positively with blood glucose levels at the end of surgery (Kotulak *et al.* 2011) indicating that FGF21 mRNA expression and serum FGF21 levels regulated glucose homeostasis, increased the insulin sensitivity and attenuated the inflammatory process.

In a cross sectional study of 189 patients who underwent cardiac multidetector coronary computed tomography, it was found that serum FGF21 levels were associated with an adverse lipid profile and pericardial fat volume only in metabolic syndrome patients (Lee *et al.* 2014). Interestingly, cardiac tissue FGF21 mRNA and antioxidant genes (UCP3 and Sod2) are upregulated in six failing human hearts which may be mechanisms to preserve myocardial function in cases of heart failure (Planavila *et al.* 2014). All of these findings indicate that FGF21 plays an important role in metabolic regulation and attenuates cardiac oxidative stress in heart failure patients. The increased FGF21 level observed in heart failure patients was due to the FGF21 resistance as shown by a previous report (Planavila *et al.* 2014). Despite the increased endogenous FGF21 under this pathological condition, its level was still not sufficient to overcome the FGF21 resistance. Therefore, the role of exogenous FGF21 is considered as a potential therapeutic strategy to provide cardioprotective effects (Lu *et al.* 2010, Cong *et al.* 2013, Planavila *et al.* 2013, 2014, Zhang *et al.* 2015a). Previous reports at least from basic studies using exogenous FGF21 demonstrating the

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Table 5 The association between FGF21 level and cardiovascular alteration in clinical reports

Models	Type of study	N	Major findings	Interpretation	References
T2DM and CV risk factor prevalence study	Cohort study	670 (158: T2DM, 502: CV risk factor prevalence study)	– FGF21 shows a positive correlation with CIMT in women – FGF21 was an independent risk factor for ↑ CIMT, hsCRP ↑ dysglycemia ↑ dyslipidemia	Serum FGF21 levels are associated and independent established risk factor with carotid atherosclerosis.	Chow *et al.* (2013)
NAFLD, CAD, non NAFLD and CAD	Cohort study	253	↑ FGF21 in NAFLD vs non NAFLD ↑ FGF21 in CAD vs non CAD – Serum FGF21 levels showed a positive correlation with TC and TG	Serum FGF21 levels increased and were associated with adverse lipid profiles in NAFLD and CAD.	Shen *et al.* (2013)
DCMP and final stage HF	Cross-sectional study	6 HF 10 donor	↑ Cardiac tissue FGF21 mRNA ↑ Cardiac tissue antioxidant genes (UCP3 and Sod2 mRNA)	Serum FGF21 expression is up-regulated in the failing human heart	Planavila *et al.* (2014)
CAD	Cross-sectional study	135 patients	– Positively correlated with TG, FBG, Apo B100, insulin, and HOMA-IR – Negative correlation with HDL, Apo A1 – Independent association with TG and Apo A1	Serum FGF-21 level is associated with adverse lipid profiles in CAD patients	Lin *et al.* (2010)
IHD (Aorto-coronary bypass surgery)	Prospective	15 male patients	↑ Serum FGF21 levels during surgery, peak at 6 h ↑ Serum glucose, insulin, CRP, IL6, IL8, MCP1, and TNFα during surgery ↑ Epicardial fat FGF21 mRNA at after surgery – Muscles FGF21 mRNA are positively correlated with BG levels at the end of the surgery	FGF21 regulated glucose homeostasis, insulin sensitivity and attenuated inflammatory process.	Kotulak *et al.* (2011)
Patients had undergone 64 slice cardiac MDCT	Cross-sectional study	189 patients	↑ Serum FGF21 in MS, but not in diabetes or CAD – FGF21 showed a positive correlation with TG, LDL, insulin, HOMA-IR and PFV – Independent association with PFV	Serum FGF21 is associated with lipid profiles, insulin resistance, PFV and MS, but not altered in DM or CAD.	Lee *et al.* (2014)
T2DM	Prospective	9697	– Higher baseline plasma FGF21 levels had ↑ Total CV events – Finofibrate did not reduce CV events	High baseline plasma FGF21 levels in T2DM patients is associated with CV events.	Ong *et al.* (2015)
AF	Cross-sectional study	113 AF patients, 60 healthy volunteers	↑ FGF21 in permanent AF > persistent and paroxysmal AF – FGF21 was positively correlated with LA diameter	Serum FGF21 levels are increased in AF patients and are an independent risk factor for AF.	Han *et al.* (2015)

