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THE ROLE OF FIBROBLAST GROWTH FACTOR 21 IN HEART FAILURE

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BACKGROUND

Heart failure (HF) is one of the leading causes of mortality worldwide, affecting millions of people every year [1]. This condition develops due to the heart's inability to meet the body's physiological demands. Due to the high energy demand of cardiac muscle cells (cardiomyocytes), HF is often associated with mitochondrial (mt) dysfunction. In patients with dilated cardiomyopathy (DCM), one type of HF, mt dysfunction results from mitochondrial DNA (mtDNA) damage and deletion [2],[3],[4]. Furthermore, disease progression exhibits increased cell death and oxidative stress induction. A cytokine known as Fibroblast Growth Factor 21 (FGF21) is produced in response to mitochondrial or oxidative stress in the body [5]. FGF21 is a metabolic regulator that is mainly secreted by the liver and plays a role in controlling energy homeostasis, lipid metabolism, and glucose regulation in the body [6]. Furthermore, FGF21 provides a cardioprotective effect in response to cardiac stresses [7].

Our prior unpublished work showed elevated s-FGF21 levels in HF patients who have undergone VAD transplantation. Immunohistochemistry (IHC) staining performed on the same HF patients showed presence of FGF21 proteins in the heart. However, the FGF21 gene expression levels in HF patients were not high enough to suggest FGF21 was being produced in the heart. As such, the signaling pathway that leads to the production and effects of FGF21 during heart failure remains unknown. Based on the data gathered, we **hypothesize that in HF, cardiomyocytes**

experience oxidative stress and initiate signaling pathways that leads to the production of FGF21 by other organs.

METHODS

To understand the origin and effects of FGF21, HF was induced in four mice by performing transverse aortic constriction (TAC) combined with coronary artery ligation, which was performed by collaborators in the Drakos laboratory. The TAC procedure involved tying a 27½ gauge blunt needle against the transverse aorta to cause partial occlusion [8]. Following the TAC procedure, the mice's coronary artery was ligated through an open chest surgery [9]. Messenger RNA (mRNA) was extracted, and FGF21 gene expression was examined via quantitative Polymerase Chain Reaction (qPCR). The mean and the standard error of the mean (SEM) of the fold change in gene expression were calculated to examine the change between the control and the experimental group. A two-tailed t-test was performed on the fold change in gene expression to determine the significance difference between the two groups. Differences were considered significant for $P<0.05$. The qPCR results were confirmed through FGF21 protein expression via western blot.

RESULTS/ DISCUSSION

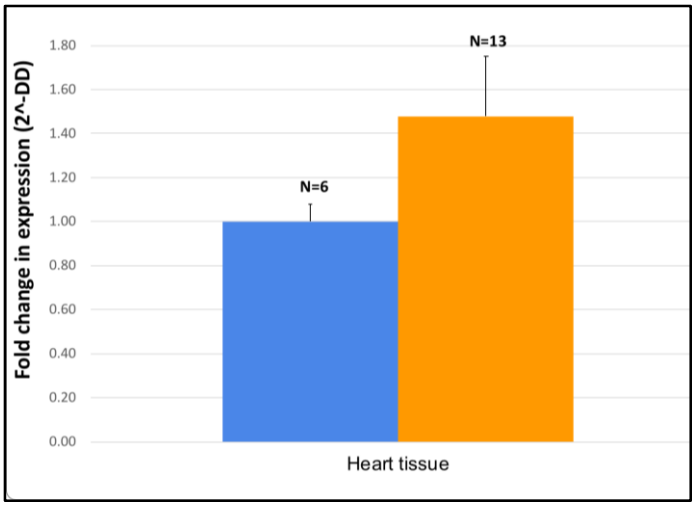

Fig 1. Fold change in FGF21 gene expression comparison between HF patients (orange) and healthy human subjects (blue) in heart tissues. HF patients (n=6, p=0.12), healthy humans (n=13).

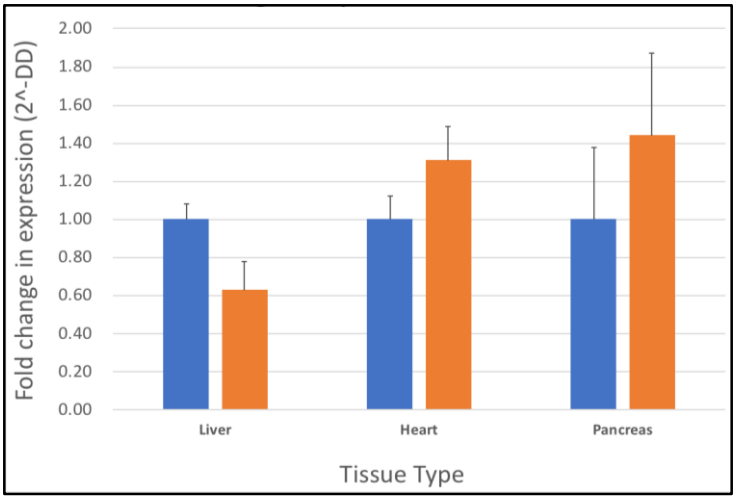

Fig 2. Fold change in FGF21 gene comparison between TAC mice (orange) and healthy mice (blue) in liver (p-value=0.09), heart (p-value=0.20), and pancreas (p-value=0.47). TAC mice n=4, healthy mice n=4.

