SUPPLEMENTAL MATERIAL

I. SUPPLEMENTAL METHODS

Blood FGF21 measurements

Serum or plasma FGF21 levels were measured using the Quantikine ELISA Human FGF21 kit (R&D Systems, Minneapolis, coefficient of variation: Intra-Assay, 3.4%, Inter-assay 7.5%) according to the manufacturer's instructions. Our control values were similar to prior studies conducted in either serum or plasma with this kit, and its accuracy has been validated in both[50-55]. Samples were run in duplicate. For each well, 50 µL standard or undiluted plasma or serum was added to 100 µL assay diluent, incubated for 2h at room temperature, washed 4X, then 200 µL conjugate was added to each well and incubated for 2h at room temperature. Wells were washed 4X, then 200 µL substrate solution was added and incubated for 30 min at room temperature, protected from light, then 50 µL stop solution was added to each well. Absorbance readings at 450 nm were recorded immediately following the addition of the stop solution, with a wavelength correction reading at 540 nm. For two samples, values were below the detection threshold, and were changed from negative values to 1 to allow log transformation.

Immunohistochemistry

The anti-FGF21 antibody (Abcam, ab171941) was used. Tissue pieces were fixed in 10% buffered formalin and embedded in paraffin. Sections 6 µm thick were made using a vibratome and mounted on coverglass. Sections were deparaffinized using Histosol (Fisher Scientific) then rehydrated by successively submerging in 100%, 95%, 70%

ethanol followed by distilled H_2O. Antigen retrieval was performed by submerging slides in antigen retrieval buffer (containing 60 mM sodium citrate and 40 mM citric acid, pH = 6.0) and heating in a microwave at a gentle boil for 10 minutes. Each section was then washed in wash buffer (containing 150mM NaCl, 50mM Tris pH=7.8, and 0.025% v/v Tween-20), permeabilized by incubation in permeabilization buffer (containing 150mM NaCl, 50mM Tris pH=7.8, 0.2% v/v Tween-20, and 1% w/v bovine serum albumin (BSA) for 15 minutes at room temperature, then washed 3X in wash buffer. Sections were then incubated in blocking buffer (containing 150 mMNaCl, 50 mM Tris pH=7.8, 0.025% v/v Tween-20, 10% v/v goat serum, and 1% w/v BSA) for 1 hour at room temperature, then in primary antibody buffer (containing 150 mMNaCl, 50 mM Tris pH=7.8, 0.025% v/v Tween-20, 1% w/v BSA, and 1:200 primary antibody) overnight at 4C. The following day, sections were washed 3X 5 minutes in wash buffer then incubated in oxidation buffer (containing 150 mMNaCl, 50 mM Tris pH=7.8, 0.025% v/v Tween-20, and 0.3%v/v H_2O_2) for 15 minutes at room temperature, followed by one wash in wash buffer. Sections were then incubated in secondary antibody buffer (containing 150 mMNaCl, 50 mM Tris pH=7.8, 0.025% v/v Tween-20, 1% w/v BSA, and 1:500 secondary antibody) for 1 hour at room temperature. Sections were then washed 3X with wash buffer and developed with DAB solution according to manufacturer's instructions, washed 3X with wash buffer, then washed 3x with MilliQ H_2O. Sections were counterstained with Mayer's hematoxylin for 1 minute at room temperature, washed 3X with 10mM NaOH, then 3X with MilliQ H_2O. Sections were then coverslipped using IHC mounting medium. Images were taken using an Olympus DSP camera mounted on an optical microscope.

Cardiac FGF21 gene expression measurement

RNA was isolated from the LV tissue samples using the miRNeasy Mini Kit (Qiagen) or Purelink RNA Mini Kit (Thermo). Single strand complementary DNA (cDNA) was generated using Superscript Vilo IV master mix (Thermo) according to the manufacturer's instructions using 1 μg RNA. cDNA was diluted 1:10 with Ultrapure distilled water (Thermo Fisher). Reactions were performed with 300 nM primers, using Power SYBR Green PCR Master Mix (Thermo) per the manufacturer's recommendations. Quantification of gene expression was performed on a 96-well CFX Real-Time PCR System (BioRad). Analysis was performed by using the 2-ΔΔCt method, using the housekeeping gene *RPLP0*. Primers were based on prior publications, designed on NCBI Primer -BLAST, or obtained from Primerbank. A list of primers is below.

Gene	Primer
ACACB	CAAGCCGATCACCAAGAGTAAA
	CCCTGAGTTATCAGAGGCTGG
BDH1	GTGTAGAAGCGTCCGGGTG
	GCAACGGGTGTTAGAATGGC
CPT1B	GAGCAGCACCCCAATCAC
	AACTCCATAGCCATCATCTGCT
FGF21	CAGCGGTACCTCTACACAGATG
	GCTTTCAGCTGCAGGAGACT
FGFR1	GTCTGCTGACTCCAGTGCAT
	ACGGTTGGGTTTGTCCTTGT
FGFR2	ACAGTTTCGGCTGAGTCCAG
	GGTGTCTGCCGTTGAAGAGA
FGFR3	CCCTACGTTACCGTGCTCAA
	CAATAGAATTGCCCGCCAGG
FGFR4	GTGGGCAGCATCCGCTATAA
	CCATGTGGGGTCCTCCTCTG
KLB	AGATGTGCAGGGCCAGTTT
	GCCACAGACTCGGGCTTA

OXCT1	TGAGGCGCTGAGAGGAACTT
	GAGAGGAGTTTGAGAGCCGC
PDK1	CTGTGATACGGATCAGAAACCG
	TCCACCAAACAATAAAGAGTGCT
PDK4	GGAGCATTTCTCGCGCTACA
	ACAGGCAATTCTTGTCGCAAA
RPLP0	TGGTCATCCAGCAGGTGTTCGA
	ACAGACACTGGCAACATTGCGG
SLC2A1	ATGGCCGGGGTCCTATAAAC
	TGTAGCCAAACTGCAGGGAG
SLC16A1	AGGTCCAGTTGGATACACCCC
	GCATAAGAGAAGCCGATGGAAAT
SLC16A7	GGGTTGGATTGTGGTTGGAG
	TCCTGCGTACATAACAGCCAG

Analysis of RNA-Seq Datasets

The NCBI Gene Expression Omnibus was searched for datasets obtained from human cardiac tissue, restricted to published studies on ischemic, nonischemic, hypertrophic, and restrictive cardiomyopathies using bulk RNA-Seq technology. We did not use single-cell RNA-Seq studies, because few were currently available and there is ongoing uncertainty about the interpretation of few or no reads per cell. We extracted metadata regarding samples, gene counting methods, and read format from the series matrix file, and raw or normalized count data for *FGF21* expression (searching for FGF21, ENSG00000105550, or ENST00000222157) from the supplementary data files. One dataset (GSE55296) was excluded as sample information was unavailable, one dataset (GSE65446) was excluded as the original study had been retracted. In these excluded studies FGF21 was either filtered out due to low expression or present in only the minority of individuals at low levels, similar to the presented data. The presented analysis includes seven datasets collected between 2014 and 2021.