T2DM, type 2 diabetes mellitus; CV, cardiovascular; CIMT, carotid intima media thickening; hsCRP, high sensitivity C-reactive protein; FGF21, fibroblast growth factor 21; NAFLD, nonalcoholic fatty liver disease; CAD, coronary artery disease; TC, total cholesterol; TG, triglyceride; LDL, low density lipoprotein; DCMP, dilate cardiomyopathy; HF, heart failure; UCPs, uncoupling proteins; Sod, superoxide dismutase; FBG, fasting blood glucose; Apo, apolioprotein; HOMA-IR, homeostatic model assessment insulin resistance; HDL, high density lipoprotein; IHD, ischemic heart disease; IL, interleukin; MCP1, monocyte chemo attractant protein 1; TNFα, tumor necrosis factor alpha; cardiac MDCT, cardiac multidetector coronary computed tomography; MS, metabolic syndrome; PFV, pericardial fat volume; DM, diabetes mellitus; CVD, cardiovascular diseases; AF, atrial fibrillation; LA, left atrium.

improved cardiac function in cardiac I/R injury (Cong *et al*. 2013, Liu *et al*. 2013, Patel *et al*. 2014), cardiac hypertrophy (Planavila *et al*. 2013), and DCM (Zhang *et al*. 2015a) supported this hypothesis.

Although previous studies indicated that PPARα is the essential downstream signaling protein in regulating the expression of FGF21 mRNA in cardiomyocytes, and preserving the myocardial metabolism through regulating the FAO (Lu *et al*. 2010, Planavila *et al*), PPARα agonist (Finofibrate) has been shown to be unable to reduce cardiovascular events in T2DM patients after a 5-year follow-up (Ong *et al*. 2015). Moreover, the risk cardiovascular events in T2DM patients showed a correlation with baseline plasma FGF21 levels. Higher baseline plasma FGF21 levels correlated with an increased risk of cardiovascular events (Ong *et al*. 2015). This suggests that plasma FGF21 levels could be used as a biomarker of metabolic dysregulation, and increased plasma FGF21 levels may be indicative of a higher risk of cardiovascular events.

Despite the fact that FGF21 can preserve the heart in several pathological conditions, FGF21 resistance may be a limitation for the potential role of exogenous FGF21 administration. However, previous studies demonstrated that exogenous FGF21 exerted its effects in a dose-dependent manner (Cong *et al*. 2013, Planavila *et al*. 2014, Yu *et al*. 2015). Moreover, it is possible that FGF21 replacement during an early stage of FGF21 resistance may be more effective than late replacement. Future studies are needed to prove this hypothesis. Furthermore, long-term FGF21 treatment (Zhang *et al*. 2015a) may provide better outcome than the acute intervention (Patel *et al*. 2014). Lastly, combined therapy of FGF21 with specific drugs may provide better efficacy than FGF21 monotherapy. All of these hypothetical strategies still need to be verified in future studies.

Conclusion

Experimental studies of FGF21 in the heart have consistently demonstrated its beneficial effects in *in vitro*, *ex vivo* and *in vivo* models. Evidence has shown that FGF21 is crucial for cardioprotection in myocardial hypertrophy, ischemia, DCM and I/R injury. FGF21 provides its therapeutic benefits by attenuating apoptosis, oxidative stress and inflammation, and improving energy supply, and therefore could be used as an indicator of metabolic dysregulation. Moreover, FGF21 could be a potential therapeutic target for metabolic disorders and CVD in the future.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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References