A trend towards mild upregulation of FGF21 gene was seen in HF patients (1.5 times, p-value=0.12) in comparison to the control group, indicating the presence of higher levels of FGF21 gene in the heart during HF (Fig 1). However, the statistical analysis showed no significant difference between the experiment and control group. Similar to the human qPCR data, there was an upregulation of cardiac FGF21 gene in the TAC mice (1.31 folds±0.18). The liver showed a downregulation in FGF21 gene expression (0.63±0.15) in the TAC mice. Although the pancreas showed an upregulation of the FGF21 gene (1.44 folds±0.43), the statistical analysis showed no significant difference between the experiment and control group.

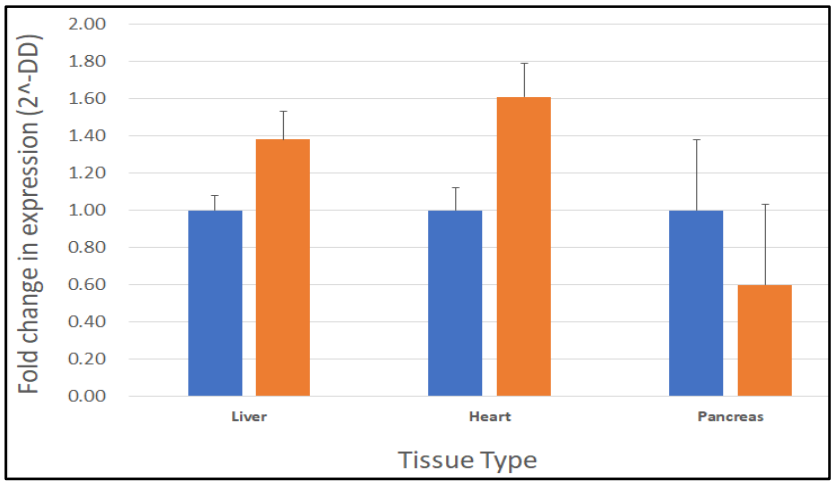

Fig 3. Fold change in KLB gene comparison between TAC mice (orange) and healthy mice (blue) in liver, heart, and pancreas. TAC mice n=4, healthy mice n=4.

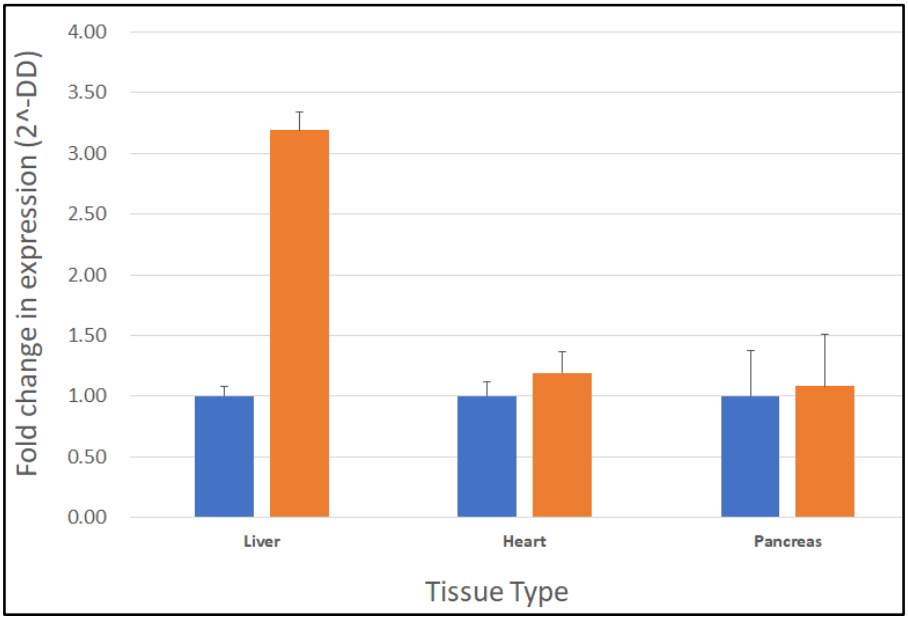

Fig 4. Fold change in FGFR1 gene comparison between TAC mice (orange) and healthy mice (blue) in liver, heart, and pancreas. TAC mice n=4, healthy mice n=4.