Badman MK, Koester A, Flier JS, Kharitonenkov A & Maratos-Flier E 2009 Fibroblast growth factor 21-deficient mice demonstrate impaired adaptation to ketosis. *Endocrinology* **150** 4931–4940. (doi:10.1210/en.2009-0532)

Beenken A & Mohammadi M 2009 The FGF family: biology, pathophysiology and therapy. *Nature Reviews. Drug Discovery* **8** 235–253. (doi:10.1038/nrd2792)

Chow WS, Xu A, Woo YC, Tso AW, Cheung SC, Fong CH, Tse HF, Chau MT, Cheung BM & Lam KS 2013 Serum fibroblast growth factor-21 levels are associated with carotid atherosclerosis independent of established cardiovascular risk factors. *Arteriosclerosis, Thrombosis, and Vascular Biology* **33** 2454–2459. (doi:10.1161/ATVBAHA.113.301599)

Cong WT, Ling J, Tian HS, Ling R, Wang Y, Huang BB, Zhao T, Duan YM, Jin LT & Li XK 2013 Proteomic study on the protective mechanism of fibroblast growth factor 21 to ischemia-reperfusion injury. *Canadian Journal of Physiology and Pharmacology* **91** 973–984. (doi:10.1139/cjpp-2012-0441)

Ding X, Boney-Montoya J, Owen BM, Bookout AL, Coate KC, Mangelsdorf DJ & Kliewer SA 2012 βKlotho is required for fibroblast growth factor 21 effects on growth and metabolism. *Cell Metabolism* **16** 387–393. (doi:10.1016/j.cmet.2012.08.002)

Fisher FM, Chui PC, Antonellis PJ, Bina HA, Kharitonenkov A, Flier JS & Maratos-Flier E 2010 Obesity is a fibroblast growth factor 21 (FGF21)-resistant state. *Diabetes* **59** 2781–2789. (doi:10.2337/db10-0193)

Gheorghiade M & Bonow RO 1998 Chronic heart failure in the United States: a manifestation of coronary artery disease. *Circulation* **97** 282–289. (doi:10.1161/01.CIR.97.3.282)

Goetz R & Mohammadi M 2013 Exploring mechanisms of FGF signalling through the lens of structural biology. *Nature Reviews. Molecular Cell Biology* **14** 166–180. (doi:10.1038/nrm3528)

Hale C, Chen MM, Stanislaus S, Chinookoswong N, Hager T, Wang M, Veniant MM & Xu J 2012 Lack of overt FGF21 resistance in two mouse models of obesity and insulin resistance. *Endocrinology* **153** 69–80. (doi:10.1210/en.2010-1262)

Han X, Chen C, Cheng G, Xie C, Yang M, Shou X & Sun C 2015 Serum fibroblast growth factor 21 levels are increased in atrial fibrillation patients. *Cytokine* **73** 176–180. (doi:10.1016/j.cyto.2015.02.019)

Heather LC, Catchpole AF, Stuckey DJ, Cole MA, Carr CA & Clarke K 2009 Isoproterenol induces *in vivo* functional and metabolic abnormalities: similar to those found in the infarcted rat heart. *Journal of Physiology and Pharmacology* **60** 31–39.

Hotta Y, Nakamura H, Konishi M, Murata Y, Takagi H, Matsumura S, Inoue K, Fushiki T & Itoh N 2009 Fibroblast growth factor 21 regulates lipolysis in white adipose tissue but is not required for ketogenesis and triglyceride clearance in liver. *Endocrinology* **150** 4625–4633. (doi:10.1210/en.2009-0119)

Ito S, Kinoshita S, Shiraishi N, Nakagawa S, Sekine S, Fujimori T & Nabeshima YI 2000 Molecular cloning and expression analyses of mouse βklotho, which encodes a novel Klotho family protein.

http://joe.endocrinology-journals.org
DOI: 10.1530/JOE-15-0289

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Mechanisms of Development **98** 115–119. (doi:10.1016/S0925-4773(00)00439-1)

Itoh N & Ornitz DM 2011 Fibroblast growth factors: from molecular evolution to roles in development, metabolism and disease. *Journal of Biochemistry* **149** 121–130. (doi:10.1093/jb/mvq121)

Itoh N & Ohta H 2013 Pathophysiological roles of FGF signaling in the heart. *Frontiers in Physiology* **4** 247. (doi:10.3389/fphys.2013.00247)

Joki Y, Ohashi K, Yuasa D, Shibata R, Ito M, Matsuo K, Kambara T, Uemura Y, Hayakawa S & Hiramatsu-Ito M 2015 FGF21 attenuates pathological myocardial remodeling following myocardial infarction through the adiponectin-dependent mechanism. *Biochemical and Biophysical Research Communications* **459** 124–130. (doi:10.1016/j.bbrc.2015.02.081)

Kelly DP, Gordon JI, Alpers R & Strauss AW 1989 The tissue-specific expression and developmental regulation of two nuclear genes encoding rat mitochondrial proteins. Medium chain acyl-CoA dehydrogenase and mitochondrial malate dehydrogenase. *Journal of Biological Chemistry* **264** 18921–18925.

Kharitonenkov A 2008 FGF-21/FGF-21 receptor interaction and activation is determined by βKlotho. *Journal of Cellular Physiology* **215** 1–7. (doi:10.1002/jcp.21357)

Kharitonenkov A 2009 FGFs and metabolism. *Current Opinion in Pharmacology* **9** 805–810. (doi:10.1016/j.coph.2009.07.001)

Kotulak T, Drapalova J, Kopecky P, Lacinova Z, Kramar P, Riha H, Netuka I, Maly J, Housa D, laha J *et al.* 2011 Increased circulating and epicardial adipose tissue mRNA expression of fibroblast growth factor-21 after cardiac surgery: possible role in postoperative inflammatory response and insulin resistance. *Physiological Research* **60** 757–767.

Lee Y, Lim S, Hong ES, Kim JH, Moon MK, Chun EJ, Choi SI, Kim YB, Park YJ, Park KS *et al.* 2014 Serum FGF21 concentration is associated with hypertriglyceridaemia, hyperinsulinaemia and pericardial fat accumulation, independently of obesity, but not with current coronary artery status. *Clinical Endocrinology* **80** 57–64. (doi:10.1111/cen.12134)

Lenart-Lipinska M, Matyjaszek-Matuszek B, Gernand W, Nowakowski A & Solski J 2013 Serum fibroblast growth factor 21 is predictive of combined cardiovascular morbidity and mortality in patients with type 2 diabetes at a relatively short-term follow-up. *Diabetes Research and Clinical Practice* **101** 194–200. (doi:10.1016/j.diabres.2013.04.010)

Lin Z, Wu Z, Yin X, Liu Y, Yan X, Lin S, Xiao J, Wang X, Feng W & Li X 2010 Serum levels of FGF-21 are increased in coronary heart disease patients and are independently associated with adverse lipid profile. *PLoS ONE* **5** e15534. (doi:10.1371/journal.pone.0015534)

Liu SQ, Tefft BJ, Roberts DT, Zhang LQ, Ren Y, Li YC, Huang Y, Zhang D, Phillips HR & Wu YH 2012 Cardioprotective proteins upregulated in the liver in response to experimental myocardial ischemia. *American Journal of Physiology. Heart and Circulatory Physiology* **303** H1446–H1458. (doi:10.1152/ajpheart.00362.2012)

Liu SQ, Roberts D, Kharitonenkov A, Zhang B, Hanson SM, Li YC, Zhang LQ & Wu YH 2013 Endocrine protection of ischemic myocardium by FGF21 from the liver and adipose tissue. *Scientific Reports* **3** 2767. (doi:10.1038/srep02767)

Lockwood EA & Bailey E 1970 Fatty acid utilization during development of the rat. *Biochemical Journal* **120** 49–54. (doi:10.1042/bj1200049)

Lu Y, Liu JH, Zhang LK, Du J, Zeng XJ, Hao G, Huang J, Zhao DH, Wang GZ & Zhang YC 2010 Fibroblast growth factor 21 as a possible endogenous factor inhibits apoptosis in cardiac endothelial cells. *Chinese Medical Journal* **123** 3417–3421. (doi:10.3760/cma.j.issn.0366-6999.2010.23.008)