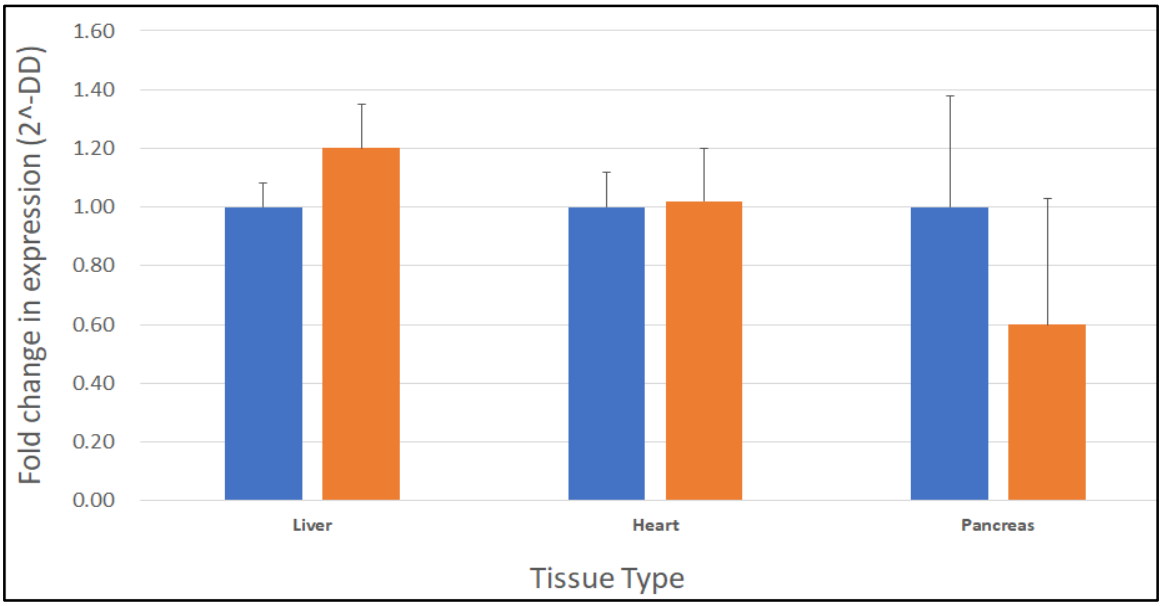

Fig 5. Fold change in CPT1B gene comparison between TAC mice (orange) and healthy mice (blue) in liver, heart, and pancreas. TAC mice n=4, healthy mice n=4.

Activation of FGF21 requires the binding of a transmembrane co-receptor, *β-Klotho (KLB),* and a fibroblast growth factor receptor 1 (FGFR1) [7], [11]. This FGFR1-mediated activation further activates the Sirt1-PPARα pathway in response to cardiac and oxidative stresses to provide a cardioprotective effect [7], [13]. Our data showed a significant increase of FGFR1 and KLB

expressions in the heart (Fig 3 and 4), which was consistent with the upregulation of FGF21. Although the low increase in cardiac FGF21 expression (Fig 1 and 2) in mice and humans indicates that the heart is not an FGF21-producing site, it confirms that cardiac cells are targets of FGF21. The western blot data also confirms the presence of FGF21 in the heart (Fig 6). Previous studies have also shown that despite the low mRNA expressions of FGF21, KLB, and FGFR1, the protein levels in cardiac cells were significant [14].

The FGF21 secretion following cardiac injury seems to be an initial step that will activate signaling pathways to prevent cardiomyocyte apoptosis [15]. In addition to its role as an anti-apoptotic agent, FGF21 also regulates energy in the heart by promoting fatty acid β oxidation (FAO) [15]. FAO involves a rate limiting step which is managed by a protein known as carnitine palmitoyltransferase 1B (CPT1*β)* [16]. Our study did not show an increase in CPT1*β* gene expression in any of the tissues examined in TAC mice (Fig 5). This might be due to the shift in energy supply from FAO in diseased hearts. Similar results have been previously reported in which the CPT1*β* mRNA expression levels were the same between the knockout and wildtype mice. However, the low CPT1*β* protein levels show that it is a post-transcriptional process [16].

In the current study, we found a down regulation of FGF21 in the liver (Fig 2) despite the upregulation of KLB and FGFR1 mRNA expressions (Fig 3 and 4). This finding contradicts previous studies which indicate that since FGF21 is a metabolic regulator, the liver is considered as its primary production site [17]. Interestingly, elevated FGFR1 mRNA values were seen in most of the tissues examined. We speculate that TAC may not have been a good model of ischemic HF and as such, we are not seeing any significance between the TAC and control mice. Future work in this field involves a different procedure to induce HF in mice. A power analysis

should be conducted to determine the suitable sample size to appropriately detect the effects of the result since one limitation of this research was the low sample size. Furthermore, this study only investigated 11 tissues and reported on 3 that showed interesting results. Investigations of other tissues could help narrow the origin of FGF21 during HF.

In summary, our study shows that the heart is an FGF21 target site despite not being a production site. Understanding the origin and effect of FGF21 in DCM could lead to the development of targeted therapies that would prevent cell death during progression of the disease. In addition, this research also opens up doors for investigating FGF21's ability to serve as a metabolic stress biomarker for heart failure in clinical settings.

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