Mohammadi M, Olsen SK & Ibrahimi OA 2005 Structural basis for fibroblast growth factor receptor activation. *Cytokine & Growth Factor Reviews* **16** 107–137. (doi:10.1016/j.cytogfr.2005.01.008)

Neely JR & Morgan HE 1974 Relationship between carbohydrate and lipid metabolism and the energy balance of heart muscle. *Annual Review of Physiology* **36** 413–459. (doi:10.1146/annurev.ph.36.030174.002213)

Neely JR, Rovetto MJ & Oram JF 1972 Myocardial utilization of carbohydrate and lipids. *Progress in Cardiovascular Diseases* **15** 289–329. (doi:10.1016/0033-0620(72)90029-1)

Nishimura T, Nakatake Y, Konishi M & Itoh N 2000 Identification of a novel FGF, FGF-21, preferentially expressed in the liver. *Biochimica et Biophysica Acta* **1492** 203–206. (doi:10.1016/S0167-4781(00)00067-1)

Ong KL, Januszewski AS, O'Connell R, Jenkins AJ, Xu A, Sullivan DR, Barter PJ, Hung WT, Scott RS, Taskinen MR *et al.* 2015 The relationship of fibroblast growth factor 21 with cardiovascular outcome events in the Fenofibrate Intervention and Event Lowering in Diabetes study. *Diabetologia* **58** 464–473. (doi:10.1007/s00125-014-3458-7)

Patel V, Adya R, Chen J, Ramanjaneya M, Bari MF, Bhudia SK, Hillhouse EW, Tan BK & Randeva HS 2014 Novel insights into the cardio-protective effects of FGF21 in lean and obese rat hearts. *PLoS ONE* **9** e87102. (doi:10.1371/journal.pone.0087102)

Pipatpiboon N, Pratchayasakul W, Chattipakorn N & Chattipakorn SC 2012 PPARgamma agonist improves neuronal insulin receptor function in hippocampus and brain mitochondria function in rats with insulin resistance induced by long term high-fat diets. *Endocrinology* **153** 329–338. (doi:10.1210/en.2011-1502)

Planavila A, Redondo I, Hondares E, Vinciguerra M, Munts C, Iglesias R, Gabrielli LA, Sitges M, Giralt M, van Bilsen M *et al.* 2013 Fibroblast growth factor 21 protects against cardiac hypertrophy in mice. *Nature Communications* **4** 2019. (doi:10.1038/ncomms3019)

Planavila A, Redondo-Angulo I, Ribas F, Garrabou G, Casademont J, Giralt M & Villarroya F 2014 Fibroblast growth factor 21 protects the heart from oxidative stress. *Cardiovascular Research* **106** 19–31. (doi:10.1093/cvr/cvu263)

Potthoff MJ, Inagaki T, Satapati S, Ding X, He T, Goetz R, Mohammadi M, Finck BN, Mangelsdorf DJ, Kliewer SA *et al.* 2009 FGF21 induces PGC-1α and regulates carbohydrate and fatty acid metabolism during the adaptive starvation response. *PNAS* **106** 10853–10858. (doi:10.1073/pnas.0904187106)

Pratchayasakul W, Kerdphoo S, Petsophonsakul P, Pongchaidecha A, Chattipakorn N & Chattipakorn SC 2011 Effects of high-fat diet on insulin receptor function in rat hippocampus and the level of neuronal corticosterone. *Life Science* **88** 619–627. (doi:10.1016/j.lfs.2011.02.003)

Razeghi P, Young ME, Alcorn JL, Moravec CS, Frazier OH & Taegtmeyer H 2001 Metabolic gene expression in fetal and failing human heart. *Circulation* **104** 2923–2931. (doi:10.1161/hc4901.100526)

Sack MN, Disch DL, Rockman HA & Kelly DP 1997 A role for Sp and nuclear receptor transcription factors in a cardiac hypertrophic growth program. *PNAS* **94** 6438–6443. (doi:10.1073/pnas.94.12.6438)

Shen Y, Ma X, Zhou J, Pan X, Hao Y, Zhou M, Lu Z, Gao M, Bao Y & Jia W 2013 Additive relationship between serum fibroblast growth factor 21 level and coronary artery disease. *Cardiovascular Diabetology* **12** 124. (doi:10.1186/1475-2840-12-124)

Suzuki M, Uehara Y, Motomura-Matsuzaka K, Oki J, Koyama Y, Kimura M, Asada M, Komi-Kuramochi A, Oka S & Imamura T 2008 βKlotho is required for fibroblast growth factor (FGF) 21 signaling through FGF receptor (FGFR) 1c and FGFR3c. *Molecular Endocrinology* **22** 1006–1014. (doi:10.1210/me.2007-0313)

Vega RB, Huss JM & Kelly DP 2000 The coactivator PGC-1 cooperates with peroxisome proliferator-activated receptor α in transcriptional control of nuclear genes encoding mitochondrial fatty acid oxidation enzymes. *Molecular and Cellular Biology* **20** 1868–1876. (doi:10.1128/MCB.20.5.1868-1876.2000)

Wente W, Efanov AM, Brenner M, Kharitonenkov A, Koster A, Sandusky GE, Sewing S, Treinies I, Zitzer H & Gromada J 2006 Fibroblast growth factor-21 improves pancreatic β-cell function and survival by activation of extracellular signal-regulated kinase 1/2 and Akt signaling pathways. *Diabetes* **55** 2470–2478. (doi:10.2337/db05-1435)

Wu X, Qi YF, Chang JR, Lu WW, Zhang JS, Wang SP, Cheng SJ, Zhang M, Fan Q & Lv Y 2014 Possible role of fibroblast growth factor 21 on atherosclerosis via amelioration of endoplasmic reticulum

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DOI: 10.1530/JOE-15-0289

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stress-mediated apoptosis in apoE mice. *Heart and Vessels* **30** 657–668. (doi:10.1007/s00380-014-0557-9)

Yan X, Chen J, Zhang C, Zhou S, Zhang Z, Chen J, Feng W, Li X & Tan Y 2015 FGF21 deletion exacerbates diabetic cardiomyopathy by aggravating cardiac lipid accumulation. *Journal of Cellular and Molecular Medicine* **19** 1157–1568. (doi:10.1111/jcmm.12530)

Yie J, Hecht R, Patel J, Stevens J, Wang W, Hawkins N, Steavenson S, Smith S, Winters D, Fisher S *et al.* 2009 FGF21 N- and C-termini play different roles in receptor interaction and activation. *FEBS Letters* **583** 19–24. (doi:10.1016/j.febslet.2008.11.023)

Yu Y, Bai F, Liu Y, Yang Y, Yuan Q, Zou D, Qu S, Tian G, Song L & Zhang T 2015 Fibroblast growth factor (FGF21) protects mouse liver against D-galactose-induced oxidative stress and apoptosis via activating Nrf2 and PI3K/Akt pathways. *Molecular and Cellular Biochemistry* **403** 287–299. (doi:10.1007/s11010-015-2358-6)

Zhang C, Huang Z, Gu J, Yan X, Lu X, Zhou S, Wang S, Shao M, Zhang F & Cheng P 2015*a* Fibroblast growth factor 21 protects the heart from apoptosis in a diabetic mouse model via extracellular signal-regulated kinase 1/2-dependent signalling pathway. *Diabetologia* **58** 1937–1948. (doi:10.1007/s00125-015-3630-8)

Zhang X, Hu Y, Zeng H, Li L, Zhao J, Zhao J, Liu F, Bao Y & Jia W 2015*b* Serum fibroblast growth factor 21 levels is associated with lower extremity atherosclerotic disease in Chinese female diabetic patients. *Cardiovascular Diabetology* **14** 190. (doi:10.1186/s12933-015-0190-7)

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